Sustainable Agriculture Reviews 31

Eric Lichtfouse *Editor*

Sustainable Agriculture Reviews 31

Biocontrol

Springer

Sustainable Agriculture Reviews

Volume 31

Sustainable agriculture is a rapidly growing field aiming at producing food and energy in a sustainable way for humans and their children. Sustainable agriculture is a discipline that addresses current issues such as climate change, increasing food and fuel prices, poor-nation starvation, rich-nation obesity, water pollution, soil erosion, fertility loss, pest control, and biodiversity depletion.

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ISSN 2210-4410 ISSN 2210-4429 (electronic)
Sustainable Agriculture Reviews
ISBN 978-3-319-94231-5 ISBN 978-3-319-94232-2 (eBook)
https://doi.org/10.1007/978-3-319-94232-2

Library of Congress Control Number: 2018947302

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Biocontrol is an ecological method of controlling pests such as insects, mites, weeds and plant diseases using other organisms.[1] This practice has been used for centuries, and the first use of an insect species to control an insect pest has been reported by the botanist Ji Han in 304 AD,[2] who wrote that people sold yellow citrus ants and their nest to protect citrus fruits from insects. The first international shipment of an insect as biological control agent was made by Charles V. Riley in 1873, shipping to France the predatory mites *Tyroglyphus phylloxera* to help fight the *grapevine phylloxera*, *Daktulosphaira vitifoliae*, that was destroying grapevines in France. *Rodolia cardinalis*, the vedalia beetle (photograph below), was imported from Australia to California in the nineteenth century, successfully controlling cottony cushion scale.

Rodolia cardinalis, the vedalia beetle. Katja Schulz 2014 CC-BY-2.0

[1] https://en.wikipedia.org/wiki/Biological_pest_control.

[2] https://en.wikipedia.org/wiki/Nanfang_Caomu_Zhuang.

Biocontrol relies on predation, parasitism, herbivory or other natural mechanisms. There are three strategies for biocontrol: importation, where a natural enemy of a pest is introduced in the hope of achieving control; augmentation, in which a large population of natural enemies are administered for quick pest control; and conservation, in which measures are taken to maintain natural enemies through regular re-establishment.

Natural enemies of insect pests, also known as biological control agents, include predators, parasitoids, pathogens and competitors. Biological control agents of plant diseases are most often referred to as antagonists. Biological control agents of weeds include seed predators, herbivores and plant pathogens. Biological control can have side effects on biodiversity through attacks on non-target species by any of the same mechanisms, especially when a species is introduced without thorough understanding of the possible consequences.

This book presents advanced ecological techniques for crop cultivation. Chapters are arranged into four sections, namely general aspects, weeds, fungi, and worms and microbes. Chapter 1 by Ginigaddara lists the ecosystem services provided by ecological rice cultivation in Asia, such as carbon sequestration, weed control and duck meat food. The mutual benefits of crop and livestock production are then explained in Chap. 2 by Aryal et al., with focus on pastoralism and transhumance. Weed control using chemical and ecological techniques is described in detail in Chaps. 3 and 4 by Pakdaman and Goltapeh, and Kaur et al. The use of fungi to control pests is presented in Chap. 5 by Pakdaman and Goltapeh, whereas the ecological management of chilli anthracnose, a fungal pest, is reviewed in Chap. 6 by Rai et al. Askary et al. discuss the management of nematode pests and the use of nematode for insect control in grapevine in Chaps. 7 and 8. Bacterial–plant interactions in soils and applications to pest control are reviewed in Chap. 9 by Crecchio et al. Chemotaxis, the movement of an organism in response to a chemical stimulus, is explained for the pollutant-degrading Pseudomonas species in Chap. 10 by Meliani and Bensoltane.

Aix-en-Provence, France Eric Lichtfouse

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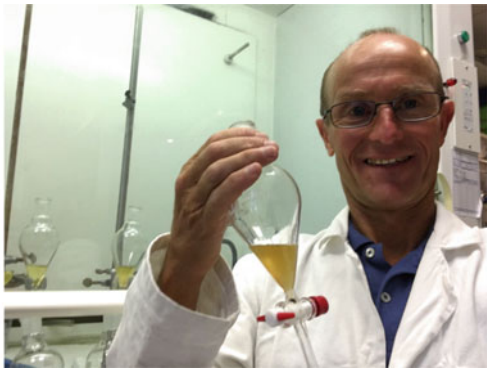
About the Editor

Left: Giving lectures and workshops on scientific writing. Right: Extracting soil organic compounds using $CHCl_3$-methanol 3/1 v/v

Eric Lichtfouse born in 1960 is a biogeochemist working on carbon sequestration and professor of scientific communication at the European Centre for Research and Education in Geosciences (CEREGE), University of Aix Marseille, Aix-en-Provence, France. He got a Ph.D. in organic geochemistry at the University of Strasbourg in 1989 for the discovery of new fossil steroids in sediments and petroleum.[3] Those molecular fossils are now widely applied as maturation parameters

[3] Occurrence of 2-methyl-, 3-methyl- and 6-methyltriaromatic steroid hydrocarbons in geological samples. *Tetrahedron Lett.* https://hal.archives-ouvertes.fr/hal-00175050. Synthesis of triaromatic steroid hydrocarbons methylated at position 2, 3 or 6: molecular fossils of yet unknown biological origin. *Tetrahedron* https://hal.archives-ouvertes.fr/hal-00175800. A molecular and isotopic study of the organic matter from the Paris Basin, France. *Geochimica et Cosmochimica Acta* https://hal.archives-ouvertes.fr/hal-00191892.

for basic research and applied petroleum exploration. He has then invented the ^{13}C-dating method allowing to measure the dynamics of soil organic molecules.[4] This method is based on the unexpected discovery of 'temporal pools' of the same organic substance of different relative ages in a same sample. As a consequence, this non-radioactive, relative dating method opens research on the origin, history and fate of organic substances in complex organic media such as waters, soils, sediments, waste, food and living organisms. The method principle can be applied to any isotope.

He is the founder and chief editor of the journal Environmental Chemistry Letters, of 3.6 impact factor, the series Environmental Chemistry for a Sustainable World and the series Sustainable Agriculture Reviews, published by Springer Nature.[5] He is also the founder and chief editor of the Newsletter *Publier La Science*, published by the French Institute for Agricultural Research (INRA).[6] As a former chief editor of the journal Agronomy for Sustainable Development, his editorial team has raised the journal impact factor from 0.3 to 4.1, ranking 4 in the Agronomy category. He has published the book Scientific Writing for Impact Factor Journal, describing the micro-article, a new tool to identify the novelty of experimental results.[7] He has given conferences and workshops on scientific writing in Australia, China, Egypt, Europe, Mexico, Tunisia and the USA.

He has 164 publications in Google Scholar and ResearchGate, with h indexes of 29 and 30. He has 95 publications in Thomson ReseacherID, with h index of 23. He has edited more than 50 books published by Springer Nature. He got the Analytical Chemistry Prize of the French Chemical Society, the Grand University Prize of Nancy University and a Journal Citation Award by the Essential Science Indicators.[8] In sport, he received the bronze medal at the World ITU Cross Triathlon Championships and he was selected 6 times for the World Championships of Ironman, Ironman 70.3 and Xterra.

He is open to collaborations with governments, universities, departments, scientists and students for performing cutting-edge science and publishing in high-rank journals.

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[4] ^{13}C-dating, the first method to calculate the relative age of molecular substance homologues in soil. *Env. Chem. Lett.* https://hal.archives-ouvertes.fr/hal-00674221. Plant wax *n*-alkanes trapped in soil humin by non-covalent bonds. *Die Naturwissenschaften* https://hal.archives-ouvertes.fr/hal-00193669. Temporal pools of individual organic substances in soil. *Analusis* https://hal.archives-ouvertes.fr/hal-00262449. ^{13}C Labelling of soil *n*-hentriacontane (C_{31}) by maize cultivation. *Tetrahedron Lett*. https://hal.archives-ouvertes.fr/hal-00192951.

[5] ECL: https://www.springer.com/journal/10311. ECSW: http://www.springer.com/series/11480. SAR: http://www.springer.com/series/8380.

[6] https://www6.inra.fr/caps-publierlascience.

[7] https://www.novapublishers.com/catalog/product_info.php?products_id=42211

[8] http://archive.sciencewatch.com/inter/jou/2010/10novAgrSusDev.

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Ecological Intensification in Asian Rice Production Systems

G. A. S. Ginigaddara

Abstract More rice is needed to feed the increasing population in Asia and beyond. Although rice breeders and scientists have produced high yielding varieties, soils have been degraded by intensive agriculture. Hence alternative intensification strategies are needed for sustaining the rice production in Asia. This chapter reviews rice production systems in Asia and potential ecological intensification stratagies. There are well adopted, genetically diverse rice groups performing under varied soil and climatic conditions. Due to the varied topography, soil types, microclimate and cultural diversity throughout, rice production systems are diverse. Ecosystem services from these diverse rice ecosystems are also unique to the location of origin, culture of the people associated, functioning style and the expected outcome of each of these rice production systems.

Keywords Asian rice production systems · Ecological intensification
Integrated nutrient management · Precision farming

1 Introduction

The whole world today is facing challenges of meeting the rapidly increasing food demand through environmentally sustainable means ensuring the world poorest population too is secured with food. Rice is the staple food for more than half of the world population while it is being the major source of energy and vitamin supplier of Asians. Asian rice production systems account for 91% of the world rice production. The present exploitable gap between average farm yields and genetic yield potential is closing in Asia and further expansion of the rice cultivation lands are also becoming extremely limited while the need of increasing the rice production is still in the top of concern.

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E. Lichtfouse (ed.), *Sustainable Agriculture Reviews 31*, Sustainable Agriculture Reviews 31, https://doi.org/10.1007/978-3-319-94232-2_1

Use of massive inputs of anthropogenic origin in rice production systems at present has started threatening to the human health, other living organisms associated with the systems and polluting the environment at length. The climatic change is menacing the rice production in Asia creating water scarcities, rising temperature and creating frequent extreme weather conditions. Hence alternative strategies are needed to address few or more of these challenges, emerging while meeting the increasing demand for rice simultaneously.

This article reviews the existing information and findings of the ecological intensification in Asian rice production systems as an strategy to face the aforesaid challenges and its future trends.

2 Significance of Rice in Asia

Rice is the life, culture, customs, traditions and spirituality of Asians. It is the staple food of them. Rice and fish is the main meal of Japan, Malaysia, Cambodia, Philippines, Indonesia, Korea, Thailand, and China while rice, vegetables, pulses and meat in Sri Lanka, India, Nepal and Bangladesh. There are over 500 ways of cooking rice while wines, beers and various forms of liqueur are also produced from it.

Rice comes in different colours such as white, brown, black, grey, red and purple and also with different shapes, sizes and aroma. Rice is the main source of energy and vitamins of majority of the Asians. The consumption per capita of rice in some Asian countries reaches 200 kg of white rice while falling approximately to 50 kg of high quality grains in rich, industrialized and high income countries like Japan and Republic of Korea. However, the one fourth of poor in developing Asian countries are still having a considerable unmet demand for rice (Papademetriou 2000).

More than 75% of rice worldwide is produced in irrigated rice lands and 90% of these irrigated rice lands are found predominantly in Asia (Bouman et al. 2006). Similarly 90% of global annual rice production comes from Asia while the largest producing countries are China, India, Indonesia, Vietnam and Thailand (Fig. 1). The central production of this percentage represents a numerous small scale family holdings who are often managing less than 1 ha of paddy lands per household in Red River Delta of North Vietnam to four hectares in southern and northern India and central Thailand (Moya et al. 2002). A large proportion of the production is self-consumed at the farming household level and the surplus ends up on the local and domestic markets (Trebuil 2011).

Two distinct types of domesticated rice, *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice) could be found in the rice history, both of which have unique domestication histories (Sweeney and Mccouch 2007). Five or six wild rice species have also been reported namely *O. rufipogon*, *O. nivara* (also considered to be an ecotype of *O. rufipogon*), *O. barthii*, *O. longistaminata*, *O. meridionalis* and *O. glumaepatula*. *Oryza sativa* has a global distribution while having highly grown

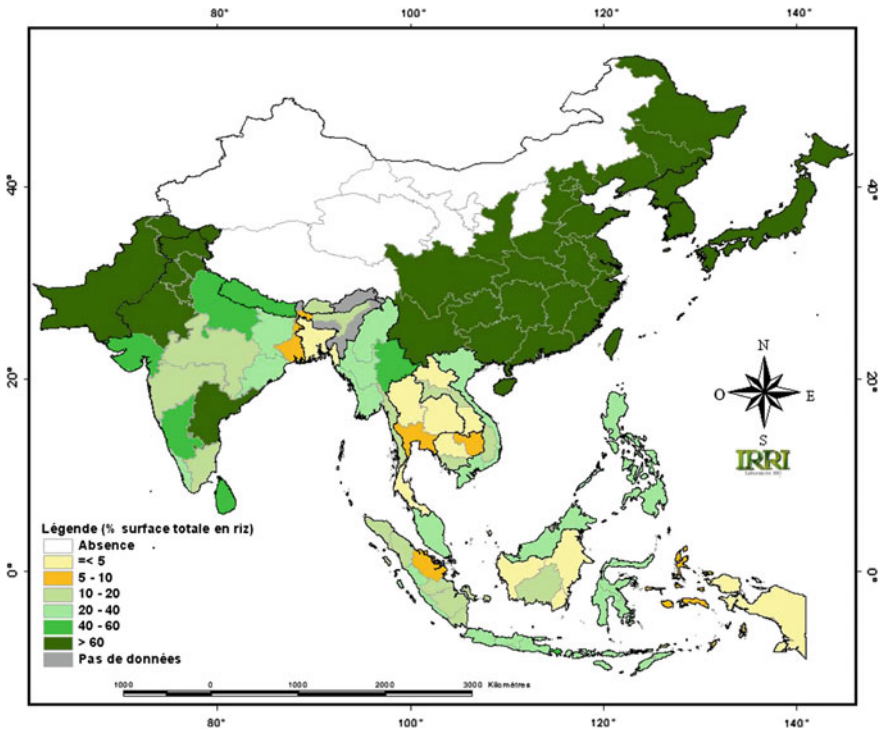

Fig. 1 Relative importance of wet season irrigated rice in Asia in 2003. Percentage of land occupied by rice cultivation. Adapted from IRRI GIS laboratory

in Asian region and the *Oryza glaberrima* has been concentrated to African region. Wild species of *Oryza* has been scattered all over the world while *Oryza rufipogon* can be found throughout Asia and Oceania and *Oryza barthii* and *O. longistaminata* are African species. These distributions establish the ancestral pools of modern rice proving the African cultivars were domesticated from *O. barthii* (formally called *O. breviligulata*) and *O. sativa* was domesticated from *O. rufipogon*. Rice breeders disclose that *Oryza sativa* was independently domesticated in China and India giving rise to the two main groups of *indicas* and *japonicas. Indicas* are widely cultivated in irrigated and rain fed lowland areas of tropical Asia, while *japonicas* are mostly found in irrigated temperate, high-elevation and upland areas in Asia. The current genetic diversity of rice is amazing and is the result of crosses between species or internal to *O. sativa* by natural or man-made selection. Currently over 100,000 rice varieties are known worldwide (Trebuil 2011).

Asia is the significant contributor for the world rice production. There are well adopted, genetically diverse rice groups performing under varied soil and climatic conditions in Asia.

3 Asian Rice Production Systems and Their Ecological Services

Rice is the staple food for at least 33 countries where 15 of these are in Asia and the Pacific. Rice production systems in Asian countries vary with different characteristics such as cultural diversity, economy, land size, water availability and etc. Rice agro-ecosystems are highly associated with livelihoods of rural subsistent farmers of the Asia. They are rich bio-diverse entities in the Asia which carry multiple benefits for local communities. Ecological services of these agroecosystems are moreover diverse. Asian rice production systems are both providers and consumers of ecosystem services.

Agroecosystems depend strongly on a set of ecosystem services provided by natural and unmanaged ecosystems (Power 2010). Humans value these systems as food providing systems. They are basically designed for obtaining higher rice production. Irrigated rice systems provide many ecosystem services such as provisioning, regulating, supporting and cultural services which are not directly valued (Bouman et al. 2006) (Fig. 2).

Paddies are important for flood control. The flooded nature of rice production practices makes the system connected to hydrologically-based ecosystem services (Garbach et al. 2014). Water retained in bunds increases groundwater recharge. Rice can be grown as a desalinization crop. There are negligible level of soil degradation and erosion occurs in rice production systems while flooded areas cool air temperature in peri-urban areas. Risks of water pollution caused by inorganic fertilizers are minimized by the particular conditions in the floodwater-soil system. Atmospheric CO_2 and N_2 fixation by aquatic flora and fauna contributes to natural fertility in these seasonally flooded systems (Dobermann et al. 2008). Fish and ducks are raised in paddies, irrigation canals and field ponds. Rice systems often serve as conservation areas for migratory waterfowl. Rice cultivation systems possesses a tremendous ecological diversity regulated by inherent, seasonal and successional processes which support effective natural control of rice pests and diseases if managed properly (Garbach et al. 2014). Asian rice production systems are occupied with aesthetic, religious, educational, cultural, and recreational values as well. However, ecosystem services provided by rice production systems depend largely on their management as well.

There are number of rice production systems in Asia providing a variety of ecological services. A single system is not adaptable to all agroecological zones and at the same time not capable of providing all the ecosystem services. Therefore, it is fair to consider the different services rendered by different rice production systems. Conservation agricultural systems, organic agricultural systems, system of rice intensification, integrated farming systems, holistic heritage rice production systems and integrated pest management represent the range of agro ecological approaches in rice production and include a range of practices that are suited for management of largescale and small scale agriculture (Garbach et al. 2014).

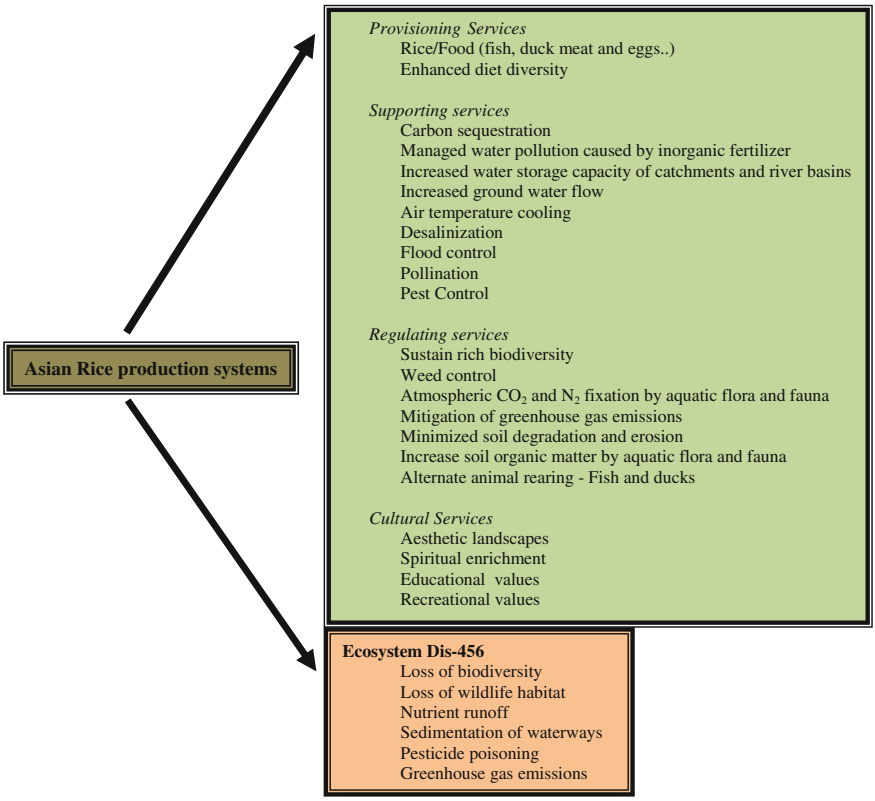

Fig. 2 Ecosystem services and dis-services provided by rice production systems in Asia (adapted and simplified from Zhang et al. 2007 and Swinton et al. 2007)

Conservation agriculture includes three core practices such as minimizing soil disturbance, maintaining permanent soil cover and integrating crop rotations (Kassam et al. 2009). Enhanced services of soil structure and fertility, carbon sequestration, and contribution to weed control, water quality, energy provision and mitigation of greenhouse gas emission together with enhanced yield are the services provided by this system (Garbach et al. 2014).

Organic rice production systems in Asia manage soil organic matter and plant nutrients, control pests and weeds through crop rotations including legumes, contribute in crop residue management, addition of animal and green manure and biological pest control. Ecosystem services provided by the system include enhanced soil structure and fertility, weed control, mitigation of greenhouse gas emissions, increased genetic diversity, and pest control (Garbach et al. 2014). Addition of straw and manure instead of burning straw which emits greenhouse gases enhances soil organic carbon (Komatsuzaki and Syuaib 2009) in the rice soil. However some researchers have pointed out on greater methane emissions in

organic systems with the application of organic manure and materials while nitrous oxide emission is being significantly reducing (Zou et al. 2003). Organic rice production systems have the ability of generating inbuilt soil fertility which secures the rice yield. George et al. in 1992 explains that biological nitrogen fixation by associated organisms is a major source of nitrogen in lowland organic rice systems. The efficiency of nitrogen uptake is higher in organic rice production systems compared to conventional rice production system which are applied with synthetic fertilizers (De Datta and Buresh 1989).

System of Rice Intensification (SRI) is an ecological approach to rice cultivation having a set of management practices which have been practiced by rice farmers over longer period of time. Use of younger seedlings, mostly 8–12 days old seedlings, alternative wetting and drying until panicle initiation and then maintaining shallow water layer, planting single seedling per hill, wider spacing, addition of plenty of organic matter, manual and mechanical weeding, and integrated pest management practices (Stoop et al. 2002) are the key management practices adopted in SRI. This system is practicing under irrigated and rain fed conditions in Asia. Ecosystem services of the system include reduced use of water, mitigation of greenhouse gases, soil structure and fertility enhancement, and pest control (Garbach et al. 2014). There are plenty of researches to prove the reduced water usage (Satyanarayana et al. 2007; Adhikari et al. 2010; Zhao et al. 2010; Sharif 2011), enhancement of water retention and absorption by improving soil capacity and quality (Adhikari et al. 2010), higher water use efficiency (Zhao et al. 2010; Lin et al. 2011; Veraputhiran et al. 2012) and enhanced productivity (Belder et al. 2002; Thiyagarajan et al. 2002; Satyanarayana et al. 2007; Ginigaddara and Ranamukhaarachchi 2009; Susi et al. 2010; Thakur et al. 2011) in the system. Scientists have also reported that reduced methane (CH_4) gas and increased nitrous oxide (N_2O) gas emissions from the system (Wassmann et al. 2000; Susi et al 2010). However, Wassmann et al. (2000) recommends site specific crop management practices in SRI as a solution for locations which would emit more greenhouse gasses.

Rice production when integrated with other components such as fish or livestock (often ducks), and/or services such as those provided by agro forestry is called as integrated rice farming systems (Garbach et al. 2014). Rice-fish systems are generally common in the Asian region. Weed control, soil structure and fertility, biodiversity habitats and pest control are the main ecosystems services from integrated rice based ecosystems (Garbach et al. 2014). Integrated rice based farming systems are highly benefited from their inherent biodiversity and different ecological niches in rich paddies by producing both rice and high-quality animal products (Bambaradeniya and Amerasinghe 2003). This ultimately facilitates diverse food baskets for the farmer families and eventually the food and nutrition security of Asians.

Smallholder rice production systems all over the Asia receive enormous validity since they are providing evidences of harmonious relationships between humans and nature. Referring to the concept on “cultural landscapes”, holistic rice systems in Asia are normally figured by the restrictions posed by local resources, and

activities developed by respecting the biophysical boundaries of the landscape (Silfwerbrand 2012). Ecosystem devices of these holistic heritage systems vary from system to system. Irrespective of the holistic system, the ecosystem services are predominantly; soil structure and fertility enhancement, carbon sequestration, pest control, and cultural services. Deep water rice is one of the flood-prone rice ecosystems where rice is submerging more than 100 cm in depth of water from 10 days to 05 months in time (Maclean et al. 2002). Deepwater rice is cultivated in the floodplains and deltas of Ganges and rivers such as Brahmaputra of India and Bangladesh, the Irrawaddy of Myanmar, the Mekong of Vietnam and Cambodia and the Chao Phraya of Thailand (Bouman et al. 2006). Enhanced cultural services, soil structure and fertility enhancement and wild biodiversity and habitat provisioning are the major ecosystem services of these systems in broad (Garbach et al. 2014). Rice production systems in Ifugao terraces in Philippines are cultivated by indigenous Ifugao people in the country. The culture and social habits of the associated community has developed deep ties with these rice terraces. Abundance of ecosystem services which have been developed for centuries in this system serves for the whole impoverished region (Charette-Castonguay 2014). Balinese *subak* rice-field gravity irrigation system in Indonesia is another cultural landscape which is providing rich ecosystem services. *Subak* is a water management system which is composed of farmers those who share water sources from a spring, a well and a stream (Silfwerbrand 2012) (Fig. 3). Both the rice terraces and the *subak* system are unique clusters of micro-watersheds that are connected to the whole mountain ecology, conserving water and acting as a filtration system at the same time. Evolution and conservation of different plant cultivars through genetic diversity is one of the most important ecosystem services of this system (Garbach et al. 2014). FAO (2013) reported that integrations between the rice systems and the society have resulted rice and another 264 species of endemic plants useful to humans in connection to the paddies. Upland rice production is another marginal ecosystem in which rice is grown in non-flooded and non-bunded fields. This system is highly fragile and poor system comparatively with lower ecological services.

Negative impacts that diminish ecosystem services due to agricultural activities can be considered ecosystem disservices (Zhang et al. 2007). Management practices also influence the potential for 'disservices' from rice production systems. Loss of biodiversity and wildlife habitats, nutrient runoff, poisoning of pesticide on humans and non-target species, including loss of habitat for conserving biodiversity, sedimentation of waterways, and greenhouse gas emissions are some of them (Zhang et al. 2007) (Fig. 2). Nevertheless, amelioration of many of these negative effects are possible with appropriate agricultural practices (Power 2010).

Asia is an unique place in the world for rice production. Due to the varied topography, soil types, micro climate and cultural diversity throughout the Asia, rice production systems are also diverse. Ecosystem services from these diverse rice ecosystems are also unique to the location of origin, culture of the people associated, functioning style and the expected outcome of each of these rice production systems.

Fig. 3 Balinese Subak irrigation system, Indonesia. *Photo Credit* Author

4 Intensifying the Rice Production in Asia

During the first half century from green revolution, traditional agroecosystems, complex in nature in whole world have gradually simplified into modern agricultural systems which rely heavily on external inputs of energy and agrochemicals and replaced biological functions, originally provided by an diverse communities of organisms (Bommarco et al. 2013). Minimized limitations of plant productivity mainly by means of supplied irrigation, added nutrients of anthropogenic origin, enhanced genetic makeup of the propagules, mechanized soil substrate preparations and replaced biological pest and diseases control by agrochemicals (Tilman et al. 2001) are prominent in these converted systems. This change has been occurred in both developed and developing countries with different paces, scales and in different production systems.

Intensification in the rice production systems during the last half decade after the green revolution has been largely responsible to meet the increasing demand for rice in the Asian region with the salient feature of greater yield per unit land and time. This giant production increase has been backed by few major factors namely (a) 'miracle' rice varieties released with higher harvest index (HI) and higher grain yield, shorter duration allowing higher cropping intensity, dwarf nature compared to traditional varieties arresting plant lodging, (b) external application of nitrogen

with no risk of lodging, and (c) improved irrigation infrastructure allowing no scarcity of water for the plant growth and development (Cassman 1999). When one crop is harvested with older traditional rice varieties with long duration, lower HI and more plant height, two to three crops are possible to harvest with these miracle rice varieties and improved management practices.

The population is exponentially increasing in the coming decades in Asia and the more food is needed to be produced to feed the increasing mouths. Rice production is given priority among other major food crops in Asia as it is the staple food of majority of Asians specially the one forth poor in the region who have higher per capita intake (Papademetriou 2000). Producing cheaper rice for the domestic market is of strategic importance in developing countries with higher rice consumption. Some possible means to increase the rice production are discussed in detail below.

4.1 Germplasm Improvement, Yield Potential and Lessening the Existing Yield Gap

Improvement of the existing germplasm might be one of the options for increasing the present yield of rice. Varietal improvement of rice has led to a higher stability of the yield in Asia during past 30 years mainly due to increased tolerance for biotic and abiotic stresses (Peng et al.1999). Accordingly, though modern rice varieties have shown a steady increase in yield, no detectable difference in yield potential could be observed during last 20–30 years of time. The average world rice yield has increased at an annual rate of 52.4 kg ha^{-1} from green revolution (1960s). There is a stabilized rice yields with the introduction of modern rice varieties in 1980s or 1990s in many countries in Asia (Cassman and Dobermann 2001). Dobermann et al. (2008) reports that there has been no significant increase in rice yield potential since the release of IR8, except hybrid rice. According to Cassman (1999) and, Peng and Khush (2003), yield gains through improved varieties in recent decades have moved towards closing yield gaps with better abiotic and biotic stresses adoptions and crop and resource management techniques than pushing yield frontiers. The current average rice yield of 5.4 t ha^{-1} in irrigated rice production systems in Asia represents 65% of the climatic-genetic yield potential. Assuming that target yields for well-managed rice systems are near 80% of the yield potential, an economically exploitable yield gap of about 1–2 t ha^{-1} exists in most intensive rice fields of Asia with good quality soils and favorable rainfall or irrigation facilities (Dobermann et al. 2008). Therefore, at the present context, increasing the theoretical yield potential of rice is uncertain.

The increased yield potential is largely contributed by the increased HI (the ratio of grain to total crop biomass) of the rice variety (Cassman 1999). Harvest Index of the rice had a quantum leap in 1960s with the introduction of dwarfing genes into rice varieties. Little further increase in HI of modern recently released rice varieties were observed by 0.50–0.55 (Peng et al. 1999). However further increase in HI is

limited by the needs of maintaining sufficient leaf area for interception of solar radiation, stem biomass for securing needed physical support to the plant and storage of assimilate N used in grain filling (Cassman 1999).

Hybrid rice which provides 7–10% yield advantage compared to the best inbred varieties (Kumar 2006) are now being testing and popularizing in Asian countries other than China. In China, hybrid rice yields on average are 15–20% more than the high-yielding inbred varieties (Kuyek et al. 2000) while costs of hybrid rice seeds are about 15 times higher than seeds from best inbred varieties (Virmani 1996). After hybrids, further increase in yield potential depends on an increase in canopy photosynthesis per unit of intercepted light or a decrease in the metabolic costs of synthesis and maintenance of carbohydrates, proteins, and lipids (Cassman 1999) which is known as transgenic rice production. Rice scientists believe that transgenic rice production would create more pronounced differences in yield potential with the manipulation of complex traits such as biochemical pathways involved in photosynthesis. However, many obstacles must be overcome until these breakthroughs in biotechnology will reach farm levels specially in developing countries (Zeigler 2001). Still there is lack of evidence on success of physiologists or breeders at increasing the assimilatory or metabolic efficiencies of the major cereal crops (Evans 1993). At the same time, researchers argue that processes governing radiation use efficiency, a parameter that integrates both photosynthetic capacity and metabolic costs are conservative and therefore offer little opportunity for improvement through genetic manipulation (Sinclair 1993).

Yield potential of a crop is explained by Evans and Fischer (1999) as yield of a cultivar when grown in environments to which it is adapted, with nutrients and water non-limiting and with pests, diseases, weeds, lodging, and other stresses are effectively controlled. The yield gap represents the difference between yield potential and the actual yield that a farmer obtains in his farm (Fig. 4). This gap might be widened by lack of water, imbalance or lack of nutrients, pest damage, weed competition, and lack of pollination; factors that, to a large extent, are modulated by ecosystem services. The investments needed is higher to regulate and support degraded ecosystem services in order to minimize this yield gap (Bommarco et al. 2013). Cassman (1999) reports that efforts to close the yield gap typically become non-economical when yields reach 80% of the yield potential. Therefore, farmers' efforts to minimize the yield gap ultimately depends on either increased conventional intensification with known negative externalities or otherwise integrating natural supporting and regulating services, such as pest control, water retention, and nutrient cycling (Bommarco et al. 2013) in the rice production systems.

4.2 Nutrient Management

Nutrient management with the combination of modern improved rice varieties in Asian rice production systems is another prospect in increasing rice yields. Rice yields in Asia has been increased at an average rate of 2.5% per year from 1967 to

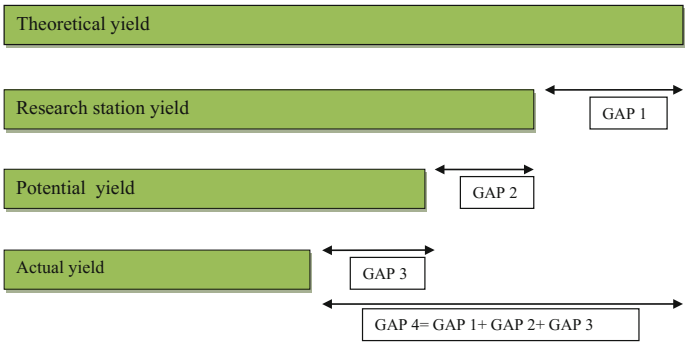

Fig. 4 Probable yield gaps at different levels of rice: Gap 1 is due to the genetic capacity of the variety. GAP 2 is due to technology transfer problems and environmental difference. GAP 3 is due to biological and socio economic constrains. GAP 4 is due to GAP1, GAP2 and GAP 3

1984 while showing dropped yield growth rates to 1.2% from 1984 to 1996 (Dawe and Dobermann 1999). This impressive increase in rice yields during the 1960s and 1970s were associated with increased use of fertilizers, particularly urea-N, while growth in efficient fertilizer consumption has slowed in recent years (Hossain and Singh 2000). Similarly, after an initial steep rise in partial factor productivity of fertilizer nutrients (PFP = kg grain yield per kg nutrient applied), due to the adoption of modern varieties and fertilizers during the same period, a steady decline in PFP of applied nutrients since the mid-1970s could be observed in irrigated rice production systems in Asia (Cassman and Dobermann 2001). This indicates that extra N remains in organic soil N forms that are less plant available than building-up of mineral N levels or indigenous N supply (Cassman et al. 1998; Olk et al. 1999). Since the green revolution in Asia, nutrient cycling in lowland rice systems has been altered threatening to the sustainability by general declining the soil quality (Greenland 1997). Hence further increase in PFP of fertilizer nutrients in Asian rice production systems with the target of increasing yields is doubtful under these disturbed rice ecosystems.

4.3 Pest and Disease Control

Pests in rice production systems in Asia has become a threatening issues at present and pest and disease control has become essential management practice in present Asian rice production systems for increased rice yields. Presently, pest management is primarily achieved through the use of pesticides, and 5 million tons of pesticides are applied annually to crops worldwide (Matson et al. 1997). Extensive adoption of semi dwarf varieties of rice after 1960s in space and time with expanded irrigated rice lands increased the heavy use of synthetic fertilizers, pesticides and weedicides and hence disturbing heavily on ecology of agricultural lands, damaging wildlife

and the environment and finally threatening human health. With continuous use of the pesticides, resistance has been developed within the rice pests which is an ever-present problem. Therefore, further reliance on pesticides for the rice pest control has reduced and chemists are searching more toxic pesticides for controlling the same pests which is an endless process and which will create more complex health and environmental problems in future.

It is an obvious fact that more rice is need to feed the increasing population in Asia and beyond. Although rice breeders and scientist are making their full efforts in producing high yielding varieties further, which are high input responsive, soil degradation with prolong cultivation with chemical inputs has made consequences for maximized use of the applied nutrients by these new varieties. The damage to the ecosystem has alerted ecosystem services ultimately creating problems of controlling rice pests and diseases. Chemical based rice production practices are ultimately threatening to the human health and their existence as well. Hence alternative intensification strategies are needed for sustaining the rice production in Asia.

5 Ecological Intensification in Asian Rice Production Systems

The prevailing dialogue among many scientists today on securing the current and future foods for increasing population and people with variety of dietary needs dominates with the debate of the need of intensifying the present agricultural production (Huang et al. 2002; Godfray et al. 2010). Scientists have estimated that there is a need to increase the agricultural production by 70% to be able to feed nine billion people by the year 2050 (Tilman et al. 2011). It is further assumed that agricultural production can only be increased through raising average crop and animal yields taking all the environmental problems that this entails into consideration. Pinstrup-Andersen (2009) stated that food security requires addressing its four pillars such as availability, access, utilization and the stability of all these over time. However the present food production techniques that don't feed all people at present cannot be expected to feed all people in 2050. Therefore, present agriculture needs to be made more productive, stable while minimizing environmental impacts to face future climatic, economic, and social challenges (Foley et al. 2005).

Asian rice production systems are facing multiple stressors including those of climate change, land degradation and water scarcity (FAO 2014) basically due to ignored ecological diversity and benefits from nature and replaced by external application of inputs. Simultaneously, many Asian countries are now recognizing and considering the need of revised rice production strategies to think beyond simple production targets (FAO 2014).

Managing service providing organisms making quantifiable direct or indirect contribution to agricultural production is the way for ecological intensification.

According to the Cassman (1999), ecological intensification of agriculture is an optimal balance of high productivity, profitability, sustainability and environmental protection which would be achieved by finetuning of management towards better exploitation of crop yield potential. The ecosystem services provided by diverse organisms can be incorporated into the rice production systems ensuring increased production while minimizing the environmental and human threats, but not necessarily avoiding anthropogenic inputs (Cassman 1999; Dore et al. 2011). Hence in order to achieve a consistent production at higher levels without causing environmental damage, improvements in soil quality and ensuring all production factors which are precisely managed in time and space are needed (Cassman 1999).

5.1 Soil Quality and Nutrient Management

Soil quality is the capacity of the soil to support biological productivity, maintain environmental quality, and promote plant and animal health (Doran and Parkin 1994). It has been elaborated as specific soil properties that support crop productivity, such as nutrient reserves, water holding capacity, and favorable structure for root growth and properties that contribute to the environmental services that soils furnish (Cassman 1999). Ecosystem services linked with rice soils are vital for better yields of rice. The rice soils are providing services such as primary production, gas regulation, nitrogen transformation, soil organic matter accumulation, water regulation, flood control and biodiversity maintenance (Xiao et al. 2011). These services are mediated by a huge, diverse, and largely unexplored biological community of mainly bacteria and fungi, and also protozoa, nematodes, arthropods, and earthworms associated with rice soils (De Deyn and Van der Putten 2005; Barrios 2007).

In rice production systems, Nitrogen as one of the essential nutrients is needed to be applied externally in most of the locations since they are not adequately present in the rice soil to meet the crop need. Nitrogen is recognized as the most important nutrient for the plant growth since it is significantly needed for tillering, leaf area growth, biomass production and grain yield (Yang et al. 2003). A total of 15–85% of fertilizer is consumed by major rice production systems in Asia (Dobermann and Cassman 2002; Heffer 2008). At the same time, fertilizers accounts for about 20% of input costs in rice production which is becoming only second to the labour cost (Clayton 2010). In most high intensive rice production systems, relatively high amounts of fertilizers are used in Asia with no consideration of the wide variation of the cultivation lands in different localities which is ultimately damaging the environment and rice ecosystems without contributing very much to the yield enhancement (Cassman and Dobermann 2001; Dobermann and Cassman 2002). A stagnation or declining of the PFP (Partial factor productivity) of fertilizer nutrients (= kg grain yield per kg nutrient applied) could be observed in rice production systems in Asia after 1970s (Dobermann and Cassman 2002). Simultaneously, Greenland (1997) suggests that, the Green Revolution in Asia has

significantly altered nutrient cycling in lowland rice systems leading to negative nutrient input–output relationship by general declining in soil quality.

In order to get rid of these unfavourable effects and declining soil quality which are threatening to the soil ecological functions, strategies might be needed in no time for the Asian rice production systems for a their sustainable production. A dynamic, site-specific nutrient management of small units such as single fields or areas within them will be required to overcome the current mismatch of fertilizer rates and crop nutrient demand at the farm level (Dobermann and Cassman 2002) which is also known as precision farming. Precision farming ensures that the required resources for crop growth are available and crop protection needs are met without deficiency or excess at each point in time during the growing season (Cassman 1999). In general, the fertilizer recommendations provided to farmers do not consider field, climate, and management-specific effects on the nutrient needs of the crop (Cassman 1999; Buresh 2006). The Site Specific Nutrient Management (SSNM) was developed in Asia by the International Rice Research Institute (IRRI) which is a low-tech, plant need-based approach for optimally applying nitrogen, phosphorus, and potassium fertilizers to rice when they are in need (IRRI 2006). On farm studies on double-crop rice systems in several Asian countries have shown that there is a tremendous field-to-field variation in native soil N supply within small production domains where soil properties are even similar (Olk et al. 1999). Then in order to optimize yield and to minimize nitrogen fertilizer losses, consideration on field-specific N fertilizer requirement is needed. Dobermann et al. in 1998 has reported that there is a large field-to-field variability in soil P and K supply. Field-specific management will produce good results for these nutrients as well.

Integrated nutrient management System for the Asian rice production systems would be another strategy which will be more environmental friendly with minimized pollution of drinking water by nitrates, eutrophication of streams, lakes, and coastal marine environments, contributions of agricultural systems to global warming and protecting of overall agro-ecosystem services (Dobermann and Cassman 2002). Integrated Nutrient Management (INM) aims optimizing the condition of the soil by improving its physical, chemical, biological and hydrological properties, for the purpose of enhancing farm productivity, whilst minimizing land degradation. With INM strategy, nutrients are added in both organic and inorganic forms enabling the soil system to perform the soil ecological services, optimum plant absorbance and enhanced yield. Wassmann et al. (2000) has reported that managing the rice field through a variety of means, including organic and inorganic amendments and crop management practices would reduce the Methane emission from the irrigated rice fields.

5.2 Ecological Pest Management

The utilization of agrochemicals for pests, diseases and weeds control in rice production systems was gradually increased starting from the green revolution era and still by far the most preferred method over others. At the same time, pesticide imports into several Asian countries including Bangladesh, Thailand, Indonesia and Vietnam are exponentially growing (FAO 2016). However the adverse effects created by the chemical based pest and weed control in rice production systems damage heavily the wildlife, environment and threaten human health (Stehle and Schulz 2015; Loos et al. 2014). The prophylactic use of insecticides has created various imbalances in rice ecosystems (Horgon et al. 2015). Simultaneous occurrences of pest outbreaks, including plant hoppers (Homoptera: Delphacidae) (Kenmore et al. 1984) and leaf folders (Lepidoptera: Pyralidae) in Asia have been reported (Spangenberg et al. 2015; Catindig et al. 2009). It has been estimated that since 2000, China has lost about 1 million tons of rice production annually, over 3 million hectares of rice in Thailand between 2009 and 2011 (Azzam et al. 2011) and an estimated 200,000 ha of rice in Central Java (Indonesia) in 2011 alone due to plant hopper damage (Horgan and Stuart undated). The 'hopper storms' are very popular in Asian rice production systems.

Development of pest populations that are increasingly resistant to insecticides and more virulent against rice varieties is very common in agricultural fields today (Kenmore et al. 1984; Matsumura et al. 2008; Horgan et al. 2015). The diversity, abundance or efficiency of the natural enemy component of the rice ecosystem are greatly reduced due to use of agrochemicals (Horgan et al. 2015). The natural enemy populations such as pollinators (Potts et al. 2010) and predatory amphibians (Collins and Crump 2009) are getting lessened from agricultural lands. Rice fields in the tropic, have a higher diversity of natural enemies than herbivores, resulting complex food webs (Cohen et al. 1994) which increase the stability and resilience of rice ecosystems (Ings et al. 2009). The biodiversity loss and the loss of services provided by the natural fauna of rice fields then reduce ecosystem stability and resilience. Dealing with the requirements for food security and climate resilience of the rice production systems in Asia, it is needed to restore biodiversity and optimize ecosystem functions. One way to achieve these goals is the use of ecologically-based pest management methods while restoring the ecology of rice landscapes (Horgan et al. 2015). The approach for achieving this is called as ecological engineering (Gurr 2009).

Ecological engineering is the deliberate manipulation of habitat for the benefit of society and the natural environment (Gurr 2009; Gurr et al. 2012). In this approach, abundance, diversity and function of natural enemies in agricultural habitats are increased by providing refuges and alternate or supplementary food resources (Gurr 2009; Lv et al. 2015). Agri-environmental schemes have been suggested for promoting biodiversity and ecosystem services in conventional agricultural systems (Bommarco et al. 2013; Wood et al. 2015). Floral and vegetation strips in rice

landscapes is one of the strategies for rice pest management (Gurr et al. 2012; Westphal et al. 2015). The physical set up of the rice fields provide a nice facility for incorporating other crops or plants into the rice ecosystems. The raised levees around the rice plots which are called as 'rice bunds' are multipurpose structures which are used as walkways by farmers, to direct and maintain water in the rice plot and to cultivate upland crops on them. Rice, being an semi-aquatic plant, will not compete with upland crops which are growing on the bunds so that becoming compatible with each other. These upland crops (bund crops) include forage crops in Indonesia (Horgan et al. 2015), field crops such as Beans and Taro (*Colocasia esculenta*) in Philippines and Indonesia (Foronda 2007) and field crops such as Long bean (*Vigna unguiculata*), Winged bean (*Psophocarpus tetragonolobus*), Common bean (*Phaseolus vulgaris*), Green gram (*Vigna radiata*) and black gram (*Vigna mungo*) and leafy vegetables in Sri Lanka (personal observation). In general, farmers are adding upland crops to the rice ecosystems for saving the space and supplement farm income but not directly knowing the supporting role of these bund crops for the pest management of the rice crop (Horgan et al. 2015). Incorporation of diverse vegetation patches (DVP) and high diversity vegetation patches (HDVP) in rice production systems have been tested in Philippines and successful results have been reported in terms of increasing pollinators percentages, attraction of more insectivorous birds and housing for beneficial insects on the vegetation (Horgan et al. 2015). Inclusion of a variety of upland crops on a randomly set platforms on the rice field bunds added with plenty of organic matter comes under HDVP whereas growing of a single crop on such set bunds come under DVP. Crops which can be incorporated in both methods include Green gram, Common bean, Cowpea (*Vigna unguiculata*), Cucumber (*Cucumis sativus*), Luffa (*Luffa* sp.), Sunflower (*Helianthus annuus*), Bottle gourd (*Lagenaria siceraria*), Winged bean (Fig. 5), Ladyfinger (Okra spp), Chili pepper (*Capsicum* sp.) and Bitter gourd (*Momordica charantia*) (Horgan et al. 2015). Researchers have further observed that some of bund crops have heavily damaged with pests in certain localities. For example Cowpeas in some localities were heavily damaged by aphids (Homoptera: Aphididae), Ladyfinger by Okra plant hoppers (*Abelmoschus esculentus* [L.] Moench.) and Bitter gourd by fruit flies (Horgan et al. 2015). Therefore selection of right bund crop for right locality is very important for better results with respect to pest management, other ecological services provision in rice production systems and earning additional income for farmers.

Gurr et al. in 2016 has reported that simple diversification of rice production systems incorporating nectar producing plants as border crops gives good results in rice pest management. Further they reported that key pest populations of rice in Asian countries such as rice brown plant hopper (*Nilaparvata lugens*) were significantly lowered in rice fields incorporated with nectar producing boarder plants compared to those which were not. Improved parasitism on the brown plant hopper eggs has been resulted for this difference in boarder plant intervention treatment. Reestablishing of ecosystem services in croplands is challenging due to high level

Fig. 5 Winged bean (a pest trap crop) vegetation patch around the rice field in Sri Lanka. *Photo Credit* Author

of chemical inputs, bigger disturbance and monoculturing behaviour (Matson et al. 1997) which are targeted for higher food production. Though switching to organic agriculture is able to provide at least some of the ecological services (Crowder 2010), it is a radical change for farmers to adopt since generally provides lower yields (Seufert et al. 2012) at the beginning.

Integrated Pest Management (IPM) has great potential for reducing the dependence of crop protection on chemical control methods (Pretty et al. 1998). Integrated pest management requires a coordinated approach integrating diverse methods, including cultural, biological, and chemical control (Dent 1991) while minimizing hazards to humans (Prokopy 1994). However, even with the implementation of IPM, the homogeneous agroecosystems are often inhospitable to natural enemies due to various reasons (Letourneau 1998). Despite the homogeneous nature of agroecosystems, biodiversity in agricultural landscapes can potentially enhance a range of ecosystem services such as breakdown and cycling of nutrients, pollination of crop plants, buffering watercourses from runoff, protection from erosion, biological control of crop pests (Gurr 2009; Costanza et al. 1997; Losey and Vaughan 2006) and potentially reduced reliance on synthetic inputs (Matson et al. 1997; Bommarco et al. 2013).

Similarly, field-specific pest management or site-specific pest management (Precision farming) is also a high-tech approach to protect crops from insect pests and diseases while minimizing the use of pesticides (Klingauf 1988). Site-specific pest management utilizes spatial information about pest distribution to apply control tactics only where pest density is economically high within a field. This is a possible technology for pest management in ecological intensification of rice production systems in Asia.

6 Conclusion

The increased food production is a vital need for ensuring the food security of the increasing populations and the agricultural intensification is one of the prime options to meet that need. The growing threats for the environment and its organisms including human beings cannot be neglected at the same time. The climate change is creating many uncertainties in fulfilling the needed requirements for the crop growth. The potential yield ceiling of the available rice varieties with optimum growing conditions under most intensified production systems is limiting the yield increasing opportunities. With all these surroundings, scientifically based ecological intensification in rice production systems is one of the best options available. Scientific understanding and application of soil quality management knowledge on growth, development and yield of rice, precision farming techniques in pest and disease control, and integrated soil nutrient management techniques can be ultimately recommended for minimizing the existing yield gaps and addressing the associated ecological problems in intensified Asian rice production systems in order to meet sustainable rice production for feeding the increasing mouths in the region and beyond.

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Transhumance, Livestock Mobility and Mutual Benefits Between Crop and Livestock Production

Suman Aryal, Tek Maraseni, Geoff Cockfield and Lisa Lobry de Bruyn

Abstract Grazing-based livestock production, named pastoralism, is classified into nomadism, transhumance and agro-pastoralism. Transhumance is characterised by the seasonal and recurring movement of livestock whereby seasonal grazing areas and routes for livestock movement are fixed. All grazing based livestock production systems including transhumance are constrained globally for a variety of reasons. The major threats to the system are globalisation, nationalisation or privatisation of rangelands, national parks and community forestry policies restricting free grazing and shortage of labour. The collapse or decline of such social-ecological systems (SESs), which have existed for over 1000s years, often induces adverse impacts on societies and ecosystems. Here we review the literature on transhumance, and discuss reasons for transhumance, and the associated advantages and disadvantages of livestock movement in transhumance. Our review also focuses on how the integration of crop and livestock production in transhumance derives mutual benefits. The review indicates that the seasonal movement of livestock is an ecological necessity in areas with harsh climates and low pasture production. Transhumance is also a herders' adaptive management to adjust to variable grazing resources and environmental conditions. The disadvantages of seasonal movement of livestock such as greater herding labour required and expenditure of more energy for livestock, are far outweighed by the ecological advantages. Some of these are: to minimise grazing competition and to protect rangeland pastures from being overgrazed. Our review also indicates that the integration of crop and livestock production derives mutual benefits and contributes for their enhanced sustainability.

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E. Lichtfouse (ed.), *Sustainable Agriculture Reviews 31*, Sustainable Agriculture Reviews 31, https://doi.org/10.1007/978-3-319-94232-2_2

Keywords Advantages · Crops · Grazing · Livestock · Movement
Social-ecological system · Transhumance

1 Introduction

Traditional social-ecological systems (SESs) such as smallholder farming systems and pastoralism are facing a range of challenges from the global social and environmental changes. The loss or decline of such traditional social-ecological systems are often irreversible (Marini et al. 2011) and is likely to affect ecosystems and livelihoods. The pastoral systems are threatened for different reasons and there is a great uncertainty about their future persistence (Mearns 2004). Transhumance; a type of pastoral system based on seasonal and recurring movement of livestock between fixed points, has also been subjected to contemporary threats (Jurt et al. 2015) and has potential for adverse impacts to the societies and ecosystems embedded in them.

Seasonal movement of livestock and people is the key feature of transhumance. On the one hand, the long distance movement of livestock has been criticised for requiring greater amount of energy and herding labour. However, on the other hand, the movement of livestock has been acknowledged in providing ecological advantages such as conserving biodiversity (Olea and Mateo-Tomás 2009) and preventing fire (Kerven and Behnke 2011). The movement of livestock also helps to utilise grazing resources, often distributed across large areas. The seasonal movement of livestock in transhumance helps to protect livestock from the harsh climate and also offers an opportunity for the integration of livestock with crop production (Bhasin 2011). Both livestock and crop production are integrated in transhumance (Aryal 2015). The integration of livestock and crop production can contribute each other in a multitude of ways. This chapter, therefore, presents a synthesis on what transhumance is, why seasonal livestock movement in transhumance is important, and how the integration of crop of livestock production in transhumance derives mutual benefits for both production systems.

2 Transhumance: A Type of Pastoralsim

Livestock production systems are classified according to use of resources, interaction with the environment, degree of intensification, grazing animal and orientation towards crop production (Bernués et al. 2011). Sere and Steinfeld (1995) provided typologies of livestock production systems combining two concepts, the agro-ecological zones and the farming systems. According to them, three major agro-ecological zones are; arid/semiarid, humid/sub-humid and tropical highland/temperate based on length of the pasture growing period. The farming systems are based on whether the system is solely livestock-based or mixed farming. Furthermore,

the solely livestock based system split to grassland based systems and landless livestock production systems, and mixed-farming systems to rainfed mixed-farming and irrigated mixed-farming system (Sere and Steinfeld 1995). Based on land use by livestock, there are three types of livestock production systems (Devendra 2007; Steinfeld et al. 2006; Thornton et al. 2009); (i) grazing systems (pastoral systems), (ii) mixed-farming systems, and (iii) industrial or landless (feedlot) systems.

Pastoral production is seen as an evolutionary stage in human history, following the hunting-gathering phase and prior to permanent settlement and agriculture (FAO 2001). Pastoralism continued in the areas not suitable for agriculture or to complement agriculture where crop production was possible, but only to a small extent. Niamir-Fuller (1998) classified pastoralism into nomadism, transhumance and semi-transhumance, based on a declining order of mobility. A similar classification based on the degree of movement was provided by FAO (2001), and has classified pastoralism into: (i) nomadism, (ii) transhumance, (iii) agro-pastoralism and (iv) enclosed system and ranching (Fig. 1).

Movements of nomads are opportunistic and follow pasture resources in a pattern that varies from year to year. In transhumance, livestock are moved seasonally between fixed points to utilize availability of grazing resources (FAO 2001; Fernandez-Gimenez and Le Febre 2006; Nyssen et al. 2009). Agro-pastoralists are settled pastoralists who cultivate sufficient areas to feed their families from their own crop production. Agro-pastoralists hold land rights, use their own or hired labour to cultivate land and grow staples crops. While livestock are still valued property in agro-pastoralism, their herds are on average smaller than other pastoral systems and graze within a finite area around their village which can be reached within a day (FAO 2001). Enclosed system or ranching includes livestock production whereby the animals are kept on large but enclosed land (owned or leased).

Transhumant pastoralists often have a permanent homestead and base at which the older members of the community remain throughout the year. Transhumance is often associated with the production of some crops, although primarily for herders' subsistence use rather than for markets (Jones 2005). The ancient Mediterranean societies developed transhumance to cope with an unpredictable and highly fluctuating climate (Oteros-Rozas et al. 2012). Common features of transhumance are flexibility, complexity and the utilisation of complementarities in space (between habitats/landscapes) and time (between seasons) (Herzog et al. 2005).

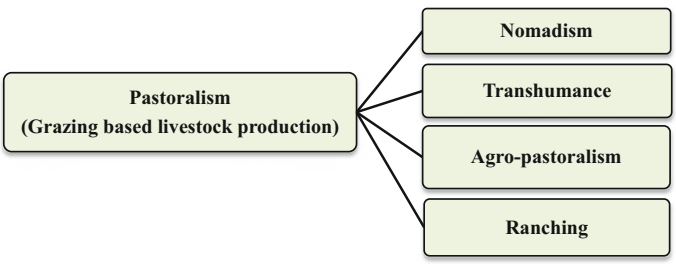

Fig. 1 Different types of pastoralism (FAO 2001)

2.1 *Seasonal Movement of Livestock: A Key Feature of Transhumance*

Livestock mobility is critical for all types of pastoral systems (Ayantunde et al. 2011). There are different factors necessitating movement of livestock. According to Aryal (2015), the most important reasons for upward and downward movement of livestock in the high Himalayas are; (i) to search for grazing resources, (ii) to avoid hot and cold temperature and (iii) to avoid overgrazing of pastures. In the Himalayas, the highest mountain system of the world, there is a considerable seasonal variation in grazing resources and climate at different altitude. The alpine and sub-alpine rangelands of the high Himalayas are covered by snow in the winter season (December–February) and grasses start to grow when snow melts with the late spring season (April–May). Responding to the growth of grasses in the rangelands, herders start ascending to higher altitudes with their livestock and graze in those rangelands. With the onset of the winter season, herders start descending to lower altitudes where they can graze in fallow agricultural fields, forests and river banks in peak winter season (Aryal et al. 2014b). Therefore, it is clear from research that the transhumant herders in the Himalayas respond to climatic variations and natural vegetation cycles at different altitude by seasonal movement of livestock (Fig. 2) (Aryal et al. 2014b).

The mobility of livestock can be viewed under two knowledge systems. In terms of local knowledge systems or from a herder's perspective, it is rational to move livestock to the area where grazing resources are located (Adriansen 2006). When grazing resources are distributed over large areas and productivity is low, it is

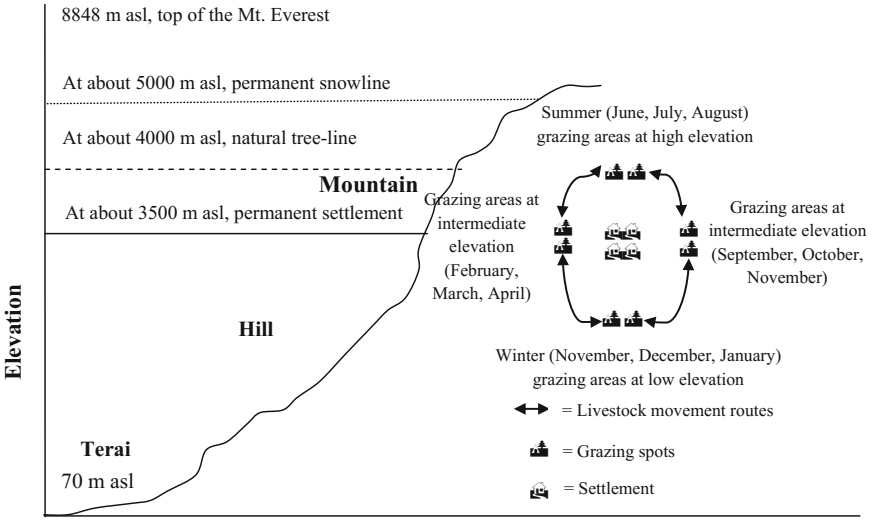

Fig. 2 Cross-section of Nepal showing ecological zones (left) and transhumance pattern (right), drawing is not in scale. asl: above sea level

uneconomical to use those lands for other purposes. In such situations, mobility offers more flexible and adaptive utilisation of grazing resources (Fernandez-Gimenez and Le Febre 2006; McAllister et al. 2006; Wang et al. 2014) and helps to convert low quality plant resources into high quality animal products. In terms of scientific knowledge systems, the livestock mobility distributes grazing pressure and avoids overgrazing. The mobility is also important from the rangelands management perspective. Therefore, livestock mobility in transhumance can be viewed both as economic and ecological necessity and an adaptive response of herders to changing conditions.

2.2 *Transhumance Pastoralism in Mountains*

Mountain areas cover one fifth of the world's terrestrial surface (Körner et al. 2005). According to Rhoades and Thompson (1975), mountains possess three major ecological and environmental features: (i) vertical biotic zonation; (ii) irregular biotic distribution; and (iii) geologic features of slope, elevation, and ruggedness of surface configuration. Adaptation by human or animal populations in mountains must involve strategies for coping with those mountains' features. Among many other adaptive strategies developed by people living in mountainous areas, transhumance is one developed by the mountain people for the optimum utilisation of low productive mountain pastures (Körner et al. 2005). In mountains, transhumance is vertical as there is a vertical stratification of resources by altitude (Galaty and Johnson 1990; Montero et al. 2009) and involves movement of livestock between high elevation pasture in the summer and low elevation valleys in the winter. Transhumant herders follow a seasonal calendar for characteristics livestock movement (Fig. 2). In high mountains, the crop cultivation is generally geographically restricted due to climatic, edaphic and topographic factors. However, transhumance links high mountain rangelands with the agricultural land in the valleys and in some cases adjacent lowlands (Herzog et al. 2005).

There is evidence that transhumance is (or was) practiced in major mountain systems of the world. In the Himalayas, transhumant agro-pastoralism is considered as an important part of the living cultural heritage (Banskota 2000; Namgay et al. 2013). It is thousands of years old (ICIMOD 1997) and sometimes it is ironically mentioned that pastoralism is 'as old as hills' (Chakravarty-Kaul 1998). Transhumance pastoralism is still the main livelihood strategy for many households in the high Himalayas (Aryal 2015; McVeigh 2004; Moktan et al. 2008; Namgay et al. 2014). As in other parts of the world, transhumance in the region is a response to harsh climatic condition (cold temperatures), low plant productivity (shortage of forage) and the search for livelihood opportunities (Moktan et al. 2008; Namgay et al. 2013). Transhumant pastoral societies inhabit the higher Himalayas and use the seasonal production of grazing areas (Bhasin 2011; Pawson and Jest 1978). The

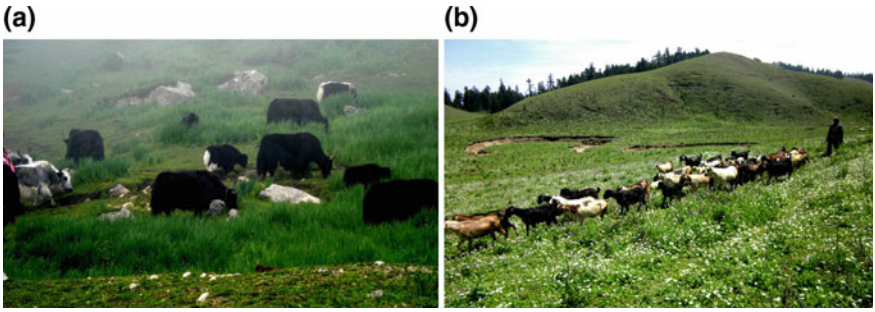

Fig. 3 Variation in livestock types in transhumance systems across the Himalayas; yaks grazing in the Langtang National Park (**a**) and a flock of goats grazing in the Khaptad National Park in Nepal Himalaya (**b**)

high elevation pastures in the Himalayas are available only for the summer months (June–August). Therefore, the herders have adjusted their livelihood activities to ecological niches at different altitudes. They use resources while synchronizing their socio-cultural activities with the seasonality of transhumant practices. There are many similarities in transhumances across the Himalayas but there are local variations in livestock types (Fig. 3), numbers and distance of summer grazing areas from the villages.

The pastoralism in Tibet has a history of around 8800 years as it started during the mid-Holocene climatic optimum (Miehe et al. 2009). Transhumance exploiting diverse areas including lowland, middle altitude and mountain environments in Pyrenees had origins in the Mesolithic era (around 15,000 years ago) (Geddes 1983). Pollen and beetle analysis showed that transhumance was practised in the high mountains near Neor of Iran at least 6500 years ago (Ponel et al. 2013).

There are some reports of inverse transhumance i.e. movement of herds to lower altitude in the summer. In some mountains of Europe such as in County Clare of Ireland where animals are taken to the higher plateau in winter (December–February) that retains the heat captured during the summer (June–August) (Biber 2010). Moreover, there is a difference in pasture availability between the tropical and the temperate mountains. In tropical mountains such as in the Andes, there is year round pasture availability for grazing, but in the temperate mountains like the Alps of Europe and the Himalayas in Asia, high elevation pastures are available only for the summer months (Rhoades and Thompson 1975). Therefore, in some tropical mountains such as in the central Andes, sedentary systems were well established because there is no pronounced seasonal variability in fodder availability. Therefore, the shift from sedentary to migratory patterns in Andes is a response to the perception of economic opportunity (Stewart et al. 1976).

3 Livestock Movement in Transhumance

3.1 Disadvantages

There are some costs associated with grazing based livestock production compared to the stall feeding. One of the most important costs of grazing based livestock production including transhumance is the greater amount of herding labour (Nyima 2014). There is also an expenditure of additional energy for the movement by livestock and humans (Osuji 1974; Turner et al. 2014). Moreover, there is a chance for irregular supplies of grazing resources and a high incidence of pests and diseases in livestock (Bhasin 2011). The movement over larger areas also increases the chance of conflict between farmers, herders and other settled communities (Banjade and Paudel 2008). Some livestock might also be lost, stolen and predated by carnivores in the movements (Bhasin 2011).

3.2 Advantages

Although there are some disadvantages associated with livestock movement across large areas, the movement of livestock as shown in the transhumance system has more advantages. First, the mobility of livestock and people helps them to adapt with resources and environmental variations and offer access to better grazing resources (Dong et al. 2009a; Moktan et al. 2008; Nyong et al. 2007). Second, livestock enjoy a high degree of freedom which ultimately benefits the health of livestock (Turner et al. 2014). Third, the movement of livestock following the seasonal calendar helps to reduce crop damage by domestic livestock (Aryal 2015; Chaudhary et al. 2007). Fourth, the mobility of herds spreads risks across larger spatial scales and reduces vulnerability to the systematic threats such as climate change (Brottem et al. 2014; McCarthy and Di Gregorio 2007).

Literature (Bauer 2000; Bhasin 2011; Bishop 1989; Moktan et al. 2008) also suggests that the movement of livestock makes the selling of livestock and livestock products easier, to purchase food and other products, and combine other livelihood activities such as collection of medicinal plants at the same time. Other motivational factors such as engaging family members in off-farm activities, schooling options for children, reducing transportation cost, avoiding animal parasites from transhumance are also described (Akasbi et al. 2012; Namgay et al. 2013).

Moreover, the mobility of livestock in transhumance has ecological rational to avoid overgrazing and rangeland degradation (Adriansen 2006; Dong et al. 2007). Therefore, from the resource management perspective, this system establishes a rotational grazing pattern which distributes livestock impact over time and space and avoids overgrazing (Brower 1992; Bruegger et al. 2014).

4 Benefits of Integrating Crop and Livestock Production

Crop-livestock interactions differ along agricultural intensification gradients (Erenstein and Thorpe 2010). Crops and livestock are specialised in developed countries where the intensification and commercialisation of the agricultural systems have weakened crop-livestock interactions (Bell and Moore 2012; Erenstein and Thorpe 2010). However, mixed crops-livestock systems tend to dominate in developing countries (Wright et al. 2012). The integration of crop and livestock is well developed in the small-scale agriculture of South-Asian region (Devendra and Thomas 2002; Yadav 1992). In entire Himalayan region, the mixed-farming is the dominant farming system. It has been reported that the integrated farming systems outperform normal or commercial farming systems in different dimensions such as food security, environmental function, economic function and social function (Tipraqsa et al. 2007).

There exists a complementary relationship between crop and livestock production while they are integrated. The use of crop residues (Fig. 4a), stubble, husk and grain to supplement cattle's feed, for instance, support livestock feed when there is a lack of grazing resources (Bell et al. 2014; Letty and Alcock 2013). The use of manure from cattle where there is no option/availability of chemical fertilisers, and the use of cattle as draught power in agricultural field where there was no access to modern technology are just a few examples of how livestock contribute to crop production (Fig. 4b). The use of draught animals in agriculture occurs in more than 50% of cultivated areas of the world (Ramaswamy 1998).

The integration of some crops and livestock is a key feature of the transhumance in high elevation areas of the Himalayas (Aryal 2015). Although the contribution of crop residues is less in mountains compared to Terai of Nepal (NARC 1996), the use of such agricultural by-products helps to lessen the severity of winter bottleneck when there is severe shortage of grasses. The use of livestock manure in the agricultural field allows circular resource flow and reduces external input for crop production (Erenstein and Thorpe 2010).

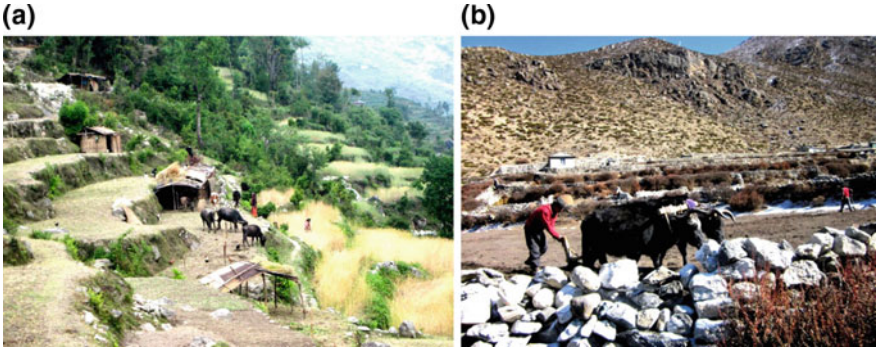

Fig. 4 Livestock grazing in fallow land after harvesting crops in Kalinchok, Dolakha (**a**) use of livestock to plough land for crop production in Khumjung, Solukhumbu (**b**) in Nepal

The integration of crops and livestock production extend beyond herder's household in transhumance system. This is because farmers who are not rearing livestock invite transhumant herders to build temporary stables in their fallow land after harvesting crops (Fig. 4a). Similar arrangements between farmers of low altitudes and transhumant herders of high altitudes also exist in other Himalayan countries such as India (Bhasin 2011) and Bhutan (Namgay et al. 2013). Farmers benefit from the improved soil fertility that increases crop yield in the next cropping season through the use of animal manure. Moreover the farmers who do not own draught animals can hire such animals for cash, kind or by human labour to prepare land for next crop. Also, herders benefit as they feed their livestock on stubble and crop residues, which would otherwise be left. The winter season (December–February) is the major grass deficit season in the Himalayan region and herders' feed agricultural products and graze in fallow agricultural land in that season.

The integration livestock and crop production in transhumance not only derive mutual benefits but also contribute for the sustainability of the system. Since both production systems are integrated and complementary to each other, it is difficult for local communities to completely switch to other livelihood activities (Aryal et al. 2014b). Moreover, a mixed strategy implies diversified livelihoods and reduces risks (Guillet et al. 1983; Wright et al. 2012). The diversification is important to combat contemporary threats such as climate change. As livestock production is integrated with the crop production in transhumance, it helps circular resource flows between livestock and crops and reduces the reliance on external inputs (Erenstein and Thorpe 2010; IFAD 2010). The mixed livelihood strategies also play a significant role for global food and soil nutrient security (Herrero et al. 2010; Wright et al. 2012).

5 Barriers to Livestock Movement in Transhumance Pastoralism

Examples from around the world indicate that the transhumance system has been declining, contracting or transforming and it is uncertain whether or not this traditional SES will persist (Mearns 2004). There is a marked decline of transhumance systems in Mediterranean Spain (Oteros-Rozas et al. 2013a) and Romania (Juler 2014), with some long distance movements gradually replaced by shorter distance movements, for example in Romania (Huband et al. 2010). In West Africa, long distance movements of people and livestock are inhibited by different climatic, socio-political boundaries and land use changes (Brottem et al. 2014; Gonin and Gautier 2015).

Similar to other SESs, there is tremendous pressure from the global contemporary changes to the transhumance system (Aryal et al. 2017; Farooquee 1998). Both aspects of globalisation; including social change as well the climate change have affected the transhumance system. The migration of young people from

Himalayan countries has created a shortage of people in livestock rearing (Aryal et al. 2017). In some areas such as in Sagarmatha National Park in Nepal the increasing number for foreign visitors (Fig. 5) has created alternate employment opportunities attracting local people into tourism business. As a result of increased tourism, the economic transformation from traditional farming and animal husbandry to tourism has been accomplished in some areas such as near trekking routes (Fisher 1990). Tourism has also opened ways to tourists who rarely possess ecological or cultural knowledge to learn more of local customs and traditions (Forbes et al. 2009). The regional tension and disruptions of trade across the Trans-Himalayan border with Tibet was another reason for the decline of transhumance systems in the Himalayan region (Bauer 2004; Bhasin 2011). The introduction of conservation and forest management policies restricting free movement and grazing has also undermined traditional grazing practices (Banjade and Paudel 2008). The establishment of protected areas (PAs) in Bhutan and Nepal has restricted resource appropriation by herders (Seeland 2000). Grazing based livestock production systems are more vulnerable to climate change than other livestock production (Thornton et al. 2009), and some of the recent studies (Aryal et al. 2014a, 2016) have indicated that the transhumance systems are no exception to this.

The decline or loss of livestock movement and transhumance systems can have many social and ecological implications (Aryal 2015). In Kenya, the pastoral sedentarisation had seriously compromised the livelihood and food security of the people (Fratkin et al. 2004). The loss of traditional grazing system led to the loss of indigenous knowledge of grazing and rangeland management in European Alps of Italy (Marini et al. 2011). There are reports from across the globe indicating that the

Fig. 5 Foreign visitors in Sagarmatha National Park. *Source* MoCTCA (2009, 2014)

abandonment or decline of traditional grazing systems resulted in changes in vegetation structure, shrub proliferation and land use (Sharma et al. 2013). When vegetation structure changes and shrub cover exceeds a certain level, it reduces plant diversity (Dullinger et al. 2003) and also alters breeding habitat and food resources of migratory birds (Boelman et al. 2015). In Cantabrian Mountains of Spain, there is a strong spatiotemporal adjustment of livestock movement in transhumance and vultures populations indicating that the loss of transhumance could be detrimental to vultures.

6 Conclusion

The seasonal and recurring movement of livestock is the key features of transhumance pastoralism. Transhumance is mainly practiced in the mountainous regions and the seasonal movement of livestock is essential to harness grazing resources distributed across large spatial scales and to adapt to variable environmental conditions. In addition the seasonal movement of livestock is ecologically beneficial as it avoids rangelands from being overgrazed, and allows them to recover when grazing pressure is reduced.

The crop and livestock production are integrated in transhumance and they are complementary to each other. Livestock are grazed in fallow cultivated land once crops are harvested enriching soil fertility. Livestock are also used as draught power to plough and prepare agricultural field for crops. Livestock benefit from crop production as they can feed on crop residue and graze in fallow land when there is acute shortage of grazing resources. Moreover, the movement of livestock in transhumance derive and/or support mutual benefits between crop and livestock production which enhances the sustainability of the transhumance system.

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Weeds, Herbicides and Plant Disease Management

Babak Pakdaman Sardrood and Ebrahim Mohammadi Goltapeh

Abstract Plants growing unwantedly in an agro-ecosystem are defined as weeds. Weeds modify crop plant growth, development and yield, not only through their competition for light, water, nutrients, but also through the establishment of ecological niches suitable for the growth and development of plant pathogens and pests, and the role they play as the host of hazardous organisms. Furthermore, some weeds produce allelopathic compounds that directly affect the growth and development of agricultural crops and even lead to their death. Such toxic compounds may be found in the leaf, flower, fruit, root, rhizome, and seed of the producing plants. To control weeds, various mechanical, agricultural, biological and chemical approaches are applied. Chemical control with herbicides is considered as the most easiest and attractive method applied in the control of weeds. Recently, compounds of biological origin have been introduced against weeds, of which mycoherbicides, of fungal origin, are the most famous group. Herbicides can impact the growth, development, reproduction, distribution, and survival of plant pathogens in several ways. Therefore, their rational use is considered as a vital part in integrated plant disease management programs.

Keywords Herbicide · Plant disease · Integrated plant disease management
Weed · Control

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E. Lichtfouse (ed.), *Sustainable Agriculture Reviews 31*, Sustainable Agriculture Reviews 31, https://doi.org/10.1007/978-3-319-94232-2_3

1 Introduction

The plants unwantedly growing in fields and gardens are called weeds. Weeds as a part of agro-ecosystems play various roles in the fluctuation of plant disease risk and are important from several different view points. While most of weeds may help in disease risk diminishment, however, there may be some weeds that considerably affect crop plant vigor and its potential for growth and development throughout its germination of seed till the end of its life in various direct and indirect manners, and necessitate weed control programs. The easiest strategy to control weeds is the application of herbicides that if applied in a rational and aware manner, can help in disease risk decrease. Therefore, any action against weeds shall be taken with enough knowledge of their direct and indirect impacts on weed-weed, weed-crop, weed-pathogen, and weed-vector, weed-beneficial organisms, pathogen-biological control agent interactions, and it is very clear that such a high level of disease management in a stable agricultural system necessitates detailed information of agro-ecosystem ecology as well as more innovative work on the development of new generations of herbicides that are environmentally non-hazarduos and toxicologically safe for crop plant.

The effects of herbicides on environment was the subject of the book "Herbicides and Environment" edited by Kortekamp (2011), and the impact of herbicides on soil biology and function has been reviewed by Rose et al. (2016). The application of herbicides requires more precise information not on groups of microorganisms, but on relevant species in agriculture and plant pathology, and their inter-relationships with other important species (such as weeds, host plant, vectors, beneficial microorganisms, biological control agents, and pathogens). Here, we intend to imply to the importance of the weeds in the increase of disease risk, and to the capacity of different herbicides of various classes for the applications in integrated disease management programs. The necessity of such a review was severely sensed because of the increased crises with the environmental pollutions and health risks related to the residues from the applied agrochemicals left on/in the food stuff ready for human as well as livestock consumption, and mounting list of pesticide resistance development in hazardous organisms, as well as the current global trend towards the establishment of stable agricultural systems. There are increasing reports of the involvement of cellular membrane Adenosine triphosphate-Binding Cassette transporters (ABC-transporters) in the development and appearance of resistant adapted microorganisms (Del Sorbo et al. 2000). Herbicides of different molecular topology and structure, and of antimicrobial activity can play a considerable role in pesticide resistance management, especially when they are applied in combination with biological control agents.

The widespread use of herbicides in agriculture fields raises serious and often controversial issues related to ambient soil pollution and changes in biological balance. Herbicides act as a "stress factor", and change soil microbiological balance. In a study on the changes of soil microbiology after herbicidal treatment, soil samples were collected from a cambic chernozem at depth ranges of 0–20 cm from

the experimental field. Herbicides used were clorsulfuron (Dacsulfuron® with doses ranging from 0.005 to 0.035 mg g^{-1} soil) and MCPB-Na also known as sodium 4-(4-chloro-2-methylphenoxy)butanoate (Butoxone®, with doses ranging from 0.5 to 3.5 MCPB-Na of 400 g L^{-1} g^{-1} soil). Potato Glucose Agar (PGA) was used for the isolation and identification of fungal colonies. Determination of mold colonies was performed up to genus level using identification keys. The following types of molds were identified in the control sample: *Penicillium* sp., *Humicola* sp., *Actinomucor* sp., *Aspergillus* sp., *Fusarium* sp. and *Mucor* sp. In the experimental variants treated with Dacsulfuron®, the presence of additional genera was noticed: *Trichoderma*, *Cladosporium*, *Chaetomium*, *Botrytis* and *Curvularia*. By comparison with the control samples, in the variants treated with Butaxone®, additional presence of the genus *Rhizomucor* was found. Some types were found in the soil samples treated with increasing doses of Dacsulfuron® and Butaxone®, others reduced their number or disappeared as the result of their sensitivity to the toxic action of xenobiotics. These xenobiotic substances disturbed soil biology and microbiology, and negatively influenced the quality and quantity of soil fungi communities. Dacsulfuronu® had lower toxicity on soil fungi communities than Butoxone® (Craciun et al. 2013). This was due to the large number of genera that occur in experimental variants treated with Dacsulfuron®. This information was confirmed by analysis of the diversity expressed by the Shannon-Weaver index (Shannon and Weaver 1949) which indicated a value of 0.84 for dacsufuron and 0.63 for Butaxone®.

Praveena et al. (2007) reported that herbicides could either negatively affect the growth and sporulation of the fungus *Fusarium pallidoroseum*, known as a potential biocontrol agent of water hyacinth [*Eichhornia crassipes* (Mart.) Solms], or had no significant effect on the fungus. They evaluated the in vitro effect of different herbicides including paraquat, pretilachlor, glyphosate, 2,4-D, and 2,4-D/anilofos mixture on *Fusarium pallidoroseum* colonies. Three treatments of the tested herbicides (pretilachlor, 2,4-D and 2,4-D/anilofos mixture) inhibited fungal growth and sporulation completely, whereas paraquat and glyphosate exhibited a relatively low level of inhibitory. Additionally, when lower concentration of the herbicides was used, paraquat, glyphosate, 2,4-D and 2,4-D/anilofos mixture could support the fungal growth (Praveena et al. 2007). These findings highlight the importance of herbicide dosage applied in order to suppress the weed‘s development. Highly concentrated herbicides not only damage the environment, but also might reduce the biocontrol effect of fungal agents.

2 The Effect of Weeds on Plant Diseases

Weeds as unwanted components of an agroecosystem interact with cultivated plants, therefore, the control of weeds is considered as an inseparatable part of the management of plant pathogens (Wisler and Norris 2005). Weeds can influence on

plant diseases through their direct and indirect effects on the components of disease triangle (host plant, pathogen, and biotic as well as abiotic environmental factors):

2.1 Direct Effect of Weeds on Plant Physiology

Weeds generally exert their hazardous effects on crop plant growth through competition for the limited water supplies, limited rates of available nutrients, space and light. Fast growing weeds can germinate and emerge earlier and reduce crop plant germination and growth through the interception of sun beams and use of water supplies. For example, the seeds of the weeds like *Datura stramonium*, and *Abutilon theophrasti* (Fig. 1) germinate synchronous to soybean seed germination, grow fast and form their well-developed canopy over soybean seedlings, the result is clear: soybean seedlings will not be able to catch light required for photosynthesis, and will consequently be etiolated and ultimately die (Merrill and Lembi 2009). In sugarcane fields, the increased growth and development of weed grasses leads to the decreased and weak growth and development of sugarcane, so that the weakened plants are difficult to discriminate from those suffering from ratoon stunting disease.

Fig. 1 Velvetleaf (*Abutilon theophrasti*), an allelopathic malvaceaous weed with velvet-like heart-shaped leaves, yellow flowers and capsular button-like fruits (*Source* http://nyc.books.plantsofsuburbia.com/) (Color figure online)

Additionally, some of weed plants actively produce and secrete toxic chemicals that after their release to soil environment impose their inhibitory effects on crop seed germination. Such a phenomenon first discovered by H. Molisch in 1937, was called "allelopathy". Dependant on the level of phytotoxicity of the secreted compounds and the concentration of the produced chemicals, environmental conditions, and the susceptibility of the crop plant in the given stage in the life cycle, it can end to the postponed germination and emergence of the seedlings, or to the death of the sensitive embryo of the crop seed or its germlings. Such allelopathic compounds may be found in the leaves, roots, flowers, fruits, and rhizomes of the producing weeds (Putnam and Weston 1986). Fischer (1986) reported that the sesquiterpene lactones found in the water after washing of the leaves of the plants belonged to the families Asteraceae, Apiaceae, and Magnuliaceae were inhibitory to the seed germination of other plants. In mixed culture systems, the allelopathic compound, juglone produced and secreted by the leaves of walnut (*Juglans regia*) trees can suppress the germination of herbaceous plant seeds.

In the evaluation of the allelopathic effects of the weeds *Chenopodium album*, *Amaranthus retroflexus* (Fig. 2) and *Cynodon dactylon* on the rapeseed germination and seedling growth, Yarnia and Rezaei (2006) found that root and shoot extracts of *A. retroflexus* and *C. dactylon* resulted in the 65% reduced seedling height, while that of *C. album* caused 38% reduction. Rapeseed seed germination percentage was decreased 30% with *C. album* extract, however, more than 90% with the extracts of other weeds. In a similar study with soybean, Farajzadeh et al. (2006) concluded that the extract prepared from the weed *C. album* reduced soybean height of seedling more than 80% (compared with control) and that of other weeds *A. retroflexus* and *C. dactylon* caused 60% reduction. They also found that soybean seed germination was decreased 70% as the result of the treatment with the extract of *C. album*, and 30% with the extracts of other two weeds. Rezaei et al. (2006) obtained similar results with the extracts of above-mentioned weeds and safflower.

Weeds that secrete their allelopathic compounds from the root system near to the depth of soil, or those that produce water-soluble compounds easily washed from the leaves and penetrate to the soil, where crop seeds are laid, will expectedly be of the most inhibitory influence on crop seed, however, the major allelopathy seems to occur by the chemical compounds released from plant residues as the result of microbial activity after weed death, the effect that has been reported by several authors (Patrick 1971; Rice 1984; Barnes et al. 1986). Although these chemicals may be non-toxic in feature, however, can potentially lead to considerable localized changes in soil pH and/other factors that can end to phytotoxic effects imposed on crop seed, germlings and seedlings. Rhizomes and residues of the graminaceous weed *Agropyron repens* affect on the growth of small-grain cereals (Ohman and Kommedahl 1960) and corn (Bandeen and Buchholtz 1967). Weeds like *Sorghum halpense*, *Setaria viridis*, *Digitaria* spp., and *Helianthus annus* impose their allelopathic effects on agronomical crops and convert environmental conditions to those favorable for their own growth and development.

Fig. 2 *Amaranthus retroflexus* (*Source* http://www.narodrecept.ru/)

2.2 *Creation of Favorable Conditions for Pathogen Growth and Development*

Some of plant pathogens require moist and humid conditions for their growth and development, and the epidemics of these diseases are in close relation to wet and high relative humid conditions and the primary foci of such diseases are found in the points of fields and gardens, where such conditions are provided. Weed plants especially those with large and broad leaves with higher densities of stomata absorb soil water and evapotranspirate it to the air, and provide the conditions conducive for pathogens.

Oomycetous airborne microorganisms like those causal of downy mildews are a good example for such a topic. Grapevine downy mildew disease caused by *Plasmopara viticola* is well encountered wherever moist conditions are provided by any biotic or abiotic factor such as irrigation water furrows, and weeds. Grey mold disease of grapes caused by *Botrytis cinerea* is more severe under humid conditions, and the same is true with the rot and blight diseases induced by the generalist pathogen *Sclerotinia sclerotiorum* (Agrios 2005). Powdery mildews another group of plant diseases develop from their foci in the points where high relative humidity exists (Agrios 2005). Bacterial pathogens need free water layer and water soaked

leaves in order to penetrate plants and cause disease (Agrios 2005), and the relative high humidity provided by weeds creates localized climates that may end to dew drop formation on sensitive host plants in dawns. Weeds grown under trees and in their crown vicinity provide humid conditions that predispose trees to the attacks by various fungi and fungus-like microorganisms. *Phytophthora* species and basidiomycetous wood-rotters like *Armillariella* spp. are among famous pathogens of tree plants to exemplify.

2.3 The Effect of Weeds on the Dissemination of Pathogens

Weeds can play their role in pathogen dissemination and disease spread by two ways, directly and indirectly.

2.3.1 Direct Effect of Weeds on the Dissemination of Pathogens

In a direct way weeds may help pathogens to be distributed through their organs like seeds which have been infected or contaminated by the pathogens such as viruses. In fact, most weed are disseminated through their wind-born seeds (Sorensen 1985), and seed dissemination crosses various groups of pathogens (Gamliel 2008). Most of the dangerous fungal diseases of sugarcane such as red rot caused by *Colletotrichum falcatum* and smut caused by *Ustilago scitaminea* survive on perennial host grasses as Kan grass (*Saccharum spontaneum*) and Munj grass (*S. munja*) often grown around sugarcane fields. Grassy weeds like wild oats (*Avena fatua* L.) and ryegrass (*Lolium* spp.) maintain cereal cyst nematode (*Heterodera avenae* Woll.) populations and should be controlled. The seed-gall nematode *Anguina tritici* parasitizes rye and the wheat ancestors, emmer, spelt, and *Aegilops* (Wiese 1987). The root-knot nematodes, *Meloidogyne* species infect an abundant number of the plants belonged to the family Solanaceae, and the produced propagules are transferred through water and soil translocations to the plots cultivated with vegetables and cucurbits. Some weeds grown among more disease resistant crop plants may be more susceptible to a pathogenic invasion, and can be infected earlier and increase the potential of pathogen inoculum that leads to further temporal and spatial distribution of the disease. The increased potential of inoculum can lead to spill over of disease and break of resistance of crops (Keesing et al. 2006). The fungal pathogen of brown leaf spot of paddy, *Bipolaris oryzae* is found also on the alternative host plants *Oryza montana* as well as *Leersia hexandra* in nature (Chattopadhyay and Chakrabarti 1953). The wounds resulted from the compact growth and harsh contacts can potentially help disease spatial progress. *Tilletia controversa*, the wheat dwarf bunt fungus can infect wheatgrass *Agropyron repens* and a variety of other wild grasses. *Bromus*, *Dactylis*, and *Poa* species are infected by *Cephalosporium gramineum*, the only true vascular fungal pathogen of wheat that cause a soilborn disease called wheat cephalosporium stripe.

Pathogens either of obligatory or facultative, specialized or non-specialized types are usually of vast host ranges. Some of the pathogens are found restricted to a plant species while others can infect very abundant plant species of different families. Highly specialized pathogens such as rust pathogens are of physiological races and they may not essentially attack from infected grasses to cultivated wheat or barley plants. However, wheat black stem rust pathogen *Puccinia graminis* f. sp. *tritici* a heteroeceous macrocyclic rust fungus infects quackgrass and wild oats as, and spreads over wheat, barley and oat cultivars grown in the farms. Thus, by this means, weeds can act as foci for the distribution and spread of the diseases. *P. g*. f. sp. *striiformis* another rust fungus causes stripe rust or yellow rust as well as glume rust on barley, wheat, and other grasses including perennial grasses that play an important role in disease establishment and development as the reservoirs for the fungus. The main problem occurs with the non-specialized pathogens of vast host ranges that take advantage of weeds, wild hosts as well as self-sown host plants for their own survival, spread and reproduction in the presence or absence of their main host plant. The direct tranfer of diseases may occur through root and/or shoot bridges where infected weeds directly involve in the spatial distribution of plant diseases. With the increase of host densities, contacts between hosts will also increase and allow more opportunities for the transmission of pathogen, which is very important in the transfer of some viruses as well as fungi and bacteria. Furthermore, the probability of a pathogen death in a host population will decrease at high population densities (Anderson and May 1991), which is of high importance in the spread of the diseases caused by biotrophic pathogens such as powdery mildews as well as facultatively saprophytic pathogens such as *Phytophthora* species which are very weak saprophytes in the absence of their hosts. Weeds may act as green bridges and facilitate the hastened development of soil-born pathogens. This is most important with the pathogens which are of vast host range. A noticeable classic example of such pathogens may be the ascomycetous fungus *Sclerotinia sclerotiorum* that invades more than 400 plant species of more than 75 plant families (Boland and Hall 1994). Similarly, *Sclerotium rolfsii* has an extensive host range, and at least 500 species in 100 families are susceptible. The most common hosts are the legumes, crucifers, and cucurbits. The fungus persists in many weed hosts as well (Punja 1985). Cucumber mosaic virus (CMV), some of powdery mildew fungi (such as *Erysiphe polygoni*), and root-knot nematodes (*Meloidogyne* spp.) each are capable to infect more than 700 host plant species. *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*; powdery mildew of cucurbits) infects 570 host plant species. The host range of the root rot causal fungus, *Phymatotrichum omnivorum* includes at least 1300 plant species (Taubenhaus and Ezekiel 1936). The main danger of the presence of additional hosts is the transfer of plant pathogens from a season to another season, as well as the provision of the conditions required for the propagation of inoculum and its development to the levels needed for an epidemic occurrence of the disease on the main culture in the early season. *P. xanthii* overwinters on wild cucurbits and also on the cultivated shade plants out of the season. Such a mechanisms seems be true with the viruses of cucurbitaceae and many other host plants. The soil-born take-all fungus

Gaeumannomyces graminis var. *tritici* although specialized for the pathogenicity on wheat, however, it also invades bromegrass (*Bromus* spp.), wheat grass, and quackgrass (*Agropyron* spp.), and the weeds can play the role of green bridges to facilitate and accelerate fungal spread in the area, wherever wheat plants have not form a dense cover of vegetation (Singh 2001).

Some pathogens may be transmitted via the absorptive organs (haustoria) of parasitic weeds involved in the suction of host plant phloem sap. Dodder (*Cuscuta*), a twining yellow, orange, sometimes tinged with purple, red, and occasionally almost white plant can feed on an infected weed and spread over crop plants in the close vicinity of its first host weed. Dodder with the very thin and threadlike or relatively stout stems is classified as a member of the Morning-Glory Family, convolvulaceae (Garcia et al. 2014). *Cuscuta* spp. have a broad host range, including many cultivated crops such as tomatoes, tobacco, clover, and dicotyledonous weeds as well as trees and shrubs, but only a few grasses or monocotyledonous weeds (Dawson et al. 1994; Albert et al. 2008). Some other dodders such as *Cuscuta campestris* (Fig. 3), and *C. pentagona* are agriculturally important, found worldwide, and can synchronously infect a broad range of higher plants (Lanini and Kogan 2005). Dodders parasitize various kinds of wild and cultivated plants, and is especially destructive to agronomic plants (alfalfa, lespedeza, flax, clover and potatoes) as well as ornamentals (chrysanthemum, dahlia, helenium, virginia-creeper, trumpet-vine, English ivy and petunias). Water, minerals and carbohydrates are absorbed from the host through haustoria (modified adventitious roots) that press up against the stem of the host plant and penetrate its tissue. Although dodder rarely ends to host plant death and leads to stunting of its host growth, however, it transfers a range of diseases. In addition to leaf hoppers, dodder can transmit phytoplasmas, the cause of more than 200 so-called yellows diseases (Swift 2010). Bennett (1940) indicated that dodder would transmit viruses from a plant to another. The transmission of the viruses probably takes place through plasmodesmata that provide transient connections sites between dodder haustorial tip and host cell cytoplasm.

Fig. 3 Vines of dodder (*Cuscuta campestris*) turned around green host stem. Note to the clusters of small white flowers each harboring five white triangular petals (*Source* http://smmflowers.org/)

Dodder-vectored transmission is similar to grafting in some aspects, however, graft compatibility is limited to quite closely related plants, usually within a genus. Dodder, on the other hand, can be used to transmit a virus between distantly related plants (Desjardins et al. 1969). While some viruses can be replicated inside dodder body (such as cucumber mosaic virus) others are transmitted in a passive manner with no replication (such as tobacco mosaic virus) (Bennett 1940). However, capability of a virus to replicate inside a dodder does not mean its ability to be transmitted by the proliferative dodder. Grapevine Leafroll associated virus 7 (GLRaV-7) can be transmitted from one host to another by *Cuscuta reflexa* and *C. europea* in which it appeared to replicate, however despite its replication in *C. campestris*, it could not be transmitted to another host via this dodder species (Mikona and Jelkmann 2010). Dodders may sometimes harbor an unsuspected virus. It has been indicated that symptomless *C. californica* is frequently infected with a virus that causes serious diseases in several unrelated plant species. The virus was called as dodder latent mosaic virus (Bennett 1944). Dodder is regarded as an insignificant factor in the transmission of economically important viruses in the field, and has rarely been used in experimental work in recent times (Hull 2014). Dodder has been shown to spread the yellows disease pear decline, aster yellows, tomato big bud, *Vinca* virescence and elm phloem necrosis. Furthermore, phloem-inhabiting "rickettsia-like" bacteria have been found to be present in dodder (Swift 2010). It is not known if the viruses can be transmitted from the holoparasitic plant bromrape (*Orobanche* sp., Fig. 4) to other hosts of this parasitic plant (Hull 2014). Cucumber mosaic virus (CMV), tomato mosaic virus (ToMV), potato virus Y (PVY), and tomato yellow leaf curl virus (TYLCV) can be translocated from infected hosts to the broomrape. CMV and possibly other viruses replicate in the parasitic plant *Phelipanche aegyptiaca* (Gal-On et al. 2009). Horizontal gene transfer between plants has been reported to take place by another parasitic plant, witchweed, *Striga hermonthica* (Yoshida et al. 2010; Fig. 4). Although there are no records of virus transmission by this important parasite in tropical countries, the capability of horizontal gene transfer indicates the parasitic weed potential as a vector (Hull 2014). The witchweed is a hemiparasitic plant that parasitizes the roots of cereals and other poaceous plants. Millets (*Panicum* spp.), finger millet (*Eleusine coracana*), rice (*Oryza sativa*), sorghum (*Sorghum bicolor*), sugarcane (*Saccharum officinarum*), and maize (*Zea mays*) are regarded as the main hosts of witchweed (www.cabi.org/isc/datasheet/51849).

2.3.2 Indirect Effect of Weeds on the Dissemination of Pathogens

Indirect role of weeds in plant disease spread is through their hosting for the organisms that feed on them and can potentially act as vectors of the pathogens, however, if weed is an alternative host for the vector but not for the pathogen, then its presence may lure vectors away from the focal host, and decrease transmission (Keesing et al. 2006). In some vector-born diseases, several vector species can transmit the pathogen, therefore, infectious diseases are considered as inherently

Fig. 4 Two examples of weeds that parasitize root of crop plants (Left) *Orobanche* sp. on tobacco (*Source* http://ephytia.inra.fr/); and (right) *Striga hermonthica* grown by its host plant, rice (*Source* Mr. Eng. HAJM Toussaint from Wageningen University & Research)

ecological systems that involve the interactions among small to large networks of species. It is then not so surprising that the potential connection between species diversity and diseases transmission has long been recognized (Keesing et al. 2006). Such a diversity can for instance be resulted either from the intercropping of different agricultural plants, or the diversity of different vectors and host weeds grown among crops, etc. The connection between diversity and disease was also recognized by Elton (1958), who suggested the possibility of plant disease amelioration in complex ecosystems provided that the existent complexity reduces host plant density for a disease. However, there are controversial results obtained by different researchers, for instance, various empirical and modeling investigations have suggested the reduction of disease risk as the consequence of the increased species diversity that occurs through regulation of the abundance of an important host species (Burdon and Chilvers 1982; Rudolf and Antonovics 2005) or through redistribution of vector meals in the case of the diseases transmitted by vectors (Van Buskirk and Ostfeld 1995; Norman et al. 1999; LoGiudice et al. 2003). However, other studies have suggested the increased disease risk resulted from the increased diversity if the added species functions as alternative sources of infection, or if the added species increase vector numbers or activity through provision of additional sources of vector meals (Holt and Pickering 1985; Norman et al. 1999; Gilbert et al. 2001; Schmidt and Ostfeld 2001; Saul 2003; Dobson 2004). High host densities are

likely to increase transmission rates both within the domestic populations and between domestic and wild or weed species (Burdon and Chilvers 1982; Anderson and May 1991; Gilbert 2002). In most multiple host systems, all host species are not equal in their epidemiologically key characters such as resistance, tolerance, and vector preference (Daszak et al. 2000; Woolhouse et al. 2001; LoGiudice et al. 2003), and consequently, the rates of transmission of a generalist pathogen with a wide host range within and between different host species are generally highly heterogenous and asymmetric (Woolhouse et al. 2001).

The effects of 21 weeds acting as hosts of tomato spotted wilt virus (TSWV), temperature, thrips population and diversity on disease progress in chrysanthemum cv. Polaris were studied. Under greenhouse conditions, only *Taraxacum officinale*, *Bidens* sp., *Reseda luteola* and *Mirabilis jalapa* were hosts for TSWV. Of 38 weeds species in the area surrounding a chrysanthemum field, *Tithonia tubaeformis* and *R. luteola* had the highest populations of adult and immature thrips. These weeds, as well as *M. jalapa* had an extensive seasonal distribution and may play a key role in the disease progress. Seventeen thrips species belonging to the genera *Bravothrips*, *Thrips* and *Frankliniella* were identified on weed flowers, with *Frankliniella occidentalis* (FOC; Fig. 5) representing 9.5% of all thrips identified. Of 123 thrips collected from chrysanthemum inflorescences, 9.75% were FOC, and only 2.5% of them transmitted TSWV. Of all the thrips species collected from chrysanthemum flowers in the field, only FOC was capable of transmitting TSWV. On 120 experimental plots established at two sites, with three transplanting dates (June, July and August), it was estimated that 1.25% of the chrysanthemum cuttings were already infected with TSWV when transplanted. Secondary spread, vectored by FOC, occurred only for the earliest transplanting date and resulted in a further 2.36% disease incidence.

Fig. 5 *Frankliniella occidentalis*, the thrips that vectors tomato spotted wilt virus, and the disease symptoms on fruits (*Source* Russell IPM Ltd, and Whitney Cranshaw from Colorado State University)

In heterogenous populations of host species, wild plants as well as weeds may act as reservoir species that maintain a relatively high population of pathogen. In such a situation, the pathogen typically reaches high prevalence in the reservoir and after then spills over into the other host maybe an agricultural crop, a process called "the spillover effect" or "pathogen spillover" (Daszak et al. 2000). The pathogen spillover may lead to the reduced abundance of the non-reservoir hosts such as of the interest agricultural crop, a pathogen-mediated phenomenon that is called "apparent competition" (Power and Mitchell 2004). There are increasing documents that support the theoretical predictions that such a phenomenon can control the outcome of the interactions among wild weed plants and crop plants that share a common natural enemy (Holt and Lawton 1994; Alexander and Holt 1998; Hudson and Greenman 1998). The pathogen might be of different kingdoms. Ergot caused by the fungus *Claviceps purpurea* is a historical disease that is repeatedly encountered on wild grasses beside its cereal hosts like wheat, rye, barley and oats, and insect vectors attracted to the secreted fungal honey dew deliver fungal conidia produced by its asexual form *Sphacelia segetum*. Grasses, especially *Agropyron repens* and *Phalaris arundinacea* are susceptible hosts for wheat chlorotic streak virus as well as for its plant hopper vector *Laodelphax striatellus* (Fallén). Maize streak virus (MSV), a *Mastrevirus* indigenous to Africa (Willment et al. 2001; Fajemisin 2003; Karavina 2014), is one of at least eight viruses that cause significant agronomic losses in maize (*Zea mays* L.) worldwide (Redinbaugh et al. 2004). It causes serious maize streak disease (MSD) of corn in sub-Saharan Africa (Martin and Shepherd 2009) and south-east Asia. A *Pennisetum* strain of MSV infects wheat in India, and others infect sugarcane, guinea grass (*Panicum maximum* Jacq.), oats, barley, and certain wild grasses. The virus is circulatively transmitted by the species of sap-feeding *Cicadulina* leaf hoppers (Homoptera: Cicadellidae: Deltocephalinae: Macrostelini) (Bosque-Perez 2000), and despite all stages of leaf hoppers transmit MSV, but transovarial passage has not been demonstrated. The virus (like other African streak viruses) is neither seed nor mechanically transmitible (Martin et al. 2008). *Cicadulina* species (Fig. 6) are generally considered as grassland leafhoppers present in wild and pasture throughout the year, but can migrate in large numbers to maize (Page et al. 1999). Agropyron mosaic virus (AgMV), a *Rymovirus* infects wheat, rye, barley, and grasses like *Elytrigia intermedia*, *Elytrigia repens* (a maintenance and propagation host in addition to bread wheat), and *Lophopyrum elongatum*. The virus is transmitted by the mite *Abacarus hystrix* distributed widely by air flows, however, it is not transmitted by seed, pollen, and even bu other mites such as *Aculus mckenziei*, and *Eriophyes tulipae* (Brunt et al. 1996; Dallwitz 1980; Dallwitz et al. 1993).

Aphids, white flies, thrips, leaf-hoppers, tree-hoppers, beetles, nematodes and mites can feed and survive on their diseased weed hosts, and increase their population feeding on them, and then transfer pathogens to crop plants. Aphids and cabbage root maggots live on mustard weed, and then attack cabbage, cauliflower, turnip, and radish plants. The barley yellow dwarf virus (BYDV) is a phloem-limited *Luteovirus* obligately transmitted in a persistent manner by several species of aphids feeding on wild grasses (Miller and Rasochova 1997) such as wild

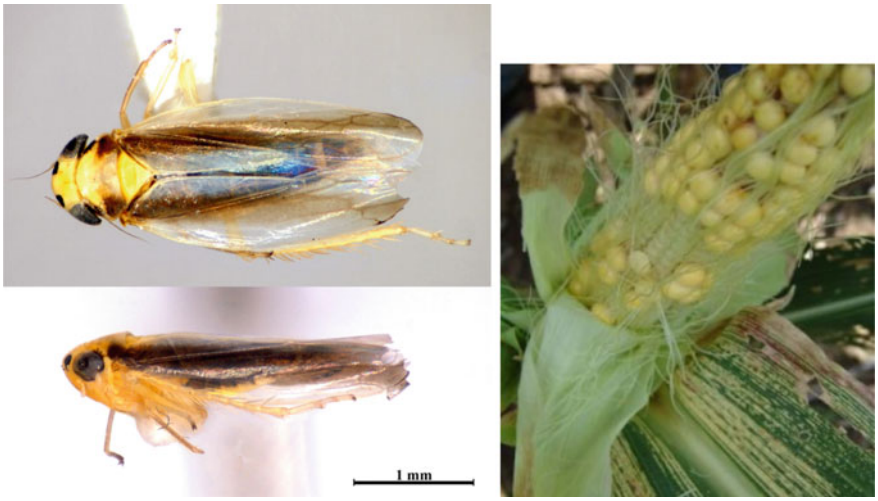


Fig. 6 From left to right: the African maize leafhopper, *Cicadulina mbila* (*Source* www.dpi.nsw.gov.au/keys/leafhop/species/cmbila.htm; credited by Holger Löcker) the most common and ubiquitous leaf hopper that transmits maize streak virus (Ruppel 1965) with a capacity higher than any other leafhopper species; Maize streak virus symptoms developed as chlorotik streaks on leaves and deformed cob development (*Source* Blackwell Publishing Ltd 2007; credited by Dr. F. Kloppers)

oats, *Avena fatua* a particularly appropriate host of BYDV that exhibits high sensitivity to infection and harbors high concentrations of the virus when infected (Power and Mitchell 2004). Power and Mitchell (2004) found no significant effect of the number of host species (richness) in the plant community on the prevalence of BYDV, but they perceived the significantly different prevalence of the virus among host communities. In particular their planned contrast indicated that the communities with *A. fatua* were of considerably higher BYDV prevalence compared with those without *A. fatua*, a result suggesting less importance of host diversity for the viral disease epidemiology versus high importance of the existence of one highly susceptible suitable host species. Furthermore, they found that in the communities with *A. fatua*, the proportion of the community made up by *A. fatua* was of no significant impact on virus prevalence among all other species, however, the simple presence of *A. fatua* affected virus prevalence in other plant species. The presence of *A. fatua* led to greatly increased prevalence of the viral pathogen in several other species demonstrating the pathogen spillover and illustrating the crucial role of host community structure in the control of the generalist pathogen dynamics. Additionally, virus spillover from wild oats decreased the abundance of two other host species *Lolium multiflorum* and *Digitaria sanguinalis* through pathogen-mediated apparent competition. Such studies indicate the role of reservoir weed species in disease prevalence. On the other hand, increased diversity by addition of host species which are less competent reservoirs may lead to reduced

disease risk if the newly added species diminish the probability of encounter between the pathogen and the focal host species that is a highly competent reservoir. The resulted encounter reduction will end to reduced disease prevalence. Such a phenomenon may occur in the multi-host plant species communities, where both weeds and crop do not effectively allow the commonly shared pathogen transmission. Most Indian wheat cultivars are susceptible to wheat mosaic streak virus transmitted by sap and by the aphids *Brachycaudus helichrysi* (Kalt.) and *Rhopalosiphum maidis* (Fitch.). The infected wheat plants occur near reservoirs of the virus in cardamom (*Amomum subulatum* Roxb.) and are predisposed to the infection by *Bipolaris sorokiniana*. Peanut mottle virus (PMV) has been isolated from a few weed hosts in nature: *Cassia obtusifolia* L., *C. leptocarpa*, *C. occidentalis*, and *Desmodium canum*. PMV is easily transmitted mechanically to and from soybean plants. Several aphid species such as *Aphis craccivora*, *A. gossypii*, *Hyperomyzus lactucae*, *Myzus persicae*, *Rhopalosiphum padi*, and *R. maidis* can readily transmit PMV in a non-persistent manner. Several other plant species, mostly legumes, can be infected with PMV: *Calopogonium mucunoides*, *Canavalia ensiformis*, *Cassia bicapsularis*, *Chenopodium amaranticolor*, *Cicer arietinum*, *Cyamopsis tetragonoloba*, *Lathyrus odoratus*, *Macroptilium atropurpureum*, *M. lathyroides*, *Phaseolus coccineus*, *Sesamum indicum*, *Trifolium hybridum*, *Trigonella foenum-graecum* L., *Vigna cylindrica*, *V. oblongifolia*, and *V. unguiculata* subsp. *unguiculata* cultigroup *sesquipedalis*. It is believed that aphids involve in the transfer of potato leaf roll virus from nightshade plants, where the virus replicates to potato plants (Hartman et al. 1999).

Hairy nightshade, *Solanum sarrachoides* is a ubiquitous weed in potato agro-ecosystems and nonagricultural lands of southeastern Idaho and the Pacific Northwest. This weed increases the complexity of the Potato leafroll virus (PLRV) (Luteoviridae: Polervirus)-potato pathosystem by serving as aphid and virus reservoir. Previous field studies showed higher densities of green peach aphid, *Myzus persicae*, and potato aphid, *Macrosiphum euphorbiae*, the two most important vectors of PLRV, on *S. sarrachoides* compared with potato plants in the same fields. Some of the *S. sarrachoides* plants sampled in these surveys tested positive for PLRV. Viral infections can alter the physiology of plant hosts and aphid performance on such plants. To understand better the potential effects of *S. sarrachoides* on the PLRV-potato-aphid pathosystem, the life histories of *M. persicae* and *M. euphorbiae* were compared on virus-free and PLRV-infected *S. sarrachoides* and potato. Individual nymphs of each aphid species were held in clip cages on plants from each treatment to monitor their development, survival, and reproductive output. Nymphal survival for both aphids across plant species was higher on *S. sarrachoides* than on potato, and, within plant species, it was higher on PLRV-infected plants than on noninfected plants. With a few exceptions, similar patterns occurred for fecundity, reproductive periods, adult longevity, and intrinsic rate of increase. The enhanced performance of aphids on *S. sarrachoides* and on PLRV-infected plants could alter the vector population dynamics and thus the PLRV-disease epidemiology in fields infested with this weed (Srinivasan et al. 2008).

In California, a kind of life and three-faced transfer of a mycoplasma disease from weeds to citrus trees has been discovered. A leaf hopper transfers the citrus stubborn disease causal microorganism *Spiroplasma citri* to the weeds periwinkle and London rocket *Sisymbrium irio*, and acquires it from them in next feeding times, so that the weeds act as the sources of the inoculum required for the infection of citrus trees. *Eragrostis*, *Bromus*, *Panicum*, and other grass genera are as susceptible as wheat plants to American wheat striate mosaic virus (AWStMV, a rhabdovirus), and durum wheat plants appear most susceptible. While the virus can not pass through eggs and plant sap, both nymphs and adults of the painted leaf hopper *Endria inimica* transmit the virus. *Elymana virescens* F. is also another vector involved in the transmission of AWStMV. European wheat striate mosaic virus (EWSMV, a tenuivirus) occurs on oats, barley, rye, corn, and grasses (*Lolium* spp.) in addition to wheat, and is transmitted persistently by plant hoppers, principally *Javesella pellucida*, and to lesser extent, by *J. dubia*, *Loadelphax striatellus*, *Psammotettix alienus*, *Delphacodes pellucida*, and *Calligypona pellucida*. The pathogen is passed through eggs and apparently is stable within its vectors for generations. Nymphs of *J. pellucida* are efficient vectors of both of oat sterile dwarf virus and European wheat striate mosaic virus, as a result, host plants may be doubly infected. Infectious nymphs overwinter on grasses and reach adulthood in early summer. Wheat infections are most numerous in autumn and spring and reflect the distribution and activity pattern of nymphs, more efficient vectors compared with adults (Wiese 1987).

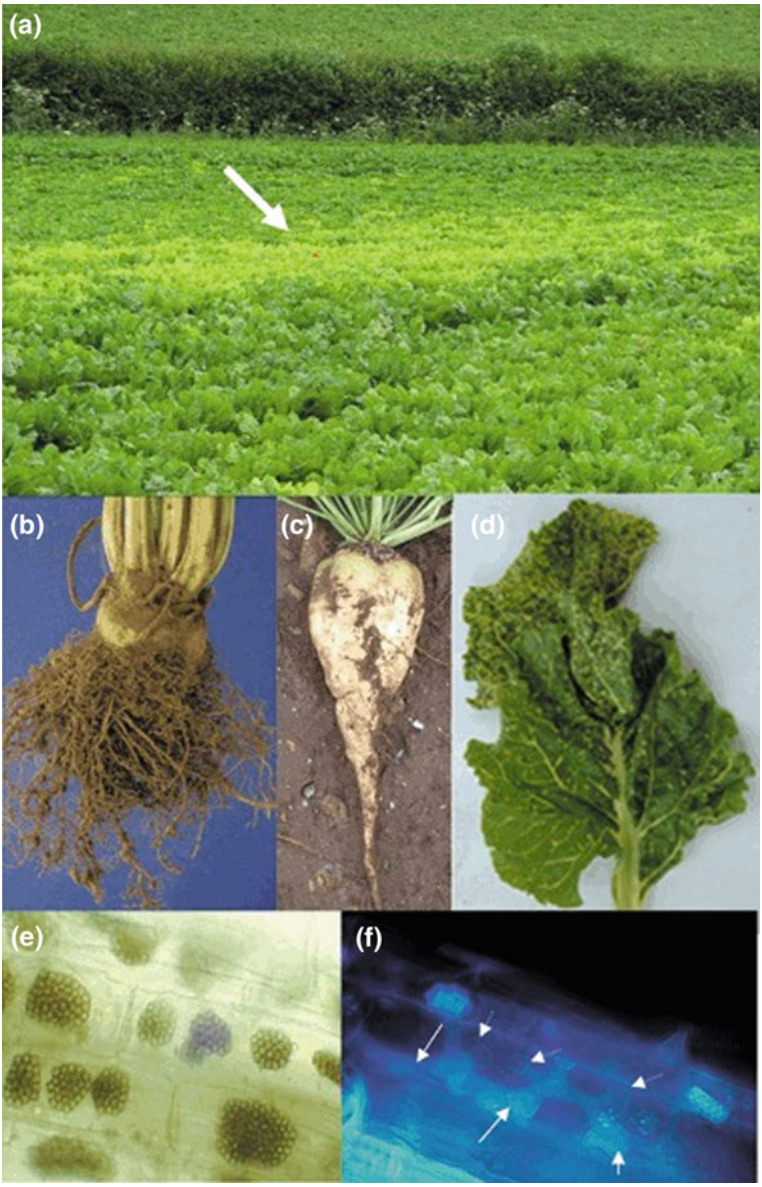
Onion thrips, *Thrips tabaci* (Fig. 7), regarded as a global pest of increasing concern in onion (Diaz-Montano et al. 2011) feeds on amarantha (*Amaranthus palmeri*), dandelion (*Taraxacum officinale*), mullin (*Verbascum thapsus*), goldenrod (*Solidago canadensis*), Kochia (*Kochia scoparia*), sage (*Salvia* sp.), sunflower (*Helianthus annuus*), smartweed (*Polygonum* spp.), yellow nutgrass (*Cyperus esculentus*), ragweeds (*Ambrosia* spp.) and mustard (*Brassica* spp.) (Chittenden 1919; Doederlein and Sites 1993), and then invades onions (Rahman and Batra 1945; Ananthakrishnan 1971).

The transmission of tobacco streak virus by thrips was investigated by Sdoodee and Teakle (1987) who mixed the adults or nymphs of *Thrips tabaci* with virus-carrying pollen from *Lycopersicon esculentum* infected with tobacco streak virus and then placed them on *Chenopodium amaranticolor* test seedlings, and found that the virus was regularly transmitted. The virus was also regularly transmitted when virus-carrying pollen was placed on the leaves of *C. amaranticolor* test seedlings and the thrips then introduced. No transmission occurred when test seedlings were exposed to virus-carrying pollen in the absence of the thrips or to the thrips without pollen. Further, no transmission occurred when the thrips were fed on virus-infected leaves and then transferred to test seedlings in the absence of virus-carrying pollen. The evidence indicates that the transmission of tobacco streak virus by *Thrips tabaci* depends on the presence of pollen-borne virus, which presumably infects via wounds made by the thrips. This method of virus transmission

Fig. 7 From left to right, *Thrips tabaci* (*Source* Thrips of California 2012, available online at keys.lucidcentral.org) the polyphagus insect known as the only vector of iris yellow spot virus; close-up of symptoms caused by IYSV on field onion leaf; and extensive leaf lesions on an onion plant heavily affected by IYSV (*Source* www.cabi.org) (Color figure online)

had not previously been reported. More importantly, *T. tabaci* is the only known vector that transmits Iris yellow spot virus (IYSV, a *Tospovirus* from the family Bunyaviridae) in many parts of the world. IYSV infects onions and induces symptoms including straw-colored, dry, tan, spindle/diamond-shaped lesions on the leaves and scales of onion plants and causes serious yield losses up to 100% in several countries (Diaz-Montano et al. 2011).

Several weed species, including the monocotyledonous plants *Alopecurus myosuroides*, *Lolium multiflorum*, *Sorghum vulgare*, *S. halepense*, and dicotyledonous plants *Calystegia sepium*, *Capsella bursa-pastoris*, *Centaurea cyanus*, *Convolvulus arvensis*, *Galinospora parviflora*, *Matricaria iodora*, and *Stellaria media* have been proved as alternative hosts for the beet necrotic yellow vein virus (BNYVV; Fig. 8), and beet soil-borne virus (BSBV), as well as their common plasmodiophorid vector, *Polymyxa betae* (Fig. 8). Also, *Chenopodium album* has been identified as a host for *P. betae*, but it is not a host for the viruses as tested by enzyme-linked immunosorbant assay (ELISA). The status of the weeds as alternative hosts has been pathologically confirmed through re-transmission of the viruses by their vector from the infected weed roots to susceptible sugar beet plants. Additionally, the use of molecular techniques (ITS sequence, and northern blot analysis) has indicated that *P. betae*, but not *P. graminis* is the vector involved in virus transmission from weed to sugar beet plants (Mouhanna et al. 2008). Several wild Hordeum species were mostly susceptible to *P. graminis* and/or barley mild mosaic virus, transmitted by *B. graminis*. An isolate of *P. betae* was used for comparison, and caused slight infection on oats but not on other cereals. The variation within and between Polymyxa spp. needs more detailed investigation (Adams and Jacquier 1994).


(a)
(b)
(c)
(d)
(e)
(f)

◂**Fig. 8** Symptoms of beet necrotic yellow vein virus (BNYVV) on sugar beet, and *Polymyxa betae*, the plasmodiophorid vector of rhizomania disease caused by the virus. **a** Rhizomania-infected patch (indicated with arrow) typical of BNYVV infection in the field; **b** classical "root madness" symptoms in BNYVV-infected sugar beet; **c** a virus-free root; **d** necrotic yellow vein symptom only observed under optimal disease conditions; **e** long-lived resting spores in sugar beet roots viewed under light microscope; **f** multilobed zoosporangium (indicated with solid arrows) inside host root cells after secondary zoospore release. Exit tubes where zoospores were released are visible (indicated with dotted arrows). The *P. betae* zoosporangium had been fixed in situ in 10% formaldehyde, pH 7.2, for at least 3 years prior to visualization and photography under the microscope with ultraviolet illumination (*Source* McGrann et al. 2009) (Color figure online)

2.4 Weeds as Alternate Hosts of the Pathogens

A number of pathogens including heteroecious rusts need two kinds of host plants in order to complete their life cycles. Some weeds have been known to play an important role in the life cycle complementation of rusts. Wheat black stem rust fungus *Puccinia graminis* f. sp. *tritici* forms its pycnia and aecidia on the leaves of barberries *Berberis vulgaris* L. and *B. canadensis*, and certain species of *Mahonia*. *Berberis* spp. are also the alternate hosts of the macrocyclic rust fungus, *P. striiformis* (Fig. 9) that causes yellow rust also known as glume rust, and stripe rust of cereals (Jin et al. 2010). With *P. g.* f. sp. *recondita* the causal fungus of a wheat disease called leaf rust, brown rust, dwarf rust as well as orange rust, aecia are formed on *Anchusa*, *Anemonella*, *Clematis*, and *Isopyrum* species (Prescott et al. 1986).

Fig. 9 Aecial pustules of the wheat yellow rust fungus *Puccinia striformis* on the abaxial side of *Berberis chinensis* leaf (*Source* Jin et al. 2010) (Color figure online)

3 Herbicides

3.1 Herbicide Groups

According to the classification system proposed by Herbicide Resistance Action Committee (HRAC) herbicides are classified in the HRAC groups (www.hracglobal.com/pages/classificationofherbicidesiteofaction.aspx) as below:

3.1.1 HRAC Group A

This group includes the herbicides that inhibit lipid biosynthesis through the inhibition of the first and key enzyme in the pathway of fatty acid biosynthesis, acetyl CoA carboxylase (ACCase). Interestingly, these herbicides only inhibit the eukaryotic form of the enzyme probably in the cytosol, but not the prokaryotic form of the enzyme found in the chloroplasts of dicotyledonous plants. As graminaceous plants lack *acc*D gene (encoding a subunit of prokaryotic form of ACCase) in their chloroplast genome, therefore do not have the insensitive prokaryotic form of the enzyme and are sensitive to the herbicides of HRAC group A (Konishi and Sasaki 1994). Malonyl-CoA, as the product resulted from ACCase activity, is both an intermediate in the de novo synthesis of primary fatty acids and also a substrate in the formation of long-chain fatty acids and flavonoids in plants (Bretschneider et al. 2007; Fig. 10).

The enzyme is important for membrane synthesis. These herbicides are primarily used in order to control post-emergence grasses in broadleaf crops. These herbicides are used solely against grasses. Broadleaf species are generally of natural resistance to the ACCase inhibitors from all three chemical families. The resistance originates from the enzyme itself which is less sensitive. However, herbicides of HRAC group A may induce symptoms on certain broadleaf crops. Natural tolerance of some grasses is because of a less sensitive enzyme or a higher rate of metabolic degradation (Mousavi et al. 2004). Some of these herbicides are active in soil to some extent, however their main activity appears on grown gramineous plants after their post-emergence application. They are effective on annual and perennial grasses, and the activity rate of each herbicide depends on the herbicide type. The translocation of these herbicides occurs through both xylem and phloem systems. The most abundant effect appears when weeds in their stage of fast growth under conditions with no stress are treated. The treated grasses decline and the complete control needs a week or more. Shoot and root growth suppression, chenages in leaf pigmentation, discoloration of leaves to red or pink colors (within 2–4 days), subsequent progressive necrosis of meristem regions and its extension to whole plant body are the symptoms encountered with the treated plants (Mousavi et al. 2004).

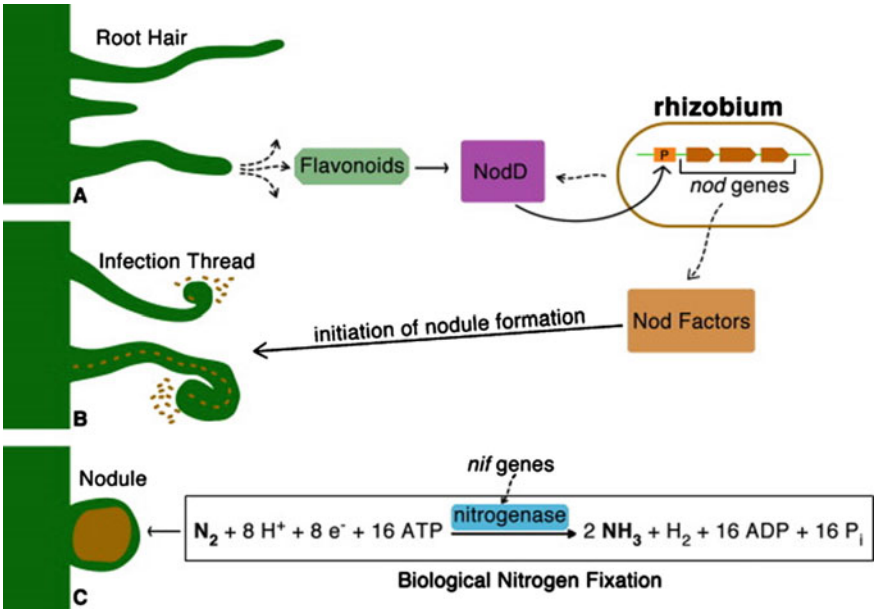


Fig. 10 The importance of the resistance of broadleaf legumes to acetyl CoA-carboxylase inhibiting herbicides (HRAC group A). Flavonoids are involved in the initial legume-*Rhizobium* recognition and subsequent induction of node genes expression (*Source* Laranjo et al. 2014)

The members of this group are from three chemically distinct families: aryloxyphenoxypropionates, FOPs (clodinafop-propargyl, cyhalofop-buthyl, diclofop-methyl, fenoxaprop-P-ethyl, fluazifop-P-buthyl, haloxyfop-R-methyl, propaquizafop, and quizalofop-P-ethyl), cyclohexanediones, DIMs (alloxydim, butroxydim, clethodim, cycloxydim, profoxydim, sethoxydim, tepraloxydim, and tralkoxydim), and phenylpyrazolines, DENs (pinoxaden). The chemical structure of three herbicides as the representatives of three chemical families has been presented in Fig. 11.

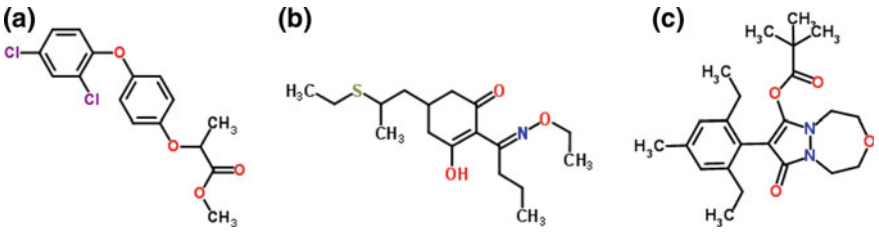


Fig. 11 Chemical structure of **a** diclofop-methyl; **b** sethoxydim; and **c** pinoxaden (*Source* www.chemspider.com)

3.1.2 HRAC Group B

The group includes those herbicides that prevent amino acid biosynthesis through the inhibition of acetolactate synthase (ALS), also known as acetohydroxyacid synthase (AHAS), in the branched-chain amino acid pathway which results in the restricted production of leucine, isoleucine, and valine. Plant death results from events that occur as the consequence of the inhibition of branched-chain amino acids, however, the actual sequence of phytotoxic process is unknown. These are potent inhibitors of plant growth effective on both dicotyledonous as well as monocotyledonous plants. The herbicides in the group exhibit either on-shoot, in-soil activity, or both activities. The growth of the treated plants is stopped after herbicidal spray. With ALS inhibitors, growing regions (terminal meristems) become chlorotic and necrotic 2–4 days after herbicide application. The treated plants are of stems with shortened internodes, reduced root growth, and converted pigments. The plant death begins from growth points and spreads to whole plant, so that it completely dies within 7–10 days (Mousavi et al. 2004).

The group includes the herbicidal compounds from five chemical families: sulfunylureas (amidosulfuron, azimsulfuron, bensulfuron-methyl, cinosulfuron, ethametsulfuron-methyl, ethoxysulfuron, flazasulfuron, flupyrsulfuron-methyl-Na, foramsulfuron, halosulfuron-methyl, imazosulfuron, iodosulfuron, mesosulfuron, metsulfuron-methyl, nicosulfuron, oxasulfuron, primisulfuron-methyl, pyrazosulfuron-ethyl, prosulfuron, rimsulfuron, sulfosulfuron, thifensulfuron-methyl, triasulfuron, trifloxysulfuron, triflusulfuron-methyl, tritosulfuron, sulfometuron-methyl, tribenuron-methyl, and cyclosulfamuron), imidazolinones (imazapic, imazamethabenz-methyl, imazamox, imazapyr, imazethapyr, and imazaquin), triazolopyrimidines (cloransulam-methyl, diclosulam, florasulam, flumetsulam, metosulam, and penoxsulam), pyrimidinyl(thio)benzoates (bispyribac-Na, pyribenzoxim, pyriftalid, pyrithiobac-Na, and pyriminobac-methyl), and sulfonylaminocarbonyltriazolinones (flucarbazone-Na, and propoxycarbazone-Na). The chemical structure of five herbicides as the representatives of the chemical families has been presented in Fig. 12.

3.1.3 HRAC Group C1

The herbicides of this HERAC group are characterized by their mode of activity, the inhibition of photosynthesis at photosystem II. Photosynthetic inhibitors control many broadleaf and some grass weeds. Generally, these herbicides inhibit photosynthesis by binding to D1 proteins of the photosystem II complex in chloroplast thylakoid membranes. Herbicide binding at D1 protein blocks electron transport and stops carbon dioxide fixation and production of energy required for plant growth. However, the death of plant is primarily not caused because of photosynthate depletion but of an indirect effect on other processes. Blockage of electron

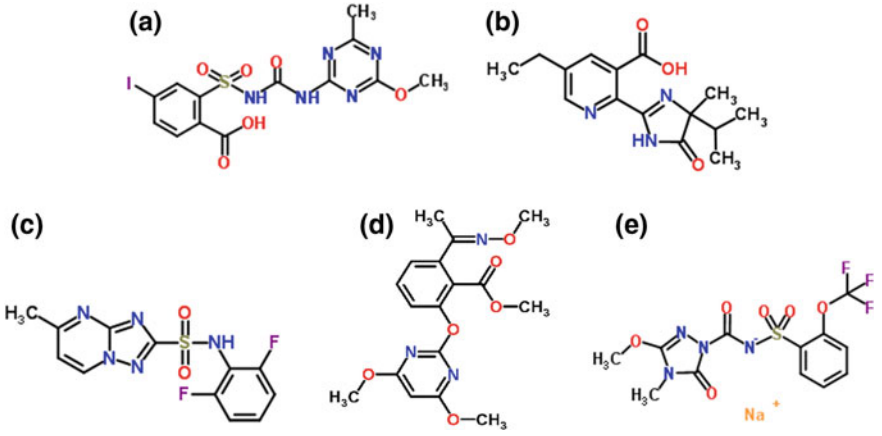


Fig. 12 Chemical structure of **a** iodosulfuron; **b** imazethapyr; **c** flumetsulam; **d** pyriminobac-methyl; and **e** flucarbazone-Na (*Source* www.chemspider.com)

transport in photosystem II promotes the formation of highly reactive molecules that initiate a chain of reactions that cause lipid and protein membrane destruction that results in membrane leakage, and leads to the desiccation and disintegration of plant cells and cellular organelles. Moreover, some PSII inhibitors affect other plant processes such as the biosynthesis of carotenoids, anthocyanins, ribonucleic acids, and proteins.

This HERAC group includes herbicidal compounds from six chemically distinct families: triazines (ametryne, atrazine, cyanazine, desmetryne, dimethametryne, prometon, prometryne, propazine, simazine, simetryne, terbumeton, terbuthylazine, trietazine, and terbutryne), triazinones (hexazinone, metamitron, and metribuzin), triazolinones (amicarbazone), uracils (bromacil, lenacil, and terbacil), pyridazinones (pyrazon, also known as chloridazon), and phenyl-carbamates (desmedipham, and phenmedipham). The chemical structure of six herbicides as the representatives of six chemical families has been presented in Fig. 13. Triazines, triazinones, uracils, and pyridazinones are soil-applied and early post-emergent herbicides. These herbicides are absorbed by both roots and shoots, but translocated only in the xylem. Phenylcarbamates are contact herbicides, primarily used as early post-emergence treatments (Mousavi et al. 2004).

3.1.4 HRAC Group C2

The group includes chemicals that inhibit photosynthesis at photosystem II but bind to protein D1 at photosystem II, however, at an attachment site different from that of the members of HERAC group C1.

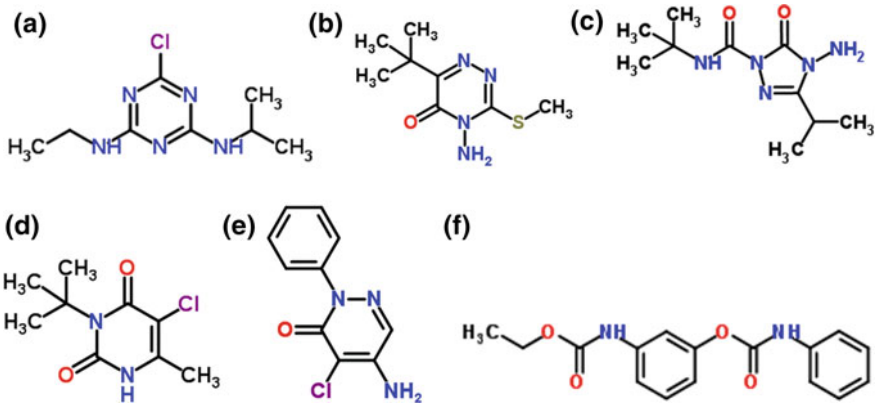


Fig. 13 Chemical structure of **a** atrazine; **b** metribuzin; **c** amicarbazone; **d** terbacil; **e** chloridazon; and **f** desmedipham (*Source* www.chemspider.com)

The members of this group are from two chemical families: ureas (chlorobromuron, chlorotoluron, chloroxuron, dimefuron, diuron, ethidimuron, fenuron, fluometuron, isoproturon, isouron, linuron, methabenzthiazuron, metobromuron, metoxuron, monolinuron, neburon, siduron, and tebuthiuron), and amides (propanil, and pentanochlor). The chemical structure of some herbicides in the group has been exhibited in Fig. 14. Ureas are soil-applied and early post-emergent herbicides. These herbicides are absorbed by both roots and shoots, but translocated only in the xylem. Amides are contact herbicides, primarily used as early post-emergence treatments (Mousavi et al. 2004).

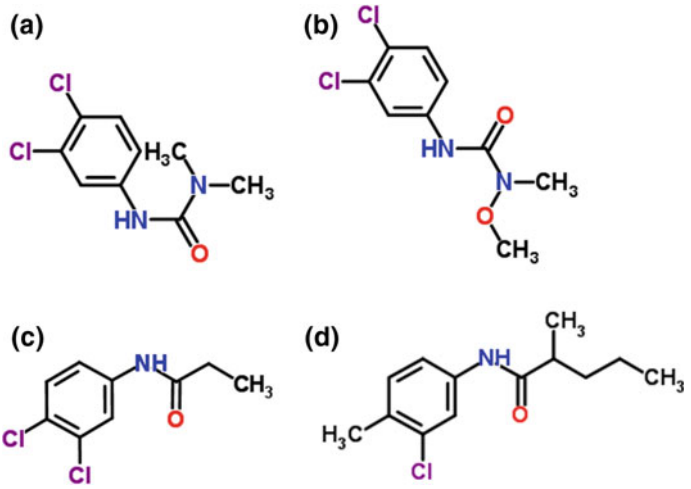


Fig. 14 Chemical structure of **a** diuron; **b** linuron; **c** propanil; and **d** pentanochlor (*Source* www.chemspider.com)

3.1.5 HRAC Group C3

The mode of action of this group of herbicides is somewhat similar to that of HRAC groups C1 and C2, however, they bind to a different attachment site of the protein D1 at the photosystem II.

The group includes members from three families of chemicals: nitriles (bromofenoxim, bromoxynil, and ioxynil), benzothiadiazinones (bentazon), and phenyl-pyridazines (pyridate, and pyridafol). The chemical structure of some herbicides in the group has been exhibited in Fig. 15. The members of all three chemical families are contact herbicides, primarily used as early post-emergence treatments (Mousavi et al. 2004).

3.1.6 HRAC Group D

This group includes the herbicide that are known by the inhibition of photosynthesis through electron diversion at photosystem I (PSI). PSI electron diverters are primarily contact herbicides that are activated with light. These relatively non-selective chemicals are used to control all existing vegetation and as pre-harvest desiccants. The group includes two members (Fig. 16) of a single chemical family: bipyridyliums (diquat, and paraquat). These herbicides accept electrons from PSI and are reduced to form an herbicidal radical that reduces other molecules to form extremely reactive and hazarduous molecules that readily destroy membrane lipids, and chlorophyll.

The disintegration of cellular membranes leads to cytoplasm leakage that results in rapid leaf wilting and desiccation (Mousavi et al. 2004). These herbicides kill plants fast when applied on shoot and leaves. In order to act effectively, these herbicides need to completely cover the weed leaves. The velocity of weed eradication is very high when it is warm and very sunny. They cause the loss of water from the tissues, and lead to the drying of the leaf tissues as the consequence of cellular membrane disruption resulted from the activity of the free radicals generated as the result of the treatment with these herbicides (Mousavi et al. 2004).

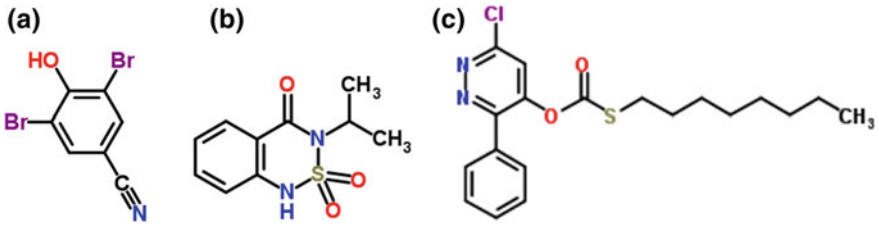


Fig. 15 Chemical structure of **a** bromoxynil; **b** bentazon; and **c** pyridate (*Source* www.chemspider.com)

Fig. 16 Chemical structure of **a** diquat; and **b** paraquat (*Source* www.chemspider.com)

3.1.7 HRAC Group E

Protoporphyrinogen oxidase (PPO) is an enzyme in the chloroplast that oxidizes protoporphyrinogen IX (PPGIX) to produce protoporphyrin IX (PPIX). PPIX is an important precursor molecule required for the biosynthesis of both chlorophyll (needed for photostnthesis) and heme (needed for electron transfer chains). The herbicides of this group inhibit PPO and block chlorophyll and heme production. Also, the inhibition of PPO results in the formation of highly reactive molecules that attack and destroy lipid and protein membranes. The affected cells and cellular organelles become leaky, dry and disintegrate rapidly (Mousavi et al. 2004). The PPO inhibitors are members of eight chemical families and one form-family: diphenylethers (acifluorfen-Na, bifenox, chlomethoxyfen, fluoroglycofen-ethyl, fomesafen, halosafen, lactofen, and oxyfluorfen), phenylpyrazoles (fluazolate and pyraflufen-ethyl), N-phenylphthalimides (cinidon-ethyl, flumioxazin, and flumiclorac-pentyl), thiadiazoles (fluthiacet-methyl, and thidiazimin), oxadiazoles (oxadiazon, and oxadiargyl), triazolinones (azafenidin, carfentrazone-ethyl, and sulfentrazone), oxazolidinediones (pentoxazone), pyrimidindiones (benzfendizone and butafenacil), and miscellaneous compounds (pyraclonil, profluazol, and flufenpyr-ethyl). The chemical structure of some herbicides in the group has been exhibited in Fig. 17. Sulfentrazone, Oxyfluorfen, and Oxadiazon are examples of the herbicides from this group that are incorporated into soil as pre-emergence soil herbicides. The herbicides of the group kill plants fast when applied on shoot and leaves. In order to act effectively, these herbicides need to completely cover the weed leaves. The velocity of weed eradication is very high when it is warm and very suny. They cause the loss of water from the tissues, and lead to the drying of the leaf tissues as the consequence of cellular membrane disruption resulted from the activity of the free radicals generated as the result of the treatment with these herbicides (Mousavi et al. 2004).

3.1.8 HRAC Group F1

This group is characterized by its particular mode of action, bleaching through the inhibition of carotenoid biosynthesis at the phytoene desaturase step (PDS). In the absence of carotenoid pigments, chlorophyll is disrupted in the presence of light, and membrane fatty acids are destructed. The treated leaves are pale white and translucent. Sometimes this paled situation is not complete but the areas between

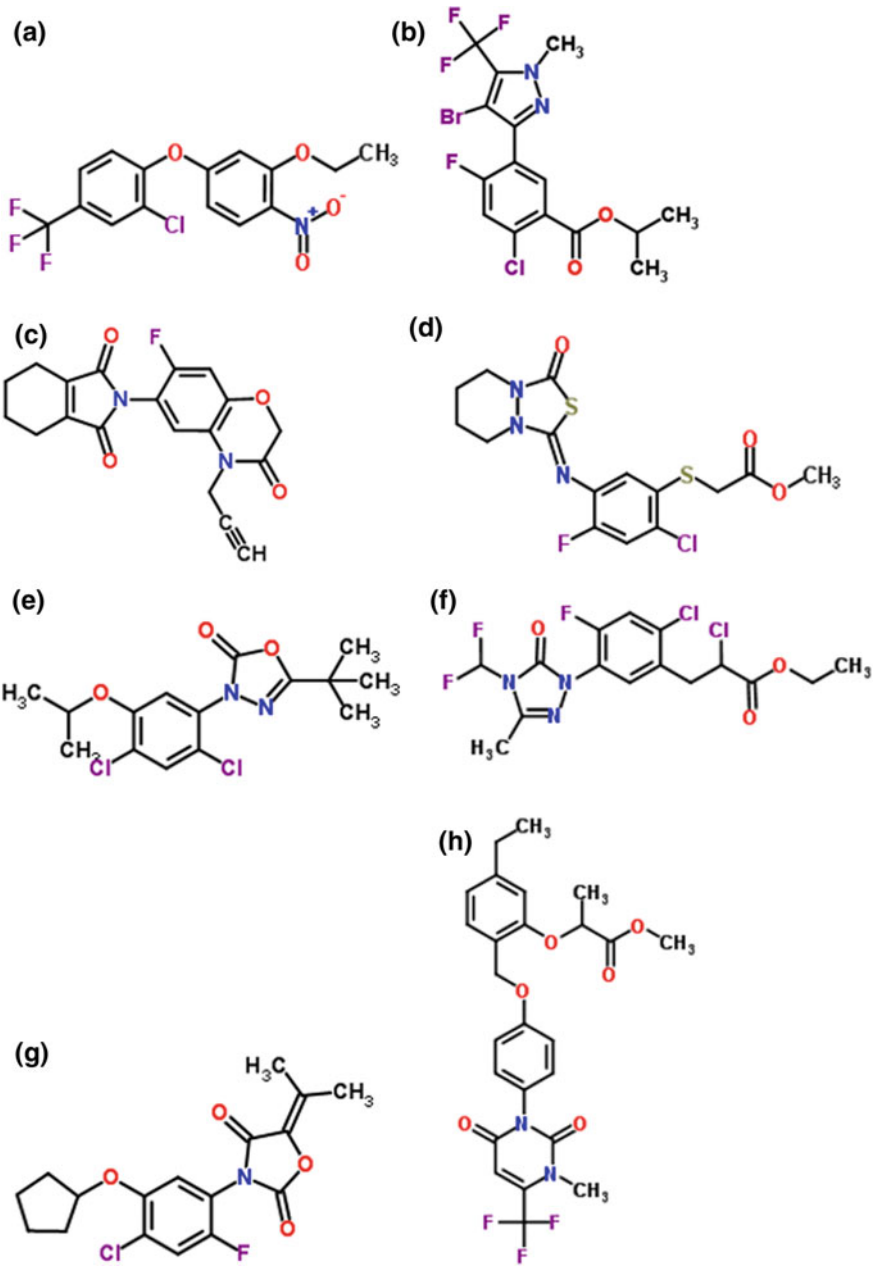


Fig. 17 Chemical structure of **a** oxyfluorfen; **b** fluazolate; **c** flumioxazin; **d** fluthiacet-methyl; **e** oxadiazon; **f** carfentrazone-ethyl; **g** pentoxazone; and **h** benzfendizone (*Source* www.chemspider.com)

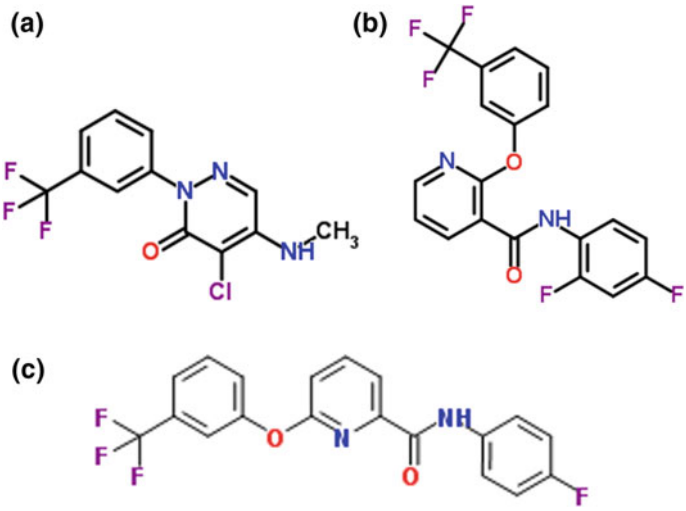


Fig. 18 Chemical structure of **a** norflurazon; **b** diflufenican; and **c** picolinafen (*Source* www.chemspider.com)

leaf veins are paled and pinkish or red hints develop in leaf margins. Norflurazon causes veinal bleaching of the affected leaves (Mousavi et al. 2004).

The HERAC group F1 includes members from two chemical families and a miscellaneous form-family: pyridazinones (norflurazon), pyridinecarboxamides (diflufenican and picolinafen), and miscellaneous compounds (beflubutamid, fluridone, flurochloridone, and flurtamone). The chemical structure of some herbicides in the group has been exhibited in Fig. 18.

3.1.9 HRAC Group F2

The group includes those chemicals that bleach treated plants through inhibition of 4-hydroxyphenyl-pyruvate-dioxygenase (4-HPPD). The enzyme catalyzes a key step in plastoquinone biosynthesis. As plastoquinone as a cofactor of phytoene desaturase, therefore, the lack of plastoquinone gives rise to bleaching symptoms resulted from an indirect inhibition of carotenoid synthesis. In the absence of carotenoid pigments, chlorophyll is disrupted in the presence of excessive light and photo-oxidation (Mousavi et al. 2004). The treated leaves are pale white and translucent. Sometimes this paled situation is not complete but the areas between leaf veins are paled and pinkish or red hints develop in leaf margins.

These herbicides are from three chemical families and a form-family: triketones (mesotrione and sulcotrione), isoxazoles (isoxachlortole and isoxaflutole), pyrazoles (benzofenap, pyrazolynate, and pyrazoxyfen) and miscellaneous compounds (benzobicyclon). The chemical structure of some herbicides in the group has been exhibited in Fig. 19.

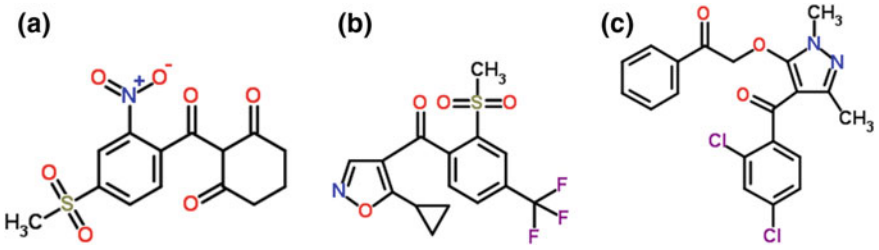


Fig. 19 Chemical structure of **a** mesotrione; **b** isoxaflutole; and **c** pyrazoxyfen (*Source* www.chemspider.com)

3.1.10 HRAC Group F3

The members of the group are from four chemical familes: triazoles (amitrole), isoxazolidinones (clomazone), ureas (fluometuron), and diphenylethers (aclonifen). The chemical structure of these herbicides has been presented in Fig. 20.

The herbicidal compounds of this group impose their bleaching effect through inhibition of carotenoid biosynthesis. In the absence of carotenoid pigments, chlorophyll is disrupted in the presence of light. The treated leaves are pale white and translucent. Sometimes this paled situation is not complete but the areas between leaf veins are paled and pinkish or red hints develop in leaf margins. Clomazone initially induces interveinal bleaching of the treated leaves (Mousavi et al. 2004). The target site of these compounds is not known, however, there is an evidence for the metabolization of clomazone to a phytotoxic form that inhibits 1-deoxy-D-xylulose-5-phosphate (DXP) synthase (Fig. 21). DXP is a key component required in plastid isoprenoid synthesis. Amitrole inhibits the accumulation of chlorophyll and carotenoids in the light (Burns et al. 1971) and it has been

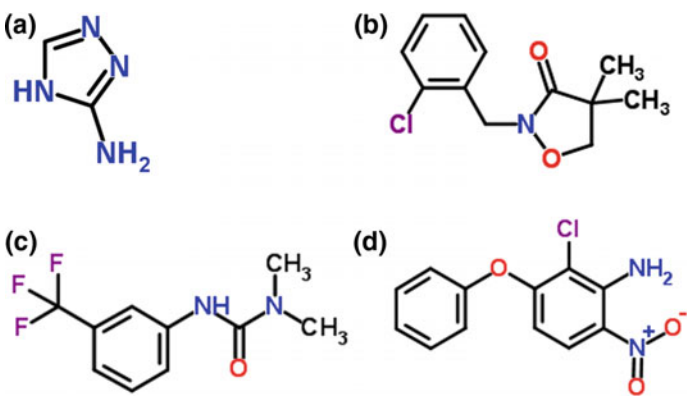
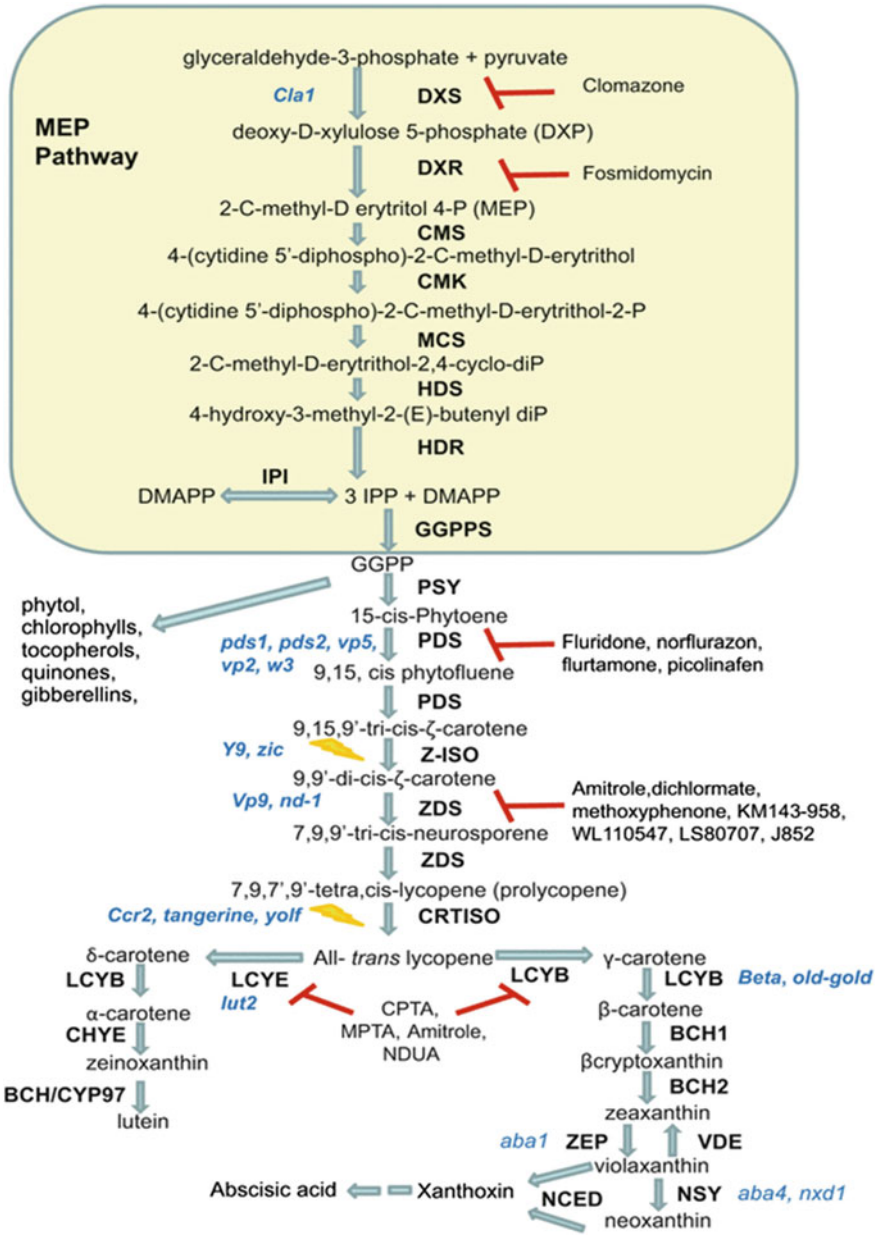


Fig. 20 Chemical structure of **a** amitrole; **b** clomazone; **c** fluometuron; and **d** aclonifen (*Source* www.chemspider.com)



reported as an inhibitor of lycopene cyclase in vivo (La Rocca et al. 1998) that can lead to the accumulation of chlorophyll precursors (La Rocca et al. 2007) and decreased salt tolerance (Chen et al. 2011). However, the specific site of its action has not been determined.

◂**Fig. 21** Isoprenoid and carotenoid pathway in plants. Schematic representation of the plastidial MEP (2-C-methyl-D-erythritol-4-P) pathway: (DXS) deoxyxylulose-5-phosphate synthase, (DXR) deoxyxylulose-5-phosphate reductoisomerase; (DXP) deoxy-D-xylulose-5-P; (CMS) 2C-methyl-D-erythritol-4-phosphate cytidyltransferase; (CMK) 4-(cytidine-5-diphospho)-2-C-methyl-D-erythritol kinase; (MCS) 2C-methyl-D-erythritol-2,4-cyclodiphosphate synthase; (HDS) 4-hydroxy-3-methylbut-2-enyl diphosphate synthase; (HDR) 4-hydroxy-3-methylbut-2-enyl diphosphate reductase; (IPP) (isopentenyl pyrophosphate); (GGPPS) geranylgeranyl pyrophosphate synthase; (DMAPP) dimethylallyl pyrophosphate; (IPI) isopentenyl pyrophosphate isomerase; (GGPP) geranylgeranyl phosphate. The relevant genes for carotenoid synthesis are: (PSY) phytoene synthase; (PDS) phytoene desaturase; (Z-ISO) ζ-carotene isomerase, (ZDS) ζ-carotene desaturase; (CRATISO) carotene isomerase; (LCYB) lycopene β-cyclase; (LCYE) lycopene ε-cyclase; (BCH) carotenoid β hydroxylase; (CHYE) carotenoid ε-hydroxylase (CYP97C1, CYP97A3); (ZEP) zeaxanthin epoxidase; (VDE) violaxanthin deepoxidase; (NSY) neoxanthin synthase; (NCED) 9-cis-epoxycarotenoid dioxygenase. Reported mutants in isoprenoid and carotenogenic genes are written in blue. Chemical inhibitors of some enzymes are included with a red T symbol. Light, referred as yellow ray can replace Z-ISO and CTISO activity in photosynthetic organs (*Source* Rosas-Saavedra and Stange 2016) (Color figure online)

3.1.11 HRAC Group G

This group is characterized by the inhibition of 5-enolpyrovyl-shikimate-3-phosphate synthase (EPSP) in shikimic acid pathway that leads to the limited production of aromatic amino acids phenylalanine, tryptophane, tyrosine, and of many important secondary compounds (Fig. 22).

The members of this group are from only a single family: glycines (glyphosate, and sulfosate). Glyphosate (relatively non-selective, Fig. 23) and sulfosate are absolutely of on-shoot activity.

The herbicides are inactivated in soil because of high soil adsorption, however, they are easily absorbed through plant foliage and translocated in the phloem to the growing points. With EPSP inhibitors, some pale colored areas may appear round the new growing regions, and plants die slowly within 1–2 weeks evenly turning to brown. The treated plants are of stems with shortened internodes, reduced root growth, and converted pigments. The plant death begins from growth points and spreads to whole plant, so that it completely dies within 7–10 days.

3.1.12 HRAC Group H

This group is known by the inhibitory impact of its members on the enzyme that converts glutamate and ammonia to glutamine, glutamine synthetase. The inhibition results in the massive accumulation of ammonia in a treated plant which destroys cells and directly inhibits both photosystem I and photosystem II reactions. Additionally, high rate of the accumulated ammonia in plants reduces the pH gradient across the membranes which inhibits energy production needed to support plant growth and development (Mousavi et al. 2004). The members of the group (Fig. 24) are from a single family: phosphinic acids (glufosinate-ammonium, and bialaphos or bilanaphos).

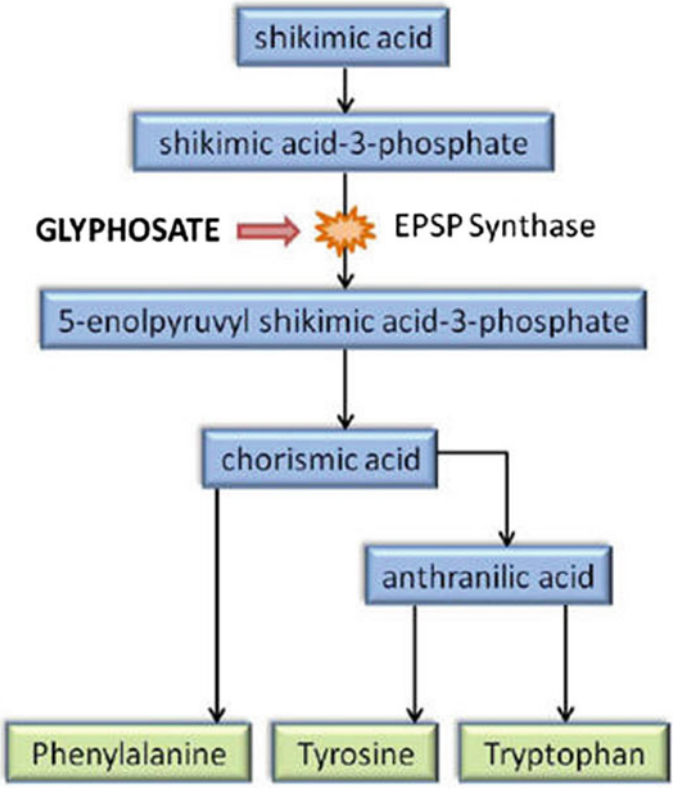


Fig. 22 The mode of action of glyphosate, a member of the HRAC group G (*Source* www.glyphosate.eu/glyphosate-mechanism-action)

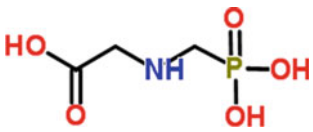


Fig. 23 Chemical structure of **a** glyphosate (*Source* www.chemspider.com)

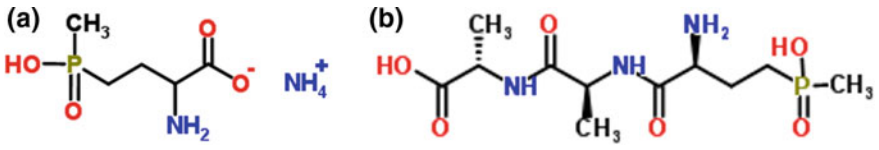


Fig. 24 Chemical structure of **a** gluphosinate-ammonium; and **b** bialaphos (*Source* www.chemspider.com)

These broad-spectrum post-emergent contact herbicides kill most annual grassy and broadleaf plants fast when applied on shoot and leaves. In order to act effectively, these herbicides need to completely cover the weed leaves. The velocity of weed eradication is very high when it is warm and severely illuminated. They cause the loss of water from the tissues, and lead to the drying of the leaf tissues as the consequence of cellular membrane disruption resulted from the activity of the free radicals generated as the result of the treatment with these herbicides (Mousavi et al. 2004).

3.1.13 HRAC Group I

The group is characterized by the inhibition of dihydropteroate (DHP) synthase. The group only includes a single member from a single family: carbamates (asulam). The chemical structure of asulam has been indicated in Fig. 25.

3.1.14 HRAC Group K1

This group includes microtubule assembly inhibitors that are generally applied pre-emergently to control annual grasses and some broadleaf weeds in many crops and turf grass. These herbicides, absorbed by both roots and shoots of emerging seedlings are not readily translocated in planta. The herbicides of HRAC group K1 are mitotic poisons that bind to tubulin, the major protein required for the polymerization of microtubules that are essential for cell division. Thus, they inhibit cell division. Hence, the meristematic regions such as growing points of stems and roots are most affected. Root tips get swollen as the cells in the region neither divide nor elongate because of the microtubule loss induced by these herbicides.

These herbicides are members of five chemical families: dinitroanilines (benefin or benfluralin, butralin, dinitramine, ethalfluralin, oryzalin, pendimethalin, and trifluralin), phosphoroamidates (amiprophos-methyl, and butamiphos), pyridines (dithiopyr, and thiazopyr), benzamides (propyzamide or pronamide, and tebutam), and benzoic acids (DCPA, also known as chlorthal-dimethyl). The chemical structure of some herbicides in the group has been presented in Fig. 26.

Fig. 25 Chemical structure of **a** asulam (www.chemspider.com)

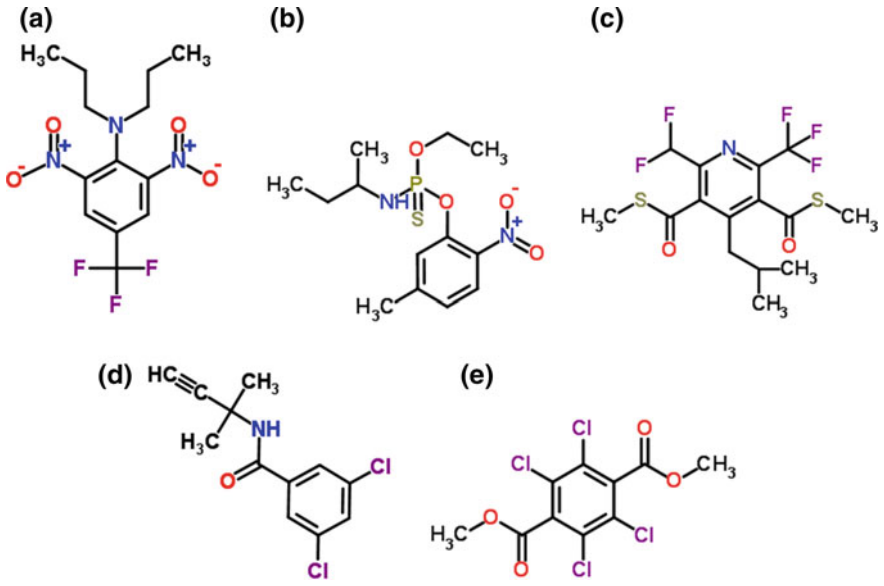


Fig. 26 Chemical structure of **a** trifluralin; **b** butamiphos; **c** dithiopyr; **d** pronamide; and **e** chlorthal-dimethyl (*Source* www.chemspider.com)

3.1.15 HRAC Group K2

The group is known through the inhibition of mitosis or microtubule organization. The members of this group are only from a single chemical family, carbamates that includes chlorpropham, propham, and carbetamide. The chemical structure of these herbicides has been exhibited in Fig. 27.

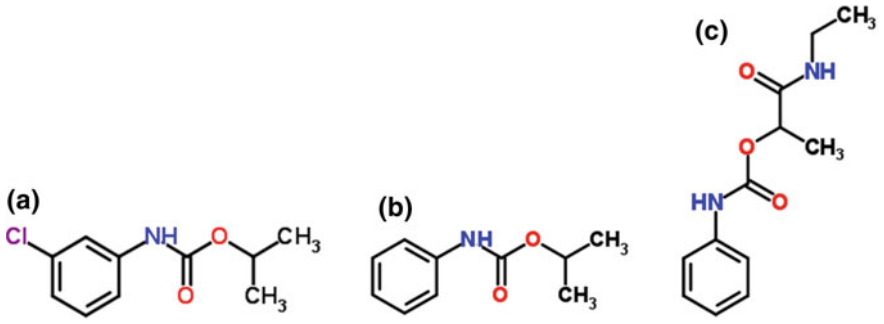


Fig. 27 Chemical structure of **a** chlorpropham; **b** propham; and **c** carbetamide (*Source* www.chemspider.com)

3.1.16 HRAC Group K3

The group is characterized by the inhibition of cell division through the inhibition of very long chained fatty acids (VLCFAs). These compounds typically affect susceptible weeds (annual grasses and some small-seeded broadleaf weeds) before emergence but do not inhibit seed germination. The primary absorption and action site of these herbicides on broadleaf species is the roots, while that on grass species is the emerging shoot (Mousavi et al. 2004).

The members of this group are from five chemical families: chloroacetamides (acetochlor, alachlor, butachlor, dimethachlor, metazachlor, metolachlor, pretilachlor, propachlor, propisochlor, thenylchlor, dimethanamid, and pethoxamid), acetamides (diphenamid, napropamide, and naproanilide), oxyacetamides (flufenacet, and mefenacet), tetrazolinones (fentrazamide), and miscellaneous compounds (anilophos, cafenstrole, and piperophos). Figure 28 represents the chemical structure of some herbicides in the group.

3.1.17 HRAC Group L

The group is characterized by the inhibition of cellulose synthesis that leads to the inhibition of cell wall synthesis. Herbicides in this group prevent cell division primarily in developing root tips and are only effective on some germinating broadleaf weeds and selected grasses. Chemically, the members of the group are classified into four families: nitriles (dichlobenil and chlorthiamid), benzamides (isoxaben), triazolocarboxamides (flupoxam), and quinoline carboxylic acid (quinclorac; for monocots; also a member of HERAC group O). An herbicide from the fifth family, alkylazines (indaziflam) is also classified in the HRAC group L (Forouzesh et al. 2015). Figure 29 represents the chemical structure of some herbicides in the group.

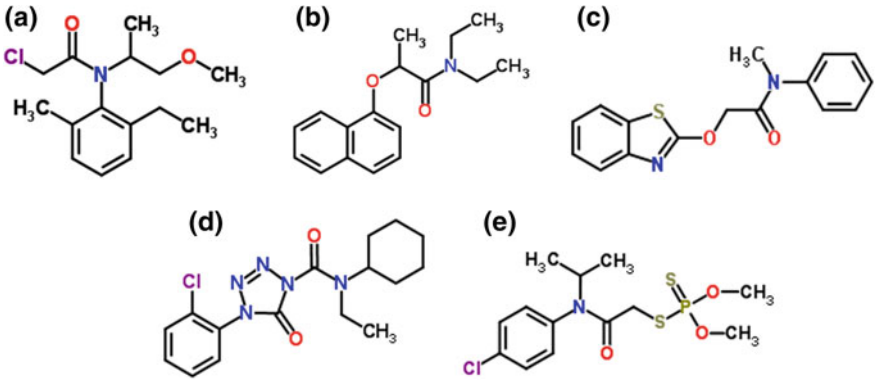


Fig. 28 Chemical structure of **a** metolachlor; **b** napropamide; **c** mefenacet; **d** fentrazamide; and **e** anilophos (*Source* www.chemspider.com)

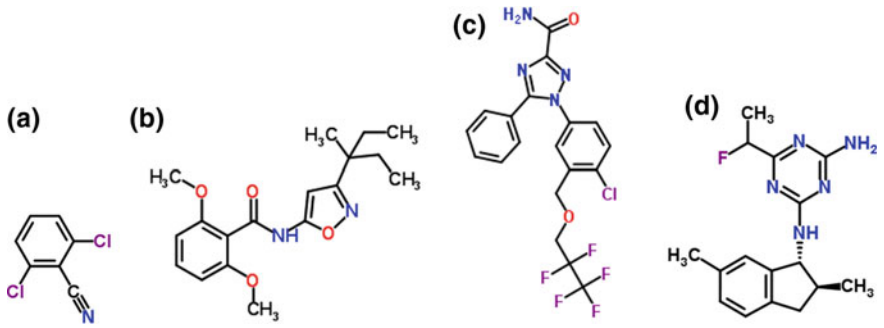


Fig. 29 Chemical structure of **a** dichlobenil; **b** isoxaben; **c** flupoxam; and **d** indaziflam (*Source* www.chemspider.com)

These compounds often used as pre-emergence herbicides include various chemical compounds that directly or indirectly inhibit the biosynthesis of cellulose and lead to reduced stability of cellular structure. They weaken the growth of weed seedlings, and induce symptoms such as root suppression and inflation. Dichlobenil and isoxaben used as pre-emergence herbicides are very effective on dicotyledonous herbicides. Quinclorac is applied in both pre-emergence and post-emergence manners and completely inhibits cellulose biosynthesis by monocotyledonous plants, while still regulates the growth of dicotyledonous plants (Mousavi et al. 2004).

3.1.18 HRAC Group M

The members of the group impose their herbicidal effect through uncoupling of oxidative phosphorylation and membrane disruption. The members of this group are classified in the chemical family of dinitrophenols (dinitro-ortho-cresol or DNOC, dinoseb, and dinoterb). Figure 30 represents the chemical structure of the herbicides in the group.

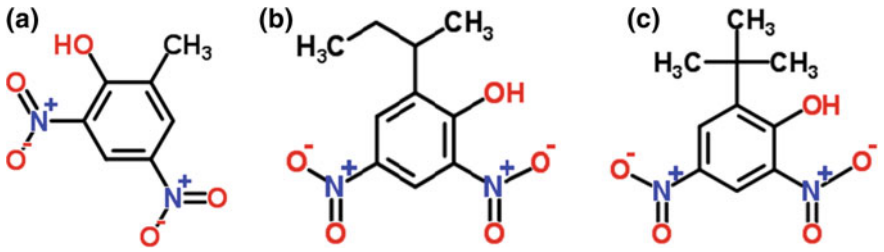


Fig. 30 Chemical structure of **a** dinitro-ortho-cresol (DNOC); **b** dinoseb; and **c** dinoterb (*Source* www.chemspider.com)

3.1.19 HRAC Group N

The members of this group inhibit lipid biosynthesis, however unlike the members of the HRAC group A and as represented in Fig. 31, they inhibit lipid biosynthesis in a target site other than acetyl coenzyme A carboxylase (ACCase). The specific mode of action of these herbicides is not well elucidated, however, there is strong evidence that these herbicides interfere with the biosynthesis of fatty acids and lipids in the newly developing shoot, which may account for the reported reductions in cuticular was deposition. Furthermore, these herbicides cause abnormal cell development or prevent cell division in germinating seedlings.

They stop the plant from growing by inhibiting cell division in the shoot and root tips while permitting other cell duplication processes to continue (Mousavi et al. 2004). These herbicides generally applied pre-planting or pre-emergence and incorporated to soil, are most effective on annual grasses and some broadleaf weeds. Most of these herbicides are volatile and need to be incorporated

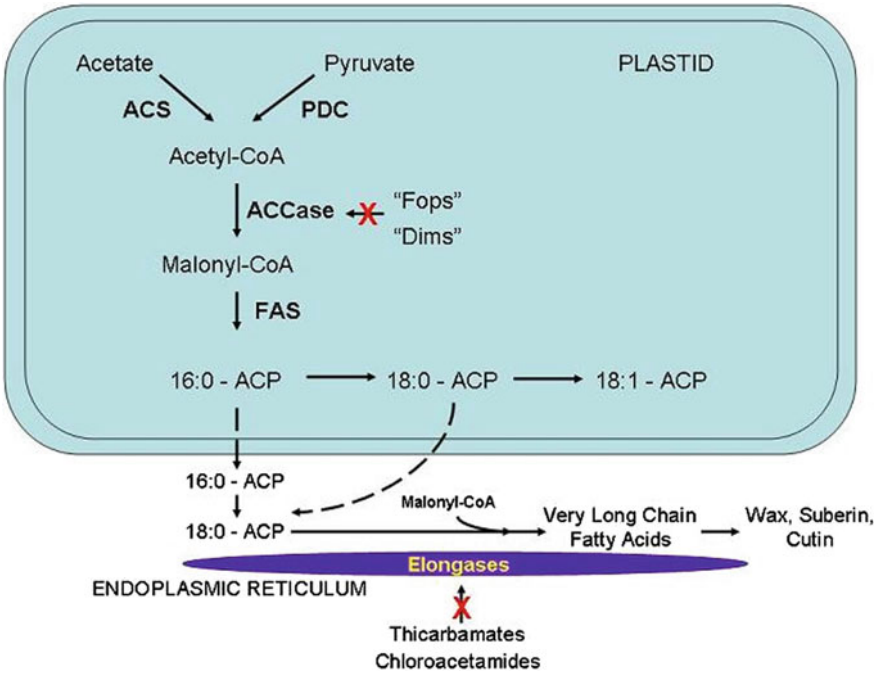


Fig. 31 Simplified schematic of fatty acid synthesis and elongation in higher plants based on the modification of Gronwald (1991). Abbreviations: ACCase, acetyl-CoA carboxylas; ACP, acyl carrie protein; ACS, acetyl-CoA synthase; CoA, coenzyme A; dims, cyclohexanedione inhibitors; FAS, fatty acid synthase; fops, aryloxyphenoxy propionate inhibitors; PDC, pyruvate dehydrogenase complex (*Source* Dyer WE, Inhibitors of fatty acid synthesis and elongation. Available: https://passel.unl.edu/pages/printinformationmodule.php?idinformationmodule=1130447055)

immediately after application to avoid excessive vapor loss. The HRAC group N herbicides are absorbed through both roots and emerging shoots but are translocated only in the xylem. The primary site of absorption and action is the emerging shoot and growing point. The members of HRAC group N are distributed in four chemical families: thiocarbamates [butylate, cycloate, diallate, dimepiperate, esprocarb, molinate, orbencarb, pebulate, prosulfocarb, thiobencarb or benthiocarb, tiocarbazil, triallate, vernolate, and S-ethyl dipropylthiocarbamate (EPTC)], phosphorodithioates (bensulide), benzofurans (benfuresate, and ethofumesate), and chloro-carbonic acids [(TCA), dalapon, and flupropanate] (Fig. 32).

3.1.20 HRAC Group O

The members of this group are known as synthetic auxins or plant growth regulators, and act as the natural plant hormone, indole acetic acid (IAA). Most of these herbicides although readily absorbable through both roots and shoots, are applied as post-emergence treatments. Their translocation to the actively growing sites occurs through both of xylem and phloem systems and their major effect is exerted on weed shoot. These herbicides include some of more effective chemicals for perennial broadleaf weed and brush control that impose their selective control effects on broadleaf weeds grown in cereal fields. With perennial weeds, most of these herbicides are transferred to the underground parts of the weed and put an end to its life. The herbicides interfere with cell formation in the meristematic regions and as the result, the primary symptoms on the newly developed leaves and shoots appear as fast turnings and shoot epinasty, cupped and shrunken leaves, inflated stems and the disruption of their phloem systems. Also, the damage in roots appears as branched and bunchy secondary roots of suppressed growth. The exact action site of this class of herbicides is not known, however, they seem to have multiple action sites and lead to hormonal imbalance, the interfered metabolism of nucleic

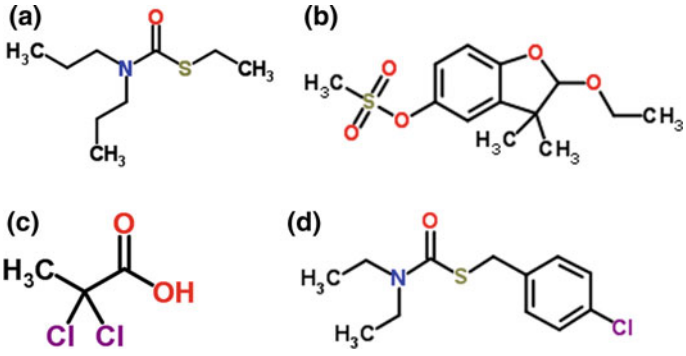


Fig. 32 Chemical structure of **a** S-ethyl dipropylthiocarbamate (EPTC); **b** ethofumesate; **c** dalapon; and **d** thiobencarb (*Source* www.chemspider.com)

acids and the interfered synthesis of proteins that end to the changes in auxine activity, weakness of cell walls, accelerated but useless cellular divisions, and final death of the plant within a few days or weeks (Mousavi et al. 2004).

The auxin-like activity is observed in the herbicidal compounds of four chemical families: phenoxy-carboxylic-acids [clomeprop, 2,4-dichlorophenoxyacetic acid (2,4-D), 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB), dichlorprop or 2-(2,4-dichlorophenoxy)propionic acid (2,4-DP), 2-methyl-4-chlorophenoxyacetic acid (MCPA), and mecoprop or meta-chlorophenylpiperazine (MCPP or CMPP)], benzoic acids [chloramben, dicamba, and 2,3,6-trichlorobenzoic acid (TBA)], pyridine carboxylic acids (clopyralid, fluroxypyr, picloram, and triclopyr), and quinolone carboxylic acids (quinmerac, and quinclorac that also belongs to the HRAC group L).

There is also an herbicidal compound, benazolin-ethyl that does not belong to the chemical families mentioned above. The chemical structure of some of the members of the HRAC group O has been shown in Figs. 33 and 34.

3.1.21 HRAC Group P

Two members (Fig. 35) of the chemical family phthalamate semicarbazones (neptalam, and diflufenzopyr-Na) belong to the group characterized by the inhibition of auxin transport. Auxine transfer inhibitors like the soil herbicide neptalam, and the on-shoot herbicide diflufenzupyr inhibit auxine activity or its distribution in

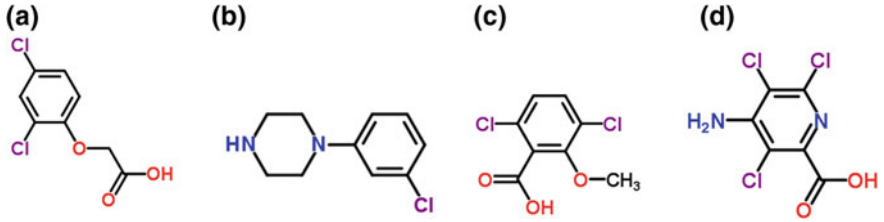


Fig. 33 Chemical structure of **a** 2,4-dichlorophenoxyacetic acid (2,4-D); **b** meta-chlorophenylpiperazine (MCPP); **c** dicamba; and **d** picloram (*Source* www.chemspider.com)

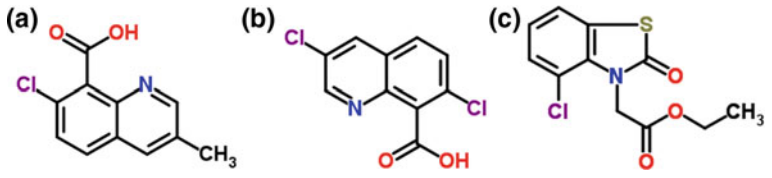


Fig. 34 Structural formula of **a** quimerac; **b** quinclorac; and **c** benazolin-ethyl (*Source* www.chemspider.com)

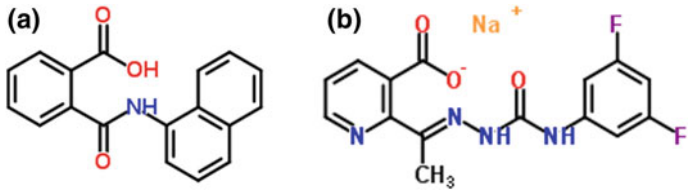


Fig. 35 Structure formula of **a** neptalam, and **b** diflufenzopyr-Na (*Source* www.chemspider.com)

plants, and as the result of the reduced growth hormone required for cellular development, bring about plant growth defects. The marked symptom of their phytoxicity in addition to reduced growth is the upward reversion of the treated plant root tips.

3.1.22 HRAC Group Z

This group includes a heterogenous collection of the herbicidal compounds of unknown mode of actions. The target site of these herbicides are not known, and may be different. The members of the group belong to three chemical families: arylaminopropionic acids (flamprop-M-methyl/-isopropyl), pyrazoliums (difenzoquat), as well as organoaresenicals including disodium methanearsonate (DSMA), and monosodium methanearsonate (MSMA). The chemical structure of these herbicides are indicated in Fig. 36.

There are also other herbicidal compounds of unknown mode of action: bromobutide, (chloro)-flurenol, cinmethylin, cumyluron, dazomet, dymron (also known as daimuron), methyl-dymron (also known as methyl-dimuron), etobenzanid, fosamine, indanofan, metam, oxaziclomefone, oleic acid, pelargonic acid, and pyributicarb. The effect of herbicides on plant diseases has been the topic of a

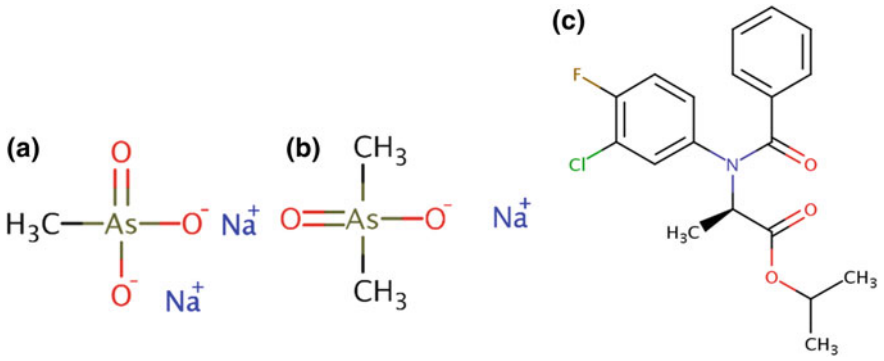


Fig. 36 Structural formulas of **a** disodium methanearsonate, **b** monosodium methanearsonate, and **c** flamprop-M-isopropyl (*Source* http://comptox.epa.gov)

number of previous reviews, either as a single topic (for example, Altman 1991; Altman and Campbell 1977a; Katan and Eshel 1973; Levesque and Rahe 1992) or as part of amore extensive review on secondary effects of pesticides (several of these are in the book by Altman 1993) or chemical effects on microbial weed biocontrol agents (Hoagland 1996).

3.2 *Effect of Herbicides on Plant Diseases*

Herbicides affect all three components of disease triangle (host plant, pathogen, and biotic as well as abiotic environmental factors). Herbicide are capable to exert their impacts on the incidence and severity of plant diseases (Altman and Campbell 1977a; Altman and Rovira 1989; Duke et al. 2007; El-Khadem and Papavizas 1984; El-Khadem et al. 1979, 1984; Heydari and Misaghi 1998; Miller et al. 1979; Moustafa-Mahmoud et al. 1993; Neubauer and Avizohar-Hershenson 1973; Pinckard and Standifer 1966; Rovira and McDonald 1986; Sanyal and Shrestha 2008). Most of the reported effects of herbicides on plant diseases have been obtained from field studies with no imply to the observed effect that if it is due to a direct herbicide-pathogen interaction or it is due to an direct effect on plant, making the plant less or more resistant to the pathogen. In general, most studies present no clue to the mechanism of the antifungal activity. In some cases, like the decrease of melon vascular wilt disease (caused by *Fusarium oxysporum*) followed by acetochlor treatment, the possibility of direct fungitoxic effects of the herbicide was rejected, but the mechanism was not revealed (Cohen et al. 1996). The decreased incidence of cotton seedling disease induced by *Fusarium oxysporum* f. sp. *vasinfectum* has been recorded as the consequence of the soil incorporation of the herbicides trifluralin, dinitramine, flumeturon, diuron, dalapon, and prometryn under field conditions (El-Khadem et al. 1984). Similarly, the use of EPTC and linuron reduces the post-emergence but not the pre-emergence incidence of cotton seedling disease caused by *Fusarium oxysporum* f. sp. *vasinfectum* (El-Khadem and Papavizas 1984). Sharma and Sohi (1983) indicated that bromacil, diuron, nitrofen, and alachlor all reduced disease severity of *Phaseolus vulgaris* induced by *Rhizoctonia solani*, but they had no data to suggest amechanism for the observed antifungal impacts. The severity of sheath blight disease of rice caused by *Rhizoctonia solani* reduced as the consequence of the application of the herbicide benthiocarb (Vasavan et al. 1980).

There are other studies showing enhancement of plant diseases by herbicides. In a survey of the effects of twelve herbicides (bentazon, acifluorfen, chlorimuron, fluazifop, diclofop, sethoxydim, imazaquin, metribuzin, oryzalin, thidiazuron, diaminozide, and mefluidide) on disease severity of four plant pathogens (*Alternaria cassiae*, *Colletotrichum coccodes*, *C. truncatum*, and *Fusarium lateritium*), all of the herbicides enhanced disease severity of at least one of the pathogens to a host plant but the mechanisms of these effects were not revealed (Caulder et al. 1987). The increased occurrence of cereal cyst disease by the nematode *Heterodera*

avenae (Altman and Rovira 1989), wheat take-all by *Gaeumannomyces graminis* var. *tritici* (Rovira and McDonald 1986), and sugar beet damping-off by *Rhizoctonia solani* (Rovira and McDonald 1986; Altman and Campbell 1977b) have been attributed to the soil application of trifluralin, chlorsulfuron, and cycloate, respectively. The application of chlorsulfuron resulted in increased levels of pythium root rot in barley seedlings (Blowes 1987). Terbacil (a uracil from HRAC group C1) increased the severity of sugarcane root rot induced by the oomycetous pathogen *Pythium arrhenomanes*, but metribuzin was found of no impact (Dissanayake et al. 1998). Heydari and Misaghi (1998) investigated the impact of herbicides on the incidence and development of cotton seedling damping-off caused by *Rhizoctonia solani*, and found that soil application of prometryn led to the significant increase of the disease incidence in soil infested at planting. Post-emergence soil infestation with the pathogen led to the considerably increased incidence of the disease in the presence of pendimethalin and prometryn, but not trifluralin. Field experiments with pendimethalin and prometryn confirmed the results obtained under controlled environmental conditions of growth chambers. Increase of various airborne as well as soilborne plant diseases have been recorded following programmed application of glyphosate (Johal and Huber 2009). These include apple canker induced by *Botryosphaeria dothidea* (Rosenberger and Fargione 2004), banana Panama disease (Fig. 37) caused by *Fusarium oxysporum* f. sp. *cubense* (Harper 2007), barley root rot induced by *Magnaporthe grisea* (Smiley et al. 1992), bean anthracnose induced by *Colletotrichum lindemuthianum* (Johal and Rahe 1984, 1988, 1990), bean damping-off, and root rot by *Pythium* spp. (Johal and Rahe 1984), pythium root rot in barley seedlings (Blowes 1987), bean root rot by *Fusarium solani* f. sp. *phaseoli* (Harper 2007), bean hypocotyl rot by *Phytophthora megasperma* (Keen et al. 1982), canola crown rot by *Fusarium* spp. (Harper 2007), canola wilt by *Fusarium oxysporum* (Harper 2007; Lange and McLaren 2002), citrus variegated chlorosis by *Xylella fastidiosa* (Yamada 2006), citrus crown rot by *Phytophthora* spp. (Yamada 2006), cotton damping-off by *Pythium* spp. (Harper 2007), cotton bunchy top manganese deficiency (Harper 2007), cotton wilt by *F. oxysporum* f. sp. *vasinfectum* (Harper 2007), grape black goo by *Phaeomoniella chlamydospora* (Harper 2007), melon root rot by *Monosporascus cannonbalus*, soybeans root rot by *Corynespora cassiicola* (Huber et al. 2005), soybeans target spot by *Corynespora cassiicola* (Huber et al. 2005), soybean sudden death syndrome by *Fusarium solani* f. sp. *glycines* (Keen et al. 1982), soybean root rot by *Phytophthora megasperma* (Keen et al. 1982), soybeans cyst nematode by *Heterodera glycines* (Geisler et al. 2002; Kremer et al. 2000), soybeans white mold by *Sclerotinia sclerotiorum* (Harper 2007), sugar beet yellows by *Fusarium oxysporum* f. sp. *betae* (Larson et al. 2006), sugar beet root rot by *Rhizoctonia solani* (Larson et al. 2006), sugarcane decline by *Marasmius* spp. (Huber, unpublished), tomato crown root rot by *Fusarium* (Bramhall and Higgins 1988), tomato wilt *Fusarium oxysporum* f. sp. *pisi* (Harper 2007), various canker diseases by *Phytophthora* spp. (Harper 2007), weeds biocontrol by *Myrothecium verrucaria* (Boyette et al. 2006), wheat bare patch by *Rhizoctonia solani* (Harper 2007), wheat glume blotch by *Septoria* spp. (Harper 2007), wheat

Fig. 37 Symptoms of Panama disease on Cavendish bananas in Jordan. External symptoms included: **a** chlorosis and/or **b** necrosis of leaves, progressing from the oldest to the youngest in a plant. Note in (**b**) that buckling of leaves also occurred before they became chlorotic or necrotic. Internally, affected xylem and the associated parenchyma was reddish to dark brown which, when viewed in **c** cross section was evident as discrete dots or contiguous sections of discoloration, and when viewed in **d** longitudinal sections encompassed much of the pseudostem length (*Source* Ploetz et al. 2015)

root rot by *Fusarium* spp. (Fernandez et al. 2005, 2007; Harper 2007), wheat head scab by *Fusarium graminearum* (Fernandez et al. 2005), and wheat take-all by *Gaeumannomyces graminis* (Hornby et al. 1998). Such an extended list of diseases affected by only an individual herbicide indicates the importance of the conscious use of herbicides in agriculture. Herbicides can directly and/indirectly affect plant diseases.

3.2.1 The Direct Impacts of Herbicides on Pathogens

Unfortunately, there are few data available on the direct effects of herbicides on plant pathogens. However, some herbicides are directly toxic to plant pathogens at the recommended rates. Three methods are conventionally used to investigate the

effects of a number of commercial herbicides on the growth of certain soil fungi: measurements of hyphal extension across agar plates; measurements of hyphal extension along sterilized plant material; and manometric techniques. In particular, three points emerged from these studies. First, that there was no stimulation of fungal growth. Herbicide interference in growth included suppression of spore germination, inhibition of the rate of linear extension of the mycelia, and abnormalities in growth habit and in patterns of spore production. Secondly, that some herbicides (for example, linuron and paraquat) were more fungitoxic than others (for instance, MCPA and simazine) to a range of organisms. Thirdly, there were differences among fungi in their sensitivity to individual herbicides. All three methods have shown consistent differences among fungi in their ability to tolerate paraquat. Pero and Owens (1971) introduced a chromatogram-based micro-method for the detection of antifungal activity of chemicals that could also be applied with herbicides.

Direct Anti-pathogenic Effects of Herbicides

The direct influence of herbicides on pathogens has also been indicated by a number of researches (such as Altman and Campbell 1977a; Black et al. 1996; El-Khadem and Papavizas 1984; El-Khadem et al. 1979, 1984; Neubauer and Avizohar-Hershenson 1973). The results from in vitro studies performed by Roberti et al. (2006) implicated the inhibitory effect of glyphosate on mycelial growth of most of the studied soilborne fungi. Abdel-Mageed et al. (2013) isolated, identified and characterized forty-five rhizofungal isolates from 11 herbicides polluted-soil. Among the isolates, 10 fungal species proved to be the most potent and promising ones in herbicides tolerance. The herbicides exhibited severe and dramatic effect and modulation on fungal DNA and protein as represented in DNA and protein profile. Severe losses were found in the rate of total soluble cell ion (SCI) and total cell protein concentration (TCPC). With the loss of SCI by glyphosate, *Aspergillus flavus* (86.30%) was the most affected one, followed by *Penicillium spiculisporus* (76.30%), *Penicillium verruculosum* (64.40%), and *Alternaria tenuissima* (64%), respectively. For pendimethalin, *A. tenuissima* (54.01%) was the most affected fungus. For diclofop-methyl, *P. spiculisporus* (74.20%) was the most affected fungus. With the loss of TCPC by glyphosate, *A. tenuissima* (64.71%) was the most effected fungus, followed by *P. spiculisporus* (57.14%), respectively. For pendimethalin, *A. terreus* (54.29%) was the most affected fungus. For diclofop-methyl, *P. spiculisporus* (60%) was the most affected fungus, followed by *A. tenuissima* (58.82%), and *Aspergillus tamarii* (55.56%), respectively. The results proved severe reductions and alteration in protein, SCI, TCPC and DNA in fungal strains exposed to these herbicides which might reflect a degree of tolerance occurred during the assimilation of those toxic compounds from the pesticides polluted-soil. The herbicidal product Galex®, the commercially available formulated mixture of two active ingredients metolachlor (from the chemical family chloroacetamides and a member of the HRAC group K3) and motobromuron (from the chemical family

ureas and a member of the HRAC group C2) affected the growth of a few plant pathogenic fungi including *Fusarium oxysporum, F. monilliforme* and *Aspergillus flavus* and reduced the incidence of soil-borne disease in legumes (Olajire and Oluyemisi 2009). Greenhouse experiments had previously demonstrated that microbial population of cowpea soil was significantly reduced by applying the normal field application rate of a pre-emergnece herbicide, Galex®, into the soil (Fawole 2000). This reductive effect was more in fungal than bacterial population. Thus among premoninant fungal genera including *Mucor*, *Fusarium*, *Aspergillus* and *Penicillium*, *Fusarium* counts were remarkably reduced upon Galex® application into the soil (Fawole 2000). Pretreatment of muskmelon seedlings with four chloracetamide herbicides at 0.1 μg g^{-1} reduced the incidence of fusarium wilt by 22–79%. Also, acetochlor applied as a seed treatment (soaking for 5 h in 50 μg acetochlor mL^{-1}) reduced fusarium wilt in muskmelon seedlings, however, it was less effective when applied as foliar spray (Cohen et al. 1992a). The herbicides oxyfluorfen (a diphenylether from HRAC group E), butachlor (a chloroacetamide from HRAC group K3), acetochlor (a chloroacetamide from HRAC group K3), cinmethylin (of a yet unknown HRAC group), and oxadiazon (an oxadiazole from HRAC group E) inhibited the mycelial growth and sclerotial germination of *Rhizoctonia solani* on potato sucrose agar (PSA) medium, where the herbicidal concentrations required for 50% growth inhibition (IC_{50}) were 2.01, 4.16, 8.12, 11.97, and 22.01 mg L^{-1}, respectively. Sclerotial germination was inhibited by oxyfluorfen, acetochlor, cinmethylin, and oxadiazon at 100 mg L^{-1} (Hua et al. 2002). Here the literature is reviewed in the context of HRAC groups of herbicides and the chemical families in which the member herbicides of antifungal activity are classified.

HRAC Group A

Studies has indicated the direct antifungal activity of some herbicidal ACCase inhibitors from the chemical families, aryloxyphenoxy propionates (FOPs) and cyclohexanediones (DIMs). Diclofop-methyl is recorder to be of direct antifungal effects on *Colletotrichum truncatum* (Caulder et al. 1987). Clodinafop-propargyl exhibited direct antifungal activity against *Ceratocystis radicicola*, *Gaeumannomyces graminis*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Verticillium dahlia*, and inhibited the growth of two oomycetous pathogens *Phytophthora* sp., as well as *Pythium* sp., however, diclofop-methyl indicated narrower range of antifungal activity and only inhibited *Bipolaris* sp., *G. graminis*, *V. dahlia*, *Phytophthora* sp., and *Pythium* sp. (Pakdaman et al. 2002). Also, studies with the herbicides applied in rapeseed or canola (*Brassica napus* var. *oleifera*) fields demonstrated that cycloxydim, sethoxydim, and haloxyfop-ethoxyethyl were of considerable antifungal impacts against *Sclerotinia sclerotiorum*, the causal fungus of canola white stem rot disease (Pakdaman and Mohammadi Goltapeh 2007). Further studies on the mechanism of antifungal activity of sethoxydim exhibited increased levels of malondialdehyde (MDA) in the biomass of the white stem rot pathogen, *S. sclerotiorum* treated with the herbicide. MDA is the main

product generated through peroxidation of polyunsaturated fatty acids and a reliable indicator of the disintegration of fungal cellular membranes (Gaweł et al. 2004). Also, hydrogen peroxide concentrations increased as the consequence of the herbicidal treatment of the pathogen (Pakdaman et al. 2007).

HRAC Group B

No information was found. However, the commercially available mixture of mesosulfuron-methyl and iodosulfuron methyl sodium (Atlantis® OD) inhibited the in vitro growth of the mycotoxigenic pathogen of fusarium head blight disease of wheat and other small grain cereals, *Fusarium graminearum* as well as the biological control agent *Trichoderma asperelloides* on potato dextrose agar when its final rate in the medium was adjusted to 5000 ppm (Pakdaman and Elahifard, unpublished data).

HRAC Group C1

There are reports of the antifungal activity of some members of the chemical families, triazines as well as phenylcarbamates. Schuldt and Wolfe (1956) proposed the toxic effects of some *s*-triazine herbicides against *Pythium* spp. as a reason for the improved emergence of soybean seedlings and decreased damping-off with the addition of atrazine. Atrazine (25, and 50 μg mL^{-1}) was of no significant effect on *Pectinobacterium carotovorum* growth (Breazeale and Camper 1972). In an study on the effect of 14 herbicides and two herbicide combinations on three microscopic soil fungus species carried out following Miller's method, Helmeczi et al. (1988) found that the triazine derivative Hungazin PK (atrazine) was of the least inhibitory impact on *Aspergillus*, *Rhizopus*, and *Fusarium*. Atrazine, simazine, and metribuzin at 500 μg mL^{-1} inhibited the mycelial development of *Sclerotinia sclerotiorum* on 1.5% Bacto agar. Additionally, the carpogenic germination of sclerotia in the soil amended with atrazine and simazine at the rate of 0.5–10.0 mg g^{-1} soil was not affected as compared with that in untreated soil, however, sclerotia in the treated soils produced abnormal apothecia, while those in the soil amended with 0.5 and 1.0 mg metribuzin g^{-1} soil could not germinate (Casale and Hart 1986). In contrast, sclerotial germination was stimulated and a higher number of stipe initials was developed in soils treated with metribuzin at 0.5 and 1.0 μg g^{-1}, and atrazine at 0.5 μg g^{-1} (Radke and Grau 1986). Atrazine reduced the colony diameter of *Macrophomina phaseolina* (Fig. 38) in sorghum (*Sorghum bicolor*; Fig. 39) but did not reduce the production and germination of microsclerotia (Russin et al. 1995). Atrazine inhibited the mycelial growth of the oomycetous species *Pythium arrhenomanes* (Dissanayake et al. 1998). Atrazine, a corn herbicide (Atrazine®, tested in vitro at two concentrations of 3 and 33 μg mL^{-1}) and cyanazine (Bladex®, tested in vitro at two concentrations of 17, and 170 μg mL^{-1}) were of no significant effect on the hatching of *Heterodera glycines* when compared with that in deionized water, however, they exerted a negative impact ($P \leq 0.05$) when compared with hatching rate in the solution of zinc sulfate (3.14 mM).

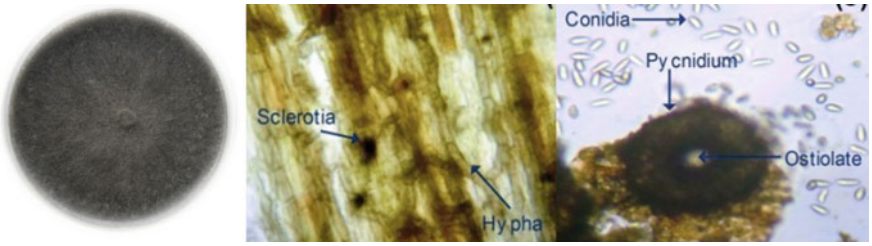


Fig. 38 Charcoal rot causal fungus, *Macrophomina phaseolina*, (Left) blackish mycelial growth on potato dextrose agar plate (*Source* Photchana Trakunsukharati, Department of Agriculture, Thailand); (Middle) hyphae and sclerotia of the fungus (*Source* Islam et al. 2012); (Right) Osteolate pycnidium of the fungus and one-celled hyaline pycnidiospores (*Source* Islam et al. 2012)

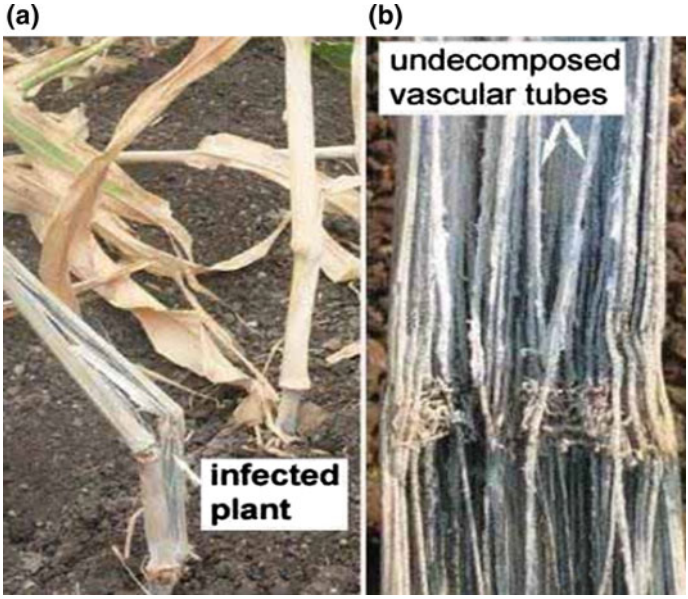


Fig. 39 Charcoal rot disease of sorghum caused by *Macrophomina phaseolina*, **a** symptoms of the disease on plant foot and foot breakage, **b** internal black rot of foot and stem and undecomposed vascular tubes (*Source* Reddy et al. 2012)

Bladex® was numerically (not statistically) a little more toxic than Atrazine® (Wong et al. 1993). Desmedipham, a phenylcarbamate, indicated direct antifungal activity against *Bipolaris* sp., *Ceratocystis radicicola*, *Fusarium graminearum*, *Gaeumannomyces graminis*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Verticillium dahliae*, and two oomycetous pathogens *Phytophthora* sp., and *Pythium* sp. (Pakdaman et al. 2002).

HRAC Group C2

The chemical compound from the family of amides, propanil (Rogue™, and Stam-F-34) was of direct fungicidal activity against brown leaf spot fungus *Bipolaris oryzae* (Sen and Kaiser 1972). Metoxuron, a member of the chemical family of ureas, was of direct antifungal activity against *Puccinia lagenophorae*, a rust fungus pathogenic on the perennial weed common groundsel, *Senecio vulgaris* (Frantzen et al. 2005; Fig. 40).

Fluometuron and diuron, tested at both concentrations of 25, and 50 μg mL^{-1} were of no Impact on *Pectinobacterium carotovorum* growth rate (Breazeale and Camper 1972). Diuron at 50 μg mL^{-1} inhibited the mycelial development of *Sclerotinia sclerotiorum* on 1.5% Bacto agar (Casale and Hart 1986). Out from five herbicides (also including isoproturon, 2,4-D ethyl ester, atrazine, and 2,4-D ethyl sodium) tested under in vitro conditions, Tribunil® (methathiabenzuron, an urea) applied at 100 ppm (equal to 100 μg g^{-1} soil; ppm: part per million) was the most effective, and completely inhibited the sclerotial germination of *Sclerotium rolfsii*. Another urea, isoproturon exhibited complete inhibition of fungal radial growth when it was applied at the concentration of 250 ppm. Soil application of 0.4% Tribunil® (equal to 4000 ppm or 4000 μg g^{-1} soil) led to complete ceasation of the fungus saprophytic activity (Harlapur et al. 1989).

Fig. 40 Aecia of *Puccinia lagenophorae*, the rust fungus pathogenic on the perennial weed common groundsel, *Senecio vulgaris* (*Source* http://www.naturefg.com/)

HRAC Group C3

Direct antifungal activity of a member of the family benzothiadiazinones, bentazon on *Colletotrichum truncatum* (Caulder et al. 1987), and of a member of the family of nitriles, bromoxynil on *Rhizoctonia cerealis* and *Pseudocercosporella herpotrichoides* (Kataria and Gisi 1990) have been recorded. Ioxynil, another nitrile herbicide, inhibited in vitro growth of *Pectinobacterium carotovorum* when applied at the concentration of 25 μg mL^{-1} (Breazeale and Camper 1972). Bentazone (Basagran® tested in vitro at two concentrations of 50 and 500 μg mL^{-1}) was of no significant effect on the hatching of soybean cyst nematode *Heterodera glycines* when compared with that in deionized water, however, it exerted a negative impact ($P \leq 0.05$) when compared with hatching rate in the solution of zinc sulfate (3.14 mM) (Wong et al. 1993).

HRAC Group D

Paraquat, a member of the chemical family bipyridyliums was found to be of direct antifungal effect on *Dreschlera teres* (Toubia-Rahme et al. 1995). Diquat (25 μg mL^{-1}), another member of the family, and paraquat (50 μg mL^{-1}) inhibited the growth of *Pectinobacterium carotovorum* (Breazeale and Camper 1972).

HRAC Group E

Acifluorfen, as a diphenylether and protoporphyrinogen oxidase (PPO) inhibitor, was found to reduce the radial growth of *R. solani* isolates of IA colonies by more than 72% under in vitro conditions (Black et al. 1996), while fomesafen, another diphenylether seemed to be of no significant direct impact on *Sclerotinia sclerotiorum* (Dann et al. 1999). Also, acifluorfen (Blazer®, applied in vitro at the rate of 50–500 μg mL^{-1}) led to a 42–67 percentile reduction (compared with that in deionized water) and to a 61–71 percentile reduction (compared with that in zinc sulfate solution) of the hatched eggs of soybean cyst nematode (SCN) *Heterodera glycines*. Therefore, this postemergence soybean herbicide may be of a potential role in the management of SCN (Wong et al. 1993). The herbicides oxyfluorfen (a diphenylether) and oxadiazon (an oxadiazole) inhibited the mycelial growth and sclerotial germination of *Rhizoctonia solani* on potato sucrose agar (PSA) medium, where the herbicidal concentrations required for 50% growth inhibition (IC_{50}) were 2.01, and 22.01 mg L^{-1}, respectively. Sclerotial germination was inhibited by oxyfluorfen, and oxadiazon at 100 mg L^{-1} (Hua et al. 2002).

HRAC Group F1

No information was found.

HRAC Group F2

No information was found.

HRAC Group F3

Fluometuron, tested at both concentrations of 25, and 50 μg mL^{-1}, was of no Impact on *Pectinobacterium carotovorum* growth rate (Breazeale and Camper 1972). An herbicide from the chemical family isoxazolidinones, clomazone (Command®) tested in vitro at the concentrations of 50, and 500 μg mL^{-1} was of no negative impact on the hatching of soybean cyst nematode *Heterodera glycines* (Fig. 41) when compared with deionized water, however, its effect was statistically significant ($P \leq 0.05$) compared with that of zinc sulfate solution (3.14 mM), which increased hatching (Wong et al. 1993).

Fig. 41 Soybean cyst nematode, *Heterodera glycines*, (Top, Left) Juvenile larva with visible stylet (*Source* Jonathan D Eisenback, Virginia Polytechnique Institute and State University, Bugwood.org), (Top, Right) Lemon-shaped female cysts (*Source* Mactode Publications, Bugwood.org); (Down) Adult male with distinct stylet and projected spicules (*Source* Jonathan D Eisenback, Virginia Polytechnique Institute and State University, Bugwood.org)

HRAC Group G

Glyphosate as the member of the chemical family, glycines, is capabale to impose its antifungal effects on *Puccinia lagenophora* (Wyse and Müller-Schärer 2001), *Dreschlera teres* (Toubia-Rahme et al. 1995), *Calonectria crotalariae* (Berner et al. 1991), *Pythium ultimum* and *Fusarium solani* f. sp. *pisi* (Kawate et al. 1992). Glyphosate also inhibited pseudothecium formation of wheat tan spot fungus, *Pyrenophora tritici-repentis* in wheat straw (Sharma et al. 1989). Glyphosate not only inhibited aeciospore formation by the biological control rust fungus, *Puccinia lagenophora* on common groundsel (*Senecio vulgaris* L.) but also inhibited the germination of its aeciospores (Wyss and Müller-Schärer 2001). Spraying glyphosate-resistant bread wheat (*Triticum aestivum*) plants with the glyphosate commercially available formulation, Roundup® WeatherMAX at 0.84 kg a. i. ha^{-1}, inhibited plant infection in growth chamber experiments where more than 75% of check plants were infected to the rust fungus after they were sprayed with water or formulation control. The rate of rust control was maximal just after glyphosate application and proportional to the herbicide concentration in planta, where the herbicide persisted at least for a 14 day period. The antifungal activity of the symplastic herbicide was attributed to its inhibitory impact on fungal 5-enolpyruvyl shikimate 3-phosphate (EPSP) synthase (Feng et al. 2005). Similar results were also obtained when glyphosate-resistant bread wheat plants were treated with the herbicide in order to study its effect on the wheat stripe rust (*Puccinia striiformis* f. sp. *tritici*) incidence, progress, and severity (Feng et al. 2005). Interestingly, the application of glyphosate on transgenic gluphosate-resistant (GR) wheat reduced leaf rust (induced by *Puccinia recondita* f. sp. *tritici*) development, and black stem rust (caused by *Puccinia graminis* f. sp. *tritici*) infection (The level of leaf rust control decreased with the reduction in the application rates of glyphosate and the transitory effect lasted for 21–35 days after application (Anderson and Kolmer 2005). These findings indicate the antifungal activity of the widely used broad-spectrum herbicide, glyphosate that provides disease control benefits in glyphosate-resistant wheat, where glyphosate exhibits both preventive and curative activities against leaf rust pathogen, *P. triticina* (Fig. 42), and stripe rust causal fungus, *P. striiformis* f. sp. *tritici* (Fig. 43) under greenhouse as well as field conditions (Feng et al. 2005, and 2008).

A similar reduction of infection has been reported with Asian soybean rust (ASR, caused by the fungus, *Phakopsora pachyrhizi*) as the result of the glyphosate-resistant soybean plants treatment with glyphosate (Feng et al. 2005). Glyphosate at the rates between 0.84 and 1.68 kg ha^{-1} delayed the onset of ASR in glyphosate-resistant soybeans under laboratory conditions. However, field trials conducted in Argentina and Brazil under natural infestations indicated variable ASR control from the application of glyphosate in glyphosate-resistant soybeans. Therefore, further field studies are ongoing in order to define the activity of glyphosate against ASR (Feng et al. 2008).

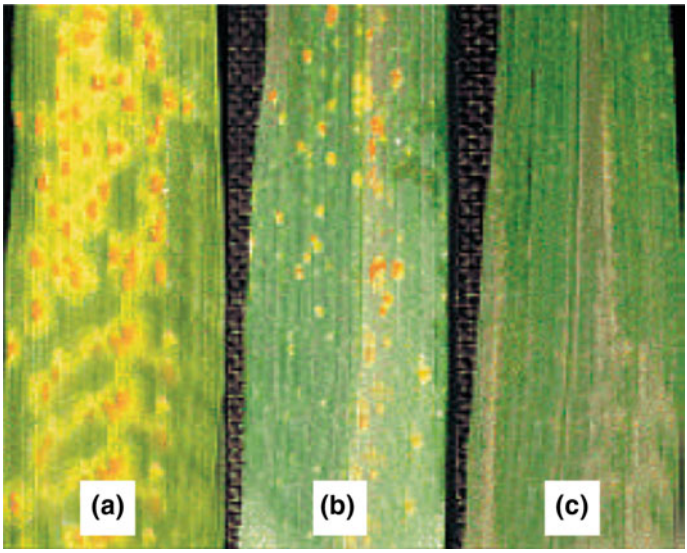


Fig. 42 Effect of glyphosate on wheat leaf rust (*Puccinia triticina*) 13 days after infection. **a** No treatment; **b** treated with 0.84 kg ae ha^{-1} Roundup® WeatherMAX 13 days before inoculation; **c** treated with 0.84 kg ae ha^{-1} Roundup® WeatherMAX 1 day before inoculation (*Source* Feng et al. 2005). Copyright 2005 National Academy of Sciences, USA

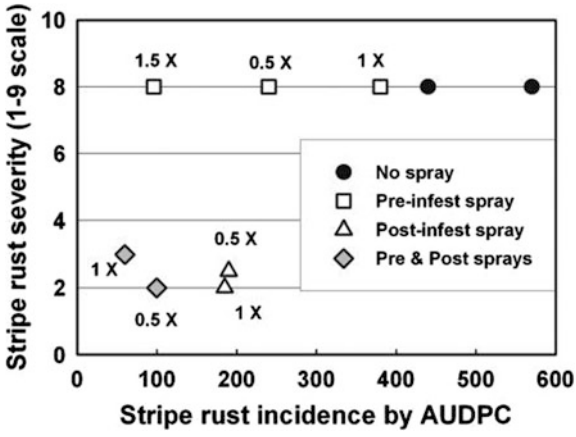


Fig. 43 Field evaluation of the effect of glyphosate treatment on stripe rust (*Puccinia striiformis* f.sp. *tritici*) in glyphosate-resistant wheat cultivar Macon from natural inoculum near Pullman, WA. Roundup® Ultra™ (0.42–1.26 kg a. i. ha^{-1}, 0.5× to 1.5× was sprayed preinfestation on May 19, 2004 and/or postinfestation on June 30, 2004. Rust symptoms first appeared on June 25, 2004. Stripe rust incidence was calculated based on Area Under Disease Progress Curve (AUDPC) from ratings on July 2nd, 8th, and 12th. The y axis describes rust severity (based on 1–9 scale) from July 12th (*Source* Feng et al. 2005)

HRAC Group H

Glufosinate and bialaphos, both glutamine synthase inhibitors belong to the single family of phosphine acids, and exhibit considerable antifungal activities against oomycetous, ascomycetous, and basidiomycetous pathogens. In a less definitive study, Ahmad and Malloch (1995) found that 1 mM glufosinate (a member of the family, phosphinic acids) reduced the bacterial population of the tested agricultural soil by 40% within 14 days after soil treatment. Additionally, it indicates direct antifungal activity against *Aspergillus flavus* (Tubajika and Damann 2002) and *Verticilium albo-atrum* (Ahmad and Malloch 1995). The effect of the non-selective herbicide glufosinate on plant diseases is known to be the result of direct fungitoxic effects. Its effects are best seen in glufosinate-resistant crops. Glufosinate has antimicrobial activity in glufosinate-resistant soybeans (Pline et al. 2001), rice (Uchimiya et al. 1993), and creeping bentgrass (Liu et al. 1998; Wang et al. 2003), protecting these crops from bacterial and fungal diseases. Liu et al. (1998) assessed the antifungal activity of the herbicides bialaphos (another member of the family, phosphinic acids) and glufosinate. Bialaphos, a natural herbicide, showed a higher level of in vitro antifungal activity against *Rhizoctonia solani*, *Sclerotinia homoeocarpa* and *Pythium aphanidermatum* than glufosinate. Glufosinate reduced the radial growth of soybean fliar blight fungus, *R. solani* by up to 61%, and reduced sclerotium formation under laboratory conditions. Glufosinate exerted a fungistatic effect on *R. solani* isolates of IA colonies, but it was fungitoxic to the isolates of IB colonies (Black et al. 1996). Glufosinate suppressed the mycelial growth of *R. solani* (Fig. 44) and *S. homoeocarpa*, but it had no inhibitory effect on *P. aphanidermatum* up to the highest tested concentration (Liu et al. 1998). In contrast, gluphosinate ammonium inhibited the growth and propagation of *Phytophthora infestanc* and *Pythium ultimum* in vitro, especially when tested under nutritionally poor conditions (Kortekamp 2008). The rate of glufosinate required for

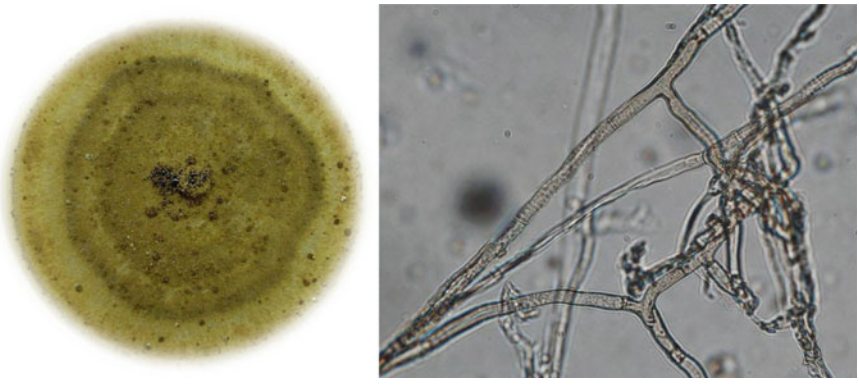


Fig. 44 *Rhizoctonia solani* (Left) mycelia and sclerotia of the fungus on agar plate (*Source* Paul Bachi, University of Kentucky Research and Education Center, Bugwood.org); (Right) mycelia of the fungus (*Source* Paul Bachi, University of Kentucky Research and Education Center, Bugwood.org)

the complete inhibition of the mycelial growth of *S. homoeocarpa* (336 mg L^{-1}) was less than that for *R. solani* (448 mg L^{-1}) (Wang et al. 2003), indicating that *S. homoeocarpa* was more sensitive to glufosinate than *R. solani* (Liu et al. 1998; Wang et al. 2003).

Additionally, glufosinate directly inhibited the mycelial growth of grapevine pathogens *Botrytis cinera*, *Guignardia bidwellii*, *Penicillium expansum*, and *Phomopsis viticola*, when it was applied at various concentrations under in vitro conditions (Albrecht and Kortekamp 2009). Interestingly, the reaction of different fungi to the presence of gluphosinate was different. Gluphosinate was found of almost a complete genostatic impact on the sporulation of *P. expansum* when it was tested at the concentration 500-fold more diluted than the recommended dose for field applications, a finding of importance in the control of late seasonal infections. However, glufosinate was less toxic to its mycelial growth, while it reduced the mycelial growth of *G. bidwellii* by about 80% at the same dilution effective on *P. expansum* sporulation. Moreover, glufosinate severely inhibited the growth of *Plasmopara viticola*, an obligate parasite and causal agent of grapevine downy mildew. While it was phytotoxic at higher doses, glufosinate hermetically stimulated chlorophyll content increase in grapevine leaves without any visible phytotoxicity at low doses (Kortekamp 2008, 2010). Various concentrations of bialaphos solutions were applied to transgenic, bialaphos- and glufosinate-resistant creeping bentgrass (*Agrostis stolonifera* var. *palustris*) inoculated with fungal pathogens. Bialaphos applications were able to significantly reduce symptomatic infection by *R. solani* and *S. homoeocarpa* on transgenic plants. Bialaphos significantly inhibited *P. aphanidermatum*, but not to the same degree that *R. solani* and *S. homoeocarpa* were inhibited. These results indicate that bialaphos may provide a means for the simultaneous control of weeds and fungal pathogens in turf areas with transgenic, bialaphos-resistant creeping bentgrass. Glufosinate ammonium specifically impeded appressorium formation of rice blast and brown leaf spot pathogens, respectively *Magnaporthe oryzae* and *Cochliobolus miyabeanus* (sexual state of *Bipolaris oryzae*) on hydrophobic surface as well as on 35S *bar* transgenic rice. In contrast, conidial germination was not affected (Ahn 2008).

HRAC Group I

No information was found.

HRAC Group K1

Studies with the herbicides applied in rapeseed or canola (*Brassica napus* var. *oleifera*) fields demonstrated that dinitroaniline herbicides trifluralin and ethalfluralin were highly toxic against *Sclerotinia sclerotiorum*, the causal fungus of canola white stem rot disease (Pakdaman and Mohammadi Goltapeh 2007). The direct antifungal activity of trifluralin against *Fusarium solani* (Yu et al. 1988), and the oomycetous pathogen *Aphanomyces euteiches* (Jacobsen and Hopen 1981) has also been recorded. Singh et al. (1999) indicated the inhibitory effect of two

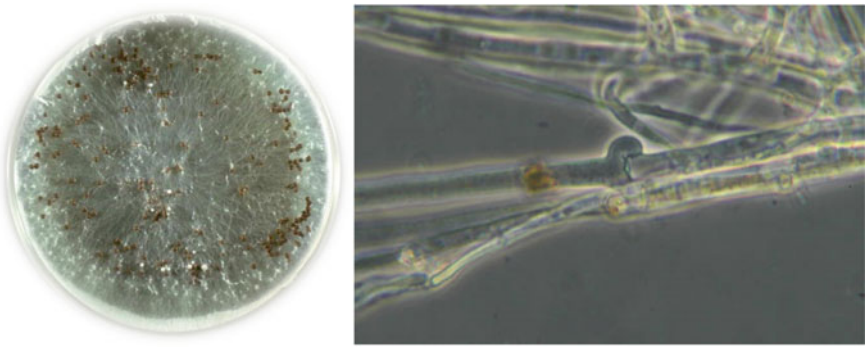


Fig. 45 *Sclerotium rolfsii*, (Left) Mycelia and sclerotia of the fungus on agar plate (*Source* Howard F Schwarz, Colorado State University, Bugwood.org); (Right) Clamp connections (hooked structures) on the mycelia of the fungus (*Source* http://www.paceturf.org/) indicating its basidiomycetous nature

pre-emergence herbicides, fluchloralin and pendimethalin on growth and development of *Phytophthora drechsleri* f. sp. *cajani*, the causal agent of pigeon pea blight. Trifluralin was found on no impact on *Pectinobacterium carotovorum* growth rate when tested at the concentrations of 25, and 50 μg mL^{-1} (Breazeale and Camper 1972). Fluchloralin drastically inhibited the growth of *Sclerotium rolfsii* (Fig. 45) and *Rhizoctonia bataticola* under in vitro conditions (Tripathi et al. 1988).

Similarly, trifluralin and basalin inhibited the growth of *S. rolfsii* in vitro (Lal and Nagarajan 1988). Trifluralin and pendimethalin were the most efficient herbicides out of the tested herbicides (also including imazethapyr, metachlor, and a mixture of acetochlor + imazethapyr) that significantly reduced the production of viable sclerotia by *S. rolfsii* under in vitro conditions (Pastro and March 1999). Also, the inhibition of the sclerotial germination of *S. rolfsii* has been reported with fluchloralin studied using soil plate technique, where its inhibitory effect (94.02%) was statistically at par ($P = 0.05$) with that of the most effective fungicides, carboxin (98.99%), and tridemorf (97.89%) (Bhoraniya et al. 2002). Trifluralin (Treflan®), and ethalfluralin (Sonalan®), both tested in vitro at two concentrations of 1 and 10 μg mL^{-1}, were of no significant effect on the hatching of soybean cyst nematode *Heterodera glycines* when compared with that in deionized water, however, they exerted a negative impact ($P \leq 0.05$) when compared with hatching rate in the solution of zinc sulfate (3.14 mM). The negative effect of Sonalan® on the nematode hatching in zinc sulfate solution was almost twice that of Treflan® (Wong et al. 1993).

HRAC Group K2

No information was found.

HRAC Group K3

In an investigation on the antifungal activity of two soil herbicides conducted by Kataria and Dodan (1983), alachlor (a chloroacetamide) was found more inhibitive to *Rhizoctonia solani* growth than fluchloralin (a dinitroaniline from HRAC group K1) in potato dextrose broth (PDB). However, the infective capacity of the pathogen was not altered by growing it in a medium containing either of the herbicides. Metolachlor inhibited of the sclerotial germination (62.78%) (Bhoraniya et al. 2002). Alachlor (LaSSO®) tested in vitro at the concentrations of 24, and 240 μg mL^{-1} was of no negative impact on the hatching of soybean cyst nematode *Heterodera glycines* when compared with deionized water, however, its effect was statistically significant ($P \leq 0.05$) compared with that of zinc sulfate solution (3.14 mM), which increased hatching (Wong et al. 1993).

HRAC Group M

The direct antifungal effect of dinoseb, an uncoupler and membrane disruptor herbicide from dinitrophenols family, on the fungal pathogens *Aspergillus niger*, and *Alternaria tenuissima* (Kovacs and Malligni 1975) as well as on the oomycetous pathogen *Aphanomyces euteiches* (Jacobsen and Hopen 1981) has been recorded.

HRAC Group N

Paul and Schönbeck (1976) studied the impact of the herbicide diallate (a thiocarbamate) on several cereal pathogens in vitro, and found that the herbicide added to agar reduced mycelial growth of *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, *F. moniliforme*, and *Bipolaris sorokiniana*. Soil-incorporated diallate reduced maize root rot caused by *F. moniliforme*, and wheat crown and root rot induced by *F. avenaceum*, and *F. culmorum*. Diallate inhibited maize root rot and also barley disease caused by *B. sorokiniana* in hydrocultural maize and barley growing systems. Diallate severely prevented *F. moniliforme* penetration in stele region, and hyphae in roots exposed to diallate often were partially deformed and of the cytoplasm seemingly very granulated. Helmeczi et al. (1988) studied the effect of 14 herbicides and two herbicide combinations on three microscopic soil fungi following Miller's method, and found that (i) none of the 14 herbicides and 2 combinations belonging to four derivative groups induced the growth of *Aspergillus niger*, *Fusarium oxysporum*, and *Rhizopus nigricans*; (ii) higher doses of the herbicides prevented the growth of all test organisms significantly, and thiocarbamate derivatives Alirox B 80 EC (EPTC), Anelda Plus 80 EC (butylate) and Anelda III (butylate) were the most inhibitory; and (iii) the sensitivity of test organisms against the examined herbicides decreased in the order of *Fusarium*, *Rhizopus*, *Aspergillus*.

The herbicides of thiocarbamates family, cycloate (Ro-Neet® tested at 15 mg mL^{-1}), pebulate (Tillam® tested at 21 mg mL^{-1}), triallate (Avadex® BW tested at 7 mg mL^{-1}), and vernolate (Vernam® tested at 12 mg mL^{-1}) eliminated the subsequent hatch of *Globodera rostochinensis* and *Heterodera schachtii* eggs in root diffusate. Tests with New Blue R (Shepherd 1962) indicated that triallate applied at the recommended dose for field application (1.7 kg a. i. ha^{-1}) killed 90% of cyst contents. The thiocarbamate family seems to be the important factor in the inhibition, and there is no link between the method of formulation and the effect on hatching of nematode eggs. Furthermore, the effect of thiocarbamate herbicides on hatch was markedly reduced by dilution, therefore, any thiocarbamate herbicide-based control strategy probably as a pre-emergence treatment would have to overcome the problem of soil incorporation to ensure that the required concentrations contact the cysts (Perry and Beane 1989).

HRAC Group O

The synthetic auxin like growth regulator, 2,4-D inhibited in vitro growth of *Ceratocystis radicicola*, *Fusarium graminearum*, *F. oxysporum*, *Gaeumannomyces graminis*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* (Fig. 46), *Verticillium dahliae*, and that of two oomycetous pathogens, *Phytophthora* sp., and *Pythium* sp. (Pakdaman et al. 2002). 2,4-D drastically inhibited the in vitro growth of *Sclerotium rolfsii* (Tripathi et al. 1988) and *Rhizoctonia bataticola* (Tripathi et al. 1988), and *R. solani* (Pathak et al. 1996).

HRAC Group P

No information was found.

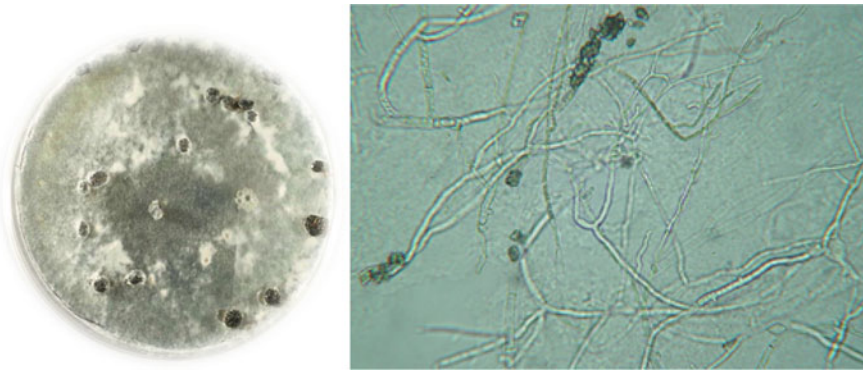


Fig. 46 *Sclerotinia sclerotiorum*, (Left) Mycelia and sclerotia on agar plate (*Source* Howard F. Schwartz, Colorado State University, Bugwood.org), (Right) mycelia of the fungus (*Source* Clarissa Balbalian, Mississippi State University, Bugwood.org)

HRAC Group Z

Because of the high diversity of the origin, chemical structure, and mode of action of these herbicides, here we ignore this group.

Direct Pro-pathogenic Effects of Herbicides

Some xenobiotic compounds such as herbicides are known as pro-pathogenic agents that either directly enhance pathogen growth and development or directly increase its virulence. Here, a new term is coined and "pathancer", the abbreviation of pathogen enhancer, is suggested. It is not unusual for low rates of herbicides to stimulate in vitro pathogen growth (for example, Yu et al. 1988). Hormesis (the stimulatory effect of a sub-toxic level of a toxin) is common with both fungicide effects on fungi and herbicide effects on plants (Duke et al. 2006). Thus, dose rates are likely to be highly important in both direct and indirect effects of herbicides on plant disease. Some of the herbicides are not of direct toxicity to the pathogenic fungi and oomycetes, and such herbicides can be used as xenobiotic sources of energy as well as materials (such as carbon, nitrogen, phosphorus, sulfur and so on) needed for the growth and development of these pathogens. For instance, 2,4-dichlorophenoxyacetic acid (2,4-D) can be biodegraded and catabolized by *Burkholderia cepacia* (Bhat et al. 1994), *B. graminis* (Dejonghe et al. 2000), *Pseudomonas* spp. (Musarrat et al. 2000), and the fungus *Phanerochaete chrysosporium* (Yadav and Reddy 1993). The effect of the herbicides, pendimethalin, and prometryn (applied to the soil at 2.4, and 3.6 μg a. i. g^{-1} soil, equivalent to the respective recommended field concentrations of 0.9, and 1.3 kg a. i. ha^{-1}, respectively) on the incidence of *Rhizoctonia solani*-induced cotton seedling damping-off was investigated in the microcosm conditions. Prometryn significantly raised up the incidence of pre-emergence damping-off, while post-emergence damping-off increased significantly after the application of pendimethalin and prometryn. Also, similar results were obtained with both herbicides, particularly with prometryn in the fields treated with the herbicides before planting (Heydari and Misaghi 1998). Arfarita et al. (2011) applied glyphosate as a screening agent used as the sole phosphorus source for the isolation of soil-born fungi from forest soil. Three fungal strains were able to grow consistently in the presence of glyphosate as the only source of phosphorus (*Fusarium* sp. strain FRP1, *Scopulariopsis* sp. strain FRP2, and *Trichoderma* sp. strain FRP3). On standard medium, ten fungal strains were isolated and identified, *Botrytis* sp. strain FR1, *Mucor* sp. strain FR2, *Acremonium* sp. strain FR3, *Trichoderma* sp. strain FR4, *Botrytis* sp. strain FR5, *Crysosporium* sp. strain FR6, *Scopulariopsis* sp. strain FR7, *Trichoderma* sp. strain FR8, *Botrytis* sp. strain FR9, and *Acremonium* sp. strain FR10. Of the three screened fungal species, *Scopulariopsis* sp. strain FRP2 and *Trichoderma* sp. strain FRP3 were of the highest ratios of growth diameter, and were selected for further studies. Some herbicides are biodegraded or bioconverted to non-toxic metabolites, and some are not lethal but restrict pathogen growth when

applied following the recommendations on the labels. These herbicides can act as pathancers. The application of glyphosate, and chlorsulfuron has been associated with increased levels of take-all fungus, *Gaeumannomyces graminis* (Mekwatanakarn and Sivasithamparam 1987). In a greenhouse study of the impact of atrazine, the populations of *Fusarium* were increased up to four folds compared with the soil not treated with atrazine added at 10, 30, and 100 μg g^{-1} soil. Additionally atrazine amendment in the soil at the rate of 10 μg g^{-1} soil led to the increased germination of *Fusarium* macroconidia, growth of germ tubes, as well as the formation of chlamydospores (Percich and Lockwood 1975). The herbicides like sethoxydim may be of potential pathancing effects on the virulence of some pathogens such as head blight causal *Fusarium* species. *Fusarium graminearum* (Fig. 47) is able to tolerate this herbicide and even to increase its rate of growth, however, its characteristic carmine red color decreases to pale pink, indicating reduced production of naphthoquinone pigments (Pakdaman and Kariman 2006) that occurs through polyketide biosynthesis pathway (Kim et al. 2005). This can lead to the fortification of trichothecene biosynthesis pathway (Pakdaman and Kariman 2006). Trichothecenes are among the main virulence factors responsible for the host plant yield loss, as well as the occurrence of mycotoxicoses in human and livestocks.

Chan Cupul et al. (2014) evaluated the effect of atrazine concentrations on mycelial growth and ligninolytic enzyme activities of eight Mexican ligninolytic macrofungi in a semi-solid culture medium. Inhibition of mycelial growth and growth rates were significantly affected ($p = 0.05$) by atrazine concentrations (468, 937, 1875, and 3750 mg L^{-1}). In accordance with the median effective concentration (EC_{50}), *Pleurotus* sp. strain 1 proved to be the most tolerant isolate to atrazine (EC_{50} = 2281.0 mg L^{-1}), although its enzyme activity was not the highest. *Pycnoporus sanguineus* (Fig. 48) strain 2, *Daedalea elegans* and *Trametes maxima* showed high laccase activity (62.7, 31.9, 29.3 U mg/protein, respectively) without

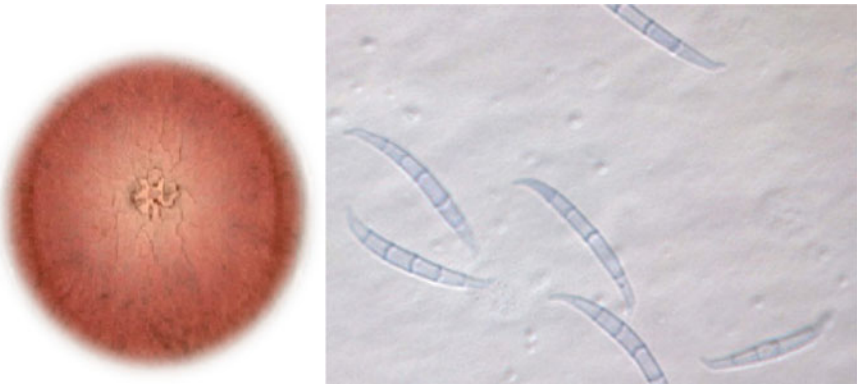


Fig. 47 *Fusarium graminearum* (Left) mycelial colony on agar plate (*Source* http://mycota-crcc.mnhn.fr/); (Right) Macroconidia (*Source* Liu et al. 2012)

atrazine (control); however, this activity considerabely increased ($p < 0.05$) (to 191.1, 83.5 and 120.6 U mg/protein, respectively) because of the effect of atrazine (937 mg L^{-1}) in the culture medium.

Pleurotus sp. strain 2 and *Cymatoderma elegans* significantly increased ($p < 0.05$) their manganese peroxidase (MnP) activities under atrazine stress at 468 mg L^{-1}. The isolates with high EC_{50} (*Pleurotus* sp. strain 1) and high enzymatic activity (*P. sanguineus* strain 2 and *T. maxima*) could be considered for future studies on atrazine mycodegradation. Furthermore, this study confirms that atrazine can increase laccase and MnP activities in ligninolytic macrofungi (Chan Cupul et al. 2014). During invasive growth of pathogenic fungi in host plants, three main enzymes get directly or indirectly involved in the modification of lignin structures that are lignin peroxidases (LiPs), manganese peroxidases (MnPs), and laccases sensu stricto (Kuo et al. 2015). In addition to lignin degradation, fungal laccases play notable roles in fungal morphogenesis (Zhu and Williamson 2004), stress defence (Thurston 1994; Gianfreda et al. 1999), as well as fungal virulence (Zhu and Williamson 2004; Kuo et al. 2015). MnP produces Mn^{3+} ions that play their major role in the oxidation and degradation of lignin (Forrester et al. 1988). The Mn^{3+} ions can directly oxidize the phenolic compounds in lignin. The Mn^{3+} ion can degrade lignin through catalysis of alkyl-aryl cleavages as well as alpha-carbon oxidation in phenols (Tuor et al. 2002). Additionally, the ions can oxidize some organic sulfur compounds and unsaturated fatty acids that leads to the formation of thiyl and peroxyl radicals. In the presence of molecular oxygen, the radicals can oxidize lignin or form hydrogen peroxide as the result of their reaction to water (Wariishi et al. 1989; Kapich et al. 1999).

Fig. 48 The laccase producing mushroom, *Pycnoporus sanguineus* (*Source* www.mycelia.be/)

3.2.2 Indirect Impact of Herbicides on Plant Pathogens

Indirect Impact of Herbicides on Pathogens Through Control of Weeds

Herbicides control of the host weeds of pathogens that otherwise could serve as survival, reservoir, alternative, alternate, and volunteer hosts and act their roles as primary infection foci, or dispersal centers for pathogens, vectors, or both. Herbicides play an important role in the control of plant diseases caused by facultative saprobes and biotrophic parasites. Soil-transient pathogens are not competitive saprophytes in a complex soil medium. Therefore, the application of non-selective herbicides will be helpful in the reduction of pathogen inoculum in the soil before the next crop plant is grown. Heteroecious rust fungi as a group of biotrophic fungi require plant hosts usually of different taxonomical groups, and soil/pre-emergence application of herbicides will be helpful in the reduction of the disease through eradication of the alternate host weeds and will lead to the plant disease cycle beakage. On the other hand, the treatment with selective post-emergence herbicides will have its own obliging effect in the combat against autoecious as well as heteroecious rust pathogen development on alternative hosts after emergence of host crop. Post-emergence herbicides would also be useful in the control of biotrophic pathogens of broad host ranges such as *Erysiphe polygoni*, and cucumber mosaic virus. However, with necrotrophic pathogens and with pathogenic facultative parasites, the conditions will be very different. The application of herbicides, either of non-selective pre-emergence type or of selective and/post-emergence type, will provide a lot of predisposed plants and dead plant materials that can easily be colonized by these saprophytically very competitive groups of pathogens. Pathogens like *Fusarium*, *Rhizoctonia*, *Alternaria*, *Botrytis cinerea* (Fig. 49) and *Pythium ultimum* lay in this group. Most of these fast-growing pathogens are armed with the genes responsible for the biosynthesis and the secretion of the toxins that are used in order to defend their territories in the competition against other microorganisms. As the result of herbicidal treatment, these necrotrophic pathogens will gain an exceptional opportunity for their fast growth and development in the treated declining weeds with disturbed defense system resistance. These conducive conditions will lead to the increased inoculum potential of the pathogen as the consequence of its increased accessibility to dead organic materials. This can result in the outbreak of these diseases if no contrivance is made with the management of the remnant weed residues.

For instance, pendimethalin and atrazine inhibit the mycelial growth of *Pythium arrhenomanes*, but neither decrease root rot severity in sugarcane. Glyphosate, pendimethalin and terbacil are injurious to sugarcane and increase root rot severity (Dissanayake et al. 1998). The increased sugarcane root rot after atrazine treatment can be explained by its especial mode of distribution *in planta* (Jachetta et al. 1986), and the method of application of atrazine in their work (spraying instead of soil application). This strongly indicates the importance of herbicide application and translocation mode in the determination of its final impact on plant disease control.

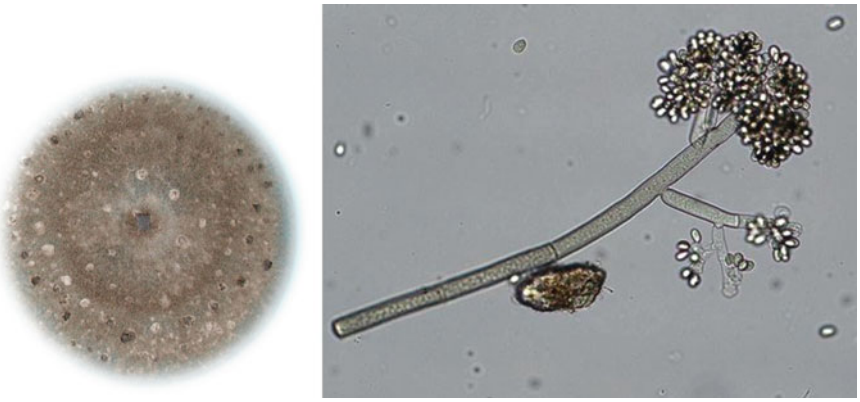


Fig. 49 Gray mold, *Botrytis cinerea* (Left) mycelial growth with gray to brown conidiation and sclerotial development on potato dextrose agar plate after 10 days of incubation at 20 C and 12 h alternating cycles of darkness and fluorescent light (*Source* Xiao 2006); (Right) Conidiophore with branches that end to fertile with denticles bearing conidia (*Source* Paul Bachi, University of Kentucky Research and Education Center, Bugwood.org)

The eradication of some weeds that host vectors of pathogens, with selective post-emergence herbicides can lead to the attack of vectors to the main crop plants. Such attacks will lead to disease transmission to the cultivated crop plants and increased damages. However, if vectors need wild weed plants to complete the primary stages of their life cycles, and if they are very vulnerable in the absence of their host weeds and mostly are in the life stages of ignorable mobility, the elimination of host weeds will create a severe break in their life cycle and will result in the reduced rate of disease transmission. Some weeds are primary attraction foci for most of the vector populations and therefore, the conscious and clever management of these weeds will lead to the synchronized extermination of weed and vectors after attraction of the first early season populations of the vectors in early growth season and before planting or seeding of the main crop or before its emergence in the field. For example, since airborne aphids are attracted to the color yellow (Agrios 2005), weeds with yellow flowers will attract the aphids, and early season control of weeds and aphids by an herbicide will be very useful in the control of the pathogens associated with aphid vectors. Furthermore, some herbicides are known to be directly entomotoxic, and may influence the insect vectors life and reproduction. For example, the mixture of glyphosate and 2,4-D was found of direct insecticidal activity and caused 100% mortality of the adult grain beetle, *Tenebrio molitor*. More beetles escaped from the herbicide as well as nitrate treatments, suggesting a kind of behavioral avoidance of toxic environments (Castilla et al. 2010). 2,4-D ethyl ester (Weedkill 80 WP) was not toxic to the larvae of *Spilarctia obliqua* (Lepidoptera: Arctiidae), while imazethapyr (Pursuit 10 EC) and quizalofop ethyl significantly reduced the pest survival at almost all doses. The concentrations required to kill 50% of the pest larvae (LC50) were calculated for

quizalofop ethyl (LC50 = 0.230%), and imazethapyr (LC50 = 0.855%) through probit analysis, and were almost equal to labeled doses (FR) of quizalofop ethyl (FR = 0.16%) and imazethapyr (FR = 0.625%). These data indicate the high toxicity of these herbicides against *S. obliqua* larvae. Additionally, imazethapyr exerted sublethal effects on the pest at almost all concentrations tested in the artificial diet. Quizalofop ethyl was of no sublethal effect, and exerted a markedly significant effect on the pest survivorship. Therefore, quizalofop ethyl incorporation into the integrated pest management module for *S. obliqua* in legumes or oilseed crops with other biorational insecticides was suggested. However, despite of entomotoxicity of imazethapyr, such a suggestion was not made with this herbicide because of its sublethal effects (Gupta and Bhattacharya 2008). Studies with the generalist endoparasitoid *Palmistichus elaeisis* (Hymenoptera: Eulophidae) indicated the negative impact of glyphosate on its performance, however it was concluded that the responsible use of this herbicide would be acceptable (Alcántara-de la Cruz et al. 2017). Glyphosate severely reduced the reproduction and fecundity of lacewing, *Chrysoperla externa* (Neuroptera: Chysopidae) the natural enemy of aphids (Schneider et al. 2009). None of glyphosate-based products (Roundup Original®, Roundup Ready®, Roundup Transorb®, Roundup WG®, and Zapp Qi®) tested at the same acid equivalent concentration under laboratory conditions according to the International Organization for Biological Control (IOBC) standard methods for testing side-effects of pesticides on egg parasitoids, were found harmful or moderately harmful to the parasitoid *Telenomus remus* (Hymenoptera: Platygastridae) when exposure occurred at the pupal or adult stages. Therefore, all mentioned glyphosate-based herbicides can be applied in agriculture with no negative influence on the parasitoid (Stecca et al. 2016), an effective biological control agent for various *Spodoptera* (Lepidoptera: Noctuidae) spp. (Cave 2000; Morales et al. 2000; Pomari et al. 2012) responsible for heavy damages in soybean, maize, and cotton crops (Cruz et al. 1999; Carmo et al. 2009; Santos et al. 2010). In addition to insects, glyphosate improved this parameter for plant mites, the broad mite, *Polyphagotarsonemus latus* (Acari: Tarsonemidae) and *Tetranychus bastosi* (Acari: Tetranychidae) at the application rate of 360 g ae ha^{-1} (Saraiva et al. 2016). On the other hand, the elimination of weeds, within or around fields, as the providers of nectar and shelter for natural enemies of insect vectors can significantly affect the abundance as well as the diversity of parasitoids and predators (Altieri 1994). The removal of natural enemies of a given pest can lead to the resurgence of vectors and the disease(s) transmitted. Map-based precision agricultural systems can be a reliable solution, especially when the primary foci of the vectors are determined and the required actions are performed in limited areas. Such an approach not only can lead to reduced crop yield losses, but also helps in the preservation of natural biodiversity and reduced environmental pollutions. With nematodes, another group of pathogenic obligate parasites of plants, the early seasonal pre-planting application of non-selective herbicides after emergence of weeds and in the absence of host plants, will result in the death of nematodes larvae because of starvation.

Indirect Impact of Herbicides on Pathogens Through the Influence on Plant Physiology

Herbicides may increase, decrease or have no effect on plant disease that depends on the herbicide, pathogen, crop and environmental conditions (Altman and Campbell 1977a). For instance, Jackson and Stack (2002) applied the herbicide dicamba (a benzoic acid from HRAC group O) to the roots of potted Siberian elm *(Ulmus pumila)* and Russian-olive *(Elaeagnus angustifolia)* trees under greenhouse conditions. They used five herbicide concentrations equivalent to the rates of 0 (0), 93 (1/12), 140 (1/8), 280 (1/4), and 1121 (1) g ha^{-1} (lb ac^{-1}) active ingredient. Two weeks after herbicide application, each tree was inoculated with a single isolate of the canker-causing fungus *Tubercularia ulmea*. The experiment was repeated using two different *T. ulmea* isolates. Leaf cupping, a symptom of dicamba exposure, was evident two to three weeks after herbicide application. Symptoms occurred at rates of 140 g ha^{-1} (1/8 lb ac^{-1}) and above in the Siberian elms and the 1121 g ha^{-1} (1 lb ac^{-1}) rate in the Russian-olives. All four *T. ulmea* isolates caused cankers, with canker size differences between fungal isolates and between tree species, however, none of the herbicide treatments increased or decreased *T. ulmea* canker size (Jackson and Stack 2002). In contrast, other herbicides applied and incorporated into soil before planting, including cycloate (a thiocarbamate from HRAC group N), diethathyl-ethyl, EPTC (a thiocarbamate from HRAC group N), and ethofumesate (a benzofuran from HRAC group N) as well as those applied after emergence, including clopyralid (a pyridine carboxylic acid from HRAC group O), desmedipham, desmedipham + phenmedipham (phenyl-carbamates from HRAC group C1), and triflusulfuron-methyl (a sulfonylurea in HRAC group B) did not affect severity of seedling disease and chronic root rot disease of sugar beet caused by the soil born oomycetous pathogen *Aphanomyces cochlioides* compared to a hand-weeded control (Roebke et al. 2002). However, herbicides may affect plant physiology and lead to changes at both physical and biochemical levels (Altman and Campbell 1977a). They may lead to the alterations in plant growth, lignin-containing substances, β-glucosides (Paul and Schönbeck 1976), wax layers on shoot and foliage (Heitefuss 1970, 1972), the rate of root exudates and the release of nutrients from plant root system (Altman and Campbell 1977b; Liu et al. 1995, 1997; Wyse et al. 1976) and altered mineral nutrition (Neumann et al. 2006). Reduced availability of important macro-, as well as micro-nutrients (potassium, magnesium, iron, and zinc) and increased availability of sodium and calcium have been reported as the result of herbicidal treatments with the recommended rates of paraquat (4 L ha^{-1}), glyphosate (4 L ha^{-1}), Primeextra (4 L ha^{-1}), and atrazine (3 kg ha^{-1}, applied for soil treatment) (Sebiomo et al. 2012). Also, low levels of residual glyphosate in soil also reduce root uptake and translocation of Fe, Mn, and Cu (Eker et al. 2006; Ozturk et al. 2008). Additionally, some herbicides may enhance defense mechanisms including the elicitation and accumulation of phytoalexin and mimicry of the hypersensitive response (Landini et al. 2003; Nelson et al. 2002a, b), and finally they may alter the level of host plant resistance to pathogens (Starratt and Lazarovits 1996).

Here, the effect of herbicides in the increase or decrease of plant diseases is discussed due to its high importance in the integrated management of plant diseases.

Increased Disease Resistance of Herbicide-Treated Crops

Increased plant resistance to disease as the consequence of certain herbicidal treatment has been reported. Huber et al. (1966) indicated that although diuron (a member of HRAC group C2) used at the rate of 1.12 kg ha^{-1} did not inhibit the initial penetration by the winter wheat foot rot disease pathogen *Cercosporella herpotrichoides*, it led to the increased winter wheat resistance to the disease. Diuron was not of direct antifungal activity when it was used at the ratio below 100 ppm on corn meal agar. Also, it was shown that the populations of soil fungi, bacteria, and actinobacteria were not affected by diuron applications. Heitefuss and Bodendörfer (1968) found that urea and triazine herbicides could significantly decrease wheat eye-spot disease caused by the fungus *C. herpotrichoides* and powdery mildew caused by *Blumeria graminis* f. sp. *tritici* that was not related to the removal of weeds, as the same reductive effect had also been observed in a weed-free crop. The extracts from simazine-treated wheat plants added to agar medium were inhibitory on *C. herpotrichoides* growth compared with control media. However, this inhibitory effect disappeared after glucose was added to the medium. They concluded that such an inhibitory fungitoxic impact was not adequate to explain the observed disease reduction resulted from the field-application of triazine and urea herbicides. Further experiments conducted by Brandes and Heitefuss (1971) on the physiological and biochemical alterations as the consequence of simazine (a triazine member of HRAC group C1) and monolinuron (an member of urea family in HRAC group C2) revealed significant changes in total nitrogen, sugar, 4-hydroxy-7 methoxy-1,4 benzoxazin-3 on-2 glucoside (DMBO-glucoside), and 2,4-dihydroxy-7 methoxy-1,4 benzoxazin-3 on-aglucan (DMBO-aglucan) that were totally related to the observed disease decrease in wheat plants exposed to the attacks by both pathogenic fungi. Two herbicides dinitrobutylacetate and ioxynil (a nitrile from HRAC group C3) were of no effect on the occurrence of both wheat diseases mentioned above.

Protoporphyrinogen oxidase inhibitors (HRAC group E) cause enough oxidative stress at sublethal levels to induce production of phytotalexins (Kömives and Casida 1983). Some protoporphyrinogen oxidase inhibitor herbicides such as azafenidin (a triazolinone; Viator et al. 2002), flumioxazin (a N-phenylphthalimide; Viator et al. 2002), lactofen (Nelson et al. 2002a), fumesafen (Nelson et al. 2002a), oxyfluorfen (Nelson et al. 2002a), carfentrazone (Nelson et al. 2002a), and sulfentrazone (a triazolinone; Nelson et al. 2002a; Viator et al. 2002) reduce the severity of sclerotinia stem rot of soybean (Dann et al. 1999; Nelson et al. 2002a, b), decrease soybean cyst nematode reproduction (Levene et al. 1998), and the severity of rhizoctonia foliar blight of soybean (Black et al. 1996) through induction of host resistance that is believed to be associated with the singlet oxygen radicals generation by PPOase inhibitor herbicides that destroy lipids in cell membranes

(Daugrois et al. 2005). High levels of glyceollin are induced by lactofen (a nitrophenoxybenzen) in soybeans leading to the protection from white mold (sclerotinia stem rot; Dann et al. 1999). The application of flumioxazin to the leaves of sugarcane resulted in the reduced severity of pythium root rot (Daugrois et al. 2005). Lactofen applied at the V3 stage of soybean, reduced the severity of sclerotinia stem rot by 40–60% in the years of high disease pressure (Dann et al. 1999). It is believed that lactofen is unlikely to directly inhibit *S. sclerotiorum* because of its rapid degradation in field, where it is of only a 3 day half-life. Therefore, the disease suppression may be because of other reasons such as alteration of the canopy environment or reduction in natural infection sites on the plant, or more possibly because of the induction of other localized defenses in plants as it was implied by the reduced lesion development resulted from the fungus in lactofen-treated detached leaflets which were of higher levels of the soybean phytoalexin, glyceollin (Dann et al. 1999). Most of the herbicides in the HRAC group E induce the synthesis of glyceollin in soybeans (Landini et al. 2003). Keen et al. (1982) indicated that glyphosate (a glycine from HRAC group G) application led to the induction of glyceollin phytoalexin production and accumulation by soybean plants, and resulted in the enhanced resistance to *Phytophthora megasperma* f. sp. *glycinea*.

Glufosinate ammonium (a phosphinic acid in HRAC group H) reduced development of rice blast and brown leaf spot diseases in *35S: bar*-transgenic rice. Both pre- and post-inoculation treatments with the herbicide decreased disease development. Glufosinate ammonium diminished mycelial growth of both pathogens however its inhibitory effect was attenuated under malnutrition conditions. Glufosinate ammonium led to slight chlorosis and lower chlorophyll content, however, these changes were almost completely restored in transgenic rice within 7 days. Glufosinate ammonium triggered transcriptions of pathogenicity-related (*PR*) genes and hydrogen peroxide accumulation in the transgenic rice and *PR1* transcription in *Arabidopsis thaliana* wild type Col-0 harboring *35S:bar* construct. All transgenic arabidopsis plants exhibited distinctive hydrogen peroxide accumulation induced by the treatment with glufosinate ammonium. Fungal infection could not alter the transcriptions of *PR* genes and the accumulation of hydrogen peroxide induced by the herbicide. Glufosinate ammonium infiltration could not affect appressorium formation of *M. grisea* in vivo, however, inhibited blast disease development. Hydrogen peroxide scavengers nullified blast protection and transcriptions of *PR* genes incited by glufosinate ammonium however, they did not influence brown leaf spot development. Both direct inhibition of pathogen infection and activation of defense systems are responsible for the protection of *bar*-transgenic rice against blast and brown leaf spot diseases (Ahn 2008; Fig. 50). Low concentrations of glufosinate, although not effective on the release and germination of the grapevine downy mildew pathogen zoospores, restricted the development of its intercellular mycelium and dramatically reduced its sporulation (Kortekamp 2010). Higher doses up to that normally applied to the field were completely effective in the inhibition of each developmental step of the disease cycle. Glufosinate ammonium indicated prophylactic as well as curative activities.

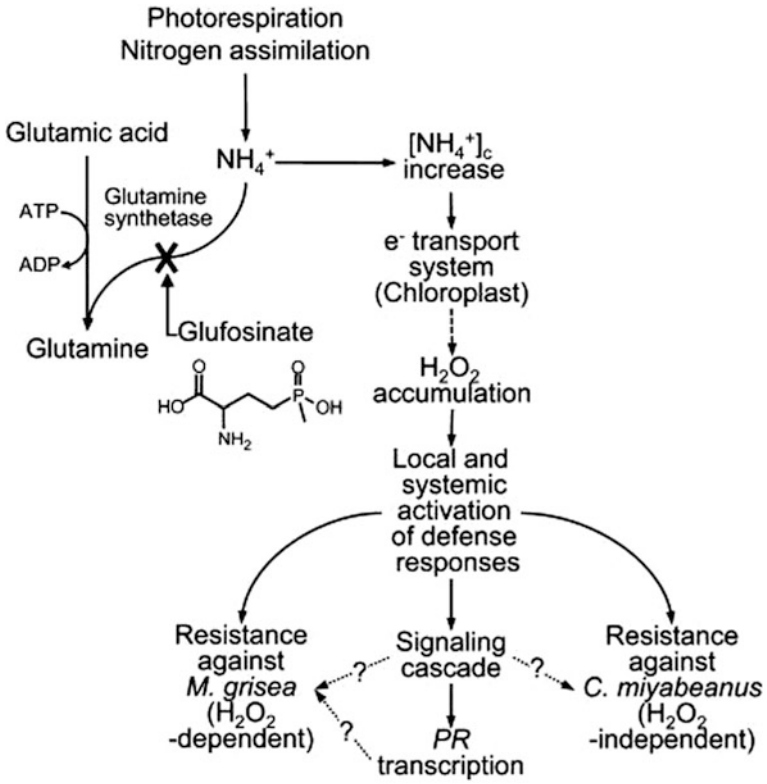


Fig. 50 Proposed model for glufosinate-induced disease resistance. Glufosinate initiates accumulation of free radicals by irreversible binding with and inactivation of Gln synthetase. Toxic ammonia derived from photorespiration or nitrogen assimilation is increased within the cell and disturbed electron transport system within chloroplast. Free radicals were produced and in turn, this molecule triggers disease resistance against *Magnaporthe oryzae* and *Cochliobolus miyabeanus* (Ahn 2008)

Pre-infectional and post-infectional treatments with the herbicide led to significant reduction of sporulation rates. The herbicide exhibited more inhibitory effect on spore production when the time intervals between inoculation and herbicidal treatment decreased, since the pathogen could not access to the opportunity required for the establishment of dense network of hyphae within the infected tissue and sporulation. However, pre-inoculation herbicidal treatment increased prophylactic impact when time intervals between treatment and inoculation increased, suggesting the activation of plant defense pathways. Alternatively and additionally, the herbicidal treatment might lead to the aggravated niche conditions due to the reduced level of amino acids, reduced nitrogen availability, an altered pH and/or an accumulation of ammonium. Especially, changes in the pH, nitrogen availability, and ammonium concentrations have been suggested as a regulatory factor for colonization of pathogenic fungi (Kortekamp 2011).

The use label for lactofen in the USA implies to its usability in soybean white mold management. Grinstein et al. (1976) reported the increased resistance to *Rhizoctonia solani*, *Fusarium*, and *Verticillium dahliae* as the result of the treatment with dinitroaniline herbicide such as fluchloralin (a dinitroaniline from HRAC group K1; Basalin®). Trifluralin decreased fusarium wilt on tomato and melon (Cohen et al. 1986), while had no effect on rhizoctonia root and crown rot of sugar beet (Ruppel et al. 1982). Trifluralin potentiates cotton and tomato to produce fungitoxic compounds treating with vascular wilt-causing fungi (Grinstein et al. 1984). Cohen et al. (1992b) found that the growth of tomato seedlings (cv. Bonny Best) in rooting substrate amended with the dinitroaniline herbicide, dinitramine (1 ppm in sand/Pro-mix, 1:1 or 1:2, V V^{-1}) caused a suppression in bacterial wilt symptoms induced by *Ralstonia solanacearum* strain K60. Dinitramine was not inhibitory to *R. solanacearum* in in vitro assays nor did it restrict the pathogen multiplication in the treated plants. Dinitroamine reduced the severity of bacterial canker caused by *Clavibacter michiganense* subsp. *michiganense* JD83-1, but had no significant impact on the severity of bacterial speck, induced by *Pseudomonas syringae* pathovar *tomato* DC894H. Resistance was not induced by similar exposure of plants to the herbicides ethalfluralin or oryzalin (Cohen et al. 1992b).

Resistance to fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici*, was induced by the exposure of seedlings to the herbicides dinitramine, ethalfluralin, and oryzalin (Cohen et al. 1992b). Exposure of plants to the dinitroaniline herbicide, trifluralin, did not induce resistance towards either bacterial wilt or fusarium wilt (Cohen et al. 1992b). A related herbicide, pendimethalin, induces the synthesis of the phytoalexin tomatine in tomato (El-Shanshoury et al. 1995). Starratt and Lazarovits (1996) demonstrated the highly induced levels of tomato seedlings to *Fusarium oxysporum* f. sp. *lycopersici* as the result of dinitroaniline herbicide application. Lotan-Pompan et al. (2007) used suppression subtractive hybridization (SSH) and cDNA-amplified fragment-length polymorphism (cDNA-AFLP) techniques in order to identify the genes involved in trifluralin herbicide-induced resistance of melon to *Fusarium oxysporum* f. sp. *melonis*. From a total of 123 clones they isolated, sequenced and annotated, 60 clones had never been isolated from melon. A significant proportion (35%) of the total 123 clones was similar to genes previously described in relation to stress, or defense. They analyzed the expression of thirty-two selected clones in detail, and found that one-third of which were up-regulated in response to trifluralin treatment and/or inoculation with the fungal pathogen. They discussed over the putative roles of seven of the clones in stress. Further studies indicated enhanced expression of four stress-related and up-regulated genes in the plants under salinity stress, suggesting that trifluralin induces a general stress response which protects the plant against fusarium wilt. Herbicides with different mechanisms of action can also stimulate production of phytoalexins and thereby influence plant disease resistance. For example, pretilachlor and butachlor (chloroacetamide members of HRAC group K3) trigger the accumulation of the phytoalexins momilactone A and sakurantetin in rice leaves (Tamogami et al. 1995). Acetochlor (a chloroacetamide in HRAC group K3) induced tomato plant resistance towards fusarium wilt but did not

towards bacterial wilt of tomato caused by *Ralstonia solanacearum* strain K60 (Cohen et al. 1992b).

In field trials, Jacobsen and Hopen (1981) demonstrated that dinoseb (a dinitrophenol in HRAC group M) and trifluralin reduced aphanomyces root rot on peas caused by *A. euteiches*. They found that both dinoseb and trifluralin were highly effective against aphanomyces root rot of peas at economical field rates, with dinoseb providing consistently superior control. Trifluralin offered better control of annual grass weeds. They recommended the consideration of the interaction of weed control, phytotoxicity, and disease control in the selection of herbicides. They suggested dinoseb as an excellent choice in the case of obtainment of excellent stands with minimal weed pressures and the occurrence of root rot as a limiting factor. Where weed pressure was as great as or greater than disease control, they recommended a combination of trifluralin + dinoseb, or trifluralin alone. They wrote that the effect of these herbicides on *A. euteiches* might be beneficial in controlling fungal pathogens with similar life cycles. Diallate (a thiocarbamate from HRAC group N) a herbicide of decreasing effect on root rot diseases of cereals caused by various *Fusarium* species reduces the abundance of the lipids, globules, and spherical bodies in the cells of the treated plants, and leads to about 25% greater β-glucosidase activity in diallate-treated cereal crops compared with controls (Paul and Schönbeck 1976).

Increased Disease Susceptibility of Herbicide-Treated Crops

Resistant as well as susceptible maize hybrids became more sensitive to maize dwarf mosaic virus (MDMV) with the increasing amounts of atrazine (a triazine in HRAC group C1) so that the application of 20 ppm atrazine led to 100% infection. Inoculation of maize plants with the virus and subsequent treatment with 1 ppm atrazine inhibited maize growth (Cole et al. 1968, 1969a, b). Some herbicides decrease crop resistance to plant diseases. For instance, the spring wheat plants malformed as swollen and bulbous because of their exposition to the herbicide mecoprop (a member of HRAC group O) were very amenable for easier penetration of the take-all pathogen fungus *Gaeumannomyces graminis* (Nilsson 1973). Hsia and Christensen (1951) recorded heavier infection with *Bipolaris sorokiniana* in the wheat plants that had been weakened, stunted, and predisposed to fungal infection due to the treatment with 2,4-D (a member of HRAC group O). The herbicide 2,4-D stimulates the accumulation of protein in corn, that has been proposed as a probable reason for the favored growth of the southern corn leaf blight pathogen *Bipolaris maydis* and resulting increased disease in corns treated with 20–200 ppm 2,4-D. Accordingly, the plants treated with only 10 ppm 2,4-D as well as untreated plants were of fewer blight lesions (Oka and Pimentel 1976). Similarly, picloram (a chloropropionic acid in HRAC group O) applied to soil increased wheat and corn seedlings root rot caused by several soil-born pathogenic fungi (Semeniuk and Tunac 1968) that was positively correlated with the significant increases in the exudation of total carbohydrates and reducing sugars that might account for the

increased root damage from soil-born root pathogens in soil treated with picloram (Lai and Semeniuk 1970).

The application of great rates of the dinitroaniline herbicides (HRAC group K1) trifluralin and nitralin under both growth chamber as well as field conditions led to increased damage to cotton crop (Fig. 51). Damage increase was observed under both field conditions of the large rates of *R. solani* and large rates of nitralin, as well as of the large rates of trifluralin and small rates of the fungus (Chandler and Santelman 1968). Neubauer and Avizohar-Hershenson (1973) found that trifluralin increased susceptibility of cotton to *R. solani* and increased the saprophytic activity of the fungus in soil.

Trifluralin (a member of HRAC group K1) increased phytophthora root rot (Duncan and Paxton 1981) and fusarium foot rot (Carson et al. 1991) on soybean as well as on rhizoctonia root rot on sugar beet (Ruppel and Hecker 1982). Cowpea seedlings grown in alachlor-treated soil were more susceptible to *R. solani* than those treated with fluchloralin (a member of HRAC group K1) and the untreated

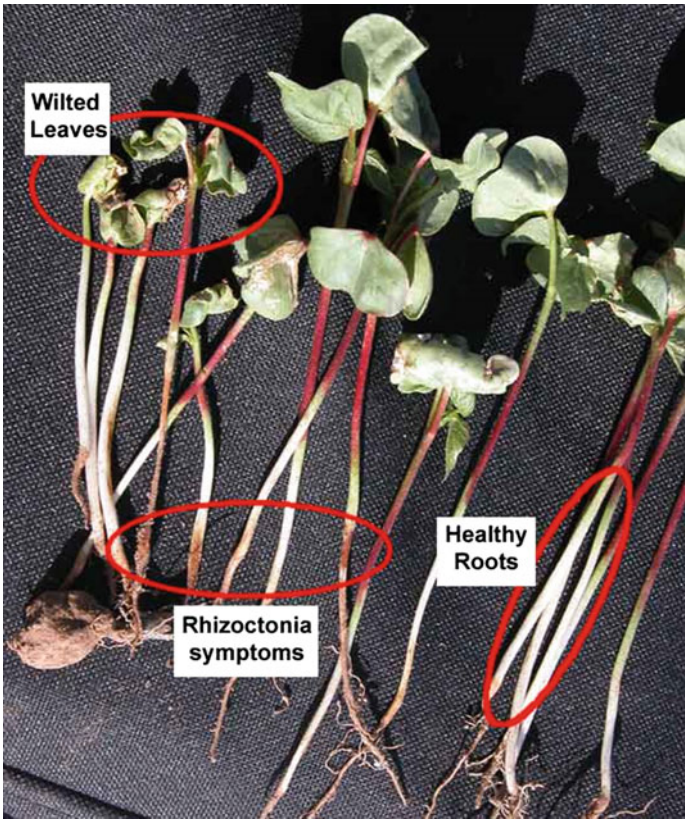


Fig. 51 Symptoms of cotton seedling infection by *Rhizoctonia solani*, root rot and wilting of leaves as the result of insufficient water uptake (*Source* http://cals.arizona.edu/)

seedlings. Pre-sowing application of alachlor (a member of HRAC group K3) in soil (5 μL a. i. kg^{-1}) aggravated damping-off whereas fluchloralin decreased the disease incidence to nearly half of that in untreated soil in greenhouse pot tests (average temperature of 31 ± 5 °C). Both herbicides reduced damping-off in pots kept at constant temperature of 30 °C and increased the disease incidence at 20 °C. Impact of fluchloralin and alachlor on *R. solani* damping-off of cowpea appears to be due to the predisposing effect by the herbicides on the susceptibility of the host and is influenced by atmospheric temperature (Kataria and Dodan 1983).

Herbicide-treated sugar beet hypocotyls leaked or exuded more substance into the soil than beet hypocotyls from beets grown in non-treated soil (Wheeler 1975). Further research indicated that greater quantities of glucose and certain mineral ions leaked out in all instances when plants were grown in soil treated with the herbicides such as cycloate (a thiocarbamate from HRAC group N) suggesting one reason for the increased disease that might be the increased availability of nutrients for the pathogens such as *Rhizoctonia solani* (Altman 1972; Altman and Campbell 1977b; Altman and Ross 1967). The herbicides EPTC (a thiocarbamate in HRAC group N) and linuron (an urea in HRAC group C2) applied to soil in the field at three different concentrations of the recommended dose reduced post-emergence, but not pre-emergence damping-off of cotton caused by *R. solani*. Both herbicides at two high concentrations considerably decreased wilt induced by *Fusarium oxysporum* f. sp. *vasinfectum*. Both herbicides reduced *F. oxysporum* f. sp. *vasinfectum* germination of chlamydospores under natural soil conditions but not in streamed soil. The 2-fold concentration of EPTC and linuron decreased *R. solani* saprophytic activity in soil. The impacts of both herbicides on both diseases were attributed to their suppressor effects on the saprophytic activity of *R. solani*, and the germinability of *Fusarium* chlamydospores in soil, respectively (El-Khadem and Papavizas 1984). The enhanced disease severity or incidence may be because of the herbicidal injury to the root system of the host plant that leads to the leakage and exudation of the nutrients put available for the soil-born pathogens. Such a phenomenon has been proved with the herbicide cycloate (a thiocabamate in HRAC group N; application rate less than 32 μg g^{-1} soil) and sugar beet damping-off pathogen, *R. solani*, where the herbicide enhances disease despite of its interference with the growth of the pathogen (Altman and Campbell 1977b).

Sharon et al. (1992) indicated glyphosate (a glycine from HRAC group G) suppression of an elicited response that led to the increased susceptibility of *Cassia obtusifolia* to a mycoherbicide. The synergistic activity of glyphosate weed control in predisposing plants to infectious organisms has been observed for many diseases, and the extensive use of glyphosate in agriculture is a significant factor in the increased severity or "re-emergence" of diseases once considered efficiently managed. Glyphosate has made crops susceptible to normally non-pathogenic isolates of *Fusarium*, and the population of *Fusarium* increases in soil after glyphosate application (Levesque et al. 1987; Kremer et al. 2000). Glyphosate predisposes tomato to fusarium crown and root rot by inhibiting the plant's structural and defense barriers (Bramhall and Higgins 1988). Cotton growers in Australia and the Western United States have seen a resurgence of fusarium wilt since the

introduction of Roundup Ready® cotton, and previously high levels of wilt resistance appear to be less effective under glyphosate management programs (Harper 2007). Glyphosate also breaks resistance to cyst nematodes in soybeans (Geisler et al. 2002). The increased fusarium yellows and *Rhizoctonia solani* diseases of Roundup Ready® sugar beets prompted Larson et al. (2006) to comment that "precautions need to be taken when certain soilborne diseases are present if weed management for sugar beet is to include post-emergence glyphosate treatments." These authors also reported that the sugar beet variety resistant to *Rhizoctonia* was as susceptible to this pathogen as the susceptible variety after glyphosate application regardless of the time of inoculation. Fusarium head scab of cereals and other diseases caused by *Fusarium* spp. increase following glyphosate applications (Fernandez et al. 2005; Larson et al. 2006), and previously established "cardinal" conditions (precipitation, flowering, and temperatures above 26°C) for head scab are modified when glyphosate is applied prior to a susceptible cereal crop (Fernandez et al. 2005, 2007). Glyphosate modifies plant nitrogen metabolism similar to high temperature-induced changes that provide susceptibility to head scab (Huber, unpublished) so that head scab and the mycotoxins produced by the causal fungi are now prevalent in cooler areas where they were rarely observed before the extensive use of glyphosate (Fernandez et al. 2005, 2007). Similar changes in nitrogen and carbohydrate metabolism provide transient resistance of wheat and soybeans to rust after glyphosate application (Anderson and Kolmer 2005; Feng et al. 2005, 2007). Plants rely on multiple components of defense to deter pathogens following infection (Hammond-Kosack and Jones 2000). Many of these active resistance components are derived from the phenylpropanoid pathway, which acquires almost all its precursors (notably phenylalanine and chorismate) from the shikimic acid pathway (Hammond-Kosack and Jones 2000; Dixon et al. 2002). A key inducible defense component associated with the shikimic acid pathway is the production of antimicrobial phytoalexins that accumulate rapidly at the site of infection. Lignification of cell walls at and around the infection site is another shikimate-derived component that functions to fortify cells and ensure isolation of the pathogen at the infection site. The production of salicylic acid (SA) following infection represents another component of inducible defense. SA is synthesized either directly from chorismic acid or indirectly through phenylalanine. Although SA is not antimicrobial per se, it functions to signal and coordinate various defenses following challenge by a pathogen; however, its direct role in plant–pathogen interactions involving root tissue remains unclear. Another defense component that relies on three final products of the shikimic acid pathway- tryptophan, tyrosine and phenylalanine- is the production of a diverse variety of pathogenesis-related (PR) proteins that function to curtail the advance of a pathogen. Many kinds of PR proteins have been identified (Hammond-Kosack and Jones 2000). Given the reliance of many plant defenses on the shikimic acid pathway, and the fact that glyphosate blocks this pathway, it is not surprising that this herbicide would render plants more susceptible to pathogens. Keen et al. (1982) were the first to show that by inhibiting the phytoalexin glyceollin, glyphosate was able to compromise resistance of soybeans to *Phytophthora sojae* (syn. *Phytophthora*

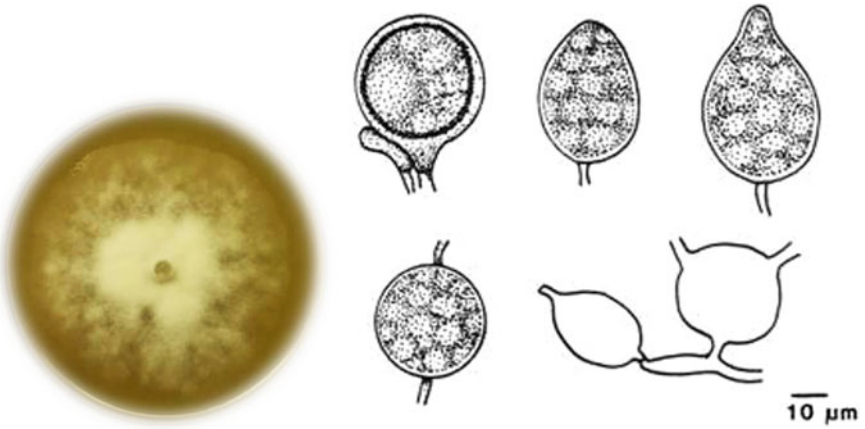


Fig. 52 The homothallic oomycetous fungus-like pathogen, *Phytophthora sajae* (Left) mycelial growth on V8 juice agar plate (*Source* Jean B Ristaino); (Right) Morphology of the oomycetous *P. sojae* (Upper row) globose and possibly subglobose oogonium with a mostly paragynous and possibly amphigenous antheridium; ovoid and obpyriform, noncaducous, nonpopillate sporangia; (Lower row) chlamydospores; empty remnants of intercalary spherical and irregular hyphal swellings formed in aqueous culture (*Source* Vaziri A, reproduced from Erwin and Ribeiro 1996)

megasperma f. sp. *glycinea*; Fig. 52). However, Ward (1984) considered the suppressive effect of glyphosate on the anti-oomycotic activity of metalaxyl as an evidence that host defence mechanisms contribute to metalaxyl inhibition of *P. sojae* to soybeans. Using the bean-anthracnose pathosystem, Johal and Rahe (1988, 1990) demonstrated that, while glyphosate did not interfere with the hypersensitive reaction (HR) of incompatible interactions, it suppressed significantly the production of all four of the bean phytoalexins. As a result, the pathogen was able to kill the plant if it escaped the localized HR, a situation that occurred only with glyphosate-treated plants.

The effect of glyphosate on the compatible bean anthracnose interaction was even more dramatic (Johal and Rahe 1990). Glyphosate almost completely suppressed the production of phytoalexins associated with susceptible lesion containment and permitted the pathogen to invade unimpededly until the entire hypocotyl collapsed. As little as 2% of the recommended herbicidal rate of glyphosate was enough to transform normally delimited lesions typical of anthracnose into constantly expanding lesions (Johal and Rahe 1990). The defense studies mentioned above were confined largely to diseases of aerial parts of host plants. There are indications that defense components may vary significantly in root tissue that are in intimate and continuous contact with potential pathogens (Hammond-Kosack and Jones 2000). For instance, roots do not rely on HR-mediated defense to contend with pathogens, although the exact defense components that keep roots pathogen-free are only partially understood. To gain an insight into what contributes to glyphosate-induced susceptibility of French beans (*Phaseolus vulgaris*) to *Pythium*, Liu et al. (1995, 1997) assessed phytoalexins as well as lignification of

root tissue in response to glyphosate treatment. By comparing phytoalexins in roots of bean seedlings grown in different media, they concluded that phytoalexins were induced by soil microorganisms. Interestingly, while phytoalexin accumulation was affected only modestly by glyphosate in response to *Pythium*, lignification (a process requiring Mn) was suppressed significantly. Thus, enhanced colonization by *Pythium* in roots of bean seedlings treated with foliar applied glyphosate occurs as a result of glyphosate interference with lignin-based defense mechanisms (Liu et al. 1997). However, these results also suggest that sustained production of phytoalexins in response to *Pythium* infection is maintained temporarily following glyphosate treatment, whereas lignification is not. The herbicide glyphosate, N-(phosphonomethyl) glycine, is a strong systemic metal chelator and was initially patented for that purpose (Bromilow et al. 1993). Its herbicidal action is by chelating with Mn, a cofactor for the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase enzyme in the shikimate pathway, to inhibit this metabolic pathway of plants and many microorganisms (Cerdeira and Duke 2006; Grossbard and Atkinson 1985; Jaworski 1972). Many cations chelate with glyphosate, thus reducing its herbicidal efficacy (Bernards et al. 2005; Hickman et al. 2002). Plants with a compromised shikimate metabolism are predisposed to variousplant pathogens (Johal and Rahe 1988; Rahe et al. 1990), and glyphosate is patented as a synergist for mycoherbicides to enhance the virulence and pathogenicity of organisms used for biological weed control (Boyette et al. 2006; Duke and Cerdeira 2005). Glyphosate is known to predispose many plants to pathogens due to its inhibition of the shikimic acid pathway (Holliday and Keen 1982), through which phytoalexins, the compounds produced by plants to defend against pathogens are produced. Mn plays an important role in plants' disease defense mechanisms (Thompson and Huber 2007). It has been proposed that glyphosate interferes with absorption and utilization of Mn, thus increasing a plant susceptibility to disease. However, the majority of research has not found reductions in Mn concentrations within plants following glyphosate applications (Bott et al. 2008; Rosolem et al. 2009; Nelson 2009). Glyphosate had no effect on charcoal rot on soybean but when plants were stressed by alachlor, chloramben or 2,4-DB and soil temperatures were greater than 26 °C, the disease increased (Canaday et al. 1986).

Effect of Herbicides Through Their Impact on Non-pathogenic Microorganisms

The effect of herbicides on soil beneficial microorganisms is of significant importance in maintainment of soil fertility, and suppressiveness against plant pathogens. Additionally, compatibility of herbicides with biological control agents is very relevant in integrated management of plant diseases and pests, as well as in development of new formulations that take advantage of compatible combination of biological as well as chemical agents. Microbial organic matter transformation is considered as a proven source of nutrient elements in nutritionally poor soils (Kang et al. 2012), and microorganisms operate as a sign of soil quality because of the key

roles they play in various soil functions (Schloter et al. 2003). Therefore, there has been long standing interest in the effects of herbicides on soil microflora (Greaves and Malkomes 1980; Simon-Sylvestre and Fournier 1979). Herbicides can affect microbial activity, soil microbiology, soil biochemistry, soil fertility, and plant disease incidence and severity. There are numerous records on the negative impacts of herbicides on soil microbiology and fertility: bromoxynil (Abbas et al. 2014; El-Ghamry et al. 2000; Pampulha and Oliveira 2006). The effect of an herbicide varies with the type and the concentration of herbicide as well as the type of soil, the experimental conditions (temperature, moisture, and the time of incubation), the method and time of herbicide application concentration, the type of herbicide formulation, the type and the rate of other materials applied in herbicide formulation. Additionally, different groups of microorganisms, and even different strains of a given species show different levels of sensitivities to an herbicide. Carfentazone-ethyl had a stimulating effect on total oligotrophic as well as organotrophic bacteria, but it inhibited the growth of *Azotobacter*, fungi, spore-forming oligotrophic bacteria and actinobacteria (Tomkiel et al. 2015). With sulfonylurea herbicides, most of the investigations carried out on the active ingredients tribenuron, primi-, tria-, and prosulfuron, metsulfuron, sulfometuron and thifensulfuron, chlorimeturon, chlorsulfuron and rimsulfuron, and nicosulfuron have indicated that the recommended field rate of sulfonylurea herbicides were generally of no significant impact on soil microbial number and activity (Allievi and Gigliotti 2001). Among the bacteria including *Bacillus*, *Pseudomonas* and *Arthrobacter*, *Bacillus* and *Arthrobacter* were primarily inhibited in the soil due to the herbicide Galex® application (Fawole 2000). *Acinetobacter calcoaceticus*, *Actinomyces bovis*, *Actinomyces viscous*, *Nocardia farcinica*, *Bacillus subtilis*, *B. licheniformis*, *B. megaterium*, *Flavobacterium aquantile*, *Pseudomonas putida*, *P. stutzeri*, *Serratia marcescens*, and *Alcaligenes eutrophs* were able to grow heavily in the soil polluted with atrazine and Primextra® (a combined formulation of atrazine and metolachlor). While the studied isolates of all the above-mentioned bacterial species were able to grow on the herbicides as the sole source of carbon, *B. subtilis* exhibited the highest growth (Sebiomo et al. 2011a). Similarly *Bacillus* spp. degrade 80–95.6% of the herbicide oxyfluorfen within 21 days, while *Pseudomonas* sp. (82.2%), *Arthrobacter* spp. (82.2%), *Aspergillus* sp. (77.8%), *Mycobacterium* sp. (75.6%), *Micrococcus* sp. (73.3%), and *Streptomyces* sp. (68.9%) were of relatively lower capabilities (Mohamed et al. 2011). The growth of *Bacillus* sp. was inhibited by diquat (25 μg mL^{-1}), paraquat (5 μg mL^{-1}), ioxynil (10 μg mL^{-1}), and pentachlorophenol (PCP, 5 μg mL^{-1}), but was not affected by the tested concentrations (25, and 50 μg mL^{-1}) of the herbicides atrazine, diuron, trifluralin, and fluometuron (Breazeale and Camper 1972). The application of the herbicides EPTC, and linuron in field soil at three doses i.e. 1/2 field recommended dose (FR), 1 FR, and 2 FR resulted in the significant reduction of the vascular wilt caused by *Fusarium oxysporum* f. sp. *vasinfectum* and of the germination rate of chlamydospores of *Fusarium* spp. in natural soil but not in the steamed soil, implying to the role of soil microflora in the disease control via

the herbicidal treatments performed with two higher doses (El-Khadem and Papavizas 1984).

Glyphosate, N-(phosphonomethyl) glycine, is the most extensively used herbicide in the history of agriculture. Glyphosate is similar to most herbicides in that when it enters the soil it differentially affects soil microorganisms (Kremer et al. 2005). Ratcliff et al. (2006) applied the herbicide glyphosate at the recommended field rate to a clay loam as well as a sandy loam forest soil that resulted in few changes in microbial community structure. Total and culturable bacteria, fungal hyphal length, bacterial: fungal biomass, carbon utilization profiles (BIOLOG), and bacterial and fungal phospholipid fatty acids (PLFA) were unaffected 1, 3, 7, or 30 days after application of a commercial formulation (Roundup®). In contrast, a high concentration of glyphosate (field rate) simulating an undiluted chemical spill substantially altered the bacterial community in both soils. Increases in total bacteria, culturable bacteria, and bacterial: fungal biomass were rapid following application. Culturable bacteria increased from about 1% of the total population in untreated soil to as much as 25% at the high concentration by day 7, indicating enrichment of generalist bacteria. Community composition in both soils shifted from fungal dominance to an equal ratio of bacteria to fungi. Functional diversity of culturable bacteria, estimated by C substrate utilization, also increased at the high glyphosate concentration, particularly in the clay loam soil. Unlike the other bacterial indices, only minor changes in bacterial PLFA resulted after the third day following the field rate application. Apparently the herbicide resulted in an across-the-board stimulation of bacteria that was not reflected by the finer-scale PLFA community structure. Changes in fungal properties (hyphae, propagules, PLFA biomarkers) were few and transient. They concluded that the commercial formulation of glyphosate had a benign affect on community structure when applied at the recommended field rate, and produces a non-specific, short-term stimulation of bacteria at a high concentration.

Using next-generation sequencing technology, it was known that glyphosate (as Roundup®) application in a soil with a high clay content (41%; favorable for sorption of the herbicide because of high surface area) and pH value of 6.9 (less favorable for sorption of glyphosate) led to no considerable change in soil diversity of bacterial populations. However, glyphosate application resulted in an increase in relative abundance of proteobacteria (in particular, the gammaproteobacterial members of Xanthomonadalean families, Xanthomonadaceae, and Sinobacteriaceae) in soybean and especially in corn cropping systems, whilst it reduced the relative abundance of acidobacteria. Further studies are required in order to get precise information on the effect of glyphosate and its metabolites, most notably, aminomethylphosphonic acid (AMPA) on soil bacteria at finer taxonomic levels. It is believed that acidobacteria are highly involved in the biogeochemical processes such as cellulose biodegradation. Decreases in the frequency of acidobacteria over long-term can lead to the impaired ability of soil to perform certain biogeochemical reactions carried out by acidobacteria, and can influence rhizosphere nutrient status. Such adverse effects in the glyphosate-tolerant (GT) corn cropping systems may be more exacerbated than in a corn-soybean rotation

(Newman et al. 2016). Further studies have shown species-dependent as well strain-dependent growth responses of *Pseudomonas* species grown on succinate (a common root exudate) and treated with different concentrations (up to 5 mM) of glyphosate. While the growth rate of both *P. protegens* Pf-5 and P. fluorescens RA12 remained unchanged, both of the tested *P. putida* strains (KT2440, and S12) exhibited growth inhibition from 0 to 100%. Monitoring disruptions in metabolic homeostasis and fluxes via application of a 13C-assisted metabolomics approach, and profiling of the whole-cell metabolome captured deviations in metabolite levels involved in the tricarboxylic acid cycle, ribonucleotide biosynthesis, and protein biosynthesis. Altered metabolite levels specifically in the biosynthetic pathwat of aromatic amino acids, the target of toxicity for glyphosate in plants, implied the same toxicity target in the soil bacterium. Kinetic flux experiments with 13C-labeled succinate revealed that biosynthetic fluxes of the aromatic amino acids were not inhibited in *P. fluorescens* Pf-5 in the presence of low and high glyphosate doses but these fluxes were inhibited by up to 60% in *P. putida* KT2440, even at sublethal glyphosate exposure. Notably, the greatest inhibition was found for the aromatic amino acid tryptophan, an important precursor to secondary metabolites. When the growth medium was supplemented with aromatic amino acids, *P. putida* S12 exposed to a lethal dose of glyphosate completely recovered in terms of both growth rate and selected metabolite levels. Collectively, the glyphosate-induced specific disruption of de novo biosynthesis of aromatic amino acids accompanied with widespread metabolic disruptions was responsible for dose-dependent adverse effects of glyphosate on sensitive soil *Pseudomonas* species (Aristilde et al. 2017).

Weed management programs in glyphosate-resiatant (GR) field crops have provided highly effective weed control, simplified management decisions, and given cleaner harvested products. However, this relatively simple, broad-spectrum, systemic herbicide can have extensive unintended effects on nutrient efficiency and disease severity, thereby threatening its agricultural sustainability. A significant increase in disease severity associated with the wide spread application of the glyphosate herbicide can be the result of direct glyphosate-induced weakening of plant defenses and increased pathogen population and virulence. Glyphosate applications to glyphosate-resistant (GR) crops alter the type and quantity of compounds released from crops roots into the rhizosphere, including the exudation of glyphosate. These changes in exudates can have a dramatic impact on the microbes found in the root zone. Manganese is absorbed by plants in the reduced state (Mn^{2+}). High soil pH limits Mn availability due to oxidation to the Mn^{4+} state under alkaline conditions. While the specific physiological mechanisms are poorly understood, many plants are able to absorb Mn from soils with limited Mn availability. This is accomplished either via associations between the plant and Mn-reducing bacteria, or alteration of the pH of the rhizosphere via root exudates (Rengel and Marschner 2005). Indirect effects of glyphosate on disease predisposition result from immobilization of specific micro-nutrients involved in disease resistance, reduced growth and vigor of the plant from accumulation of glyphosate in meristematic root, shoot, and reproductive tissues, altered physiological efficiency, or modification of the soil microflora affecting the availability of nutrients

involved in physiological disease resistance. Furthermore, even low levels of residual glyphosate in soil can decrease root uptake and translocation of iron (Fe), manganese (Mn), and copper (Cu) (Eker et al. 2006). Thompson and Huber (2007) reported that glyphosate application to GR soybean altered the balance of Mn reducing and oxidizing bacteria associated with soybean roots in a manner that suggested that Mn would be immobilized in the soil. However, published data documenting reduced soil availability of Mn due to the activity of glyphosate on soil microorganisms is lacking. Furthermore, there have been no documented reports of Mn crop deficiency symptoms in Iowa.

Manganese (Mn) deficiency symptoms occur in some regions of the cornbelt, and these areas are where interactions between glyphosate and Mn nutrition have been reported. Microorganisms proposed for biological control of this disease such as *Bacillus cereus* and *Trichoderma konigii* are all strong Mn reducers that increase Mn availability in the rhizosphere (Huber and McCay-Buis 1993; McCay-Buis 1998; Rengel et al. 1996). In contrast, the addition of Mn-oxidizing organisms increases take-all (Crowley and Rengel 1999; McCay-Buis 1998; Rengel 1999; Thompson et al. 1998; Fig. 53).

The virulence mechanism of some pathogens such as *Gaeumannomyces*, *Magnaporthe*, *Phymatotrichum*, *Corynespora*, and *Streptomyces* involves Mn oxidation at the infection site to compromise plant resistance mechanisms involving the shikimate pathway (Thompson and Huber 2007). Isolates of these pathogens that cannot oxidize physiologically available Mn^{2+} to the non-available Mn^{4+} are

Fig. 53 More severe take-all root rot of wheat grown following Roundup Ready® soybeans sprayed with glyphosate (left) than following Roundup Ready® soybeans grown with a non-glyphosate herbicide (right) (*Source* Johal and Huber 2009)

avirulent and disable to cause significant tissue damage (Roseman et al. 1991). Production of the Mn oxidizing enzyme(s) occurs soon after spore germination and during epiphytic growth (Cheng 2005; Schulze et al. 1995; Thompson et al. 2005). The damage from corynespora root rot, previously considered minor, may become economically damaging in Roundup Ready® soybeans since application of glyphosate to Roundup Ready® soybeans greatly increases severity of the disease. This fungal root rot is more severe when glyphosate is applied to soybeans under weedy conditions even though the weeds may not be hosts for *Corynespora cassiicola*. The weeds serve to translocate and release more glyphosate into the rhizosphere environment to reduce the population of Mn-reducing organisms and increase Mn-oxidizing organisms. This change in soil biology limits manganese availability for plant uptake and active defense reactions, and acts synergistically with *Corynespora* to increase disease (Huber et al. 2005). Similarly, various diseases caused by *Fusarium* spp. are increased by glyphosate (Fernandez et al. 2005; Sanogo et al. 2000, 2001). Here, the impact of herbicides on the microbial population of importance in agricultural soil fertility and plant pathology is reviewed.

Effect of Herbicides on Nitrogen Cycle

Herbicides affect nitrogen cycle through their impacts on different groups of microorganisms involved in nitrogen transformation in nature:

Effect of Herbicides on Symbiotic and Nitrogen-Fixing Rhizobium Species

Effect of herbicides on rhizobial nitrogen fixation depends on the variety of legume crop, soil type, environmental conditions, the strain of a rhizobial species, the herbicide, its dose and its application method and time. The effect of herbicides on rhizobial nitrogen fixation can be considered from two viewpoints, their effects on the process of legume host rhizobial nodulation, and on the process of nitrogen-fixation in the developed nodules (Singh 2005; Fig. 54). Herbicides can exert their adverse effects on legume plants and thereby prevent rhizobial nodulation of their roots. For example, terbutryn/terbutylazine, trietazine/simazine and prometryn negatively affect rhizobial nodulation of legume plants due to their adverse effects on plants, and not on the rhizobial bacterium *Rhizobium leguminosarum*, as these herbicides were of negative impact on the growth of *R. leguminosarum* only when they were tested at the concentration unexpected to occur under normal field conditions, and bentazone was safe to rhizobia even under these conditions (Singh and Wright 2002). Here, the effect of herbicide on rhizobial bacteria is the subject of our focus. Out from three rhizobial species, *Rhizobium* sp. IC 3342 (a pigeonpea nodulator), *R. leguminosarum* 2001 (nodulating lentil), and *R. meliloti* 4013 (nodulating alfalfa), the former was found sensitive to all the herbicides tested, butachlor, simazine, and oxyfluorfen. The herbicides were of more adverse effects compared to tested insecticides and fungicides. Also, the plants ifested with herbicide treated *Rhizobium* were of less dry weight and less total nitrogen content because of the decreased growth and nitrogen fixing capacity

Fig. 54 Nodules induced by rhizobial bacteria in legume roots (*Source* Jeremy Kemp, San José State University, Wikimedia.org)

of the rhizobial bacteria (Madhavi et al. 1994). Different strains of *R. leguminosarum*, *R. meliloti*, and *R. loti* were able to reproduce when they were exposed to the equivalent to bentazon (a benzothiadiazinone from HRAC group C3; 1–3 kg ha^{-1}) and glyphosate (1–3 kg ha^{-1}), and MCPA (2-methyl-4-chlorophenoxyacetic acid, 0.5–3.5 kg ha^{-1}) and bacterial exposure to the herbicides exerted no imact on the nodulating capacity of the bacteria except for glyphosate (Mårtensson 1992). Bacterial growth in aseptic culture was not influenced by the amendment of 0.55 and 5.50 μM chlorsulfuron (Mårtensson and Nilsson 1989) and it was concluded that the inhibition of nodulation and nitrogenase activity of alfalfa as well as red clover nodules probably occurred as the result of the adverse impacts of the herbicides on the plant growth and development rather than on the rhizobia. The significantly inhibiting effect of glyphosate (10 mg a. i. L^{-1}), paraquat (2 mg a. i. L^{-1}), and chlorsulfuron (2 mg a. i. L^{-1}) on the growth of *R. trifolii* in nutrient broth is recorded (Eberbach and Douglas 1989).

The application or residues of acetolactate synthase inhibitor (ALS) herbicides can reduce the growth and nitrogen-fixing ability of legumes (Anderson 2001), and further studies indicate that common ALS herbicides such as Spinnaker® (imazethapyr as ammonium), Broadstrike™ (flumetsulfam), and Glean® (chlorsulfuron) can reduce the amount of biologically fixed nitrogen in chickpeas by up to 70%, leading to evidently slowed growth and reduced grain yields in alkaline soils. Reduced productivity has also been observed for pea, medic, subclover, and Lucerne. Apparently, the herbicides can interfere with rhizobial nodule formation process through their influence on *Rhizobium* bacteria, rather than the growth of the bacteria, or legume plant (Anderson 2001). Imazatapyr was rather non-toxic to *Rhizobium* growth and higher doses more than 0.34 mM were required to cause only slight effects on the bacterium growth in a defined medium. Additionally, imazethapyr did not affected the nodulation ability of *Rhizobium* on pea roots. Therefore, it was concluded that the herbicide would not inhibit rhizobial growth under normal field conditions (Gonzalez et al. 1996). It was proposed that the high acetolactate synthase (ALS) activity expressed by *Rhizobium*, both as free-living

bacteria and as bacterioids, was related to the growth tolerance of rhizobia to imazethapyr and it was related to the relative tolerance of symbiotic pea plants (Royuela et al. 1998). Herbicides can affect *Rhizobium*-legume symbiosis in four ways (Kalia and Gosal 2011), of which three ways are related to their effects on *Rhizobium* symbionts: (i) herbicides can negatively affect the growth and survival of the rhizobia, and reduce their nodulation capabilities (Anderson et al. 2004), (ii) herbicides can reduce the efficacy of rhizobia in terms of their ability to nodulate or form an effective symbiosis (Anderson et al. 2004), and (iii) herbicides can reduce the nitrogen-fixing effectiveness of the symbiosis through inhibition or interruption of biochemical pathways in the bacterioids (Drew et al. 2007). Sawicka and Selwet (1998) indicated that seed treatment with imazethapyr (an imidazolinone from HRAC group B) could lead to the reduced nitrogenase activity of root-nodule rhizobia and stimulate the development of resistant bacteria. Also, they reported that the effect depended on the herbicide, its concentration, as well as on the weather conditions. Accordingly, a reduction in nitrogenase activity of the active strain KGL of *R. leguminosarum* biovar *trifolii* was reported in red clovers as the result of Pivot® 100 SL (imazethapyr) application under both pot and field conditions. The herbicide inhibited the proliferation of the microorganisms in the soil under red clover plantations in the first days after treatment, but later stimulated their multiplication (Niewiadomska 2004).

Yueh and Hensley (1993) reported that trifluralin though not significantly effective on nitrogen fixation of soybean and limabean, adversely influenced nodulation. Trifluralin was not toxic to *Bradyrhizobium* and *Rhizobium* species in a study carried out using disc inhibition method (Durgesha 1994). The inhibition of nodulation as the result of the application of trifluralin (a dinitroaniline from HRAC group K1) and metribuzin (a triazinone from HRAC group C1) has been recorded (Bertholet and Clark 1985). The triazine herbicides of the HRAC group C1 (photosystem II inhibitors), terbutryn, propyzamide, terbutryn + propyzamide, and metribuzin were not of any significant effect on the rhizobial nodulation of pea plants, however, carbetamide significantly increased the number of nodules per plant. Hand weeding with no use of the herbicides also significantly raised rhizobial nodulation rate (Zaid et al. 2014). Thiobencarb (a thiocarbamate from HRAC group N), when applied at the rates of 2 and 4 mg kg^{-1} soil, inhibited *Azospirillum* populations, anaerobic nitrogen fixers, and *Azotobacter* in an alluvial soil (Jena et al. 1990).

Investigation of the in vitro effect of nine glyphosate commercial formulations (Zapp QI®, Roundup®, Roundup Multiação®, Roundup Transorb®, Roundup® WG, Trop®, and Agrisato®) on three *Bradyrhizobium* strains (*B. japonicum* SEMIA 5079, and two strains of *B. elkanii*, SEMIA 5019, and SEMIA 587) revealed the different toxicity of the formulations and different vulnerability of the strains. Zapp Qi® was the least toxic formulation to the strains, while Roundup Transorb® was of the highest toxicity and reduced growth over 94% for all the strains tested. No correlation was found among the type of salt, isopropylamine, ammonium or potassic present in the formulations, and the toxicity degree to the strains. *B. elkanii* strain SMIA 587 was known as the least tolerant to most formulation while the

strain SEMIA 5019 was the most sensitive to the control treatment N-(phosphonomethyl) glycine, without salts or other additives (Santos et al. 2004). Reduction in growth of the strains in the lowest tested glyphosate concentration (5.4 μg L^{-1}) was 18% for SEMIA 5079, 29% for SEMIA 5019, and 35% for SEMIA 587. In general, the higher the concentration of the herbicide in the culture medium, the greater the growth inhibition. The strains depicted differential sensitivity only at the lowest concentration of glyphosate, and all exhibited undifferentially severe growth reduction in the presence of the highest concentration of the herbicide (43.2 μg L^{-1}) (Jacques et al. 2010).

Effect of Herbicides on Free-Living Nitrogen-Fixing Bacteria

There are a number of free-living bacterial species from different genera that are able to fix the atmospheric nitrogen (Lal 2006) out of which, the species from two genera *Azospirillum* and *Azotobacter* have been regarded as potential nitrogen fertilizers (Martín et al. 1993) and tested as plant growth promoting bacteria (Baltensperger et al. 1978; Tejera et al. 2005; Yasari et al. 2008; Mirzae et al. 2010; Naderifar and Daneshian 2012).

Effect of Herbicides on Azotobacter Species

Azotobacter is a genus of usually motile, oval or spherical bacteria that form thick-walled cysts and may produce large quantities of capsular slime. The bacteria in the genus are aerobic, free-living soil microbes that play an important role in atmospheric nitrogen (a form of nitrogen inaccessible to plants) fixation and its release as ammonia into soil (Kizilkaya 2009). The common characteristics of the species in the genus *Azotobacter* are presented in Table 1.

Table 1 Biochemical characteristics of *Azotobacter* species

Biochemical tests	Azotobacter species
Gram-staining	Positive
Motility	Motile
Catalase test	+
Nitrate reduction	+
Pigment production	+
H_2S production	+
Urease test	+
Citrate test	+
Utilization of carbon source	+
Glucose	+
Fructose	+
Maltose	–
Indole test	+

Source Kasa et al. (2017)

The significance of the bacterium contribution to nitrogen fixation was understood knowing that atmospheric nitrogen fixation by the bacterium occurs much more efficiently in the rhizosphere, and the bacterium exists in larger numbers in the rhizosphere than in "open" non-rhizospheric soil (Abd-El-Malik 1971). Beside nitrogen fixation, *Azotobacter* spp. also synthesize some biologically active substances including some phytohormones (auxins, cytokinins, and gibberellin-like substances) (Azcorn and Barea 1975) and stimulate plant growth (Jnawali et al. 2015). Additionally, the bacteria facilitate the mobility of heavy metals in the soil, and help in the bioremediation of soil from heavy metals such as cadmium, mercury, and lead. *Azotobacter* spp. produce siderophores that bind to the available form of iron (Fe^{3+}) in the rhizosphere, and make it unavailable to plant pathogens and protect the plant health. Also, most of *Azotobacter* spp. produce and secret antimicrobial compounds such as hydro cyanine (HCN, Althaf and Srinivas 2013), and an antifungal antibiotic (Jnawali et al. 2015). *Azotobacter* in sufficient numbers will outcompete pathogens for food. Some of the pathogens controlled by *Azotobacter* spp. in the soil and on the leaf include: *Alternaria*, *Fusarium*, *Colletotrichum*, *Rhizoctonia*, *Macrophomina*, *Diplodia*, *Botryodiplodia*, *Cephalosporium*, *Curvularia*, *Helminthosporium*, and *Aspergillus* (Jnawali et al. 2015). *Azotobacter* spp. can also biodegrade chlorine-containing aromatic compounds such as 2,4,6-trichlorophenol (Li et al. 1991), a mutagenic and carcinogenic compound previously applied as an insecticide, fungicide, and herbicide. However, the bacteria in the genus *Azotobacter* are extremely sensitive to soil contamination and they are considered as the effective indicators of the pollution with crop protection products (Milošević and Govedarica 2002). It is, therefore, a reliable indicator of the biological value of soil. The numbers of this group of nitrogen-fixing bacteria decrease considerably in the period of 7–14 days after herbicide application (Milošević and Govedarica 2002). Some herbicides such as carfentrazone-ethyl (the most inhibitory effect at only 42.240 μg kg^{-1} soil; Tomkiel et al. 2015), a mixture of diflufenican + mesosulfuron methyl + iodosulfuron-methyl-sodium (4.560 mg kg^{-1} soil; Baćmaga et al. 2015), Successor T (pethoxamid + terbuthylazine; Wyszkowska et al. 2016), sulfometuron (0.41 mM; Burnet and Hodgson 1991), chlorsulfuron (0.56 mM; Burnet and Hodgson 1991) inhibit the growth of *Azotobacter*. The herbicides Agronex 50SC, Agroxone, and 2,4-Damine were toxic to *Azotobacter vinelandii*, and the latter was of the most toxicity. Also, there was a reduction in LC_{50} of herbicides with increased number of days, and the percentage survival decreased with increased concentration of herbicides and days (Adeleye et al. 2004). Dalapon and 2,4,5-T were neither effective on growth of *A. vinelandii*, nor on its nitrogenase activity in pure culture (Mackenzie and Macrae 1972). The reaction of Azotobacter towards different herbicides was highly variable. Some herbicides such as metribuzin 70% WP (applied at the rate of 300 g ha^{-1}) are significantly toxic to *Azotobacter*. Similar results on the toxicity of metribuzin to *Azotobacter* were also reported (Radivojevic et al. 2003). Certain herbicides such as clodinafop propargyl 15% WP (applied at the rate of 400 g ha^{-1}; Hussain et al. 2014), and quizalofop (Das et al. 2012) induce its growth as if they are favourably exploited by the bacterium. 2,4-D (Balajee and Mahadevan 1990; Musarrat et al.

2000), p-chlorophenoxy-acetic acid (Balajee and Mahadevan 1990), p-chlorophenol (Balajee and Mahadevan 1990), and simazine (Martinez-Toledo et al. 1991) were biodegraded by *Azotobacter chroococcum* as carbon sources, which ultimately stimulated nitrogenase enzyme. Other herbicides such as isoproturon 75% WP (applied at the rate of 1333 g ha^{-1}) are neutral and do not influence it at all (Hussain et al. 2014). Similarly, linuron did not inhibit the growth of any of fourteen strains of *A. chroococcum* (Lenart 2012), and pendimethalin (Stomp 330 EC, applied at the rate of 400 mL da^{-1}) as well as s-metolachlor (Dual Gold 960 EC, applied at the rate of 150 mL da^{-1}) were of no negative impact on the development of *A. chroococcum* (Kalinova et al. 2014). Furthermore, the sulfonylurea herbicides such as metsulfuron-methyl (He et al. 2006), mesosulfuron-methyl and iodosulfuron methyl sodium (Atlantis® 3.6% WG, 400 g ha^{-1}; Hussain et al. 2014), and Sulfosulfuron® 75% WG (applied at the rate of 33.33 g ha^{-1}; Hussain et al. 2014) initially enhance its growth, and then depress it. With UPH-110 54% WG (clodinafop propargyl and metribuzin) applied at the rates of 400, 500, 600, and 1000 g ha^{-1}, at all the doses barring the lowest dose, a fall in the population of *Azotobacter* was observed at varying time intervals. With UPH-110, the magnitude of toxicity and duration of hazard increased as with the increase of the dose. The lower concentration of UPH-110 might have been metabolized rapidly leading to no toxic effect, while higher concentration might have persisted for a longer period and thereby led to the inhibition of *Azotobacter* population (Hussain et al. 2014). A similar behavior has been reported with bentazon that was not detected in soil after a few months when applied at 10 ppm concentration, whereas higher amounts persisted for several months (Drescher and Otto 1973; Marsh et al. 1978; Gaynor and Hamill 1983). With UPH-110 (500 g ha^{-1}), the *Azotobacter* count was well short of control at 3rd and 7th day of incubation. The inhibitory impact of the higher doses of UPH-110 (600, and 1000 g ha^{-1}) was observable up to one month after which it neutralized and paralleled with control (Hussain et al. 2014). Glyphosate not only inhibits nitrogen fixation process in *A. chroococcum* but also reduces the bacterium respiration rate by 40–60% and thereby precludes its positive effects (Chennappa et al. 2014). Cinosulfuron, a sulfonylurea herbicide (applied at recommended field rate of 42 μg kg^{-1} of soil) influenced acetolactate synthase (ALS) activity and inhibits the biosynthesis of three branched chain amino acids, however, its effect was limited even at the doses higher than that recommended for field applications. Also, there were apparent differences between the sensitivity of *A. chroococcum* strains (Allievi and Gigliotti 2001).

Effect of Herbicides on Azospirillum Species

Azospirillum species are found in much larger numbers in the rhizosphere of some cereals and grass seed crops than in the soil itself (Döbereiner and Pedrosa 1987; Cárdenas et al. 2010). Newer findings have indicated the possibility of its adaptation in the soils of the highland (Aguirre-Cadena et al. 2014). Furthermore, the beneficial impact of *Azospirillum* spp. is not restricted to monocotyledonous crops,

and they seem as universally well-distributed general root colonizers and not the plant-specific bacteria restricted to poaceous crops and grasses. In contrast to *Azotobacter* spp., these bacteria are not so sensitive to environmental pollutions (Bashan and Holguin 1997). *Azospirillum* spp. enhance the growth of various plants including sorghum, sugarcane, triticale, barley, wheat, corn (in a genotype-dependent manner), rice, sunflower, carrot, mustard (in a genotype-dependent manner), tomato, eggplant, cotton, pepper, chick-pea, and oak seedlings (Bashan and Holguin 1997; Bashan et al. 1989; Del Gallo and Fabbri 1990; Pedraza et al. 2009; Saha et al. 1985). *Azospirillum* spp. are of other interestingly positive characters (Bashan and Holguin 1997). Seed application of cobalt and molybdenum together with seed inoculation with *A. brasilense* promoted the highest leaf nitrogen content, hundred-grain weight, yield, and profitability with soybean crop (Galindo et al. 2017). Several studies have marked the ability of *Azospirillum* spp. to stimulate plant growth, yield, and nitrogen content (Díaz-Zorita and Fernández-Canigia 2009), dinitrogen fixation, radical proliferation, and hormone development activities (Dart 1986; Bashan and de-Bashan 2010). Inoculation of corn seed with *A. brasilense* reduced iron concentration in leaf and increased leaf chlorophyll index (LCI), and leaf concentration of phosphorus, agronomic efficiency, and grain yield. Therefore, the use of *A. brasilense* was found viable even when high rates of nitrogen were applied (Galindo et al. 2016). Similarly, plant growth promotion was observed with potato seed tubers inoculated with an *Azospirillum* sp. studied beside other plant growth promoting bacteria (Naqqash et al. 2016; Fig. 55).

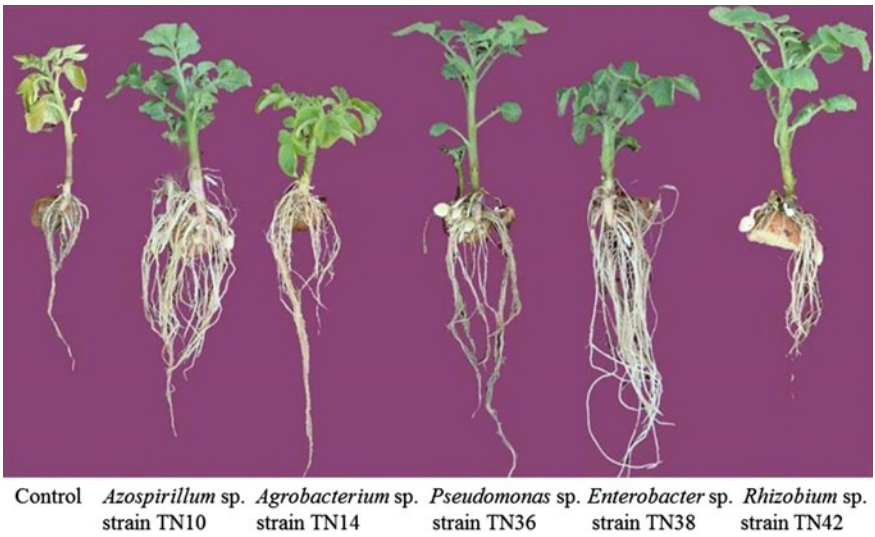


Fig. 55 Effect of bacterial inoculation of seed tubers on the health and root system of potato (variety Kuroda) plants. Plants were harvested 60 days after sowing (Naqqash et al. 2016)

A number of factors such as plant type and age, soil type, composition of microbial community, agricultural practice, root constituents and chemical compounds applied to the soil and plants, may influence the ecology and dinitrogen fixation of *Azospirillum* (Balandreau 1986). Herbicides may influence the growth and activity of *Azospirillum* in vitro, and *in soilum*. For instance, in an in vitro assay, all tested herbicides led to an initial decrease in the growth of *A. lipoferum* (after 24 h) followed by a recovery in the later stages of incubation. The negative effect of butachlor on the growth of the *A. lipoferum* in N-free malic acid broth (5.128 log CFU mL^{-1}) was recorded compared with 2,4-diphenoxy-2-ethylhexyl ester (2,4-DEE; 5.188 log CFU mL^{-1}), pretilachlor (5.226 log CFU mL^{-1}), and pyrazosulfuron ethyl (5.258 log CFU mL^{-1}). Among the different concentrations, the maximum inhibition of growth was recorded at a dose 100 fold of the recommended dose for field application (100 FR; 5.059 log CFU mL^{-1}), while the dose of 1 FR caused the least inhibition (5.244 log CFU mL^{-1}). 2,4-DEE, butachlor, pretilachlor, and pyrazosulfuron ethyl were found to reduce the growth and nitrogenase activity of *A. lipoferum* when the herbicides were applied at the concentrations of 0.375, 0.50, 0.15, and 0.125 μg mL^{-1} (Latha and Gopal 2010). It seems that no of the tested herbicides including butachlor can exert a hazardous impact on *A. lipoferum* populations *in soilum*, where herbicides are biodegraded by some members of soil microflora, or they lose their bioactivity being sequestered by organic and inorganic components *in soilum*. With *A. brasilense*, the reduction of dinitrogen fixation and adenosine triphosphate (ATP) content of the bacterium has been reported in a chemically defined medium as well as in a dialyzed soil medium as the result of their exposure to either of the herbicides, alachlor or metolachlor. However, the adverse effects due to metolachlor disappeared after 48 h indicating the tolerance of high concentrations of metolachlor by *A. brasilense* (Salmeron et al. 1991). The sulfonylurea herbicides, chlorosulfuron and rimsulfuron inhibited the growth of *Azospirillum*, and the significantly enhanced toxicity of the commercial formulations of rimsulfuron was attributed to the surfactants applied (Forlani et al. 1995). Another in vitro study indicated the inability of 2,4-diphenoxyacetic acid (2,4-D) to affect the growth and nitrogenase activity of *A. brasilense* when the herbicide was amended at 100, 200, and 300 μg mL^{-1} (Martinez-Toledo et al. 1990). However, 2,4-D influenced growth and protein, DNA and RNA synthesis of *A. brasilense*. At a concentration of 1 mM, 2,4-D inhibited bacterial cell growth tat was reversed via transferring the bacteria to a control 2,4-D-free medium or to a 2,4-D treated medium supplemented with polyamines. The inhibitory effect of 2,4-D on in vitro protein synthesis was also reversed by the addition of polyamines to the 2,4-D treated medium (Rivarola et al. 1992). The influence of the herbicides metamitron, metribuzin, ethiozin, and paraquat on the growth and nitrogenase activity of *A. lipoferum* and *A. brasilense* indicated that metamitron (35, and 70 μg mL^{-1}) and ethiozin (20 μg mL^{-1}) were not of any inhibitory effect on nitrogenase activity of the tested bacteria, while metribuzin (7, and 14 μg mL^{-1}) and ethiozin (50 μg mL^{-1}) caused a marked decrease in the enzyme activity (Gadkari and Klingmuller 1988). In vitro screening of the field recommended doses of the herbicides applied on sugarcane for those

that do not affect neither growth nor the biological nitrogen fixation (BNF) process of the diazotrophic bacterium *A. brasilense* indicated that out of eighteen herbicides including paraquat (8.49 μg L^{-1}), ametryn (56.60 μg L^{-1}), amicarbazone (19.81 μg L^{-1}), diuron (45.28 μg L^{-1}), metribuzin (27.17 μg L^{-1}), hexazinone + diuron (5.60 + 19.87 μg L^{-1}), clomazone (15.57 μg L^{-1}), hexazinone + clomazone (3.54 + 14.15 μg L^{-1}), isoxaflutole (3.71 μg L^{-1}), sulfentrazone (11.32 μg L^{-1}), oxyfluorfen (16.98 μg L^{-1}), imazapic (3.47 μg L^{-1}), imazapyr (7.08 μg L^{-1}), trifloxysulfuron-sodium + ametryn (0.52 + 20.70 μg L^{-1}), S-metolachlor (27.17 μg L^{-1}), glyphosate (25.47 μg L^{-1}), MSMA (40.75 μg L^{-1}), and 2,4-D (14.22 μg L^{-1}), some herbicides could reduce the bacterial growth in liquid DIG medium. This effect was found with the herbicides paraquat, oxyfluorfen, trifloxysulfuron-sodium + ametryn, and glyphosate. With (trifloxysulfuron-sodium + ametryn), the effect was associated with increases in both the length of lag phase as well as the generation time, whereas with glyphosate, it was only associated with the increased generation time. MSMA, amicarbazone, and specially paraquat reduced the BNF of *A. brasilense*. Other herbicides were effective on neither growth nor the BNF of *A. brasilense* (Procópio et al. 2011). In a similar study, nitrogenase activity of *A. lipoferum* was assayed in the presence of glyphosate, 2,4-D, mecoprop + dichlorprop, and the commercial products Roundup® (glyphosate), Nurmikko-Hedonal® (2,4-D), Mepro® (mecoprop), and Dipro® (MCPA) tested at different doses of 0, 5, 25, and 100 mg L^{-1}. In average field soils, where d = 1 kg dm^{-3}, the highest recommended dose of herbicides, affecting the 5 cm-thick top layer is 5 mg L^{-1}. Apart from Mepro® and mecoprop, no phenoxy acid herbicide inhibited the nitrogenase activity of *A. lipoferum*. Glyphosate and Roundup® were of no impact on the growth of *A. lipoferum*, but stimulated its nitrogenase activity (Haahtela et al. 1988).

Studies performed under greenhouse conditions indicated the occurrence of a significantly positive synergistic effect on the growth of a local cultivar of maize, Galal, when the potted soil inoculated with *A. lipoferum* strain ATCC29145 (2 mL of a bacterial suspension with the concentration of 3.2×10^8 cells mL^{-1}) was treated with one of the herbicides bromoxynil (0.42 L ha^{-1}), or particularly afalon S (a mixture of linuron and monolinuron; 0.42 kg ha^{-1}). The incorporation of the recommended field dose of either bromoxynil or afalon S into the soil was seemingly of no significant effect on nitrogenase and dehydrogenase activities (Fayez et al. 1983). In another study under greenhouse conditions, the seed of wheat cultivar, Altiplano were inoculated with *A. brasilense* before planting, and the herbicides were applied 40 days after planting. The treatments including (i) uninoculated plants (absolute control), (ii) inoculated with *A. brasilense* (inoculated control), (iii) inoculated with *A. brasilense* + application of an organochlorine herbicide, 2,4-D, (iv) inoculated with *A. brasilense* + application of an organophosphate herbicide, glyphosate, (v) treatment with 2,4-D without any inoculation, and (vi) treatment with glyphosate without any inoculation were applied in a completely randomized design with ten replicates. The survival of the bacteria in the root system was recorded via the use of the most probable number technique, recording plant height and weight. Two herbicides were of no significant

influence on the population of *A. brasilense*. Wheat plants inoculated with *A. brasilense*, with or without herbicide treatment indicated increased biomass compared with the non-inoculated plants. The application of organochlorine herbicide, 2,4-D (250 g ha^{-1}) resulted in the increased biomass production of the wheat cultivar planted in an unsterilized sandy (Bouyoucus) soil mixed with organic soil waste and filled into 1 kg pots with perforated bases to promote drainage. The pH of the mixture was 7.5 (2:1 potentiometer), and its total nitrogen and phosphorus contents were respectively 0.13 ppm (Kjeldahl), and 7.5 mg kg^{-1} (Olsen/spectrophotometer). While both herbicide induced *Azospirillum* proliferation in inoculated as well as uninoculated unsterilized soil mixture, glyphosate was more inductive than 2,4-D, but still of more negative impact on wheat growth (Aguirre-Cadena et al. 2014). In contrast, the herbicides, 2,4-D and atrazine negatively influenced *Azospirillum* populations in the rhizospheric soil of groundnut crop, as well as soil enzymatic activities (Mohiuddin and Mohammed 2014). In another study on the effect of fertilizers (urea, diammonium phosphate and potassium chloride) and herbicides (glyphosate and 2,4-D) on the viability of *A. brasilense* strain C16 performed with three different concentrations for each agrochemical in a minimal salt medium at 0, 12, and 24 h of growth, the herbicides indicated more toxicity than fertilizers. In addition to type of the tested agrochemical, the concentration and time of exposure for each agrochemical were directly correlated with their antibacterial effects. After 24 h exposure to the maximal concentration, glyphosate, 2,4-D and potassium chloride reduced C16 viability by 43, 27, and 26%, respectively. Instead, urea (23%), and diammonium phosphate (50%) promoted its growth by 12 and 6% (Romero-Perdomo et al. 2015). The study on the effect of thiobencarb (also known as benthiocarb) on two *Azospirillum* spp. in pure cultures as well as in association with rice under both laboratory and field conditions indicated that the herbicide was of no negative effect on *A. lipoferum* 4B, but it inhibited the growth of *A. brasilense* N040. Also, despite of its negative impact on the growth of aseptically grown rice plantlets, thiobencarb did not influence dinitrogen fixation when rice plantlets were first inoculated with *Azospirillum* strains in gnotobiotic conditions containing the herbicide (Omar et al. 1992). However, *Azospirillum* populations were inhibited with low levels of thiobencarb applied to three tropical rice soils incubated under non-flooded conditions (Jena et al. 1990). The inoculation of corn seeds with *A. brasilense* together with the application of nitrogen fertilizer (140 kg urea ha^{-1}; applied at 26 days after sowing) minimized the harmful effects of the herbicide mesotrione (192 g ha^{-1}; applied at 21 days after sowing) in the initial development of corn (Bulegon et al. 2017).

Effect of Herbicides on Cyanobacteria (Green-Blue Algae)

Heterocystous and filamentous cyanobacteria are considered as a considerable part of soil microflora of paddy fields in tropical countries that significantly contribute to soil fertility (Singh 1961; Venkataraman 1981) through photosynthetic and photoheterotrophic dinitrogen fixation (Stewart et al. 1975). Biofertilization with

cyanobacteria and green algae is able to increase rooting of grapes cuttings and germination of sunflower seeds, and improve plant growth as observed in rice, barley, oats, tomato, radish, cotton, sugarcane, maize, chili, lettuce, wheat, gilly-flower, grapevine (Spiller and Gunasekaran 1990; Romanowska-Duda et al. 2004, 2010; Saadatnia and Riahi 2009; Tajuddin and Subramanian 2005; Song et al. 2005; Nilsson et al. 2005; Karthikeyan et al. 2007; Abd El-Moniem and Abd-Allah 2008; Shanan and Higazy 2009; Sahu et al. 2012; Shariatmadari et al. 2013; Grzesik and Romanowska-Duda 2014, 2015). Additionally, the effectiveness of the foliar application of cyanobacteria and green algae in enhancing the photosynthetic performance and growth of willow (*Salix viminalis* L.) plants has newly been indicated under limited synthetic fertilizers application (Grzesik et al. 2017). Cyanobacteria possess photosynthetic machinery similar to that of the chloroplasts of higher plants (Fogg et al. 1973; Stewart 1973) and therefore, can be influenced by the certain groups of the herbicides that affect photosynthesis or respiration (Dodge 1975). The herbicides can also exert an indirect negative influence on cyanobacterial dinitrogen fixation through inhibition of cyanobacterial photosynthesis (Vaishampayan 1984). The effect of herbicides amitrole, a derivative of amitrole (3,5-diamino-1,2,4-triazole), diquat, paraquat, linuron, MCPA, and monuron on nitrogen-fixing blue-green algae, *Anabaena cylindrica*, *Aulosira* sp., *Calothrix elenkenii*, *Chlorogloeae frischii*, *Cylindrospermum muscicola*, a phycobiont *Nostoc* sp. from the lichen *Collema tenax*, *N. muscorum*, *Tolypothrix tenuis*, and *Westiellopsis* sp. was studied. The responses were recorded as an initial period of depression followed either by an increased activity, or by a distinct decrease on prolonged incubation. Some herbicides could severely restrict the nitrogen-fixing capacities of the cyanobacteria, and thereby generally influenced the overall nitrogen economy of soils (DaSilva et al. 1975).

Pre-emergence herbicides applied at field recommended doses, 3 days after transplanting (DAT) rice plants inhibited cyanobacterial growth and dinitrogen fixation inoculated 10 DAT. The inhibition lasted up to 20 DAT. Butachlor and oxadiazon were more toxic than benthiocarb and pendimethalin. The application of 0.5 kg ha^{-1} active ingredient of 2,4-DNa (Na-2,4-dichlorophenoxy acetate) inhibited cyanobacterial growth, but the post-emergence herbicide 2,4-DEE (2,4-dichlorophenoxyacetic acid ethyl ester) applied 30 DAT inhibited cyanobacterial growth as well as dinitrogen fixation. The inoculation of 10 kg ha^{-1} of dry mixture of blue green algae 10 DAT could produce the maximum biomass 60 and 80 DAT in control and herbicide treated plots, respectively. The biomass and nitrogen produced by cyanobacteria were higher in controls than in the herbicide treated plots. The application of cyanobacterial biofertilizer along with herbicides increased the grain and straw yields as well as panicle number and nitrogen uptake by rice over no cyanobacterial treatment. The cyanobacterial treatment even without weeding resulted in the increased rice yield up to that of herbicide and biofertilizer treatments (Singh et al. 1988). The herbicides Saturn and Knockweed inhibited the growth of blue-green alga at relatively higher concentrations, while exerted no significant effect on heterocyst frequency. Saturn depicted more toxicity than knockweed as reflected by their lethal doses of 20 ppm and 1000 ppm, respectively

(Ahluwalia 1988). Herbicides chlorotoluron and diuron effectively induced mutants of *Nostoc muscorum* to 50–100 times more than background level. The mutants indicated a range of characters including pigment deficiency, no fixation of dinitrogen, as well as cyanophage resistance (Amla and Kochhar 1982). Prolonged cultivation of the cyanobacterium *A. variabilis* in the presence of sublethal concentrations of diuron indicated a two-stage adaptation to the herbicide action. In the relatively short-time first stage, physiological adaptation was observed as the increased ratio of phycobilin/chlorophyll. In the more prolonged second stage, cells with low sensitivity to diuron were selected in the population. These cells were of a low ratio of phycobilin/chlorophyll (Andreev and Maslov 1988). Monuron inhibited growth and heterocyst formation in the nitrogen-fixing blue-green alga *Nostoc muscorum* (Fig. 56), where the inhibitory impact was reversible in both nitrogen-free and nitrate media (Vaishampayan 1984).

The herbicides DCMU [3-(3,4-dichlorophenyl0-1,1-dimethylurea], isopropturon, and ioxynil not only blocked photosynthetic photosystem II in the aquatic cyanobacterium, *Synechocytis salina*, but also more strongly inhibited the photosynthetic oxygen evolution. Phenylurea herbicides (DCMU, and isoproturon) were found of stronger impact on cyanobacterial PSII activity compared to the phenol-type ioxynil (Yotsova et al. 2017). Interestingly, herbicides can increase the rate of nitrogen fixation, wherever they omit the impact of green algae interactions with the effective herbicide-resistant cyanobacterial strains. This has been indicated by developing a variant of the nitrogen-fixing cyanobacterium, *Aulosira* strain 68 resistant to the herbicide simetryne at the concentrations that prevented the development of indigenous algae. Interference from indigenous algae may hamper the development of nitrogen-fixing cyanobacteria introduced into rice fields in attempts to increase biological nitrogen fixation (Wilson et al. 1979). Monosulfuron, applied at 0.03–0.3 nMol L^{-1} under laboratory conditions, stimulated the growth of the nitrogen-fixing cyanobacteria *Anabaena flos-aquae* (a

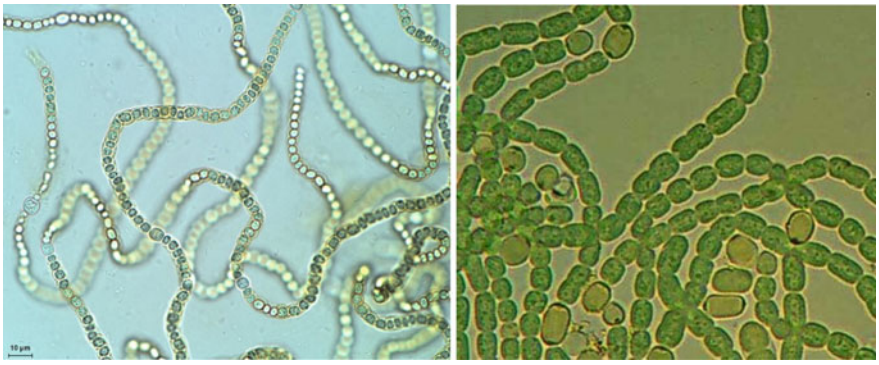


Fig. 56 (Left) *Nostoc muscorum*, filamentous structures called trichomes with enlarged cells called heterocysts (*Source* http://dbmuseblade.colorado.edu/); (Right) *Nostoc* (*Anabaena*) *azollae* trichomes and heterocysts (*Source* http://www.pinterest.com/)

free-living soil cyanobacterium that mat be toxic to some organisms; Fig. 57), *A. azollae* (a cyanobacterial symbiont with the water fern, *Azolla* spp., Fig. 56), and *A. azotica* (a free-living soil cyanobacterium with a high nitrogen-fixing capability; Fig. 57), but applied at higher concentrations of 30–300 nMol L^{-1} inhibited protein. The production of 16 amino acids was reduced in *A. flos-aquae* from 7 to 69% with increasing the herbicide concentration. The application of monosulfuron at 3–300 nMol L^{-1} substantially inhibited in vitro activity of acetolactate synthase (ALS) enzyme as indicated by 50% inhibition index values of 3.3, 65.2, and 101.3 nMol L^{-1} for *A. flos-aquae*, *Nostoc (Anabaena) azollae*, and *A. azotica*, respectively. Apart from *A. flos-aquae* at higher concentrations of 30–300 nM L^{-1}, the activity of the extracted ALS was not affected in the algal species treated with monosulfuron when applied at the rate of 0.03–300 nMol L^{-1}. Thus, *A. flos-aquae* was found as the species most sensitive to monosulfuron.

The toxicity of the herbicide to three cyanobacterial nitrogen-fixers was attributed to its interference with protein metabolism via the inhibition of branch-amino acid biosynthesis and particularly ALS activity. In rice cropping systems where monosulfuron is applied at low concentration (3 nMol L^{-1}), the use of the beneficial nitrogen-fixing cyanobacteria *A. azotica* and *A. azollae* may be suitable as "biofertilizers" given their high tolerance to the sulfonylurea herbicide (Shen et al. 2009). The sensitivity of cyanobacteria to PSII inhibitors such as s-triazines, and phenylurea has been reported (Lockert et al. 2006), and blue-green algae exhibit less sensitivity than green algae to the phenylura herbicide diuron applied at 0.16 mg L^{-1} (Abdel-Aty and El-Dib 2016).

Effect of Herbicides on Nitrifying Bacteria

Studies on the effects of selected herbicides on the parameters of soil nitrification processes (Fig. 58) led to the separation of four distinct groups of herbicides. The formulated octanoates of bromoxynil and ioxynil (NPH1320 and Totril, respectively) were the most toxic herbicides. Because of their very high toxicity, ioxynil and bromoxynil might also, in spite of their low practical field rates of

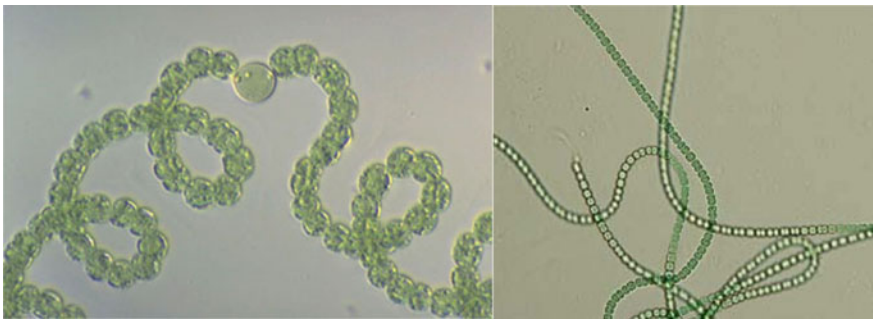


Fig. 57 Trichome of (Left) *Anabaena flos-aquae* with heterocyst, and (Right) *A. azotica*

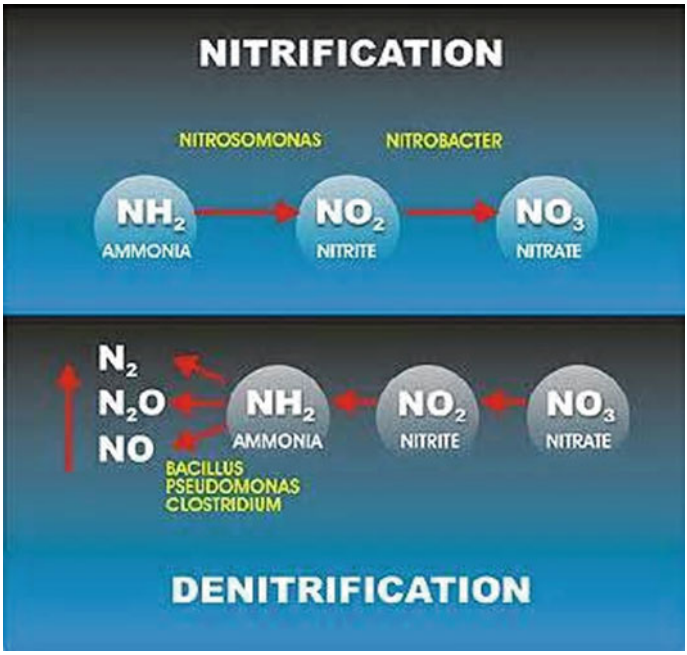


Fig. 58 Nitrification and denitrification and the bacteria involved in the phenomena (*Source* http://nsspo.com/p1/Nitrification.htm/)

0.25–0.5 kg ha^{-1}, exert a small though temporary depressing effect (Debona and Audus 1970). Next in order of toxicity were chlorbufam, phenmedipham, formulated oxadiazon, formulated legurame, ioxynil, formulated trifluralin, and bromoxynil. Terbacil, dicamba, and tricamba were of low toxicity, whereas asulam and related experimental herbicide MB9555 influenced some parameters only when they were tested at the very high concentrations. Bromoxynil application resulted in the decreased population of the bacteria involved in nitrification. *Nitrobacter winogradskii* was more sensitive than *Nitrosomonas europaea* to four groups of herbicides.

Legurame and oxadiazon were relatively more toxic to *N. europaea* in culture, but this differential toxicity was not found in the soil. Dicamba, tricamba, trifluralin and chlorbufam were more toxic to *N. winogradskii* than to *N. europaea* in the soil. However, the formulated trifluralin seemingly stimulated the growth of both nitrifiers but only in the soil, suggesting the suppression of antagonistic organisms as a possible reason. It was extrapolated that terbacil was the only herbicide that might cause small inhibition of nitrification at the rates recommended for field applications. However, terbacil was found disproportionately toxic at low concentration. The formulated octanoates of bromoxynil and ioxynil, and possibly dicamba and oxadiazon can cause small inhibitions when applied at the rates somewhat in excess of normal (Ratnayake and Audus 1978). The bacteria involved

in nitrification process show highest sensitivity to bromoxynil herbicide (Topp et al. 1992) and the inhibition of the activity of ammonium oxidation by the bacteria has also been reported in soils treated with the herbicide (Pampulha and Oliveira 2006). The actions of these two herbicides are nearly similar, and both effectively inhibit nitrification to the 50% level even at the concentrations much lower than 50 ppm (Debona and Audus 1970). Chlorflurazole was not an inhibitor of nitrification as complete as propanil, and even at 100 ppm inhibited only a little more than 80% of nitrification. Chlorflurazole had much more striking effect on the growth of bacterial nitrifiers and no growth was detected at only at 50 ppm. No indication of adaptation was found with chlorflurazole in contrast to propanil, bromoxynil and ioxynil. Chlorflurazole might exert a small depressing effect under field conditions, however unlike bromoxynil and ioxynil, its effect might be more persistent (Debona and Audus 1970). Chlorthiamid depicted a marked suppression of nitrification, 50% inhibition when tested at the concentration of 100 ppm. The herbicide was found to be of a strict impact on the proliferation of nitrifiers. Its effect on the proliferation of nitrifying bacteria was more than that of propanil, bromoxynil, ioxynil, and chlorflurazole, and led to 50% inhibition of proliferation at the concentrations below 50 ppm. The inhibition of growth clearly occurs through an action independent of that through nitrification itself. Under field conditions, chlorthiamid with application rates up to 10 kg ha^{-1}, might lead to a very slight depression of nitrification processes at the highest rates (Debona and Audus 1970).

2,3,6-TBA completely inhibits nitrification processes at the application rate of 1000 ppm, however, the toxicity of this herbicide is much less in soil (50% inhibition at the rate of 1000 ppm). Despite of the inhibition of proliferation, the nitrifying bacteria extensively adapt to 2,3,6-TBA in soil (Debona and Audus 1970). The lowest concentrations used in the perfusers (50 ppm) correspond with field rates of approximately 25 kg ha^{-1} (see Fletcher 1960), therefore, it seems very unlikely that endothal at its normal field rate could have any significant impact on nitrification (Debona and Audus 1970). Picloram had a much less impact on nitrification process, and its virtually total inhibitory impact was found only at its concentration of 500 ppm, while it reached the 50% level at 150 ppm. Picloram, even in lower concentrations, significantly inhibited the proliferation of bacterial nitrifiers. No growth was detected at the rate of 1000 ppm, where nitrification was completely inhibited. Picloram influenced *Nitrosomonas* activity and the process of ammonia to nitrite conversion (Debona and Audus 1970). Similarly, partial inhibition of the conversion of ammonia to nitrite was found in soil when picloram was applied at the rate of 1000 ppm. However, the treatment was of no effect on nitrite to nitrate conversion suggesting that *Nitrosomonas* was much more sensitive than *Nitrobacter* (Goring et al. 1967). The lowest concentrations used in the perfusers (50 ppm) correspond with field rates of approximately 25 kg ha^{-1} (see Fletcher 1960), therefore, it seems very unlikely that endothal at its normal field rate could have any significant impact on nitrification (Debona and Audus 1970). The toxicity of paraquat is low in soil because of its extensive adsorption on clay colloids. Biologically, the adsorbed paraquat is totally inactive, therefore, only a relatively

small fraction of unadsorbed herbicide in equilibrium in the soil solution is effective in inhibition of nitrification in the soil (Debona and Audus 1970). However, paraquat is highly inhibitory of the oxidation of ammonia to nitrite and nitrate by *Nitrosomonas* and *Nitrobacter* in culture (Debona 1967). The effect of paraquat on the nitrification seems to be a progressive inhibition, with increasing concentrations reaching 50% level of inhibition at about 750 ppm. Paraquat was of no significant effect on the rate of nitrifying bacteria proliferation, and its action would seem to be only on nitrification, not directly on cell growth (Debona and Audus 1970). Paraquat (4 M) inhibited the growth of *Nitrobacter agilis* but did not influence the growth of *N. europaea*. Also, nitrite did not accumulate in the presence of paraquat (4 M) when ammonia was oxidized by a mixed culture of the two nitrifiers (Yamanaka 1983). As the toxicity of paraquat on *N. gracilis* is known as the result of superoxide anion radical formation (Moody and Hassan 1982), the difference in sensitivity to paraquat between the two nitrifiers may be attributable to the differences in the content of superoxide dismutase in the two bacteria (Yamanaka 1983). Additionally, the relative insensitivity of *N. europaea* to paraquat has been attributed to hydroxylamine (Yamanaka 1983), a metabolic intermediate of ammonia (Hofman and Lees 1953), which may function as a scavenger of superoxide anion radicals (Elstner et al. 1975). The lowest concentrations used in the perfusers (50 ppm) correspond with field rates of approximately 25 kg ha^{-1} (see Fletcher 1960), therefore, it seems very unlikely that endothal at its normal field rate could have any significant impact on nitrification (Debona and Audus 1970). Dichlobenil inhibited nitrification but its effect was somewhat smaller than that of paraquat, and the 50% level of inhibition was reached at the application rates over 1000 ppm. In contast to paraquat, dichlobenil not only reduced nitrification, but also significantly reduced their proliferation. The lowest concentrations used in the perfusers (50 ppm) correspond with field rates of approximately 25 kg ha^{-1} (see Fletcher 1960), therefore, it seems very unlikely that endothal at its normal field rate could have any significant impact on nitrification (Debona and Audus 1970).

Endothal stimulated nitrification, and the greatest effect was obtained at the highest concentration tested (1000 ppm). Endothal only caused a slight reduction in the rate of cell proliferation at all its concentrations tested. The rapid microbial breakdown of endothal in the soil (Jensen 1964), the ability of soil bacterial nitrifiers in the complete breakdown of the herbicide to carbon dioxide and in the use of carbon dioxide as the principal carbon source, and the possible existence of other growth-stimulants in the formulated product have been discussed as the reasons for stimulatory impact of endothal. The lowest concentrations used in the perfusers (50 ppm) correspond with field rates of approximately 25 kg ha^{-1} (see Fletcher 1960), therefore, it seems very unlikely that endothal at its normal field rate could have any significant impact on nitrification (Debona and Audus 1970). Studies seven herbicides, indicated that sodium pentachlorophenate (PCP), 4,6-dinitro-ortho-secondary-butyl phenol (DNBP), isopropyl N-(3-chlorophenyl)-carbamate (CIPC), and monuron were the strongest inhibitors. Sesone (sodium 2,4-dichlorophenoxyrthyl sulfate also known as 2,4-DES) and dalapon were of the least inhibitory impact on the respiration of nitrifying bacteria, while PCP exhibited

the greatest level of inhibition. A comparison of the rates of nitrification as measured by formation of nitrate in the percolation method and oxygen uptake in the monometric method showed fair agreement for CIPC, but not for monuron. A comparison of the approximate concentrations of herbicides used in the field for chemical weed control and concentrations which caused a 50% inhibition of oxygen uptake by the nitrifying microorganisms indicated that the concentrations of herbicides which inhibited respiration by 50% would not occur at usual field application rates and that there would be little or no detrimental effect on soil nitrification (Hale et al. 1957). Propanil extremely inhibits nitrification, and gives virtually complete inhibition even in the lowest concentrations (50 ppm). Propanil, with normal practical rates of 5–15 kg ha^{-1}, is likely to cause serious and persistent depressions of nitrification in the field (Debona and Audus 1970).

Effect of Herbicides on Mycorrhizal Fungi

Mycorrhiza (plural, Mycorrhizae) is a term that means "fungus root", widely applied to describe the mutually beneficial symbiotic relationship between fungal partner (mycobiont) and plant partner (phytobiont) roots. These mycobionts colonize the root of their phytobionts and then form a vast hyphal network throughout the soil and thereby greatly increase absorptive surface area, and improve phytobiont nutrition through increased absorption of phosphorus (P), zinc (Zn), manganese (Mn), copper (Cu), and water. The phytobiont supplies carbohydrates for mycorrhizal mycobiont. More than 150 species of mycorrhizal mycobionts have been identified in all types of soils and climates throughout the world. There are several classes wherein these fungi are categorized to, however, here we will pay to the effect of herbicides on mycobionts involved in the two most common classes of mycorrhizae, endomycorrhizal and ectomycorrhizal fungi.

Effect of Herbicides on Endomycorrhizal Fungi

Endomycorrhizal fungi are regarded as obligate mycobionts that cannot aseptically be cultivated on growth media. These glomeromycotan true fungi are morphologically identified with chitinized walled aseptate (without cross-walls) hyphae, that form arbuscules (dendroid haustorium-like structures, Fig. 59) in the periplasmic region of plant root cortical cells, and reproduce asexually through production of thick-walled and long-surviving spores that are very resistant to freezing and intense heat. Also, some genera produce intercalary or terminal sac-like cellular structures in their hyphae that contain lipids and primarily serve as storage organs for the fungus. These structures called vesicles (little sacs, Fig. 59) can also act as propagules and colonize other parts of the plant root. As the formation of arbuscules is a common morphological character, these fungi are also known as arbuscular mycorrhizal fungi (AMF).

AMF are of interest for their reported roles in preserving soil fertility in agroecosystem, which form mutualistic symbiosis with the roots of most agricultural plants. These associations are common in terrestrial ecosystems, including

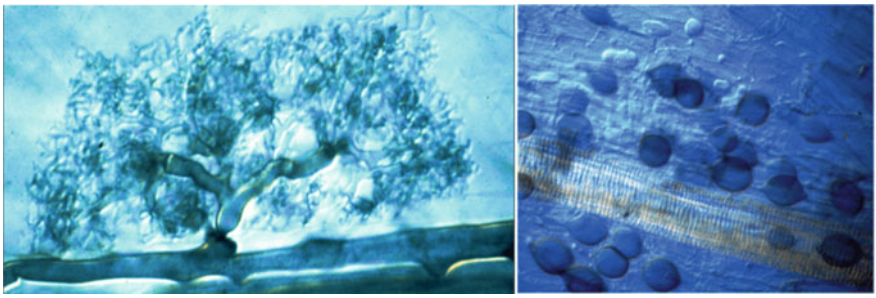


Fig. 59 Morphological characters of arbuscular fungi. (Left) an arbuscule (a little tree-like organ) formed by the fungus in the periplasmic region of plant root cortex cell and involved in bidirectional exchange of metabolites between two symbionts; (Right) vesicles, round sac-like structures developed by the fungus in the intercellular spaces in plant root (Buechel and Bloodnick 2016)

those of anthropogenic origin, such as agroecosystems (Smith and Read 2008). AMF stimulate host plant growth, and improve its resistance to hazardous biotic agents such as pathogens, as well as pests, as well as abiotic agents such as drought, salinity, heavy metals, hot temperatures, and chemical stressors (Smith and Read 2008; Gianinazzi et al. 2010; Vannette and Hunter 2009). AMF secret a glycoproteinaceous substance, glomalin and thereby play an important role in the formation and stabilization of soil aggregates (Smith and Read 2008; Gianinazzi et al. 2010). Additionally, they act as crop nutrition facilitators through increment of nutrient uptake and crop growth promotion that results in non-mycorrhizal weed suppression. Therefore, AMF represent potential alternatives to costly and environmentally hazardous herbicides (Cameron 2010; Rinaudo et al. 2010). Hence, AMF are crucially relevant in the maintenance of soil sustainability and plant production (Gosling et al. 2006; Gianinazzi et al. 2010; Raviv 2010). In the past, most research on AMF has been focused on possible responses to fungicides, rather than on the potential effects on mycorrhizal symbiosis following herbicide application. Because of obligately endosymbiotic nature of endomycorrhizal associations between host plants and AMF, any severe herbicidal phytotoxicity to the host can result in a severely damaged AMF and endomycorrhizal association. Pasaribu et al. (2013) studied the effect of three application rates of two widely used herbicides (1.8, 3.6, and 5.4 μg a. i. g^{-1} for alachlor and 1.08, 2.16, and 3.24 μg a. i. g^{-1} for glyphosate) on sporulation and infection of peanut plant by *Glomus mosseae* under greenhouse conditions. The result of their study indicated that the mycobiont *G. mosseae* responds in a differential way to two different herbicides applied, and the fungus sensitivity to alachlor proved significantly higher than glyphosate. None of the herbicide treatments could affect the external hyphal length and succinate dehydrogenase activity. However, the spore number, total and active infection intensity of internal hyphae was significantly reduced with the increasing rates of alachlor application, while glyphosate had no significant impact at all tested application rates. Consequently, phosphorus inflow through mycorrhizal hyphae

was considerably increased with the application rates of glyphosate, with the highest value (41.48 and 479.72 $\times$ 10^{-13} mol Phosphorus m^{-1} s^{-1} hyphal inflow and hyphal uptake, respectively) obtained at recommended rates for field application of glyphosate (2.16 μg a. i. g^{-1}). Therefore, symbiotic functions of *G. mosseae* with host plant could be affected by the depressive effects of the herbicides, apparently related to the types of herbicides and their application rates (Pasaribu et al. 2013). Similarly, the application of glyphosate (Roundup®) in a model ecosystem resulted in 40% reduction in mycorrhization in the soil amended with *G. mosseae* (Zaller et al. 2014). The reduced total and active mycorrhizal infection intensity following certain herbicidal treatments is attributed to the decreased supply of host photosynthates as the result of negative effect of the herbicide on plant photosynthesis (Smith 1980). Accordingly, glyphosate did apparently not influence the spore germination of *G. mosseae* even when applied at highest doses but when added to culture medium, it reduced AMF spore germination and germ tube growth only at the concentrations higher then those recommended for application in field (Giovannetti et al. 2006). However, the negative impact of glyphosate on endomycorrhization of carrot roots by *Rhizophagus irregularis* has been observed in vitro (Wan et al. 1998). Moreover, the evaluation of three rates of glyphosate (0, 0.26 and 1 $\times$ recommended field rate) 10 and 30 days after treatment (DAT), indicated that spre viability in herbicide untreated control soils was between 5.8-and 7.7-fold higher than in treated soils, even when the lower rate was applied. Significant reductions in root mycorrhization were found in *Lolium multiflorum* plants grown in glyphosate treated soil than in control soils. The reduced number of arbuscules (but not vesicles) indicated the affected functionality of symbiosis 30 DAT (Druille et al. 2013). Arbuscules are considered as the main sites for nutritional exchanges between host plant and endomycorrhizal mycobiont (Smith and Gianinazzi-Pearson 1988). The results indicated that soil residence time of glyphosate and/or its degradation products were enough to reduce AMF spore viability and their ability to colonize host roots. The decrease in the viability and symbiotic capability of AMF propagules may affect plant diversity, taking into account that different plant species are of different degree of mycorrhizal dependencies (Druille et al. 2013). Similarly, soil treatment with glyphosate (0.4, and 4 L ha^{-1}) notably reduced endomycorrhizal colonization of pepper (*Capsicum annuum* L.) roots (Ronco et al. 2008). In contrast, no adverse impact on soybean root endomycorrhization was found when glyphosate was applied in high dose of 10 L ha^{-1} (Malty et al. 2006). These controversial differences are possibly due to the differences in the studied host plant species, AMF species, dose and formulation of the herbicide, agricultural practices (such as irrigation), soil texture, soil chemistry and microbiology, and weather conditions. Herbicide effects on vesicular AMF in *Citrus* spp. under greenhouse as well as grove conditions indicated that the mixture of bromacil + diuron was not of significant impact on mycorrhization of *Citrus* spp. in cultivated plots. Trifluralin, bromacil, and diuron were of no apparent effect on *Glomus etunicatus*, and plant growth. However, the mixture of simazine + paraquat led to a slightly reduced mycorrhization of grove trees roots. Paraquat, and simazine were of adverse impacts on plant growth as well as the

mycobiont (Nemec and Tucker 1983). Simazine did not influence the mycorrhizal hyphal elongation in vitro, but paraquat, and dichlobenil, even at the lowest concentrations, were found to be of significantly inhibitory impact on hyphal elongation, however, different application rates of these three herbicides could not affect mycorrhizal root colonization under greenhouse conditions (Hamel et al. 1994). The significant inhibition of endomycorrhization of plant root as well as reduction of the number of spores in legumes have been reported following treatments with Brominal® (bromoxynil) and Gramoxone® (paraquat; Abd-Alla et al. 2000). Terbuthylazine (5.2, 10, 20.4, 40.4, and 81.2 mg L^{-1}), and MCPA (2-methyl-4-chlorophenoxyacetic acid; 1.2, 2.8, 5.2, 10.8, and 21.2 mg L^{-1}) significantly inhibited the mycelial growth of *G. mosseae* in vitro, and the fungus indicated a clear dose-effect response exclusively in the presence of the herbicidal active ingredient, pendimethalin (9.6, 19.2, 38, and 76 mg L^{-1}). Pendimethalin, when applied at the rate of 4.8 mg L^{-1}, exhibited a hormetic effect and induced the mycelial growth of *G. mosseae* under in vitro conditions (Giovannetti et al. 2006).

Ocampo and Barea (1984) found that carbamate herbicides which are responsible for inhibited photosynthesis mostly did not have any negative effect on arbuscular mycorrhiza (AM). Among three carbamate herbicides including chlorpropham, sulfallate and phenmedipham, only phenmedipham caused the reduction of root sugars and fungal metabolism when applied as foliar spray. However, these herbicides were of no effect on the amount of AM infection when applied through either foliar spraying or direct addition to the soil. Although the application of high concentration of phenmedipham greatly reduced plant growth, plants could recover with the help of AM demonstrating the beneficial effects of AM for its host plant. The results strongly suggest that plants which benefit from AM are less affected by the deleterious effects of herbicides, especially when used at high concentrations, than those without AM. Moreover, it seems that carbamate herbicides cannot significantly affect the infection of AM which makes them proper choices in the chemical control of weeds. The negative impact of sulfentrazone (0.7 mg kg^{-1} soil) application on soybean symbiotic processes (Vieira et al. 2007), and of soil treatment with herbicides on crop root colonization by AMF (Santos et al. 2006) have been indicated. The pre-emergence herbicide, isoxaflutole (Converge Pro®; applied at the rate of 19.8, 39.6, 79.2, and 158.4 μg a. i. L^{-1} of soil) did not influence corn root colonization by the mycorrhizal fungus, *Rhizophagus irregularis* (Stoklosa et al. 2011).

In vitro studies on the effect of two herbicides, prometryn and acetochlor on the endomycorrhization of carrot hairy roots [induced through transformation by root inducing tumor-inducing DNA (Ri T-DNA)] by the AM fungus, *G. etunicatum* indicated that acetochlor at all tested concentrations (0.1, 1, and 10 mg L^{-1}) and low concentrations of prometryn (0.1, 1 mg L^{-1}) were not of any significant impact on spore germination, while all treatments notably inhibited hyphal growth of the fungus. When exposed to the herbicides at certain concentrations, the initial growth of the fungus spores was directly affected. Prometryn and acetochlor detrimentally influenced the formation and function of AM, se well as the rate of mycorrhizal colonization, regardless of concentration. The colonization of host plant roots is a

prerequisite for growth and development of AMF, whereas, the rate of mycorrhizal colonization is an index of the affinity of AMF for the host plants. Hence, the limitation of hazardous effects of agricultural chemicals (such as various types of pesticides as well as fertilizers) on mycelial growth and metabolism, as well as the formation, function, and colonization of AM, is a key factor in maximizing the positive effects of AM symbiosis on plants (Li et al. 2013). Also, both herbicides significantly reduced succinate dehydrogenase activity at all concentrations (but not 0.1 mg acetochlor L^{-1}), indicating their inhibitory impact on the respiratory electron transfer within fungal mitochondria, where the enzyme forms a part of complex II. Prometryn and acetochlor also inhibited the activity of alkaline phosphatase (Li et al. 2013), the enzyme associated with active metabolism of phosphates (Tisserant et al. 1993).

Interestingly, AMF help host plants to escape from the phytotoxic impacts of herbicides. Bethlenfalvay et al (1996) conducted a greenhouse study to determine if AM fungi, whose hyphae interconnect the roots of adjacent plants, modify herbicide effects by enhancing nutrient fluxes between associated plants. Soybean (*Glycine max* (L.) Merr.), and common cocklebur (*Xanthium strumarium* L.) plants were grown together in pots (1.5 L) in a high-P (28 mg kg^{-1}) soil. They were sprayed with the herbicide, bentazon (BEN, 3-isopropyl-lH-2,1,3-benzothidiazine-(4)3H-one 2,2-dioxide), at dose rates of 0, 1/3, 2/3, 3/3, and 4/3 of field recommendation (FR, 1.12 kg a. i. ha^{-1}) while in the 7- to 8-leaf stage. Labelled N (1 mL of 100 mM tSNHaNO_3, 98 atom percent 15 N) was applied to the cocklebur leaves 4 days before spraying and then assayed in the soybean leaves at harvest. Growth and nutrient contents of +AM soybean shoots were enhanced only at the intermediate FR levels, while shoot growth in adjacent cocklebur was inhibited beyond the extent measured in −VAM plants. Labelled N was at natural abundance in both +AM and −AM soybean leaves at 4/3-FR, but at 1/3-FR to 3/3-FR 15 N abundance was significantly higher in +AM than in −VAM plants. These results suggest that shifts in source-sink relations occurred both within each plant and between plants as a result of the selective stress imposed on cocklebur. Moreover, this shift in competitiveness permitted an AM-mediated flux of nutrients from weed to crop.

It is interesting that the inhibitory effect of an herbicide on a given AM fungus can differ in a host-dependent manner. For instance, diclofop inhibited root colonization by *G. deserticola* in wheat, but not in ryegrass. Its inhibitory effect on wheat root endomycorrhization directly increased as its dose (10%, 50%, 100%, and 1000% of the dose recommended for field application, 0.9 kg ha^{-1}) rose (Rejon et al. 1997).

Effect of Herbicides on Ectomycorrhizal Fungi

Ectomycorrizal fungi (EMF) can help in plant establishment in nutritionally poor or degraded soils. They increase volume of the exploited soil by roots through the improvement of water and nutrient absorption, in special those with low mobility such as phosphorus (Marx and Cordell 1989). EMF can also provide great

resistance to high temperatures and extreme pH values (Marx and Cordell 1989), increase root longevity (Allen 1991), stimulate dry matter production (Souza et al. 2004), and provide greater tolerance to soil toxicity conditions and pathogens (Allen 1991; Grazziotti et al. 2003). Weeds harm seedling establishment and growth via allelopathy (Toledo et al. 2003) or competition over water, nutrient and light. Additionally, weeds can raise fire risks, interfere with, and even hinder other forestry practices (Tuffi Santos et al. 2006). Therefore, herbicides are broadly applied in silviculture and forestation, where the use of EMF requires some knowledge on the impact of herbicides on these fungi (Fernandes et al. 2014). Ectomycorrhizal associations depend on interaction with soil, environment, and host plants and can be affected by adopted crop management (Campos et al. 2011). Herbicide can directly affect EMF in three ways: (i) some herbicides may exert no influence on EMF (Wardle and Parkinson 1991); (ii) some herbicides may stimulate the growth and/sporulation of EMF (Roslycky 1982); and (iii) some herbicides may inhibit EMF growth and/sporulation as well as ectomycorrhization of host plant roots (Iloba 1978; Culdlin et al. 1983; Trappe et al. 1984). A few herbicides commonly used in foresty were tested for their possible effects on ectomycorhizal development and seedling growth of lodgepole pine (*Pinus contorta* var. *latifolia*) and white spruce (*Picea glauca*), and it was shown that hexazinone, glyphosate and triclopyr are able to reduce seedling growth and mycorhizal development of the plants (Sidhu and Chakravarty 1990). Among these foresty herbicides, triclopyr exhibited the most toxic effect and hexazinon was significantly effective when applied at high concentrations (2 and 4 kg ha^{-1}). Recovery from adverse effects of hexazinone was observed over the time and even no negative effect could be found when the herbicide was applied at low concentration (1 kg ha^{-1}) highlighting the importance of natural adaptation and herbicide dosage applied to the soil. Interestingly, it was found that seedlings with mycorrhizal fungus (*Suillus tomentosus*) are more sensitive to herbicides than those without any type of mycorrhiza. The adverse effect of hexazinone was less in field experiments as only high concentration (4 kg ha^{-1}) of the herbicide could reduce the seedling growth and mycorrhizal infections (Sidhu and Chakravarty 1990). Triclopyr, imazapyr, and sulfometuron methyl did not inhibit ectomycorrhizal formation at concentrations as high as twice the recommended field rates (Busse et al. 2004). Experiments with the isolates of a *Pisolithus* sp. indicated that they were of different tolerances to the herbicides glyphosate as well as isoxaflutole, and the rate of an isolate tolerance depended on the tested herbicide, its concentration, and physical status of the culture medium (liquid or solid). A given herbicide exhibited more toxicity on the growth of a given isolate of the fungus when it was amended into a solid culture medium. Also, no uniformity was found in the behavior of different isolates of the fungus. While the isolate Pt24 was the most tolerant of glyphosate, the isolate UFVJM04 exhibited the highest tolerance to isoxaflutole. The lowest effect of isoxaflutole suggested the possibility of its use in the areas where seedlings inoculated with EMF are applied (Fernandes et al. 2014). Furthermore, glyphosate was found more toxic to the isolates of *Pisolithus* than isoxaflutole.

The growth of *Pisolithus tinctorius* (Fig. 60) on a solid medium decreased because of 1 mg L^{-1} of the herbicides triclopyr, glyphosate, hexazinone, and 2,4-dichlorophenoxyacetic acid and was totally inhibited in 5000 mg L^{-1} of the same herbicides (Estok et al. 1989). On a solid medium, an isolate of *P. tinctorius* exhibited growth reduction by 59% at 50 mg L^{-1} concentration of glyphosate, and complete growth inhibition in same concentration of oxyfluorfen (Paula et al. 1995). However in an experiment with another isolate of *P. tinctorius*, no growth reduction was found in broth medium with glyphosate concentrations up to 10 mg L^{-1} (Lake et al. 1981). Triclopyr and 2,4-D when tested at the rate of 1000 mg L^{-1} completely inhibited the growth of *Hebeloma logicaudum*, while hexazinone and glyphosate were found more toxic and inhibited its growth in 55% at the concentration of 100 mg L^{-1} (Estok et al. 1989). Studies on the effect of herbicides on the growth of ectomycorrhizal fungi under in vitro conditions revealed that hexazinone was highly toxic to *P. tinctorius* (ED_{50} = 1 μg a. i. mL^{-1}), *Suillus hirtellus* (ED_{50} = 1 μg a. i. mL^{-1}), and *Suillus cothurnatus* (ED_{50} = 5 μg a. i. mL^{-1}). Bifenox decreased the in vitro growth of *P. tinctorius* (ED_{50} = 1 μg a. i. mL^{-1}), and

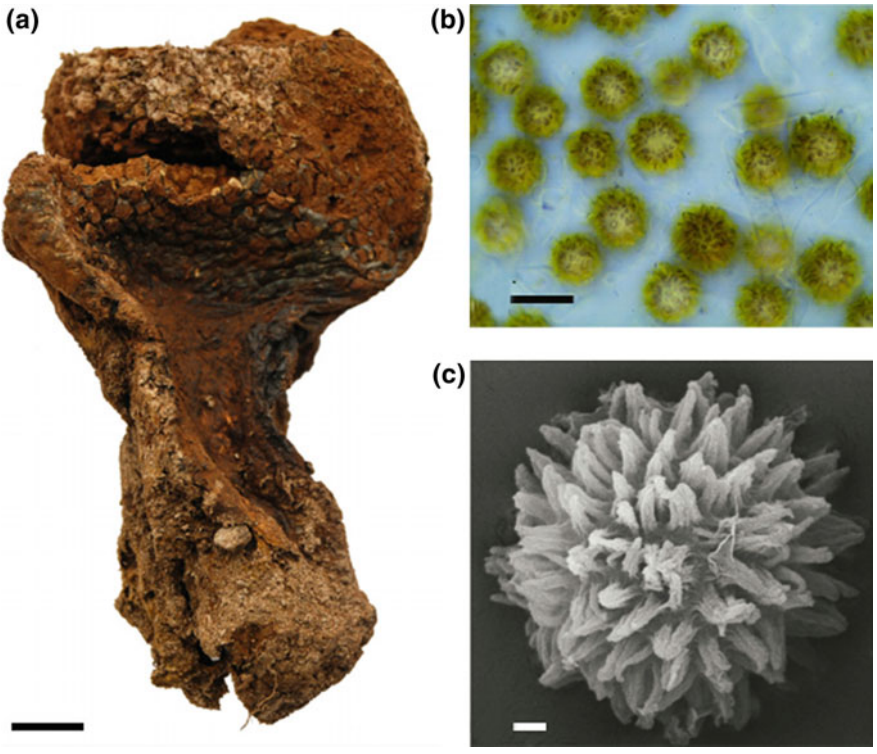


Fig. 60 *Pisolithus tinctorius* (05MCF5506). **a** Basidioma, scale bar = 1 cm; **b** spores under light microscope, scale bar = 10 μm; **c** spores under scanning electron microscope, scale bar = 1 μm (*Source* Rusevska et al. 2015)

S. cothurnatus (ED_{50} = 5 μg a. i. mL^{-1}). Oxyfluorfen had an inhibitory impact on the growth of *P. tinctorius* (ED_{50} = 1 μg a. i. mL^{-1}), *S. cothurnatus* (ED_{50} = 1 μg a. i. mL^{-1}), and *Laccaria laccata* (ED_{50} = 3 μg a. i. mL^{-1}). Oryzalin had a negative impact on the growth of *L. laccata* (ED_{50} = 3 μg a. i. mL^{-1}), *S. cothurnatus* (ED_{50} = 3 μg a. i. mL^{-1}), and *P. tinctorius* (ED_{50} = 10 μg a. i. mL^{-1}). Nitrofen (ED_{50} = 3 μg a. i. mL^{-1}), dipropetryn (ED_{50} = 3 μg a. i. mL^{-1}), trifluralin (ED_{50} = 10 μg a. i. mL^{-1}), and napropamide (ED_{50} = 10 μg a. i. mL^{-1}) were effective in the inhibition of the growth of *P. tinctorius*, while atrazine, propazine, simazine, and perfluidone did not seem to affect its growth at the doses recommended for field applications. Propazine (ED_{50} = 1 μg a. i. mL^{-1}) had a considerable effect on the growth of *S. luteus* under in vitro conditions. Interestingly when applied at different rates of 1, 3, and 500 μg a. i. mL^{-1}, simazine stimulated the growth of *P. tinctorius* and *S. hirtellus*, whereas perfluidone induced the growth of *Thelefora terrestris* (Kelley and South 1980). The ectomycorrhizal species *Rhizopogon vulgaris* and *Gautieria crispa* could metabolize and biodegrade atrazine (Donnelly et al. 1993). *Cenococcum geophilum* was known as an ectomycorrhizal fungus of high potential to biodegrade hexazinone (Donnelly and Fletcher 1994), and its was stimulated in 53% up to 10 mg L^{-1} dose of triclopyr and hexazinone (Estok et al. 1989). The ability to degrade an herbicide apparently depends on the herbicide as well as the mycorrhizal fungus (Donnelly et al. 1993; Donnelly and Fletcher 1994) and the rate of bidegradation of an herbicide by an ectomycorrhizal fungus varies with the ratio of C/N and pH (Donnelly and Fletcher 1994). The ectomycorrhizal fungi *Hebeloma cylindrosporum*, *Suillus bellini*, and *Suillus variegatus* were known as potent biodegraders of the herbicide chlorpropham (Rouillon et al. 1990).

Recently the ectomycorrhizal species *Hebeloma cylindrosporum* (Fig. 61) has been introduced as a model fungus (Fig. 62). Its full genome has been sequenced and annotated at the Joint Genome Institute, and is now available at http://genome.igi-psf.org/Hebcy2.home.htm for public information. Functional studies on the symbiosis are carried out using transcriptomic and proteomic approaches, while the fungus is still useful in secretomic studies and targeted analysis of secreted proteins carried out to evaluate the controversial "saprotrophic potential" of symbiotic fungi and their capacity to mobilise nutrients from forest soils. Its entire life cycle, from spore to spore, can be obtained under in vitro conditions (Debaud and Gay 1987). Just recently, a protocol has been developed for the establishing a symbiotic interface between cultured ectomycorrhizal fungi and plants to follow fungal phosphate metabolism (Becquer et al. 2017). It can easily be transformed using *Agrobacterium tumefaciens* (Combier et al. 2003), and a collection of mutant strains is available, including non-mycorrhizal ones (Combier et al. 2004). Furthermore, the fungus harbors a dominant carboxin resistance gene (*Hc.Sdh*^{R}; Ngari et al. 2009) that may be useful in development of selective media for the fungus. Therefore, the fungus provides an excellent opportunity for studies on the effect of agrochemicals (such as herbicides) on the biology of ectomycorrhizal fungi.

Fig. 61 *Hebeloma cylinrosporum*, an ectomycorrhizal fungus that is frequently and abundantly born in autumn and easily located under pines and holm oaks in pastures of sandy soil. Its descriptive characteristics are as follow: caps, 2–4 cm of diameter, at beginning convex, soon flattened, slightly umbonate, margin slightly striated and somewhat incurved; cuticle, viscous in wet weather, smooth, brown in the center with the lightest edge; stalks, cylindrical, relatively long, of the same color or somewhat lighter than cap, and without appreciable remnants of the curtain, when tearing it retains the sand; meat, thin, whitish with a weak smell; basidiospores (*Source* Mythological Society Extremena, MICOEX, http://micoex.org/2016/09/17/hebeloma-cylindrosporum/) (Color figure online)

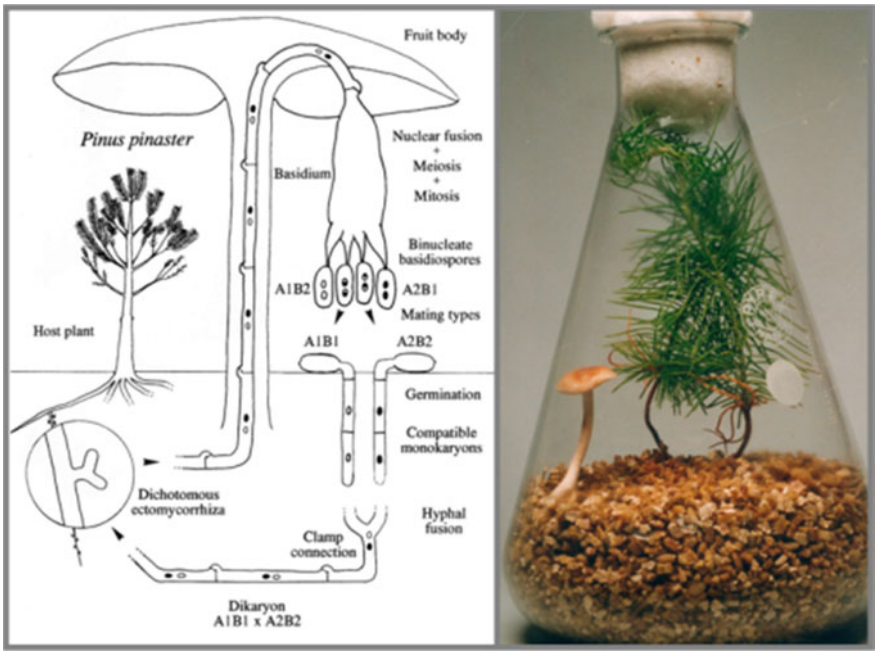


Fig. 62 The basidiomycetous fungus, *Hebeloma cylindrosporum* associated with *Pinus pinaster* as a model to infer the genetic and molecular bases of differentiation and functioning of the ectomycorrhizal symbiosis (*Source* http://www.ecologiemicrobiennelyon.fr/)

Effect of the Herbicides on Microbial Biological Control Agents

Effect of the Herbicides on Antagonistic Fungi

As *Trichoderma* spp. are the most important fungi applied in the biological control of plant diseases, therefore, here we focus on the impact of herbicides on *Trichoderma* spp. Studies with *Clonostachys rosea* 47 (CR47), *Trichoderma atroviride* 59 (TA59), *T. atroviride* 312 (TA312), *T. harzianum* 24 (TH24), *T. longibrachiatum* 9 (TL9), *T. longibrachiatum* 144 (TL144) and *T. viride* 15 (TV15) to evaluate their in vitro sensitivity to four herbicides (chlorsulfuron, chlorotoluron, flufenacet and pendimethalin) indicated that except the isolate TL144, none of the antagonists were sensitive from the stand point of their mycelia radial growth inhibition in the presence of the herbicides applied at field doses. Most antagonists did not exhibit any inhibition of conidial germination as the result of herbicide fungitoxicity. The predisposing impact of diphenamid on the host has been shown to be due to its adverse effect on soil microbial antagonists (Eshel and Katan 1972). Meriles et al. (2006) transplanted bean (*Phaseolus vulgaris* L.) seedlings to soil with previous corn crop residues, previous groundnut crop residues, and no agricultural soil, treated with a range of glyphosate concentrations. Population dynamics of soilborne fungi *Trichoderma*, *Gliocladium*, *Fusarium* and *Pythium* were monitored during a 24-day period after glyphosate treatment to study the effects of the herbicide and previous crop residue on fungal populations. In addition, those genera of soilborn fungi were tested to study in vitro fungitoxicity of glyphosate. Independent on glyphosate concentration, the highest population of *Trichoderma* spp. and *Gliocladium* spp. were noted on soil with previous corn residue. *Fusarium* and *Pythium* populations increased proportionally to the increase in glyphosate concentration. Glyphosate did not have any effect on *Trichoderma* and *Gliocladium* populations. *Trichoderma* isolates can be adversely affected by some herbicides commonly used for tea plantation (Islam et al. 2008). Among the tested herbicides, Bimaster was the only one which completely inhibited the growth of *Trichoderma.* Other herbicides including glyphosate, paraquat, kem-Amin and butachlor also negatively affected the fungal growth in the media. As *Trichoderma* isolates are of well-known antagonistic fungal microorganisms in tha soil, it is necessary that to precisely manage the incorporation of these herbicides into the soil. The in vitro effect of the herbicide ohinol 50 SC (50% napropamide) on *Trichoderma harzianum* and in vivo influence on colony forming units per gram of soil, also, the interaction between its quantity and the intensity of the root rot disease induced by *Rhizoctonia solani* were studied by Gveroska (1999). Different doses and methods were tested. The inhibition of dry biomass yield and radial growth proportionally increased with the increase of the applied dose of the herbicide. The highest decreases of *T. harzianum* quantity was found in 0.2 g m^{-2} before +0.3 g m^{-2} herbicide application after sowing. The greater quantity led to reduced intensity of the disease. The most suitable approach of *Trichoderma* application with tobacco seedlings was determined as the sowing of the seeds

already kept in pure culture of the biocontrol agent followed by after sowing application of the herbicide in a dose of 0.5 g m^{-2} (Gveroska 1999). Additionally, our simple experiments exhibit the possibility of the application of some of biological control agents such as *Talaromyces flavus* and various *Trichoderma* species in integrated disease management programs synchronous to or following the intelligent application of herbicides. Furthermore, our unpublished data from the experiments with sethoxydim and *Fusarium graminearum* and *Trichoderma* isolate No. 100 have well indicated that despite of the increased growth rate of *F. graminearum* on herbicide-amended culture media, the pathogen is still amenable to the biological control with the *Trichoderma* isolate No. 100, inferring to high capacity of its application in the integrated plant disease management programs against two major pathogens of wheat and rapeseed crops, namely *F. graminearum* and *Sclerotinia sclerotiorum*. To apply herbicides, the positive effects of some weeds shall also be considered, for example their role in the preservation of the beneficial parasitoids useful in control of pests. An experiment with the commercially available herbicidal formulation, Atlantis® OD 42 (mesosulfuron + iodosulfuron) was performed in order to study its potential effect on the interaction of the biological control fungus, *Trichoderma asperelloides* and the mycotoxigenic fungal pathogen *Fusarium graminearum* on potato dextrose agar (PDA) medium amended with the Atlantis® OD 42 to the final concentrations of 0, 50, 500, and 5000 ppm (Pakdaman and Elahifard, unpublished data). Interestingly, Atlantis® OD 42 inhibited the growth of both fungi when it was applied at the highest concentration (a dose higher than that recommended for field applications). *T. asperelloides* could finally control the mycotoxigenic fungus in the presence of lower concentrations of the herbicide (Fig. 63) under in vitro conditions (darkness, 26 °C). Also, the herbicide increased the pathogen resistance (R) and its linear growth (p) parameter in the presence of *T. asperelloides*, and reduced Pakdaman's Biological Control Index (PBCI), an index already introduced to study the effect of various biotic and abiotic factors (Pakdaman et al. 2013).

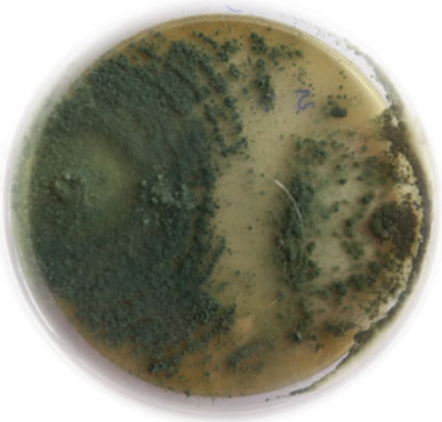


Fig. 63 Growth and sporulation of *Trichoderma asperelloides* over the mycotoxigenic pathogen *Fusarium graminearum* in potato dextrose agar medium amended with Atlantis® OD 42 (500 ppm) (*Source* Pakdaman and Elahifard, unpublished data)

In another study, it was shown that fungal strains from three genera including *Penicillium* and *Trichoderma* could use an herbicide, metsulfuron-methyl (MM) as a sole source of carbon and energy (Vázquez and Bianchinotti 1999). Among these genera, *Trichoderma* isolates exhibited the highest capacity for using the herbicide. These isolates could use the herbicide during their growth. Fungal spores could germinate in the media containing MM and use it as a carbon and energy source. These isolates were introduced as promising bioremediation agents (mycoremediants) to eliminate the herbicidal pollution from the environment (Vázquez and Bianchinotti 1999). *Trichoderma viride*, in particular, has been found to be sensitive to paraquat. The inhibitory effects were observed at concentrations well within the range likely to be experienced in the field (Wilkinson and Lucas 1969). Romero et al. (2014) indicated that a few soil fungi such as *Gliocladium roseum,* were able to degrade one of the widely used herbicides, atrazine in both pure cultures and polluted soil sediments. As atrazine residue in soil and water imposes lasting environmentally noxious effects, using living microorganisms to degarade this chemical would be promising in the removal of these pollutant residues from the natural habitats.

Effect of the Herbicides on Antagonistic Actinobacteria and Bacteria

Actinobacteria with their capacity to produce a broad spectrum of antibiotics and extracellular enzymes represent a high proportion of the soil microbial biomass (Doumbou et al. 2001), especially in suppressive soils (Postma et al. 2008). As an important part of soil microflora, actinobacteria are regarded as (i) a source of agroactive biological compounds, (ii) a group of plant growth promoting soil microorganisms, and (iii) tools for the biological control of plant diseases (Doumbou et al. 2001; Palaniyandi et al. 2013). Taxonomy, physiology, and natural products of *Actinobacteria* have recently been reviewed (Barka et al. 2016). Microbial community is significantly affected by the application of herbicides into the soil. When a few herbicides including atrazine, Primeextra (a combination of atrazine plus metolachlor), paraquat and glyphosate were incorporated into soil, bacterial, and actinobacterial populations decreased immediately. However, the soil microorganisms adapted to this chemical stress as their population was recovered over time (Sebiomo et al. 2011a). Microorganisms degrade herbicides and they may serve as bioindicators of soil changes following herbicide application. Simultaneously, the numbers of actinobacteria and less so of fungi increase, indicating that these microorganisms use herbicides as sources of biogenous elements. Rate of herbicidal decomposition depends on the properties of the preparation applied, herbicide dose as well as on the physical and chemical soil properties, soil moisture and temperature, ground cover, agrotechnical measures applied and the resident microbial population (Milošević and Govedarica 2002). Some herbicides such as isoproturon (Hussain et al. 2014), carfentrazone-ethyl (Tomkiel et al. 2015), sulfosulfuron (Kucharski and Wyszkowska 2008), terbutryn (Zaid et al. 2014), carbetamide (Zaid et al. 2014), propyzamide (Zaid et al. 2014), terbutryn + propyzamide (Zaid et al. 2014), metribuzin (Zaid et al. 2014; Hussain et al. 2014), Successor T (pethoxamid

and terbuthylazine; Wyszkowska et al. 2016), ametryn (Bera and Ghosh 2013), atrazine (Sebiomo et al. 2011b), Primextra (a mixture of atrazine plus metolachlor; Sebiomo et al. 2011b), paraquat (Sebiomo et al. 2011b), glyphosate (Sebiomo et al. 2011b), and glufosinate (Pampulha et al. 2007) are of negative impact on the growth of actinobacteria. Interestingly, hand weeding reduced actinobacterial populations highly significantly, and its effect on actinobacteria was higher than terbutryn, but less than that of other tested herbicides (Zaid et al. 2014). However, pre-emergent herbicides atrazine, metsulfuron methyl, metolachlor, and anilofos were of no negative impacts on soil actinobacteria in rain-fed maize field (Ramesh and Nadanassababady 2005). Other herbicides, for instance sulfentrazone (Martinez et al. 2008), glyphosate (in a dose of 2.16 mg kg^{-1} soil dry weight; Araújo et al. 2003), a mixture of diflufenican + mesosulfuron-methyl + iodosulfuron-methyl-sodium (Baćmaga et al. 2015) stimulate the growth of actinobacteria. The stimulation of the growth and development of actinobacteria by herbicides has been known as a general phenomenon by Milošević and Govedarica (2002) occurring with dimethenamide and metolachlor applied to sugarbeet (Govedarica et al. 2001), prometryn applied to soybean and sunflower (Milošević and Govedarica 2001), flumetsulam + trifluralin (Rival®), and alachlor + linuron (Linuron®), imazethapyr and clomazone (Milošević and Govedarica 2002). The population of actinobacteria did not increase significantly with any type or concentration of the employed herbicides including clodinafop propargyl 15% WP (applied at the rate of 400 g ha^{-1}), metribuzin 70% WP (applied at the rate of 300 g ha^{-1}), isoproturon 75% WP (applied at the rate of 1333 g ha^{-1}), mesosulfuron-methyl and iodosulfuron methyl sodium (Atlantis® 3.6% WG, 400 g ha^{-1}), Sulfosulfuron® 75% WG (applied at the rate of 33.33 g ha^{-1}), and UPH-110 54% WG (clodinafop propargyl and metribuzin) applied at the rates of 400, 500, 600, and 1000 g ha^{-1}. At field recommended dose, isoproturon (up to 15th day) and metribuzin (up to 7th day) reduced the actinobacterial population but other treatments did not alter the populations. UPH-110 was not of any significant impact at lower application rates, but it could significantly depress actinobacterial populations at higher doses, persisting up to 7th and 15th day of application. The toxicity of UPH-110 was directly correlated with its dose of application (Hussain et al. 2014).

Paraquat (25 μg mL^{-1}), pentachlorophenol (PCP, 25 μg mL^{-1}), and picloram (50 μg mL^{-1}) inhibited *Pseudomonas fluorescens*, while nitralin (25 μg mL^{-1}), and gluphosate (Gloria 2010) stimulated its growth. Atrazine, diuron, trifluralin, and fluometuron were found of no significant impact on *P. fluorescens* growth rate when tested at the concentrations of 25, and 50 μg mL^{-1} (Breazeale and Camper 1972). Sulfometuron methyl (0.41 mM) was found more toxic to in vitro growth of *P. aeruginosa* than *P. fluorescens* (93% compared to 33% growth inhibition; Burnet and Hodgson 1991). Similarly, chlorsulfuron (0.56 mM) was of more toxicity to in vitro growth of *P. aeruginosa* than *P. fluorescens* (93% compared to 24% growth inhibition; Burnet and Hodgson 1991).

Effect of Herbicides Through Their Interactions with Pesticides

Herbicides interact with each other as well as with other pesticides. Here, we focus on the interactions of herbicides with other group of pesticides. Such interactions may lead to synergistic, additive, neutral, or antagonistic effects on the pathogens, vectors, as well as biological control agents. Therefore, these interactions can influence plant disease incidence and severity.

Effect of Herbicides Through Their Impacts on the Antifungal Activity of Fungicides

Herbicides may have enhancing impact on the antifungal activity of the fungicides applied in the chemical control of fungal plant diseases, and vice versa. The effect of two herbicides (paraquat and simazine on the antifungal activity of two fungicides (captan and mounsrin) against *Rhizoctonia solani* was studied. When the herbicides paraquat and simazine were applied to soil they altered the effectiveness of both fungicides in controlling *R. solani*, thus causing damping-off of cotton. Both herbicides increased the toxicity of both fungicides against mycelial growth of the fungus. In pot tests, seed/soil treatment with captan or mounsrin gave better control of *R. solani* damping-off disease when the soil treated with paraquat or simazine was compared to untreated soil. Captan was, however, found to be more effective in controlling the disease than mounsrin (Awadalla and El-Refaie 1994).

The herbicides fluchloralin and alachlor applied to soil altered the effectiveness of fungicide treatments to seed and soil for controlling cowpea damping-off. These herbicides also modified the in vitro toxicity of the fungicides to the mycelial growth of *Pythium butleri* and *Rhizoctonia solani* in a nutrient medium. Both herbicides reduced the toxicity of 2-methoxyethylmercury chloride (MEMC) and propamocarb to the growth of *P. butleri*, and of carbendazim to the growth of *R. solani*, but enhanced the toxicity of captafol and quintozene to *P. butleri* and *R. solani*, respectively. In pot tests, quintozene gave better control of *R. solani* damping-off in soil treated with fluchloralin or alachlor than in untreated soil, whereas disease control by carbendazim was decreased in similarly treated soils. Both herbicides attenuated the effectiveness of MEMC and captafol applied to control the damping-off caused by *P. butleri*. The efficacy of propamocarb was increased by alachlor but was decreased by fluchloralin. The implications of herbicide-fungicide interactions are discussed in the context of fungicidal control of root diseases in herbicide-treated soil (Kataria and Dodan 1982). In vitro studies have proved the influence of herbicides on the toxicity of fungicides. Ward (1984) found that glyphosate suppresses the anti-oomycotic activity of metalaxyl. Singh et al. (1999) indicated the synergistic effect of fluchloralin on the toxicity of matalaxyl against the oomycetous fungus-like *Phytophthora*, despite it reduced the fungicidal activity of captafol. Singh et al. (1999) indicated that two pre-emergence soil fungicides, fluchloralin and pendimethalin affected the in vitro toxicity of some fungicides against pigeaon pea blight pathogen, *Phytophthora drechsleri* f. sp. *cajani*. Both herbicides enhanced the antifungal activity of Apron® 35 WS, and Ridomil® MZ-72, while reduced the fungitoxicity of captafol. Moreover, when

combinations of the herbicides and pesticides were used, most of them (10 combinations) acted synergistically while only two combinations acted antagonistically. The researchers concluded that these herbicides can be mixed with these fungicides in order to efficiently achieve both herbicidal and fungicidal effect.

The combination of the fungicide cyproconazole and four herbicides DNOC (dinitro-ortho-cresol), dicamba, ioxynil and bromoxynil were tested singly as well as in mixtures against *Rhizoctonia cerealis* and *Pseudocercosporella herpotrichoides*, and cyproconazole-herbicide mixtures exhibited synergistic activity against both fungi in vitro as well as in bread wheat (*Triticum aestivum* L.), where the level of synergism depended on the relative proportion of fungicide and herbicide components in the mixture (Kataria and Gisi 1990).

Effect of Herbicides Through Their Impacts on the Insecticidal Activity of Insecticides

The search of literature for the interactive effect of herbicides and insecticides on insect pests did not lead to any result. The only study on the interactive effects of herbicides and insecticides have been carried out with the honey bee, *Apis mellifera*, where no additive/synergistic interaction was found with Advise® (58.6 mg a. i. L^{-1} imidacliprid) + Roundup® (1217.5 mg a. i. L^{-1} glyphosate). However, this does not mean the infeasibility of the incidence of herbicide-insecticide interactive effects on insect vectors of plant diseases, and there are reports on the direct entomotoxicity of at least some herbicides (Castilla et al. 2010).

Effect of Herbicides Through Their Impacts on the Nematicidal Activity of Nematicides

The effects of nematicides carbofuran and fenamiphos and the herbicides metribuzin and trifluralin, alone and in combination, on hatching, penetration, development, and reproduction of root knot nematode *Meloidogyne incognita* race 3 was studied under laboratory conditions. To study hatching, entire egg masses were exposed to nematicides (6 μg mL^{-1}), herbicides (0.5 μg mL^{-1}), and their combinations over a 16 day period. The hatched juveniles were extracted and counted every 24 h. Second-stage juveniles that hatched from day 6 to day 8 were used as inoculum to determine the effect of the chemicals on penetration, development, and reproduction of the nematode on tomato 4, 16, and 32 days after inoculation. Fenamiphos, alone and combined with either of the herbicides effectively inhibited hatching, therefore, it was not possible to get enough juveniles for post-infection development studies. However, carbofuran, trifluralin, metribuzin, carbofuran combined with either of the herbicides were of no impact on hatching, penetration, and development of females, or reproduction. Apparently, the herbicides were of no antagonistic effect on the nematicidal activity of both nematicides (Payan et al. 1987). Interestingly, while alachlor, vernolate, metribuzin, and trifluralin were of pathancing effects on the population development of *Heterodera glycines* when applied at recommended rates under field conditions (Bostian et al. 1986; Kraus

et al. 1982), metribuzin and trifluralin improved the efficacy of aldicarb, when the nematicide was combined with each of the herbicides (Kraus et al. 1982). The combined application of the herbicide alachlor with phenamiphos led to reduced efficacy of phenamiphos against the number of eggs of *H. glycines*. Also, linuron and alachlor synergistically increased the nematicidal activity of aldicarb, where application of the nematicide in bands was of higher impact than in furrow (Schmitt et al. 1983). In an experiment with sugarcane plants grown in two irrigated, and non-irrigated systems, no interaction was found between herbicides (diuron, ametrin, oxyfluorfen, and pendimethalin) and systemic nematicides (aldicarb, and terbufos) in the sense of reducing nematicide efficiency, except in an irrigated experiment for the number of stalks in herbicide treatments and the number of stems and stalks in nematode treatments. Nematicides and herbicides did not affect (Pol) and (PCC) levels at harvesting. However, a synergistic interaction between nematicide and herbicide was found with *Pratylenchus zeae* in the rhizosphere in irrigated system, where aldicarb and pendimethalin interactive effect brought down the nematode population to the lowest rate (Barros et al. 2006). While incorporation of Avadex® (40% diallate) into the soil at 3.5 L ha^{-1} just before planting led to high levels of soil infestation with *Heterodera schachtii* in sugar beet field, the combined application of the herbicide together with the nematicide Temik® 10 G (10% aldicarb) applied at 10 kg ha^{-1} in the furrow at planting time resulted in the greatest reduction in cyst production at all sampling dates ($P = 0.05$) and stabilized the enhanced effect of Temik® 10 G against sugar beet cyst nematode (Kraus and Sikora 1983). The pathancing effect of Avadex® has been attributed to its effect on the lipid layer of the nematode egg shell (Perry and Beane 1989), the presence of which was demonstrated by Perry and Trett (1986).

4 The Share of Herbicides in the Integrated Plant Disease Management

There are two point of views in the control of plant diseases. One emphasizes on the control of a disease on a plant species, and the other intends to control major diseases of a plant in an area (Singh 2001). Here, a third view point is introduced, that is to control of the diseases of crop plants that are grown in rotation in a given area. While the previously introduced viewpoints focus on a crop, the new viewpoint focus on the disease of the crops in a given altering agroecosystem wherein different crops are grown in rotation. With an eye to what mentioned above, there remains no doubt that herbicides, if intelligently chosen, can play an importantly great role in the integrated plant disease management programs. With enough ecological and toxicological information on the consequences of a special herbicide application in an agricultural program, it will be possible to control weeds of the grown crop in field and in the mean time, to reduce disease risks imposed by different pathogens, and the population of vectors through the removal of their wild

host plants. Herbicides can be selected in a manner that reduce the inocula of the pathogens expected to be problematic for the current and/next crop due to be grown in a given rotation program. All these will help farmers to reduce their costs for the production of agricultural products, and will provide a way to diminish the probability of the rapid emergence of resistant pathogenic populations, and to reduce the need for more repeated application of antimicrobials. Herbicides with a mode of action different from that of fungicides can help in the decrease of fungal pathogen population and the risk of fungicide-resistance development. The herbicides of chemical structures other than those of antimicrobials are expected to be useful in the control of the pathogenic microbes adapted to the pesticides (in its general meaning) due to the increased activity of the plasma membrane transporters. It seems possible to decrease population of some important pathogens through the intelligent application of well-selected herbicides against weeds.

The formulation/and co-application of the herbicides together with the biological control agents not only will help to control weeds but also will aid plant residue management for pest and disease risk diminishment as well as recyclization of the nutrients and their re-incorporation into soil environment for the preservation of soil fertility. The application of mycoherbicides and other biological products may help to the control of the plant diseases caused by the pathogenic microorganisms of close taxonomic relationship, although their mixed application with chemical herbicides will ensure higher control output against resistance development in weeds. The herbicides effective against necrotrophic pathogens but still allowing the bioactivity of biological control agents are suitable for crop residue management. Alternatively, such herbicides can be co-formulated/mixed with proper fungicides that are more toxic to pathogens than biological control agents. However, more studies are required in this neglected research area, and a research performed with imazapic and the biocontrol fungus *Pyrenophora semeniperda* in order to control winter annual grass, *Bromus tectorum* (Ehlert et al. 2014) well indicates the necessity of further information required for the integrated control of weeds. The compatibility of herbicidal formulation and biological control agents, the timing as well as method of application, the host range of the biological control agent are among important factors that determine the result. Herbicides effective against pathogens or pests are of advantage and can lead to reduced control costs and environmental damages such as soil erosion. Such a view point is expected to have an effective impact in the subsequent conversion of disease conducive soils to disease suppressive and or ideally composite soils, as has been discussed before (Higa and Parr 1994).

With the herbicides like glyphosate, there are strategies to ameliorate their predisposing effects on disease. These strategies include judicious selection of herbicide application rates, micro-nutrient amendment, glyphosate detoxification in meristematic tissues and soil, changes in cultural practices to enhance micro-nutrient availability for plant uptake, and biological amendment with GR microbes for nitrogen fixation and nutrient availability. Given that the recommended doses of glyphosate are often many times higher than that needed to control weeds, it is believed that the most prudent method to reduce the detrimental effects

of glyphosate on GR crops will be to use this herbicide in as small a dose as practically needed. Such a frugal approach will not only curtail disease predisposition of GR crops, but will also benefit the grower and the environment. Toxicity of glyphosate to Mn-reducing and synergistic nitrogen-fixing organisms in the rhizosphere can have serious consequences for sustainability of legume production. Regular inoculation of legume crops with synergistic nitrogen-fixing organisms may be required in many areas for maximal productivity where extended applications of glyphosate have eliminated them from the soil profile. Development of glyphosate-tolerant nitrogen-fixing and Mn-reducing organisms would be beneficial in many of these situations, and especially for perennial Roundup Ready® legume crops such as alfalfa (Huber et al. 2004).

There is little information on the precise effects of herbicides on the pathogens, biological control agents, and their interactions under in vitro, in vivo, and *in soilum* conditions. Such information is very essential for effective integrated management of plant diseases as well as for the companies that intend to continuously improve their products in the world of increasing concerns, knowledge, and competitive marketing intensified by continuous decrease of agricultural lands due to increasing population and climatic changes. It is a global need to not only preserve the current fertility of agricultural lands but also increase the fertility of the ever-decreasing available lands, and keep them free of pollutants. Finally, I want here to finish this

Fig. 64 An old Indian proverb that well indicates the way we should take for future (*Source* http://www.geckoandfly.com/)

chapter with an Indian proverb (Fig. 64) once I knew it by a poster on the wall in the office room in Martin Luther University, Halle-Wittenberg, Halle (Saale), Germany.

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Ecological Methods for Weed Management

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Abstract Weed ecology is influenced by the plant community composition, the evolution of weeds, allelopathy and competition. Climatic, edaphic and biotic factors determine the distribution of weed species, their type, prevalence and intricate relationships resulting in association of weeds whose ecological requirements tally with the crop. Environmental factors such as temperature, osmotic potential, pH, salinity, light, burial depth and management practices affect seed germination and emergence of weeds. These factors are important for the development of integrated weed control strategies. We review ecological weed association, germination ecology of weeds, crop-weed competition, weed flora in different crops and ecological approaches for weed management. We discuss shifting the focus from weed control to weed management and how the integration of cultural, mechanical and chemical practices can reduce weed establishment. Weeds reduce crop yield by competition for light, water, nutrients and space, interfering with harvesting operations and increasing the cost involved in crop production. Cultural practices can be manipulated for eliminating or reducing the population of major weeds. Overall, knowledge of weed ecology can be used as a tool for effective weed management.

Keywords Cultural practices · Germination · Temperature · Weeds

1 Introduction

Nature has provided the adaptability to weeds in the form of certain mechanisms that they emerge besides all odds to survive in whatsoever environment it is. Weed ecology refers to the study of the interaction or relationship between a weed and its environment. The major difference between a crop and a weed is that weeds simply do not know to grow in lines. The ecological role of weeds can be seen in many

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E. Lichtfouse (ed.), *Sustainable Agriculture Reviews 31*, Sustainable Agriculture Reviews 31, https://doi.org/10.1007/978-3-319-94232-2_4

different ways, depending on one's perspective. Most commonly, weeds are perceived as unwanted intruders into agro-ecosystems that compete for limited resources, reduce crop yields and force the use of large amounts of human labor and technology to prevent greater crop losses. Excessive weed growth is also a nuisance on uncultivated/wastelands.

Weeds are perceived as plants that possess adaptive characteristics which allow them to invade, survive and reproduce in cropping systems. In developing countries, farmers may spend 25–120 days hand-weeding in one hectare of cropland, yet still lose a quarter of the potential yield from the crops due to severe weed competition (Akobundu 1991; Parker and Fryer 1975). There are many limitations of hand weeding in addition to high labour cost like many weed plants may escape due to their close morphological similarity with the crop plants. During initial growth stages, barnyard grass (*Echinochloa crus-galli*) and wrinkle grass (*Ischaemum rugosum*) resemble morphologically with rice crop, whereas litteseed canary grass (*Phalaris minor*) and wild oats (*Avena ludoviciana*) with wheat plants. Apart from morphological similarities of weeds with the crops during vegetative stage, some weeds produce seeds morphologically similar to that of crops. For example, seeds of blue daisy (*Cichorium intybus*) resemble with seeds of Egyptian clover (*Trifolium alexandrinum* L.) with respect to size, shape and colour. So, this weed is disseminated into new areas with the seeds of Egyptian clover crop. Other problems encountered during manual weeding are that some weeds like canada thistle (*Cirsium arvense*) and mexican poppy (*Argemone mexicana*) bear thorns on different plant parts and the plants are difficult to uproot. Another very important characteristic possessed by weeds is their quick growing habit even under adverse climatic conditions and their tendency to germinate ahead of the crop due to which they take lead in competition.

2 Losses Due to Weeds

Among various pests that infest crops, weeds account for about 45% reduction in yield while the insects 30%, diseases 20% and other pests 5%. If a crop produce contains weed seeds, it is to be rejected especially when the crop is grown for seed. The wild oat weed seeds have very similar size and shape as that of crops like barley, wheat and its admixture may lead to rejection of crop seed lot for seed purpose. Weeds also serve as food sources for some animals and are themselves susceptible to many pests and diseases. They may serve as important reservoirs or alternate host of pests and diseases because of their close association with crops. When crop is heavily infested with certain weed, it will limit the growth of a particular crop. Weeds reduce human efficiency through physical discomfort caused by allergies and poisoning. Weeds such as congress grass (*Parthenium hysterophorus*) causes itching, bronchitis and dermatitis.

In India, unchecked growth of weeds causes 60–70% yield loss in fababean (Nehra and Malik 1999), 62% in grain cowpea (Mathew et al. 1995), 46.6% in

lentil, 40.6% in wheat, 28.1% in barley (Pandey et al. 1998) and 63.9–76.5% losses in rhizome yield of *Curcuma longa* L. (Kaur et al. 2008). In few crops like sugarcane weeds germinate 30–40 days before the emergence of crops and if weeds are not controlled properly, these may reduce crop yields from 10–50% or even more depending upon the crop variety and associated weed species. Only broadleaf weeds cause 17%, only *Avena* spp cause 36.6% and both broadleaf and grass weeds cause 45.1% reduction in wheat yield (Walia and Brar 2001). In corn, the yield reduction of about 77.4% by grass weeds, 44.2% by broadleaf weeds and 38.4% by sedges has been reported (Pandey et al. 2002). Weeds were reported to cause up to one-third of the total yield losses. Indian weed scientists estimated 10–100% losses in some surveys due to weeds (DWSR 2013). About 10% loss by weeds (Bhan et al. 1999) would cause approximately US$13 billion loss of food grains (Yaduraju 2012). Losses of this magnitude due to weeds may occur in plantation crops, fruits, vegetables, grasslands, forestry and aquatic environments. The potential yield loss due to weeds recorded in different major crops are viz. chickpea 10–50, pea 10–50, cotton 40–60, pearlmillet 16–65, fingermillet 50, pigeonpea 20–30, greengram 10–45, potato 20–30, groundnut 30–80, rice 10–100, horsegram 30, sorghum 45–69, jute 30–70, soybean 10–100, lentil 30–35, sugarcane 25–50, corn 30–40, vegetables 30–40 and wheat 10–60% in India (Rao et al. 2014).

3 Useful Aspects of Weeds

In spite of all the losses caused by weeds, they can offer some beneficial properties, particularly when occurring at low densities. In India, people consume smooth pigweed (*Amaranthus viridis*), false amaranth (*Digera arvensis*), purslane (*Portulaca*) and nettle leaf (*Chenopodium album*) species as nutritious food in vegetables and are also used as fodder for cattle. *Chlorella pyrenvoides* is used in Japan and China for extracting good quality protein for human consumption. Seeds of *Cichorium intybus* are used for increasing the taste and flavour of coffee. In western Rajasthan, yields of sesame and pearl millet can be increased by allowing the crops to grow in association with the leguminous weed *Indigofera cordifolia* (Bhandari and Sen 1979). Some weeds have medicinal properties viz. *Leucas aspera* for curing snake bites; *Argemone mexicana* seeds for healing skin diseases. Certain weeds may limit insect damage to crops by interfering with pest movement or by providing habitat for natural enemies of pests (Nentwig et al. 1998). Quack grass (*Agropyron repens*) serve as an important source of fodder and medicine (Chacon and Gliessman 1982) and provide habitat for birds and other desirable wildlife species (Sotherton et al. 1989). Reduction of root knot nematode population has been reported by incorporation of *Crotolaria, Parthenium, Calotropis* and *Eichhornia* spp. Some weeds have other commercial uses like *Cyperus rotundus* nuts are used for making perfumes and *Saccharum* spp. for making thatches. *Cactus* and *Opuntia* spp. are used for fencing the fields against wild animals. *Croton sparsiflora* when incorporated into the soil add to organic matter of the soil.

Weeds can also be used as mulch for reducing the evaporation losses of water from soil surface. Some weeds accumulate metals in their tissues in excess concentrations than those present in the environment and act as metal indicators. For example, boron is concentrated in *Salsola nitrata*, cobalt in *Crotolaria cobaltica*, copper in *Commelina* spp., iron in *Acacia patens*, manganese in *Crotolaria florida*, gold, cadmium and uranium in *Artemsia tridentata*, copper, lead, zinc and cadmium in *Eichhornia crassipes*. *Saccharum spontaneum* is used for breeding purposes in sugarcane; *Datura* in genetic studies for understanding the phenomenon of inheritance and *Chorella* to understand the process of photosynthesis. Weed species can conserve soil moisture and reduce soil erosion by soil binding effects of their roots. Weeds can serve as an important source of food for wildlife, especially birds. These types of beneficial effects indicate that weeds are not just agricultural pests, but can also play beneficial roles in agro-ecosystems.

4 Ecological Weed Association

Weeds possess many growth characteristics and adaptations which enable them to successfully exploit the numerous ecological niches left unoccupied by crops. Among the important adaptations relevant to competitive advantage are synchronization in germination, rapid establishment and growth of seedlings, tolerance to shading effects by the crop or by other weeds at the time of establishment, quick response to available growth factors like soil moisture, light and nutrients, adaptation to the most severe climatic situations of the habitat such as extremes of temperature, salinity, moisture stress and pH, adaptations to the edaphic regime and herbicide resistance. In the initial stages of habitat invasion by weeds in exposed ecological niches, only a very limited competition for resources by the crop and weed may occur; but as establishment of the crop-weed association is completed, competition for the available resources is more. Intense competition occurs when the demand of the plants for moisture, nutrients, light etc. exceed the available supply. The ultimate outcome of crop-weed competition usually results in the adverse effects of presence of weeds on growth and yield of crops.

Some weeds have very strong association with a particular crop because of ecologically similar requirements of both crop and weeds. So, by changing crop, ecological requirement of the associated weeds are not met with and as a consequence, weed growth will be less. For instance, *Phalaris minor* had strong association with wheat and *Echinochloa crus-galli* with rice and these weeds can be seen in abundance if rice-wheat rotation is adopted continuously on the same field. Similarly parasitic weeds have specific host crop like *Cuscuta chinensis* in Egyptian clover (*Trifolium alexandrinum*) and these weeds can be controlled by not sowing the host crop in the infested field. Few parasitic weeds like *Cuscuta chinensis* die after germination if suitable host like lucerne/alfafa (*Medicago sativa*) is not available. *Striga* spp. is a parasitic weed of sugarcane and its occurrence can be avoided by rotating sugarcane with any other summer season crop. Quick growing

crops can also smother the weeds very effectively. For example, growing of pearl millet (*Pennisetum glaucum*) for fodder or pigeon-pea (*Cajanus cajan*) during summer season smother weeds very effectively and growing of *Brassica* crops or Egyptian clover (*Trifolium alexandrinum*) during winter season can suppress problematic weeds like *Phalaris minor*.

Thus, the climatic, edaphic and biotic factors of environment determine the distribution of weed species, their type, prevalence and intricate relationships resulting in association of weeds whose ecological requirements tally with the crop.

4.1 Germination Ecology of Weeds

The important factors that determine a weed's ecological interactions are light, temperature, soil pH, salinity, burial depth and soil moisture. By understanding weed ecology, the weaknesses of particular weed can be known, which can be exploited as opportunities for weed management.

4.1.1 Light

It plays an important role in germination because seeds of some weed species buried in the soil undergo transition from primary to secondary dormancy (Benvenuti and Macchia 1995). However, the germination response of different weed species to light and darkness varies. Weed species can be classified into three different groups based on their response to light. Non-photoblastic weed species, such as *Mimosa invisa* Mart. ex Coll (giant sensitive plant) and *Melochia corchorifolia* L. (redweed) can germinate equally in light and dark and positively photoblastic species, such as *Cyperus iria* L. (rice flatsedge) and *Eclipta prostrata* (L.) that do not germinate in the dark at all and light is essential for their germination. The third category is intermediate group of weed species, such as *Echinochloa colona* (L.) Link (jungle rice) and *Amaranthus viridis* L. (slender amaranth) in which light is not a requirement for germination but light stimulates germination. Germination of *Conyza canadensis* seeds is independent of light and seeds can germinate either under no light or when subjected to alternate periods of light/dark (13/11 h) (Nandula et al. 2006). Seeds of *Delairea odorata* (Cape ivy) do not persist in areas exposed to intense and prolonged sun exposure, but can survive in areas with reduced light (Robinson et al. 2011). Light is not a pre-requirement for seed germination of *Tagetes minutas* (Kumar and Sharma 2012). Seeds of *Delonix regia* germinated better in an environment with longer period of darkness than light; Seeds of *Corchorus olitorius* and *Amaranthus cruentus* germinated efficiently in light than in dark environment (Ologundudu et al. 2013). The seeds buried deep in the soil may not be able to germinate as light cannot penetrate beyond a few millimeters into the soil (Woolley and Stoller 1978). The quality and quantity of

Fig. 1 Effect of light (24 h) and dark (24 h) on germination and growth of *P. minor*

light that penetrates in the soil may also depend on the soil type and its physical state. Species that require light for germination could be reduced by stale seedbed practices before crop sowing (Fig. 1).

4.1.2 Temperature

Temperature is one of the key environmental factors that influences cellular metabolism and control the growth processes like seed germination and dormancy (Mohammed and Tarpley 2010). However, there is no optimum and uniform temperature for all species. Alvarado and Bradford (2002) reported that germination rate usually increases with temperature linearly within a well defined range and then declines sharply at higher temperatures. Gorai et al. (2011) reported that *Salvia aegyptiaca* L. can germinate in temperature range between 10 and 40 °C with maximum germination at 30 °C. Norsworthy and Oliveira (2006) observed that germination of *Senna obtusifolia* (sicklepod) occurred over a range of constant temperatures from 15 to 50 °C, with optimum germination between 15 and 30 °C. Tanveer et al. (2012b) found that seeds of *Carthamus oxycantha* showed maximum germination (75%) at 20 °C and minimum germination was recorded at 30 °C. Seeds of *Convolvulus arvensis* germinated over wide range of temperatures from 15 to 40 °C, while complete inhibition was observed at 45 °C (Tanveer et al. 2013). Burke et al. (2009) found that *Dactyloctenium aegyptium* (crowfootgrass) germinated over wide range of temperature (15–40 °C) with optimum germination at 30 °C. Fang (2012) reported that germination of *Aegilops tauschii* (tausch's goatgrass) seeds occurred at temperatures ranging from 0 to 40 °C with optimum germination between temperatures 15 and 25 °C. *Amaranthus retroflexus* prefer

temperatures above 25 °C for optimum germination while *Chenopodium album* and *Ambrosia artemisiifolia* both prefer temperature below 11 °C. Chachalis et al. (2008) reported that under constant temperatures of 10, 15, 20, 25, 30, 35, 40 and 45 °C germination in seeds of *Hibiscus trionum* (venice mallow) was <40%. However, a fluctuating day/night temperature of 30/20 °C resulted in highest germination percent. Chauhan et al. (2006b) found that freshly harvested seeds of *Sonchus oleraceus* germinated over a broad range of fluctuating day/night temperatures of (25/15, 20/12, and 15/9 °C) with maximum germination (93%) at 20/12 °C.

4.1.3 Moisture

Water availability is another important abiotic factor responsible for the germination of plants. Seed germination and early seedling growth are stages in the life cycle of plant that are more sensitive to water shortage. Lack of water is an important environmental stress limiting seed germination. Moisture stress delays the onset of seed germination, slows the rate of germination and decreases final germination percentage (Patanè et al. 2013). Some weeds like common cocklebur (*Xanthium strumarium*), barnyardgrass (*Echinochloa crus-galli*) and crabgrass (*Digitaria sanguinalis*) mostly grow in humid regions, whereas Kochia (*Kochia scoparia*) and Russian thistle (*Salosal iberica*) can grow in dry conditions (Weise and Vandiver 1970). Germination of *Oenothera laciniata* (cutleaf evening primrose) seeds was drastically decreased when subjected to increased water stress (Clewis et al. 2007).

Harris et al. (2002) reported that the first and foremost effect of moisture stress is impaired germination and poor seedling establishment. Germination of horseweed (*Conyza canadensis*) decreased from 25 to 2% as osmotic potential decreased from 0 (distilled water) to −0.8 MPa indicating that germination can still occur under moderate water stress conditions (Nandula et al. 2006). Ali et al. (2013) exposed the seeds of *Rhynchosia capitata* to PEG solutions with six levels of osmotic potential (0, −0.2, −0.4, −0.6, −0.8 and −1.0 MPa) and found that germination of *R. capitata* was completely inhibited at osmotic potential of −1.0 MPa. Germination decreased from 100 to 15% as osmotic potential decreased from 0 to −0.6 MPa. However, more than 10% germination at an osmotic potential of −0.6 MPa indicates that some seeds of *R. capitata* can germinate under marginal water-stress conditions. Rezvani et al. (2014) reported that seeds of *Capsella bursa-pastoris* were not able to tolerate high drought stress conditions as germination was completely inhibited at an osmotic potential level of −1.0 MPa. Delachiave and Pinho (2003) observed that decrease in osmotic potential from 0 to −0.6 MPa caused a decrease in percent germination and the speed of germination in *Senna occidentalis.* Larson and Kiemnec (2005) exposed seeds of Russian knapweed (*Acroptilon repens*) and perennial pepperweed (*Lepidium latifolium*) to polyethylene glycol (PEG) solutions with four levels of osmotic potential (0, −0.2, −0.4 and −0.6 MPa) and found that

germination of Russian knapweed was completely inhibited at all the levels of osmotic potential, whereas germination of perennial pepperweed occurred at −0.2 MPa. Germination of *Emex spinosa* decreased from 68.3 to 0.83% as the osmotic potential decreased from 0 (distilled water) to −0.8 MPa indicating that it is highly sensitive to moisture stress (Shoab et al. 2012).

4.1.4 Salinity

Salinity is an environmental stress that limits growth and development of plants and response of plants to excess NaCl is complex involving changes in their morphology, physiology and metabolism (Kaya et al. 2001). High salt concentration prevents water from entering the seed and inhibits the germination. Chauhan et al. (2006c) reported that germination of *Lolium rigidum* (rigid ryegrass) decreased linearly as NaCl concentration increased from 0 to 200 mM. Tanveer et al. (2012a) found that seed germination of *Cucumis melo* decreased with increasing concentrations of NaCl solution and only 15% seed germination was recorded at 150 mM NaCl solution. Wei et al. (2009) observed that germination of *Solanum rostratum* (buffalobur) seeds decreased as NaCl concentration increased from 0 to 320 mM. However 2% germination at NaCl concentration of 320 mM suggested that buffalobur seeds were fairly tolerant to salt stress in the soil. Germination was greater than 95% at NaCl concentration less than 40 mM and decreased to 52% at 160 mM concentration of NaCl. Pahlevani et al. (2008) observed that *Cynanchum acutum* (swallowwort) seeds were not able to germinate at 300 mM NaCl, however, 12% germination was recorded at 200 mM NaCl concentration. Lu et al. (2006) found that *Eupatorium adenophorum* (crofton weed) germination was 65% at 100 mM

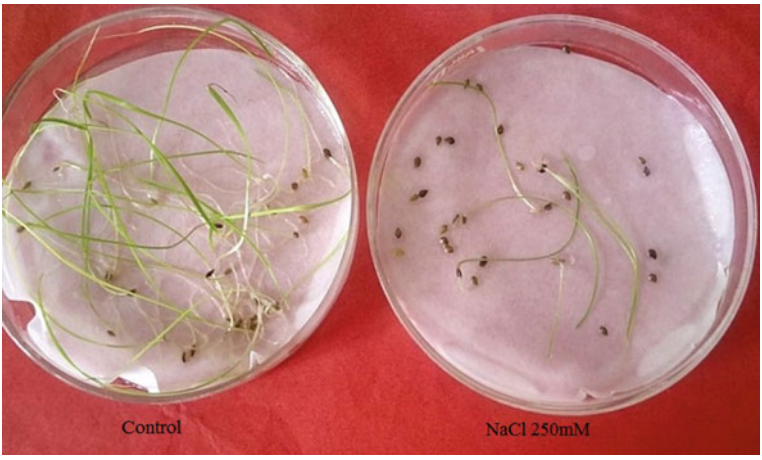

Fig. 2 Effect of NaCl 250 mM on germination and growth of *P. minor*

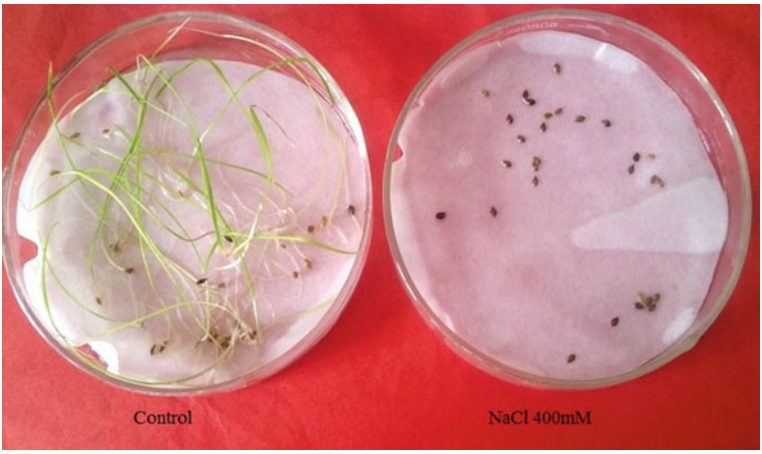

Fig. 3 Effect of NaCl 400 mM on germination and growth of *P. minor*

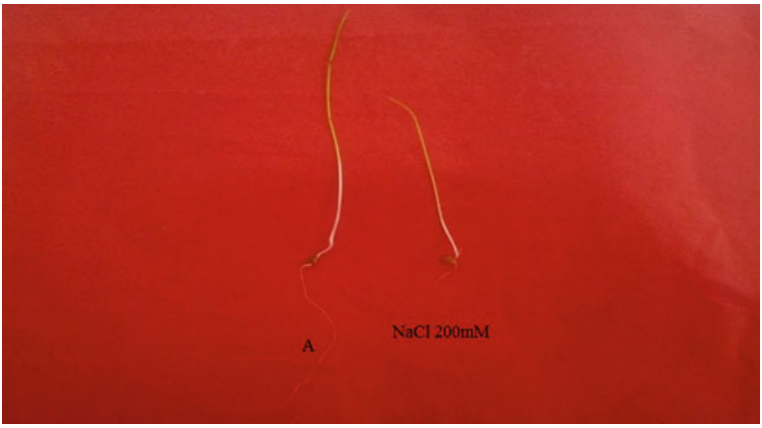

Fig. 4 Effect of NaCl on seedling of *P. minor*

NaCl concentration and only 10% seeds germinated at 250 mM concentration of NaCl And completely inhibited at 320 mM. Chauhan and Johnson (2008) observed that *Eclipta prostrata* was able to tolerate salinity and showed 83% germination at 150 mM NaCl and germination was declined to 37% at 200 mM NaCl with complete inhibition at 250 mM NaCl concentration (Figs. 2, 3, and 4).

4.1.5 pH

Some weed species can grow only on particular soil environment conditions and attention to amend these conditions has a major function in weed management. Weed distribution pattern can be influenced by the ability of weed to tolerate pH of the soil (Nandula et al. 2006). *Synedrella nodiflora* (synedrella) showed 84% germination between pH 4 and 8, however, germination declined to 73% when pH increased from 8 to 10 (Chauhan and Johnson 2009). Norsworthy and Oliveira (2005) observed that optimum pH for germination of *Cassia occidentalis* (coffee senna) was 6. Increasing pH from 3 to 6 increased the germination, however, pH > 6 decreased the germination. At pH 3, germination ranged from 9 to 12%, but was completely inhibited at pH 10, indicating that *Cassia occidentalis* germination was more tolerant to acidic than basic solutions. Wang et al. (2009) reported that pH did not affect the germination of *Urena lobata* and maximum germination (88%) occurred at pH 6 and germination was declined to 70 and 67% at pH 4 and 9, respectively. Vanijajiva (2014) reported that seeds of *Tridax procumbens* germinated over pH range of 4–10 with higher germination in the pH range of 6–7. Thomas et al. (2006) exposed the seeds of *Amaranthus viridis* to buffer solutions of pH 3–10 and observed that seeds germinated best at pH 7 and 8 and germination was declined to 28% at pH 3 and 11. Germination of *Beckmannia syzigachne* (American sloughgrass) was unaffected by pH and >80% germination was recorded at all tested pH values from 4–10 (Rao et al. 2008). *Melinis repens* (natalgrass) seeds do not successfully germinate under extremely acidic or basic pH conditions (Strokes et al. 2011). Clewis et al. (2007) observed that germination of *Oenothera laciniata* decreased as pH increased from 3 to 9, with greatest germination occurring at pH 4 suggesting preference to acidic soil conditions (Fig. 5). Germination of *Urochloa subquadripara* (tropical signalgrass) was greatly reduced

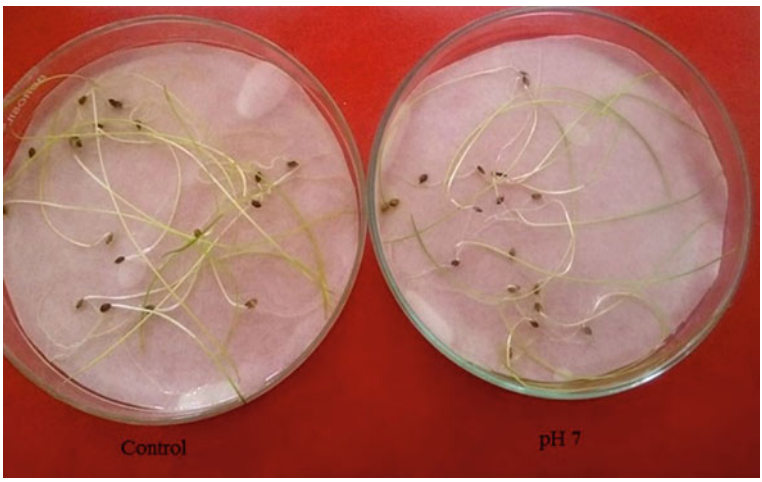

Fig. 5 Effect of pH 7 on germination and growth of *P. minor*

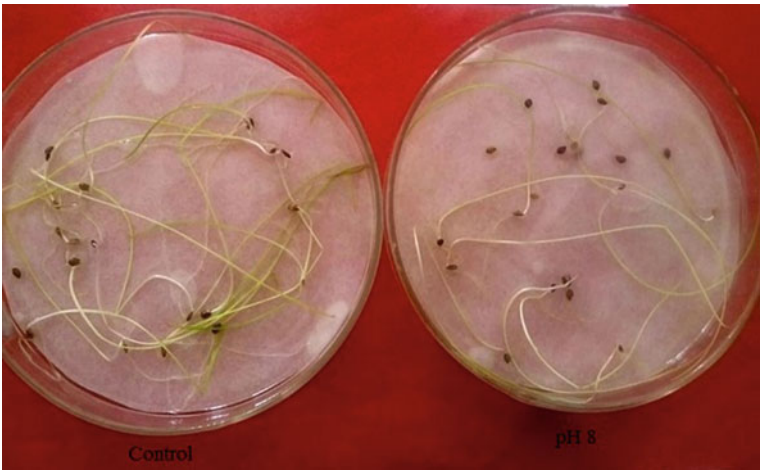

Fig. 6 Effect of pH 8 on germination and growth of *P. minor*

at pH less than 5 or greater than 6 with complete inhibition at pH 10 (Teuton et al. 2004). Seeds of *Rapistrum rugosum* (turnipweed) subjected over a broad pH range of 4–10 showed germination >76% indicating that pH was not a limiting factor for germination in most soils (Chauhan et al. 2006d) (Fig. 6).

Detailed knowledge about the environmental factors required for weed seed germination is an important prerequisite for the development of integrated and biological weed control strategies. Environmental factors such as temperature, osmotic potential, pH, salinity, light, burial depth, crop residues and management practices affect seed germination and emergence of weeds (Ganepour et al. 2014) (Figs. 7 and 8).

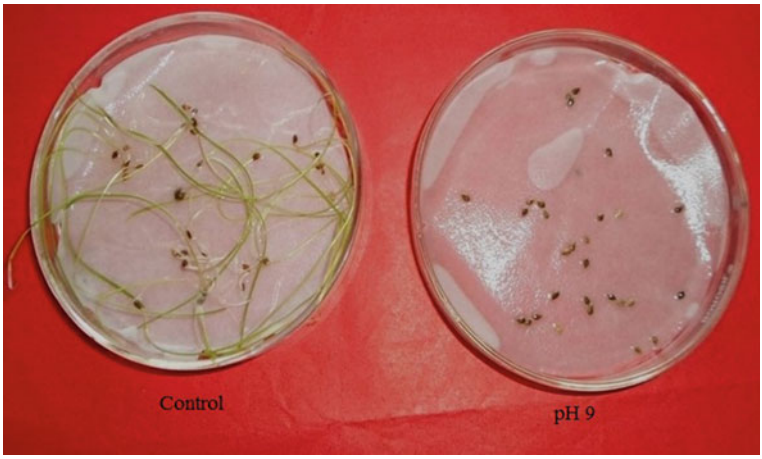

Fig. 7 Effect of pH 9 on germination and growth of *P. minor*

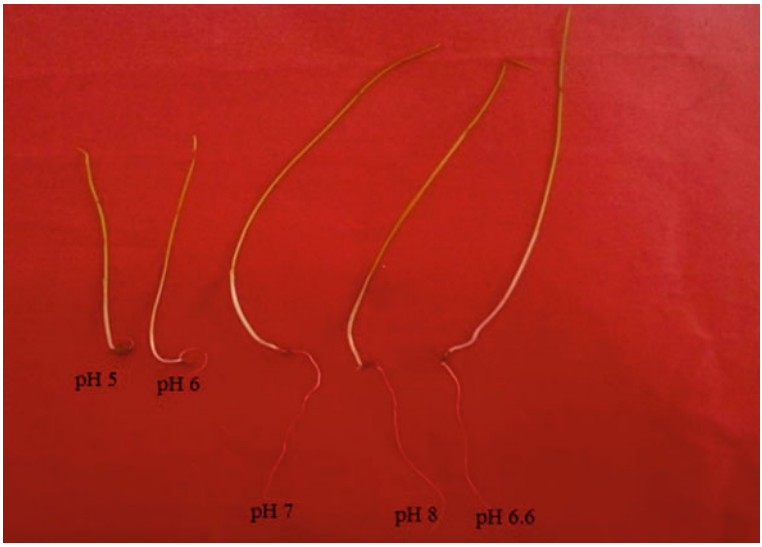

Fig. 8 Effect of pH on *P. minor* seedlings

4.1.6 Seed Burial Depth

Germination and emergence of seeds is reduced with increasing depth of planting. The main reason for lack of germination at greater depth is induction of secondary dormancy in seeds. Highest germination percentage of fieldbind weed (*Convolvulus arvensis*) is observed at a depth of 1.5 cm (Asgharipour 2011). Maximum germination of *Ipomoea purpurea* seeds occur when it is sown at 0 and 2 cm depth in soil (Singh and Singh 2009). Seeds of *Urena lobata* placed on the soil surface exhibited highest germination (Awan et al. 2014). Seedling emergence of little mallow (*Malva parviflora*) was greatest at burial depths of 0.5–2 cm but declined progressively with further increase in depth and no emergence occurred from seeds placed at depth of 8 cm or deeper (Chauhan et al. 2006a). Seed burial inhibited germination of *Rumex obtusifolius* also in proportion to depth. Maximum seedling emergence occurred when the seeds were placed at 2 cm depth and decreased with increasing depth of planting and no seed emerged from depth of 10 cm (Benvenuti et al. 2001). Ivyleaf speedwell (*Veronica hederifolia*) seed germination gradually increased with decreasing burial depth (Grundy et al. 2003). Li et al. (2015) reported that seedling emergence of *Bromus japonicas* was greatest (98%) when seeds were placed on the soil surface but increased burial depth significantly decreased the emergence with only 7% of seedlings emerging from a depth of 5 cm. Different soil depths vary in availability of moisture, temperature and light (Chauhan et al. 2006a) which affects the behavior of weed seed banks from different soil profiles. Zero-till systems leave most of the weed seeds on or close to the soil surface. In a study of direct seeded rice (DSR), 77% of the seeds were retained in

the top 2-cm soil layer under zero-till systems, whereas the consequence of soil disturbance under conventional tillage resulted in 62% of the seeds being buried to a depth of 2–5 cm (Chauhan and Johnson 2009).

Soil texture, soil fertility, soil moisture and soil reaction greatly influences the crop growth. However, weeds are adapted to grow well in moisture stress and ample moisture conditions under wide pH range. Apparently, adverse weather conditions like drought, excessive rains, extremes of temperature, will favour growth of weeds but most of our crops are susceptible to climatic stresses. In general, when the time of sowing of crop coincides with the emergence of first flush of weeds, it leads to intense crop-weed interference. Weed seeds germinate most readily from 0 to 2 cm of soil but few weeds can germinate even from 15 cm depth. Therefore, planting method that dries the top 3–5 cm of soil more rapidly to deny weed seeds opportunity to absorb moisture for their germination usually postpones weed emergence until the first irrigation. By this time, the crop plants are well established to compete with late germinating weeds.

Weed seed banks are pools of viable weed seeds present in the soil layers which may compete with a crop during the growing season (Forcella 1993). Here the proverb "One year seeding seven years weeding" holds very true. Weed seed banks have strong deposits of seeds produced in the field itself and also dispersed into it from elsewhere by different means like wind, water, farmyard manure, animals, contaminated crop seeds and agricultural machinery or equipments used for different field operations. In Asia, the use of contaminated rice seeds and harvesting machinery are mainly responsible for weedy rice. Different tillage practices also influence the weed seed distribution in the soil profile (Chauhan et al. 2006a), germination and emergence greatly affect the relative abundance of weed species in the field (Mohler 1993).

5 Crop-Weed Competition

Competition is a negative interaction where individuals make simultaneous demands that exceed limited resources. The competition does not start as long as the growth factor is abundant in supply. But the competition starts when two or more plants present in a given area demand particular growth factor and instantaneous supply of that factor falls below their combined demand. Crop-weed competition in a natural ecosystem in response to struggle for resources for their existence and superiority is often reflected on the growth and yield of the crop. Weeds reduce crop yield by competing for light, water, nutrients and space, interfere with harvesting operations and increase the cost involved in crop production. The yield reductions are generally in proportion to the amount of light, water or nutrients used by the weed plants at the expense of a crop (Zimdahl 1999).

5.1 Water

Water is primary environmental factor limiting crop production and is probably the most critical of all the plant growth requirements. Weeds compete for water, reduce its availability to immediate crop and contribute to water stress. Weeds consume water intended for crops, cause water loss by seepage through root channels, transpire water and cut water flow in irrigation ditches, leading to higher water consumption by weeds and more evaporative losses of water (Zimdahl 2013). Competition for water is greater when roots of crop and weeds are closely intermingled and obtain their water from the same volume of soil. Water extraction pattern of weeds are more close to root zone volume of a species rather than the above ground biomass. Weeds have higher transpiration coefficient than crop plants and thus, remove more water from soil as compared to many crops. Competition for water varies from season to season, year to year, site to site and even from species to species. Under water stress condition, weeds can reduce crop yields by more than 50% through moisture competition depending on weed density, the plant's physical characteristics and root distribution within soil profile (Abouziena et al. 2014). In general, for producing equal dry matter, weeds transpire more water than do most of the crop plants. *Echinochloa crus-galli* and crab grass (*Digitaria sanguinalis*) severely deplete soil moisture and pose intense competition to corn crop (Wiese and Vandiver 1976). The consumptive use of water for lambsquarter weed (*Chenopodium album*) has been estimated about 550 mm against 479 mm for wheat crop. Weeds can remove moisture from deeper layers of soil than crops. Common lambsquarter require 658 lb of water to produce one pound of dry matter, common sunflower 623 lb and common ragweed 912 lb, compared with 349 lb for corn and 557 lb for wheat (Parker 2003). In a study of competition for water between palmer amaranth (*Amaranthus palmeri* S. Wats.) weed and irrigated corn; it was found that total water use by *A palmeri* continually rose as densities rose from 0 to 8 plants per meter of corn row. Water use efficiency (WUE) of corn continued to decrease with rising *A palmeri* density resulting in corn yield losses from 11 to 91% as density increased from 0.5 to 8 plants per meter, respectively. Although raising weed density decreases soil water, the competitive ability of different weed species at similar densities may not have the same influence on water use (Massinga et al. 2003).

5.2 Nutrients

Weeds usually absorb higher mineral nutrients at a faster rate than many of crop plants and accumulate these in their tissues in relatively large amounts. Uncontrolled weeds removed 52.3, 9.6 and 38.9 kg of N, P, K per ha, respectively in corn (Sreenivas and Satyanarayana 1996). Many weeds are high nitrogen consumers thus limiting nitrogen for crop growth. *Amaranthus* spp. often accumulate

over 3% nitrogen in their dry matter (Hans and Johnson 2002). Weeds compete for essential nutrients and decrease the crop yield even at high rate of fertilization (Vengris et al. 1955). Nitrogen content of weeds in the zero N plot was 31 and 39% less than the weeds grown in 50 and 90 kg N per ha, respectively (Jalali et al. 2012). Redroot pigweed (*Amaranthus retroflexus*) dry weight contribution to total weed dry weight and N uptake was the highest at early corn growth stage. It has been also reported that all the weed species examined contained more copper in their biomass than corn, but their percent share in total uptake was as small as 1.7%. The content of zinc in corn biomass was similar, except for *Cirsium arvense* L. which accumulated considerably less zinc than corn as well as the other weed species. The percent share of weeds in zinc uptake was only 1.4 of total uptake by the corn crop. Competitiveness of weeds in the accumulation of manganese and iron showed high species specificity. *Chenopodium album* L. and *Galinsoga parviflora* Cav. were the most competitive in accumulating manganese, while *Cirsium arvense* L. showed high ability to accumulate iron that was considerably much higher than corn and other weeds species (Glowacka 2012). In an another study, Glowacka (2011) reported that *Galinsoga parviflora* Cav. was the most competitive species with corn for potassium; *Cirsium arvense* L. for calcium; *Chenopodium album* L. and *Polygonum lapathifolium* L. *subsp. lapathifolium* for magnesium. Presence of velvetleaf (*Abutilon theophrasti*) had negative effect on corn growth and yield at the lower phosphorous rates; whereas presence of this weed at the higher phosphorous application rates had no significant effect on corn growth (Aghaie et al. 2013). Thus, weeds absorb nutrients and transpire large quantities of water that would have otherwise available for crop growth.

5.3 Space

Weeds compete for space and reduce the yield of crop plants. Faster growth of weeds in terms of height and leaf development results in suppressed crop growth by limiting the space and light availability. This effect can be overcome to some extent by reducing the row spacing and increasing the crop densities. Reduction in row spacing has been reported to increase the yield of corn by narrowing down the competition for space (Tharp and Kells 2001). Weed infestation significantly reduced the photosynthetically active radiation available to the lower leaves of the corn plants leading to yield reduction (Assemat and Allirand 1996). Greater net photosynthesis of lambsquarters (*Chenopodium album* L.) may be partly attributed to its earlier emergence and higher leaf area relative to corn (Colquhoun et al. 2001). Exposure to low red to far red light ratio caused by the presence of *Amaranthus retroflexus* delayed leaf appearance and reduced shoot dry weight of corn and it was concluded that plant competition is triggered initially by the red to far-red light ratio (R:FR) originating from neighbouring plants, followed by a series of complex physiological processes which exclude direct resource competition (Liu et al. 2008). Competition from weeds may be reduced by stimulating quick

germination of crop plants so that crop can form a dense canopy quickly that can shade emerging weed seedlings. Thus, it is apparent that weeds compete with crop plants for space and ultimately through shading deprive the crop of sunlight and hence restrict the crop growth due to less photosynthetic activity.

Critical Crop-Weed Competition Period

The critical period has traditionally been defined in days or weeks after emergence, not stages of crop growth. The time of weed control is as important as competitiveness of weeds. It depends upon the duration of interference with the crop (Akhtar et al. 2000). The longer the weeds remain in competition with crop, the greater is the damage caused to the crop (Anderson 2000). The identification of critical period of weed control is essential for successful weed management in various crops. Critical period of weed competition is defined as the shortest time span during the crop growth when weeding results in highest economic returns. Critical period of weed control may also be defined as the time length during which crop must be kept weed free to prevent yield loss due to weed interference (Martin et al. 2001). However, the critical period of weed competition is not necessarily the time of most intense interference (Hall et al. 1992). Therefore, it is better to use the term critical period of weed control instead of critical period of weed competition. The critical period of weed control is determined by calculating the time interval between two components of weed interference. These are (1) the critical weed interference period or the maximum length of time during which weeds emerging soon after crop planting can coexist with the crop without causing unacceptable yield loss, and (2) the critical weed free period or the minimum length of time required for the crop to be maintained weed free before yield loss caused by late emerging weeds is no longer a concern (Evans et al. 2003; Hall et al. 1992). Critical crop-weed competition is determined by characterising functional relationships between two separately measured competition components–crop yield as a function of the duration of weed interference to identify the beginning of critical period of weed control and crop yield as a function of duration of the weed free period to identify the end of critical period of weed control (Williams and Masiunas 2006).

Corn and cotton being widely spaced crop with slow early growth, allows the weeds to compete easily as compared to other cereals, emphasizing the need to start weed control at early growth and development stages. Critical period for weed control in corn has been reported from second to third week after seeding where heavy infestations of *Echinochloa crus-galli, Chenopodium album, Digitaria sanguinalis* and *Portulaca oleracea* can reduce corn yield by 23% (Ferrero et al. 1996). Weeds germinated 10 days after emergence were able to compete with corn as compared with the weeds that germinated 20 days after corn emergence which did not have any noticeable effect on corn green matter yield (Adzgauskiene and Jakstaite 1997). Weeds that emerge simultaneously with the crop or shortly after the crop can cause severe yield losses at very low densities. There is great loss in crop yield if weeding is practised after the critical period of weed competition (Bhutada 2015; Kanteh et al. 2014). In assessment of determining critical period of

crop-weed competition in sesame, it was reported that when crop was kept weed free for first 30 days after sowing of crop, then there was significant increase in seed yield as compared to weedy condition during that period in sesame (Duary and Hazra 2013). In India, critical period of crop-weed competition (CPCWC) for transplanted rice is 20–40 days after transplanting (Mukherjee et al. 2008), in wet seeded rice is 15–60 days after seeding, in wheat is 32–40 days after sowing (DAS) (Chopra et al. 1999) and in spring maize is 30–60 DAS (Kiranjit et al. 2016). Climate, crop genetics and cultural practices like row spacing are some of the several factors that may influence the critical period of weed control by directly affecting weed composition, weed density, time of weed emergence relative to the crop. Therefore, determining the critical period of weed control could help in reducing yield losses due to weed interference. The critical period of weed control could be used to enhance the efficacy of various methods of weed management. It is useful in making decisions on need and timing of weed control for maintaining optimum crop yield. Understanding of the critical period of weed control is also one of the most important tool in integrated weed management.

6 Weed Flora in Different Crops

Out of the total 826 weed species reported in India, 80 are considered as very serious and 198 as serious weeds (Anonymous 2007). Several studies were conducted on weed flora in India which include: corn in Punjab (Sandhu et al. 1999); potato in Haryana (Punia et al. 2007); rice-wheat system in Indo-Gangetic plains (Singh et al. 2005); wheat in Punjab and Haryana (Singh et al. 1993; Singh et al. 1995; Brar and Walia 2007); soybean in Madhya Pradesh and Himachal Pradesh (Jain and Tewari 1993; Rana and Angiras 1994); sunflower in Delhi (Wanjari et al. 1999); pointed guard in Assam (Barua et al. 2002); and tea in southern part of India (Ilango and Sharma 2008) (Table 1). Weedy rice is emerging as a major problem in direct-seeded rice (Rao et al. 2007; Rao and Nagamani 2007).

7 Ecological Approaches for Weed Management

There are many ecological approaches to tackle the weed problems in field by devising effective and viable management techniques such as crop rotation (Martin and Felton 1993), variable plant density (Kappler et al. 2002), crop geometry (Sharma and Angiras 1996), manipulation of sowing time (Virk et al. 2003) that allow the crop weed competition in favour of crop.

Among the non-chemical methods, cultural control can play an important role in reducing weed population pressure for providing an edge to crop over the weeds. The cultural weed management is based on agronomic manipulations which are non-monetary and are also environment friendly. These techniques help to modify

Table 1 Weed flora of major crops in India

Crop	Weed flora		
	Grasses	Broadleaved weeds	Sedges
Wheat	*Avena ludoviciana, Phalaris minor, Poa annua, Polypogon monspeliensis, Sorghum halepense*	*Chenopodium album, Coronopus didymus, Fumaria parviflora, Medicago neglecta, Rumex dentatus, Rumex spinosis, Medicago denticulata, Melilotus indica, Anagalis arvensis, Solanum nigrum, Convolvulus arvensis, Cirsium arvense*	
Rapeseed & Mustard	*Phalaris minor, Avena ludoviciana*	*Medicago denticulata, Sonchus arvensis, Anagallis arvensis*	
Berseem	*Poa anua, Polypogon monspeliensis*	*Cichorium intybus, Rumex dentatus, Coronopus didymus, Trianthema portulacastrum, Cuscuta chinensis*	
Rice	*Echinochloa crus-galli, Echinochloa colona, Dactyloctenium aegyptium, Leptochloa chinensis, Ischaemum rugosum, Paspalum distichum*	*Ammania baccifera, Alternanthera, Caesulia axillaris, Digera arvensis, Eclipta alba*	*Cyperus rotundus, Cyperus iria, Cyperus compressus, Cyperus difformis, Fimbristylis tenera, Scirpus tuberosus, Scirpus roylei*
Direct seeded rice	*Echinochloa colona, Dactyloctenium aegyptium, Leptochloa chinensis, Eragrostis tenella, Commelina benghalensis*	*Digera arvensis, Phyllanthus niruri, Ammannia baccifera, Alternanthera spp.*	*Cyperus rotundus, Cyperus difformis, Cyperus compressus*
Corn	*Acrachne racemosa, Brachiaria reptans, Dactyloctenium aegyptium, Digitaria sanguinalis, Digitaria ciliaris, Eleusine indica, Eragrostis tenella, Commelina benghalensis, Echinochloa colona*	*Trianthema portulacastrum, Portulaca oleracea, Solanum nigrum, Amaranthus viridis, Tribulus terrestris, Cleome viscosa, Phyllanthus niruri, Physalis minima, Mollugo* spp, *Digera arvensis*	*Cyperus rotundus, Cyperus compressus*

(continued)

Table 1 (continued)

Crop	Weed flora		
	Grasses	Broadleaved weeds	Sedges
Cotton	*Dactyloctenium aegyptium, Digitaria sanguinalis, Eragrostis tenella, Echinochloa colona, Eleusine indica, Cynodon dactylon, Sorghum halepense*	*Ipomoea* spp., *Trianthema portulacastrum, Digera arvensis, Celosia argentia, Portulaca oleracea*	*Cyperus rotundus*
Sugarcane	*Dactyloctenium aegyptium, Digitaria sanguinalis, Brachiaria reptans, Eleusine indica, Sorghum halepense, Cynodon dactylon*	*Physallis minima, Ipomoea pestigridis, Ipomoea nil, Celosia argentea, Conyza stricta*	*Cyperus rotundus*
Summer Pulses	*Dactyloctenium aegyptium, Eragrostis tenella, Digitaria sanguinalis, Acrachne racemosa*	*Trianthema portulacastrum, Digera arvensis, Celosia argentea, Physalis minima*	*Cyperus rotundus*
Groundnut	*Eragrostis pilosa, Dactyloctenium aegyptium, Digitaria ciliaris, Commelina benghalensis*	*Tribulus terrestris, Mollugo spp,. Bulbostylis barbata, Trianthema portulacastrum, Celosia argentea, Digera arvensis*	*Cyperus rotundus, Cyperus compressus*

the crop environment in favour of crop by inducing quick canopy growth of crop or by early establishment of crop plants to smother the weeds. Competitive ability of crops against weeds is affected by number of factors such as weed density, crop density, weed species, crop species and variety, soil factors, moisture, pH, climate, time of sowing, crop rotation etc. With the increase in density of weeds, there is decrease in yield. Increase in plant population of crop decreases weed growth and reduce competition until they are self competitive. Dense weed growth is usually noticed in wider row spaced crops like cotton (Fig. 9). In such cases, square planting of crops with equal row and plant spacing or bi-directional sowing are ideal for reducing intra-crop plant competition. Different crop varieties differ in their competing ability with weeds. Fast canopy forming, tall and vigorous crops suffer less from weed competition than the slow growing and short statured crops.

Ecological weed control approaches are becoming popular among the farmers as they will not only reduce the cost of crop production but will also save the environment from the hazardous effects of herbicides. Different cultural practices can be manipulated for eliminating/reducing the population of major weeds. Rice-wheat is very popular cropping system in the Indo-Gangetic Plain Region and the major weeds of these crops i.e. *Phalaris minor* (little seed canary grass) in wheat and

Fig. 9 Infestation of weeds in cotton crop

Echinochloa crus-galli (barnyard grass) in rice can be effectively controlled with the adoption of certain agronomic practices.

7.1 Crop Cultivars

Crop species and cultivars are known to differ in their competitiveness with weeds (Lemerle et al. 1995) as they have variations in morphological features, different growth habits, canopy structure, competitive ability, relative growth rate and rate of maturity leading to weed suppression. Appropriate cultivar selection has the potential to significantly influence the level of weed control. Competitive cultivars can be adopted by the farmers as a part of integrated weed management at little or no additional cost. So, correct choice of cultivar is not only essential in exploiting the crop's ability to compete with weeds but also in maintaining crop quality (Fig. 10).

Growth and development of *P minor* can also be suppressed by selecting quick growing wheat varieties like PBW 621 and PBW 550 as compared to DBW 17 and WH 542 due to more leaf area index (Sharma and Kaur 2012). Cultivars with quick initial growth and higher leaf area must be preferred in order to reduce crop weed competitions. Crop varieties that are tall with higher tillering capacity, leaf area and particularly higher initial vigour are more competitive against weeds.

Fig. 10 Effect of crop cultivars on weeds

7.2 Crop Rotation

Weed infestation can be minimized by rotating the crops because weeds are associated with certain crops due to their identical ecological requirements. Crops can be harvested before weeds set seed to break the life cycle. Adoption of rice-potato-wheat, rice-potato-sunflower and rice-Egyptian clover (*Trifolium alexandrinum*) rotations resulted in significant reduction in dry matter accumulation by *Phalaris minor* as compared to rice-wheat and rice-mustard rotations (Walia and Brar 2004). Inclusion of Egyptian clover–a fodder crop in cropping system can help in reducing the seed bank of *P. minor* within a lesser period of time because of the mowing of emerged plants of *P. minor* with each cutting of fodder. Similarly in potato-based rotations, uprooting of germinated *P. minor* plants takes place with earthing up or digging operations. The left over *P. minor* plants are uprooted during land preparation for succeeding crops and hence resulted in reduced soil seed bank status. Inserting crop having different seeding and maturity time can break the life cycle of some important weeds which prevents the build-up of particular weed specie.

7.3 Time of Sowing

After studying biology of a troublesome weed, the sowing of the crop can be manipulated in such a way that ecological conditions for germination of weed seeds

Fig. 11 Effect of date of sowing in wheat on *P. minor*

are not met due to escape mechanism. For instance, under North Indian conditions, temperature conditions during the last week of October are less conducive for the germination of *P. minor* and its infestations would be low if wheat crop is sown during this week (Fig. 11). Under such situations, seeds of *P. minor* will germinate with first irrigation and these plants will be poor competitor as wheat plants have taken lead of about one month during competition. Adjustment of sowing date of wheat is a non-monetary approach which helps in reducing not only dry matter of *P. minor* due to its poor germination but also improves wheat grain yields.

Weeds are less problem in basmati rice as compared to coarse rice which may be due to the reason that basmati rice is transplanted late than coarse rice providing sufficient time. Problem of horse purslane (*Trianthema portulacastrum*) can be solved with slight delay in sowing of Egyptian clover (*Trifolium alexandrinum*) due to prevalence of low temperature at which the seeds of this weed will not germinate.

7.4 Planting Pattern and Geometry

Weeds can be suppressed by manipulating crop density and geometry. The main objective of this technique is to distribute the crop plants per unit area with same seed rate for providing better crop architect uniformly. Another aim of this technique is to provide minimum space to weeds and maximum to the crop so that it can provide better smothering effect on weeds. Cross sowing leaves very little space for

the emergence and growth of weeds especially *P. minor* along with reduced population of other weeds.

Row orientation is an important factor influencing light penetration within the crop rows with significant influence on the growth and development of weeds. Wheat sown by cross sowing method reduced *P. minor* population by 59.6, 23.4 and 39.0% and dry weight by 59.2, 23.1 and 37.5% than broadcast, closer (15.0 cm) and normal sowing (22.5 cm), respectively (Singh and Singh 1996). Bi-directional sowing of wheat (22.5 cm × 22.5 cm) registered significantly less dry matter accumulation by *P. minor* over uni-directional sowing (22.5 cm), while uni-directional sowing at closer rows (15.0 cm) proved to be equally effective as bi-directional sowing for suppressing the weeds (Singh 1996). Increased weed biomass under wider spacing might be due to the better environment in terms of available space and solar radiation interception for weed growth (Brar and Singh 1997). Narrow row spacing and high seeding rates may help in suppressing weeds (Chauhan 2012).

Bi-directional and north-south sowing resulted in higher grain yield of wheat than east-west row orientation (Angiras and Sharma 1996). In bi-directional and north-south row orientation, there was higher light penetration by the crop, which increases the photosynthetic efficiency, yield attributes and suppressed the weed growth and ultimately increases the grain yield. Biomass of *P. minor* was significantly lowest in bi-directional row orientation followed by north-south row orientation. The principle of planting pattern technique is to provide minimum space for the weeds to grow without enhancing the seed rate. The minimum space to weeds can only be provided by uniform distribution of crop plants in the field so that these can cover maximum space within minimum period of time. In this way, crop plants will compete better with the weeds for space and consequently for nutrients, soil moisture, light etc. and also self competition between crop plants will be minimized. This method is more applicable in tillering crops like wheat and the two important options are cross sowing or closer sowing at 15 cm by keeping constant seed rate. Combination of bi-directional (22.5 cm × 22.5 cm) sowing along with wheat varieties with more tillering capacity and leaf area index like PBW 621 and PBW 550 provide significantly higher smothering potential against *P. minor* (Sharma and Kaur 2012).

7.5 Method of Sowing/Planting

Bed planting is another technique of reducing weed infestations. Most of the weed seeds lying in the top soil layer are buried deep at the time of bed preparation. The weed seeds lying on the top of bed will show germination and their growth will be comparatively less due to less availability of irrigation water on the top of bed. Weed control in bed planted wheat, corn is very easy and economical which can be done

with tractor and weeds also get uprooted during reshaping of beds. Secondly, grown up weeds can be easily uprooted in bed planted wheat without any loss to the crop.

7.6 Seed Rate and Plant Density

Increasing plant density by using higher seed rate tends to provide minimum space to weeds to grow due to overcrowding of crop plants. Heavy stand of crop will cover ground in a shorter period of time, thereby reducing the penetration of light on the ground where weeds are present. This creates shading which prevents weed seed germination, emergence and establishment. Hence, increased seed rate helps in smothering weeds particularly *P. minor* by providing early competition due to more number of crop plants per unit area. The higher seed rate (150 kg ha^{-1}) significantly decreased population of *P. minor* (Yaduraju and Ahuja 1997). Wheat sown with 50% higher seed rate (150 kg ha^{-1}) produced more crop dry matter thus reducing the dry matter accumulation by *P. minor* by 35.4% and resulting in increased grain yield of wheat by 12.3% over recommended seed rate (100 kg ha^{-1}) (Bhullar and Walia 2004a).

For better yield and effective weed control in rice, 33 plants per square metre are recommended; however, some farmers transplant only 18–20 plants per square metre. This low plant population not only provides less smothering effect on weeds but also encourages prolonged tillering resulting in poor quality produce. Plant population of 44 plants per square metre increased grain yield of paddy as compared to 22 plants per square metre (Brar and Walia 2001). Less dry matter accumulation by weeds under higher plant density may be attributed to more smothering effect of crop as compared to low plant density. Seed yield of rice tended to increase significantly with increase in plant population per unit area. Increased grain yield at higher seeding rate can be attributed to thick crop stand and the production of more effective tillers, more leaf area as well as reduced dry matter accumulation by weeds. Higher crop density helps in reducing uptake of nutrients and moisture by weeds and also results in less space availability and light interception by weed plants. So, overall growth and development of weeds will be poor, when crop density is on the higher side.

7.7 Fertilizer Use and Method of Application

Application of fertilizers in adequate quantities improve crop growth but when these are applied uniformly to soil through broadcast method, they may benefit the crops and weeds alike. Placement of fertilizer near crop plants is another agronomic technique by which more opportunities could be provided to the crop plant for the

uptake of applied fertilizers. Placement of fertilizers near to the crop plants could be very beneficial in providing good start to the crop due to their quick and more availability to crop as compared to weeds. Increased nitrogen application is known to increase the ability of cereals to suppress the weeds (Walia 1983). The wheat crop supplied with 175 kg N ha^{-1} reduced the population and dry matter of *P. minor* by 51.3 and 26.5%, respectively as compared to the dose of 125 kg N ha^{-1} with 20.3 and 14.6% increase in effective tillers and grain yield, respectively (Bhullar and Walia 2004b). Due to early availability of band placed fertilizers by crop plants and that too in higher amounts, the crop will make early good growth and will take lead in competition. Significantly less *P. minor* plants per square metre in placement method (2.5 cm below the seed) as compared to broadcast method of fertilizer application have been reported (Ahuja and Yaduraju 1989). Placement of nitrogenous fertilizers near the crop row either half dose or full dose reduced the intensity of *P. minor* and consequently helped in achieving higher grain yields of wheat (Walia and Kaur 2004).

7.8 Zero Tillage

Lowest density and dry matter accumulation of *P. minor* and broad leaf weeds was observed in zero till wheat as compared to conventional till crop (Bisen et al. 2006). Fewer weeds in zero tillage treatments can be attributed to the reason that germination of weed seeds present on the soil surface was stimulated with pre-sowing irrigation to wheat followed by killing of germinated seedlings with the application of contact herbicide (paraquat). However, the weed seeds present in the deeper layer do not get chance to germinate. Different tillage practices did not influence the weed population, dry matter of weeds and grain yield of wheat. However, significant influence of tillage operations was recorded on the vertical distribution of winter weeds in the wheat field with greater number of weed seeds in the upper 0–5 cm soil layer in zero tillage than conventional and deep tillage; but at higher depths of 5–10 cm and 10–15 cm, the number of weed seeds were significantly less in zero tillage as compared to other tillage techniques. The population of weeds especially *P minor* can be minimized both in zero tillage and deep tillage technologies. The seeds of *P minor* buried very deep during deep tillage operations are unable to germinate inspite being viable (Namrata et al. 2007).

7.9 Irrigation Management

Irrigation management has direct or indirect effect on weed intensity. The frequency as well as method of irrigation is known to influence the crop-weed competition. The studies have shown that with increase in the number of irrigations there is significant improvement in the crop yields. But more availability of moisture in the

soil can also enhance germination and growth of weeds. In drip irrigation, the weed intensity is generally lower than in furrow irrigation method. Similarly, in alternate furrow irrigation low weed intensity is observed in dry furrows than in irrigated furrows.

Application of four irrigations at 22, 65, 85 and 105 days after sowing had significantly more populations of *P. minor* as compared to one irrigation at 22 days after sowing and two irrigations at 22 and 85 days after sowing. However, yield improvement was observed due to four irrigations with increase in grain yield by 28.2 and 62.0% over two and one irrigations, respectively (Singh and Yadav 1998). The depth of irrigation also greatly effects the weed growth and crop yield. Minimum number of panicles and dry matter of *P. minor* were recorded in the crop receiving 7.0 cm depth of water in both first and second irrigations. Crop receiving both heavy irrigations (10 cm) recorded significantly higher panicle number as well as dry matter of *P. minor.* Notably, higher moisture level during initial stages will encourage growth and development of *P. minor* and will discourage growth of wheat especially in medium to heavy textured soils (Kumar 1998).

Application of light irrigations play significant role in reducing weed pressure and improving grain yield of wheat. Heavy irrigations also lead to delayed application of herbicide and yellowing of crop due to creation of oxygen scarcity in soil especially in medium to heavy textured soil. So, application of light and frequent irrigation results in improvement of herbicide efficacy and higher crop yields.

7.10 Soil Solarization

Soil solarization is a method of heating soil surface by using plastic sheets placed on moist soil to trap solar radiation. This process increases the soil temperature by 8–12 °C. Weed seeds are stimulated to germinate in the moist and warm soil and the young seedlings are killed by the heat. This technique is applicable on small scale and is very effective method for raising weed free nurseries as well as vegetables and flowers. But the major drawback of this method is that it cannot be adopted on large scale for weed control in field crops. Soil solarization with 50 μm thick transparent polythene even for a period of 3 weeks significantly reduced the emergence of all the weeds compared to non solarization and summer ploughing. Significant reduction in dry weight of weeds was recorded when duration of solarization was increased from 3 to 5 weeks (Singh et al. 2004).

In baby corn, thorough land preparation and irrigation up to field capacity for solarization was found effective in suppressing weeds followed by one ploughing with harrowing and 40 mm of irrigation followed by one hand weeding at 30 days (Thimmegouda et al. 2007). Soil solarization was also observed to record the highest system productivity in the soybean-wheat cropping system closely followed by wheat straw incorporation and repeated tillage with irrigation (Das and Yaduraju 2008).

7.11 Stale Seed Bed

A stale or false seed bed is a useful weed control technique which involves creating a seedbed some weeks before seed is due to be sown. After applying pre-sowing irrigation twice, preparation of such seedbed makes sure that any weed seeds that have been disturbed and brought to the soil surface during cultivation will have a chance to germinate which can then be eliminated by cultivation or with the use of herbicides before sowing of the actual crop is carried out. If the sowing is to be done as zero tillage, then the weeds can be cut with operating planker having attachment of dent behind.

7.12 False Seedbed

In this method, weeds emerging in response to tillage are killed by two or more additional shallow cultivations at weekly intervals. The crop is planted immediately after the final cultivation.

7.13 Seedbed Preparation

For significant reduction of weed infestation, properly prepared seedbed is required. If possible, the first flush of weeds is allowed to germinate before beginning tillage operations. Ploughing should be done as deeply as possible to break up soil compaction and reduce the risk of herbicide carryover. The final tillage should be just before planting wheat so that any germinated weeds do not get a competitive advantage. There should be no delay between seedbed preparation and planting/sowing of the crop.

7.14 Flooding

Weeds have association with different crops i.e. they may be more troublesome in a specific crop. Few weeds like *Saccharum spontaneum*, *Asphodelus tenuifolius, Carthamus oxycantha* etc. prefer to grow in dry habitats that have eliminated from today's agriculture due to the creation of assured irrigation facilities and with change of crop rotation from groundnut (*Arachis hypogaea)/*pigeonpea (*Cajanus cajan*)-wheat to rice-wheat in Northern India. Their place has been taken up by *Echinochloa crus-galli*, sedges and few broadleaf weeds in rice and *P minor*, *Rumex* spp in wheat.

Fig. 12 Effect of flooding for control of weeds in transplanted rice

Puddling is the best method for controlling non-paddy weeds that can control even severe infestations of troublesome weeds like *Trianthema portulacastrum, Eleusine spp. Eragrostis spp., Cyperus rotundus, Sorghum halepense, Cynodon dactylon* etc. in corn, cotton, pulses and oilseed crops as these weeds cannot tolerate anaerobic conditions. However, typical weeds of paddy will appear with continuous cultivation of puddled transplanted paddy. Ponding of field especially during first fifteen days can provide excellent control of most of the weeds in rice (Fig. 12). Sometimes, weeds can appear even after the application of pre-emergence herbicides in those patches of field where water do not stand after puddling. For effective weed control, water stagnation for at least one week after the application of herbicide is very important.

7.15 Mulch

During summer season, soil mulch plays more important role for better growth and development of crops especially those with wider row to row spacing like sugarcane and corn. Soil mulch reduces soil temperature during hot months, conserves soil moisture by reducing evaporation and prevents weed seeds to germinate. Mulch is also a rich source of nutrients after its decomposition. The use of mulch has lot of benefits and this practice should be encouraged for sustainable and higher productivity. Turmeric (*Curcuma longa* L.), a spice crop is gaining popularity these days due to its higher economics. Farmers are planting this crop in poplar

(*Populus tremula*) tree as intercrop. Being a slow growing crop, weeds are a big problem in turmeric as this crop takes more than a month to emerge. So, weeds germinate first and take lead in competition and if left uncontrolled, can cause huge losses may be cent percent for rhizome yield. Rice straw mulching has shown good promise in this crop due to its many benefits like enhancing its germination by 7–10 days as compared to no-mulch treatment, reducing soil temperature during very hot months, prolonged retention of soil water due to reduction in evaporation, reduction in weed Primary>Weed population as weed seeds are unable to germinate/emerge under mulching, addition of many nutrients in soil after its decomposition, improvement in the physico-chemical properties of soil and provision of better environment for growth and development of soil micro-organisms and earth worms.

Mulching with polyethylene plastic sheets is also very effective method of controlling weeds. Use of plastic mulch is very common and practically possible approach in orchards due to the availability of lot of open space. Integration of herbicide use with plastic mulch can provide excellent weed control for a longer period. Intercropping of short duration leguminous crops like cowpea for fodder purposes in orchards or any other less exhaustive crops like greengram/blackgram can be selected for intercropping in orchards. These intercrops will not only help in controlling the weeds in orchards but will also provide additional profits to the farmers. Application of straw mulch (8 cm, 15.5 t ha^{-1}) resulted in higher weed control efficiencies in peach at 6 weeks after treatment (WAT). The weed control efficiencies with treatments having diuron as pre-emergence herbicide did not differ significantly from black polythene at 6 WAT. Straw mulch having thickness of 8 cm did not differ significantly from straw mulch with 6 cm thickness, black polythene mulch, and diuron treatments for fruit yield (Thakur et al. 2012).

8 Integrated Weed Management

Integrated weed management requires strategy for number of years. Due to less persistence behavior of dinitroaniline group of herbicides, integration of one hand hoeing with the pre emergence/pre plant application of these herbicides was made in cotton, oilseeds, pulses, vegetables, etc. On the other hand, due to high persistence nature of atrazine/simazine in corn, band application of these herbicides was recommended, so that half to two-third dose of herbicide can be saved. These are sprayed in bands on crop rows only and weeds can be controlled with interculture from the inter-row area. In transplanted paddy, integration of flooding with the use of herbicides provides very effective control of weeds. Without stagnation of water particularly for 8–10 days, the efficacy of rice herbicides gets decreased. It was also observed that integration of herbicides with increased number of rice seedlings from 33 to 44 per square metre provided better weed control and higher yields.

Integrated methods of weed management have proved very beneficial for the control of weeds in wheat. For instance, isoproturon resistant *P minor* can be eliminated within a period of 2–3 years by rotating wheat with Egyptian clover or potato-based systems. Extensive research work also indicated that *P minor* population can be reduced/eliminated from the wheat by sowing of wheat in the end of October (early sowing), with closer sowing (15 cm row to row spacing), use of higher seed rate, placement of fertilizers, selection of quick growing crop cultivars like PBW 621 etc. Population of weeds especially *P minor* can be minimized either by deep ploughing or with the adoption of zero till technology of wheat sowing. Possibilities of controlling *Trianthema portulacastrum* (horse purslane) in Egyptian clover by delaying sowing time or mixing egyptian clover seed with raya (*Brassica juncea*) were explored. Delay in sowing of egyptian clover by mid October drastically reduced the density of *T portulacastrum* (horse purslane). Similarly, sowing of Egyptian clover mixed with raya (*Brassica juncea*) helped in suppressing the weeds. Apart from these cultural practices, stale seed bed technique also helps in reducing intensity of winter season weeds. It was also observed that raya (*Brassica juncea*) is very smothering crop for *P minor* and other weeds as compared to wheat.

Integration of herbicides like pendimethalin 1.0 kg ha^{-1}, atrazine 0.75 kg ha^{-1}, metribuzin 0.70 kg ha^{-1} etc. with mulch at 9.0 t ha^{-1} resulted in significant improvement in rhizome yield of turmeric as compared to alone application of mulch or integration of one hand hoeing with the pre-emergence application of these herbicides. Higher rhizome yield in the treatments where pre-emergence application of herbicides was integrated with straw mulching may be due to season long weed control attained with the integration of herbicidal treatments with straw mulching. Moreover, crop in these treatments was also benefited with the direct or indirect effect of rice straw mulching (Kaur et al. 2008) (Figs. 13 and 14).

Fig. 13 Effect of paddy straw mulch in wheat

Fig. 14 Integrated use of herbicide and paddy straw mulch provided better control of weeds than mulch alone in Japenese mint

9 Conclusion

The objective of this review was to identify strategies that can eliminate or minimize the need for herbicides to manage weeds. When neither tillage nor herbicides are used, successful weed management will require identification and integration of numerous tactics that can be flexibly applied to meet a wide range of soil and environmental conditions (Kurstjens 2007). Much more research efforts are needed on weed ecology. Weed ecology of important weeds need to be studied with respect to their management so that IWM approaches can be explored. Detailed information on weed ecology is very limited. Information about mechanisms of weed interactions with crops and responses of weeds to various production systems is limited. Choosing the most effective strategies for a particular field requires sufficient knowledge of the field's weed seed bank. Weed species differ widely in seed dormancy and longevity, season in which they emerge, depth from which they can emerge, and seed responsiveness to light and other stimuli. All these characteristics can help the farmers to select the best strategies for managing a particular weed seed bank. Weed management plan can be devised effectively by understanding the general biology of weeds, understanding the general principals of ecological weed management, identifying the critical weed problems.

The gist of the manuscript lies in the golden words that without studying ecology of weeds, weed management ultimately may fail and make the weed problems

even more worse. Farmers often rely on single and easy solutions to manage weeds. The main obstacle with a single weed control strategy is that weeds adapt to management. Although herbicides are excellent tools for the management of weeds but continuous use over the years can lead to problems such as residual carry-over, cropping restrictions, ground water contamination or have unintended and detrimental environmental and economic consequences.

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Effect of Agricultural Chemicals and Organic Amendments on Biological Control Fungi

Babak Pakdaman Sardrood and Ebrahim Mohammadi Goltapeh

Abstract The demand for food and energy of the rising population and unfavorable climatic changes necessitate yield increase in the reducing cultivable areas. This requires a reduction in environmental pollution that not only exerts its hazardous effects on plants but also on poultry, livestock and humankind. Integrated pest and disease management is a solution that not only can lead to the increased crop yield and reduced environmental pollution, but also to the ecofriendly growth of economy in less developed countries. Integrated management requires more precise information on the effects of agricultural inputs on the population and biological activity of biological agents used in biological control of plant pests and diseases, biostimulation, as well as biofertilization. Here, we focus on the effect of pesticides on biological control fungi.

Keywords Fungi · Pesticide · Insecticide · Fungicide · Herbicide

1 Introduction

Plants not only provide food and energy for humankind, livestock and poultry, but also the the raw materials for dependent textile, pharmaceutical, wood and recently developed natural biodegradable plastics industries. However, increasing population means further extension of house building that decreases the area available for agricultural production. The result is further destruction of wild plantations and subsequent disappearance of dependent floras, and the most serious crisis, the loss

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E. Lichtfouse (ed.), *Sustainable Agriculture Reviews 31*, Sustainable Agriculture Reviews 31, https://doi.org/10.1007/978-3-319-94232-2_5

of biodiversity. Use of various types of agrochemicals and pesticides has aggravated the problem and led to the extensive water and soil pollutions.

Unfortunately, the situation has got more worsened because of increased global warming and severe changes in atmospheric precipitations (Massachusetts Institute of Technology 2017; Pfahl et al. 2017). Considering that most of already hungry and thirsty people of current world live in arid subtropical and tropical regions of the planet, therefore, these problems will expectedly get worse in these areas, where poor, less-developed and developing (if any) countries known as the third world countries are located. The farmers of these countries suffer from poverty and ill-education imposed by geopolitical factors, and this leads to the most worsened crises at different social, environmental, economical, and political levels. The misuse of pesticides not only has led to environmental pollutions, but also to medical problems such as cancers like leukemia (Hernández and Menéndez 2016) caused by acute and chronic effects of pesticides some of which although have already been removed from the markets in the developed countries, but still are prevalently sold in ill-managed areas of the third world countries. The increasing gap between the economies of the third countries and well-developed countries imposes more unavailability of agricultural inputs and technologies that leads to increased extension and severity of the problems. One way to improve life in such countries, is to develop biological and ecofriendly biofertilizers and biopesticides based on the native isolates of beneficial microorganisms and/organisms. Although this strategy may not completely eliminate the need for pesticides and agrochemicals but still can help farmers to establish an ecofriendly sustainable agriculture relied on native products. At least some of the biological control fungi such as *Trichoderma* spp. are known as bioremediators (Pakdaman et al. 2013a, b) and the use of the appropriate isolates can not only lead to reduced use of pesticides, but also to the reduced pollution of the environment through bioremediation.

Furthermore, the chemical control of some pests and pathogens is very difficult or even impossible due to special ecological niches of their activity (such as vascular wilt pathogens, bark beetles, wood and stem borers) or the necessity of monitoring that requires professional education and periodic inspections (such as white flies, bugs, and mites). However, these pests and pathogens are more amenable to the biological control measures. On the other hand, the biological control mainly relied on the native biological control agents rather than exotic products, provides a way for poor and undeveloped countries to step forward toward economic bloom. In such a system, the application of bioproducts requires some management to help the beneficial microorganisms and organisms to establish/preserve their primary active populations and to build up themselves and make new effective populations. In this chapter the effect of different agrochemicals and agricultural amendments on biological control fungi is discussed. Such information not only can lead to more wise application of biological control fungi in an integrated pest/disease management program, but also will be useful in other aspects like the development of selective media for the isolation of peculiar biological control fungi from the environment, and the development of better formulations that can promote better establishment of a biological control fungus in an

environment. Such information can open a new humanistic field for international cooperations where the final result is stable growth and development of developed as well as developing countries.

2 Pesticide Effect on Biological Control Fungi

There are two major viewpoints in the control of plant diseases. The first considers the control of a single disease in a single crop, while the second attempts to control a range of single crop diseases (Singh 2001). Both of these viewpoints narrowly consider only a single crop either in a short period of time (throughout a growing season, where crop rotation is applied) or in a restricted place where monoculture system is applied. However, there can be another viewpoint that is more broad and holistic, and makes it possible to take advantage of both above-mentioned viewpoints and in the meantime, to eliminate their defects. This holistic approach considers the management of the diseases of the plants grown in a crop rotation program in a place. Therefore, it is attempted to apply the methods that not only control the diseases of a present crop but also decrease the population of the pathogens of the next crop/s. Implementation of such a vision requires accurate and precise information of the effects of various agricultural inputs on the important pathogens in the area as well as on the biological control fungi applied. Hence, this chapter may be at least partially useful from this stand of view.

Various species of biological control fungi have been studied against plant diseases and pests, and some of them have been formulated and introduced to markets (De Faria and Wraight 2007; Leng et al. 2011; Reddy et al. 2013; Woo et al. 2014). Klingen and Haukeland (2006) provided a comprehensive review of the effects of agrochemicals on entomopathogenic fungi and concluded that insecticides and herbicides were not generally harmful to fungal growth, while fungicides were sometimes harmful. A review of some data of 20 year research was carried out by Lo (2010), where the effects of twenty-one pesticides on the soil microorganisms associated with nutrient and cycling processes were presented in Sect. 1, and the applications of denaturing gradient gel electrophoresis (DGGE) for studying microbial diversity were discussed in Sect. 2. Most of the studies cited were performed in vitro and extrapolation of laboratory results to the field are difficult to make. None of the fungicides tested in these studies had any significant impact of the number of colony forming units (CFUs) in bulk soil. This was true even for the fungicides captan and triflumizole both of which had reapplication intervals of 14 days or less and significantly reduced germination and mycelial growth in vitro. It was expected that the repeated application of these fungicidal (inhibiting germination of fungal spores) and fungistatic (retarding development of mycelia while in contact with the chemical) chemicals in vitro and in a short period of time would result in the reduced fungal populations. However, this was not the case for fungal populations in the bulk soil.

While some pesticides stimulate the growth of microorganisms, other pesticides have depressive effects or no effects on microorganisms. Such effects will be reflected by biological and biochemical changes in the environment. For examples, an herbicide simazine, even at normal rates of application, showed toxicity to bacteria and fungi. It was less toxic to actinomycetes, since toxicity up to 20 ppm of the herbicide was not observed. On the contrary, the normal rate of simazine stimulated both *Azotobacter* and actinomycetes population. The interaction of simazine with soil ecological factors, such as temperature, moisture, pH, and organic matter, affected soil microbial population differently. Simazine was relatively less toxic to bacteria under acidic and alkaline conditions of soil; they were not affected at 15 °C. Actinomycetes were comparatively not adversely affected even with 200 ppm of simazine under high soil moisture regime. The stimulatory effect of simazine on *Azotobacter* was also confirmed under different ecological conditions. The incorporation of 2% of organic matter in soil mitigated the toxicity of simazine in respect to soil fungi.

Simazine also appeared to be less toxic to soil fungi at lower temperatures, under acidic and alkaline conditions of soil, as well as under high moisture regime (Gaur and Misra 1978). Additionally, a pot experiment was run for studying the effect of Temik (insecticide and nematocide), Orthocide® (fungicide), and Treflan® (herbicide) on certain desirable soil micro-organisms. Total counts of bacteria, actinomycetes or fungi were mostly lower in the treated than in the untreated soil. Aerobic cellulose-decomposers were either stimulated or depressed. Despite the temporarily slight stimulation, observed in some instances at the initial periods, the effect of the pesticides on counts of *Azotobacter*, N-fixing clostridia, and ammonifiers was afterwards depressed. The period of retardation or stimulation differed according to type of pesticide and type of micro-organisms under study. However, the autotrophic nitrifying bacteria, especially ammonium-oxidizers, seemed to be more sensitive and their counts in treated soil were sharply depressed (Makawi et al. 1979).

Also, carbofuran stimulated the population of *Azospirillum* and other anaerobic nitrogen fixers in flooded and non-flooded soil, but butachlor reduced the population of *Azospirillum* and aerobic nitrogen fixers in non-flooded soil. Diuron and chlorotoluron showed no difference between treated and non-treated soil, and linuron showed a strong difference (Lo 2010). The effect of selected pesticides, monocrotophos, chlorpyrifos alone and in combination with mancozeb and carbendazim, respectively, was tested on nitrification and phosphatase activity in two groundnut (*Arachis hypogeae* L.) soils. The oxidation of ammonia nitrogen was significantly enhanced under the impact of selected pesticides alone and in combinations at 2.5 kg ha^{-1} in black soil, and furthermore, increase in concentration of pesticides decreased the rate of nitrification, whereas in the case of red soil, the nitrification was increased up to 5.0 kg ha^{-1} after 4 weeks, and then decline phase was started gradually from 6 to 8 weeks of incubation. The activity of phosphatase was increased in soils, which received the monocrotophos alone and in combination with mancozeb up to 2.5 and 5.0 kg ha^{-1}, whereas the application of chlorpyrifos singly and in combination with carbendazim at 2.5 kg ha^{-1} profoundly increased

the phosphatase activity after 20 days of incubation, in both soils. But higher concentrations of pesticides were either innocuous or inhibitory to the phosphatase activity (Srinivasulu et al. 2012). Phosphorus (P)-containing herbicide (glyphosate) and insecticide (methamidophos) stimulated soil microbial growth, but other P-containing insecticide (fenamiphos) was detrimental to nitrification bacteria (Lo 2010).

The use of fungicide is the best known and popular strategy method in the Integrated Disease Management (IDM). However, inspite of their efficient role in the control of plant pathogens, fungicides are expensive and the continued or repeated application of them may lead to ecological imbalance, and result in dramatic disease outbreaks, widespread development of pathogens resistant to one or more chemicals, toxicity to non-target organisms and environmental problems (Dłużniewska 2003; Lee et al. 2009). Sometimes, fungicides accumulate in the food chain as residues (Lee et al. 2008) and lead to environmental and health-related hazards to men and also adversely affects the beneficial microorganisms in soil (Dłużniewska 2003). Some soilborne root infecting fungi are difficult to eradicate because they produce resting structure like sclerotia, chlamydospores or oospores for their survival for a longer period of time under adverse environmental conditions (Baker and Cooke 1974). With these cases, biological control fungi can be a reliable choice. The use of microorganisms that antagonize plant pathogenic fungi is risk free (Benítez et al. 2004).

The term phytotoxic effect could be expressed as an injurious effect of any fungicide or their mixture in any form under any environmental condition when they are applied at their recommended doses on field crops or on any other crops. In view of such phytotoxic effect use of a chemical even being an excellent fungicide, is limited by such undesirable characters. Reduced rates of some fungicides resulted in loss of efficiency to control disease but increased rates of application caused phytotoxicity (Litterick et al. 1993). A number of symptoms like reduced emergence, twisted and thickened leaves, stunted roots and tops have been observed using different fungicides on different crops (Goulart and Paiva 1993; Seymour et al. 1994). Similarly, many fungicides showed phytotoxicity to different field crops under various conditions of their application (Singh et al. 2003, Ali and Archer 2003). There is hardly any fungicide, which is not injurious to field or nursery crops at any stage of their growth and conditions of treatment. It therefore, could be considered that study of phytotoxic effects of pesticides is very important for their safer application on crop plants. In addition to plants, fungicides, compared with various groups of pesticides, are expected to be of the highest effect on the fungi used as biological control agents. For instance, the effect of five fungicides, benomyl (1 mg L^{-1}), dodine (50 mg L^{-1}), manzate (100 mg L^{-1}), cupric sulphate (200 mg L^{-1}) and thiabendazole (4 mg L^{-1}) was tested under in vitro conditions on the development of 15 isolates of fungi pathogenic for insects and other invertebrates (*Beauveria brongniartii*, *Culicinomyces clavisporus*, *Duddingtonia flagrans*, *Hirsutella thompsonii* (Figs. 1 and 2), two *Metarhizium anisopliae*, *Nomuraea rileyi*, two *Isaria/Paecilomyces* spp., and *Sporothrix insectorum*) and 13 isolates of contaminant fungi (five *Aspergillus* spp., *Cladosporium cladosporioides*,

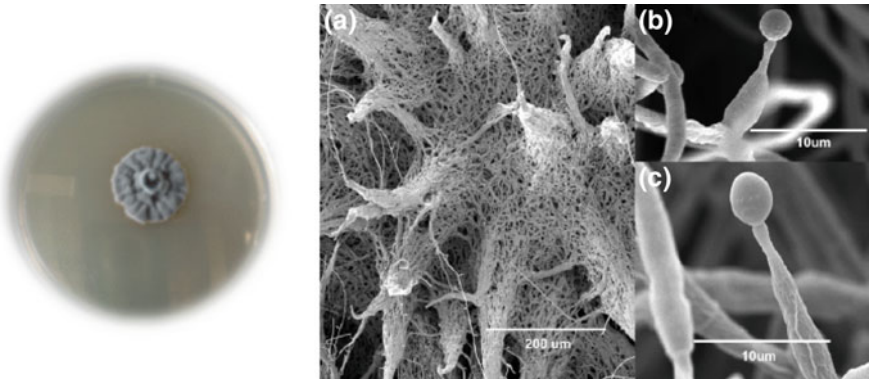

Fig. 1 *Hirsutella thompsonii*. (Left) Colony morphology on agar plate (*Source* Montesinos et al. 2015); (Right) Scanning electron micrograph of a synnematous strain of the fungus, **a** initial synnemata on medium, **b** a high magnification of the basally inflated phialidic conidiogenous cell and the primary spherical with a verrucose surface phialospore, and **c** a high magnification of a polyblastic phialidic conidiogenous cell with an elongated stalk bearing a smooth, ellipsoid phialospore (*Source* Boucias et al. 2007)

Cunninghamella echinulata, *Fusarium roseum*, *Gliocladium* sp., *Mortierella isabellina*, *Mucor plumbeus*, *Rhizopus arrhizus* and *Trichothecium roseum*) originating mostly from tree-hole breeding sites of mosquitoes. Most pathogenic and contaminant fungi had clear patterns of susceptibility or resistance to tested concentration of the fungicide. Development of both pathogenic and contaminant fungi on fungicide-supplemented medium varied among fungi and fungicides tested. Minimal inhibition of pathogenic fungi was found for cupric sulphate, benomyl, dodine, thiabendazole < manzate.

The highest inhibition of contaminants was obtained with thiabendazole > benomyl and dodine > manzate and cupric sulphate. Thiabendazole was the most appropriate fungicide to isolate fungi pathogenic to invertebrates from substrates with high water contents and rich in organic material. The results underlined the importance of adapting both a fungicide and its concentration for a selective medium for isolating specific target fungi and while selecting against possible contaminants (Luz et al. 2007).

The effect of fifteen commercially available fungicides on the germination, growth and virulence of *M. anisopliae*, *Beauveria bassiana*, *Isaria fumosorosea*, and *Lecanicillium longisporum* was studied (Shah et al. 2009). The fungicide influence on conidial germination was dependent upon its type and dose. Most fungicides retarded conidial germination of all the tested fungi at 10× and at the recommended rate of application, however, their toxicity declined at lower concentration. Most of the fungicides inhibited the mycelial growth of *B. bassiana*, whereas the growth of *L. longisporum* was not influenced. Only two and eight fungicides affected the mycelial growth of *I. fumosorosea* and *M. anisopliae*, respectively. None of the fungicides influenced the virulence of *B. bassiana* and

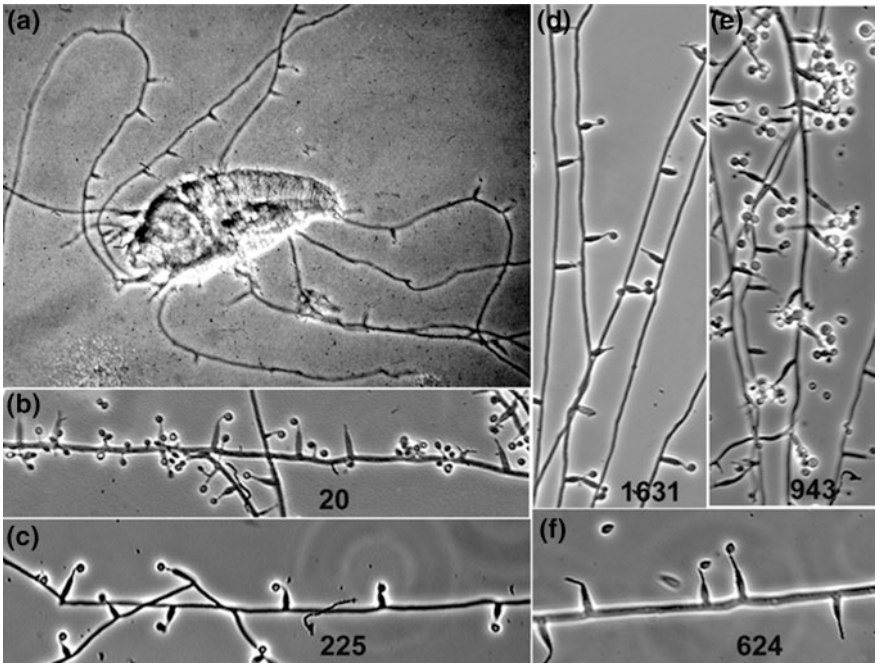

Fig. 2 The mononematious *Hirsutella thompsonii* strain (**a**) infecting citrus rust mite *Phyllocoptruta oleivora* with extensive hyphal network bearing phialides; Panels **b–f** are light micrographs of Ridell mounts of *H. thompsonii* strains from different mite species including that from (**b**) *Artocarpus heterophyllus*, and (**e**) *Dipterocarpus alatus* producing multi-neck phialides bearing primary conidia, and that from (**c**) *Strebus asper*, and (**d**) *Millingtonia hortensis* producing typical well-spaced phialides bearing primary conidia, and a strain from (**f**) *Elaeocarpus hygrophilus* producing elongate phialides. *Source* Maimala and Bouicias (2004)

L. longisporum, while tolyfluanid and azoxystrobin reduced the virulence of *M. anisopliae* and *I. fumosorosea*, respectively. Clearly, certain fungicides had the potential to inhibit the germination of entomopathogenic fungi in vitro but appeared to have little or no effect on their virulence against target insects (Shah et al. 2009). Fungicides were more damaging on *Neozygites floridana* (Weiser and Muma) Remaudiere and S. Keller (Zygomycetes: Entomopthorales) (Fig. 3) survival and efficacy in the control of the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae) than methiocarb (acaricide, insecticide, molluscicide) in strawberry production (Klingen and Westrum 2007). However, it is interesting that one fungicide (fosetyl-aluminium) appeared to stimulate mycelial growth of another entomopathogenic fungus, *Lecanicillium longisporum* (Fig. 4).

Such information not only can lead to more wise application of biological control fungi in an integrated pest management program but also will be useful in other aspects like the development of selective media for the isolation of peculiar biological control fungi from the environment, and the development of better

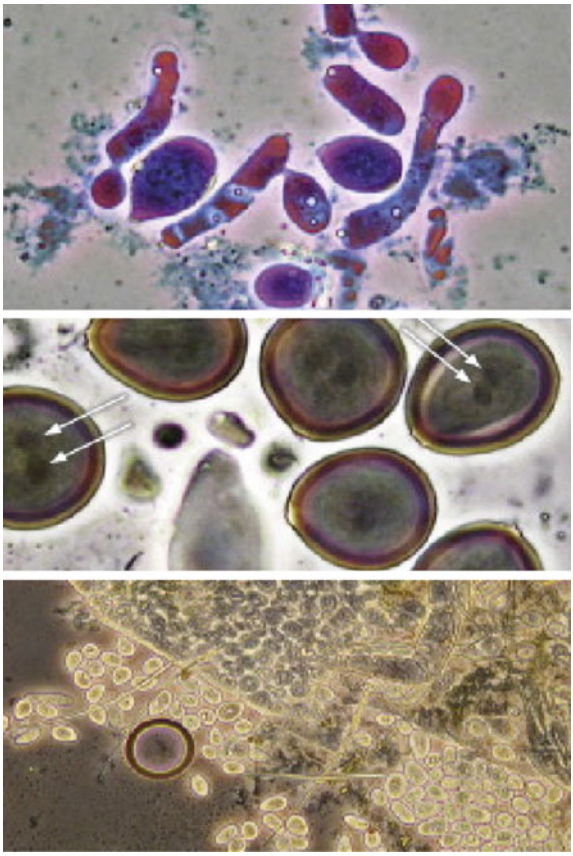

Fig. 3 Formation of resting spores of *Neozygites floridana* in *Tetranychus urticae* in the Brazilian strain ESALQ 1420. (Top) Azygospores budding from hyphal bodies; (Middle) Mature resting spores with two nuclei (arrows); (Down) Squash mounted cadaver of *T. urticae* totally filled with immature resting spores (Westrum et al. 2014)

formulations that can promote better establishment of a biological control fungus in an environment. The effect of pesticides applied in the field on the occurrence and abundance of entomopathogenic fungi in soils is difficult to evaluate, because fungi are indirectly or directly influenced by numerous abiotic and biotic environmental factors (Roberts and Campbell 1977). Miêtkiewski et al. (1997) have suggested that pesticides may have a direct impact on the natural occurrence, infectivity, and population dynamics of fungal entomopathogens, as also have direct impact on other macro- and micro-organisms in soils which affect the entomopathogenic fungi indirectly.

Certain key biotic and abiotic factors influencing the joint action of fungi as insecticides include type of formulations, carriers, emulsifiers, dosage, soil types, and condition of host plants. Any suppression of these fungi may be detrimental to their capacity as natural enemies. Previous researchers have indicated that pesticides used under field conditions are unlikely either to kill all the entomopathogenic fungi present in the treated area or to limit their recolonization. It seems likely, therefore, that pesticides have the potential to be used in conjunction with

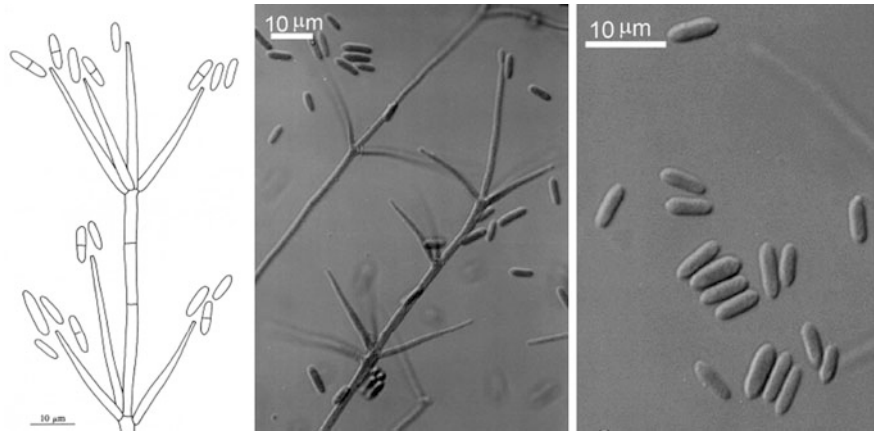

Fig. 4 *Lecanicillium longisporum*. Conidiophores and conidia from two different isolates. *Source* Zare and Gams (2001)

mycoinsecticides in integrated pest management systems. Fungal entomopathogens can be applied as an important component in integrated pest management either alone or in combination with reduced amounts of insecticides. Mycoinsecticides containing viable conidia and sublethal doses of insecticides have been reported to be more effective (Anderson et al. 1989). The resultant enhanced action in combination between pathogens and pesticides has been referred to as insect specific economic synergism (Benz 1971). Fargues (1975) illustrated that the effects of the components in these combinations are additive.

Many experiments have been carried out aiming to detect pesticides side effects on entomopathogenic fungi (Clark et al. 1982; Gardner and Storey 1985; Neves et al. 2001; Olmert and Kenneth 1974). Neves et al. (2001) pointed out the importance of conidial germination in compatibility studies, later confirmed by Todorova et al. (1998). In addition, the inhibition of vegetative growth is not the most important index of compatibility for fungi and insecticides. Under field conditions, inhibition of vegetative growth is not a good indication of fungicidal effects, but conidial germination seems to be the key factor (Loria et al. 1983; Neves et al. 2001), so the effect of insecticides on conidial germination should be considered as one of the most important factors (Anderson and Roberts 1983). Integrated pest management (IPM) programs the compatibility between entomopathogenic fungi and pesticide used in the fields as a major concern (Todorova et al. 1998). Conidial survival can be affected due to agrochemicals, environmental factors or by biopesticide and/or chemical products used to protect plants (Anderson and Roberts 1983).

The impact of pesticides on the processes of germination, fungal growth and sporulation vary depending on the fungal species and strain (Vänninen and Hokkanen 1988; Anderson et al. 1989). Majority of work on join action of microbials-synthetic insecticides indicate that fungal sporulation is synergized at

subnormal insecticide concentrations (Todorova et al. 1998; Ambethgar et al. 2009). The most important issues that need to be addressed while considering insecticide resistant management through co-application of insecticide-fungus combinations include: (i) resurgence of less important insect pests, (ii) effect on non-target organisms, and (iii) speed of action on target species. Many researchers have examined the factors which influence the synergism (Hassan and Charnley 1989; Boucias et al. 1996; Kaakeh et al. 1997; Quintela and McCoy 1997, 1998a). Chemical insecticides, botanicals, insect growth regulators and mineral oils at subnormal doses have been combined with entomopathogenic fungi to enhance control of certain insect pests (Reddy et al. 2013), and biorational insecticides are being increasingly emphasized for inclusion in integrated pest management programs for invasive insects.

Synergism has been identified between entomopathogenic fungi and insecticides (Shah et al. 2007, 2008). Olmert and Kenneth (1974) tested 9 fungicides 14 insecticides and acaracides were in vitro on various isolates of the entomopathogenic fungi, *Beauveria bassiana*, *Lecanicillium lecanii* (Fig. 5) and *Verticillium* sp. The fungicides included benomyl (Benlate^{TM} 50 a. i. WP applied at the rate of 0.1%), thiabendazole (Tecto^{TM} 100 a. i. WP applied at the rate of 0.1%), captan (Merpan^{TM} 50 a. i. WP applied at the rate of 0.25%), maneb (Manebgan^{TM} 80 a. i. WP applied at the rate of 0.12%), daconil (Daconil 2787^{TM} 75 a. i. WP applied at the rate of 0.25%), copper oxychloride (Cuprantol^{TM} 50 a. i. WP applied at the rate of 0.25%), dinocap (Karathane^{TM} 25 a. i. WP applied at the rate of 0.1%), binapacryl (Morocide^{TM} 25 a. i. WP applied at the rate of 0.1%), and zineb (Zidan^{TM}75 a. i. WP applied at the rate of 0.15%). The insecticides and acaricides studied were endosulfan (Thionex^{TM} 35 a. i. EC applied at the rate of 0.4%), omite (Omite^{TM} 30 a. i. WP applied at the rate of 0.15%), trichlorfon (Danex^{TM} 80 a. i. WP applied at the rate of 0.4%), chlorobenzilate (Chlorobenzilate^{TM} 25 a. i. WP applied at the rate of 0.12%), diazinon (Diazinon^{TM} 25 a. i. EC applied at the rate of 0.3%), ethion (Itopaz^{TM} 50 a. i. EC applied at the rate of 0.15%), azinphosmethyl (Cotnion^{TM} 25 a. i. WP applied at the rate of 0.2%), chloropyrifos (Dursban 4^{TM} 40.8 a. i. EC applied at the rate of 0.4%), narrow-range paraffinic oil (B.C.L. 99^{TM} Oil applied at the rate of 1.5%), dichlorvos (Divipan 100^{TM} 100 a. i. EC 0.1%), carbaryl (Ravion^{TM} 50 a. i. WP 0.2%), and white summer oil (Levanola^{TM} 80 a. i. EC applied at the rate of 2.0%).

Two techniques were employed: for *Verticillium*, a "poisoned-bait" method, showing growth inhibition; for *B. bassiana*, a poisoned-agar disc method, showing inhibition of conidial germination. All chemicals except white summer oil caused some inhibition to *Verticillium* growth at 10^{-4} of the recommended dosages for use. Of the fungicides, benomyl (a benzimidazole fungicide like carbendazim) and maneb (a dithiocarbamate fungicide like mancozeb) caused the greatest inhibition at recommended dosages and at one-tenth concentrations. Great variations in sensitivity toward a particular fungicide (e.g., captan, copper oxychloride, dinocap, binapacryl) were found among isolates of the same fungal species of both genera, and were also observed with some insecticides (e.g., trichlorfon and narrow-range paraffinic oil on *Verticillium*). Among the fungicides, benomyl (methyl

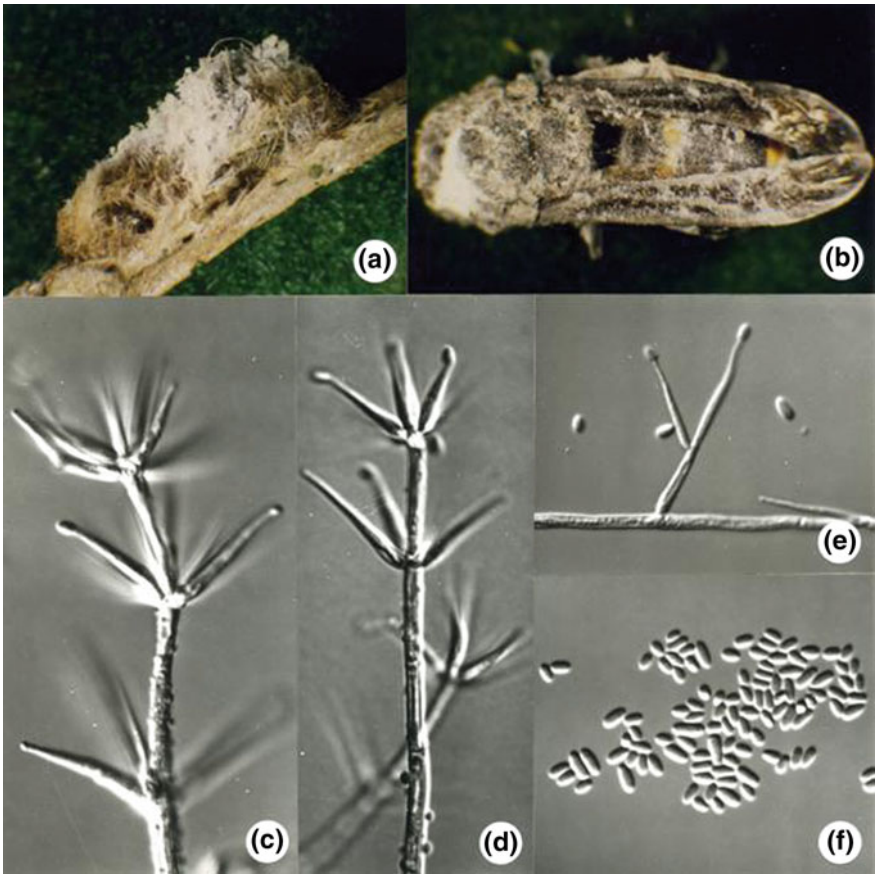

Fig. 5 *Lecanicillium lecanii*, the anamorphic state of *Cordyceps confragosa*. **a**, **b** Cadaver of an insect infected with the fungus; **c**, **d** conidiphore with verticilliate arrangement of phialides; **e** a conidiophore with only two phialides developed from one common point and each harboring a single phialospore; **f** hyaline, ellipsoid, unicellular phialospores. *Source* http://www.bcrc.firdi.org.tw/fungi/

1-[butylcarbamyl]-2-benzimidazole carbamate) and maneb caused the greatest inhibition of all isolates of both genera at recommended dosages and at 10^{-1} concentration, indicating that their use in field or grove would endanger the fungi. At recommended dosages, copper oxychloride and dinocap were the most innocuous to all the fungi (with the exception of 1 isolate of *B. bassiana*). Binapacryl had no effect on spore germination of *B. bassiana* but moderately inhibited growth of most isolates of *Verticillium* spp. Daconil 2787™ (tetrachloroisophthalo-nitrite) proved moderately fungitoxic to *Verticillium* and less so to *B. bassiana* (Olmert and Kenneth 1974). Among insecticides, sodium fluorosilicate, dichlorvos, and chloropyrifos at recommended dosages were strongly

inhibitory to growth of *Verticilium* spp., but only sodium fluorosilicate inhibited germination of *B. bassiana* (Olmert and Kenneth 1974). The possibility to use simultaneously biological and chemical control products as efficient and environmental friendly seed treatment for pest and disease control could provide a decrease of the chemical dose needed for plant protection. This phytosanitary strategy promotes microbial strain from *Beauveria*, *Bacillus* and *Pseudomonas* genera that can be used together with some chemical products for plant protection in order to decrease the amount of chemical product per unit area. Therefore, Constantinescu et al. (2014) chose two chemical insectofungicides based on the ecotoxicological risk assessment and legislation related to plant protection products (Regulation (EC) No 1107/2009 and Directive 2009/128/EC). The compatibility studies between the biocontrol microorganisms and chemical pesticides were based on the microbial strains interaction with the active substance from the chemical insecticide and insectofungicides (imidacloprid 600 g L^{-1} and mix of imidacloprid 460 g L^{-1} with thiram 176 g L^{-1}, respectively). The viability of the microbial strains was studied under laboratory conditions.

A greenhouse experiment was designed with soybean plants. Seeds belonging to soybean cultivars PR91M10 (susceptible to *Botrytis cinerea* attack) and PR92B62 (resistant to *B. cinerea* attack) have been seeded in vegetation pots with 5 kg soil in 3 replications per variant. Experimental variants included: untreated control, chemical contact pesticides Captan®, Teldor® and Batron® administrated on plant; chemical systemic pesticides Topsin®, Rovral® and Topsin® M administrated in soil; biological control agent E1 (monostrain extract of selected *Botrytis* isolate administrated on leaves and in soil); biological control agent E2 (multi-strain extract of selected *Botrytis* isolates administrated on leaves and in soil); biological control agent E3 (multi-strain extract of selected *Trichoderma* and *Penicillium* isolates administrated on leaves and in soil); biological control agent E4 (multistrain extract of selected *Botrytis*, *Trichoderma* and *Penicillium* isolates administrated on leaves and in soil).

Soybean plants were experimentally infected with inoculum of *B. cinerea*. Four treatments with chemical or biological agents of two day intervals were done. Three days after, rhizospheric soil samples were collected from pots. Soil crumbs were plated on water-agar in Petri dishes 10 cm diameter and incubated at 25 °C for 5 days. The developed fungal structures were taxonomically identified under optic microscope according to Domsch and Gams (1970) and Samson and Hoekstra (1988). The average number of colonies/variant and the frequency of each taxa registered allowed their grouping in 3 classes of constancy, respectively in constant, accessory and accidental taxa. A total number of 18 fungal genera were identified in samples collected from rhizosphere of resistant soybean cultivar PR92B62 and 21 from the cultivar PR91M10 susceptible to gray mold (Tables 1, and 2).

With the cultivar PR91M10 the average number of colonies developed from a soil crumb ranged between 9 and 16 as a function of treatment nature and method of administration and with the cultivar PR92B62 this number ranged between 6 and 13. In soybean cultivar PR91M10 the number of colonies was lower with both chemical treatments, biological preparations E2 and E3, and both application

Table 1 The structure of fungal communities in rhizosphere of soybean cultivar PR91M10 treated with various pathogen control agents. Extract 1: mono-strain extract of selected *Botrytis* isolate administrated on leaves and in soil; Extract 2: multi-strain extract of selected *Botrytis* isolates administrated on leaves and in soil; Extract 3: multi-strain extract of selected *Trichoderma* and *Penicillium* isolates administrated on leaves and in soil; and Extract 4: multi-strain extract of selected *Botrytis*, *Trichoderma* and *Penicillium* isolates administrated on leaves and in soil (Matei and Matei 2010)

No	Fungal genera	Experimental variants										
		Control	Chemical treatment		Extract 1		Extract 2		Extract 3		Extract 4	
			Contact	Systemic	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil
1	*Actinomycetes*											
2	*Harposporium*											
3	*Mortierella*											
4	*Alternaria*											
5	*Fusarium*											
6	*Papulaspora*											
7	*Humicola*											
8	*Curvularia*											
9	Sterile mycelia											
10	*Acaulopage*											
11	*Aspergillus*											
12	*Acremonium*											
13	*Cunninghamella*											
14	*Myrothecium*											
15	*Botrytis*											
16	*Actinomucor*											
17	*Nematoctonus*											
18	*Rhinocladiella*											
19	*Arthrobotrys*											
20	*Trichoderma*											
21	*Geotrichum*											
	No. colonies	12	9	10	15	12	10	9	9	9	16	13

constant genera
accessory genera
accidental genera

methods as compared with control. With the treatments made by E1 and E4, especially when applied on plant, the number of fungal colonies was higher than in control rhizosphere.

Generally, the communities were formed by 5–8 genera, slowly varying from the 7 taxa identified to control. The greatest diversity of microflora was recorded for E4 administrated on plants. In soybean cultivar PR92B62 the number of colonies isolated from rhizosphere of plants treated with E2 applied to soil exceeded the others including the control. Systemic and contact fungicides induced reductions of number and diversity in fungal communities more than biological control agents E1–E4. As a general characteristic, at this cultivar, the treatment with biological preparations administrated in soil preserved better the number and diversity of rhizosphere fungal communities. With few exceptions, actinomycetes for PR91M10 and *Humicola* for PR92N62 had the status of constant genera in all experimental variants, their very high frequency being connected with general and stable conditions in rhizosphere. They were less influenced by experimental factors

Table 2 The structure of fungal communities in rhizosphere of soybean cultivar PR92B62 treated with various pathogen control agents. Extract 1: mono-strain extract of selected *Botrytis* isolate administrated on leaves and in soil; Extract 2: multi-strain extract of selected *Botrytis* isolates administrated on leaves and in soil; Extract 3: multi-strain extract of selected *Trichoderma* and *Penicillium* isolates administrated on leaves and in soil; and Extract 4: multi-strain extract of selected *Botrytis*, *Trichoderma* and *Penicillium* isolates administrated on leaves and in soil (Matei and Matei 2010)

No	Fungal genera	Experimental variants										
		Control	Chemical treatment		Extract 1		Extract 2		Extract 3		Extract 4	
			Contact	Systemic	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil
1	*Humicola*											
2	*Nematoctonus*											
3	*Mortierella*											
4	*Actinomicete*											
5	*Botrytis*											
6	*Cylindrocarpon*											
7	*Rhinocladiella*											
8	*Arthrobotrys*											
9	Sterlie mycelia											
10	*Alternaria*											
11	*Cunninghamella*											
12	*Acremonium*											
13	*Dactylaria*											
14	*Gliocladium*											
15	*Fusarium*											
16	*Rhizopus*											
17	*Aspergillus*											
18	*Harposporium*											
	No. colonies	11	8	6	8	9	10	13	9	9	9	10

constant genera
accessory genera
accidental genera

than other fungal taxa. A number of 1–3 genera were common with those from control rhizosphere, but 1–4 differential genera occurred in rhizosphere of plants under the influence of chemical or biological control agents for each soybean cultivar. *B. cinerea* was present in the rhizosphere of control at cultivar PR92B62 and in the communities from plants treated with E1 and E3 with the cultivar PR91M10, with status of accidental genus, at frequencies below 25%. A special group developed on water-agar was that of nematophagous (predaceous) fungi, which feed with soil nematodes.

With the cultivar PR91M10, *Harposporium anguillulae* (Fig. 6) was constant species in control rhizosphere. It was also present in the rhizosphere of plants treated with E1 administrated in soil (as accidental species) and with E4 administrated on plants (as accessory species). *Acaulopage* appeared as a constant species in rhizosphere of plant treated with systemic fungicides and as accessory species under the influence of E2 administrated on plants. The same preparation administrated in soil, as well as both E3 variants were favorable to *Nematoctonus* (Fig. 7) isolated as accidental species.

In the rhizosphere of the plants treated with E4, *Arthrobotrys* was identified as accessory genus when control agent was administrated on plants and as accidental genus when it was administrated in the soil. With the cultivar PR92B62, *Nematoctonus* had accessory status in control and accidental in variant E3

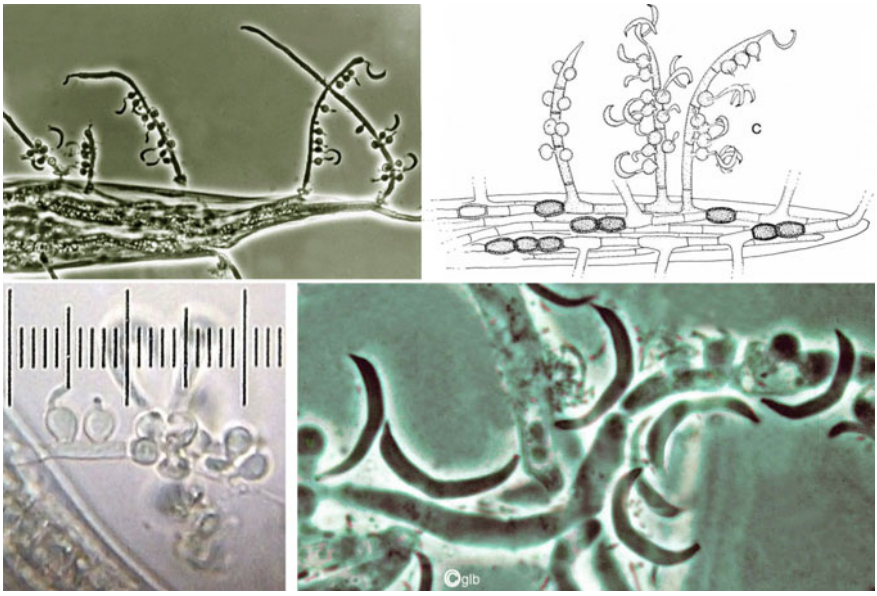

Fig. 6 *Harposporium anguillulae*. (Top) Microscopic and schematic view of the endoparasitic fungus conidiophores, sub-spherical to spherical conidiogenous phialides, hyaline crescent phialospores, septate hyaline hyphae in nematode body, and dark chlamydospores (resting spores) developed as individual, paired, and chained cells with thickened walls. (Down) Microscopic view of erect conidiophores with swollen phialides with narrow necks and hyaline, crescent phialospores. *Source* Barron (1977)

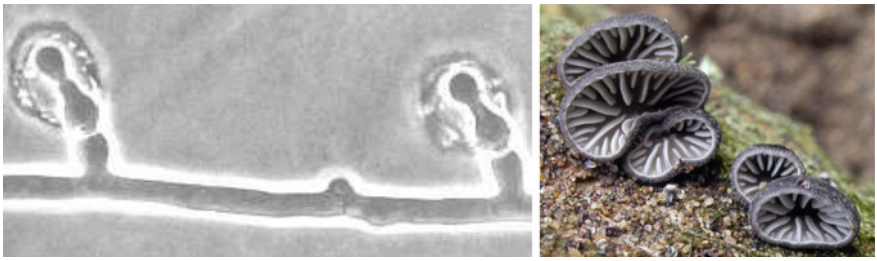

Fig. 7 *Nematoctonus* sp. (Left) The adhesive knobs of a nematophagous hyphomycetous anamorph of a basidiomycetous fungus as revealed by the presence a clamp connections beside a septum of a dikaryotic hypha (*Source* Bryce Kendrick, http://www.mycolog.com); (Right) *Hohenbuehelia grisea*, a telemorphic basidiomycetous stage of *Nematoctonus* sp. (*Source* Fred Stevens, http://www.mycoweb.com/)

administrated in soil. *Arthrobotrys*, which developed adhesive three-dimensional network traps, immobilized nematodes and consumed their bodies by enzymatic lysis, has the status of constant species in E1 administrated in soil, accessory species in variant with contact pesticides and accidental species in variant with

systemic fungicides. *Dactylaria* had constant status in variant with E1 on plant and *Harposporium* developed as accessory species in rhizosphere of soybean treated with E4 in soil. The structure of fungal communities in rhizosphere of soybean varied as a function of cultivar, the nature of control agent and the method of administration. Eighteen fungal genera were identified from variants of soybean susceptible to pathogens PR91M10 and 21 genera from soybean cultivar PR92B62 resistant to pathogens.

With the resistant cultivar PR92B62, the treatments with biological control agents E1 and E4 administrated to soil supported numerical increments of fungal structures and E4 administrated on plants stimulated the fungal diversity. In susceptible cultivar PR91M10, the biological control agents administrated to soil preserved better the number and diversity of fungal communities in rhizosphere. A number of 1–3 genera were common with control, but 1–4 differential genera occurred in rhizosphere of plants under the influence of chemical or biological control for each soybean cultivar. In susceptible cultivar PR91M10, genus *Botrytis* was present accidentally in variants with E1 and E3 administrated on plants and in resistant cultivar PR92B62 only in control, the other treatments with biological or chemical control agents being efficient in limiting pathogen proliferation. The presence of four fungal nematophagous species recorded with status of constant, accessory or accidentally taxa in experimental variants and control, too. In agriculture, both chemical and biological methods of control act on pathogen population as well as on rhizospheric microflora.

A greenhouse experiment was designed to compare the structure of fungal rhizospheric microflora of soybean cv. PR91M10 susceptible to gray mold and PR92B62 resistant, treated with systemic and contact fungicides, as well as with four fungal preparations from genera *Botrytis*, *Trichoderma* and *Penicillium* administrated on plants and in soil. The taxonomic composition of fungal community was assessed by plating soil fragments on water agar and identifying the developed structures. A total number of 21 genera were identified for susceptible soybean cultivar PR91M10 and 18 genera for resistant cultivar PR92B62. The frequency registration allowed the genera to be classified as constant accessory and accidental. Thus, the status of each taxon was the same or modified as a function of the nature of treatment (with biological or chemical control agents) or method of administration (on leaves or in soil). Apart of significant influence of fungal extracts on improving plant health, the ecological analysis of the rhizospheric fungal communities assessed the influence of biological control agents provided by selected microbial strains, on natural microflora composition and number. The use of water agar substrate allowed the isolation and identification of the species of nematophagous genera *Arthrobotrys*, *Dactylaria*, *Nematoctonus* and *Harposporium*, some of them rarely isolated on other usual media. The development of ring form fungal traps or adhesive knobs and haustoria-like hyphae was possible to be monitored as well as trapped nematodes digestion by predaceous fungi, demonstrating the dynamic relationships between microflora and fauna in the rhizosphere of cultivated plants such as soybean under the influence of control agents for gray mould (Matei and Matei 2010).

Here it is attempted to discuss the impact of pesticides on the fungal genera and species, which are of importance in the biological control of plant pests and diseases.

2.1 Arthrobotrys *spp.*

Few management options are available for the suppression of soilborne plant-parasitic nematodes in established golf course putting greens. The exploitation of nematophagous fungi to suppress nematodes may be inhibited by pesticides used to manage plant diseases. For instance, the effect of various pesticides (diflubenzuron, malathion, mancozeb and carbendazim), disinfectants (calcium hypochlorite and formaldehyde) and oil cakes (sunflower and soybean oil cakes) commonly used as supplements in mushroom cultivation on the growth of the nematophagous fungus, *Arthrobotrys oligospora* (Fig. 8, and 9), was studied under in vitro conditions. Carbenazim caused 99% inhibition of radial mycelial growth in Petri dishes at all concentrations tested (10–40 µg a. i. mL^{-1}) in comparison to non-treated dishes.

Mancozeb caused 43 and 23% inhibition at 250 and 500 µg a. i. mL^{-1} respectively and 99% inhibition at concentration of 1000 µg a. i. mL^{-1} and above. Diflubenzuron and malathion at 10–40 µg a. i. mL^{-1} caused 30–41% and 24–54% inhibition, respectively. Formalin (0.5–2.0% v v^{-1}) inhibited growth of *A. oligospora* completely. However, calcium hypochlorite, sunflower and soybean oil cake at concentrations of up to 2.0% w v^{-1} caused less than 3.5% inhibition (Mohammadi Goltapeh et al. 2008). Hyphal growth of *A. oligospora* was limited at chlorothalonil concentrations in excess of 10 ppm, and ceased at myclobutanil concentrations in excess of 1 ppm. Conidia did not germinate in the presence of chlorothalonil concentrations in excess of 1 ppm and Myclobutanil concentrations in excess of 5 ppm. These studies suggest that continued application of chlorothalonil and Myclobutanil to manage fungal pathogens of plants such as bentgrass may inhibit the ability of *A. oligospora* to survive in soil (Woodward 2002). Furthermore, it has been shown that some fungicides, like maneb, and mancozeb are toxic to certain nematode species, such as *Caenorhabditis elegans*, whereas their potentially toxic metabolite, ethylenethiourea (ETU) was of minimal anti-nematode toxicity only at high concentrations (Easton et al. 2001). The effect of fungicides, benomyl, copper oxychloride and mancozeb on the rhizosphere microflora of potato has been studied. The fungal and bacterial populations of rhizosphere and non-rhizosphere soils dropped significantly following application of the fungicides and throughout the study treated plots harbored lower population than the control plots. The effect of fungicides was more pronounced in the rhizosphere region than the non-rhizosphere.

The species composition was also altered and dominant species were more severely affected. *Acremonium rutilum*, *Aspergillus flavus*, and *Necteria ventricosa* were isolated only from the fungicide treated plants. Irrespective of treatment,

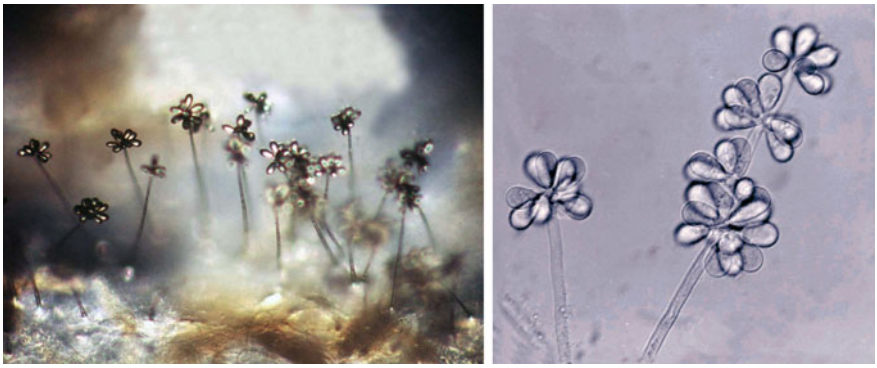

Fig. 8 *Arthrobotrys oligospora*. (Left) Erect condiophores and hyaline conidia (*Source* George Barron, University of Guelph, http://hdl/handle/10214/6011); (Right) Closer view of conidiophores and conidia (*Source* George Barron, University of Guelph, http://hdl/handle/10214/5663)

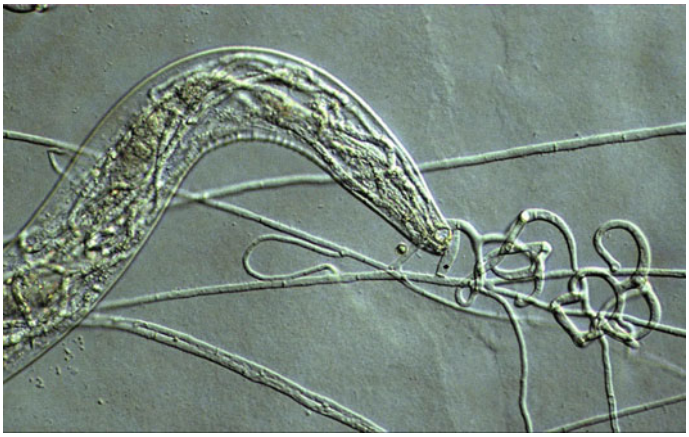

Fig. 9 *Arthrobotrys oligospora*. *Source* George Barron, http://hdl.handle.net/10214/5661

A. rutilum and *N. ventricosa* were isolated only from the rhizosphere soils while *Arthrobotrys conoides*, *Athrobotrys oligospora*, *Oidiodendron echinulatum*, and *Rhizopus oryzae* were isolated from non-rhizospheric soils only. *Cladosporium cladosporioides*, *Fusarium minutissima*, *Mucor plumbeus*, *M. racemosus*, *O. echinulatum*, *Penicillium brevicompactum*, *P. chrysogenum*, *Trrichoderma harzianum*, and *T. viride* indicated sporadic appearance and did not show any treatment or habitat (rhizosphere, non-rhizosphere) preference. Species composition of fungi isolated from rhizosphere and non-rhizosphere soils during the study was similar to that of Sudha (1979) and Chandra et al. (1982). Srivastava and Dayal (1981) also reported change in structure of fungal community following fungicidal treatment. The population of dominant rhizosphere fungi like *C. cladosporioides*,

Fusarium poae, *Mucor hiemalis*, *M. plumbeus*, *M. racemosus*, *P. brevicompactum*, and *P. chrysogenum* was drastically reduced in the fungicide treated sets. On the other hand, fungi with lower population were least affected. This also suggests that the fungicidal treatment adversely altered the rhizosphere environment for the fungi community. Their results clearly demonstrated the possibility of exudation of fungicidal substances from the roots of fungicide treated potato plants. The fungal and bacterial populations of rhizosphere of fungicide treated plants are drastically reduced and significantly altered in species composition. It appears the fungicide spray changes fungal community structure and thus adversely affects various fungi mediated processes of soil and rhizosphere. This may harmfully affect the process of nutrient release and aggravate soil pathogens due to indiscriminate killing of antagonistic saprotrophs of the root region (Shukla et al. 1987).

Application of fertilizers such as urea, diammonium phosphate (DAP) and muriate of potash in soil adversely affected the spore germination of *Arthrobotrys dactyloides*. Amendment of soil with urea at the concentrations of 1.0, 0.5 and 0.1% completely inhibited spore germination and direct trap formation on the conidium, whereas muriate of potash delayed and reduced the spore germination even at the lowest concentration. DAP also inhibited spore germination at 1.0% concentration, while at lower concentration the percentage of spore germination was reduced. Application of neem cake at the concentration of 0.5% also inhibited spore germination after 24 h of amendment. The inhibitory effect of neem cake was reduced after 15 days of amendment, while after 30 days after amendment the inhibitory effect was completely lost and the spore germinated by direct trap as in non-amended soil. Nematodes were not attracted to non-germinated spores after 24 h of amendment. After 15 days of amendment nematodes were attracted to agar blocks containing fewer germinated spores after 24 h of incubation but after 48 h of incubation large number of nematodes were attracted and trapped by the germinated spores with direct traps. After 30 days of amendment, larger number of nematodes were attracted and trapped by direct traps. Among the bran media, pea bran agar medium supported maximum growth of all the isolates except isolate B. Gram and rice bran agar media were next best. However, the growth of isolate B on the gram bran agar medium was more or less equal as other isolates. On pigeon pea bran agar medium, isolate E failed to grow while other isolates recorded poor growth. On lentil bran agar medium, only isolate B and D recorded little growth, whereas other isolates failed to grow. All the isolates recorded good sporulation on bran agar media except pigeon pea and lentil bran agar media. The grain agar media supported moderate to very good growth of all the isolates. In general isolate B remained slow growing on these media except gram grain and sorghum grain agar media on which growth of this isolate was comparable to other isolates. Sporulation in general, was good on all the grain agar media. Among different substrates screened, barley grain and pea bran were found superior to others for mass culture of isolate A of *A. dactyloides* (Kumar et al. 2005a).

Jaffee (2004) used soil cages (polyvinyl chloride pipe with mesh-covered ends) to determine how the quantity of two organic amendments affected the nematode-trapping fungi *Dactylellina haptotyla* and *Arthrobotrys oligospora*, which were

studied independently in two different vineyards. Each cage contained 80 cm^3 of field soil (120 g dry weight equivalent), fungal inoculum (two alginate pellets, each weighing 1.9 mg and containing assimilative hyphae of one fungus), and dried grape or alfalfa leaves (0, 360, or 720 mg equivalent to 0, 4500, or 9000 kg ha^{-1}) with a C:N of 28:1 and 8:1, respectively. Cages were buried in the vineyards, recovered after 25–39 days, and returned to the laboratory where fungus population density and trapping were quantified. *Dactylellina haptotyla* population density and trapping were most enhanced by the smaller quantity of alfalfa amendment and were not enhanced by the larger quantity of alfalfa amendment. *Arthrobotrys oligospora* population density was most enhanced by the larger quantity of alfalfa amendment, but *A. oligospora* trapped few or no nematodes, regardless of amendment. Trapping and population density were correlated for *D. haptotyla* but not for *A. oligospora* (Jaffee 2004).

Variability in growth and sporulation of five isolates of *Arthrobotrys dactyloides* was studied on five agar, 6 bran and 5 grain media. Potato dextrose agar (PDA) supported maximum growth of isolate A, C and E, while growth of isolate B and D was significantly lower on this medium. On Czapek's agar and yeast glucose agar media the differentiation in the isolates in relation to growth was poor than PDA. The other two media showed much poorer differentiation. On Czapek's agar medium, sporulation was recorded in isolate B only, whereas other isolates showed rare sporulation. Among the bran media, pea bran agar medium supported maximum growth of all the isolates except isolate B. Gram and rice bran agar media were next best. However, the growth of isolate B on the gram bran agar medium was more or less equal as other isolates. On pigeon pea bran agar medium, isolate E failed to grow while other isolates recorded poor growth. On lentil bran agar medium, only isolate B and D recorded little growth, whereas other isolates failed to grow. All the isolates recorded good sporulation on bran agar media except pigeon pea and lentil bran agar media. The grain agar media supported moderate to very good growth of all the isolates. In general isolate B remained slow growing on these media except gram grain and sorghum grain agar media on which growth of this isolate was comparable to other isolates. Sporulation in general, was good on all the grain agar media. Among different substrates screened, barley grain and pea bran were found superior to others for mass culture of isolate A of *A. dactyloides* (Kumar et al. 2005b).

2.2 Beauveria Bassiana *(Balsamo) Vuillemin*

Biological control, particularly by entomopathogenic fungi, is important for reducing the population density of pests in Integrated Pest Management (IPM) programs. *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) (Figs. 10 and 11) is a registered biopesticide with a broad host range of approximately 700 insect species used for management of several crop insect pests. The integration of microbial pesticides with chemical pest management practices

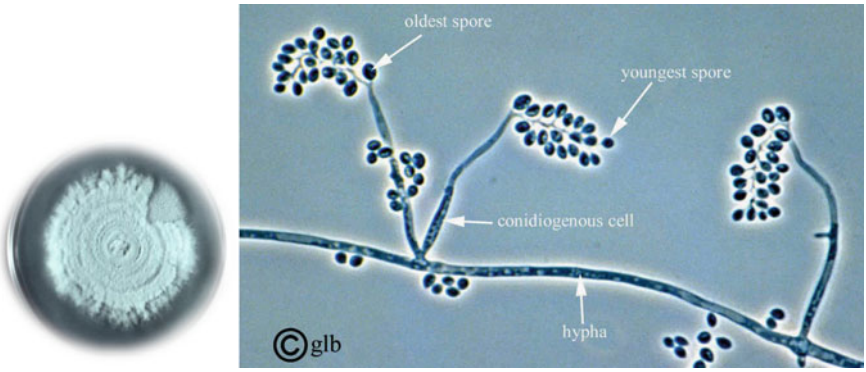

Fig. 10 *Beauveria bassiana*. (Left) Colony growth on agar plate (*Source* http://www.indiamart.com/); (Right) Fungal hypha with hyaline conidiophores and hyaline, sympodially produced conidia (*Source* George Barron, University of Guelph)

Fig. 11 Synnemata of *Beauveria bassiana* developed from hawk moth pupa. *Source* George Barron, http://hdl.handle.net/10214/6030/

requires detailed compatibility studies. Data from such studies would enable farmers to select appropriate compounds and schedule microbial and chemical pesticide treatments so that benefits from compatible sets can be accrued and, with non-compatible pairs, the deleterious effect of the chemical on the microbe in the biopesticide can be minimized (Butt et al. 2001; Inglis et al. 2001; Lacey et al. 2001).

The knowledge of the compatibility between entomopathogenic fungi and pesticides may facilitate the choice of proper products for integrated pest management

(IPM) program considering the fungus as an important pest control agent (Neves et al. 2001). Combined utilization of selective insecticides in association with fungus pathogens can increase the efficiency of control by reduction of the amount of applied insecticides, minimizing environmental contamination hazards and pest resistance (Moino Jr and Alves 1998; Quintela and McCoy 1998a, b). Conidial survival can be effected by interaction with agrochemicals, environmental factor (Benz 1987) or by biopesticide and/or chemical product used to protect plants (Alves and Lecuona 1998; Anderson and Roberts 1983; Loria et al. 1983). Fungal biocontrol agents and selective insecticide may act synergistically increasing the efficacy of control, allowing the lower doses of insecticides, preservation of natural enemies, minimizing environmental pollution and decreasing the likelihood of development of resistance to either agent (Ambethgar 2003). Chemical insecticides, herbicides and fungicides are usually applied in conventional farming practices. These compounds, especially fungicides applied against plant pathogens, might also negatively affect the populations of entomopathogenic fungi with reduced pest regulation potential as a consequence (Meyling and Eilenberg 2007). Klingen and Haukeland (2006) provided a detailed review of published studies of effects of chemical pesticides on entomopathogenic fungi and nematodes. Their main conclusions were that insecticides and herbicides were not very harmful to fungal growth while fungicides were sometimes harmful (Klingen and Haukeland 2006). However, most studies performed in vitro with fungal cultures and extrapolation from studies in laboratory experiments to field conditions may not be straightforward (Meyling and Eilenberg 2007). In the UK, for example, previous field application of the fungicide benomyl correlated with a lower incidence of *B. bassiana* in soil samples (Miêtkiewski et al. 1997; Chandler et al. 1998). The entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. exhibit good compatibility with imidacloprid insecticide (600 g L^{-1} a.s. in comercial product), which does not affects the biological parameters of the beneficial microorganism. However, *B. bassiana* exhibits high sensitivity towards insectofungicide mixture, which inhibits completely the spore germination at the recommended concentrations. The tested chemical phytosanitary products (at different concentration) did not inhibit completely the bacterial growth. The insecticide based on imidacloprid 600 g L^{-1}, did not affect the growth of any *Bacillus* biocontrol strains, when it was tested at 20% concentrations. Only the insectofungicides mixture, in 20% concentration, caused moderate growth inhibition (less than 5 mm) to some of the bacterial strains tested (Constantinescu et al. 2014).

In vitro experiments further showed that the fungicide triadimefon inhibited the growth of *B. bassiana*, but fields previously treated with this product showed a higher frequency of occurrence of the fungus in soil samples than in samples from untreated control soils (Miêtkiewski et al. 1997; Chandler et al. 1998). The fungicidal product albicarb even increased activity of in vitro cultures of *B. bassiana* (Miêtkiewski et al. 1997). This emphasizes that due to the complex interactions and composition of agroecosystems applications of specific fungicides are not necessarily detrimental to the occurrence of entomopathogenic fungi in the soil. Selected compounds could thus possibly be used in integrated pest

management (Miêtkiewski et al. 1997). Durán et al. (2004) mentioned that benomyl, dimethomorph-mancozeb, chlorothalonil, propineb, mancozeb, and mancozeb-cymoxanil mixture fungicides significantly affected germination and growth of *B. bassiana* while fosetyl-Al, propamocarb, and copper oxychloride did not. Mancozeb and copper oxychloride were not compatible and caused complete or strong inhibition of vegetative growth as well as sporulation (Shafa et al. 2012). Four fungicides used commercially for control of foliar diseases of potato were evaluated in vitro and under field conditions for their effects on the survival of spores of *Beauveria bassiana* (Balsamo) a pathogen of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), (Coleoptera: Chrysomelidae). Mancozeb, the most detrimental of the fungicides, substantially reduced survival in both laboratory and field studies. Metiram was only slightly less inhibitory to *B. bassiana* than was mancozeb in vitro but was not different from the control under field conditions. Chlorothalonil and metalaxyl were not detrimental to spore survival under any of the conditions examined (Loria et al. 1983). The effect of seven fungicides on the entomopathogen fungus *B. bassiana* strain LBb-1. (Bals.) Vuil. was studied in vitro with fungicide concentrations of 10, 100, 200, 500, 1000, and 2000 mg L^{-1}. The inhibition of fungus colony growth, the effect on spore production capacity and conidia germination were evaluated. LD_{50} and LD_{95} of the fungicide tebuconazole were considered below 10 mg L^{-1}. LD_{50} of difenconazole was considered in 30.45 mg L^{-1}. For mancozeb it was 161.49 mg L^{-1}; for folpet it was 398.92 mg L^{-1}, and for azoxstrobin it was 1463.11 mg L^{-1}. LD_{50} for copper oxide and zineb were >2000 mg L^{-1}. LD_{95} for difenconazole was considered as 1019.04 mg L^{-1}, while for the rest of the fungicides it was considered above 2000 mg L^{-1}. Tebuconazole was classified as toxic for *B. bassiana* by the OILB scale, while the rest were classified as lightly toxic. The seven studied fungicides were classified as very toxic for the fungus according to their T values, and they were not compatible with the fungal isolate. Conidia germination of *B. bassiana* was not occurred in none of tebuconazole, difenconazole, mancozeb, azoxystrobin, and folpet concentrations assayed, while in the presence of zineb and copper oxide they germinated up to 1000 and 2000 mg L^{-1}, respectively (González et al. 2011). The three fungicides tested for their compatibility with *B. bassiana* isolates exhibited varying results. Mancozeb was found detrimental to all the isolates of *B. bassiana* tested in the present study. It totally inhibited conidial germination. In fact, mancozeb is used for preventing *B. bassiana* infection in silkworm rearing (Kumar et al. 2011). Bavistin is a conversion product of benomyl which is used in germination bioassays to slow down the growth of germ tubes (Goettel et al. 2000). Counts of germinated conidia can be made more accurately when the germ tubes of nearby conidia do not anastomose. The germination pegs in most of the *B. bassiana* isolates showed an abnormal bent appearance bursting at the tip in the presence of bavistin. Only three isolates (ARSEF 1315, ARSEF 1316, and ITCC 913) showed normal germ tube morphology in the presence of bavistin and showed growth in the growth bioassay. Thus three bavistin tolerant isolates were identified when 30 isolates were screened. The basis for decreased sensitivity to MBC in the isolates of *B. bassiana* was reported by Butters et al. (2003). The *B. bassiana* isolates differed

in their response to copper oxychloride fungicide. While germination was completely inhibited in only one of the thirty isolates, growth was completely inhibited in 8 isolates. With some isolates, growth was stimulated in the presence of copper oxychloride while with others, growth was inhibited—inhibition ranging from slight to significantly less than the control. Martins et al. (2012) reported that copper oxychloride inhibited the growth of *B. bassiana* but found less toxic when compared to other copper-based compounds. In fact copper is used in the medium for selective growth of entomogenous fungi (Akello et al. 2007). Though some highly sensitive to copper oxychloride *B. bassiana* isolates have been found (Challa and Sanivada 2014), the majority of the isolates ($\sim$2/3rds of the 30 tested) were found tolerant to copper oxychloride. Copper oxychloride was found as the best fungicide formulation in combination with entomopathoenic fungi *B. bassiana* to use in integrated pest management program. Further field trails under greenhouse should be done to confirm the synergistic performance of *B. bassiana* and fungicides or insecticides with integrated pest management approach. Except mancozeb, all the insecticides and fungicides showed a significant inhibitory effect on the germination of *B. bassiana* conidia. There was significant inhibition of mycelial growth in few isolates with insecticides as well as fungicides except copper oxychloride. Copper oxychloride promoted the mycelial growth of many isolates whereas mancozeb inhibited the mycelial growth of all the isolates tested. They suggested that the most appropriate insecticide and fungicide for use in integrated pest management programs in combination with *B. bassiana* isolates (Challa and Sanivada 2014). Kouassi et al. (2003) found that simultaneous application of the fungicides metalaxyl, macnozeb and copper oxide with *B. bassiana* reduced insect infection, suggesting that the fungicides inhibited germination on the cuticle. Background information about the different degrees of entomopathogenic fungi showing fungicide tolerance was reported by Maribel and France (2010). Tamai et al. (2002) classified sulphur as compatible and tebuconazole, mancozeb and copper oxychloride as highly toxic to this mycopathogen. Mani et al. (1995) found that exposure to copper oxychloride reduced the longevity and fecundity of the citrus mealybug parasitoid *Leptomastix dactylopii* (Hymenoptera: Encyrtidae). Durán et al. (2004) mentioned that benomyl, dimethomorph-mancozeb, chlorothalonil, propineb, mancozeb, and mancozeb-cymoxanil mixture of fungicides significantly affected germination and growth of *B. bassiana* while fosetyl-Al, propamocarb, and copper oxychloride did not. Jaros-Su et al. (1999) evaluated four fungicides used for controlling foliar diseases of potato (*Solanum tuberosum*) under field and laboratory conditions for their effects on the infectivity and sporulation of *Beauveria bassiana* when used as a control for the Colorado potato beetle, *Leptinotarsa decemlineata*. The direct effects of fungicides on *B. bassiana*-induced *L. decemlineata* mortality and the effect of time between fungicide and *B. bassiana* application was investigated. Effects of fungicide on conidial survival in the soil and on foliage were examined in the field. Significantly more larval mortality was observed when larvae were sprayed with *B. bassiana* than with the water control. Fungicide had no significant effect on larval mortality in the field. In the laboratory, survival of larvae was significantly lower among larvae fed fungicide-treated foliage.

B. bassiana-induced mortality in the laboratory was observed only when larvae were fed foliage treated with copper hydroxide or water. Larvae fed mancozeb- or chlorothalonil-treated foliage experienced high mortality regardless of *B. bassiana* treatment. While there was no significant effect of fungicide on *B. bassiana* sporulation on cadavers in the field, a pattern emerged that indicated higher proportions of cadavers producing conidia in plots sprayed with water or copper hydroxide than in plots sprayed with chlorothalonil or mancozeb. Survival of *B. bassiana* conidia in the soil and on foliage was significantly greater in plots treated with copper hydroxide or water than in plots treated with mancozeb or chlorothalonil. Fungicides such as copper hydroxide may be less deleterious to the fungus than mancozeb and chlorothalonil (Jaros-Su et al. 1999).

The in vitro fungicidal effects of six commonly used fungicides, namely fluazinam, propineb, copper (II) hydroxide, metiram, chlorothalonil and mancozeb, and herbicides, namely isoxaflutole, fluazifop-P-butyl, flurochloridone, foramsulfuron, pendimethalin and prosulfocarb, on mycelial growth, sporulation and conidial germination of entomopathogenic fungus *B. bassiana* (ATCC 74040) were investigated. Mycelial growth rates and sporulation at 15 and 25 PDA plates containing 100, 75, 50, 25, 12.5, 6.25 and 0% of the recommended application rate of each pesticide. The tested pesticides were classified in four scoring categories based on reduction in mycelial growth and sporulation. All pesticides, herbicides and fungicides tested had fungistatic effects of varying intensity, depending on their rate in the medium, on *B. bassiana*. The most inhibitor herbicides were flurochloridone and prosulfocarb, and fluazinam and copper (II) hydroxide were most inhibitory among the fungicides, while the least inhibitory were isoxaflutole and chlorothalonil. Sporulation and conidial germination of *B. bassiana* were significantly inhibited by all tested pesticides compared with the control treatment. Flurochloridone, foramsulfuron, prosulfocarb and copper (II) hydroxide inhibited sporulation entirely at 100% rate (99–100% inhibition), and the lowest inhibition was shown by fluazifop-P-butyl (22%) and metiram (33%). At 100% dosage, all herbicides in the test showed a high inhibitory effect onconidial germination. Conidial germination inhibition ranged from 82% with isoxaflutole to 100% with fluorochloridone, pendimethalin and prosulfocarb. At 200% dosage, inhibition rates even increased (96–100%). All 12 pesticides tested had a fungistatic effct on *B. bassiana* of varying intensity, depending on the pesticide and its concentration. *B. bassiana* is highly affected by some herbicides and fungicides even at very low rates. Flurochloridone, foramsulfuron, prosulfocarb and copper (II) hydroxide stopped sporulation. Of all tested pesticides, isoxaflutole, fluazifop-P-butyl and chlorothalonil showed the least adverse effects and therefore probably could be compatible with *B. bassiana* in the field (Celar and Kos 2016). The effects of 6 fungicides (chlorothalonil, maneb, thiophanate-methyl, mancozeb, metalaxyl + mancozeb and zineb) and two herbicides (diquat and glufosinate-ammonium) used commercially in potato fields were evaluated in vitro on *B. bassiana*, a fungal pathogen of the Colorado potato beetle, *L. decemlineata* (Say) (Fig. 12). All 6 fungicides tested along with the herbicide glufosinate-ammonium inhibited *B. bassiana* mycelial growth and sporulation. However, the second herbicide tested, diquat, had no noticeable effect on

Fig. 12 Colora potato beetle, *Leptinotarsa decemlineata*. (Left) Healthy beetle (*Source* http://bugguide.net/); (Right) Cadaver of Colorado potato beetle, *Leptinotarsa decemlineata*, infected to the entomopathogenic fungus, *Beauveria bassiana* (*Source* http://www.potatobeetle.org/)

B. bassiana mycelial growth and sporulation. Remarkably, diquat synergized the insecticidal activity of *B. bassiana* in the simultaneous treatments on Colorado potato beetle adults and caused 50–76.6% mortality (Todorova et al. 1998). Oryzalin was the only one of 21 herbicides that caused no significant inhibition of in vitro germination and growth of the entomogenous fungus *Beauveria bassiana* (Balsamo) Vuillemin. Twelve of the herbicides significantly inhibited germination and growth at the lowest concentration tested (6 mg a. i. L^{-1}).

Others caused significant inhibition at higher concentrations. Diuron, pronamide, simazine, and terbacil inhibited germination but either did not affect or only moderately inhibited mycelial growth, thus indicating possible fungistatic rather than fungicidal activity. Mortality due to *B. bassiana* was significantly reduced when *Spodoptera frugiperda* (J. E. Smith) larvae were exposed to conidia + soil treated with either alachlor or oryzalin. Numbers of viable colony-forming units extracted from the conidia + soil treatments were significantly reduced by the alachlor application but not by oryzalin (Gardner and Storey 1985). Celar and Kos (2012) studied the in vitro effect of five commonly used herbicides viz., pyridate (Lentagran 45 WP®, 45% a. i., Belchim Crop Protection), fluazifop-P-butyl (Fusilade Forte® 150 EC, 15% a. i., Syngenta), foramsulfuron (Equip®, 2.25% a. i., Bayer CropScience), tembotrione (Laudis®, 4.4% a. i., Bayer CropScience) and Smetolachlor (Dual® Gold 960 EC, 96% a. i., Syngenta) on mycelial growth of entomopathogenic fungus *Beauveria bassiana* (ATCC 74040). They evaluated each herbicide at different concentrations: 100, 75, 50, 25, 12.5, 6.25 and 0% of recommended field application rate on PDA agar plates at 15 and 25 °C. The herbicides tested were classified in 1–4 scoring categories based on the reduction in mycelial growth: 1 = harmless (<25% reduction), 2 = slightly harmful (25–50%), 3 = moderately harmful (51–75%), harmful (>75%) in toxicity tests. All the five

herbicides had fungistatic effect to *B. bassiana* at varying intensities depending on their concentrations in medium. They showed that *B. bassiana* was very sensitive to the herbicides tested, particularly at the recommended as well as lower field dosages. The ratios of 50–100% of the recommended doses of the selected herbicides foramsulfuron, tembotrione and S-metolachlor had strong fungistatic effects on mycelial growth (>75% inhibition) at 15 °C. Foramsulfuron had fungicidal effect at 100% concentration. Foramsulfuron, tembotrione and S-metolachlor were less inhibitory at 25 than at 15 °C, but the temperature had no statistically significant influence on the reduction of mycelial growth at pyridate and fluazifop-P-butyl. Of the herbicides tested, pyridate and fluazifop-P-butyl showed less adverse effects and were found to be probably compatible with *B. bassiana* in the field. However, extensive field studies complemented by parallel laboratory experiments should consider assessing the interaction between selected herbicides and *B. bassiana* isolates to evaluate their ecological impact in cropped environments. The in vitro effect of six commonly used herbicides viz., amidosulfuron, dicamba, metribuzin, pyridate, S-metolachlor and tembotrione on mycelial growth of entomopathogenic fungus *B. bassiana* (ATCC 74040) was investigated by Kos and Celar (2013).

Mycelial growth rates at 15 and 25 °C were evaluated on PDA plates containing 100, 75, 50, 25, 12.5, 6.25 and 0% of the recommended application rate of each selected herbicide. The tested herbicides were classified in 4 scoring categories based on reduction of mycelial growth in toxicity tests. All six herbicides had a fungistatic effect of varying intensities, dependent on their rate in medium, on *B. bassiana*. They showed that *B. bassiana* was sensitive to all tested herbicides, particularly at recommended as well as lower field rates. Metribuzin, S-metolachlor and tembotrione had a strong fungistatic effect on mycelial growth even at rates 25 and 12.5%. Pyridate was slightly harmful, depending on the rate and temperature. Dicamba and amidosulfuron had slight effect on mycelial growth. Sporulation and conidial germination of *B. bassiana* were significantly inhibited by all tested herbicides. Amidosulfuron and dicamba, both at 100% rate, had the lowest inhibitory effect on sporulation, i.e. 24 and 44%, respectively. Other herbicides in test showed much higher inhibitory effect on sporulation (69–95%). With exception of dicamba with 33% of conidial germination inhibition all other herbicides in test inhibited conidial germination for 70–100%. At 200% dosage, inhibition rates even increased. Of all tested herbicides, amidosulfuron and dicamba showed the least adverse effects and were therefore probably compatible with *B. bassiana* in the field (Kos and Celar (2013).

Storey and Gardner (1986) studied the antifungal activity of four plant growth regulators and eight spray adjuvants in vitro to test their potential use in turf, pasture, or orchard systems where *B. bassiana* could be used as a microbial insecticide. They tested plant growth regulators included fluprimidol (Cutless 50 W), mefluidide (Embark 2 S), paclobutrazol (Cutlar 4 E), and (2-chloroethyl)methylbis (phenylmethoxy)silane (Silaid 4 E) applied in six concentrations ranging from 0 to 30 mg L^{-1}. Also, they evaluated five concentrations (0–10%) of each of eight adjuvants possessing spreader-sticker, surfactant, or antitranspirant properties,

including Ortho X-77, Plyac, Miller-Aide, Nu-Film 17, ProStik, Triton Ag-98, Triton CS-7, and Tween 80. Mefluidide was the only one of four plant growth regulators that caused little to no significant inhibition of in vitro germination and growth of the entomogenous fungus *B. bassiana*. Silaid, paclobutrazol, and flurprimidol significantly inhibited germination and growth of the fungus. Mortality of fall armyworm, *Spodoptera frugiperda*, resulting from *B. bassiana* (Fig. 13) was notably reduced when larvae were exposed to conidia plus soil treated with paclobutrazol. Larval mortality resulting from conidia plus soil treated with mefluidide did not differ from mortality resulting from untreated conidia. Triton CS-7 was the only one of eight spray adjuvants that significantly inhibited *B. bassiana* conidial germination.

Six *Beauveria bassiana* isolates and 13 insecticide formulations were examined to find compatible combinations for *L. decemlineata* (Say) management. Few significant differences were found between *B. bassiana* isolates. Emulsifiable-concentrate insecticide formulations using xylene-based, aromatic solvents were most inhibitory toward Bb. Wettable-powder formulations often increased colony counts. Pyrethroids (permethrin and fenvalerate) were inhibitory as both formulated and technical materials. Most Bb inhibition occurred within 4 h of mixing. Separate application of *B. bassiana* and insecticides greatly mitigated *B. bassiana* inhibition (Anderson and Roberts 1983). De Oliveira and Neves (2004) evaluated compatibility of *B. bassiana* with 12 acaricides and showed that avermectin and the pyrethroids were more compatible with *B. bassiana* than the others. Amutha et al. 2010 suggested use of Quinolphos and chloripyriphos in combination with *B. bassiana* which were proved to be less toxic to the fungal pathogen in their studies on compatibility. Monocrotophos, endosulfan and deltamethrin were the most harmful insecticides to *B. bassiana* development. Thiamethoxam, diafentiuron and acephate were compatible with *B. bassiana*, with no effect on reproductive or vegetative growth. Carbosulfan was classified as incompatible, significantly affecting conidial production, and imidacloprid was moderately compatible. Shafa et al. (2012)

Fig. 13 Fall armyworm, *Spodoptera frugiperda*. (Left) Adult male (*Source* Goergen et al. 2016); (Right) Healthy Larva (*Source* http://www.chemtica.com/) and a fall armyworm, *Spodoptera frugiperda* larva killed and overgrown by the entomopathogenic fungus, *Beauveria bassiana* (*Source* http://entopcastillo.blogspot.com/)

concluded that imidacloprid, monocrotophos and quinalphos were highly safe and most compatible to *B. bassiana* and *M. anisopliae* whereas chlorpyriphos was found to be highly deleterious. Alizadeh et al. (2007) reported that imidacloprid (Konfidur® SE 35%) was compatibile with *B. bassiana* and could be used simultaneously in IPM programs, while the insect growth regulator flufenoxuron (Cascaid® EC 5%) was found totally imcompatible. Monocrotophos was reported to be compatible to *B. bassiana* by Devi et al. (2004). Challa and Sanivada (2014) investigated the effects of three commonly used chemical insecticides, quinolphos, monocrotophos, cypermethrin on germination, mycelial growth of thirty isolates of *B. bassiana*. Quinolphos inhibited the mycelial growth of *B. bassiana* in 24 of 30 isolates but was of no significant inhibiting impact on the conidial germination in 26 isolates. Among thirty isolates of *B. bassiana* five isolates showed tolerance with monocrotophos in mycelial growth assay. No significant inhibition of conidial germination was found with all isolates tested. In association with these results, Devi et al. (2004) reported that few isolates of *B. bassiana* showed tolerance towards organophosphates. Also, they explained the mechanism underlying the tolerance to organophosphates. Ambethgar et al. (2008) reported that quinolphos and monocrotophos showed a moderate fungistatic effect on *B. bassiana* isolates at field recommended doses. Cypermethrin, a synthetic pyrethroid had no significant effect on conidial germination in 28 isolates, whereas it inhibited the mycelial growth of 25 isolates. Additionally, Cazorla and Morales (2010) reported that cypermethrin was not found compatible with field recommended dose by inhibiting conidial germination. Insecticide permethrin was found synergistic with *B. bassiana* in controlling West African insecticide-resistant *Anopheles gambiae* mosquitoes (Farenhorst et al. 2010). However, Barci et al. (2009) reported that cypermethrin and *B. bassiana* were compatible. Rashid et al. (2012) evaluated the compatibility of *B. bassiana* isolate DEBI 002 (Atashgah) with fipronil, pyriproxyfen and hexaflumuron insecticides recommended against subterranean termites (Remmen and Su 2005). To determine the impact of the insecticides on the germination of the fungal spore, different concentrations of the compounds were added to the culture medium (malt agar). In order to calculate the mycelial growth in different treatments, each colony diameter was measured and the spores were counted in the surface area to assess its sporulation. They found that pyriproxyfen (1500 ppm) and hexaflumuron (80 ppm) completely inhibited mycelial growth, while the inhibitory effect of fipronil (1600 ppm) remained at 76.6%. The inhibitory effect of lower concentrations of all three insecticides was between 10 and 20%. All tested insecticides inhibited the spore production between 80 and 100% at the highest concentrations without any significant differences among them. Pyriproxyfen (400 ppm) and hexaflumuron at all tested concentrations completely inhibited spore germination, significantly different from the rest of the treatments. They indicated that hexaflumuron had the highest inhibitory effect on the spore germination and could not be recommended for simultaneous applications together with *B. bassiana* against the insect pests.

Antony et al. (2011) evaluated the fungitoxic effect of commonly used chemical insecticides (endosulfan, chlorpyriphos, dimethoate and quinalphos) and fungicides

(bordeaux, hexaconazole and triadimefon) on germination, vegetative growth and sporulation of *B. bassiana*. The insecticides and fungicides were tested at three concentrations (Field Recommendation (FR), half FR, and twice FR). All the tested concentrations inhibited the germination (9.0–81.19% and 19.3–100%), vegetative growth (0.5–62.9% and 37.1–100%) and sporulation (7.0–99.9% and 99–100%) of *B. bassiana* by the insecticides and fungicides respectively, but dimethoate exhibited minimum inhibitory effect. Dimethoate showed better compatibility to *B. bassiana* in all the three concentrations. They concluded that dimethoate because of being safer to the biological control agent could be used as an integrated management of coffee berry borer, *Hypothenemus hampei* (Fig. 14) in coffee. Antony et al. (2011) indicated that the fungicides, bordeaux and hexaconazole were so highly toxic to *B. bassiana* that completely inhibited its germination, vegetative growth and spore production. Furthermore, they found that triadimefon inhibited 99–99.8% spore production, while the vegetative growth inhibition was less than 40%. Similar results were found in the earlier reports of Rachappa et al. (2007) that fungicides hexaconazole, carbendazim, propiconazole and chlorothalonil were highly toxic (100%). Loureiro et al. (2002) revealed that the fungicides viz. thiophanate methyl, tebuconazole, metalaxyl and mancozeb, as well as cartap inhibited the germination, vegetative growth and sporulation of *B. bassiana*. It was reported that fungicides were found to be more toxic to entomopathogenic fungi (Tamai et al. 2002).

There has been an attempt to replace the synthetic insecticides with less expensive, locally available, ecologically safe and socio-friendly options including botanicals (Isman 2007), however, these natural products can also affect biological control fungi. Hirose et al. (2001) observed 45% reduction in spore germination of *B. bassiana* when mixed with neem oil at 2%. *B. bassiana* activity was enhanced by agroneem (a commercial neem insecticide) (Al-Mazra'awi et al. 2009). When an IPM strategy is devised, it is important to take into account the compatibility of

Fig. 14 Coffee berry borer, *Hypothenemus hampei*. (Left) healthy coffee berry borer (*Source* Régis Babin, International Center for Insect Physiology and Ecology, Nairobi); (Right) Adult coffee berry borer of killed and colonized by *Beauveria bassiana* (*Source* José Nilton Medeiros Costa, Agroavanes.com)

products sprayed on the crop, avoiding the use of the most toxic, or using them during seasons when the effect over a natural control agent is minimized. Therefore, the toxic effect on the control agent will be smaller, contributing indirectly to control the host pest-insect and, consequently, to reduce damage in the cultivated field (Islam and Omar 2012). Usha et al. (2014) evaluated the in vitro effect of commercial pesticides, fungicides and botanicals on conidial germination, vegetative growth and sporulation of selected isolates of entomopathogenic fungus *B. bassiana,* an important biocontrol agent used in integrated pest management programs. By using isolates of *B. bassiana*, compatibility assessment was made with insecticides, fungicides and botanical, at three concentrations (0.1×, 0.5× and 1×) in the laboratory based on the recommended dose for field application by food poison technique and their effect on conidial germination, vegetative growth and sporulation. They indicated that all the four pesticides tested had different compatible levels with all the isolates at different concentrations. Among the pesticides tested, chlorpyrifos (Hilban®) was proved to be highly detrimental to all the isolates at 1 and 0.5× concentrations. Similarly, the other pesticides monocrotophos (Monodhan®36) and quinalphos (Ekalux®) were also categorized as either highly toxic or moderately toxic to all the isolates at all concentrations but Conversely, B55 displayed its compatibility with all pesticides except chlorpyrifos where it showed moderate toxicity. Moreover, B55 was highly compatible with imidacloprid (Media®) even at higher concentrations showing least inhibition of sporulation less than 3% at lower concentration. quinalphos drastically inhibited sporulation of all the isolates in spite of showing vegetative growth and the percentage reduction of sporulation varied among isolates but followed a similar decreasing tendency on the concentration of the pesticide amended in the medium. All the fungal isolates showed differential sensitivity to the fungicides tested at the different concentrations. The conidial germination of all the isolates was completely inhibited at 1× concentration of carbendazim (Bavistin®) amended in the SDA medium whereas the germination rates were drastically reduced at 0.5 and 0.1× concentration ranging from 86 to 24%. Isolate B57 showed complete inhibition of germination with all concentrations of mancozeb (Indofil®M-45) and carbendazim tested. Isolate B56 displayed 40% inhibition at higher concentration, but only 9% at lower concentration of copper oxychloride (Cuprogarb®500). Sulfur (Sulfex®) showed no inhibition at 0.1× concentration. Copper oxychloride was compatible to all the isolates at lower concentration but showed toxicity at higher levels, nevertheless sulfur displayed high compatibility to all isolates at higher concentration except B57 which was moderately toxic even at low concentrations. The spore output was highly inhibited in all the isolates regardless of the concentration used, conversely B55 displayed less than 5 and 11% reduction respectively at lower concentrations. As far as vegetative growth was concerned except isolate B55 all others showed significant inhibition at 1× concentration and the percent reduction ranged from 30 to 74% at other concentrations with Bavistin®. The mycelial growth was highly reduced even at lower concentrations (more than 43%) for all isolates. The fungicides Bavistin® and Indofil®M-45 were highly deleterious totally inhibiting the reproductive growth of the fungal isolates irrespective of the concentration of

the active ingredient present in the culture medium. Fungicides which retarded fungal germination resulted in relatively slower growth i.e., mycelial mat was observed 4–6 days post inoculation, whereas in plates where fungicides had no significant effect on germination, plates were fully covered with mycelia mat within 2 days post inoculation (Usha et al. 2014). Insecticides have potential to affect the various developmental stages of entomopathogenic fungi.

In the study made by Usha et al. (2014) all tested insecticides displayed varying degree of potential to inhibit growth and conidial germination. Fungal germination is an important factor of pesticide compatibility evaluation with entomopathogenic fungi considering the pest management, because the beginning of epizootics is conditioned by the capacity of conidia to germinate on the host (Alizadeh et al. 2007; Anderson and Roberts 1983).The entomopathogenic fungus success, however, depends on conidial viability (De Oliveira and Neves 2004). Their research showed that, in general the pesticides tested (except imidacloprid) significantly affected *B. bassiana* germination, vegetative growth and sporulation in vitro at higher concentrations. The isolate B55 was compatible with imidacloprid even at higher concentrations (Usha et al. 2014). According to James and Elzen (2001), imidacloprid had no negative effect on *B. bassiana*. Synergistic interaction of imidacloprid with fungal agents in insect control has previously been reported (Kaakeh et al. 1997; Quintela and McCoy 1998a, b; Lacey et al. 1999; Ramakrishnan et al. 1999; Furlong and Groden 2001; Ying et al. 2003) and imidacloprid, deltametrin and trichlorfon have been classified as compatible with *B. bassiana.* In earlier reports, chlorpyriphos and monocrotophos were found to be slightly harmful to *B. bassiana* at normal field dose (Ambethgar 2009). Chlorpyriphos had been reported to strongly inhibit the growth and sporulation of *B. bassiana* (Batista et al. 2001) in a dose-dependent manner even at concentrations lower than recommended rates of field use (Rao 1989; Masarat 2009) reported that chlorpyriphos and endosulfan strongly inhibit the growth of *B. bassiana*. De Oliveira et al. (2003) reported triazophos, chlorpyriphos and endosulfan formulations inhibited 100% of the germination of *B. bassiana*. These reports were in good agreement with the results obtained via the study made by Usha et al. (2014) in which chlorpyriphos and monocrotophos at higher concentrations inhibited the growth of all fungal isolates. Quinolphos was found to be toxic to all the fungal isolates tested. Among the fungicides tested, sulfur was found to be compatible with isolate B55 at all concentrations.

With respect to botanicals, neemgold, biospark and exodon showed compatibility to all the isolates in the study and neemgold displayed synergism with B55 which was manifested by enhanced vegetative growth of the isolate when grown in combination. Sahayaraj et al. (2011) also observed that the commercial plant based pesticides were well tolerated by *B. bassiana.* Neemgold and biospark were relatively safe for combined use. Jayaraj (1988) hinted the possibility of combining botanicals with microbial for enhanced efficacy against insect pests. The compatibility of isolates of *B. bassiana* with azadirachtin formulations has been

investigated previously (Rodriguez-Lagunes et al. 1997; Bajan et al. 1998; Gupta et al. 1999; Depieri et al. 2005). However, a few isolates had been tested and contradictory results reported. For example, neem oil was found compatible with *B. bassiana* by Rodriguez-Lagunes et al. (1997) but was reported to be inhibitory by Bajan et al. (1998) and Depieri et al. (2005). The observed difference could be due to inherent variability of chemicals to biological creatures. Mohan et al. (2007) studied the compatibility of AZA and neem oil extract (0.15% AZA) with 30 different isolates of *B. bassiana.* Of those studied 23 combinations were found to be compatible. *B. bassiana* activity was enhanced by agroneem (a commercial neem insecticide) as observed by Al-Mazra'awi et al. (2009). The germination percentage of *B. bassiana* was slightly affected (reduction percentage was not more than 12%) by various neem concentrations (Islam et al. 2010). It is evident that the action of all insecticides was mainly dependent on the chemical nature of the compounds as well as concentrations used and different fungal isolates utilized in the experiment (Usha et al. 2014).

Park et al. (2012) studied the influence of chemical pesticides and environmentally friendly agricultural materials (EFAMs) used in tomato cultivation on the pathogenicity of the entomopathogenic fungus, *Beauveria bassiana*. *B. bassiana* mycelium didn't grow on PDA media containing 13 fungicides including chlorothalonil and colonies were not formed on PDA media containing 12 fungicides. *B. bassiana* mycelium grew and colonies were formed on all PDA media containing insecticides and EFAMs, but mycelial growth and colony formation on most PDA media were significantly inhibited compared to the control. The insecticidal activity of *B. bassiana* against *Trialeurodes vaporariorum* (Figs. 15 and 16) was decreased when fungicides (polyoxin B, mandipropamid) and EFAMs containing sulfur were added, but insecticides (pyridaben, dinotefuran) and EFAMs originated from plant extracts did not have any influence on the insecticidal activity of *B. bassiana*. The pathogenicity of a mixture of *B. bassiana* and polyoxin B against *T. vaporariorum* was lower than that of *B. bassiana* alone under greenhouse conditions. Sahayaraj et al. (2011) also observed that the commercial plant based pesticides were well tolerated by *B. bassiana.* Neemgold and biospark were relatively safe for combined use.

Kouassi et al. (2003) reported that the simultaneous use of mancozeb and copper oxide with *B. bassiana* reduced the mortality of adults of *Lygus lineolaris* (Hemiptera: Miridae) (Fig. 17). Gatarayiha et al. (2010) studied effects of a triazole fungicide, flutriafol, on *Beauvaria bassiana* in vitro and its efficacy against *Tetranychus urticae*. It was determined that flutriafol inhibited mycelial growth and conidial germination of the fungus and also its infection capacity in bioassay. Gatarayiha et al. (2010) stated that azoxystrobin showed little effect on conidial germination and mycelial growth of *B. bassiana* and that the application concentration of the fungicide did not reduce activity of *B. bassiana* against mites in bioassays.

Fig. 15 Different stages of the life cycle of the whitefly, *Trialeurodes vaporariorum*. *Source* Surendra Dara and Jack Kelly Clark, http://cesantabarbara.ucanr.edu/

Fig. 16 Eliminated colony of whitefly, *Trialeurodes vaporariorum* by *Beauveria bassiana*. *Source* Eran Finkle, http://www.flickr.com/

Fig. 17 Tarnished plant bug, *Lygus lineolaris*. (Left) Young tiny and light green neemphs easily confusable with aphids, and older nymphs larger and with noticeable black dots on their back and well-developed wing pads, and an adult with reddish brown markings on the wings (*Source* University of Gerorgia Archive, University of Georgia, Bugwood.org); (Right) Cadaver of a tarnished plant bug killed and colonized by the fungus *Beauveria bassiana* (*Source* Antonio Castro, http://es.slideshare.net/)

2.3 Isaria *spp.*

2.3.1 *Isaria farinosa* (Holmskjold) Fries

Isaria farinosa (Figs. 18, and 19) has shown promising entomopathogenic effects on the citrus mealybug *P. citri* (Demirci et al. 2008). The conidia of *I. farinosa* are capable of surviving for a long period in vitro without germination. Conidia viability is very long at a humidity between 0 and 34% (Roberts and Yendol 1971). This provides an important advantage for the fungus as a biocontrol agent against insect pests. *I. farinosa* is a well known entomopathogenic fungus with a worldwide distribution in temperate and tropical zones and a relatively wide host range with Lepidoptera dominating, which makes them interesting agents for the development of biocontrol methods. For more than 30 years, it was named *Paecilomyces farinosus* and recently transferred to the genus *Isaria* (Zimmermann 2008). The species was also isolated from *Saissetia coffeae* (Hemiptera: Coccidae) in Sri Lanka (Evans and Hywel-Jones 1997).

Demirci et al. (2011) determined the effects of some fungicides used against citrus diseases, on mycelial growth and conidial germination of *Isaria farinosa* (Holmsk.) Fries (Sordariomycetes: Hypocreales) and also on the pathogenicity of the fungus on citrus mealybug, *Planococcus citri* (Risso). Systemic fungicides such as tebuconazole (Folicur® EC 250 g L^{-1}), penconazole (Topas® EC 100 g L^{-1}) and nuarimol (Trimidal® SC 90 g L^{-1}) were the most effective regarding both conidial germination and mycelial growth. Protective fungicides such as captan (Safa Captan® WP 50%), chlorothalonil (Hektanil® WP 75%), mancozeb (Penncozeb® WP 80%) and propineb (Antracol® WP 70%) inhibited conidial germination at between 1 and 5 μg mL^{-1} concentration, but captan, chlorothalonil and propineb did not inhibit the mycelial growth at 5000 μg mL^{-1}. Mancozeb inhibited mycelial growth between 2500 and 5000 μg mL^{-1}.

Fig. 18 *Isaria farinosa*. *Source* M Valentine, http://wildaboutbritain.co.uk/

Fig. 19 *Isaria farinosa*. (Left) Synnematal condiophores and (Right) Closer view of conidiophores with verticilliate biseriate phialides (*Source* Atlas of Invertebrate-Pathogenic Fungi of Thailand, Thai Science Biodiversity, http://www.thai2bio.net/museum/) (Right) Conidiophores (**a**) on the natural substrate, (**b**, **c**) in pure culture; and conidia

Sulphur (Power Sulphur® H WG 80%) and copper oxychloride (Newbakir® WP 50%) did not inhibit the fungus even at very high concentrations. Sulphur, copper oxychloride, fosetyl-Al (Aliette® WP 80%), chlorothalonil and carbendazim did not decrease the mortality percentage caused by *I. farinosa*. Tebuconazole, penconazole and mancozeb were the most effective and respectively reduced the mortality from 83% to 33%, 28% and 30% in the ovisacs, from 81% to 29%, 27 and 29% in the 1st instar larvae, and from 84 to 34% in the adult females. In control petri plates, mean conidial germination was 99.5% in 24 h. Triazole (tebuconazole and penconazole) and pyrimidine (nuarimol) fungicides were the most effective fungicides against both conidial germination and mycelial growth. Tebuconazole was the most effective fungicide against conidial germination, with the fungicide inhibiting the germination at 1 μg mL^{-1} concentration. MIC values of penconazole and nuarimol were under 10 and 50 μg mL^{-1}, respectively. The application concentration of nuarimol was 27 μg mL^{-1}, but for penconazole the concentration was 350 μg mL^{-1}. Penconazole is supposed to be more effective against the fungus than nuarimol, because of its higher application rate. In this study, tebuconazole was the most effective fungicide against conidial germination. The triazole fungicides, tebuconazole and penconazole, were the most effective against mycelial growth, with EC_{50} values of 3.29 and 0.871 μg mL^{-1}, and conidial germination with EC_{50} values of 0.0186 and 0.490 μg mL^{-1}, respectively. Captan, chlorothalonil, mancozeb and propineb inhibited conidial germination at 5 μg mL^{-1}. None of the fungicides except mancozeb inhibited mycelial growth of the fungus at 5000 μg mL^{-1}. Mancozeb inhibited mycelial growth between 2500 and 5000 μg mL^{-1}. Mancozeb was one of the most effective fungicides on conidial germination of *I. farinosa* in our study, but the effect of carbendazim was low. It inhibited conidial germination between 500 and 1000 μg mL^{-1}, the EC_{50} value of the fungicide being 63.096 μg mL^{-1} on germination. However, carbendazim was not effective against *I. farinosa* in this study.

Azoxystrobin (Quadris® SC 250 g L^{-1}), captan, copper oxychloride and sulphur were the least effective fungicides against conidial germination. Azoxystrobin could not inhibit conidial germination totally at 5000 μg mL^{-1}, but inhibited 50% of the germination at 15.31 μg mL^{-1} Azoxystrobin, chlorthalonil, copper oxychloride, fosetyl-Al, propineb and sulphur could not inhibit the mycelial growth of the fungus at 5000 μg mL^{-1}. The germination of conidiospores was more severely affected than was mycelial growth of the fungus in the presence of fungicides. The entomopathogen caused 83.85, 81.1 and 84.14% death on ovisacs, 1st instar larvae and adults, respectively. The fungicides did not show any toxic effect on any of the three life stages of the mealybug ($P < 0.05$). Tebuconazole, penconazole and mancozeb were the most effective on the entomopathogenic activity in all three life stages of the mealybug ($P < 0.05$). The fungicides reduced the mortality percentages to under 35% in all life stages. These fungicides inhibited the conidial germination of *I. farinosa* totally at 5 μg mL^{-1}. The application concentrations of tebuconazole, penconazole and mancozeb were 62.5, 350 and 2000 μg mL^{-1}, respectively; therefore, the fungicides might decrease the infection capacity of the entomopathogen in nature. Mancozeb significantly reduced the mortality of citrus

mealybug inoculated with *I. farinosa* in our research ($P < 0.05$). The effectiveness of nuarimol on *I. farinosa* was higher than carbendazim in vitro. Mortality percentage of nuarimol, applied to mealybugs, was lower than that of carbendazim, and tebuconazole was one of the most effective fungicides. Azoxystrobin, captan and nuarimol also reduced significantly the mortality to between 40% and 60% in all life stages of the mealybug ($P < 0.05$). Of these fungicides, the MIC values of captan and nuarimol range from 1 to 5 μg mL^{-1} and from 10 to 50 μg mL^{-1} for the conidial germination, respectively. In contrast, azoxystrobin could not completely inhibit germination at 5000 μg mL^{-1}. However, the EC_{50} value of the fungicide was 15.31 μg mL^{-1}; thus, the fungicide reduced the germination rate of the conidia. This may explain the inhibitory effect of the fungicide on the pathogenic activity of the fungus. Copper oxychloride did not cause a significant reduction in mortality in any of the studied three life stages of mealybug. Carbendazim, chlorothalonil, fosetyl-Al and sulphur caused a minimum reduction in mortality percentage. These fungicides, except chlorothalonil, did not have high fungicidal activity in vitro too. In spite of the high inhibitory effect of propineb on conidial germination of *I. farinosa*, the fungicide could reduce the mortality approximately to 60%. The triazole fungicides tebuconazole and penconazole, and the dithiocarbamate fungicide mancozeb, exhibited high inhibitory effects on pathogenic activity of *I. farinosa*. Azoxystrobin, captan and nuarimol also inhibited mortality, but their inhibitory effects were less than tebuconazole, penconazole and mancozeb. Triazole, dithiocarbamate, pyrimidine, dicarboximide and strobilurine fungicides have been preferred by the farmers because of their effectiveness on a broad spectrum of fungal plant diseases. The fact that the triazole and dithiocarbamate fungicides have high inhibitory effects on entomopathogenic fungi can cause suppression of biocontrol activity of the fungi in nature. Furthermore, the inhibitory effect of triazole fungicides on both conidial germination and mycelial growth of *I. farinosa* may interfere with the persistence of the entomopathogenic fungus in farmlands. This aspect may restrict the usage of the fungicides in IPM programs harmoniously with *I. farinosa*. Sulphur and copper oxychloride were determined as compatible fungicides for IPM programs (Demirci et al. 2011). Majchrowicz and Poprawski (1993) evaluated the effects of nine fungicides in vitro and stated that mancozeb completely inhibited conidial germination of some entomopathogenic fungi including *I. farinosa*. In spite of its effectiveness on conidial germination, captan could not inhibit the mycelial growth of the fungus at 5000 μg mL^{-1}. The inhibitory effect of the fungicide on conidial germination of *I. farinosa* has also been recorded by Majchrowicz and Poprawski (1993).

2.3.2 *Isaria fumosorosea* Wize

The triazole fungicide, propiconazole, inhibited mycelial growth and sporulation of *I. fumosorosea* (Figs. 20, and 21) and *I. farinosa* (Vänninen and Hokkanen 1988). Triazole fungicides have also been used effectively for opportunistic fungal infections in humans caused by *Isaria* species (Aguilar et al. 1998).

Fig. 20 *Isaria fumosorosea* colony on Czapek yeast extract agar, CYA. *Source* http://www.docplayer.cz/44370651-Hypocreales-dalsi-anamorfy.html/

Fig. 21 *Isaria fumosorosea*. (Left) peripheral formation of synnemata on agar plate; (Middle) Close view of synnemata; (Right) Conidiophores with verticilliate branches ending to phialides and phialospores produced individually or in chain. *Source* http://www.docplayer.cz/44370651-Hypocreales-dalsi-anamorfy.html/

Sapieha-Waszkiewicz et al. (2004) reported that *I. fumosorosea* was more resistant than *B. bassiana* and *M. anisopliae* to cyproconazole (a triazole), dithianon, captan and fenarimol, which were often applied in apple orchards. They also found that fenarimol (a pyrimidine) was the most effective fungicide on growth of *I. fumosorosea*, cyproconazole had limited effects on the growth of the fungus at its application dose, and captan and dithianon did not affect the growth rate of *I. fumosorosea*. Sterk et al. (2002) stated that captan was moderately toxic to mycelial growth of *I. fumosorosea*. In contrast to that report, Er and Gökçe (2004) recorded that one-tenth of the recommended application concentration of captan inhibited completely the conidial germination and mycelial growth of two *I. fumosorosea* isolates. Sterk et al. (2002) found that azoxystrobin was moderately toxic to mycelial growth of *I. fumosorosea* while sulphur was harmless to mycelial growth of *I. fumosorosea*.

Expectedly, negative impacts of fungicides in vitro on entomopathogenic fungi are widespread. Several fungicides completely inhibited the conidial germination of two *I. fumosoroseus* (Wize) Brown and Smith (Deuteromycota: Hyphomycetes) isolates at recommended rates, while captan and dichlofluanid completely inhibited germination of both isolates at one tenth the recommended rate. All fungicides tested completely inhibited germination of both isolates at recommended rates (Er and Gökçe 2004). Captan and dichlofluanid also completely restricted mycelial growth of both *I. fumosoroseus* isolates. Pirimicarb was the only fungicide tested at recommended or reduced rates that did not significantly inhibit mycelial growth of *I. fumosoroseus* (Er and Gökçe 2004).

Zimmermann (1975) studied the effects of some systemic fungicides on the conidial germination and mycelial growth of *Beauveria tenella*, *B. bassiana*, *M. anisopliae*, *I. farinosa* and *I. fumosorosea*. The author mentioned that conidial germination was severely suppressed by benomyl and triforine, and mycelial growth was inhibited by benomyl, triforine and tridemorph. The least effective fungicides were carboxin and ethirimol. Nuarimol and ethirimol are pyrimidine fungicides. Nuarimol was more effective against both conidial germination and mycelial growth than a benzimidazole fungicide, carbendazim, like benomyl in the study conducted by Demirci et al. (2011). The author emphasized that the fungicides were far less effective against conidial germination than against mycelial growth.

The entomopathogenic fungus, *Isaria fumosorosea*, can be used to help manage the Asian citrus psyllid with minimal impact on beneficial arthropods, but its effectiveness may be compromised by agrochemicals used to control concurrent arthropod pests and diseases.

Results of laboratory and greenhouse tests showed a range of responses of the fungus to the different materials, including compatibility and incompatibility. Overall, *I. fumosorosea* growth in vitro was reduced least by petroleum-based materials and most by botanical oils and borax, and some of the copper-based fungicides, suggesting that tank mixing of *I. fumosorosea* with these latter products should be avoided. However, equivalent negative effects of test materials on fungal pathogenicity were not always observed in tests with adult psyllids. They hypothesized that some oils enhanced adherence of blastospores to the insect cuticle, overcoming negative impacts on germination. Their data indicated that care should be taken in selecting appropriate agrochemicals for tank-mixing with commercial formulations of entomopathogenic fungi for management of citrus pests.

2.4 Metarhizium anisopliae *(Metschnikoff) Sorokin*

The genus *Metarhizium* Sorokin is composed of anamorph entomopathogenic fungi that generally are greenish when conidiating on the corpses of their arthropod hosts or in axenic culture. They frequently are isolated from soils, parasitize a broad range of insect species representing numerous orders and are found throughout the tropics and temperate regions (Bischoff et al. 2009). Species from this genus are used as biological control agents to manage and prevent infestations of various species of superfamily Acridoidea, including locusts and grasshoppers (Lomer et al. 1997; Milner 1997; Milner and Pereire 2000, Hunter et al 2001, Lomer et al 2001). In addition *M. anisopliae* (Metschn.) Sorokin, the type species of the genus, has been shown to be effective in the control of malaria-vectoring mosquitoes (Culicidae, Diptera), and that applications of *M. anisopliae* (Figs. 22 and 23) can reduce the intensity of malaria transmission by 75% (Scholte et al. 2005). Liang et al. (1991) were the first to confirm the connection of *Metarhizium*, long considered to be asexual, to the teleomorph genus *Cordyceps* (Fr.) Link (Hypocreales: Clavicipitaceae). Bischoff et al. (2009) employed a multigene phylogenetic approach using near-complete sequences from nuclear encoded EF-1a, RPB1, RPB2 and β-tubulin gene regions and evaluated the morphology of these taxa, including ex-type isolates whenever possible. The phylogenetic and in some cases morphological evidence supported the monophyly of nine terminal taxa in the *M. anisopliae* complex that we recognize as species. Bischoff et al. (2009) proposed to recognize at species rank *M. anisopliae*, *M. guizhouense*, *M. pingshaense*, *M. acridum* stat. nov., *M. lepidiotae* stat. nov. and *M. majus* stat. nov. In addition, Bischoff et al. (2009) described the new species *M. globosum* and *M. robertsii*, and resurrected the name *M. brunneum* and showed that *M. taii* was the later synonym of *M. guizhouense*.

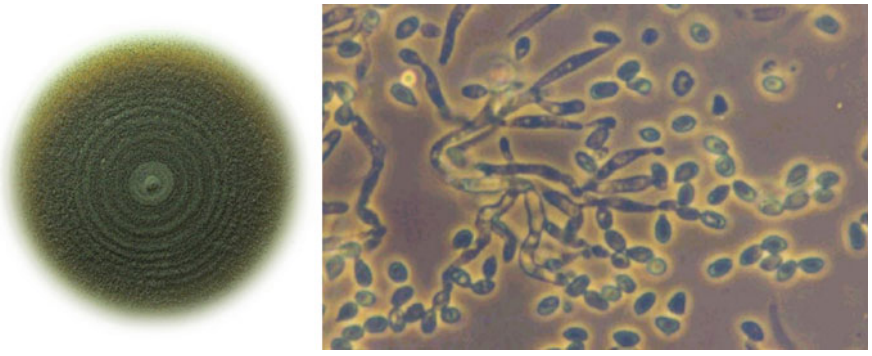

Fig. 22 *Metarhizium anisopliae*. (Left) colony growth on agar plate (*Source* Fumio Ihara, National Institute of Fruit Tree Science, Tsukuba); (Right) Conidiophores and conidia of the fungus, causal pathogen of green muscarine of insects (*Source* Svetlana Y Gouli, University of Vermont, Bugwood.org)

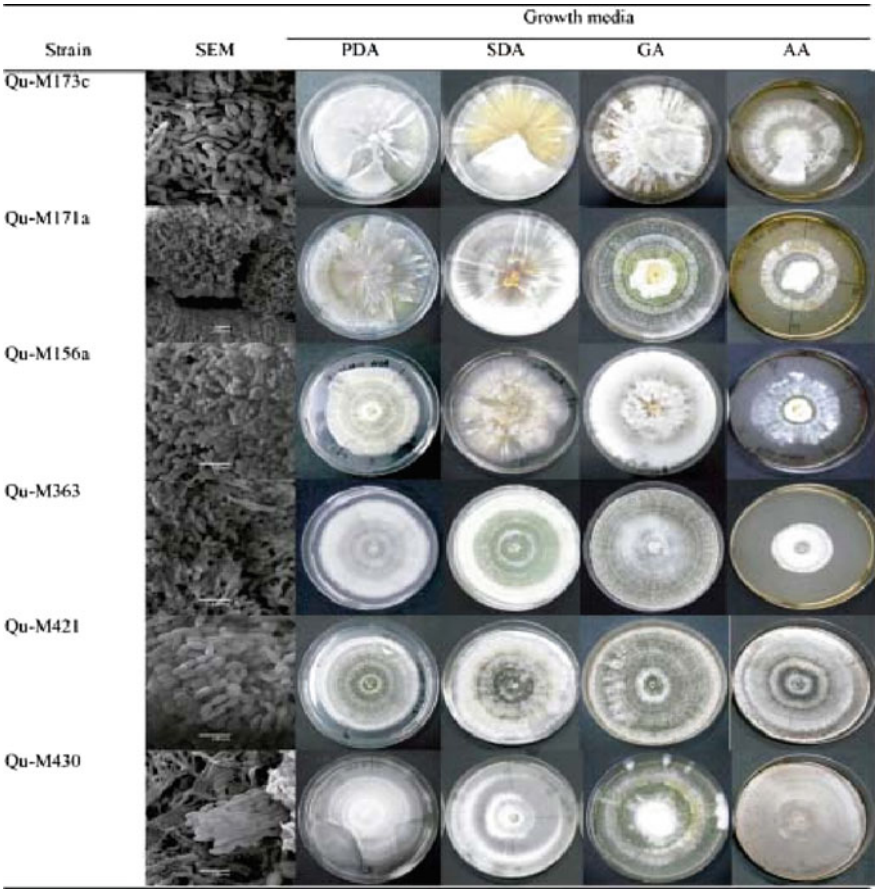

Fig. 23 Morphology of conidia and colonies of six strains of *Metarhizium anisopliae* in different growth media 14 days after inoculation. *Source* Sepũlveda et al. (2016)

M. anisopliae (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) has been studied extensively for the biological control of a wide range of insect pests, including the black vine weevil, *Otiorhynchus sulcatus* (Moorhouse et al. 1992, 1993a, b; Booth and Shanks 1998; Bruck 2005; Bruck and Donahue 2007). The success of the fungal entomopathogen can be affected by chemical pesticides, the toxicological impact of which on fungal entomopathogens and their use in tank-mixing can be directly measured through in vitro compatibility tests. Da Silva et al. (2013) reported the in vitro toxicity of eight insecticides, four fungicides and five herbicides in the conidial germination, vegetative growth and conidiation of *Metarhizium anisopliae* (strain CG 168). A conidial suspension containing the pesticide at recommended field dosage was subjected to constant agitation in a rotary shaker for 3 h to simulate a tank mixing. Then, aliquots of each suspension were used to determine conidial germination, vegetative growth and conidiation on

potato dextrose agar (PDA). The fungicides difenoconazole (69 mL ha^{-1}), propiconazole (75 mL ha^{-1}), trifloxystrobin (313 g ha^{-1}) and azoxystrobin (56 mL ha^{-1}) were the most harmful productsto all biological stages of *M. anisopliae* and they should not be applied together with this fungus in tank mixing. The insecticides exhibited the least degree of toxicity to this fungal pathogen, whereas the herbicides had the greatest impact on mycelial growth. The agrochemicals compatible with *M. anisopliae* were the insecticides methyl parathion (240 mL ha^{-1}), thiamethoxam (31 g ha^{-1}), and lambda-cyhalothrin (6.3 mL ha^{-1}) and the herbicides glyphosate (1560 mL ha^{-1}), bentazon (720 mL ha^{-1}), and imazapic + imazapyr (84 g ha^{-1}). The compatible pesticides could be simultaneously used with this biocontrol agent for integrated pest management in rice production systems. Tkaczuk et al. (2013) studied the inhibitory effect of a range of fungicides on the growth of *M. anisopliae* culture. They indicated that Score® 250 EC and Antracol® 70 WG were respectively of the highest and lowest inhibitory impact on the fungus growth. Also, they found that the addition of soil extracts to the culture medium with fungicides increased the inhibitory action, and the inhibition was stronger when the extract from organic soil, and not from sandy soil, was added to culture medium.

All insecticides affected conidial germination at 20 h in contrast to the untreated control ($F = 261.98$; $df = 8, 45$; $p < 0.0001$). The highest detrimental effect on conidial germination was caused by fipronil (25% w v^{-1}) and cypermethrin (reduction of 54 and 100%, respectively). At 48 h, thiamethoxam and methyl parathion had not affected conidial germination ($p > 0.05$), while the other insecticides reduced germination compared to control ($F = 266.7$; $df = 8, 45$; $p < 0.0001$). The lowest germination rates were observed for fipronil (25% w v^{-1}) and cypermethrin which caused a significant inhibition of conidial germination when compared to thiamethoxam, thiamethoxam + lambda-cyhalothrin, methyl parathion, lambda-cyhalothrin, fipronil (20% w v^{-1}) and methamidophos after 20 and 48 h of incubation ($p < 0.05$). Mycelial growth was reduced in all insecticide treatments when compared to the untreated control ($\chi = 62.14$; $df = 8$; $p < 0.0001$), except methyl parathion which allowed normal fungal growth ($p > 0.05$). No *M. anisopliae* mycelial growth was observed with cypermethrin which was different from the other treatments. Conidiation of *M. anisopliae* was inhibited by all insecticides ($\chi = 63.05$; $df = 8$; $p < 0.0001$), except methyl parathion which did not differ from the control ($p > 0.05$). Thiamethoxam + lambda-cyhalothrin, fipronil (20% w v^{-1}), fipronil (25% w v^{-1}) and methamidophos were highly antagonistic (>73.7% of reduction) to *M. anisopliae* sporulation ($p < 0.05$). Since cypermethrin inhibited mycelial growth, no conidial production was observed in this treatment. According to the Biological Index, methyl parathion, thiamethoxam and lambda-cyhalothrin were compatible with *M. anisopliae* CG 168, while fipronil (20% w v^{-1}), fipronil (25% w v^{-1}), thiamethoxam + lambda-cyhalothrin and methamidophos were classified as moderately toxic. Among the insecticides tested only cypermethrin was not compatible with *M. anisopliae*. All herbicides inhibited germination at 20 h and no germination was observed when *M. anisopliae* conidia were exposed to pendimethalin and 2,4-Dichlorophenoxyacetic acid (2,4-D) ($F = 255.22$; $df = 5, 29$;

$p < 0.0001$). After 48 h, the germination rate of the treatments with imazapic + imazapyr and bentazon were similar to the control ($p > 0.05$), whereas the other herbicides considerably reduced the conidial germination ($F = 715.18$; $df = 5, 29$; $p < 0.0001$). In addition, glyphosate delayed conidial germination with only 8.2% germination at 20 h but 96.4% at 48 h. Most herbicides reduced mycelial growth in comparison with the control ($\chi^2 = 39.47$; $df = 5$; $p < 0.0001$), except for glyphosate ($p > 0.05$). A complete suppression of vegetative growth of *M. anisopliae* was caused by 2,4-D, and consequently no conidia were produced. All herbicides had a deleterious effect on conidial yield compared to the control ($\chi^2 = 36.53$; $df = 5$; $p < 0.0001$), with pendimethalin and 2,4-D causing the highest reduction in fungal conidiation (81.8 and 100%, respectively). According to the Biological Index, glyphosate, Bentazon and imazapic + imazapyr were compatible with *M. anisopliae* CG 168, while pendimethalin was classified as moderately toxic, and 2,4-D as toxic. Similar to other chemical groups, all fungicides negatively affected *M. anisopliae* germination at 20 h ($F = 1246.20$; $df = 4, 20$; $p < 0.0001$) and 48 h ($F = 256.62$; $df = 4, 20$; $p < 0.0001$) compared to the controls. Trifloxystrobin and azoxystrobin were the most deleterious fungicides for fungal germination at 20 h, since no germinated conidia were observed. However, some germination occurred after 48 h incubation (48–52%) indicating that trifloxystrobin and Azoxystrobin delayed conidial germination. Vegetative growth and conidial yield were inhibited by all fungicides ($F = 54.4$; $df = 4, 32$; $p < 0.0001$; $F = 107.51$; $df = 4, 32$; $p < 0.0001$, respectively). Difenoconazole, propiconazole and trifloxystrobin caused more than 50% of reduction of *M. anisopliae* conidiation. According to the Biological Index, azoxystrobin was the only compatible fungicide with *M. anisopliae* CG 168, whereas difenoconazole, propiconazole and trifloxystrobin were classified as moderately toxic. No fungicide was scored as toxic to this fungus. For the analysis of the chemical groups through a comparison of pooled means, it was shown that all tested products reduced the biological parameters of *M. anisopliae* CG 168 to some extent. Insecticides comprised the least deleterious products to *M. anisopliae*. Fungicides and herbicides inhibited in a higher degree the mycelial growth of *M. anisopliae* than insecticides ($\chi = 36.97$; $df = 3$; $p < 0.0001$), whereas all pesticides considerably reduced the conidial production compared to the control ($\chi^2 = 33.06$; $df = 3$; $p < 0.0001$) at the same extent. Fungicides inflicted the most harmful effect on germination at 20 h ($\chi^2 = 64.28$; $df = 3$; $p < 0.0001$) and 48 h incubation ($\chi^2 = 33.31$; $df = 3$; $p < 0.0001$). Moreover, although herbicides reduced the germination at 20 h in comparison to insecticides, there was no difference among chemical groups after 48 h. None of the tested agrochemicals at label rate recommendations promoted positive effects on developmental stages of *M. anisopliae*. It is possible that those products compatible with *M. anisopliae*, especially the insecticides, might be used at sublethal doses in combination with the fungus in further studies aimed at improving its virulence against the rice stalk stink bug, *Tibraca limbativentris* (Heteroptera, Pentatomidae) (Fig. 24) or even to broaden its host range in rice crops. Although the insecticides methamidophos, methyl parathion, thiamethoxam + lambda-cyhalothrin, Karate™, fipronil (20% w/v), thiamethoxam and the

Fig. 24 (Left) The rice stalk stink bug, *Tibraca limbativentris* (*Source* Lucas Rubio, https://www.ecoregistros.org); (Right) Sporulation of Metarhizium anisopliae on the mycosed *Tibraca limbativentris* (*Source* Kruger and Eduardo, https://inta.gob.ar/)

herbicide imazapic + imazapyr differed from the control, conidial germination in these treatments was high, ranging from 84.6 to 95.8%.

These levels of germination suggest that these insecticides would be suitable for using in combination with *M. anisopliae* for insect control as part of an integrated pest management strategy. Increasing fungus concentration did not increase insect susceptibility when combined with thiamethoxam either at 0.77 or 0.38 ppm active ingredient. In a field experiment, the combination of *M. anisopliae* at 1×10^{12} viable conidia ha^{-1} with thiamethoxam at 12.5 g (a. i.) ha^{-1} (1/4 full dose) synergistically increased mortality and mycosis of adults of *T. limbativentris*. Therefore, enhanced *T. limbativentris* control could potentially be achieved with label rates of the fungus (5×10^6 conidia mL^{-1}) and sublethal dose of thiamethoxam (0.77 ppm). The strategy of using sublethal doses of chemical insecticides in combination with entomopathogenic fungi was introduced as a promising approach to battle the rice stalk stink bug under field conditions. The method is of additional importance considering the natural resistance of the pest to the fungus, where the adult bugs exhibit higher susceptibility to thiamethoxam than to lambda-cyhalothrin (Quintela et al. 2013). Some of the agrochemicals tested delayed conidial germination of *M. anisopliae* as seen in the increased germination from 20 to 48 h incubation. As example, Fipronil (25% w/v), glyphosate, difenoconazole, propiconazole, trifloxystrobin and azoxystrobin had fungistatic Activity on conidial germination at 20 h; however by 48 h germination of the fungus was still occurring after mixing it with these products. The fungicide azoxystrobin inhibited conidial germination at 20 h but allowed larger mycelial growth and greater conidiation compared to the other fungicides, and thus was classified as compatible according to the Biological Index formula. The effect of these products on conidial germination was probably due to the active ingredient and/or to some components of the inert carriers in the formulation. Quintela and McCoy (1998a, b) observed that one component of the inert carrier of the insecticide Admire™ 2 F (Bayer Corporation) affected conidial germination of *M. anisopliae*. The fungicidal

activity of the chemical group strobilurins (azoxystrobin and trifloxystrobin) relies on the ability of the active ingredient to disrupt energy production in fungal mitochondria and consequently prevents spore germination. In the triazole fungicides (difenoconazole and propiconazole), ergosterol biosynthesis is inhibited; consequently preventing fungal cell membranes formation (Bartlett et al. 2002). These results are in agreement with the mode of action of the strobilurins azoxystrobin and trifloxystrobin as they negatively affected conidial germination at 20 and 48 h, meanwhile the triazoles difenoconazole and propiconazole reduced conidial germination, mycelial growth and conidiation. Herbicide 2,4-D and the insecticide cypermethrin were the most harmful to *M. anisopliae* CG 168 as they completely inhibited conidial germination, vegetative growth and conidiation. The compound 2,4-D acts by inhibiting the enzyme acetyl-CoA carboxylase, and as a result, prevents the biosynthesis of fatty acids and glucosylceramides, which are components of membrane lipids of animals, plants, and fungi (Leipelt et al. 2001). It remains unclear how the insecticide cypermethrin or other cypermethrin-based insecticides act on fungi. In contrast to the results obtained in the present study, Rachappa et al. (2007) pointed out that pyrethroids were safer to the developmental stages of *M. anisopliae*, but this interaction depends on methodological procedures, pesticide formulations and fungal strains. The compatibility of *M. anisopliae* with thiamethoxam and lambdacyhalothrin is in agreement with other studies (Batista et al. 2001; Cavalcanti et al. 2002; Loureiro et al. 2002; Neves et al. 2001; Rampelotti-Ferreira et al. 2010). Despite the insecticide thiamethoxam + lambda-cyhalothrin is a combination of two compatible insecticides, it was scored as moderately toxic in this study. The combination of two or more active ingredients in an agrochemical may reduce or increase the degree of compatibility via synergism or antagonism (Alves 1998). In the present study, none of the agrochemicals tested were synergistic to *M. anisopliae*. At doses of 160 and 240 mL ha^{-1} the insecticide fipronil (25% w v^{-1}) was not detrimental to *M. anisopliae* mycelial growth and conidiation; therefore, it was scored as compatible (Rampelotti-Ferreira et al. 2010). However, in our study, this product was classified as moderately toxic to *M. anisopliae* CG 168 when using the same Biological Index. These results cannot be compared because the experimental methodology and fungal isolates were different. These authors as well as Schumacher and Poehling (2012) incorporated the test pesticides into PDA medium. Conversely, in our study the pesticides were added to an aqueous suspension of conidia for three hours of exposure under constant agitation in a rotary shaker. We provided a novel and more reliable compatibility method based on a tank mixing with fungal conidia and agrochemicals for an integrated application approach, enabling conidia to be totally exposed to the chemical product for a certain period of time, and thus can be considered a more realistic method than the other in which the pesticide is incorporated into the solid medium. The precipitation of some chemicals, especially those in granular and wettable powder formulations, cannot be avoided with the previous method (solid medium). Therefore, these pesticides may not have an even dilution in the solid medium due to differences in density, which would compromise the direct contact between the chemical and conidia (Da Silva et al. 2005). According to the

Biological Index, the fungicide azoxystrobin was classified as compatible with *M. anisopliae,* enabling normal vegetative growth and sporulation, although there were no germinated conidia after 20 h of incubation. This result seems anomalous, because a compatible pesticide should not affect conidial germination. Any chemical pesticide preventing conidial germination up to 20 h should not be used in combination with this fungus, since the conidial germination is the first step to initiate the fungal infection process in the insect. If conidial germination is delayed or inhibited, the potential for the fungus to infect insects in the field will be greatly reduced. In the field, fungal conidia must cope with abiotic and biotic factors detrimental to survival and most of conidial survival on the leaves is lost after 24 h because of environmental constraints such as solar radiation, high temperature, low relative humidity, rainfastness, and plant allelochemicals (Jaronski 2010). In addition, the high and fast germination rate for fungal entomopathogens has a positive relationship with their virulence toward a host (Altre et al. 1999; Hassan et al. 1989; Rangel et al. 2008). Faster conidial germination indicates non-stressed conidia which play an important role for the success of biological control of insects and should be the major concern in quality control protocolsfor conidia-based mycoinsecticides (Faria et al. 2010). Faria et al. (2010) assumed that only vigorous conidia of *Beauveria* and *Metarhizium* which germinated quickly within 24 h incubation should be considered suitable for insect control. On the other hand, conidia germinating after 24 h should be considered debilitated spores (low vigor), as they may cause low insect mortalities and thus should not be used for insect control. As a result, the outcome from conidia that germinated at 20 h instead of at 48 h to calculate the Biological Index was used (Table 2), since those vigorous and non-stressed conidia are able to germinate faster and thus are much more likely to have success in germinating and penetrating through the insect cuticle and escape from environmental stresses. The fact that insecticides, fungicides and herbicides inhibit mycelial growth of a fungal entomopathogen is not necessarily indicative of reduction in sporulation and conidial germination and vice versa (Zimmermann 1975). Some agrochemicals can delay mycelial growth, although they can stimulate higher conidiation later, presumably in response to stresses caused by the chemical product, perhaps due to the reduced early mycelial growth. In other cases, the fungus grows well during its vegetative development, but later conidiation does not take place for reasons which are not understood. There is no positive relationship between vegetative growth and conidial yield and the factors that govern this outcome warrant further detailed investigations (Tamai et al. 2002). In the current Biological Index proposed by Rossi-Zalaf et al. (2008), there is a low weight attributed to the germination parameter (i.e., 10%) in comparison to the other variables (vegetative growth and conidiation). As a result, the Biological Index may in some cases mask the real toxic impact of an agrochemical on entomopathogenic fungi, since 90% of the formula is attributed to vegetative growth and sporulation. Conidial germination is more important than vegetative growth and sporulation on cadavers, because the former corresponds to the first step that triggers an epizootic, and the fungus relies on it to infect the host successfully (Alizadeh et al. 2007; Khalil et al. 1985). Thereby, if an agrochemical causes substantial decrease in

conidial germination, it may reduce the effectiveness of the entomopathogen toward its target. As mycelial growth develops inside the insect host and the concentration of agrochemicals, especially those with systemic mode of action, are usually found in low titer in the hemolymph, there is little chance of this developmental stage to be negatively affected (Khalil et al. 1985). On the other hand, vegetative growth and conidiation are also important in regards to secondary infections caused by fungi and thus they should be considered for fungal virulence and persistence in the environment (Schumacher and Poehling 2012). The current Biological Index for toxicological classification of pesticides has merit regarding the ability of a fungus to grow saprophytically in the environment and to produce secondary inoculum sources through sporulation on cadavers. Such factors are related to the conservational approach for entomopathogens in agroecosystems. Based on all the facts mentioned above, we have identified a situation where this formula is not reliable since it produced a classification of compatible for an agrochemical that reduced conidial germination. Therefore, we strongly contend that the formula of Biological Index proposed by Rossi-Zalaf et al. (2008) must be revised carefully by researchers giving special attention to improving the incorporation of information regarding conidial germination. For successful control of *T. limbativentris* in rice crops adopting integrated pest management, the awareness of such compatible agrochemicals is very useful to ensure safety fungal application and to facilitate the combination of the fungus with chemical products. On the other hand, for those chemicals not compatible with the fungus, there are two strategies which might be employed to prevent *M. anisopliae* from harmful exposures: (i) plan fungus application for two to four days before the chemical spraying (the exact time depends on the residual effect of each product), so that the fungus would have enough time to infect the host (Kouassi et al. 2003); and (ii) use of a conidial formulation in oil to reduce the fungistatic effect of pesticides on fungal performance. Lopes et al. (2011) showed that an oil-based formulation afforded protection to aerial conidia of *M. anisopliae* against products with fungistatic or fungicide activity and this formulation also enhanced fungal virulence on sugarcane borer, *Diatraea saccharalis* (Lepidoptera: Crambidae) larvae (Fig. 25).

In addition, there may be some compatible insecticides which can be used at low label rates in combination with the fungus aiming to lower the insect immunity and consequently increase fungal effectiveness. In summary, our findings from in vitro compatibility indicate that the tested fungicides are more detrimental to conidial germination, mycelial growth and sporulation of *M. anisopliae* CG 168 than herbicides and insecticides, and they should not be applied together with this fungus in tank mixing. The agrochemicals compatible with *M. anisopliae* CG 168 are: insecticides methyl parathion (240 mL ha^{-1}), thiamethoxam (31 g ha^{-1}), and lambda-cyhalothrin (6.3 mL ha^{-1}); herbicides glyphosate (1560 mL ha^{-1}), bentazon (720 mL ha^{-1}), and imazapic + imazapyr (84 g ha^{-1}). Although the results of our in vitro study did not consider the effect of many variables associated with field use of pesticides, our findings are of paramount importance to guide and advice farmers to use safely this entomopathogen in combination with pesticides registered for rice crops without affecting fungal virulence and germination (Da Silva et al. 2013).

Fig. 25 (Left) Larvae of sugarcane borer, *Diatraea saccharalis* affected by *Metarhizium anisopliae* (*Source* De Romero et al. 2008); (Right) Columns of spore chains produced by *M. anisopliae* (*Source* De Romero et al. 2008)

In a study on the compatibility and synergy in the efficacy of the termiticide fipronil with *M. anisopliae*, alone or in combination, against the subterranean termite, *Coptotermes curvignathus*, sublethal doses of fipronil were found relatively less detrimental to fungal growth in a compatibility test. The fungus-insecticide bait formulation showed the greatest synergistic eefect that increased termite mortality as well as reduced the lethal time at a sublethal dose of 0.05 mg a. i. L^{-1} fipronil with conidia concentration of 10^7 conidia g^{-1} bait ($\chi^2 = 48.80$) at LT_{50} value of 6.46 days, followed by 10^8 conidia g^{-1} bait ($\chi^2 = 5.09$) at LT_{50} value of 4.89 days compared to the use of these control agents alone. The insecticidal stress caused by sublethal fipronil in the formulated bait may weaken the termites and reduce their defense mechanism, which facilitates fungal infection on termites. The observed synergism treatmeants exhibit the potential for integrated fungus–insecticide control method and need to be further investigated on termite infested oil palm trees (Yil et al. 2016).

Rachappa et al. (2007) studied the compatibility of 10 fungicides, 27 insecticides and 8 weedicides, which are presently in use were studied with *M. anisopliae* in vitro In general, the results indicate that fungicides were highly inhibitory (78.18%) and toxic to the mycopathogen followed by insecticides (44.235). Weedicides were less toxic (20.33%) to fungus. All the 10 fungicides inhibited the growth of fungus significantly. Among them carbendazim, propiconazole, chlorothalonil and hexaconazole were found highly detrimental to fungus by retarding the growth totally. Whereas the degree of inhibition in other tested fungicides ranged from 33.51 to 83.12%. Captan and wettable sulphur were found to be comparatively safe to the fungus (53.13 and 33.53% inhibition) followed by triadimefon (64.94%). Spore count per plate was assessed in all the treatments. As there was no growth in carbendazim, propiconazole, chlorothalonil and hexaconazole no spores were produced whereas, wettable sulphur recorded highest conidial load of 1.434×10^8 conidia per plate among the fungicides. Captan,

wettable sulphur and triadimefan were comparatively safe to the *M. anisopliae* under laboratory studies needs confirmation under field situation. It would be better if they are used in sequence with time lag either before or after the mycopathogen application. Toxicity of fungicides will also gradually reduce after application. This leads to the assumption that application of the mycopathogen is possible after certain safe period, which again needs more detailed study under field conditions. Eleven organophosphates, six carbamates, two chlorinated hydrocarbons, six pyrethroids and also spinosad and imidacloprid were assayed for their fungicidal property. Over all inhibition of fungal growth by insecticides ranging from 5.13 to 69.23%. Chlorinated hydrocarbons (endosulfan and dicofol) were highly detrimental to the fungus (60.69% inhibition) than organophosphates (46.66% inhibition) carbamates (45.45% inhibition). On the other hand, neonicotinoid (Imidacloprid) and spinosad were found safe to the fungus by inhibiting only 11.10 and 5.10% growth, respectively. Among the organophosphates, chlorpyriphos found more detrimental by retarding its growth significantly (69.23%) over others, followed by dichlorvos (59.82%) and malathion. Conversely, dimethoate, phosphamidon, profenophos and monocrotophos were found comparatively less detrimental (30.77–33.77% inhibition) to the fungal growth. With the carbamates, fenobucarb and indoxycarb were relatively less detrimental (30.36–39.74%). In general, significantly lesser growth inhibition was noticed in pyrethroids (36.11%). Spore yield among insecticides depends upon its inhibitory action on colony growth. It varied from 0.179×10^8 to 4.834×10^8 conidia plate^{-1}. Obviously conidial count was less in chlorpyriphos treated plate has its inhibitory action was strong and it did not allow fungus to grow and sporulate. On the contrary in spinosad treated plates spore load was highest among insecticides. Spore yield in other insecticides was proportionate to colony growth. The safety of imidacloprid observed in the study is in corroboration with the findings of Boucias et al. (1996); Quintela and McCoy (1997); Kruger and McCoy (1997); Kaakeh et al. (1997); Gardner and Kinard (1998). They found that this insecticide is not only safe to conidial germination but also to mycelial growth. It was also found to exert synergistic effect on insects at sublethal or lethal dose either as spray mixture or as bait with conidia of the fungus. The reasons/mechanisms for enhanced efficacy of *M. anisopliae* in combination with imidacloprid against insects is yet to be unravelled. However, Quintela and McCoy (1997) based on their study on sugarcane root stalk borer weevils opined that the sluggishness caused by the imdacloprid to the insects probably reduced the mechanical removal of conidia from the surface of cuticle and thus making it more vulnerable to the fungal attack. Results of the present study thus reveal that except few (chlorpyriphos, endosulfan, dicofol, diclorvos and malathion) all other insecticides can be safely used along with the mycopathogen. However, laboratory results on artificial media may not be reproducible in field as there will be degradation of toxicants. Imidacloprid and spinosad can be mixed with the fungus to get enhanced effect but needs field confirmation. For others, as discussed earlier with fungicides, safe interval between insecticide spray and the mycopathogen inoculation decides the effectiveness and strategy development for the conjunctive or supplementary use of mycopathogens.

The present study aimed to analyze the action of some acaricides, fungicides, insecticides and herbicides containing different active ingredients on *M. anisopliae* Metsch. (Sorokin) inoculated into autoclaved soil. The action of the pesticides was evaluated based on the fungal respiratory activity. The first assessment was done at 48 h after inoculation. The pesticides were then added and respiratory activity was determined nine times every 48 h and an additional five times every 4 days. Except for the fungicides, no significant effect ($P > 0.05$) of the pesticides on *M. anisopliae* was observed. A reduction in CO_2 production was observed for the mancozeb treatment from day 4 to day 6 of incubation, and for tebuconazol between days 4 and 6, 8 and 10, and 32 and 36. The same was observed for copper oxychloride between days 10 and 12 and 32 and 36, and for chlorothalonyl between 8 and 10, 10 and 12, and 32 and 36 days of incubation. Identical effect occurred for the acaricides abamectin and fenbutatin oxide, with a reduction in CO_2 production between 20 and 24 days of incubation. The herbicides glyphosate, trifluralin and ametrin reduced the respiratory activity of the fungus between days 10 and 12, while the insecticide trichlorfon reduced respiratory activity only from 32 to 40 days of incubation. The results indicate that the toxic action of pesticides on the fungus in soil is small, suggesting that this pest control bioagent can be used in combination with pesticides without compromising its activity (Mochi et al. 2005). Mochi et al. (2006) investigated if the presence of pesticides in the soil could affect the pathogenicity of *M. anisopliae* to Mediterranean fruit fly (Fig. 26), *Ceratitis capitata* (Wied.) (Diptera, Tephritidae) and assess the effect of conidia application as suspension or dry conidia. The fungicides chlorothalonyl and tebuconazol, the acaricide abamectin, the insecticide trichlorfon, and the herbicide ametrin were applied at the manufacturer-recommended doses. Soil samples were placed in glass flasks and were given the fungus as conidial suspension or dry.

Fig. 26 Mediterranean fruit fly (medfly), *Ceratitis capitata*. *Source* Gastón Zubarán, https://www.ecoregistros.org

After pesticide application, 20 3-instar larvae were placed in the soil. The flasks were sealed with voile fabric and incubated at 27 ± 0.5 °C for nine days, until adult emergence; incubation continued for four more days at room temperature. The total insect survival was significantly affected and pathogenic activity was detected from the pupa stage on. Pupa survival was reduced ($P < 0.05$); the same occurred during the adult phase. No effect was observed at the larval stage. The pesticides applied to the soil affected the activity of *M. anisopliae* slightly: Only in the dry conidia assay the fungicides chlorothalonyl and tebuconazole reduced (86.2 and 82.5%, respectively) the survival period of *C. capitata* compared to the control (95.0%). The techniques used for conidia application did not influence the total insect survival rate, but conidial suspension applied on soil surface reduced survival during the pupae and adult phases (Mochi et al. 2006). Weedicides also appeared to be toxic but level of toxicity was low as evidenced from inhibition of fungal growth from 10.26 to 26.07%. There was no significant variation between attrazin, nitrofen, glyphosate and pendimethalin (23.07%), but the toxicity was more than two times of 2,4-D and butachlor inhibited 10.26 and 12.39% respectively (Rachappa et al. 2007). Li and Holdom (1994) also reported absolute safety of 2,4-D. Chemical constituents of weedicides, mostly being selective to specific group of plants may not exert toxicity to fungal spores.

Moorhouse et al. (1992) also found little correlation between in vitro laboratory studies and in situ applications of fungicides and insecticides for *M. anisopliae*. In their studies, there were some indications that reduced germination in vitro may be linked to reduced infection rates in soil, but this relationship was not significant. This was not the case in the acaricide, insecticide and herbicide treatment (Mochi et al. 2006). Based on fungal respiratory activity, the toxic action of a range of pesticides (acaricides, fungicides, insecticides and herbicides) on *M. anisopliae* in the soil is small, suggesting little negative impact on the fungal activity resulting from their use (Mochi et al. 2005). In the rhizosphere soil, some fungicides tested in these studies significantly reduced the number of *M. anisopliae* CFU. To my knowledge, this is the first study to consider the impact of fungicides on entomopathogenic fungal populations in the rhizosphere. The biology of entomopathogenic fungi outside of their role as entomopathogens is becoming an increasingly important area of study. The potential of utilizing rhizosphere competent entomopathogenic fungi is great and any adverse effect that chemical fungicides have on the fungal population in the rhizosphere must be considered. If the fungus is not able to persist and proliferate in the rhizosphere it may not be present at levels adequate to control BVW larvae feeding on the roots. There was no significant increase in the *M. anisopliae* populations in the rhizosphere in this study relative to the bulk soil populations as was observed by Bruck (2005). It can take as long as 8–10 weeks for fungal populations to increase significantly in the rhizosphere (unpublished data). It appears that the duration of this study was too short for *M. anisopliae* to become established and proliferate in the rhizosphere. The fungicides (captan and triflumizole) which significantly reduced fungal populations in the rhizosphere were fungistatic and fungicidal in vitro and had short (7–14 days) reapplication intervals. The impact of other chemicals has been shown to reduce

entomopathogen infection rates when applied with short reapplication intervals (Hall 1981; Anderson and Roberts 1983). There were a number of other fungicides, thiophanate methyl (Cleary's 3336F®, Cleary Chemical; Banrot® 40 WP, Scotts Sierra Crop Protection), triflozystrobin (Compass® WG, Bayer Environmental Science), pyraclostrobin (Insignia® WG, BASF Corp) and azoxystrobin (Heritage® WG, Syngenta Crop Protection) with similar qualities in vitro with longer reapplication intervals (14 days) that had no significant impact on fungal populations in the rhizosphere in situ. Iprodione (Iprodione Pro 2SE, BASF Corp) was fungistatic in vitro and while it did not significantly reduce fungal populations in the rhizosphere, the resulting fungal population was also not significantly different than those for triflumizole (Terraguard® 50 WP, Chemtura Corp). Propamocarb (Banol® WP, Bayer Crop Science) and dimethomorph (Stature DM® WP, BASF Corp) were the only fungicide tested with a 7–14 days reapplication interval that did not have any effect on *M. anisopliae* populations in situ. Propamocarb (Banol® WP, Bayer Crop Science) was also the only fungicide with a 7–14 days reapplication interval that had no impact in vitro on *M. anisopliae*. The results of the in vitro bioassays of fungicides with <14 days reapplication intervals were somewhat predictive of the impact that these fungicides had on *M. anisopliae* populations in the rhizosphere. The use of *M. anisopliae* in the rhizosphere is a potentially new approach for BVW management in container-grown ornamentals that may be negatively impacted by some fungicides labeled for use in the industry. It appears from these studies that the impact is limited to fungicides with fungicidal and fungistatic effects in vitro and reapplication intervals of less than 14 days. Fungicides with similar effects in vitro and reapplication intervals >14 days were not detrimental to rhizosphere populations, presumably due to the length of time between fungicide applications. It may be that reapplication intervals of greater than 14 days are adequate for the fungal population in the rhizosphere to rebound. In soils not treated with fungicides, *P. abies* can support rhizosphere populations of *M. anisopliae* (F52) up to 10× the level in surrounding bulk soil (Bruck 2005). *M. anisopliae* (F52) clearly has a great deal of potential as a microbial control agent for BVW (Bruck 2005, 2006, 2007; Bruck and Donahue 2007). *M. anisopliae* is currently labeled for use as a soil incorporant, and when used as such is compatible with all of the fungicides tested. As researchers develop and growers begin to implement rhizosphere competence as an alternative management strategy for BVW, the fungicides captan and triflumizole should be avoided. *M. anisopliae* (Metschnikoff) Sorokin is an entomopathogenic fungus used for controlling different insect pests. It is most frequently applied to berry fruit crops, where fungicides are also used for disease control. Fungicides: azoxystrobin, benomyl, captan, chlorothalonil, fenhexamid, fludioxonil, iprodione, and metalaxyl in concentrations of 0.01, 0.1, 1.0, 10, and 100 mg L^{-1} were evaluated in this research study. Vegetative growth, conidia germination, and conidia germination tube length were measured on the Qu-M82, Qu-M151b, Qu-M253, Qu-M430, and QuM984 *M. anisopliae* var. *anisopliae* fungus strains. Those strains were selected because of their present use against different insect pest in bramble fruits. Vegetative growth was measured through the colony rate growth in agar media, and those reaching up to 50% of the check

growth were considered compatible. Results indicate that the benomyl and fenhexamid fungicides were compatible with the five isolates whereas, azoxystrobin and fludioxonil were incompatible. Furthermore, benomyl and fludioxonil reduced conidia germination by 53 and 91%, and germination tube length by 18 and 37%, respectively (Yáñez and France 2010). Six fungicides used in pecan culture were evaluated in vitro against the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *M. anisopliae* (Metschnikoff) Sorokin, both of which attack the pecan weevil, *Curculio caryae* (Horn). Triphenyltin hydroxide was the most toxic fungicide to both pathogens, followed by: benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate; zineb, zinc ethylene bis [dithiocarbamate]; and dodine, *n*-dodecylguanidine acetate. Sulfur and dinocap, 2-(1-methylheptyl)-4, 6-dinitrophenyl crotonate, were the least toxic (Tedders 1981).

The biocontrol agent *M. anisopliae* is efficient to combat more than three hundred species of insect pests and can be used in biological-chemical combinations with chemical defensives maintaining the inoculum source of fungi in the field. Studies of conidiogenesis in *M. anisopliae* are fundamental, considering that conidia are the main form of inoculum for biological control. Among the pesticides applied in pastures for pest control, to prevent and control plant diseases, is thiophanate-methyl. Due to the importance of *M. anisopliae* as a microbial agent of a wide variety of insect pests, it is of critical importance to evaluate the effect of chemical products on this fungus, considering the conidia germination speed parameter, which is directly associated with virulence. Therefore, this study aimed to verify the effect of different concentrations of thiophanate-methyl on the conidia germination speed of MT (Mato Grosso) strain of *M. anisopliae*. Conidia were incubated with thiophanate-methyl in concentrations of 200 μg ml^{-1} (T1), 20 μg ml^{-1} (T2), 2 μg ml^{-1} (T3) and 0.2 μg ml^{-1} (T4) at 28 °C and sampled throughout 24 h. The control was performed without the pesticide. Bayesian analysis showed an inhibition of conidia germination in the presence of 200 and 20 μg ml of thiophanate-methyl. The curve of conidia germination speed showed that until 14 h of incubation, there was an increase in the germination speed of control and all treatments. A stronger inhibition of conidia germination was caused by T1. The compatibility observed in concentrations of 20 and 2 μg ml^{-1} indicates that this fungicide could be mixed with *M. anisopliae* in biological-chemical combinations, maintaining the viable fungal inoculum after its application. To confirm it, third-instar larvae of sugarcane borer (*Diatraea saccharalis*) were infected with a combination of a conidia solution of MT strain and thiophanate-methyl in concentration of 20 μg ml^{-1}. As controls, water, conidia solution and the fungicide were applied separately. Food was offered ad libitum and larvae were monitored daily throughout 7–12 days at 25 °C and the factors of living larvae, larvae in pupal stage and dead larvae were evaluated. As results, the thiophanate-methyl did not affect the *D. saccharalis* larvae, but when these larvae were treated with *M. anisopliae* conidia mixed with the pesticide, it was observed a reduction of larvae mortality of 26.8% when compared with the use of *M. anisopliae* only (without pesticide) (Fabrice et al. 2013). The entomopathogenic fungus *M. anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) is registered in the

United States and The Netherlands for black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) control in container-grown ornamentals. These studies were conducted to determine the compatibility of *M. anisopliae* (F52) with a wide range of fungicides commonly applied to container-grown ornamentals for the management of soil-borne plant pathogens. The impact of fungicides on spore germination and mycelial growth were determined in vitro. In addition, *M. anisopliae* persistence in bulk and rhizosphere soil was determined 30 days following dual application of each fungicide at 7–28 days intervals as prescribed. A number of fungicides (thiophanate-methyl as Cleary's 3336F® and Banrot® 40 WP, dimethomorph as Stature DM®, captan as Captan® 50 WP, triflumizole as Terraguard® 50 WP, triflozystrobin as Compass®, pyraclostrobin as Insignia®, and azoxystrobin as Heritage®) inhibited spore germination in vitro. A larger number of fungicides (fosetyl-Al as Alliette®, thiophanate-methyl as Cleary's 3336F® and Banrot® 40 WP, dimethomorph as Stature DM®, captan as Captan® 50 WP, quintozene as Terraclor® 75 WP, triflumizole as Terraguard® 50 WP, fludioxanil as Medallion®, triflozystrobin as Compass®, pyraclostrobin as Insignia®, fludiox-mefanox as Hurricane®, iprodione as Iprodione Pro® 2 SE, azoxystrobin as Heritage®, phosphorus acid/K-salts as Agri-Fos®) inhibited mycelial growth in vitro. Only three fungicides, etridiazole (Terrazole® 35 WP, Chemtura Corp), propamocarb (Banol® WP, Bayer Crop Science) and mafanoxam (Subdue® MAXX MC, Syngenta Professional Prod), had no significant impact in vitro on spore germination or mycelial growth. While a number of fungicides had a detrimental impact in vitro, there was no impact on *M. anisopliae* populations in bulk soil following dual application of any fungicide. However, the fungicides captan and triflumizolet, which have a short reapplication interval, had a detrimental impact on *M. anisopliae* populations in the rhizosphere. As researchers develop rhizosphere competence as an alternative management strategy for black vine weevil, the fungicides captan and triflumizole should be avoided (Bruck 2009). A number of soil fungicides labeled for use as a drench application on ornamental nursery plants had an adverse effect on *M. anisopliae* (F52) germination and mycelial growth in vitro. Mycelial growth of *M. anisopliae* was more sensitive to fungicides than spore germination. Moorhouse et al. (1992) also found the impact of several fungicides and pesticides more pronounced on mycelial growth than spore germination of *M. anisopliae*. The impact of applied soil fungicides to *M. anisopliae* (F52) spores incorporated into soilless potting media was much less profound than observations in vitro. Studies in which the impact of fungicides in the field was determined in addition to in vitro effects highlights this fact. Chandler and Davidson (2005) also found *M. anisopliae* to be compatible with iprodione and tebuconazole under glass house conditions for the control of *Delia radicum* (L.) (Diptera: Anthomyiidae) even though these fungicides were inhibitory to fungal growth in vitro. Iprodione was also inhibitory to fungal growth in this study while having no adverse effect on CFU numbers when applied to potting media incorporated with *M. anisopliae* spores. Fungicides used in sugarcane production (prochloraz, propiconazole, flusilazole and methyl ethyl mercuric chloride) also significantly inhibited mycelial growth of *M. anisopliae* in vitro, but were found be

compatible in commercial practice (Samson et al. 2005). The activity of *M. anisopliae* against *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) was significantly reduced when the fungicides chlorothalonyl and tebuconazol were applied to soil. The fungicidal action of three fungicides, Sphere® (containing trifloxystrobin and cyproconazole as active agents), Nativo® (containing a combination of trifloxystrobin and tebuconazole as active ingredients), and Bendazol® (containing carbendazim as the only active ingredient formulated) used in soybean for control of fungal diseases on the lineages CG-28 and CG-30 of *M. anisopliae* var. *anisopliae* was evaluated. It was found that the fungicides inhibited the vegetative growth of the lineages at the concentrations indicated for the field, thereby showing its antifungal effect (Onofre et al. 2011). The effect of two isolates of the entomopathogenic fungus *M. anisopliae* (Metchnikoff) Sorokin (389.93 and 392.93) on root-feeding stages of cabbage root fly, *Delia radicum* (L.), was studied under glasshouse and field conditions. In glasshouse studies, the effect of drenching a suspension of conidia (concentration $I \times 10^8$ spores mL^{-1}, 40 mL $plant^{-1}$, applied on four occasions) onto the base of cabbage plants infested with *D. radicum* eggs was compared with mixing conidial suspension into compost modules (concentration $I \times 10^8$ spores mL^{-1}, 25 mL $plant^{-1}$) used to raise seedlings. Drench application reduced the mean number of larvae and pupae recovered per plant by up to 90%, but the compost module treatment had no statistically significant effect. Both application methods reduced the emergence of adult flies from pupae by up to 92%. Most conidia applied as a drench application remained in the top 10 cm layer of compost. Applications of the fungicides iprodione and tebuconazole, which are used routinely on brassica crops, were compatible with using *M. anisopliae* 389.93 against *D. radicum* under glasshouse conditions, even though these fungicides were inhibitory to fungal growth on SDA medium. In a field experiment, drench applications of *M. anisopliae* 389.93 to the base of cauliflower plants at concentrations of 1×10^6 to $I \times 10^8$ conidia mL^{-1} did not control *D. radicum* populations, although up to 30% of larval cadavers recovered supported sporulating mycelium. Drench applications often exhibited considerable lateral movement on the soil surface before penetrating the ground, which may have reduced the amount of inoculum in contact with *D. radicum* larvae (Chandler and Davidson 2005). Believing that the basic method of pest control in crops was still the use of pesticides that are building up in the soil and were the cause of changes in microbial activity, usually in the quantitative composition of the soil microflora, Tkaczuk et al. (2013) investigated the effect of three selected fungicides and soil extracts obtained from the sandy and organic soil on the growth of *M. anisopliae* under in vitro conditions. Among the tested fungicides, mancozeb (Dithane Neo Tec™ 75 WG), difenconazole (Score® 250 EC), and propineb (Antracol® 70 WG) the strongest growth inhibition of *M. anisopliae* caused Score® 250 EC (difenconazole) formulation, and relatively least Antracol 70 WG (propineb). The results indicate that the addition of soil extracts to the culture medium with fungicides increased the inhibitory action on the growth of the fungus. Extract from organic soil added to the culture medium with fungicides strongly inhibited the growth of *M. anisopliae* colonies than an extract obtained from the sandy soil (Tkaczuk et al. 2013).

In the laboratory, the fungicides chlorothalonil and zineb prevented germination of *M. anisopliae* conidia when incorporated into Sabouraud dextrose agar (SDA) at the commercial concentration based on the manfacturers' recommended rates for horticultural crops. Twelve other fungicides and six insecticides had no effect on spore germination when applied at the same rate. Mycelial growth of *M. anisopliae* on SDA plates containing the recommended rate of all the pesticides (except propamocarb) was reduced compared with SDA alone. Two fungicides, benomyl and carbendazim, totally inhibited growth at 0.1 times the recommended rate. Growth was also completely prevented by the fungicides etridiazole, triforine, and zineb, and the insecticides dichlorvos and hostathion, at 10 times the recommended rate. In glasshouse experiment, a prophylactic drench of *M. anisopliae* conidia reduced vine weevil (*Otiohynchus sulcatus*) populations on Impatient plants by 88%. This level of control was not significantly reduced by subsequent application (7 days after egg infestation) of any of the pesticides at the recommended concentration. Larval control in pots treated with *M. anisopliae* plus any one of the 12 fungicides and four insecticides examined, ranged from 82 to 98%. The insecticide diazinon applied alone reduced larval numbers by 100%. Two other insecticides, dichlorvos and cypermethrin, and the fungicide pyrazaphos, also reduced weevil populations by over 50%. These experiments demonstrate the limitations of laboratory based in vitro screening programs for assessing the chemical compatibility of *M. anisopliae* (Moorhouse et al. 1992). Prolonged conidial survival is probably to be a relevant characeristic or a successful microbial control agent for *O. sulcatus* and any reduction in survival would reduce the effective control period. Application of tolcofos-methyl and zineb to peat compost containing *M. anisopliae* conidia reduced the number of colony forming units (CFUs) per pot after 10 weeks by more than 56%. This reduction could have resulted from either mortality of the original conidia or inhibition of sporulation on infected larvae (Moorhouse et al. 1992). The entomopathogenic fungus, *M. anisopliae* (Metschnikoff) Sorokin is used for control of white grubs (Coleoptera: Scarabaeidae) in sugarcane fields in Queensland, Australia. Eight fungicides and three liquid insecticides are currently registered for application to sugarcane at planting, and may come into contact with *M. anisopliae* during its application from cane planters. Seven of these were tested for deleterious effects on two isolates of *M. anisopliae*, FI-147 and FI-1045, in laboratory and field experiments. In growth studies on medium, the four most commonly used fungicides were much more harmful to growth of *M. anisopliae* than the three insecticides. In a field experiment, where rice granules covered with spores of *M. anisopliae* were sprayed with each of four fungicides and two insecticides at very high rates and then covered with soil, only the fungicide Shirtan (methoxy ethyl mercuric chloride) showed any toxic effect on spore viability, with a reduction from 82 to 69%. No harmful effect of any chemical was detected in counts of colony-forming units in soil samples or in bioassays of treated soil using white grubs. Spore viability was not reduced when FI-1045 on rice granules (BioCane™) was applied with fungicides through nine commercial planters, including five using Shirtan, compared with granules buried in untreated soil. Thus, we believe that *M. anisopliae* is compatible with these chemicals in commercial practice

(Samson et al. 2005). High toxicity of Mancozeb towards all the isolates in the present study was in accordance with Durán et al. (2004) who mentioned that benomyl, dimethomorph-mancozeb, mancozeb, and mancozeb-cymoxanil mixture of fungicides significantly affect germination and growth of *B. bassiana* while fosetyl-Al, propamocarb, and copper oxychloride do not. Isolates of the entomopathogenic fungus *M. anisopliae* were tested for their compatibility with insecticides, fungicides and botanical pesticides, which are being used in the field, as a prerequisite for developing as mycopesticides and their use in IPM programmes. Three concentrations (0.1, 0.5 and 1×) of each chemical were evaluated in the laboratory based on the recommended dose for field application by food poison technique. Variation in vegetative growth and sporulation of *M. anisopliae* appeared to be related to the chemical nature of the formulations, its concentration and the fungal isolates in study. M19 and M48 isolates showed compatibility with imidacloprid at 0.5 and 0.1× and with fungicide sulfur at all the concentrations tested. All the four botanicals tested were found to be compatible to all the four fungal isolates and neem gold displayed maximum tolerance, at all the concentrations. M19 displayed an enhancement in the vegetative growth with imidacloprid (2%) and HIT (2–18%). 2% increase in the spore output was also recorded by M19 with chloropyrifos and sulfur. M19 and M48 isolates demonstrated compatibility with pesticides, fungicides and botanicals as well as with a cockroach management pesticide, HIT. The two isolates of *M. anisopliae* tested emerged as prospecting candidates for use as mycopesticide component in the combined application with pesticides like imidachloprid and fungicide, sulfur as well as botanicals in the IPM programmes (Babu et al. 2014). Akbar et al. (2012) designed a study to assess the toxicity of insecticides and fungicides on mycelial growth and spore production of *M. anisopliae*. All insecticides significantly inhibited mycelial growth and spore production of the fungus. Chlorpyrifos, match, profenofos and metalaxyl + mancozeb were the most toxic chemicals to mycelial growth and conidial germination followed by emamectin, cypermethrin, acetameprid, imidacloprid and fosetyl-aluminium (Sinophos®) which were relatively less toxic to mycelial growth and spore production ($P = 0.05$) of the fungal pathogen. On the contrary, spinosad (Tracer®, DOW Agrosciences, Indianapolis) and indoxacarb (Steward®) were significantly compatible and were found safe to conidial germination and growth of the fungi. Further studies related to their field evaluation are needed to confirm the findings (Akbar et al. 2012). Kotwal et al. (2012) tested the efficacy of *M. anisopliae* against *Spodoptera litura* (Fig. 27). The spore suspension at 2.5×10^9 spore mL^{-1} recorded highest mortality (90%) after ten days of spraying whereas lower concentration resulted less mortality. While the cultural filtrate resulted maximum mortality 26.66% after ten days. The compatibility studies of insecticides and fungicides by poisoned food technique revealed that spinosad, diamethoate and copper oxychloride were found most compatible. Whereas carbendazim, thiram and wettable sulfur were toxic to the *M. anisopliae* (Kotwal et al. 2012).

The entomopathogenic fungus conidia viability and sporulation may be affected by different environmental factors or by biopesticides and chemical products used to protect cultivated plants. In this research, we investigated the compatibility of

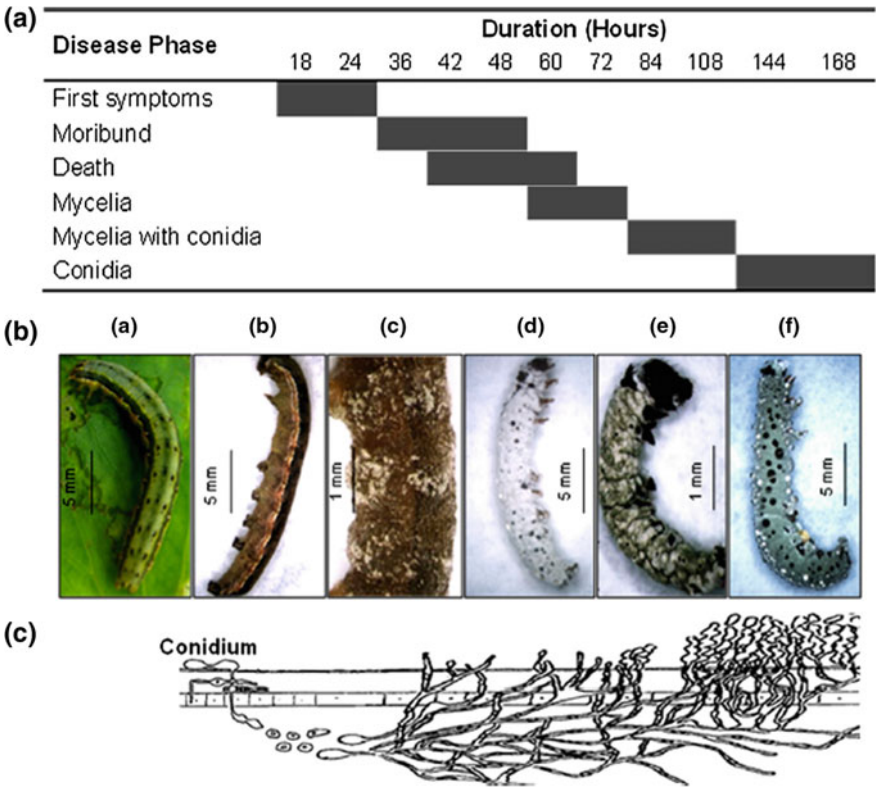

Fig. 27 Progressive infection as observed in fifth instars of *Spodoptera exigua* by *Metarhizium anisopliae*. **a** Duration, in hours, of progressive infection; **b** Gross morphology of the infection progression in (a) healthy larva, (b) dead infected larva, (c) mycelia emergence from insect cadaver, (d) insect cadaver mummified with mycelia, (e) emergent mycelia with few conidia, (f) cadaver mummified with conidia; **c** An artistic impression of the infection cycle in the larva (*Source* Small and Bidochka 2005) presented in parallel to the panel **b** (*Source* Javar et al. 2015)

M. anisopliae DEMI 001 isolates with three insecticides: fipronil (Agenda® EC 2.5%), and two insect growth regulators, pyriproxyfen (Admiral® EC 10%) and hexaflumuron (Consult® EC 10%). The effect of fipronil, pyriproxyfen and hexaflumuron on sporulation, vegetative growth and conidial germination of the fungus was studied based on measuring the vegetative growth and sporulation. SDA medium was mixed with fipronil (10, 40 and 120 ppm), pyriproxyfen (10, 500 and 1 000 ppm), hexaflumuron (20, 50 and 120 ppm). SAD medium excluding insecticides was designated as blank. To determine the sporulation rate, spores was counted on surface area and vegetative growth was measured on the basis of colony diameter. The results showed that hexaflumuron at the concentration of 120 ppm reduced the vegetative growth to 0% showing the highest reduction effect compared to pyriproxyfen (24.59%) and fipronil (24.31%). All three insecticides reduced

drastically the conidial germination at the highest concentrations (0–15%). Hexaflumuron treatment with 0% germination at all three concentrations and fipronil and pyriproxyfen with 32.36 and 9.7% germination at 200 ppm were not significantly different. Regarding the highest negative effect of hexaflumuron on germination at all three concentrations, this insecticide should not be applied together with the entomopathogenic fungus (Rashid et al. 2010).

The fungitoxic effects of imidacloprid and fipronil on the entomopathogenic fungi *Beauveria bassiana* and *M. anisopliae* were evaluated. The colony radial growth and conidial production were measured in the presence of the insecticides added to the culture medium. Imidacloprid was less toxic to the fungi than fipronil, and *M. anisopliae* was less affected by either insecticide than *B. bassiana*. Using scanning electron microscopy, conidia of *B. bassiana* and *M. anisopliae* were observed on the surface of the insect tegument at different times after inoculation. Imidacloprid at sublethal concentrations affected the grooming behavior of *H. tenuis*, while fipronil did not alter this behavior (Moino Jr and Alves 1998). An experiment was conducted to assess the in vitro effect of spinosad 45 SC (Tracer) on the entomopathogenic fungi viz., *M. anisopliae* and *B. bassiana.* The insecticide was applied at various concentrations and the field dose and higher doses adversely affected colony development, sporulation and spore germination. The effect was significantly higher on *B. bassiana* than on *M. anisopliae* and the effect increased with the dosage of insecticide used. Since the response is seen as dose dependent by lowering the dose in field use it may be possible to nullify the adverse effect of combining treatments. Regarding *B. bassiana* which shows high growth suppression and 100% suppression on sporulation and spore germination even at field level of the insecticide, a combined use may not be advantageous (Gowrish et al. 2013). *Metarhizium anisopliae* has been considered a promising alternative with low environmental impacts for the biological control of a variety of insect pests. Another alternative is the use of biological pesticides such as insect growth regulators, including lufenuron. Alves et al. (2011) evaluated the effect of different concentrations of lufenuron on the conidia germination speed of *M. anisopliae*. Conidia were incubated at 28 °C and sampled throughout 12 h. Bayesian analysis showed an inhibition of conidia germination in the presence of 2.0 mg mL^{-1} of lufenuron, whereas their compatibility was observed in the concentrations of 1.0 mg mL^{-1} and 700 μg mL^{-1}. It indicated that in these last two concentrations, the insect growth regulator and fungicidal compound, lufenuron had no toxicity on *M. anisopliae*, therefore, it could be employed in biological-chemical combinations, maintaining viable the fungal inoculums after its application in the field, with a low environmental impact (Alves et al. 2011). Shafa et al. (2012) recommended imidachloprid to be highly safe and most compatible to *M. anisopliae.* Imidacloprid was found to be compatible to M19 and M48 at all the three concentrations teste and in some cases demonstrated synergistic effects. On the other hand chlorpyrifos was compatible to M19 and monocrotophos against M52 isolate. Chlorpyrifos along with *M. anisopliae* at sub lethal doses was tested for mortality studies on German cockroach by Pachamuthu and Kamble (2000) and found a significant interaction between the entomopathogenic fungi and commercial pesticide.

On the other hand, Asi et al. (2010) reported detrimental effects of chloropyrifos to *M. anisopliae*. Virulence of *M. anisopliae* (Metschnikoff) Sorokin strain ESC-1 against the German cockroach, *Blattella germanica* (L.), was determined using 5 concentrations ranging from 8×10^7 to 2×10^9 spores mL^{-1}. The calculated LD_{50} value was 4.18 by 10^8 spores mL^{-1} (4.18×10^5 spores per cockroach). In vitro study was conducted to determine the compatibility of *M. anisopliae* strain ESC-1 with chlorpyrifos, propetamphos, and cyfluthrin. Insecticides did not affect conidial germination but did adversely affect the growth and sporulation of *M. anisopliae* strain ESC-1. The growth of *M. anisopliae* colonies on media amended only with 50 and 500 ppm of chlorpyrifos and 500 ppm of propetamphos treatments at 3, 6, and 9 d was significantly inhibited compared with the control. Similarly, sporulation was significantly reduced in treated colonies exhibiting partial colony growth. The colonies cultured on SDAY media amended with 50 ppm of chlorpyrifos had significantly reduced sporulation compared with the control and no sporulation was observed in colonies cultured on media amended with 500 ppm of chlorpyrifos and propetamphos (Pachamuthu et al. 1999). Mortality of German cockroaches, *Blattella germanica* (L.), caused by *M. anisopliae* strain AC-1 alone and in combination with different formulations of boric acid, was evaluated in laboratory bioassays. Topical application of *M. anisopliae* alone (8.96×10^9 conidia/m^2) required 28 days to cause > 92% cockroach mortality (LT_{50} = 10 days). In contrast, in combination with boric acid (H_2BO_3, topically applied as a dust or in drinking water), *M. anisopliae* killed cockroaches significantly faster than without boric acid. *M. anisopliae* conidial dust (8.96×10^8 conidia m^{-2}) with either 12.5% (w w^{-1}) boric acid dust or 0.1% (w v^{-1}) boric acid in drinking water killed 100% of the cockroaches in only 8 days (LT_{50} = 5 days) and 10 days (LT50 = 6 days), respectively, without compromising the fungus emergence from cadavers. Replacement of *M. anisopliae* with flour dust or heat-killed *M. anisopliae* conidia eliminated this effect, demonstrating that it was not the consequence of greater boric acid ingestion due to more extensive cockroach grooming upon exposure to *M. anisopliae* conidia. Moreover, injections of a low dose of *M. anisopliae,* which caused only 30% mortality, together with sub-lethal concentrations of boric acid into the cockroach hemocoel resulted in a doubling of mortality. Statistical analysis demonstrated a synergistic interaction between these two insecticides (Zurek et al. 2002). Ericsson et al. (2007) determined that spinosad interacts synergistically with the biocontrol agent *M. anisopliae* to increase the mortality of two wild-collected wireworm species, *Agriotes lineatus* (L.), and *Agriotes obscurus* (L.). Bioassays were performed using a *M. anisopliae* isolate originally acquired from a local wireworm cadaver. *M. anisopliae* was applied as a soil drench at 3.3×10^2 and 10^4 conidia/gram sand, respectively. Soil drenches also were prepared using a commercial formulation of the actinomycete toxins spinosyn-A and spinosyn-D (common name spinosad) at sub-lethal doses of 1.5, 3, and 6 ppm active ingredient per gram sand. Combined treatments of spinosad (Tracer®, DOW Agrosciences, Indianapolis) and *M. anisopliae* were synergistic in causing mortality for all spinosad concentrations. Wireworm feeding activity was reduced after exposure to both spinosad and *M. anisopliae* and was found to be concentration dependent.

The high mortality and reduced rate of wireworm feeding suggest that spinosad and *M. anisopliae* treatment combinations should be tested in the field (Ericsson et al. 2007). Li and Holdom (1994) observed chlorinated hydrocarbon insecticides as more deleterious than other insecticide groups to the mycopathogen. They observed extremely detrimental effect of chlorpyriphos, tempephos and malathion to mycelial growth and sporulation of *M. anisopliae*, while carbamate insecticides like carbofuran, methomyl and oxamyl were moderately toxic. Pyrethroids and insect growth regulators were safe to the development stages of fungus. The compatibility of entomopathogenic microorganisms with thiamethoxam and other insecticides was studied in vitro and under field conditions. The microorganisms tested were: *Aschersonia aleyrodis*, *Bacillus thuringiensis*, *Baculovirus anticarsia* (NPVAg), *Beauveria bassiana*, *Hirsutella thompsonii*, *Metarhizium anisopliae*, *Nomuraea rileyi*, *Paecilomyces farinosus*, *Sporothrix insectorum,* and *Lecanicillium* (*Verticillium*) *lecanii*. Two concentrations of each product were tested in the laboratory, based on the maximum and minimum recommended rates for application in the field. The products were added to specific culture medium for growth of the entomopathogens. Reproductive and vegetative growth were evaluated for fungi, and colony forming units (CFUs) were evaluated for bacteria. For the field test, CFUs were considered for both fungi and bacteria and caterpillar mortality for the NPV of *Anticarsia gemmatalis* (Hueb.). Results showed that: (1) the action of the pesticides on the vegetative growth and sporulation of the microorganisms varied as a function of the chemical nature of the products, its concentration and the microbial species; (2) thiamethoxam was compatible with all microorganisms studied; (3) endosulfan, monocrotophos and deltamethrin were the insecticides that most affected *B. thuringiensis*, *B. bassiana*, *M. anisopliae* and *S. insectorum*; (4) thiamethoxam did not affect the inoculum potential of *B. thuringiensis*, *B. bassiana* or *M. anisopliae* when applied to bean crop (*Phaseolus vulgaris*); and (5) thiamethoxam did not affect the efficiency of the nuclear polyhedral virus of *A. gemmatalis* (Batista et al. 2001). Batista et al. (2001) also observed that pesticides can also act in a positive manner in combination with entomopathogens. At sub lethal doses, they interact with the latter causing or activating infectious disease by stress and making the insects more susceptible to the action of microbial infection. Inconsistency prevailed in the compatibility relationship between the isolates of *M. anisopliae* and type of pesticides tested as reported by different workers.

Vyas et al. (1992) reported that, neemark, a biopesticide of neem was well tolerated by *M. anisopliae*. HIT and lakshmanrekha, among the pesticides used to control cockroaches, displayed compatibility with M19 while other isolates showed lack of tolerance to HIT, lakshman rekha as well as boric acid. Kumar et al. (2008) studied the in vitro compatibility of *M. anisopliae* with representative pesticides, plant oils, and a fungal antagonist. The pesticides tested were: endosulfan, acephate, abamectin, ethion, chlorothalonil, iprodion + carbendazim, thiophanate methyl, and dinocap, an antagonist, *Trichoderma harzianum*, and plant oils: coconut oil, groundnut oil, gingili oil, sunflower oil, neem oil, pongamia oil, and castor oil. Each product was tested in laboratory at 27 ± 1 °C and 65% RH, based on the

recommended rates for application in the field. The products were added to potato dextrose agar (PDA) culture medium for growth of the entomofungal pathogen and the dual culture technique was followed in the case of *T. harzianum*. Reproductive and vegetative growth was evaluated for *M. anisopliae*. Results showed that the action of the pesticides on the vegetative growth and conidial spore formation of *M. anisopliae* varied as a function of the chemical nature of the products. Thiophanate methyl recorded maximum vegetative growth of 2.46 cm diameter, whereas the maximum conidial spore formation of 2.45×10^7 spores mL^{-1} was observed in chlorothalonil. iprodion + carbendazim suppressed *M. anisopliae* completely. In the case of plant oils, sunflower oil yielded 5.77×10^7 spores mL^{-1} with a vegetative growth of 4.40 cm diameter indicating a synergistic effect when compared to all other treatments. However, 3.38 cm diameter and 3.4×10^7 spores mL^{-1} were recorded in the untreated control. Background information about the different degrees of entomopathogenic fungi tolerance of fungicides was reported by Maribel and France (2010). With respect to botanicals, neem gold, biospark and exodon showed compatibility to all the isolates in the study and neemgold displayed synergism with M19 which was manifested by enhanced vegetative growth of the isolate when grown in combination.

2.5 Nomuraea rileyi *(Farlow) Samson*

The fungus *Nomuraea rileyi* (Fig. 28) is regarded as a dimorphic hyphomycetous species that causes fungal epizootics in the population of several noctuid pests (Ignoffo et al. 1989; Tang and Hou 1998; Vargas et al. 2003). The fungus was more effective on ealy larval instars of *Spodoptera litura* and leads to highest rates of mortality (96.25%) under experimental conditions, while the lowest mortality (20%) was recorded with the fifth instar larvae. The median lethal time (LT_{50}) values for the 1st to 5th instars were 130.71, 137.77, 148.04, 235.55, and 263.10 h, respectively. The pathogenicity of the fungus against *S. litura* starts with the adhesion of conidia on the insect cuticle (Srisukchayakul et al. 2005), therefore, the effect of pesticides on the sporulation as well as germinability of the condia is of peculiar importance in the efficiency of the biological control mediated by the fungus.

In laboratory assays, benomyl, difenoconazole, sulphur, and carbendazim affected conidial germination of *N. rileyi*, while carbendazim was less deleterious (Sosa-Gómez et al. 2003). The effect of different pesticides on the spore germination of *N. rileyi* has been investigated. Five fungicides, eight insecticides and nine herbicides, commonly used in corn fields, were evaluated for inhibition of conidial germination by a paper disk test performed on Sabouraud maltose agar amended with 0.2% yeast extract (SMA + Y). Among them, only two fungicides, viz., maneb and propineb were highly inhibitory, while insecticides and herbicides tested were not inhibitory to the fungus (Table 3; Tang and Hou 1998).

In another study on the compatibility of the fungus with synthetic as well as botanical pesticides tested on potato dextrose agar plates, fungicides were found

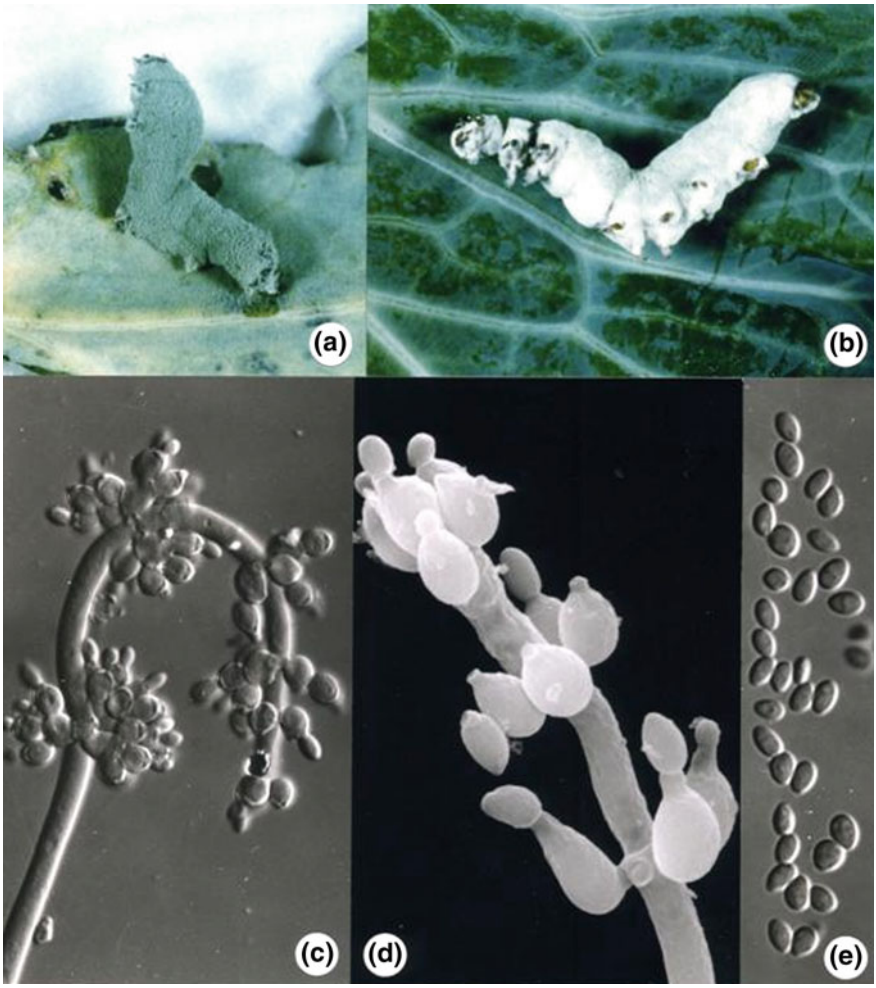

Fig. 28 *Nomuraea rileyi*, **a**, **b** habitat on larvae of cabbage looper, *Trichoplusia ni*; **c**, **d** conidiophores bearing metulae, phialides, and conidia; **e** Conidia (*Source* http://www.bcrc.firdi.org.tw)

more toxic to the fungus than insecticides (Patil et al. 2014). All tested fungicides significantly inhibited fungal growth, where carbendazim and mancozeb were highly detrimental at both tested concentrations. They did not allow any growth of the fungus, while others led to 55.25–76.92 percentile growth inhibition. Tridemefon and cyperconazole were comparatively safe to *N. rileyi* with less inhibition even at the field recommended doses. These were followed by hexaconazole, propiconazole, and chlorothalonil (Table 4; Patil et al. 2014).

Out of the tested insecticides, endosulfan and dichlorvos were of highly noxious impact on the fungal growth. Endosulfan inhibited fungal growth at both recommended as well as reduced doses, while dichlorvos inhibited its growth when applied

Table 3 Diameter of inhibition ring formed on *Numuraea rileyi* cultures 10 days after treatment with fungicides, insecticides, and herbicides recorded on Sabouraud maltose agar amended with 0.2% yeast extract (SMA + Y) at 25 °C

Treatments	Formulation	Dilution factor	Diameter of inhibition (cm)
Fungicides			
Maneb (80%)	WP	400	7.8 ± 0.73 a
Propineb (70%)	WP	500	5.4 ± 0.55 b
Copper oxychloride + Zineb + Maneb (63%)	WP	500	2.3 ± 0.19 c
MALS (16.5%)	EC	2500	2.2 ± 0.39 c
Iprodione (50%)	WP	1500	1.6 ± 0.12 d
Insecticides			
Chlorpyrifos (25%)	WP	800	1.9 ± 2.05 a
Bifenthrin (20.8%)	EC	1000	0 ± 0 b
Carbofuran (75%)	WP	1500	0 ± 0 b
Methomyl (90%)	WP	2000	0 ± 0 b
Buprofezin (25%)	WP	1500	0 ± 0 b
Mevinphos (25.3%)	EC	500	0 ± 0 b
Decamethrin (2.8%)	EC	1500	0 ± 0 b
Sumicidin (20%)	EC	4000	0 ± 0 b
Herbicides			
Oxyfluorfen (23.5%)	EC	600	0 ± 0 b
Fluroxypyr (29.64%)	EC	700	0 ± 0 b
Glyphosate + fluroxypyr (33%)	S	600	0 ± 0 b
Pendimethalin (34%)	EC	300	0 ± 0 b
2,4-D (40%)	WP	150	0 ± 0 b
Glyphosate (41%)	S	600	0 ± 0 b
Trifluralin (44.5%)	EC	300	0 ± 0 b
Butachlor (58.8%)	EC	1000	0 ± 0 b
Glyphosate (74.7%)	SG	600	0 ± 0 b

Source Tang and Hou (1998)

at field recommended dose. The rate of growth inhibition by these highly toxic insecticides was circa 50.58 to 52.77%. The inhibitory effect of other insecticides ranged from 15.98 to 35.42% at recommended doses (Table 5; Patil et al. 2014).

Out of the thirteen plant products evaluated, *Annona squamosa* seed extract (5%), *Polyalthia longifolia* (5%), and *Parthenium hysterophorus* (10%) inhibited growth to the extent of 50–55% after seven days of inoculation. The inhibitory effect of other botanicals varied in the range of 24–17.58%. The least inhibitory effect (2.4%) was recorded with *Vinca rosea* followed by *Stachytarphita indica* (5%), NSKE (5%), *Vitex negundo* (5%), neem oil (1%), *Argemone mexicana* (5%), pongamia oil (1%), *Clerodendron inerme* (5%), and *Acorus calamus* (5%) (Table 6; Patil et al. 2014).

Table 4 Effect of fungicides on colony growth of the entomopathogenic fungus, *Nomuraea rileyi*

Treatments	Colony size of *Nomuraea rileyi* (mm)					
	3 DAI	Inhibition (%)	5 DAI	Inhibition (%)	7 DAI	Inhibition (%)
Carbendazim (0.05%)	0.00 j	100.00	0.00 g	100.00	0.00 g	100.00
Carbendazim (0.025%)	0.00 j	100.00	0.00 g	100.00	0.00 g	100.00
Mancozeb (0.20%)	0.00 j	100.00	0.00	100.00	0.00 g	100.00
Mancozeb (0.10%)	0.00 j	100.00	0.00 g	100.00	0.00 g	100.00
Cyperoconazole (0.10%)	11.67 e	53.32	14.00 c	57.14	18.67 d	60.83
Cyperoconazole (0.05%)	12.67 d	49.32	15.67 b	52.03	21.33 c	55.25
Propiconazole (0.10%)	9.33 g	62.68	11.00 de	66.33	11.00 f	76.92
Propiconazole (0.05%)	10.67 f	57.32	12.00 d	63.26	14.00 e	70.63
Chlorothalonil (0.20%)	6.67 i	73.32	8.67 f	73.46	12.00 f	74.48
Chlorothalonil (0.10%)	8.00 h	68.00	10.33 e	68.38	14.33 e	69.94
Tridemefon (0.10%)	14.33 c	42.68	14.33 c	56.14	21.33 c	55.25
Tridemefon (0.05%)	15.33 b	38.68	15.67 b	52.00	24.00 b	49.65
Hexaconazole (0.10%)	10.00 fg	60.00	12.00 d	63.26	14.00 e	70.63
Hexaconazole (0.05%)	12.00 de	52.00	13.67 c	58.15	15.33 e	67.84
Untreated Check	25.00 a	–	32.67 a	–	47.67 a	–

DAI Days After Inoculation; Means marked by the same alphabet do not differ significantly ($P = 0.05$) as known through Duncan's Multiple Range Test
Source Patil et al. (2014)

2.6 Purpureocillium lilacinum *(Thom) Luangsa-Ard, Houbraken, Hywel-Jones & Samson*

Arun and Ramya (2014) studied the effect of some fungicide such as blue copper (copper oxy chloride 0.3%), difenoconazole (0.07%), propiconozale (0.05%) and herbicides like gramaxone (0.5%) and fernoxone (0.2%) with *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*; Fig. 29), a fungus from Ophiocordycipitaceae family used as one of the most promising and commercialized biological nematicide

Table 5 Effect of insecticides on colony growth of the entomopathogenic fungus, *Nomuraea rileyi*

Treatments	Colony size of *Nomuraea rileyi* (mm)					
	3 DAI	Inhibition (%)	5 DAI	Inhibition (%)	7 DAI	Inhibition (%)
Monocrotophos (0.05%)	18.67 cd	25.32	24.67 c	24.48	36.33 e	24.31
Monocrotophos (0.025%)	21.00 b	16.00	26.33 b	19.40	37.67 d	21.52
Chloropyriphos (0.04%)	16.00 e	36.00	21.67 d	33.67	30.00 h	35.42
Chloropyriphos (0.02%)	17.67 d	29.32	22.33 d	31.65	31.33 g	34.73
Endosulfan (0.07%)	13.67 fg	45.32	16.33 e	50.00	22.67 i	52.77
Endosulfan (0.035%)	14.33 f	42.68	16.67 e	48.97	22.67 i	52.77
Dichlorvos (0.04%)	13.00 g	48.00	16.33 e	50.00	23.67 i	50.58
Dichlorvos (0.02%)	18.00 d	28.00	21.67 d	33.67	35.00 e	27.08
Methomyl (0.025%)	19.33 c	22.68	24.33 c	25.52	40.33 c	15.98
Methomyl (0.0125%)	20.67 b	17.32	25.33 bc	22.46	42.33 b	11.81
λ-Cyhalothrin (0.01%)	13.33 f	46.68	24.33 c	25.52	40.33 b	11.81
λ-Cyhalothrin (0.005%)	12.67 g	49.32	22.67 d	30.60	33.00 fg	33.33
Phosphamidon (0.04%)	13.33 f	46.68	24.33 c	25.52	36.33 e	24.31
Phosphamidon (0.02%)	20.67 b	17.32	25.67 bc	21.43	39.33 c	18.06
Untreated Check	25.00 a	–	32.67 a	–	48.00 a	–

DAI Days After Inoculation; Means marked by the same alphabet do not differ significantly ($P = 0.05$) as known through Duncan's Multiple Range Test
Source Patil et al. (2014)

(Wang et al. 2016). The optical density values, serial dilution method, and radial growth technique were carried out to find the compatibility of some agrochemicals with the entomopathogenic fungus, *P. lilacinum*. The concentration of the agrochemical used under field recommendation was not compatible. The study indicated that this concentration highly inhibit the growth of the fungus. If the concentration of the agrochemical is reduced, the fungi may grow. However as the final result, the tested fungicides and herbicides were non-compatible with the fungus. Further study was

Table 6 Effect of botanicals on colony growth of the entomopathogenic fungus, *Nomuraea rileyi*

Treatments	Colony size of *Nomuraea rileyi* (mm)					
	3 DAI	Inhibition (%)	5 DAI	Inhibition (%)	7 DAI	Inhibition (%)
Annona squamosa (5%)	12.00 e	50.68	14.00 e	57.14	24.33 g	55.76
Parthenium hysterophorus (10%)	19.67 c	19.15	27.67 bc	15.30	27.67 f	49.69
Parthenium hysterophorus (5%)	20.67 bc	15.04	27.67 bc	15.30	27.67 f	49.69
Adhatoda vasica (5%)	23.67 a	2.70	31.67 a	3.06	52.33 bc	4.85
Vinca rosea (5%)	24.33 a	0.00	32.67 a	0.00	53.67 ab	2.40
Polyalthia longifolia (5%)	15.33 d	36.99	21.33 d	34.71	24.33 g	55.76
Vitex negundo (5%)	21.00 b	13.68	36.67 bc	18.36	51.33 c	6.67
Acorus calamus (5%)	19.33 c	20.55	26.33 c	19.40	45.33 e	17.58
Clerodendron inerme	19.67 c	19.15	27.67 bc	15.30	45.67 e	16.96
Argemone mexicana (5%)	20.67 bc	15.04	28.67 b	12.24	51.30 c	6.72
Stachytarphita indica (5%)	20.33 bc	16.44	27.33 bc	16.34	52.33 bc	4.85
Neem oil (1%)	21.33 b	12.32	30.67 a	6.72	51.33 c	6.67
Pongamia oil (1%)	19.67 c	19.15	27.33 bc	16.33	47.33 d	13.94
NSKE (5%)	27.67 a	2.71	32.00 a	2.00	51.67 c	6.05
Untreated Check	24.33 a	–	32.67 a	–	55.00 a	–

DAI Days After Inoculation; Means marked by the same alphabet do not differ significantly (P = 0.05) as known through Duncan's Multiple Range Test
Source Patil et al. (2014)

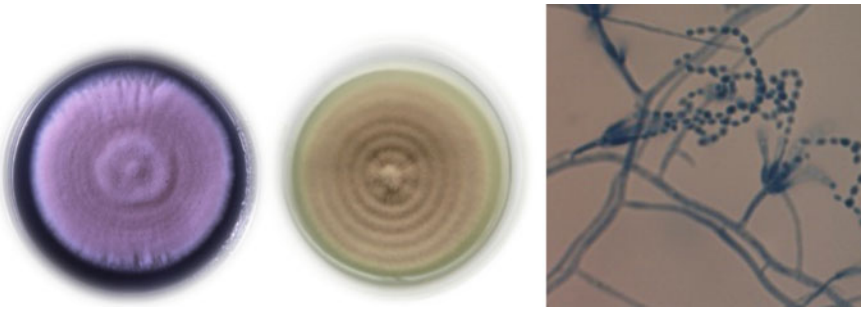

Fig. 29 *Purpureocillium lilacinum* (Left and Middle) colony morphology on potato dextrose agar (*Sources* K. Nishimura, Chiba University Research Center for Pathogenic Fungi and Microbial Toxicoses; Lê Thi Mai Châm, Center of Biotechnology Ho Chi Minh City); (Right) conidiophores and conidia of the fungus (*Source* Lê Thi Mai Châm, Center of Biotechnology Ho Chi Minh City)

needed to find the compatibility level at low concentration. In vitro effects of some fungicides on *P. lilacinu*m, which was pathogenic on apple rust mite, were investigated (Demirci and Denizhan 2010). Protectant fungicides, captan, propineb, and mancozeb were effective against conidial germination. Systemic fungicides such as benzimidazole and triazole were effective against mycelial growth. Carbendazim, penconazole and tebuconazole were found to be the most effective fungicides on mycelial growth of *P. lilacinum*, with EC_{50} values <3 μg ml^{-1}.

Chan Cupul et al. (2014) assessed the in vitro toxicity of the herbicides atrazine and paraquat on vegetative growth and sporulation of saprobic soil fungi. In thirteen strains of fungi isolated from soil, dose-response bioassays were performed with four concentrations of herbicides: atrazine (468, 937, 1875 and 3750 mg L^{-1}) and paraquat (93, 187, 375 and 750 mg L^{-1}). The fungi were inoculated with 2 mL of a spore suspension (1×10^6 spores mL^{-1}) in Petri dishes with potato dextrose agar (PDA) supplemented with herbicides. Daily growth rates (DGR), percent inhibition of mycelial growth (% IMG), sporulation and the median effective concentration (EC_{50}) were evaluated. *Paecilomyces carneus* (Fig. 30) significantly showed the highest DGR (0.26 cm^2 day^{-1}) and maintained its sporulation rate (3.7×10^5 spores mL^{-1}) at 468 and 937 mg L^{-1} of atrazine.

The % IMG of *P. carneus, P. marquandii* (Fig. 31) and *P. lilacinum* at 3750 mg L^{-1} of atrazine in APD were: 22.6, 44.4 and 46.3%; with an EC_{50} of 6820, 4736 and 3633 mg L^{-1}, respectively. Paraquat was more fungitoxic than atrazine; *P. carneus* significantly maintained its DGR (0.17 cm^2 day^{-1}) under 93 and 187 mg L^{-1} of paraquat. The EC_{50} of paraquat showed the lowest values compared to atrazine; *Aspergillus tamarii* obtained the highest EC_{50} (256.4 mg L^{-1}) in paraquat. The genus *Paecilomyces* spp. and *A. tamarii* were the most tolerant to atrazine and paraquat, respectively. These strains are candidates to be included in studies regarding the biodegradation of both herbicides in environmental biotechnology.

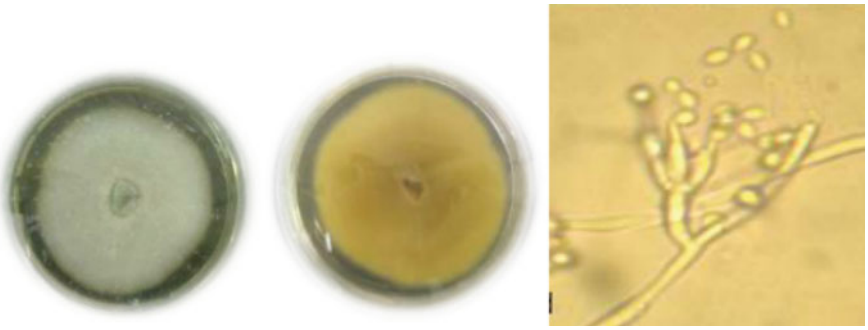

Fig. 30 *Paecilomyces carneus*. (Left) colony growth on malt extract agar (MEA); (Middle) Pink-brown reverse color on MEA; (Right) Conidiophore and conidia 1000× maginified. *Source* Bakeri et al. (2007)

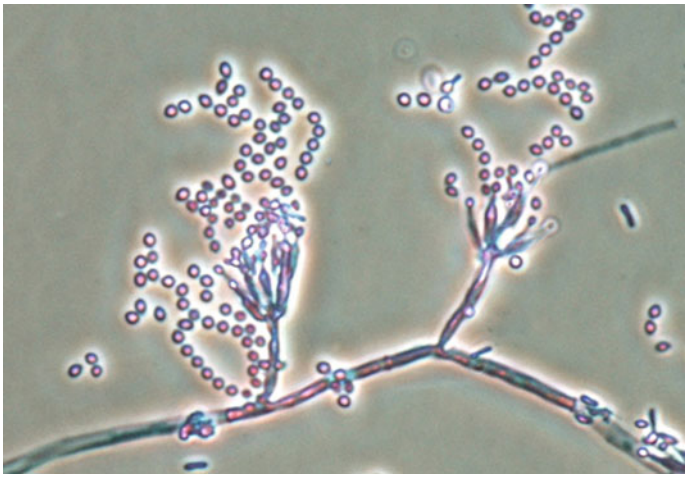

Fig. 31 *Paecilomyces marquandii* conidiophores, phialides, and conidia (*Source* http://mycology.adelaide.edu.au/descriptions/hyphomycetes/paecilomyces)

2.7 Trichoderma *Spp*

Trichoderma species are imperfect filamentous fungi, with telomorphs belonging to the hypocreales order of the division Ascomycota. *Trichoderma* spp. are among the most frequently isolated soil fungi, well known for their biocontrol ability against a wide range of plant pathogenic fungi (Howell 2003). *Trichoderma* spp. are fungi that occur worldwide. Previous studies have shown that they are not only parasites of fungal plant pathogens but also can produce antibiotics. In addition, certain strains can induce systemic and localized resistance to several plant pathogens. Moreover, some strains may enhance plant growth and development (Ha 2010).

Species of the *Trichoderma* genus are able to inhibit the growth of variety of potentially pathogenic fungi. A recent list of mechanisms are viz., mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, solubulization and sequestration of inorganic nutrients, induced resistance and inactivation of the pathogens enzymes (Lewis and Lumsden 2001). Growth promotion due to *Trichoderma* spp. is also reported in several crop species (Manoranjitham et al. 1999).

Trichoderma spp. have received the most attention for control soil borne pathogens. *Trichoderma* species are known to suppress infection of root by soil-born pathogens like *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium* species and *Pythium* species on various crops (Ehtesham et al. 1990; Benítez et al. 2004; Adekunle et al. 2001; Lutchmeah and Cooke 1985; Howell 1982). Species of *Trichoderma* also have growth promoting capabilities that may or may not be integral to biological control (Benítez et al. 2004; Dubey et al. 2007; Yedidia et al. 1999). *T. harzianum* has shown effective control of root infecting fungi and root-knot nematodes (Spiegel and Chet 1998; Sun and Liu 2006). *T. harzianum* isolated from rhizome rot suppressive soils reduced the disease and increased plant growth and yield (Ram et al. 1999). Although not normally associated with foliar surfaces, *Trichoderma* spp. have been widely investigated also for the control of foliar diseases, particularly gray mold caused by *Botrytis cinerea* on lettuce (Card 2005), snapbeans (Nelson and Powelson 1988), cucumber (Lee et al. 2006), tomato (Hmouni et al. 2006; Lisboa et al. 2007), Eucalyptus globulus (Zaldúa and Sanfuentes 2010) and faba bean (Bendahmane et al. 2012). *Trichoderma* has been successfully applied to aerial plant parts for the biocontrol of decay fungi in wounds on shrubs and trees (Papavizas 1985), brown blotch caused by *Colletotrichum truncatum* on cowpea (Bankole and Adebanjo 1996), anthracnose induced on strawberry by *Colletotrichum acutatum* (Freeman et al. 2004), *Bipolaris oryzae* causal agent of brown spot of rice (Mouria et al. 2003), four species of *Botrytis* (*B. maydis*, *B. sorokiniana*, B. *sorghicola*, and *B. tetramera*) on sorghum (Berber et al. 2009). Actually among the aspects that future research should focus on to make better use of *Trichoderma* as a biocontrol agent for the management of crop diseases, the first is the ability of *Trichoderma* for control of foliar pathogens/air (Ramanujam et al. 2010). Additionally, some *Trichoderma* spp. are expected to infect and kill insect pests (Fig. 32) and mites because of the rich arsenal of bio-chemical they produce, including chitinases (Seidl et al. 2005), glucanases (Kubicek et al. 2011), proteases (Seidl et al. 2009), peptaibols (Degenkolb et al. 2007, 2008), and toxic compounds (Dix and Webster 1995). Compared with *B. bassiana* with hyaline spores, *Trichoderma* spp. produce a huge number of green spores that are expected to be of more tolerance to solar ultraviolet rays. *Trichoderma* spp. can behave in a way similar to a snake pursuing a rat inside the tunnels, and find the humid niches of stem/wood borers as well as bark beetles, where they can effectively control both vector and the pathogen harbored (Pakdaman 2013).

Although the use of biocontrol agents could reduce chemical application to a limited extent, it is less reliable and less efficient, however, the combination of

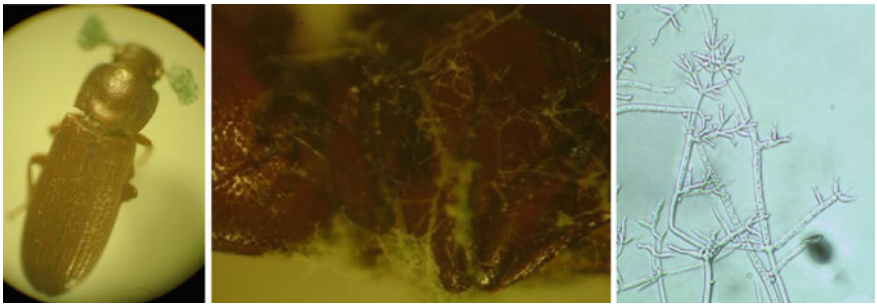

Fig. 32 *Tribolium confusum*. (Left–Middle) Infected and killed by *Trichoderma asperelloides*; (Right) Conidiophores and conidia of *T. asperelloides*. *Source* Pakdaman (2013)

fungicide tolerant biological control agents with reduced levels of fungicide integrated control strategies would promote the degree of disease suppression similar to that achieved with full dosage of fungicides (Monte 2001). Although the use of biocontrol agents could reduce chemical application to a limited extent, it is less reliable and less efficient, however, the combination of fungicide tolerant biological control agents with reduced levels of fungicide integrated control strategies would promote the degree of disease suppression similar to that achieved with full dosage of fungicides (Monte 2001). Reduced amount of fungicide can stress and weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonist (Hjeljord and Tronsmo 1998). Integration of biocontrol agents with fungicides gave significantly higher disease control in several crops than that obtained either by biocontrol agent or by fungicide alone (Sawant and Mudhopadhay 1990; Dubey 1997). Also, the seed and seedling treatment with captan, metalaxyl and carboxin may eradicate the wilt causing pathogens or other microflora thereby less competition for biological control agent to colonize the seed and root surface and proliferate (Chet et al. 1982; Ram et al. 2000). Moreover, the subsequent application of compatible fungicides may support the growth of antagonists and may also prevent the plant form the attack of pathogens. The other workers also observed the additive effects of compatible fungicides and antagonists (Chattopadhyay and Sen 1996).

The combined use of biocontrol agents and chemical pesticides has attracted much attention as a way to obtain synergistic or additive effects in the control of soil-born pathogens (Locke et al. 1985), and the most promising possibility for the application of *Trichoderma* strains is within the framework of integrated plant protection management, based on the combined application of physical, chemical and biological means of control (Kredics et al. 2003). This leads to an emerging necessity for the information on *Trichoderma*, as a prerequisite for their application in combination of chemical and biological control (Hatvani et al. 2006). The tolerance of these fungi to chemical fungicides is a prerequisite for biocontrol agents for their application to suppress the growth of some soilborne pathogens (Joeniarti et al. 2014), and the compatibility of fungicides has been considered to be a most

important criterion with regards to integrated pest management. The compatibility is desirable not only with other fungicides but also with agrochemicals including fertilizers and biocontrol agents with a view to reduce their cost of application. Sangeetha et al. (1993) reported that rice bran yielded 35% higher seed germination in brinjal and wheat as well as rice bran as the best substrate for the formulation of *Trichoderma*. The differential response of antagonistic flora to various fungicides might be due to their inherent resistance to most fungicides and their ability to degrade chemicals (Lifshitz et al. 1985; Papavizas 1985). It has been reported that many *Trichoderma* species has an innate and/or induced resistance to many fungicides but the level of resistance varies with the fungicide (Omar 2006).

For successful combined application of biological and chemical control of pathogens, biocontrol agents that tolerate chemical fungicides are needed. The integration of both control strategies has showed a positive association, by reducing infection, compared to their individual applications (Srinivas and Ramakrishnan 2002), and some strains of *Trichoderma* show compatibility with fungicides as they are tolerant to fungicides and successfully used in the IPM strategy (Dutta and Chatterjee 2004; Khan and Shahzad 2007).

Abd-El Moity et al. (1982) found that some wild strains of *Trichoderma* were tolerant to high concentrations of chlorothalonil during germination. Mukhopadhyay et al. (1986), Sharma and Mishra (1995); Abha Agarwal and Tripathi (1999), found good growth of *Trichoderma* isolates at low and medium concentrations of various fungicides. De Cal et al. (1994) reported that the biocontrol agents, which could tolerate fungicides up to a certain level, were mixed with fungicides and resulted in eradication of diseases. Mukhopadhyay et al. (1992) reported the insensitivity of *Trichoderma* and *Gliocladium* spp. against the carboxin. The differentiating response of antagonistic flora to various fungicides might be due to their inherent resistance to the fungicides and their ability to degrade chemicals (Papavizas 1985). Also, through studies in glasshouse, they indicated that seed + seedling + spraying of carbendazim + metalaxyl and captan + metalaxyl were more effective than seed + seedling + soil treatment of *Trichoderma virens* (Gv3) and *T. viride* (Tv) under glasshouse conditions. The treatment captan + metalaxyl recorded significantly higher wilt incidence (38.8%) than observed in carbendazim + metalaxyl (31.9%), however, when *T. viride* (Tv2) and *T. virens* (Gv32) were integrated with captan + metalaxyl it resulted in lesser wilt incidence which was at par with carbendazim + metalaxyl. The effectiveness of carboxin + metalaxyl was also observed when used in combination with fungal antagonists. The maximum disease reduction (70.9%)) over control was observed each in captan + metalaxyl + *T. viride* and captan + metalaxyl + *T. virens*. Carboxin + metalaxyl also recorded disease reduction of 47.3% over control. Under present investigation, *T. viride* (Tv) and *T. virens* (Gv3) showed more resistance against the fungicides rather than *T. harzianum* (Th) which might be due to their inherent resistance to fungicides and their ability to degrade chemicals as described by Papavizas (1985).

Caron et al. (1994) have established the compatibility chart of *Trichoderma* MAUL-20 with the most commonly used pesticides in greenhouses. They showed that fenhexamid was among the active ingredients compatible with *Trichoderma*

MAUL-20. Fenhexamid, a hydroxyanilide, is an inhibitor of sterol biosynthesis. When the fungus *B. cinerea* was grown in the presence of fenhexamid, the ergosterol content was decreased, and three 3-keto compounds, 4-α-methylfecosterone, fecosterone, and episterone accumulated, suggesting an inhibition of the 3-ketoreductase involved in C-4 demethylation (Debieu et al. 2001). The sensitivity of the fungal antagonists (*Chaetomium globosum* and *Trichoderma* species) of onion white rot, caused by *Sclerotium cepivorum*, to captan, mancozeb, thiram, benomyl and two dicarboximides was also evaluated, and dicarboximide-resistant biotypes were selected (Kay and Stewart 1994). Singh et al. (1995) reported that selected isolates of *T. harzianum*, *T. viride*, *T. reesei* and *T. koningii* were tested with Captaf® 500 ppm, Dithane M-® at 500 ppm and Thiram® at 200 ppm. The fungicides were highly inhibitory to *T. reesei* and they were compatible to *T. koningii*.

Latorre et al. (1997) suggested that antagonistic activity of biocontrol agents might be effective if it is integrated with other control practice and may result in acceptable levels of disease control with reduce level of chemicals use. The result of the present screening would help in the selection of biocontrol agents, which can be used, with reduced dose of selected fungicides for the control of plant pathogenic fungi. The influence of mancozeb, benomyl and vinclozolin on the antagonistic effect of four *Trichoderma* strains against *Sclerotinia minor* was investigated by Naár and Kecskés (1998), and vinclozolin and mancozeb were proposed for combined application with *Trichoderma* against *Sclerotinia*. In vitro action of 5 mixtures of fungicides and 11 insecto-fungicides to different antagonistic fungi, among them to *T. viride*, was tested by Sesan and Oprea (1999), and a restricted group of the examined pesticides with low inhibitory action was suggested to be applicable in the integrated protection of different crops. Domondon and Poppe (2000) reported that benomyl, prochloraz, and imazalil inhibited *Trichoderma* growth, while krezoxim-methyl and azoxystrobin inhibited sporulation. Bhatt and Singh (2000) reported that carbendazim (0.075%) was most effective against *Trichoderma* spp.

Sushir and Pandey (2001) reported that fluchloralin, oxadiazon and pendimethalin adversely affect the growth of *Trichoderma* spp. 42.22, 37.77, and 55.55% even at 125, 250 and 250 µl/mL, respectively. Srinivas and Ramakrishnan (2002) have reported that integration of biocontrol agents and commonly used fungicides showed positive association by reducing the seed infection compared to fungicide and the fungal antagonists individually (Khan and Shahzad 2007). The effects of three fungicides (benomyl, carbendazim and dicloran) and four herbicides (fenuron, fluometuron, monuron and diuron) on the growth of *T. aureoviride* T122, *T. harzianum* T66 and T334, and *T. viride* T124 and T228 strains (Kredics et al. 2002) were examined. In the case of diuron, 50% inhibition could not be reached. For the other herbicides and dicloran the IC concentrations were found to be so high that their values cannot be present in the soil during their application. The effect of certain fungicides and herbicides on *Trichoderma* spp. was reported earlier with an emphasis on practical applications (Kredics et al. 2003).

Rinker and Alm (2008) evaluated four fungicides, benomyl, chlorothalonil, thiabendazole, and thiophanate-methyl against 10 isolates of *Trichoderma*. Thiabendazole was the most effective, followed by benomyl, and thiophanate-methyl. Chlorothalonil was ineffective. The compatibility of *Trichoderma* spp. with metalaxyl has also been reported (Anonymous 2008). Khosla and Gupta (2008) observed the toxicity of carbendazim against the *Trichoderma* spp. In addition, their field studies revealed the similar trend in results as under pot culture studies. Superiority of carbendazim + metalaxyl, captan + metalaxyl + Gv3 and captan + metalaxyl + Tv was confirmed as higher disease reduction of 59.8, 58.6 and 58.0% over control and maximum yield of 138.6, 137.0 and 135.6 q ha^{-1} was observed, respectively. The integration of BCAs with carboxin + metalaxyl resulted lesser wilt incidence compared to the individual treatment of biological control agents and fungicides.

Gabriolotto et al. (2009) investigated the effectiveness of control strategies against *B. cinerea* in vineyard and evaluation of the residual fungicide concentrations and indicated that the application of three treatments of a *Trichoderma* spp. was really ineffective against gray mold, even though in association with pyrimethanil. Pyrimethanil, whose mode of action is not fully understood, interferes at amino acid level and mainly with methionine biosynthesis (Heye et al. 1994). Polygalacturonase, cellulose, proteinase, and laccase activities were all decreased in the medium of three day-old cultures grown of *B. cinerea* in the presence of pyrimethanil. No significant growth inhibition was observed at the pyrimethanil concentrations tested. Pyrimethanil did not inhibit the enzymes directly, nor did it inhibit the synthesis of cytosolic proteins. Therefore, it apparently inhibits protein secretion at a post-translational stage in the secretory pathway. Accordingly, pyrimethanil seems most efficient in the media where the fungus has to utilize extracellular enzymes to mobilize the nutrients required for its growth (Milling and Richardson 1995). Pervez et al. (2009) evaluated formalin, bavistin, and combination of formalin and bavistin against mycoflora of oyster mushroom substrates. The combination of formalin and bavistin (500 ml + 75 ppm) was found of the most inhibiting effect on the radial growth of all the identified fungi.

Bagwan (2010) conducted compatibility tests under in vitro condition to find out safer fungicides, pesticides, different cakes and botanicals against *Trichoderma*. For this different fungicide, pesticides, cakes and botanicals were tested against *Trichoderma harzianum* (Th 09) and *Trichoderma viride* (Tv 11). Results indicated that among the fungicides tested, thiram (0.2%; Thiram®), copper oxychloride (0.2%; Cobox®) and mancozeb (0.2%; Milzeb M-45®) were found comparatively safer against *T. harzianum* and *T. viride* as compared to other fungicides. Orthocide (Captan®) exhibited intermediate effect on both species of *Trichoderma*. *Trichoderma* was most sensitive to benomyl (Benlate®), tebuconazole (Raxil®, and Folicur®), Vitavax® (carboxin 37.5% + thiram 37.5%), propiconazole (Tide®), chlorothalonil (Kavach®), and hexaconazole (Control total®). These seven fungicides effectively suppressed 100% growth of both *Trichoderma* spesies. *Trichoderma* was tolerant to all the pesticides and weedicides tested. None of the pesticides (imidachloroprid as Amida® 17.8 SL, chloropyriphos as Ankurban® 20%

EC, profenofos as Carina® 50% EC, and carbosulfan as Aayudh® 25% EC) and weedicides (glyphosate as Rulout® 41% SL, quizalofop-ethyl as Turga super® 5% EC, and fluchloralin as Basalin® 45% EC) inhibited the growth of *Trichoderma* above 10%. Among the botanicals tested, 10% fresh leaf extract of karanj leaves (*Pongamea pinnata*) inhibited 46.7 and 54.4% growth of *T. harzianum* and *T. viride*, respectively. Other all botanicals and cakes were found to be compatible with *Trichoderma*. Cumin leaves inhibited 34.1%, and 25.2% growth of *T. viride* and *T. harzianum*, respectively as compared to control. Another intresting thing observed that, neem (*Azadirachta indica*) oil (5%), neem leaves extract (10%), wild sorghum leaf extract (10%), neem cake, castor cake and mustard cake extract (10%) enhanced the growth of *Trichoderma*. This finding indicates that seed treatment or furrow applications of *Trichoderma* would be compatible with thiram, copper oxychloride, mancozeb, pesticides, weedicides, neem oil, neem leaves extract, wild sorghum leaves extract, neem cake, castor cake and mustard cake extracts for the integrated management of soil borne diseases of groundnut (Bagwan 2010). Bagwan (2010) showed that *Trichoderma* was very sensitive to chlorothalonil, and the strains of *Trichoderma* spp. tested could not grow in the presence of thiram. The active ingredient thiram belongs to the family of dithiocarbamates that are effective on many pathogenic fungi including *B. cinerea* (Hmouni et al. 2003). Dithiocarbamates inhibit a variety of enzymes, such as those of glycolysis (Ragsdale and Sisler 1991). Gaur and Sharma (2010) also integrated *T. viride*-1 or *T. harzianum* (TG-1) with metalaxyl and cymoxanil 8% + mancozeb 64% to control root rot in cotton. On the basis of present investigation it may be concluded that seed + seedlings + spraying of carbendazim + metalaxyl or captan + metalaxyl + *T. virens* or captan + metalaxyl + T. *viride* can be applied to manage the devastating wilt disease in *Capsicum*.

Ranganathswamy et al. (2012) evaluated eighteen selected fungicides for their compatibility to *Trichoderma* based on in vitro senstitivity of *T. harzianum* and *T. virens*. Observations on radial growth indicated that, carbendazim, benomyl, carboxin, propiconazole, hexaconazole, tricyclozole, tridemorph, chlorothalonil were incompatible with *Trichoderma* spp. showing 100% inhibition of radial growth at field concentration. While dinocap, copperoxy chloride, fosetyl-Al captan, thiram and metalaxyl were found to be least compatible showing more than 70% inhibition of radial growth. Bordeaux mixture, azoxystrobin and mancozeb were moderately compatible with radial growth inhibition in the range of 20–45%. Only wettable sulphur was found to be highly compatible with least inhibition of radial growth (2.2%) of test *Trichoderma* isolates (Ranganathswamy et al. 2012). Some insecticides viz., Ekalux® (quinolphos), Marshal® (carbosulfan), and Rocket® (profenophos + cypermetrin) inhibited the growth of *Trichoderma* spp. (Vinit Pratap Singh et al. 2012).

Nandeesha et al. (2013) reported that, out of four systemic and two non-systemic fungicides tested under in vitro for compatibility with potential bioagent, mancozeb was found highly compatible with *Trichoderma* spp. Therefore, they developed an integrated management strategy to manage the collar rot disease of groundnut under green house conditions, where a selected isolate of *Trichoderma* sp. (TAG-2, an

effective isolate compatible to mancozeb) was incorporated in soil at the rate of 8 g kg^{-1}, and mancozeb was used in order to treat seed at the rate of 2 g kg^{-1}. Out of 12 treatment combinations, this treatment not only resulted in the maximum disease reduction with the disease incidence of only 7.16%, but also in the maximum plant height (37.64 cm), root length (28.50 cm), maximum dry weight of groundnut shoot (6.84 g) and root (0.71 g) (Nandeesha et al. 2013). Nongmaithem (2015) tested two isolates of *Trichoderma* spp. in vitro for their compatibility with different concentrations of commonly used pesticide and their antagonistic activity against four pathogenic soils borne pathogens (*Fusarium oxysporum* f. sp. *lycopersici*, *Sclerotium oryzae*, *Rhizoctonia solani* and *Pythium ultimum*). Dual culture of pathogens and *Trichoderma* spp. revealed that IBT-II reduced the growth of *Fusarium oxysporum* f. sp. *lycopersici* by 30.05%, *Sclerotium oryzae* by 58.33%, *Rhizoctonia solani* by 47.92%, and *Pythium ultimum* by 59.17%. The pesticides tested at different concentrations of 0.1, 0.2 and 0.3% showed that a progressive increase in percent inhibition of radial growth in the bioagents was observed as the concentrations of pesticide increased. Fungicide was found to be more toxic than biopesticide. Biopesticide is found to be compatible at lower concentration i.e. at 0.1% as it showed percent inhibition of only 33.36, 38.00 and 38.22% at 24, 48 and 72 h period of incubation respectively. Fungicide was found to be incompatible with bioagents as it exhibited toxicity even at lower concentration. It showed maximum inhibition (100%) against isolate MT-4 at 0.1, 0.2 and 0.3% concentration at 24 and 48 h period of incubation while other isolate IBT-II, showed 87.44% inhibition at 0.3% at 72 h period of incubation. Biopesticide was found to be less toxic than fungicide which indicates the compatibility with bioagents tested. The present result will help delineate the possibility of combining biocontrol agents and agrochemical for use in integrated pest management approach (Nongmaithem 2015). Azadirachitin has less inhibitory growth effect as compared to chemical fungicide carbendazim. Least growth inhibition of 33.64% was recorded in *Trichoderma*. Azadirachtin resistant isolates of *Trichoderma* in this study may be used in integrated pest management (IPM). *Trichoderma* isolate not affected by azadirachtin might have a synergistic effect in controlling plant disease when both are used together at the same time and may serve as low monitory input in integrated plant disease management. Hence the isolate MT-4 and IBT-II which were less affected by Azadirachtin may be applied along with azadirachtin to manage disease and pest (Nongmaithem 2015). As some of *Trichoderma* species grow as weed molds on mushroom cultivation substrates and/parasitize edible mushrooms, therefore, the information on the differential behaviors of these fungi in their confrontation with various inorganic as well as organic chemicals would be of high value in practical mushroom cultivation. *Trichoderma* species, the causal agents of green mold disease, induce great losses in *Agaricus bisporus* farms. Fungicides are widely used to control mushroom diseases although green mold control is encumbered with difficulties. The aims of this study were, therefore, to research in vitro toxicity of several commercial fungicides to *Trichoderma* isolates originating from Serbian and Bosnia-Herzegovina farms, and to evaluate the effects of pH and light on their growth. The majority of isolates demonstrated optimal growth

at pH 5.0, and the rest at pH 6.0. A few isolates also grew well at pH 7. The weakest mycelial growth was noted at pH 8.0–9.0. Generally, light had an inhibitory effect on the growth of tested isolates. The isolates showed the highest susceptibility to chlorothalonil and carbendazim (ED_{50} less than 1 mg L^{-1}), and were less sensitive to iprodione (ED_{50} range of 0.84–6.72 mg L^{-1}), weakly resistant to thiophanate-methyl (ED_{50} = 3.75–24.13 mg L^{-1}), and resistant to trifloxystrobin (ED_{50} = 10.25–178.23 mg L^{-1}). Considering the toxicity of fungicides to *A. bisporus*, carbendazim showed the best selective toxicity (0.02), iprodione and chlorothalonil moderate (0.16), and thiophanate-methyl the lowest (1.24), while trifloxystrobin toxicity to *A. bisporus* was not tested because of its inefficiency against *Trichoderma* isolates (Kosanović et al. 2015).

Of a range of fungicides tested, tolclofos-methyl and captan gave the best control of rhizoctonia disease on heathers (Calluna vulgaris), caused by *Rhizoctonia solani* and binucleate *Rhizoctonia* spp., when applied at the rates recommended by the manufacturers. Reduced fungicide rates resulted in the loss of efficiency and increased rates caused phytotoxicity (Litterick et al. 1993). Elshahawy et al. (2016) investigated the in vitro compatibility of ten *Trichoderma* spp. isolates (three of *T. harzianum*, three of *T. viride*, one of *T. virens* and three of *Trichoderma* spp.) with seven fungicides viz., carbendazim, flutolanil, mancozeb, metalaxyl M + mancozeb, pencycuron, thiram + tolclofos-methyl and thiophanate-methyl under different concentrations ranged from 50 to 800 ppm using poisoned food technique. Results revealed that *Trichoderma* spp. isolates were compatible with thiophanate-methyl, mancozeb, metalalaxyl M + mancozeb, pencycuron and flutolanil. While it was incompatible with carbendazim and thiram + tolclofos-methyl. All *Trichoderma* spp. isolates had antagonistic effect against *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Sclerotinia sclerotiorum* by reducing the growth in agar assays. Significant differences were observed among pathogenic fungi in their response to fungicides. Carbendazim completely inhibited the radial growth of all pathogenic fungi even at 100 ppm, while other fungicides showed increased efficacy with the increase of concentration. Each of flutolanil, pencycuron and thiophanate-methyl when separately combined with *Trichoderma* spp. isolates reduced the growth of the tested soil borne pathogens in the ranges of 22.2–100%, 43.7–100% and 50.4–100%, compared to the reduction of 0.0–21.1%, 0.0–18.9% and 15.6–18.9% resulted by the same fungicides when used alone, respectively. Results suggested that the fungicides-*Trichoderma* spp. isolates combination may be effective in controlling soil borne pathogenic fungi than individual treatment and reduced the amount of fungicide used (Elshahawy et al. 2016). Khirallah et al. (2016) tested the compatibility between strains of *T. harzianum* (Tcomp, TH1 and TH3) and *T. viride* (TV1) and different active ingredients used as fungicides against seedborne and foliar diseases of some cultures under in vitro conditions. The activities of the formulations, Signum® WG (pyraclostrobin 6.7% + boscalid 26.7%), Switch® 62.5 WG (cyprodinil 37.5% + fludioxonil 25%), Teldor® 50 WG (fenhexamid 50% WG) and Frupica® 50 WP (mepanipyrim 50% WP) were null to low on the mycelial growth of the strains tested. The combination cyprodinil + fludioxonil had moderate effect

on mycelial growth of *Trichoderma* strains tested but showed good compatibility from the second week. However, only the TH1 strain gave a moderate compatibility on the conidia production whose germination was completely inhibited. The dual action of cyprodinil and fludioxonil is due to its containing active ingredients of two different anilinopyrimidines (cyprodinil) (Heye et al. 1994; Hilber and Hilber-Bodmer 1998) and phenylpyrroles (fludioxonil) (Gehmann et al. 1990; Nyfeler and Ackermann 1992) families. Biochemical studies have indicated that anilinopyrimidines inhibit methionine biosynthesis (Masner et al. 1994; Leroux 1996) through blockage of cystathionine-β-lyase (Rosslenbroich and Stuebler 2000). However, phenylpyrroles stimulate intracellular accumulation of glycerol and thereby blockage of fungal cell growth (Leroux 1996; Pillonel and Meyer 1997). The compatibility estimated on mycelial radial growth was moderate between the combination pyraclostrobin + boscalid and *Trichoderma* spp. (Khirallah et al. 2016). In the first week and become very important from a second week. This compatibility was moderate with conidia germination and low with the conidia production. Pyraclostrobin and boscalid belong respectively to the strobilurins and carboxamides chemical groups. The fungitoxic activity of strobilurins is exercised at the reduction site ubihydroquinone Qo, by blocking electron transfer between cytochrome b and c1 of the mitochondrial respiratory chain, by inhibiting the production of ATP (Becker et al. 1981; Von Jagow and Link 1986) and therefore the fungus growth (Zheng et al. 2000; Bartlett et al. 2002; Bahous et al. 2005). Caboxamides are inhibitors of complex II (Leroux 2003).

The compatibility pecentages were low to moderate the first week, respectively 35.9, 10.0, 28.7 and 34.4% for TH3 and 59.1, 32.8, 61.9 and 84.1% for Tcomp at the recommended doses 501, 375, 750 and 400 ppm. They become high during the second week respectively 88.1, 75.4 and 100%. Seven days after incubation, the compatibility of Tcomp and TH3 was moderate (51.4%) at 200 ppm of pyrimethanil and become important after 16 days, respectively 65.0 and 78.7%. 32 days after incubation, this fungicide showed a moderate activity at the recommended dose (800 ppm), the compatibility percentages varied between 33.3 and 68.5%. After the second week, the chlorothalonil at 500 ppm showed good compatibility with Tcomp and TV1 (51.9 and 48.2%) and all the strains tested except TH1 become moderately compatible (43.5–72.2%) with the different concentrations after 24 days of incubation. However, thiram was able to completely inhibit mycelial growth of the *Trichoderma* strains during 4 weeks. No compatibility was observed between them. After one month, the *Trichoderma* strains produced conidia in the presence of low concentrations of chlorothalonil (Clortosip® 75% WP) and in the recommended dose of fenhexamid (Teldor® 50 WG), showing compatibility respectively ranging from 50.5 to 89.7% and 51.5 to 97.1%. The conidia production of the tested strains was inhibited at the recommended doses of the other fungicides tested. However, thiram (Basultra® 80% WP) showed good compatibility at 500 and 666.6 ppm ranging from 45.5 to 84.9%. Similary, boscalid + Pyraclostrobin (Signum® WG: boscalid 26.7% + pyraclostrobin 6.7% WG) at 125.5 ppm and cyprodinil + fludioxanil (Switch® 62.5% WG: cyprodinil 37.5% + fludioxonil 25%) at 93.7 ppm were compatible respectively with TH3 and

TV1 (50.6–56.1%) and TH1-TH3 (90.4–79.3%). After 24 h of incubation, the *Trichoderma* conidia could germinate in the presence of different concentrations of mepanipyrim and fenhexamid showing compatibility respectively ranging from 45.0 to 98.0% and 37.5 to 97.1%. The Tcomp strain showed good ability to germinate in presence of low doses of thiram (500 ppm), chlorothalonil (375 ppm), boscalid + pyraclostrobin (125.2 ppm) and cyprodinil + fludioxonil (93.7 ppm) with respective compatibility percentages of 54.3, 54.2, 75.5 and 57.2% (Khirallah et al. 2016). Stains of *Trichoderma* spp. grew well in the presence of mepanipyrim, while conidia germination was moderately reduced, and the production of conidia was completely inhibited (Khirallah et al. 2016). Mepanipyrim has shown an excellent activity against *B. cinerea* (Nagata et al. 2004), *Venturia* spp. on grapes, vegetables, apples and pears, as well as *Monillinia fructicola* on fisheries (Maeno et al. 1990). The fungicide, 2-anilino-4-methyl-6-(1-propynyl) pyrimidine affect the intracellular transport of the proteins in the protein secretion process (Miura et al. 1994). This fungicide showed no significant phytotoxicity (Nagata et al. 2004). This fungicide tended to affect both mycelial growth and pectinase secretion of younger *B. cinerea* mycelia more strongly than older cultures, which is suggestive of its mode of action. Mepanipyrim was more effective in inhibiting pectinase secretion and in disease control activity against *B. cinerea* in the early stages of growth and infection (Miura and Maeno 2007). *Trichoderma* strains tested showed significant compatibility in the presence of fenhexamid (Khirallah et al. 2016). The pyrimethanil inhibited moderately mycelial growth and strongly production of conidia and germination of all strains of *Trichoderma* spp. tested (Khirallah et al. 2016).

As for chlorothalonil, *Trichoderma* strains (TH1 and TV1) have appeared very sensitive and two others (Tcomp and TH3) have proved moderate resistance to this active ingredient (Khirallah et al. 2016).

The incorporation of of fungicides into soil affects *Trichoderma* populations, and such population dynamics are expected to be of significant importance in the management of soil biology and maintainment of soil suppressiveness in suppressive soils, as well as in the gradual development of suppressive soils evolved from conducive soils. The population of indigenous *Trichoderma* was influenced by fungicide drenching. The population of *Trichoderma* in soils drenched with tridemorph (Calixin®) and propiconazole (Tilt®) was lower than in the untreated soil. However, the differences were only significant for propiconazole (Tilt®) at three and four months. Within the first four months, the population of *Trichoderma* in soils drenched with triadimefon (Bayleton®) was slightly lower in the first month, higher in the second and third month and lower in the fourth month as compared to the control. These differences were not significant. Dazomet and sulphur promoted population of *Trichoderma* but these differences were also not significant (Hashim and Chew 1997).

2.7.1 Trichoderma Asperellum Samuels, Lieckfeldt & Nirenberg

Conidia germination of *T. atroviride* (Fig. 33) was highly sensitive to Signum but *T. asperellum* was not sensitive to low concentrations of this fungicide (Shovan 2012). The conidia germination of *T. atroviride* and *T. asperellum* were moderately sensitive to switch (Shovan 2012).

Joeniarti et al. (2014) conducted a study in order to evaluate the tolerance of *Trichoderma asperellum* local isolates TK and TS from Batu-East Java, to mefenoxam fungicide, as well as to identify the in vitro antagonistic activity of *T. asperellum* against the plant pathogen *Phytophthora infestans* by using dual culture method. Thus, an integrated approach of chemical and biological methods was used to control the growth of *P. infestans*. *T. asperellum* isolates TK and TS were evaluated in vitro for their efficacy against *P. infestans* and tolerance to 5000 ppm mefenoxam. The results showed that the growth of the isolates of *T. asperellum* on potato dextrose agar with 5 mL/L mefenoxam was up to 67%, while their antagonistic activity against *P. infestans* in dual culture was 5.08% and 16.37%. The results were expected to help in selection of potential biocontrol agents (Joeniarti et al. 2014).

2.7.2 *Trichoderma* harzianum Rifai

Trichoderma harzianum (Fig. 34) is a fungal biocontrol agent that attacks a range of pathogenic fungi. It can be used either alone or in combination with other *Trichoderma* species in biological control of several plant diseases (Papavizas 1985; Chet 1987; Samuels 1996). A number fungicides have been tested for their compatibility with a most commonly used fungal antagonist namely, *Trichoderma* sp. of which many of them have been reported to be compatible with *T. hazianum*

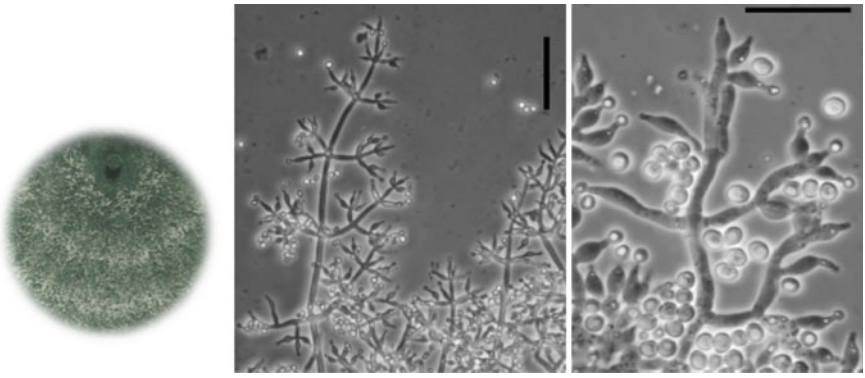

Fig. 33 *Trichoderma asperellum*. (Left) Growth and sporulation of the fungus on potato dextrose agar (*Source* Samuels et al. 2010); (Right) Conidiophores and conidia of the fungus, bar: 20 μm (*Source* Samuels et al. 2010)

(Desai and Schlosser 1993; Sharma et al. 2001). The effects of the herbicide propyzamide and five fungicides (benomyl, quintozene, vinclozolin, thiram and prothiocarb) on the colonisation of substrates by *T. harzianum* were investigated by Davet, and discussed with a view to practical applications (Davet 1981). Abd-El Moity et al. (1982) observed that the combination of iprodione and iprodione-tolerant isolate of *T. harzianum* gave significantly higher control of white rot of onion caused by *Sclerotium cepivorum* than did iprodione or *T. harzianum* alone. Field experiments evaluating three isolates of *T. harzianum* Rifai and a white spored *Trichoderma* sp. alone and in combination with fungicides and fungicide-insecticide/nematicide combinations were conducted during 1979–81 in an area with a history of high incidence of *Sclerotiurn rolfsii* Sacc. None of the *Trichoderma* sp. treatments alone, mixed with wheat middlings, or combined with carboxin reduced disease or increased yield over the control; however, disease was reduced when *T. harzianum* was applied with PCNB at 11.2 kg ha^{-1}. *Trichoderma* spp. appeared to be active only over a 3–8 day period, which was inadequate for control of *S. rolfsii* for the entire season. Treatments containing PCNB (11.2 kg a. i. ha^{-1}) alone or with the insecticide/nematicides ethoprop (3.4 kg a. i. ha^{-1}), fensulfothion (3.4 kg a. i. ha^{-1}), and aldicarb (1.7 kg a. i. ha^{-1}) significantly increased yields 9 of 12 times with an average increase of 790 kg ha^{-1}; and significantly reduced disease loci at harvest 5 of 12 times with an average reduction of 36%. Ethoprop 10G alone at 3.4 kg a. i. ha^{-1} increased yield one of 3 times, but did not reduce disease. Aldicarb and phenamiphos alone did not decrease disease or increase yield. Carboxin 4G decreased disease and increased yield only when applied at 1.12 kg a. i. ha^{-1} six times on an as required basis. Carboxin 3F at 0.84 kg a. i. ha^{-1} applied 6 times and carboxin 75W at 1.27 kg a. i. ha^{-1} applied one time did not increase yield or reduce disease at harvest (Csinos et al. 1983).

Khattabi et al. (2001) investigated the effect of three fungicides (benomyl, hymexazol, oxyquinoleine) on the viability of sclerotia of *Sclerotium rolfsii* in natural and sterilized soils. Also, they carried out a similar experiment in natural

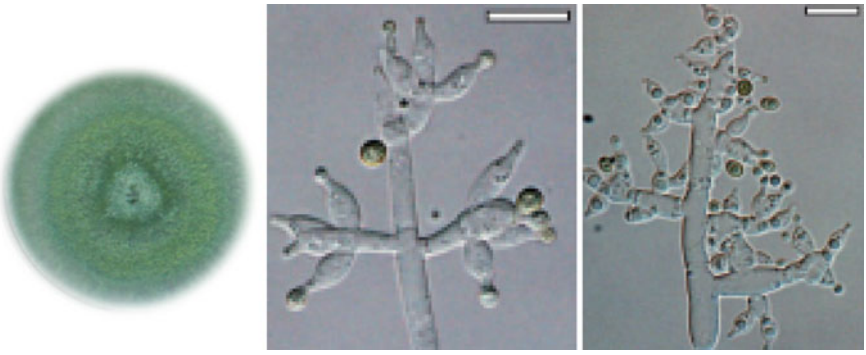

Fig. 34 *Trichoderma harzianum*. (Left) colony growth and sporulation on potato dextrose agar (*Source* Błaszczyk et al. 2014); (Right) Conidiophores and conidia of the fungus, bar = 10 μm (*Source* Jaklitsch 2009)

soil combining each of these fungicides with one of four isolates of *T. harzianum*. In addition, the mycelial growth of the *T. harzianum* isolates and *S. rolfsii* was monitored on agar media amended with these fungicides at three concentrations. Benomyl reduced the antagonistic ability of *T. harzianum* isolates in the soil, oxyquinoleine yielded variable results, while hymexazol improved the antagonism of *T. harzianum* isolates. In an agar medium, benomyl inhibited all *T. harzianum* isolates, as did oxyquinoleine. By contrast, hymexazol had only a negligible impact on the growth of the antagonist (Khattabi et al. 2001).

Mukhopadhyay and Kaur (1990), as well as Dubey (1997) recorded better control of disease through integration of bicontrol agents with fungicides than the individual application of either biocontrol agent or fungicide. This synergism might be due to partial suppression of the pathogen by the chemical without disturbing much the activity of the fungicide tolerant antagonist. Application of *T. harzianum* and soil drenching with 0.2% carbendazim reduced stem rot (Asghari and Mayee 1991). Muthamilan and Jeyarajan (1996) reported that *T. harizanum*, *Rhizobium* and carbendazim reduced the groundnut root rot caused by *Sclerotium rolfsii*. A glasshouse pot trial by McLean et al. (2001) confirmed that *T. harzianum*, an effective biocontrol agent of the onion white rot pathogen *Sclerotium cepivorum*, was sensitive to mancozeb. Mclean et al. (2001) testing spore germination in vitro indicated that *T. harzianum* was very sensitive to this fungicide. Parakhia and Akbari (2001) reported that pendimethalin, fluchloralin, butachlor, paraquat, 2,4-D and oxydiazon showed no adverse effect on radial growth of *T. harzianum*. Desai et al. (2002) also reported that mancozeb at 500 ppm recorded a lower inhibition of hyphae (5.70%) and sporulation (11.02%) of *T. harzianum*. The triazole fungicides hexaconazole, propiconazole and penconazole were found highly inhibitory against *T. harzianum* at various concentrations (Narayana and Srivastava 2003). On the other hand difenoconazole was found less inhibitory against *T. harzianum* (Cilliers et al. 2003). The triazole fungicides hexaconazole, propiconazole and penconazole were found highly inhibitory against *T. harzianum* at various concentrations (Narayana and Srivastava 2003). On the other hand difenoconazole was found less inhibitory against *T. harzianum* (Cilliers et al. 2003). Tiwari et al. (2004) studied non-target effect of insecticide on mycelial growth of *T. harzianum* and reported that chlorpyriphos and carbosulfan was highly inhibitory to the growth of *T. harzianum* while imidachloprid was found to be highly compatible at 500 and 1000 ppm concentrations.

T. harzianum showed growth on medium containing benomyl at 1 and 10 ppm but no growth was observed when benomyl was used at 100, 1000 and 10,000 ppm. In 1 and 10 ppm treatments, growth of biocontrol agent showed negative correlation with concentration of benomyl for up to 4 days but plates were filled on fifth day (Fig. 35). Topsin® M proved to be highly toxic and very little growth of *T. harzianum* was observed in treatments containing Topsin® M at 1 and 10 ppm. No growth was observed at 100 ppm or higher concentrations (Fig. 35).

Carbendazim was found to be more effective since it completely inhibited the growth of *T. harzianum* even at 10 ppm where very minute mycelial growth was observed after 3 days of incubation where carbendazim was used at 1 ppm

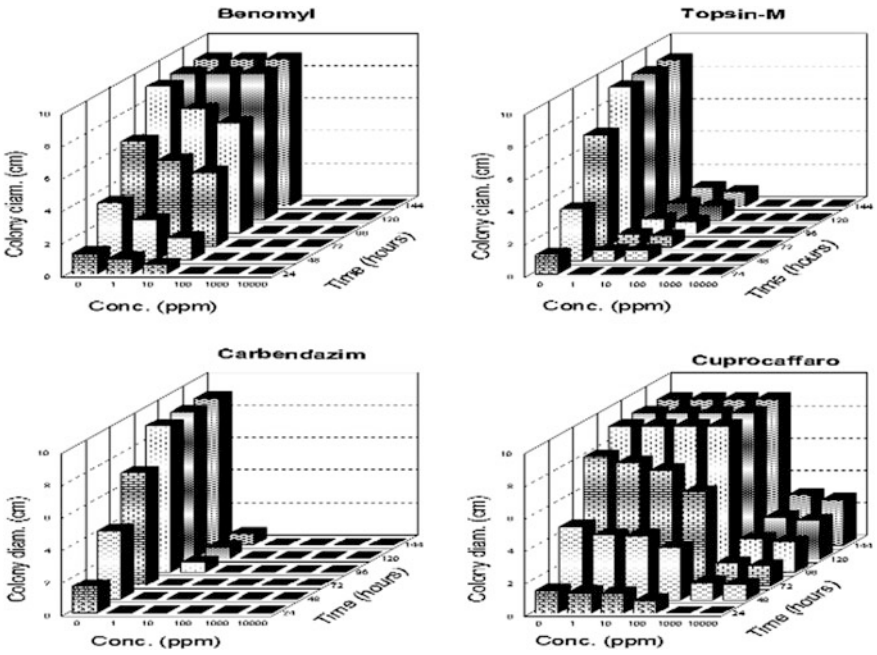

Fig. 35 Effect of fungicides on *in vitro* growth of *Trichoderma harzianum* (Khan and Shahzad 2007)

(Fig. 35). *T. harzianum* grew easily in medium containing Cuprocaffaro® at 1, 10 and 100 ppm and plates were filled on 4th day of incubation. Growth of *T. harzianum* was significantly reduced where Cuprocaffaro® was used at 1000 and 10,000 ppm (Fig. 35). A laboratory experiment was conducted to test the possibility of combining *T. harzianum* a potential biocontrol agent with fungicides commonly recommended viz., captan, thiram, carbendazim and thiophanate methyl for seed treatment against soil borne diseases. Maximum inhibition (100%) was recorded with carbendazim at 0.1 and 0.2% concentration at 24 h. incubation followed by 96.88 and 88.44% inhibition at 24 h. with thiophanate methyl at 0.1 and 0.2 per cent concentration, respectively. All the tested concentrations of captan and thiram at 0.1 and 0.2% concentration at 72 h. incubation period recorded less than 20% inhibition of *T. harzianum* (Gowdar et al. 2006). Fungicides viz., benomyl, Topsin® M, carbendazim and Cuprocaffro® were used at different concentrations i.e., 0, 1, 10, 100, 1000 and 10,000 ppm a. i. to evaluate *T. harzianum*, for tolerance to fungicides. Topsin® M and carbendazim were the most effective fungicides that inhibit the growth of *Trichoderma* species even at low concentration. Topsin® M completely suppressed the growth of *T. harzianum* at 10 ppm. Among the four *Trichoderma* species, the growth of *T. harzianum* was mostly inhibited by the fungicides except Cuprocaffaro®; the most effective fungicide was carbendazim followed by Topsin® M (Khan and Shahzad 2007).

An experiment was conducted to study the effect of antagonist *T. harzianum* and different fungicides for the control of guava decline (*Psidium guajava*) in vivo at the research area of Department of Plant Pathology University of Agriculture Faisalabad. Guava decline is a serious disease caused by different pathogens *Botryodiplodia theobromae*, *Fusarium oxysporum* f. sp. *psidii*, *Phytophthora parasitica* and *Fusarium solani* f. sp. *psidii*. The disease can be controlled effectively through antagonists but effectiveness increases when fungicides are used as soil drenching along with antagonists. This paper reports in vivo evaluation of fungal antagonist *T. harzianum* and the fungicides Topsin® M, Alert plus and Reconil-M for the control of guava decline. Various experiments were conducted to analyze the effectiveness of antagonist and fungicides for the control of disease. It was found that in control, where no fungicide and antagonist was added in soil, it showed ample disease intensity i.e. 24–57 and 44.15% in sterilized and unsterlized soil respectively. Statistical analysis revealed that disease intensity was zero and 0.74% when treated with *T. harzianum* and Topsin® M as drenches in sterilized and unsterlized soil and showed maximum control of disease (Bokhari et al. 2008).

Khalko and Pan (2009) found that propineb (70 WP), a member of the dithiocarbamate group of fungicides was compatible with *T. harzianum* and might be an easy selection for integrated disease management. The in vitro effect of certain fungicides, insecticides, and biopesticides on mycelial growth of *T. harzianum* was evaluated. Seven systemic fungicides, 2 contact fungicides, and 4 biopesticides were tested. A progressive increase in % inhibition of radial growth in the fungus was observed as the concentrations of the fungicides increased. Among the systemic fungicides, hexaconazole (Contaf® 5 EC) was the most toxic, followed by propiconazole (Tilt® 25 EC) and triflumizole (Procure® 30 WP). Toxicity of the contact fungicides was lower than that of the systemic fungicides, among which copper oxychloride (Fytolan® 50 WP) and copper hydroxide (Excel® 46.1% DF) were highly compatible; no inhibition was observed at lower concentrations. In terms of the insecticides tested, quinalphos (Ekalux® 20 EC) and dicofol (Ditol® 18.5 EC) exhibited toxicity (quinalphos more so), even at the low concentration of 10 ppm. Endosulfan (Endocel® 35 EC), fenpropathrin (Meothrin® 30 EC), and propargite (Omite® 57 EC) were less toxic, which indicated the compatibility of these insecticides with *T. harzianum*. A varying level of inhibition was observed in response to the biopesticides. Although neem-based biopesticides completely inhibited the growth of the biocontrol strain, ovis was compatible with the growth of *T. harzianum*. The present results will help delineate the possibility of combining *T. harzianum* biocontrol agents and agrochemicals for use in an integrated pest management approach (Sarkar et al. 2010). Bagwan (2010) indicated that thiram (0.2%) was compatible with *T. harzianum*, and mancozeb was comparatively safer against *T. harzianum*. Copper oxychloride was found as highly compatible with *T. harzanium* (Suseela and Thomas 2010).

Divya et al. (2011) showed that the wild strain of *T. harzianum* was insensitive to the half of the pyraclostrobin recommended dose. Tolerance of *Trichoderma* species collected from tea plantation to fungicides was evaluated in vitro using poisoned food method. Fungicides like carbendazim (50 WP), copper oxychloride

(50 WP), hexaconazole (5 EC), and propiconazole (25 EC) were used at two lower and two upper of recommended doses. The growth of *T. harzianum* was mostly inhibited by carbendazim (50 WP) and propiconazole (25 EC) with the recommended doses; while *T. viride* could grow easily with the said doses. Both species of *Trichoderma* grew easily in medium containing copper oxychloride even at highest doses. Hexaconazole (5 EC) proved to be highly toxic with no growth of *Trichoderma harzianum* in treatments containing 40.0 and 42.05 ppm. At highest concentration (42.5 ppm) of hexaconazole (5 EC), *T. viride* grew after 72 h of incubation. It was 14.44% over the control on 4th day of plating (Islam et al. 2011). Hilmida® (imidachloprid) was found to be most compatible with V1 strain of *Trichoderma harzianum* in both the liquid media as it showed nil percentage reduction of mycelium. It was concluded that Decis® (deltamethrin), Hilcron® (monocrotophos), Hilmida® (imidachloprid) and Rogar® (dimethoate) were compatible insecticides with *T. harzianum* (Shukla 2011).

The fungal antagonist, *T. harzianum* was used in the present study and poisoned food technique was adopted for evaluation with respect to nature of compatibility of four fungicides viz., propineb 70 WP, prochloraz 45 EC, difenoconazole 25 EC and flusilazole 40 EC at five concentrations (8.0, 16.0, 32.0, 64.0 and 128.0 ppm). All the fungicides were found to have inhibitory effect towards the antagonist except propineb 70 WP irrespective of its concentrations assayed. The antagonist showed little growth (21.00 mm) at 32.0 μL L^{-1} concentration against difenoconazole 25 EC, but no growth was recorded above this concentration. Prochloraz 45 EC and flusilazole 40 EC totally inhibited the growth of *T. harzianum* even at 8.0 μL L^{-1}. Difenoconazole 25 EC appeared to be partly tolerated by *T. harzianum* only at lower concentrations. Spore formation was delayed in 48 h in case of propineb 70 WP as compared to control. Although spore appeared in difenoconazole 25 EC much later than the appearance of spore in propineb 70 WP, most of the spores (phialospore) failed to germinate in 0.1% sugar solution. Both in the cases of propineb 70 WP and difenoconazole 25 EC initiations of numerous branches in germ tube at short intervals were noted (Ahmad et al. 2012). The combinations of antagonists and fungicides showed different performance. *T. harzianum* with Dithane M 45® showed better performance and reduced the *R. solani* growth by 76.1–100% (at 100, 200 and 300 mg L^{-1}) than check. This reduction was 52.9–100 and 65.5–100% when *T. harzianum* + Benlate® and *T. harzianum* + Ridomil® were applied (Ahmad et al. 2012).

The synergism mechanism of *T. harzianum* combined with boscalid to *Botrytis cinerea* determined by microscopy method, mycelium growth rate and disc filter method was showed by the mycelium partial dissolution of *B. cinerea*, the beneficial to *T. harzianum* to occupy nutrition and space and enhanced the antifungal activity of volatile compounds of *T. harzianum* to *B. cinerea* (Fangsheng et al. 2013). Seven systemic fungicides (tebuconazole as Folicur® 250 EC, tridemorph as Calixin® 80 EC, propiconazole as Tilt® 25 EC, hexaconazole as Contaf® 5 EC, triflumizole as Procure® 30 WP, bitertanol as Baycor® 25 WP, and azoxystrobin as Amistar® 25 SC) and 2 contact fungicides (copper oxychloride as Fytolan® 50 WP and copper hydroxide as Excel® 46.1% DF) were tested. In addition, 5 insecticides

(propargite as Omite® 57 EC, endosulfan as Endocel® 35 EC, fenpropathrin as Meothrin® 30 EC, dicofol as Ditol® 18.5 EC, and quinalphos as Ekalux® 20 EC) were evaluated. Among the biopesticides, 3 neem-based formulations viz. nimbecidine® (0.03%), ponneem® (0.05%), neem kernel aqueous extract (NKAE®), and a formulation containing a mixture of plant extracts (Ovis®) was tested at 2.5, 5, and 7.5% concentrations. The in vitro bioefficacy of the test compounds was determined using the poisoned food technique (Dhingra and Sinclair 1985). Inhibition of radial growth was measured based on control plate colony diameter using Sundar et al.'s formula (Sundar et al. 1995): Inhibition % = $[(X - Y)\ X^{-1}] \times 100$, where X is growth of the control plate and Y is growth of the treated plate. Among the systemic fungicides tested, hexaconazole was the most toxic to the growth of *T. harzianum*, followed by propiconazole and triflumizole at the lowest concentration (5 ppm) tested. At the 5 ppm concentration growth inhibition caused by hexaconazole-, propiconazole-, and triflumizoleamended medium was 87.7, 56.4, and 36.2%, respectively. No growth was observed at the 10-ppm level with hexaconazole, the 25-ppm level with propiconazole, or the 50 ppm level with triflumizole. The antifungal activity of hexaconazole and propiconazole (both of the triazole group of fungicides) have been reported to be the result of their ability to inhibit ergosterol biosynthesis in fungi (Anonymous 1985).

Mizuno (1988) reported the antifungal activity of triflumizole, which acts as a selective inhibitor of demethylation during ergosterol biosynthesis. A progressive increase in percent inhibition of radial growth in *T. harzianum* was observed as the concentration of all the fungicides increased. Although most of the systemic fungicides were able to completely suppress the growth of *T. harzianum* at the highest concentration (300 ppm) used in the present study, azoxystrobin and bitertanol were more compatible with the biocontrol strain. At the highest concentration of azoxystrobin and bitertanol, inhibition of the fungus was 41.1 and 71.3%, respectively. In the case of the contact fungicides, both copper oxychloride and copper hydroxide showed compatibility with *T. harzianum*. No inhibition of mycelial growth was noted at the 5 and 10 ppm levels of both chemicals. A gradual increase was observed in percent inhibition as the concentration increased, but inhibition was lower than that of the systemic fungicides. At the highest concentration inhibition was 60% and 33.1%, respectively, for the 2 chemicals. This can be explained in terms of the variation in sensitivity of the test fungus to the fungicides (Nene and Thapliyal 1993). Earlier reports suggest that biocontrol agents that can tolerate a certain level of fungicides were mixed with agrochemicals, resulting in eradication of diseases (De Cal et al. 1994). In terms of the insecticides tested in the present study, quinalphos exhibited the highest toxicity, followed by dicofol at the lowest concentration; at the 10 ppm level the percent inhibition of mycelial growth was 17.5 and 11.3%, respectively. No inhibition was observed in response to the other pesticides at this concentration. Although a gradual increase in inhibition was observed as the concentration of pesticides increased, none of the chemicals completely suppressed the growth of the fungus, even at the highest concentration, except dicofol. At the 300 ppm level dicofol completely suppressed fungal growth, followed by quinalphos (75.5%). Other pesticides, viz. endosulfan, fenpropathrin,

and propargite, exhibited a lesser degree of toxicity towards *T. harzianum*, which indicates their compatibility with the test fungus. The compatibility of endosulfan with beneficial fungi was reported earlier (Isaiah et al. 2005). The biopesticides tested in this study exhibited varying levels of inhibition on the growth of *T. harzianum*. The results indicate that neem-based biopesticides (nimbecidine and ponneem) were incompatible with *T. harzianum*. Both biopesticides completely inhibited growth at the 5% concentration. On the other hand, lower sensitivity to the formulation containing other plant extracts (Ovis) was observed. At the 5% concentration the percent inhibition was only 13.1%. Neem kernel aqueous extract (NKAE) exhibited 80.8% inhibition at the 5% level, which may have been due to the azadirachtin present in the kernels (Harlapur et al. 2007). High antimicrobial activity with extracts of different parts of neem has been reported (Biswas et al. 2002). In conclusion, the present study reported the in vitro influence of some common agrochemicals and biopesticides used on tea plantations on the growth of *T. harzianum*. Data on combining agrochemicals and biocontrol agents for developing an integrated approach to pest and disease management on tea plantations are limited. The current findings will provide base-level data in this context. Further research is needed to evaluate the practical application of these chemicals and biopesticides with *T. harzianum* biocontrol agents in the field.

The effect of *T. harzianum* and *T. viride* and three fungicides i.e. Benlate®, Ridomil® and Dithane M-45®, was investigated on the management of fusarium root rot in okra under screen house conditions. The disease incidence and percent mortality were significantly reduced ($p \leq 0.05$) by all the fungicides and antagonists when compared with untreated check plants. *T. harzianum* and Ridomil® increased the yield by 83.6 and 80.2%, respectively. Under in vitro study, Dithane M-45® proved to be more effective than Ridomil® and Benlate® used alone or integrated with any of the antagonists. Maximum colony diameter of the pathogen (6.9 cm) was recorded in control treatment. *T. viride* was less effective when used alone or with any fungicide, while *T. harzianum* reduced the colony diameter by 43.5% under in vitro (Ahmad et al. 2012).

Green mold (*Trichoderma* spp., Figure 36) is a devastating disease in the crop production of mushrooms. In India, it has been reported to cause serious crop losses. It is also common contaminant, occurring in mushroom houses in the Kashmir valley. Shah et al. (2013) investigated the in vitro and in vivo efficacy of fungicides against Green mold (*T. harzianum*) associated with the cultivation of *Pleurotus sajor-caju*. *P. sajor-caju* is the third most commercially important edible mushroom worldwide. Five fungicides namely carbendazim, bitertanol (Baycor®), hexaconazole (Anvil®), captan (Captain®) and mancozeb (Dithane M-45®) were evaluated in vitro against Green mold (*T. harzianum*) and against the mushroom mycelium as well, by following Poison food technique. The results revealed that the maximum average inhibition of *T. harzianum* was recorded in carbendazim (90.8%), followed by bitertanol (40.0%), captan (36.6%) and hexaconazole (16.1%). The least inhibition (11.7%) of *T. harzianum* was exhibited by mancozeb. It was further observed that carbendazim exhibited the least inhibition (24.9%) of *P. sajor-caju*, followed by captan (45.5%), bitertanol (63.0%) and hexaconazole

(74.5%). The maximum inhibition (87.4%) of *P. sajor-caju* was exhibited by mancozeb. Fungicides showing maximum efficacy against the pathogen (*T. harzianum*) and minimum efficacy against mushroom (*P. sajor-caju*) were further tested against *T. harzianum* during an in vivo test in mushroom house. It was observed that all tested fungicides reduced the disease intensity of *T. harzianum* and produced more yield than control polybags. Maximum increase in yield (36.9%) over control and minimum mean disease incidence (9.3%) was recorded in treatment which received carbendazim (Bavistin®) as the fungicide. carbendazim (Bavistin®) was found to be best fungicide, against the infection of Green mold (*Trichoderma* spp.) disease of mushrooms (Shah et al. 2013). The maximum inhibition of *T. harzianum* (56.2%) by bitertanol was recorded at the highest concentration of 1000 µg mL^{-1}, while the percent inhibition of *P. sajor-caju* by this fungicide ranged from 52.1% at the lowest concentration (25 µg mL^{-1}), and 71.1% at the highest concentration (1000 µg mL^{-1}). The maximum inhibition of *T. harzianum* (35.5%) by hexaconazole was recorded at 1000 µg mL^{-1} concentration. *T. harzianum* remained unaffected at the lowest dose of hexaconazole (25 µg mL^{-1}). Hexaconazole inhibited mycelial growth of *P. sajor-caju* by 63.6–85.1%. There was a significant difference between the concentrations of fungicides in inhibiting the radial growth of both *T. harzianum*, and *P. sajor-caju*, i.e. with the increase in concentration, the percent inhibition also increased. It was also observed that the interaction between the treatment (fungicide) and concentration was recorded as significant in case of *T. harzianum*, but there was no significant difference in the interaction between the fungicide and concentration in the case of *P. sajor-caju* (Shah et al. 2013). In vitro evaluation of the fungicides against both *T. harzianum* and *P. sajor-caju* was carried out. Among the systemic fungicides tested by food poisoning technique, carbendazim exhibited the maximum inhibition of *T. harzianum*, followed by bitertanol. The least inhibition of mycelial growth of *T. harzianum* was expressed by hexaconazole. Carbendazim exhibited minimum efficacy against mushroom, indicating that *P. sajor-caju* was slightly resistant to this fungicide. Among the two non-systemic fungicides tested, captan was found to be effective against *T. harzianum*. The minimum inhibition against *T. harzianum* was exhibited by mancozeb and it showed the maximum inhibition of mushroom mycelium. Out of five fungitoxicants tested in vitro, carbendazim, bitertanol, and captan were further assessed for in vitro trial in mushroom house. The selected fungicides expressed the minimum inhibition of mushroom mycelium but strong inhibition of *T. harzianum* as compared to the other fungicides. Carbendazim was the most inhibiting against *T. harzianum*, but it expressed the least inhibitory potential against *P. sajor-caju* (Shah et al. 2013).

Shandliya and Guloria (1984) reported that carbendazim was the most effective fungicide for the treatment of green mold. Jhune et al. (1990) reported that maximum green mold control was obtained when 2 or 5 g m^{-2} of thiabendazole was applied to the substrate before pasteurization. Grogan et al. (1996) reported that carbendazim applied to spawn grains gave the best control of *T. harzianum*, responsible for serious yield reductions of *Agaricus bisporus*. The species pathogenic on *Pleurotus* spp. and *Agaricus* spp., are now considered as three different

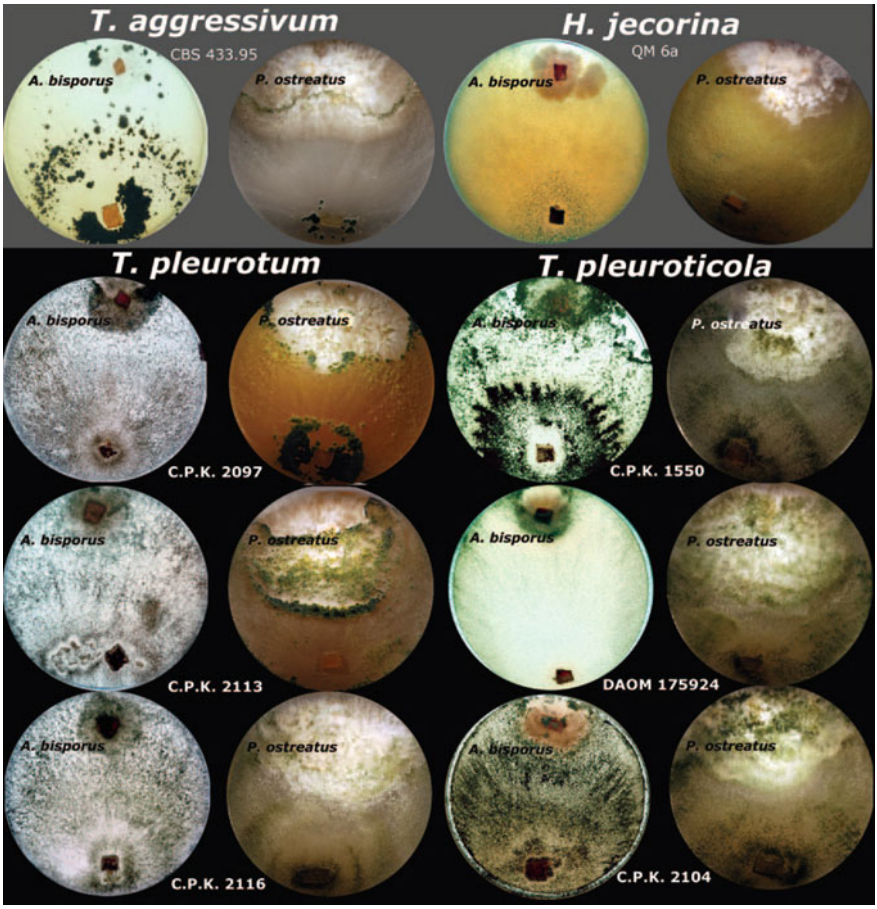

Fig. 36 Dual confrontation assay between cultures of *Agaricus bisporus* and *Pleurotus ostreatus* and mushroom green mold species observed after 10 days of incubation on potato dextrose agar. *Hypocrea jecorina*/*Trichoderma reesei* QM 6a was used as a negative control. *T. pleurotum*, and *T. pleuroticola* are found on the cultivated *Pleurotus* and its substrate, and the latter has also been isolated from soil and wood, and displayes pachybasium-like morphological characteristics typical of its neighbors in Harzianum clade, whereas *T. pleurotum* is characterized by a gliocladium-like conidiophore morphology uncharacteristic of Harzianum clade. Both species are of antagonistic activity against *Agaricus* equal to that of another member of Harzianum clade and the causative agent of green mold disease of *Agaricus*, *T. aggressivum*. *Source* Komoń-Zelazowska et al. (2007)

species, *T. aggressivum*, *T. pleuroticum*, and *T. pleuroticola*, all of which are members of Harzianum clade (Komoń-Zelazowska et al. 2007; Samuels et al. 2002).

Efficacy of eight different carrier materials and their combinations were tested to formulate a suitable *T. harzianum* based bio-fungicides for controlling foot and root rot diseases of brinjal caused by *S. rolfsii* in tray soil as well as seed bed soil under

net house condition of Bangladesh Agricultural Research Institute (BARI). The results from a series of experiments revealed that four combination of carrier materials based *T. harzianum* bio-fungicides such as (1) wheat bran + rice bran, (2) wheat bran + mustard oil cake (MOC) + rice bran, (3) khesari bran + rice bran, and (4) khesari bran + MOC + rice bran were suitable for controlling the soil borne foot and root rot disease (*S. rolfsii*) of brinjal in tray soil as well as seed bed soil conditions (Faruk et al. 2014a). Eight different organic matters were tested for their suitability as carrier materials to prepare *T. harzianum* based bio-fungicides for controlling foot and root rot disease of tomato caused by *Sclerotium rolfsii*. Four independent experiments were conducted and found that the carrier materials used singly or in combinations were suitable to prepare the bio-fungicides. Mixed use of carrier materials gave better results as compared to single ones. When wheat bran + rice bran, wheat bran + mustard oil cake (MOC) + rice bran, grasspea bran + rice bran, and grasspea bran + MOC + rice bran were used as carrier materials. *T. harzianum* based bio-fungicides reduced seedling mortality of tomato by 20.33, 19.33, 24.33, and 19.34%, respectively. Treatment of soil with those bio-fungicides previously infested with *S. rolfsii* caused considerable increased in shoot and root growth of tomato. Based on the findings of investigation, the above mentioned carrier materials might be used to prepare *T. harzianum* based bio-fungicides (Faruk et al. 2014b).

The in vitro effect of some commonly used fungicides, insecticides and herbicides on the mycelial growth of *T. harzianum* PBT 23 were evaluated taking four concentrations of all agrochemicals under study (25, 50, 75, and 100 µg a. i. mL^{-1}). Nine fungicides, 6 insecticides and 7 herbicides were tested. A progressive increase in percent inhibition of radial growth in the fungus was observed as the concentrations of the fungicides increased. Among fungicides, Captaf® (50 WP), thiram (75 WP), chlorothalonil (75 WP) and copper hydroxide (46.1 WP) were found compatible with the test antagonist up to 100 µg a. i. mL^{-1}, while mancozeb (75 WP) up to 250 µg a. i. mL^{-1}, as these did not adversely affect the growth of test antagonist. However, benomyl (50 WP), thiophanate methyl (70 WP), Bayleton (25 WP) and ipridione (50 WP) were found incompatible with the test antagonist even at 25 µg a. i. mL^{-1}.

Among insecticides, monocrotophos (36 SC), dichlorvos (76 EC), profenophos (50 EC) and triazophos (40 EC) were found compatible up to 250 µl a. i. mL^{-1}; while deltamethrin (2.8 EC) and quinalphos (25 EC) up to 100 µl a. i. mL^{-1}. Seven herbicides, 2,4-D ethyl ester (38 EC), pretilachlor (50 EC), aniliofos (30 EC), alachlor (50 EC), butachlor (50 EC), fluchloralin (45 EC) and pendimethalin (30 EC) were found compatible with the test antagonist even at higher concentration (250 µl a. i. mL^{-1}). The present results would be helpful to delineate the possibility of combining *T. harzianum* PBT 23 as biocontrol agent with agrochemicals under integrated pest management approaches (Saxena et al. 2014). The effects of several pesticides on *T. harzianum* were studied using the growth rate and spore germination test methods. The results suggested that the nine pesticides tested at different concentrations showed different controlling effects on the mycelial growth and spore germination of *T. harzianum*. Phosalone, amitraz and ethalfluralin

showed the maximum inhibition of spore germination and the lowest colony growth rate was observed in the presence of ethalfluralin, amitraz, and malathion pesticides. The efficacy of the pesticides on the inhibition of the mycelial growth and spore germination showed that they were both reduced with the increasing concentration of the insecticide. The results showed that the chemical control of pests during the growing season may be greatly reducing the population of the biocontroler (Mohammadi and Amini 2015). Commercial formulations of ethalfluralin and amitraz reduced the mycelial growth and spore germination of *Trichoderma* even at the lowest concentration (Figs. 37 and 38). Ethalfluralin effectively controls foxtail, barnyardgrass, fall panicum, crabgrass, pigweed, kochia, and black nightshade when applied prior to planting or to the plant seedlings (Thriveni et al. 2009). In the presence of Ethalfluralin, spore germination zeroed and the growth rate of the *Trichoderma* colony was less than 1 cm per week. We found that the Ethalfluralin not only controlled the weeds, it also suppressed the *Trichoderma* growth in the fields (Figs. 37 and 38). Amitraz is an insect repellent, insecticide and a pesticide synergist. Its effectiveness is traced back to the alpha-adrenergic agonist activity, interaction with octopamine receptors of the central nervous system and inhibition of the monoamine oxidases and prostaglandin synthesis (Lee et al. 2013). Amitraz inhibited spore germination completely at the all of the concentrations tested and the growth rate of the *Trichoderma* colonies was reduced to below 2 mm day^{-1} (Figs. 37 and 38). Based on the results of this experiment, the application of amitraz reduces the *Trichoderma* populations in the agricultural ecosystems and acts as a biocontrol inhibitor. Phosalone, a phosphorodithioate acaricide and insecticide, is used to control the various insect species in/on almonds, apples, apricots, cherries, grapes, peaches, pears and plums (Colinese and Terry 1968). Phosalone inhibited spore germination in all the concentrations tested and significantly ($P < 0.05$) reduced the *T. harzianum* growth when pesticide-amended treatments were compared with the control (Figs. 37 and 38). Thiodicarb acts as an insecticide against the major lepidopterous pests, and suppresses the coleopterous and some hemipterous insect pests. Thiodicarb is used primarily on cotton, sweet corn and soybeans (Ammon et al. 1995). Thiodicarb significantly ($P < 0.05$) reduced spore germination in *T. harzianum* at 2000 and 2500 ppm concentrations when compared with the control treatment (Figs. 37 and 38). No significant differences were observed in the *T. harzianum* mycelial growth between concentrations of 1500, 2000 ppm of Thiodicarb (Figs. 37 and 38). Haloxyfop is in the pyridine chemical family and is used as a pre- and post-emergent selective herbicide. Haloxyfop controls the annual and perennial grasses in sugar beet, oilseed, potatoes, leaf vegetables, onions, sunflowers, strawberries and other crops (Zhang et al. 2004). Haloxyfop inhibited spore germination at 2000 ppm completely and significantly ($P < 0.05$) the control spore germination and colony growth of *T. harzianum* at other concentrations (Figs. 37 and 38). Fenpropathrin is a highly effective, broad-spectrum insecticide and acaricide of synthetic pyrethroids. The widely used insecticide fenpropathrin in agriculture has become a public concern because of its heavy environmental contamination and toxic effects on mammals (Khazanchi and Handa 1989). No inhibition of *T. harzianum* spore germination and mycelial

growth was observed with 2000–2500 ppm of fenpropathrin and there was no significant difference between the control and fenpropathrin pesticide treatment (Figs. 37 and 38). Chlorpyrifos is a broad spectrum organophosphate insecticide and acts by interfering with the activities of cholinesterase. It is used as an insecticide on grain, cotton, field, fruit, nut and vegetable crops, and well as on lawns and ornamental plants (Domagalski and Munday 2003). Chlorpyrifos significantly ($P < 0.05$) reduced the spore germination and growth of *T. harzianum* mycelia at different concentrations when compared with the control treatment and conidial germination was inhibited 100% at 3000 ppm concentration of this pesticide (Figs. 37 and 38). Malathion is an organophosphate pesticide that is used to kill insects on agricultural crops, stored products, in home gardens as well as to kill mosquitoes and Mediterranean fruit flies in large outdoor areas (Russell-Manning 1991). *Trichoderma* growth in media amended with Malathion decreased for all test rates and spore germination was reduced at 30–85 when Malathion was used in the 2500–3000 ppm concentration (Figs. 37 and 38). Binapacryl, a member of the dinitrophenol family, acts as a contact miticide with ovicidal action and as a fungicide against powdery mildews (Unger 1996). Binapacryl did not show significant statistical differences, regarding the control of the *Trichoderma* growth germination throughout the experiment, but a reduction in the spore germination of *Trichoderma* was observed at 2000 mg L^{-1} of the fungicide rate (Figs. 37 and 38). In vitro effect of four fungicides on mycelial growth of *T. harzianum* was evaluated. Among the systemic fungicides, myclobutanil was the more toxic, followed by cymoxanil. Toxicity of the contact fungicides was lower than that of the systemic fungicides, among which copper oxychloride and sulphur were highly incompatible, no inhibition was observed at lower concentrations. The present results will help to delineate the possibility of combining *T. harzianum* biocontrol agents and agrochemicals for use in integrated pest management approach (Bhosale and Borade 2015). Sensitivity test of *T. harzianum* against systemic fungicides revealed metalaxyl MZ (Ridomil MZ® 72 WP) as the safer fungicide for mycelial growth that showed 42.59% growth inhibition with ED_{50} value of 2553 µg mL^{-1}, followed by triadimefon (Bayleton® 50 WP) 0.1% of recorded ED_{50} value of 1531 µg mL^{-1}. Whereas, benomyl (Benlate® 50 WP), penconazole (Topas® 10 EC), propiconazole (Tilt® 25 EL) and prochloraz (Octare® 50 WP) were proved highly toxic for mycelial growth with ED_{50} values of 1, 11, 29, and 34 µg mL^{-1}, respectively. Among the fungicides metalaxyl MZ showed no inhibition to the bioagent up to 50 µg mL^{-1} and little inhibition (<10%) up to 250 µg mL^{-1}. However, it grow safely up to 2000 µg mL^{-1} with radial growth of 51.67 mm and growth inhibition of 42.59%. Similarly, triadimefon was found a little inhibitory in comparison to metalaxyl MZ, wherein the bioagent growth of 57.330 mm and growth inhibition of 36.30% was recorded at 1000 µg mL^{-1}. However, propiconazole and prochloraz were next in order of the toxicity to bioagent is at 25 µg mL^{-1} both showed 58.33 and 50.67 mm radial growth and 35.19 and 43.70% growth inhibition, respectively. Among the fungicides tested, benomyl was found to be most toxic as no no growth of bioagents was observed at any concentration tested. This was followed by penconazole wherein 11.33 mm mycelia growth with 87.41% growth inhibition

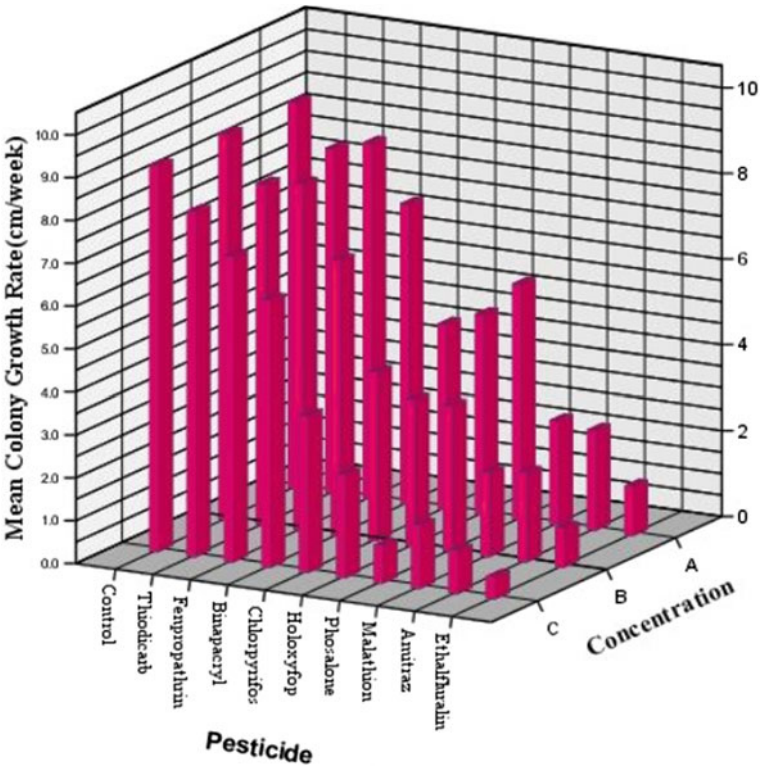

Fig. 37 Effect of pesticide on growth of *Trichoderma harzianum* colonies (Mohammadi and Amini 2015)

was obtained at 25 μg mL^{-1} (Sushir et al. 2015). Khirallah et al. (2016) demonstrated the compatibility of *Trichoderma* strains with the tested fungicides. Thus, Tcomp *T. harzianum* strain showed high compatibility both for the mycelial growth and for the germination of the conidia in the presence of most active ingredients. Their data could be applied as part of an integrated strategy against *B. cinerea* by *T. harzianum* with compatible fungicides.

The fungal antagonist, *Trichoderma harzianum* was used in the present study and poisoned food technique was adopted for evaluation with respect to nature of compatibility of four fungicides viz., propineb 70 WP, prochloraz 45 EC, difenoconazole 25 EC and flusilazole 40 EC at five concentrations (8.0, 16.0, 32.0, 64.0 and 128.0 ppm). All the fungicides were found to have inhibitory effect towards the antagonist except propineb 70 WP irrespective of its concentrations assayed (Fig. 37 and 38). The antagonist showed little growth (21.00 mm) at 32.0 μL L^{-1} concentration against difenoconazole 25 EC, but no growth was recorded above this concentration. Prochloraz 45 EC and flusilazole 40 EC totally inhibited the growth of *T. harzianum* even at 8.0 μL L^{-1}. Difenoconazole 25 EC appeared to be partly

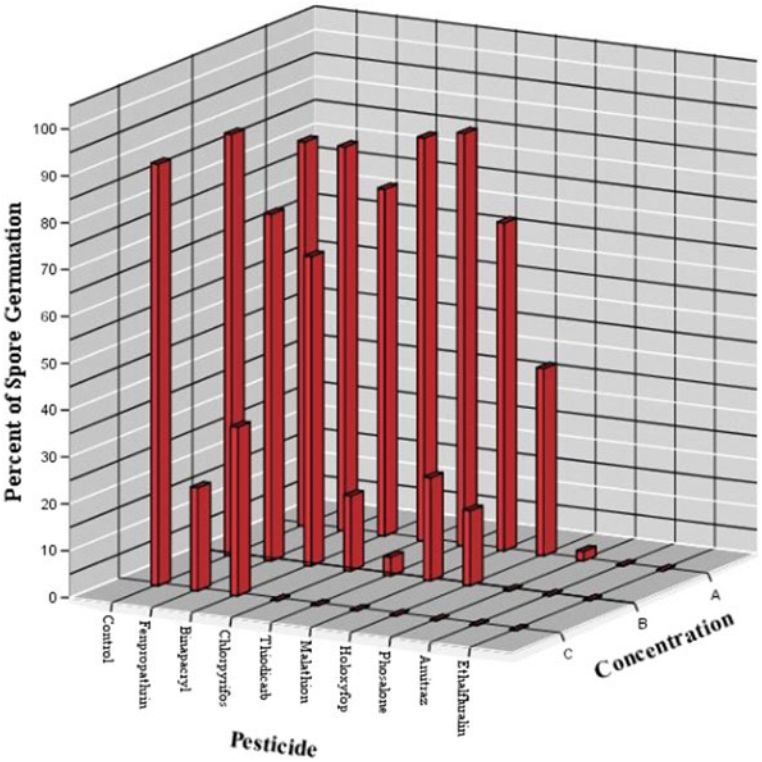

Fig. 38 Effect of pesticide on spore germination of *Trichoderma harzianum* (Mohammadi and Amini 2015)

tolerated by *T. harzianum* only at lower concentrations. Spore formation was delayed in 48 h in case of propineb 70 WP as compared to control. Although spore appeared in difenoconazole 25 EC much later than the appearance of spore in propineb 70 WP, most of the spores (phialospore) failed to germinate in 0.1% sugar solution. Both in the cases of propineb 70 WP and difenoconazole 25 EC initiations of numerous branches in germ tube at short intervals were noted.

2.7.3 *Trichoderma koningii* Oudemans

Singh et al. (1995) investigated the compatibility of *T. koningii* (Fig. 39) with Captaf® 500 ppm, Dithane M-45® at 500 ppm and Thiram® at 200 ppm, and found that the fungicides were compatible to *T. koningii*. Hashim and Chew (1997) studied the effects of integration of fungicide with *T. koningii* on the control of white root disease of hevea rubber. Control of white root disease with *Trichoderma* was erratic and not persistent. Similarly, the effects of *Trichoderma* on infected

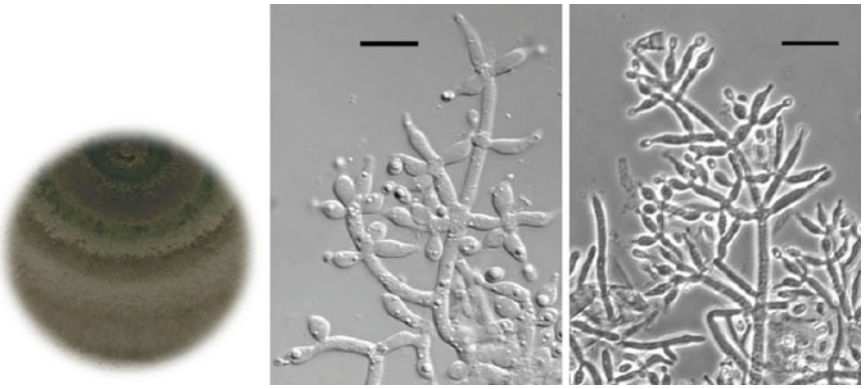

Fig. 39 *Trichoderma koningii*. (Left) colony growth and sporulation on potato dextrose agar 96 h after inoculation (*Source* Samuels et al. 2006); (Right) Conidiophores and conidia, bar = 10 μm (*Source* Samuels et al. 2006)

nursery plants drenched once with fungicides were also inconsistent. The addition of *Trichoderma* to plants in field plantings, which had been twice drenched with fungicides, did not improve control. In fact, addition of *Trichoderma* reduced control by propiconazole (Tilt®).

Triadimefon (Bayleton®) and propiconazole (Tilt®) were effective for the control of white root disease. Triadimefon (Bayleton®) was less toxic to *Trichoderma* than tridemorph (Calixin®), propiconazole (Tilt®), penconazole (Topers®) and cyproconazole (Alto®). At 10 mg L^{-1}, triadimefon (Bayleton®) inhibited radial growth by 10.6% while the % inhibition by the other fungicides was 71.6% for penconazole (Topers®) and 81.2, 91.2 and 86.4% for tridemorph (Bayleton®), propiconazole (Tilt®) and cyproconazole (Alto®), respectively. At 100 mg L^{-1}, triadimefon (Bayleton®) inhibited radial growth by 46.6% while the other fungicides totally inhibited growth.

2.7.4 Trichoderma Longibrachiatum Rifai

Complete inhibition of growth of *T. longibrachiatum* (Fig. 40) was observed where benomyl was used at 10,000 ppm. No growth was observed after first 48 h of incubation in benomyl at 10 and 100 ppm treatments. In 1000 ppm treatment, the growth of *T. longibrachiatum* started after 96 h of incubation. Plates were filled in benomyl at 1 ppm treatment after 6 days of incubation, however, the growth of *T. longibrachiatum* suppressed significantly where benomyl was used at 10 ppm or more (Fig. 41). Topsin® M at 10,000 ppm completely prevented the growth of *T. longibrachiatum*. Growth started after 72 h of incubation where Topsin® M was used at 1000 ppm treatment and after 48 h in 100 ppm treatment. Plates were filled after 5 days of incubation at 1 ppm treatment. The only significant suppression in

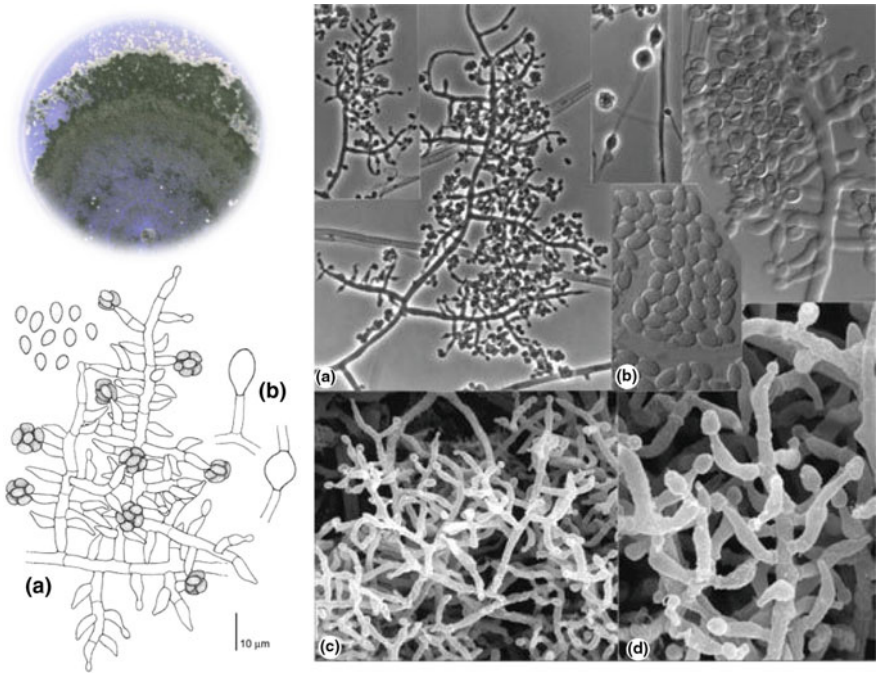

Fig. 40 *Trichoderma longibrachiatum*. (Left) colony growth and sporulation on potato dextrose agar (*Source* Choi et al. 2010), and **a** a schematic presentation of the fungus conidiophores and conidia, and **b** chlamydospores (*Source* http://www.mycobank.org/); (Right) Micrographs of conidiophores (**a**, **c**, **d**) and conidia (**b**) (*Source* http://www.mycobank.org/)

growth of *T. longibrachiatum* was observed where Topsin® M was used at 1000 and 10,000 ppm (Fig. 41). Growth of *T. longibrachiatum* was completely suppressed where carbendazim was used at 10,000 ppm. There was a negative correlation between the growth of *T. longibrachiatum* and the concentration of carbendazim. The plates were not filled after 144 h even in 1 ppm treatment (Fig. 41).

There was a sharp decline in growth of *T. longibrachiatum* where Cuprocaffaro® was used at 1000 and 10,000 ppm. Growth started after 24 h in 1000 ppm and after 48 h in 10,000 ppm treatments. Growth of *T. longibrachiatum* was not inhibited where Cuprocaffaro® was used at 1, 10 and 100 ppm and plates were filled after 72 h of incubation (Fig. 41).

Fungicides viz., benomyl, Topsin® M, carbendazim and cuprocaffro were used at different concentrations i.e., 0, 1, 10, 100, 1000 and 10,000 ppm a. i. to evaluate *T. longibrachiatum* for tolerance to fungicides. Topsin® M and carbendazim were the most effective fungicides that inhibit the growth of *Trichoderma* species even at low concentration. Except benomyl which inhibited the growth of *T. longibrachiatum*, no other fungicide was able to suppress the growth of this fungus (Khan and Shahzad 2007).

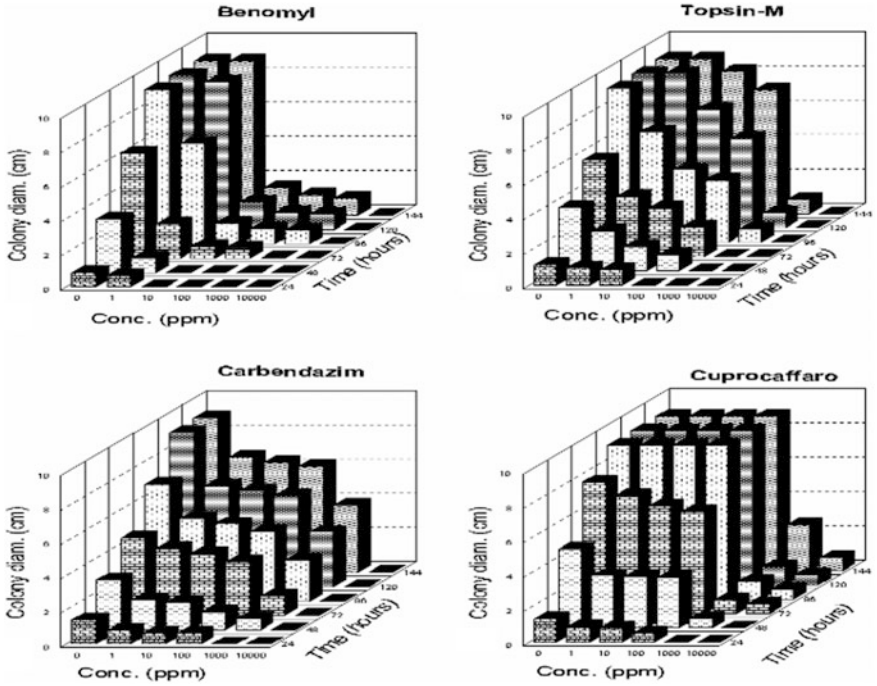

Fig. 41 Effect of fungicides on in vitro growth of *Trichoderma longibrachiatum* (Khan and Shahzad 2007)

2.7.5 *Trichoderma pseudokoningii* Rifai

A negative correlation was observed between concentration of benomyl and the growth of *T. pseudokoningii* (Fig. 42) for up to 3 days of incubation. Growth in 100 and 1000 ppm treatments started after 48 h and in 10,000 ppm treatment after 72 h of incubation. Growth of the fungus in plates containing benomyl at 10 ppm or higher concentration was significantly less than the control even after 6 days of incubation (Fig. 43). Concentration of Topsin® M showed a negative correlation with growth of *T. pseudokoningii* during 6 days of incubation. Growth in 10 and 100 ppm treatments started after 48 h, in 1000 ppm after 72 h and in 10,000 ppm after 96 h of incubation. Suppression in growth was less in 1 ppm treatment but use of Topsin® M at 10 ppm or more showed significant suppression in growth of *T. pseudokoningii* (Fig. 43). No growth of *T. pseudokoningii* was observed in carbendazim at 10,000 ppm treatment. Growth in 10 ppm started after 48 h and in 100 and 1000 ppm after 72 h of incubation. A significant negative correlation between the growth of *T. pseudokoningii* and the concentration of carbendazim was evident during 6 days of incubation but effect in 10, 100 and 100 ppm treatments was less than the effect of Topsin® M (Fig. 43).

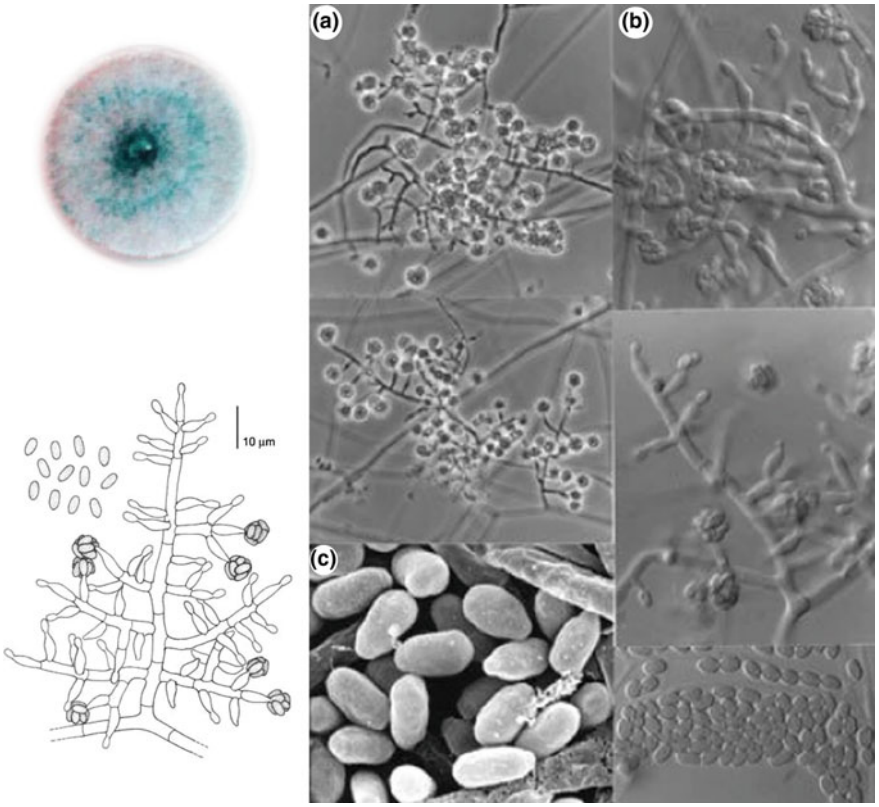

Fig. 42 *Trichoderma pseudokoningii*. (Left) colony growth and sporulation on potato dextrose agar (Rahman et al. 2011), and a schematic presentation of the fungus conidiophores and conidia (*Source* http://www.mycobank.org/); (Right) Micrographs of conidiophores (**a**, **b**) and conidia (**c**) (*Source* http://www.mycobank.org/)

Use of Cuprocaffaro® at 1, 10 and 100 ppm showed a negative correlation with the growth of *T. pseudokoningii* for up to 5 days but plates in all the treatments were filled after 6 days of incubation. Growth in 1000 and 10,000 ppm treatments started after 72 h of incubation and was very much reduced as compared to control (Fig. 43). Fungicides viz., benomyl, Topsin® M, carbendazim and Cuprocaffaro® were used at different concentrations i.e., 0, 1, 10, 100, 1000 and 10,000 ppm a. i. to evaluate *T. pseudokoningii* for tolerance to fungicides. Topsin® M and carbendazim were the most effective fungicides that inhibit the growth of *Trichoderma* species even at low concentration. *T. pseudokoningii* was suppressed by benomyl and Topsin® M (Khan and Shahzad 2007).

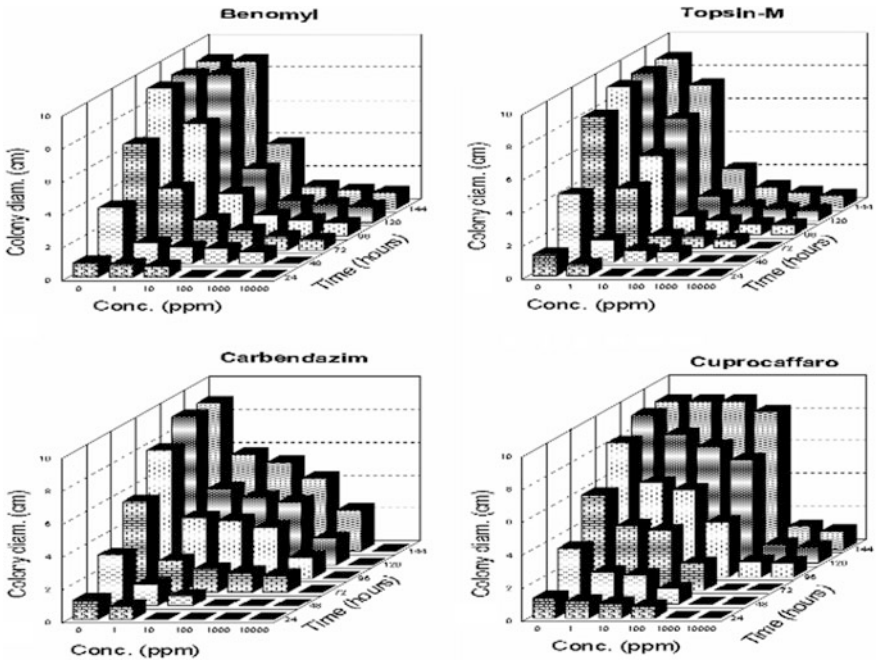

Fig. 43 Effect of fungicides on in vitro growth of *Trichoderma pseudokoningii* (Khan and Shahzad 2007)

2.7.6 *Trichoderma viride* Persoon

The beneficial effects of the *Trichoderma viridae* (Fig. 44) is that it establishes symbiotic rather than parasitic relationships with the plant, by increasing plant growth and productivity, helping to overcome stress stimulations, and improving nutrient absorption (Harman et al. 2004). Thapa and Seth (1977) reported that the best control of *T. viride* was given by carbendazim, without affecting the growth of mushrooms. Martinez-Toledo et al. (1992) also reported inhibition percentage of both hyphae and sporulation of native isolate of *T. viride* with chlorpyriphos in addition to methyl pyrinofos. Accordingly, Rai and Vijay (1992) reported that carbendazim stimulated the mycelial growth of *Pleurotus sajor-caju* at low concentration but inhibited it at higher concentrations. However, they found that carbendazim at 5 and 10 ppm concentration inhibited *T. viride*. Gupta et al. (1995) studied in vitro effect of chemicals against *T. viride* isolated from the button mushroom, *Agaricus bisporus*. They reported that mancozeb and carbendazim inhibited *T. viride* isolates in vitro. Thiram at 200 ppm inhibited *T. viride* while Captaf® 500 ppm, Dithane M-45® at 500 ppm were compatible with *T. viride* (Singh et al. 1995). Ghewande and Savaliya (1998) reported seed treatment with carbendazim 2 g/kg seed, *T. viride* at 4 g/kg seed gave maximum control (48%) of stem rot. Soil application of castor cake gave 19% control of stem rot

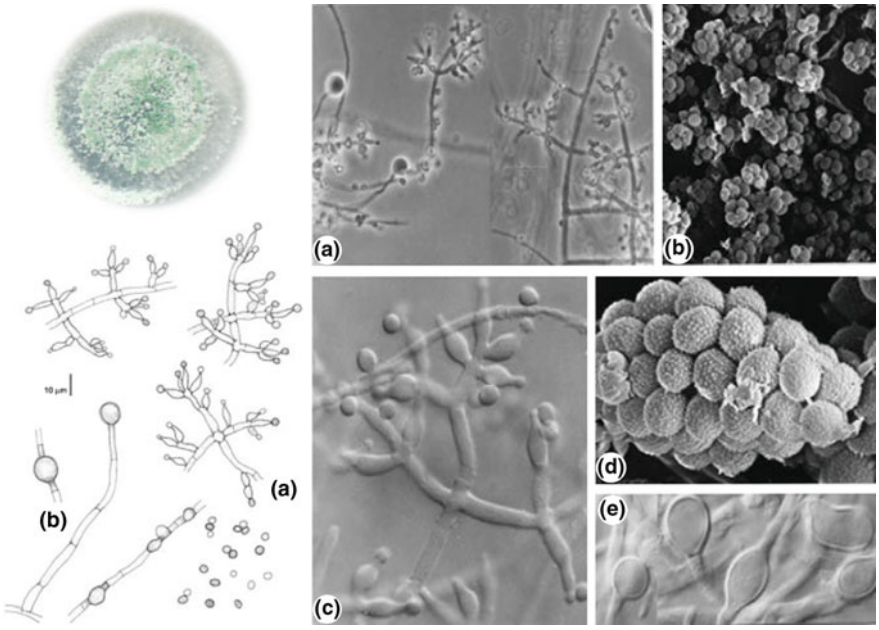

Fig. 44 *Trichoderma viride*. (Left) Colony growth and sporulation on potato dextrose agar (*Source* Błaszczyk et al. 2014), and a schematic presentation of the fungus conidiophores and conidia, and **b** chlamydospores (*Source* http://www.mycobank.org/); (Right) Micrographs of conidiophores (**a**, **c**), conidia (**b**, **d**), and terminal as well as intercalary chlamydospores (**e**) (*Source* http://www.mycobank.org/)

(Ghewande and Savaliya 1998). Kumar (1998) reported that there was significant reduction in the radial growth and sporulation of *T. viride* when tested at 7000 ppm of imidachloprid. Anita et al. (2001) screened carboxin and metalaxyl against fungal and bacterial antagonists in the laboratory and found that carboxin and metalaxyl did not inhibit the growth of *Trichoderma viride*.

Ramarethinam et al. (2001) reported that the fungicides like carbendazim (50% WP), hexaconazole (5% EC) completely inhibited the growth of *Trichoderma viride* centration in vitro. Lovkesh and Pahil (2006) reported that growth inhibition of *T. viride* and *Pleurotus* species increased with an increase in the concentration of different fungitoxicants. Maximum yield was obtained when carbendazim was applied, and the increase in carbendazim concentration enhanced the yield of mushroom and reduced the disease incidence. Khan and Shahzad (2007) applied fungicides viz., benomyl, topsin® M, carbendazim and cuprocaffro at different concentrations i.e., 0, 1, 10, 100, 1000 and 10,000 ppm a. i. to evaluate *T. viride*. They found that no fungicide was able to inhibit the growth. Carbendazim suppress the growth to some extent but not completely. Growth of *T. viride* showed negative correlation with the concentration of benomyl, plates were filled after 72, 96 and 120 h in 1, 10 and 100 ppm treatments, respectively. Reductions in growth

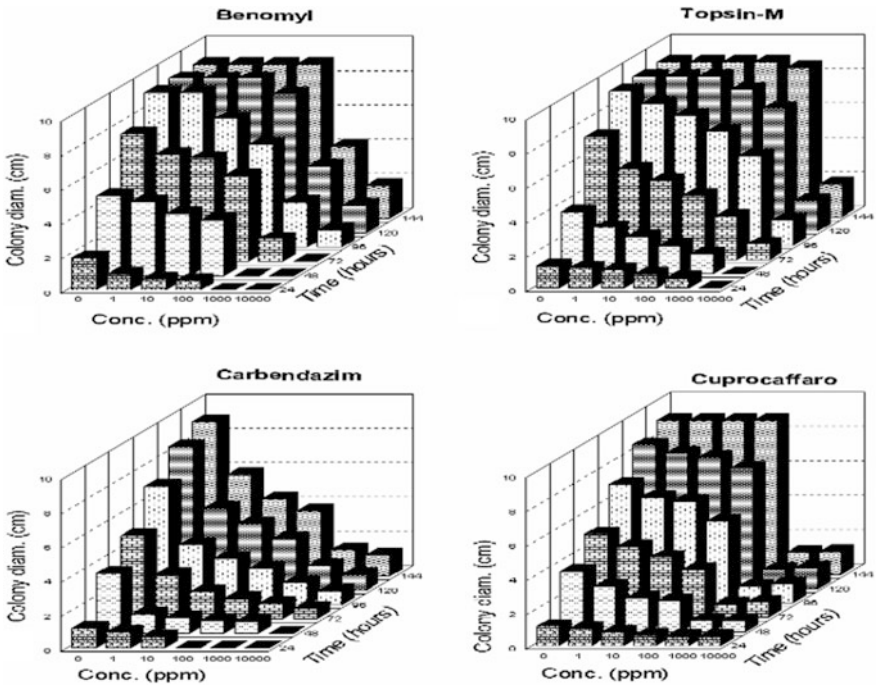

Fig. 45 Effect of fungicides on in vitro growth of *Trichoderma viride* (Khan and Shahzad 2007)

were observed in benomyl at 1000 and 10,000 ppm treatments as compared to control. Growth started after 48 h of incubation in 1000 ppm and after 72 h in 10,000 ppm treatments (Fig. 45). Topsin® M used at 1, 10, 100 ppm was not able to suppress the growth of *T. viride*. Plates were filled at 1 and 10 ppm treatments after 96 h and at 100 and 1000 ppm treatments after 120 h of incubation. There was sharp decline in growth of *T. viride* when Topsin® M was used at 10,000 ppm (Fig. 45). There was a significant negative correlation between the growth of *T. viride* and the concentration of carbendazim even after 144 h of incubation. Growth in 100 and 1000 ppm treatments started after 24 h and in 10,000 ppm treatment after 48 h of incubation. Overall growth of *T. viride* was slow as compared to control (Fig. 45).

Cuprocaffaro® was also not able to inhibit the growth of *T. viride* when used at 1, 10 and 100 ppm and plates were filled after 120 h of incubation. However, there was a sharp decline in growth of *T. viride* in 1000 and 10,000 ppm treatments where growth started after 24 h of incubation and was very much reduced as compared to control (Fig. 45).

Study on the compatibility of diafenthiuron with antagonistic microorganisms of plant pathogens viz., *T. viride* and *Pseudomonas fluorescens* revealed that diafenthiuron had some inhibitory effect on the mycelial growth of *T. viride*.

Diafenthiuron did not affect the growth of *P. fluorescens* (Stanley et al. 2010). The moderate to good compatibility of *T. viride* with copper oxychloride, mancozeb, fosetyl-Al and cymoxanil 8% + mancozeb 64% mixture fungicides was shown by exhibiting tolerance limits (ED) of 848, 710, 578 and 448 μg mL^{-1} respectively (Gaur and Sharma 2010). Bagwan (2010) indicated that thiram (0.2%) was compatible with *T. viride*, while mancozeb was found comparatively safer against *T. viride*. Using copper oxychloride, mancozeb, fosetyl-Al and cymoxanil 8% + mancozeb 64% mixture fungicides, Gaur and Sharma (2010) showed that the tested fungicides were of moderate to good compatibility with *T. viride* by exhibiting tolerance limits (ED_{50}) of 848, 710, 578 and 448 μg mL^{-1}, respectively.

Madhavi et al. (2011) who reported that *T. viride* showed a high compatibility with the insecticide imidachloprid (7.6 cm mycelial growth) followed by mancozeb (6.3 cm) and tebuconazole (3.7 cm). Shukla (2011) reported the compatibility of *T. viride* with Bavistin® (0.1%) and carbosulfun (0.05%) recorded percent inhibiting in the growth and sporulation of the fungus. While carbendazim, captan and propiconazole completely inhibited radial mycelial growth hence, were not compatible. Among insecticides evaluated at 0.1 and 0.2% concentration, imidacloprid showed little compatibility with isolates. But, chlorpyriphos, carbofuran and indoxacarb were highly incompatible. The compatibility of *T. viride* with aqueous extract of two medicinal plants (*Argemone mexicana* and *Nyctanthes arbortristis*) and three synthetic insecticides and three fungicides at their recommended does were evaluated under in vitro condition. Bavistin® (50 WP; 0.1%) and carbosulfun (0.05%) recorded cent percent inhibiting in the growth and sporulation of the fungus (Shukla 2011).

Rather et al. (2012) showed that mycelial growth of *T. viride* (Tv2) was completely inhibited at all tested concentrations of carbendazim (50, 100, 250 ppm). The best compatibility of tested Biocontrol agent (BCA) was observed with carboxin. The mycelial growth of Tv2 observed at 50 and 100 ppm concentrations (carboxin) was statistically at par with untreated check, however, at 250 ppm concentration significantly lower growth (72.3 mm) was recorded in Tv2 compared to untreated check (90 mm). The growth of antagonist was slightly inhibited by metalaxyl at its higher concentration, however, significantly lesser growth of the antagonist was observed at all concentration of captan. *T. viride* also showed poor performance in combination with fungicides. Dithane M 45® (at 100, 200 and 300 mg L^{-1}) and *T. viride* reduced the growth of *F. solani* by 57–100%, while *T. viride* + Benlate® and *T. viride* + Ridomil® by 21.3–100 and 52.9–100 (Ahmad et al. 2012). In order to devise a proper integrated management of soilborne plant diseases, Tapwal et al. (2012) conducted laboratory tests to work out the possibility of combining fungicides and botanicals with *T. viride*. Five fungicides, viz. Dithane M-45®, Ridomil®, Captaf®, Blue Copper®, Bavistin®, and five botanicals, viz. *Parthenium hysterophorus*, *Urtica dioeca*, *Cannabis sativa*, *Polystichum squarrosum*, and *Adiantum venustum* were evaluated at different concentrations. Among fungicides only Captaf® and blue copper were recorded as to some extent compatible with *T. viride*. Apart from *C. sativa*, other aqueous extracts of the tested botanicals were found quite compatible with *T. viride* while were of some inhibitory

impact on the growth of pathogens. The results indicated that the compatible fungicides and botanicals could be used with *T. viride* in an integrated disease management program to control soilborne plant pathogens (Tapwal et al. 2012).

Benomyl and BCM gave better in vivo control of mushroom (*Pleurotus sajor-caju*) pathogens, *Verticillium malthousei*, *Mycogone perniciosa*, and *T. viride* than maneb. Benomyl, or BCM at 0.5 g a. i. m^{-2} applied 3 days after casing gave satisfactory disease control (Shah et al. 2013). The effect of three synthetic insecticides, viz, malathion (50 EC), quinalphos (25 EC), carbosulfan (25 EC) and aqueous leaf extract of two medicinal plants, viz., *Argemone mexicana* and *Nyctanthes arbortristis* and three common fungicides viz., Dithane M-45®, Bavistin® (50 WP), Fytolan® (50 WP), at recommended does on the mycelial growth of *T. viride* was studied by poisoned food technique. Statistical analysis of the mean radical growth and spore count of *T. viride* revealed that there was significant difference among the treatments. Bavistin® (0.1%) and carbosulfan (0.05%) completely inhibited the mycelial growth and sporulation of *T. viride*, whereas quinolphos completely inhibited the sporulation and recorded 80% inhibition over control on the mycelial growth of the fungus. This indicates that carbosulfan, quinolphos and bavistin were lethal to the test fungus. Among the three fungicides tested Fytolan® had the least inhibitory effect on the growth (29.8%) and sporulation (52.33%) of the fungus. There are reports on the incompatibility of this fungus with thiram (Patibanda et al. 2002). In this study, the aqueous extract of two medicinal plants, viz., *Argemone mexicana* and *Nyctanthes arbortristis* was tested. In the laboratory experiments it is observed that aqueous leaf extract of *Nyctanthes arbortristis* have less inhibitory effect on the growth of the fungus. Thiram at 200 ppm inhibited *T. viride* while the rest of the two fungicides were compatible with *T. viride* (Valarmathi et al. 2013). Valarmathi et al. (2013) conducted an experiment to study the compatibility of copper hydroxide (Kocide® 3000) with bacterial and fungal biocontrol agents under in vitro conditions. Bacterial biocontrol agents, viz. *Pseudomonas fluorescens* and *Bacillus subtilis* were compatible with copper hydroxide (Kocide® 3000) even at a high concentration of 300 ppm. Fungal biocontrol agent, *T. viride* was inhibited by copper hydroxide at a concentration above 2500 ppm. The fungal biocontrol agent was highly compatible with the fungicide than the bacterial biocontrol agents.

In vitro experiments were conducted to check the compatibility of two insecticides, three fungicides and their combinations on *T. viride*. It shows variable responses against the tested pesticides (fungicides and insecticides) and their combinations at recommended concentrations for field studies. The treatments of mancozeb (Indofil® M-45 75% WP, 3000 ppm), imidacloprid (Confidor® 17–18% SL, 2000 ppm) and combination of mancozeb (3000 ppm) + imidacloprid (2000 ppm) showed high compatibility with *T. viridae* by showing 7, 11 and 11% growth inhibition respectively. The treatments viz. carbendazim (Bavistin® 50% WP, 1000 ppm) + chlorpyriphos (Force® 20% EC, 6000 ppm), tebuconazole (Raxil® 2% DS, 1000 ppm) + imidacloprid (2000 ppm) and tebuconazole (1000 ppm) + chlorpyriphos (6000 ppm) showed high incompatability with 100% growth inhibition. While moderate compatibility were recorded in the treatments of

chlorpyriphos (6000 ppm) with 68%, tebuconazole (1000 ppm) with 60%, carbendazim (1000 ppm) + imidacloprid (2000 ppm) with 57%, mancozeb (3000 ppm) + chlorpyriphos (6000 ppm) with 55%, carbendazim (1000 ppm) alone with 55% growth inhibition respectively (Vasundara et al. 2015). Their findings indicated that seed treatment of *T. viride* would be high compatable with fungicide mancozeb at 3000 ppm concentration, followed by combination of mancozeb with imidacloprid (3000 + 2000 ppm), imidacloprid (2000 ppm), respectively. High incompatability was observed in the treatments of carbendazim (1000 ppm) + chlorpyriphos (6000 ppm), tebuconazole (1000 ppm) + imidacloprid (2000 ppm) and tebuconazole (1000 ppm) + chlorpyriphos (6000 ppm). Moderate compatibility were recorded in the treatments of chlorpyriphos (6000 ppm), tebuconazole (1000 ppm), carbendazim (1000 ppm) + imidacloprid (2000 ppm), mancozeb (3000 ppm) + chlorpyriphos (6000 ppm), carbendazim (1000 ppm) alone (Vasundara et al. 2015). Among the treatments the mean radial growth of *T. viride* varied from 0.0 to 9.0 cm. Mancozeb showed more compatibility with *T. viride* and luxuriant growth of antagonist was found in all plates containing poisoned medium and the observed mean radial growth of *T. viride* was 8.4 cm with 7% growth inhibition, combination of mancozeb and imidacloprid, imidacloprid alone are also showed compatibility by recording radial growth of 8.0 cm and 8.0 cm, growth inhibition percentage in both treatments is 11%. All these three treatments mancozeb, imidacloprid and combination of mancozeb + imidacloprid treatments are on par with control agent *T. viride* and were significantly superior over all other treatments. Combination of the treatments carbendazim and chloropyriphos, tebuconazole and imidachloprid, tebuconazole and chloropyriphos, showed high incompatible with *T. viride* and the observed mean radial growth was of 0.0 cm and 100% growth inhibition was recorded. Carbendazim, tebuconazole, chlorpyriphos, alone and combination of mancozeb and chlorpyriphos, carbendazim and imidachloprid, showed moderate compatibility with *T. viride*. The mean radial growth recorded in these treatments were 4.1 cm with 55% growth inhibition, 3.6 cm with 60% growth inhibition, 2.9 cm with 68% growth inhibition, 4.0 cm with 55% growth inhibition, and 3.9 cm with 57% growth inhibition, respectively (Vasundara et al. 2015). Desai and Srikant (2002) had previously reported the fungistatic effect of chlorpyriphos on the growth of *Trichoderma viride*. An attempt was made in the present investigation to formulate an integrated management strategy against wire stem of cabbage caused by *Rhizoctonia solani* Kuehn. Induction of *T. viride* to carbendazim was done through repeated exposure to the fungicide. Out of the four selected induced strains of *T. viride* to the systemic fungicide carbendazim, all the strains were found sensitive to the fungicide at different levels of concentration. However, one of the carbendazim tolerant strain of the antagonist (TV-S-4) could tolerate the fungicide at 10 µg a. i. mL^{-1} indicating that *T. viride* and carbendazim could be successfully integrated against the pathogen at low concentrations of the fungicide. Higher antagonistic activity, faster growth and increased production of antifungal volatile substance were also observed in TV-S-4. The pot trial conducted with different treatment combinations of carbendazim (reduced dose) with *T. viride* revealed

maximum (90.72%) control of the disease could be achieved when the fungicide (at 0.05%) was integrated with the bioagent TV-S-4 at 5 g kg^{-1} of pot soil followed by application of another carbendazim tolerant strain of the antagonist (TV-S-3) and carbendazim at 0.05%. when carbendazim was applied at 0.1% the percent plant mortality was 23.64 as compared to 85.21% in the untreated inoculated control. Amongst the treatments tested, application of the bioagent (TV-W) alone was found least effective (Bhowmick et al. 2015).

2.7.7 Trichoderma virens Arx

Anita et al. (2001) screened carboxin and metalaxyl against fungal and bacterial antagonists in the laboratory and found that carboxin at 0.1% concentration caused little inhibition of the growth of *Trichoderma* (formerly *Gliocladium*) *virens* (Fig. 46). Propineb was found effective against several pathogens but was non-inhibitory to the antagonist, *T. virens* (Mukherjee and Tripathi 2000). Girija and Umamaheswaran (2003) reported the compatibility of *T. virens* with carbendazim in vitro at three concentrations (100, 500 and 1000 ppm) concentrations and observed that the antagonist *T. virens* was compatible with carbendazim at 100 ppm concentration. In order to see the possibility of the application of *T. virens* strain G-6 in an integrated system of management of cotton pre-, and post-emergence damping-off diseases, Howell (2007) applied the fungicides Baytan® 30 FL (a.i. 30% triadimenol, 14.8 g 45.4 kg^{-1}), chloroneb 65 WP (147.9 g 45.4 kg^{-1}), Deltacoat AD® (a.i. 30% chloroneb plus 3.5% metalaxyl, 347.8 g 45.4 kg^{-1}), Dividend® 32.8 FL (a.i. 32.8% difenoconazole, 29.6 g 45.4 kg^{-1}), Flint® (a.i. 50% trifloxystrobin, 2.22 g 45.4 kg^{-1}), Maxim® 42 FL (a.i. fludioxonil, 2.4 g 45.4 kg^{-1}),

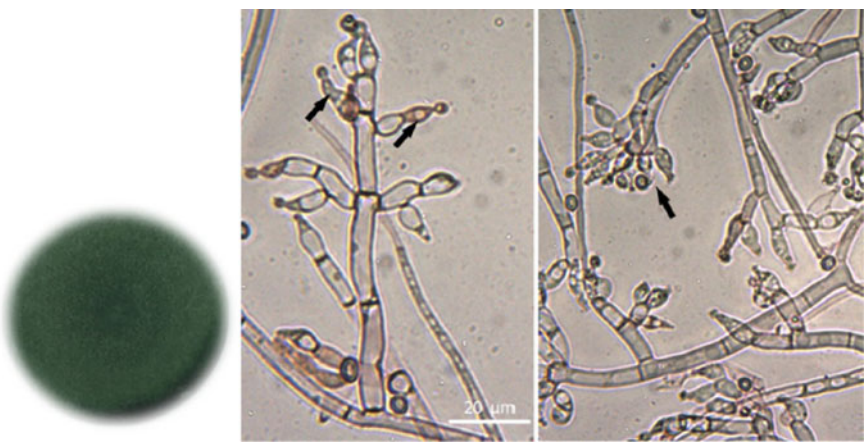

Fig. 46 *Trichoderma virens*. (Left) Colony growth and sporulation on potato dextrose agar seven days after inoculation (*Source* Vargas et al. 2009); (Right) Verticilliate conidiophores, conidiogenous cells (phialides), and conidia (phialospores) of the fungus (Armando et al. 2017)

Nuflow M® 40 FL (a.i. 40% myclobutanil, 37 g 45.4 kg^{-1}), Vitavax ® 34 FL (a.i. 34% carboxin, 88.8 g 45.4 kg^{-1}), Vitavax® 17 FL plus PCNB® 17 FL (a.i. 17% carboxin plus 17% pentachloronitrobenzene, 177.6 g 45.4 kg^{-1}), and Vortex® (a.i. ipconazole, trade secret, 2.7 g 45.4 kg^{-1}) to the seed in a volume of water equal to one tenth the weight of the seeds, and dried the seeds with the filter-sterilized air flow under the hood. The control was treated with water only. The seedling survival data derived from planting nontreated, fungicide-treated, or G-6-treated SG747 seeds in soil naturally infested with *Rhizopus oryzae* and *Pythium* spp. showed that fungicide treatment of seeds planted in this soil resulted in little or no improvement in seedling stand over the non-treated control (0–13%).

Seed treatment with preparations of the biocontrol strain G-6, however, gave 93% seedling survival in the pathogen-infested soil. The planting of SG747 seeds, nontreated, fungicide-treated, or G-6-treated, in naturally infested cotton field soil amended with *Rhizoctonia solani* inoculum produced somewhat different results. The percent survival of nontreated and fungicide-treated seeds remained at low levels, but survival of the G-6-treated seeds was reduced to 40%. The numbers of surviving seedlings from seeds planted in naturally infested soil amended with *R. solani* inoculum were vastly improved by some of the seed treatments with combinations of fungicides and the biocontrol agent preparation. Other combinations were no better than the individual treatments (Fig. 47). The most effective chemical combinations with strain G-6 were chloroneb (93%) and Deltacoat AD® (80%), followed by Vitavax® (60%) and Vitavax-PCNB (53%). The results of the disease control assays of chloroneb, biological seed treatments, and the combination of chloroneb plus biological seed treatments for efficacy in the control of pre- and post-emergence damping-off in cotton indicated that chloroneb in combination with any of several biological control preparations led to effective disease control (Fig. 48). Neither chloroneb nor the biocontrol agents alone controlled all of the pathogens involved. Poor quality seeds were very susceptible to seedling diseases and might require a fungicide, a biological seed treatment, or a combined seed treatment in order to survive. The appropriate treatment would depend on the kinds of pathogens present in the soil.

The results of this study indicated that in soils containing several different kinds of pre-emergence damping-off pathogens and a post-emergence pathogen, seed treatment with a fungicide and a biocontrol agent would be required to control cotton seedling diseases in poor quality cotton seed. In soils where only pre-emergence pathogens such as *Pythium* spp. and *Rhizopus oryzae* were present, the biocontrol agent strain G-6 of *T. virens* gave excellent disease control. Seed treatment with the fungicides used in this study did not give adequate control of cotton seedling disease when the seeds were planted in soil containing only pre-emergence pathogens or where both pre- and post-emergence pathogens were present. In soil containing both pre- and post-emergence pathogens, neither the fungicides nor the biocontrol agents alone gave adequate seedling disease control. This was because the fungicides, although effective in controlling *R. solani*, did not have a wide enough activity spectrum to control both *R. oryzae* and the *Pythium* spp. (Thomson 1997). Strain G-6 of *T. virens* controls the pre-emergence pathogens

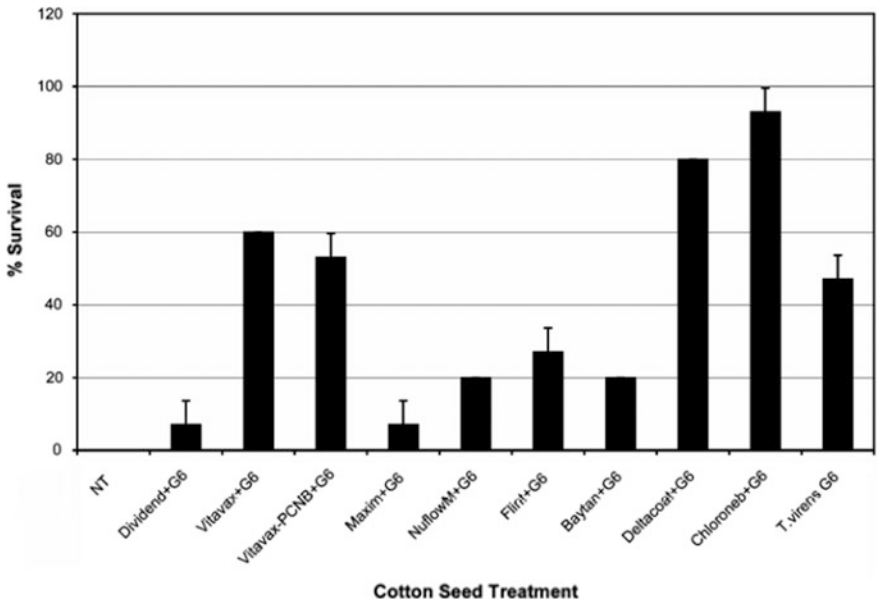

Fig. 47 Effect on cotton seedling survival of seed treatments with combinations of fungicides and *Trichoderma virens* strain G-6 in soil naturally infested with pre-emergence damping-off pathogens and amended with *Rhizoctonia solani*. Yhe cotton cultivar was poor quality SG747. NT = nontreated. Values represent the mean ± standard error of three replicate. Fischer's least significant difference = 17% (Howell 2007)

by metabolizing pathogen germination stimulants excreted by the seedling and by inducing phytoalexin synthesis in the roots (Howell 2002; Howell et al. 2000). Induction of phytoalexin synthesis, however, does not extend to the hypocotyls, and that area is susceptible to postemergence damping-off. Combination of seed treatments consisting of the biocontrol agent and a systemic fungicide can control both phases of cotton seedling disease. The biocontrol agent suppresses the activities of the pre-emergence pathogens, while the fungicide inhibits those of the postemergence pathogen. Although all of the fungicides used in this test were toxic to *T. virens* in vitro, and may have inhibited the activities of the biocontrol agent in vivo, much of the systemic chemical was likely absorbed by the germinating seedling, and the biocontrol agent was shielded from the remaining fungicide by the latex coating on the seed. Optimum disease control in soil containing both pre- and post-emergence pathogens was achieved with a combination of chloroneb and any one of several *Trichoderma* spp., indicating that the mechanisms employed to control the pre-emergence phase of cotton seedling disease may be common within the genus. Deltacoat AD®, in combination with *T. virens* strain G-6, was also effective in controlling cotton damping-off.

However, the Deltacoat AD® formulation is mostly chloroneb, and this may account for its success. All of the fungicides applied in the recommended doses for

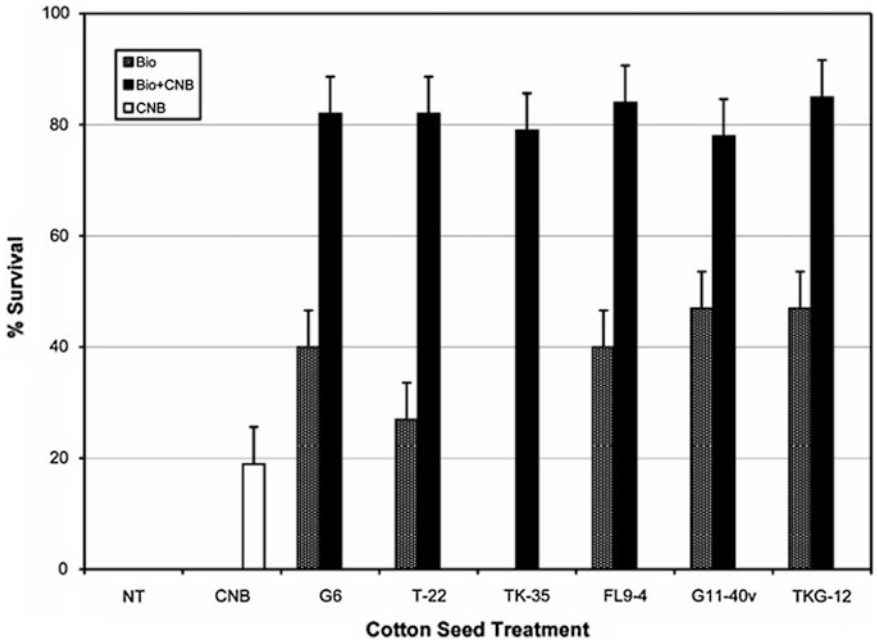

Fig. 48 Effect of chloroneb, biological (*Trichoderma* spp.) and biological plus chloroneb seed treatments on cotton seedling survival in soil naturally infested with pre-emergence damping-off pathogens and amended with *Rhizoctonia solani*. CNB = chloroneb alone; Bio = biological alone; Bio + CNB = biological and chloroneb in combination; G6 = *T. virens* Q strain; T-22 = *T. harzianum*, TK-35 = *T. koningii*; FL9-4 = *T. virens* Q strain; G11-40v = *T. virens* Q strain; and TKG-12 = *T. virens* × *T. koningii* hybrid. The cotton cultivar was poor quality SG747. NT = nontreated. Values represent the mean ± standard error of three replicates. Fischer's least significant difference = 14% (Howell 2007)

seed treatment and assayed for toxicity to G-6 proved to be inhibitory to the fungus in vitro, with some less inhibitory than others. However, there was no positive correlation between fungicide resistance and disease control efficacy of the combined fungicide/biological treatments. Microscopic examination of chloroneb plus G-6-treated cotton seeds harvested from sterile soil tubes after 3 days incubation at 25 °C gave evidence of fungal hyphae growing from the seed surface. After 72 h incubation at 25 °C on PDA containing rifampicin (50 μg mL^{-1}), sporulating cultures of *T. virens* were observed growing from the seeds. Good quality seeds of cotton cultivars often escaped pre-emergence damping-off incited by *Pythium* spp. and *Rhizopus oryzae*, and they were resistant to postemergence damping-off incited by *Rhizoctonia solani*. Poor quality seeds, however, were highly susceptible to both phases of seedling disease and required seed treatment in order to survive. Preemergence damping-off incited by *Pythium* spp. and *Rhizopus oryzae* could be controlled by seed treatment with biocontrol preparations of a number of *Trichoderma* spp., but these treatments were much less effective in controlling

postemergence disease incited by *Rhizoctonia solani*. Postemergence seedling disease can be controlled by fungicides, but they were much less effective in controlling the pre-emergence phase of the disease. Combination seed treatments of poor quality cotton seeds with fungicides and *Trichoderma* spp. preparations, followed by planting in pathogen-infested soil, indicated that this technique will control both phases of seedling disease. Seed treatment with either the fungicides or the biocontrol agents alone did not achieve this goal. The optimum combination treatment for disease control was that of chloroneb plus *Trichoderma* spp., followed by chloroneb plus metalaxyl (Deltacoat AD) plus *T. virens* strain G-6 (Howell 2007). Rather et al. (2012) showed that mycelial growth of *T. virens* (Gv) were completely inhibited at all tested concentrations of carbendazim (50, 100, 250 ppm). The best compatibility of tested Biocontrol agent (BCA) was observed with carboxin. The growth of the antagonist was slightly inhibited by metalaxyl at its higher concentration, however, significantly lesser growth of *T. virens* (GV) was observed at all concentration of captan.

3 Strategies for the Integrated Application of Biological Control Fungi and Agrochemicals

The integrated management of plant pests and diseases necessitates the co-application of biological control agents and other agrochemicals such as pesticides (fungicides, insecticides, acaricides, herbicides, etc.). However, biological control fungi may be exposed to the negative impacts of the agrochemicals on their spore germination, hyphal growth, antagonistic activities, and sporulation. Despite of negative effect of fungicides on the biological control potential of the entomopathogenic fungi reported as the result of co-application of these fungi with the fungicides (Clark et al. 1982; Loria et al. 1983; Saito and Yabuta 1996; Pell et al. 2010; D'Alessandro et al. 2011), the integrated management of plant pests and diseases is fascinating. Better control of disease through integration of biological control agents with fungicides than the individual application of either biological control agents or fungicide has been recorded (Mukhopadhyay and Kaur 1990; Dubey 1997). This synergism might be due to partial suppression of the pathogen by the chemical without disturbing much the activity of the fungicide tolerant antagonist. Abd-El Moity et al. (1982) observed that combination of iprodione and iprodione-tolerant isolate of *Trichoderma harzianum* gave significantly higher control of white rot of onion caused by *Sclerotium cepivorum* than did iprodione or *T. harzianum* alone.

Four approaches have been taken in order to overcome the incompatibility between biological control agents and agrochemicals:

3.1 Temporal/Spatial Separation of the Application of Incompatible Agrochemical(S) and Biological Control Fungi

One of the simplest approaches taken in order to avoid the problem of incompatibility between biological control fungi and agrochemicals is the temporal separation of their applications so that biological control fungi are applied after a period past chemical treatment with incompatible compound(s). This strategy not only eliminates hazardous targets and reduces their population in the environment, but also weakens, predisposes, and makes them more amenable to biological control applied after an extended interval in an integrated management program (Gardner et al. 1984; Bruck 2009) when high noxious doses of the agrochemicals decrease to the residues tolerable/non-toxic for incompatible fungal biological control agent(s). However, such intervals may increase the input of energy, and the final product cost, and complicate the effective and practical use of both products in the field (Shinohara et al. 2013.

In some cases, spatial separation of biological control fungi and agrochemicals can be an efficient solution. For instance, spraying plants with apoplastic xylem-mobile agrochemicals is not expected to be of considerable hazardous impact on the biological control fungi in the rhizosphere and underground parts of the treated plants. The upward motion of the agrochemical in plant xylem will not allow the incompatible chemical to contact the fungi in a biologically effective dose.

3.2 Application of the Biological Control Fungi Well-Adapted to Given Incompatible Agrochemical

This approach requires subsequent growth and adaptation steps on the gradually increasing concentrations of the chemical amended into the culture media. Therefore, the procedure is time-consuming. Additionally, adaptation, as a process related to the increased expression of the involved genes/and the increased activity of some gene products, is not a genetically inheritable characteristic, and therefore, it's maintain necessitates a continuous effort. Furthermore, the active process is energetically and materially high-demanding and imposes high fitness costs on the adapted fungus vitality reflected as diminished growth rate and sporulation. Another disadvantage of this approach is that adaptation cannot end to complete safeness of the adapted fungus to the hazardous effects of the considered chemical(s). Indeed, the residual concentrations of an incompatible chemical are expected in the cell of adapted fungus. These concentrations although not sufficient to kill the cell, but still may affect chemical pathways as those involved in antagonism. Consequently, the final effect on fitness and biological control potential seems to depend on the collective impact of the individual effects each of a particular value. Tolerance in

isolates of *Trichoderma* was developed by exposing two strains of *T. harzianum* and three of *T. asperelloides* to increasing concentrations of chemical fungicides. This isolation of *Trichoderma* was exposed to three fungicides: captan, thiabendazol and the mixture captan-carboxin. Some selected lines of these strains reached tolerance to captan and partial tolerance to the mixture captan-carboxin. The biological and genetic changes in these tolerant lines were monitored by determining the relative growth rate of the fungus, inhibition of *Fusarium* and by analyzing the genomic changes through UP-PCR. The results showed that the tolerance to fungicides could be developed without affecting the parameters of biological activity in these lines of *Trichoderma* (growth and parasitism against *Fusarium*). Chemical tolerance to the fungicide was verified by means of changes at the DNA level (UP-PCR), mainly in the lines tolerant to captan. This suggests that *Trichoderma* survives in environments with remnants of fungicide molecules (Chaparro et al. 2011). *Trichoderma* strains used in this study were isolated from different geographical areas and from different sources. All of them were also naturally tolerant to the recommended concentration of the chemical fungicide captan, and exposure of *Trichoderma* strains to increasing concentrations of this fungicide allowed for the selection of tolerant lines. Growth of *T. asperelloides* and *T. harzianum* strains in liquid medium with the fungicides captan and captan-carboxin confirmed that the selected lines have developed a mechanism to tolerate the exposure to homogenous concentrations of the chemical fungicides. Tolerance to The fungicide mixture captan-carboxin was obtained in the treated lines of *T. asperelloides* strains T-19, T-84 and T-109, while some degree of tolerance to thiabendazole was only obtained with the *T. asperelloides* strains T-19 and T-84. These data suggested detoxification mechanisms are restricted to particular strains, and are not present in all the specimens of a taxa. In some cases, growth rate and inhibition rate of the *Trichoderma* tolerant lines were affected by the exposure to the chemical fungicides. The antagonism capacity under in vitro conditions was only negatively affected in one out of the 10 tolerant lines obtained. A similar phenomenon was found in *Penicillium* on imazalil-resistant and sensitive strains on which no difference in spore production and radial growth was found (Holmes and Ecker 1995). Differences in the number of genetic changes observed in the *Trichoderma* strains treated with chemical fungicides could be due to their mode of action or to the approach used for tolerance development. It has been described that protectant fungicides such as captan, induce mutations in several genes, contrary to systemic fungicides in which a particular gene or gene product is targeted (Deyle et al. 1997; Goldman et al. 1993; Yamamoto and Baird 1999; Yan and Dickman 1996). Accordingly, high genetic changes were observed in the captan tolerant *Trichoderma* lines as compared to the wild type strains. The results suggest that it is possible to develop *Trichoderma* tolerant lines to some chemical fungicides. Most importantly, the changes induced by this tolerance, in most cases, did not negatively affect the antagonistic activity of the biological control strains, and in some other cases, the growth rate and the inhibition rate increased. The molecular study led to recognition of the changes at the genomic level, which in most cases were not related to the loss of biological fitness of the fungal strains

(Chaparro et al. 2011). Ruocco et al. (2009) explained that the ability of *Trichoderma* to withstand relatively high concentrations of a variety of synthetic and natural toxic compounds, including its own antibiotics, depends on efficient cell detoxification mechanisms supported by a complex system of membrane pumps. Now it is well known that the genome of *Trichoderma* includes ABC transporters (ATP-binding cassette (ABC) transporters), which are members of a protein superfamily that effluxes drugs from cells of target organisms. Thus transporters may provide a mechanism of protection against cytotoxic drugs and xenobiotic agents. The natural function of ABC transporters in plant pathogenic fungi may relate to transport of plant-defense compounds or fungal pathogenicity factors (De Waard 1997). The ABC transporters may explain the natural tolerance of fungicides on *Trichoderma*, and their ability to successfully to survive in extreme environments.A correlation between fungicide resistance and antagonistic activity is suggested by Marra et al. (2006), affirming that the up-regulated expression of ABC transporter genes of *T. atroviride* during the three-way interaction with various plants and fungal pathogens, possibly supports both antagonistic activity and root colonization. Saikia (2000) recorded the enhanced radial and mycelia growth of UV irradiated strains of *Trichoderma viride*. An attempt was made to formulate an integrated management strategy against wire stem of cabbage caused by *Rhizoctonia solani* Kuehn. Induction of *Trichoderma viride* to carbendazim was done through repeated exposure to the fungicide. Out of the four selected induced strains of *T. viride* to the systemic fungicide carbendazim, all the strains were found sensitive to the fungicide at different levels of concentration. However, one of the carbendazim tolerant strain of the antagonist (TV-S-4) could tolerate the fungicide at 10 µg a. i./mL indicating that *T. viride* and carbendazim could be successfully integrated against the pathogen at low concentrations of the fungicide. Higher antagonistic activity, faster growth and increased production of antifungal volatile substance were also observed in TV-S-4. The pot trial conducted with different treatment combinations of carbendazim (reduced dose) with *T. viride* revealed maximum (90.72%) control of the disease could be achieved when the fungicide (at 0.05%) was integrated with the bioagent TV-S-4 at 5 g/kg of pot soil followed by application of another carbendazim tolerant strain of the antagonist (TV-S-3) and carbendazim at 0.05%. when carbendazim was applied at 0.1% the percent plant mortality was 23.64 as compared to 85.21% in the untreated inoculated control. Amongst the treatments tested, application of the bioagent (TV-W) alone was found least effective (Bhowmick et al. 2015). The increased radial and mycelia growth of the carbendazim tolerant strains may be attributed to the production of more amount of beta-1, 4-glucosidase as compared to the wild strain which enables them to utilize substrate more efficiently (Ahmed and Baker 1987). The possible mechanisms involved in *T. viride* antagonism against *R. solani* were studied in vitro and several stages of interactions were observed. The attachment of spores of *T. viride* and subsequent coiling of hyphae of this mycoparasitic fungus around the pathogen hyphae were observed. Hyperparasitism and volatile metabolites may be involved in the inhibition of growth of *R. solani*. Higher biological control activity of TV-S-3 and TV-S-4 against *R. solani* may be due to higher release of enzymes like

chitinase, glucanase and proteases closely related to the mycoparasitism of *Trichoderma* strains (Chet 1987; Harman 2006). The inhibition of the pathogen by *T. viride* strains may also be attributed to the production of secondary metabolites such as glioviridin, viridin and gliotoxin (Shabir and Rubina 2010). The release of volatile substances by *Trichoderma* spp. against the fungal pathogens has been reported (Zeilinger and Schuhmacher 2013). Inhibitory volatile substances such as alkyl pyrons may also contribute to the biological control activity of some *Trichoderma* strains (Claydon et al. 1987). *Trichoderma* spp. produce both volatile and non-volatile metabolites that adversely affect the growth of different fungi (Horvath et al. 1995).

3.3 Application of Naturally Resistant Strains of the Biological Control Fungi that Resist to Given Incompatible Agrochemical(s)

The approach relies on the natural genetic diversity in the population of a biological control fungus and the selection of the resistant strains naturally found in the population. The fungicide can in fact control sensitive isolates, causing natural resistant isolates to potentially may become dominant in populations under selection pressure of fungicide. This phenomenon happens in assays with fungi like *Trichoderma*, evidencing the fact that such fungi have a natural ability to tolerate fungicides, which is called 'natural' or 'inherent resistance'. Resistance is as a response to repeated use of the fungicide, or to the repeated use of another chemically related fungicide and/or by a biochemical mechanism of antifungal action (Brent and Hollomon 1995). Fungicide resistance is a stable, inheritable adjustment by a fungus to a fungicide, resulting in reduced sensitivity of the fungus to the fungicide. Resistant isolates are less affected or not inhibited at all by application of a fungicide (Ma and Michailides 2005). The method, although simple and in the meantime fascinating, is seemingly species-dependent. The populations of some fungal species include such naturally resistant strains that may be also of high antagonistic potentials beneficial in biological control of weeds, plant pests and diseases. However, such a natural resistance may not found with other fungal species.

Also, dependent on the type of chemical resistance (vertical/horizontal), the number of the genes involved in the development of resistance (unigenic/multigenic), and the biochemical feature of the resistance development, such a natural resistance may be of different practical values. Not all, but most of the practically valuable natural resistance are vertical conferred by a change (mutation) in asingle nucleotide of/the nucleotide sequence of single gene, the single product of which is the only target of the chemical. Dependent on the role of the gene product, such a genetic change may lead to different outcomes. It may lead to different hazards and even death of the fungal cell, or it can lead to vertically

resistant strain of high fitness. With multi-target-site chemicals that exert their effects on more than one gene product, the chance of resistance development and getting access to an acceptable fitness rate decreases with the increase in the number of the targeted gene products in the biological control fungus. So it will be more rational to screen for the strains naturally resistant against single-target-site chemicals. Shapiro-Ilan et al. (2002) demonstrated enhanced fungicide resistance in *B. bassiana* through artificial selection. However, it was not clear if the enhanced resistance was because of improved germination, vegetative growth, or both. Additionally, the enhanced fungicide resistance had only been demonstrated in *B. bassiana*, and therefore it was of interest to investigate the potential to enhance resistance in other fungi. Thus, Shapiro-Ilan et al. 2011 extended their studies in order to determine the potential to enhance fungicide resistance in *M. brunneum* through artificial selection, and investigate if selection is based on germination, vegetative growth, or both in *B. bassiana* and *M. brunneum*. Selection for resistance to fenbuconazole, and triphenyltin hydroxide was assessed through inhibition evaluations on solid media, and germination and mycelial growth in liquid media. Increased resistance after selection was observed for all fungicide-fungus combinations on solid and or liquid media. Selection resulted in increased resistance to fenbuconazole in both fungi in solid and liquid media; in liquid culture fungicide resistance in *B. bassiana* was manifested by increased germination and mycelial growth, whereas in *M. brunneum* fungicide resistance concerned only mycelial growth. Selection for resistance to triphenyltin hydroxide varied in the different media. For *B. bassiana*, triphenyltin hydroxide resistance was enhanced on solid media but not in liquid, whereas enhanced resistance of *M. brunneum* was detected in both media. Fungicide sensitivity and selection potential differs based on the medium and fungal species. Selection for fungicide resistance, had negative effects on other beneficial traits when fungicide pressure was removed, for example, some selected populations showed decreased germination or growth, relative to their non-selected control populations. Additionally, reduced virulence to the greater wax moth, *Galleria mellonella* (L.), was observed in all fungal populations that were exposed to fungicide resistance regimes. Shapiro-Ilan et al. (2011) concluded that it was possible to use genetic selection to enhance fungicide resistance in *B. bassiana* and *M. brunneum*, but before use the resulting populations should be screened for inadvertent negative impacts on beneficial traits (Shapiro-Ilan et al. 2011).

3.4 Application of Artificially Developed Resistant Strains of the Biological Control Agents that Resist to Given Incompatible Agrochemical(s)

According to the definitions of unigenic and multigenic chemical resistance, and the consequences of the development of these resistance types, the fourth approach is practically more feasible and easier when biological control strains resistant to

given incompatible chemicals are artificially developed in laboratory. The development of unigenic resistance can be achieved through treatments with physical and chemical mutagens, as well as via genetic engineering techniques.Physical mutagens applied range from irradiation of ultra-violet (UV) (Boyd 1950), X-rays (Babalola 2008–2009), ion-beams (Matuo et al. 2006; Tanaka et al. 2010; Toyoshima et al. 2012), and gamma rays (Vu et al. 2009). Additionally, more than one physical mutagen have also been applied for instance, gamma-ray and then UV (Vu et al. 2009).

Chemical mutagens applied with fungi include $NaNO_2$ (Zou et al. 2006; Song et al. 2011), colchicine (Khan et al. 2015; Khanam and Prasuna 2014), acridine orange (Moturi and Charya 2010), N′-methyl-N′-nitro-N-nitroso guanidine (MNNG) (Bapiraju et al. 2004; Ho and Ho 2015), diethyl sulfonte (DES) (Xu et al. 2006), ethyl methylsulfonate (EMS) (Moturi and Charya 2010; Ho and Ho 2015), dimethoite (Saued et al. 2013), nitrous acid (Justin et al. 2010), ethidium bromide (EtBr) (Chand et al. 2005; Reddi et al. 2012). Additionally, the mixtures of more than one chemical mutagen have been applied, for instance MMNG and EtBr (Chand et al. 2005).

Also, the mixed application of physical and chemical mutagens has been made: UV and MNNG (Kuhad et al. 1994), and UV and EtBr (Chand et al. 2005), MNNG, EtBr and UV (Chand et al. 2005). Furthermore, sequential application of physical and chemical mutagens (for example, gamma ray, UV, and MNNG) as well as sequential application of a physical mutagen and then simultaneous treatment with the mixture of both physical and chemical mutagens have been made (Vu et al. 2009). The creation of biodiversity through treatment with physical and/or chemical mutagens, although technically cheaper and simpler, however suffers from randomness of achievement. Most of the mutations are not those wanted, and are of deleterious impacts on biological control fungus. This may lead for more labor and time consumption before the favorite results are obtained. Mutagenic treatments have been applied in the improvement of biological control fungi, for example, exposure of the entomopathogenic fungus *Cordyceps militaris* to ion beams successfully generated a mutant isolate capable of enhanced production of cordycepin, a medicinal adenosine analogue (Das et al. 2008, 2010). However, there is relatively little information in the literature on the use of ion-beam irradiation to induce mutations in entomopathogenic fungi and none considering induction of mutations conferring resistance to fungicides. Gamma-ray irradiation has also been demonstrated as a successful mutagenic agent in entomopathogenic fungi (Paccola-Meirelles and Azevedo 1991; Kava-Cordeiro et al. 1995), although there are no reports describing enhanced fungicide resistance induced by gamma-ray irradiation.

Shinohara et al. (2013) induced *Isaria fumosorosea* mutants with enhanced resistance to the fungicide benomyl by irradiation using either ion beams or gamma rays, or a combination of the two. When grown on agar containing benomyl, mycelial growth was observed for five of the six mutant isolates at benomyl concentrations that were more than 2000-fold those observed for the wild-type isolate (EC_{50} >5000 mg L^{-1} c.f. EC50: 2.5 mg L^{-1} for the wild-type isolate). The mutant isolates evaluated also showed enhanced resistance to other fungicides at

recommended field application rates. No differences were observed at the β-tubulin locus between the wild-type and the mutant isolates, suggesting that the enhanced benomyl resistance was not attributable to mutations in that gene. Ion beams and gamma rays are thus potentially useful tools for inducing beneficial fungal mutations and thereby improving the potential for application of entomopathogenic fungi as microbial control agents. UV-mutagenesis was found to be a useful method for the isolation of benomyl-resistant *Trichoderma* strains (Papavizas et al. 1982).

Similarly, Yuan et al. (2007) reported that the strain of *Trichoderma* T-21 has shown pyrimethanil resistance and that the growth and the conidia production of the strain were good. Two mutants tolerant to pyramethanil were obtained by UV-light induction on PDA amended with pyrimethanil from a wild-type *Trichoderma* strain. Tolerance level was observed 20 times compared with that of wild-type mother strain. The mutants could maintain their tolerance after 8 times of transfer on the fungicide-free PDA. Two tolerant strains kept their ability of antagonism against *Botrytis cinerea* in vitro and in vivo (Hongman et al. 2005). Mukherjee et al. (1999) found that the mutants of benomyl-tolerant strains of *T. pseudokoningii* were superior to the wild type in biocontrol potential on *S. rolfsii*.

Among 22 Iraqi *Trichoderma harzianum* and *T. viride* isolates, T3, T5, D5, S1 and S6 related to *T. harzianum* showed a higher antagonistic activity against the plant pathogenic fungi *Fusarium oxysporum* and *Rhizoctonia solani*. These isolates were selected for improvement of their biocontrol activity by using UV-irradiation. The optimum exposure time to ultraviolet irradiation was 30 min, in which, the percentage of kill was 98–99%. After UV-irradiation treatment (Quartz lamps 30 Wat 240–280 nm, peak 254 nm, distance from the source was 20 cm), antagonistic capability against *R. solani* and *F. oxysporum* was improved in mutants MT3, MT5, MS1 and MS6, in which antagonistic reaction score were 3.6, 3.9, 3.2 and 3.6 against *F. oxysporum* and 3.7, 4, 3.4 and 4 against *R. solani* compared to 3.2, 2.8, 2.6 and 2.8 against *F. oxysporum* and 2.7, 2.9, 3.1 and 3 against *R. solani* in the wild types T3, T5, S1 and S6, respectively. The growth of *T. harzianum* isolates (estimated as colony diameter) was significantly increased in all *T. harzianum* mutants except MT3 and MD5 mutants, CMCase activity was increased from 13.2, 16.2, 10 and 15.8 U mL^{-1} mg^{-1} protein in the wild isolates T3, T5, S1 and S6 to 18.6, 20.3, 17.7 and 22.5 U mL^{-1} mg^{-1} protein in the mutants MT3, MT5, MS1 and MS6, respectively. Chitinase activity was also increased in these mutants to 88.4, 132.3, 86.1 and 136.7 U mL^{-1} mg^{-1} protein, compared to 82.7, 93.2, 74.5 and 103.2 U mL^{-1} mg^{-1} protein in their wild, respectively. As comparison between wild types and mutants, the maximum growth was 5.2 and 4.8 cm (as colonies-diameters) by MT5 in present of 25% of the recommended field rate of Topsin® and Benomyl®, compared to 3.3 and 2.2 cm in the wild type T5, respectively, followed by MS6, in which, themaximum growth in this mutant was 4.8 and 4.2 cm in the same concentration of these two fungicides,compared to 3.4 and 2.5 cm in the wild type S6, respectively.The mutants MT5 and MS6 developedfrom UV-irradiation treatment, showed as promising *T. harzianum* mutants through their antagonistic activity (at mycoparasitism level), fast growth, higher chitinase activity and growth in 25% of the recommended field rate of Elsa® (Carbendazim 50%,

Dupont de Nemours, France), 25 and 50% of the recommended field rate of Diathane M-45® (Dithane, Dow Agrosciences, Rohm & Haas) and Mizab® (Mancozeb 80%, Agria, Bulgaria) and all concentrations of Topsin® (Thiophanate methyl 70%, Nippon-Soda, Japan) and Benomyl® (Benomyl 50%, United Phosphorus, India) with complete inhibition of pathogenic fungi *F. oxysporum* and *R. solani* (Abdullah 2011). Although not discussed by Abdullah (2011), such mutations of a vast range of effects may impose their multiple impacts because of the resulted changes in transcription factors, or in the secretion potentials of cell wall/plasma membrane.

Sometimes, apparently unexpected results have been got with the application of mutagens in the improvement of biological control fungi. For example, the results of the diagnostic test designed by Cañas (2004) indicated that there were not changes in the β-tubulin gene level. Nevertheless benzimidazole resistance was conferred by point mutations in the β-tubulin gene in most phytopathogenic fungi. However, exceptions have also been noticed through via site-directed mutagenesis, a mutation that confers benomyl tolerance to other fungi does not impart resistance in *Trichoderma viride* (Mukherjee et al. 2003). Kawchuk et al. (2002) established that the amino acid sequences of the β-tubulin genes from several thiabendazole-resistant and sensitive isolates were identical in *Gibberella pulicaris*. This analysis confirmed that the β-tubulin gene was not linked to thiabendazole resistance. These results suggest that there must be other genomic regions involved in the resistance to benzimidazoles, but the exact molecular mechanism for this resistance is still unknown. DNA changes were observed in *T. asperelloides* lines T-19 and T-84 treated with thiabendazole (benzimidazole group). However, these apparently inept and ambiguous results are clearly in accordance with the results previously obtained by Allen and Gottlieb (1970) that the terminal electron transport system was probably the primary site of inhibition with thiabendazole.

Genetic engineering is more direct approach, but it requires primary information about the gene and genetic construct applied, and about the codon preference of the biological control fungus. Also, this approach needs more accurate and professional work, and more abundant and in the same time more expensive materials and instruments. Disregarding other factors that determine the success of the approach, targeted insertion of the applied genetic construct is also very important. If the insertion of the genetic construct occurs randomly, the integration might lead to the disruption of the nucleotide sequences which were vital for the transformed fungus, and to unwanted defected transformants. Therefore, targeted insertion is required to overcome this problem, a task that requires information on the genetic map of the fungus of interest. Genetic transformation of fungi has been applied in the development of fungicide-resistant fungal transformants (Pfeifer and Khachatourians 1992; Inglis et al. 1999).

Unfortunately, the traditional method of confrontation test applied in order to study the interaction of biological control fungi and pathogenic fungi and oomycetes in vitro may lead to inaccurate results. Recently a new method has been introduced that makes it possible to statistically analyze the data, to collectively study the different mechanisms of antagonism (production and release of soluble as well as volatile antimicrobials, parasitism, and competition) in detail in a single test,

to determine the effect of studied conditions on the resistance of the fungus that is biologically controlled as well as on the biological control potential of a biological control fungus, and to synchronously consider both temporal points and growth inhibition. The method seems very useful in the studies on the effects of various exterior (such as physical and chemical environmental factors, growth media, agrochemicals, formulation components) as well as interior factors (genotypes, genetic mutations, adaptation-related changes, and transgenes) on the biological control potential of mycoparasitic fungi like *Trichoderma* (Pakdaman et al. 2013a, b). The method has successfully been applied in the comparative screening of *Trichoderma* isolates and its sensitiveness as well as preciseness have been proved compared to the traditional confrontation (dual culture) test (Toghueo et al. 2016).

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Ecology, Population Biology and Management of Chilli Anthracnose

Pallavi Rai, Prem Lal Kashyap, Sudheer Kumar, Alok K. Srivastava and Mala Trivedi

Abstract Anthracnose caused by *Colletotrichum* spp. induces extensive damage to chilli, *Capsicum annuum* L. Management of chili anthracnose is done by integrated management practices such as cultural, chemical and biological control. Fungicide applications are only partially effective under environmental conditions that favour pathogen infection. Fungicides are also costly and hazardous to the environment. The use of resistant cultivars is the most effective means of controlling crop diseases. Unfortunately, no resistant cultivars of chilli have been developed and commercialized so far. Therefore, better understanding of pathogen's lifestyle, ecology and population biology can provide valuable information required to develop targets for developing resistant varieties of chilli against the pathogen. This article reviews the ecology, population biology and management of chilli anthracnose.

Keywords Anthracnose · Chilli · Colletotrichum · Diagnosis · Epidemiology Management

1 Introduction

Anthracnose is an economically important disease that affects chilli (*Capsicum annuum* L., family Solanaceae) production worldwide. The disease affect almost all aerial parts of the plant, primarily, causes fruit rot at both green and red fruit stages,

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E. Lichtfouse (ed.), *Sustainable Agriculture Reviews 31*, Sustainable Agriculture Reviews 31, https://doi.org/10.1007/978-3-319-94232-2_6

hence is also known as ripe fruit rot of chilli (Saxena et al. 2016). Under congenial environment, characteristic symptoms of anthracnose disease appear on the ripe chilli fruit, which appear as sunken circular or angular lesions (Fig. 1). Often multiple lesions coalesce to form severe fruit rot. Generally, the lesions are characterized by the presence of black colored spots in concentric rings at maturity. Initially, orange to pink conidial masses may be visible on the fruit surface. The dark spots when observed under microscope are the acervuli structures containing setae hairs entrapping the conidia of the pathogen. Further, the pathogen produces micro sclerotia in plant debris, seed and soil, which act as mode of survival under unfavorable conditions (Manandhar et al. 1995). The pathogen infects all parts of the host plant, including stems and leaves (Fig. 1). Lesions on stems and leaves appear as small sunken grayish brown spots with dark margins, further on which development of acervuli in concentric rings could be easily seen. The disease is seed-, soil-, water- and air-borne and hence may lead to damage at the seedling stage or on the aerial parts of the plants. Several species of *Colletotrichum*

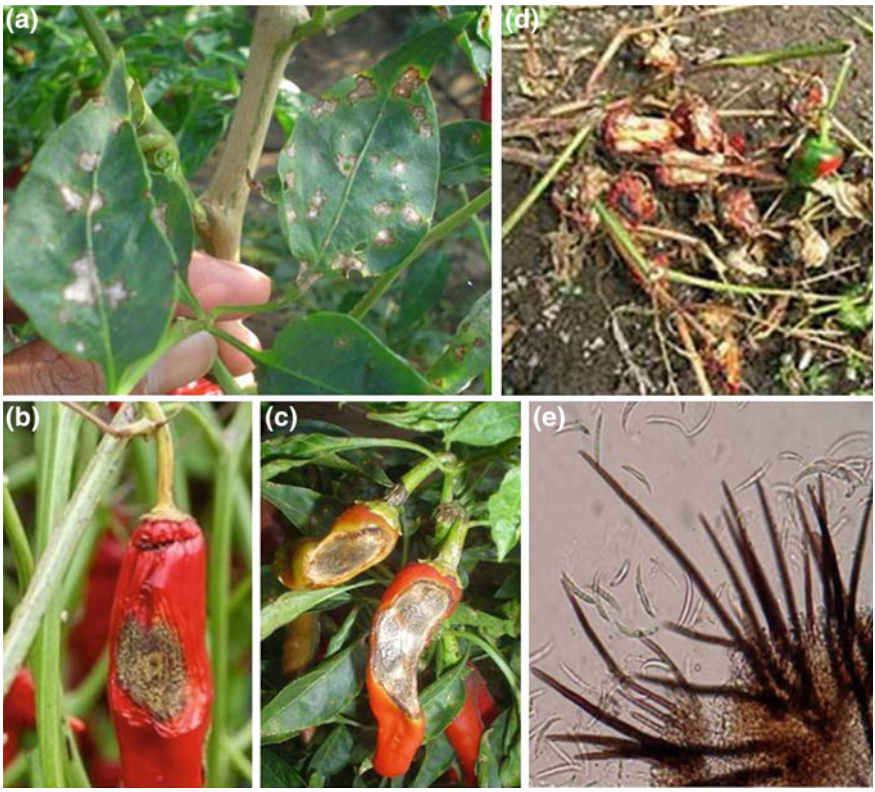

Fig. 1 Characteristic features of chilli anthracnose. **a** Irregular brown coloured spots on leaves; **b** spores arranged in concentric rings; **c** symptom are extending in long axis; **d** die-back symptoms observed under field; **e** acervulli form on infected plant tissue

associated with the chilli anthracnose include: *C. fructicola* and *C. siamense* in India (Sharma and Shenoy 2014); *C. gloeosporioides* in Korea (Kim et al. 1999), Thailand (Than et al. 2008), Indonesia (Voorrips et al. 2004); *C. truncatum* in Australia, China, India, Thailand (Sharma et al. 2005; Ranathunge et al. 2012; Diao et al. 2015, 2017); *C. acutatum* from almost all chili-growing countries, including China, India, Korea, New Zealand, Sri Lanka, Thailand, the USA and Indonesia (Simmonds 1968; Harp et al. 2008; Than et al. 2008; Damm et al. 2012b); *C. coccodes* in New Zealand and India (Johnston and Jones 1997; Sharma et al. 2011; Cannon et al. 2012); and *C. queenslandicum*, *C. simmondsii* and *C. siamense* in Australia (De Silva et al. 2016). An estimated annual loss of about 29.5%, amounting US$491.67 million has been reported from India alone (Garg et al. 2014). In India, an estimated loss of 10–54% has been reported in yield of the crop due to the anthracnose disease (Saxena et al. 2016). Besides this, significant losses also reported from other parts of the world (Table 1). The loss is high owing to the post and pre harvest involvement of the pathogen causing a loss of 10–80% of the marketable yield of chilli fruits (Than et al. 2008).

Impact on global economic loss by anthracnose disease of chilli has triggered extensive studies on diverse aspects of the biology of the pathogen for better understanding of its infection process and host interaction mechanisms. Anthracnose causes extensive pre- and post-harvest damage to chili fruits, causing anthracnose lesions. Even small anthracnose lesions on chilli fruits reduce their marketable value (Ramdial and Rampersad 2015; Ramdial et al. 2016). Many post-harvest diseases of fruit exhibit the phenomenon of quiescence in which symptoms do not develop until the fruit ripens. *Colletotrichum* species are the most important pathogens that cause latent infection (Jeffries et al. 1990). Appressoria are known to form adhesive disks that adhere to plant surfaces and remain latent until physiological changes occur in fruits (Bailey and Jeger 1992). Appressoria that form on immature fruits may remain quiescent until ontogenic changes occur in the fruits (Prusky and Plumbley 1992). Anthracnose disease can occur on leaves, stems, and both pre- and post-harvest fruits (Gautam 2014). Typical fruit symptoms are circular or angular sunken lesions, with concentric rings of acervuli that are often wet and produce pink to orange conidial masses (Fig. 1). Under severe disease pressure, lesions may coalesce. Conidial masses may also occur scattered or in concentric rings on the lesions (Roberts et al. 2001; Saxena et al. 2016). There is a distinction between anthracnose on immature fruit caused by *C. acutatum*, which has been termed “early anthracnose”, and disease caused by *C. gloeosporioides* sensu lato on mature red fruit (Harp et al. 2014; Lewis-Ivey et al. 2004). *Colletotrichum* species infecting chilli have a high degree of pathogenic variability. Therefore, knowledge of population genetic structure, levels of intra species divergence, gene flow among wide geographic populations of the species help in understanding of biogeographic history, evolutionary and adaptive potential of the pathogenic species (McDonald 1997; Rampersad et al. 2016). Information on variation in the species at wide geographical level is a prerequisite for the development of disease management strategies i.e. identification of resistance sources, predicting resistance breakdown, development and deployment of disease resistant varieties and in streamlining cultural practices (McDonald and Linde 2002;

Table 1 Yield losses due to *Colletotrichum* spp. associated with anthracnose of chilli in different parts of the world

Country	Yield loss (%)	Species associated	References
Australia	–	*C. brisbanense*	Damm et al. (2009), De Silva et al. (2016)
Brazil	–	*C. boninense*	Tozze and Massola (2009)
China	–	*C. acutatum*, *C. aenigma*, *C. cliviae*, *C. endophytica*, *C. hymenocallidis*, *C. incanum*, *C. karstii*, *C. viniferum*, *C. fioriniae*, *C. fructicola*, *C. gloeosporioides*, *C. scovillei*, *C. truncatum*	Xia et al. (2011), Diao et al. (2015, 2017), Liu et al. (2016)
India	10–54	*C. acutatum, C. capsici, C. coccodes, C. dematium, C. gloeosporioides, C. siamense*, *C. karstii*, *C. fructicola*	Kaur and Singh (1990), Lakshmesha et al. (2005), Ramachandran and Rathnamma (2006), Ranathunge et al. (2012), Saxena et al. (2014), Saini et al. (2016), Pakdeevaraporn et al. (2005), Roat et al. (2009), Sharma and Shenoy (2014)
Indonesia	75	*C. acutatum, C. gloeosporioides, C. nymphaeae, C. capsici*	Kusandriani and Permadi (1996), Damm et al. (2009), Voorrips et al. (2004)
Korea	10–15	*C. acutatum*, *C. gloeosporioides*, *C. coccodes*, *C. dematium*	Park and Kim (1992), Kim and Park (1988), Byung (2007), Kim et al. (2008a, d)
Malaysia	50	*C. truncatum*	Sariah (1994), Mahmodi et al. (2013)
Mexico		*C. capsici*	Damm et al. (2009)
New Zealand	–	*C. karstii*, *C. novae-zelandiae*, *C. nigrum*, *C. coccodes*	Damm et al. (2012b), Liu et al. (2013)
Pakistan	–	*C. truncatum*	Tariq et al. (2016)
Papua New Guinea	–	*C. capsici*, *C. gloeosporioides*	Pearson et al. (1984)
Sri Lanka	21–47	*C. capsici, C. gloeosporioides*	Rajapakse (1998), Damm et al. (2012a)
Taiwan	–	*C. acutatum*, *C. capsici*, *C. gloeosporioides*	Manandhar et al. (1995)
Thailand	50–100	*C. acutatum*, *C. capsici*, *C. gloeosporioides*, *C. siamense*, *C. scovillei*, *C. asianum*	Than et al. (2008), Damm et al. (2009), Phoulivong et al. (2012), Weir et al. (2012), Pakdeevaraporn et al. (2005)
United States	–	*C. capsici*, *C. gloeosporioides*, *C. acutatum*, *C. coccodes*	Harp et al. (2008)

(continued)

Table 1 (continued)

Country	Yield loss (%)	Species associated	References
Vietnam	20–80	*C. acutatum*, *C. capsici*, *C. gloeosporioides*, *C. nigrum*	Don et al. (2007)
Zimbabwe	–	*C. nymphaeae*	Damm et al. (2009)

Rampersad et al. 2013). Several methods have been practiced to control the diseases such as inter-cropping, adjustment of time of sowing, rouging, physical methods like seed selection, hot water treatment, solar heat treatment and chemical treatments etc. Use of plant extracts like neem (*Azadirachta indica*), mahogany (*Swietenia mahagoni*), koromcha (*Carissa carandas*), garlic (*Allium sativum*), ginger (*Zingiber officinale*), marigold (*Tagetes erecta*), allamanda (*Allamanda cathartica*) (Ashrafuzzaman and Khan, 1992; Freeman et al. 2001; Rashid et al. 2015; Masuduzzaman et al. 2008; Amin et al. 2009; Perello et al. 2013; Kabir et al. 2014) and the use of bio-fungicide containing strains of *Trichoderma* spp. (Kashyap et al. 2017a, Imtiaj and Lee 2008, Solanki et al. 2011, Srivastava et al. 2012, Rai et al. 2016a), *Bacillus* spp. (Fitsum et al. 2014; Solanki et al. 2015; Singh et al. 2014) and *Pseudomonas* spp. (Solanki et al. 2014, Sharma et al. 2018) might be useful in controlling different fungal diseases of various agricultural crops. The effect of certain plant extracts on the management of some diseases of chilli such as dieback, fruit rot and anthracnose were reported (Roat et al. 2009). Interestingly, some entomopathogenic fungi, such as *Cordyceps sobolifera*, have been reported for use as a biocontrol agent against *C. gloeosporioides* and *C. miyabeanus* (Imtiaj and Lee 2007; Jaihan et al. 2016). Overall, the present article is not intended to be a thorough review of the literature on general aspects of anthracnose disease of chilli. Rather, the current prospects for its management based on the critical assessment of available knowledge on the disease ecology, epidemiology, and control strategies and measures have been discussed.

2 Pathogenic Diversity in the Pathogen Populations

Colletotrichum species belongs to the Kingdom Fungi; Phylum Ascomycota, Class Sordariomycetes; Order Phyllachorales; and Family Phyllachoraceae. Anthracnose of chilli was first reported from New Jersey, USA, by Halsted (1890) who described the causal agents as *Gloeosporium piperatum* and *Colletotrichum nigrum*. These taxa were then considered as synonyms of *C. gloeosporioides* by von Arx (1957). Kim et al. (2004) reported that different species of *Colletotrichum* affect different organs of the chilli plant; for instance, *C. acutatum* and *C. gloeosporioides* infect chilli fruits at all developmental stages, but not usually the leaves or stems, which are mostly damaged by *C. coccodes* and *C. dematium*. Leaf anthracnose of chilli

seedlings attacked by *C. coccodes* was first reported from Chungnam Province of Korea in 1988 (Hong and Hwang 1998).

Sharma et al. (2005) reported the existence of fifteen pathotypes of *Colletotrichum capsici* from Northern India based on quantitative differences in lesion development on inoculated fruit of *Capsicum annuum* genotypes. Than et al. (2008) showed pathotype differences within *Colletotrichum acutatum* isolates from infected strawberry and chilli fruit. Isolates from chilli were able to infect inoculated fruit of the resistant *Capsicum chinense* (cv. PBC932), whereas isolates from strawberry were unable to infect this genotype. Both isolates were able to infect the susceptible chilli (cv. Bangchang). Pathotype in *Colletotrichum* were differentiated based on the size of lesions that developed on inoculated fruit (Than et al. 2008). However, fruit size and shape of genotypes from different *Capsicum* spp. vary considerably; therefore, to standardize host reactions between *Capsicum* spp., Kanchana-udomkan et al. (2004) and Pakdeevaraporn et al. (2005) measured host reactions based on lesion size as a proportion of fruit size. The development of disease scales for measuring host reactions that incorporated fruit size and severity of infection (lesion size, presence of acervuli, and necrosis or water soaked tissue) would be appropriate for assisting in screening many isolates across a range of genotypes from different *Capsicum* spp. Three pathotypes (PCc1, PCc2, and PCc3) of *C. capsici* in Thailand have been identified according to differential qualitative infection on *C. chinense* (cv. PBC932 and C04714) fruit by Montri et al. (2009).

Molecular markers have been widely used to examine genetic diversity, genetic structure, and virulence in populations of plant pathogens. Random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), inter simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), and restriction fragment length polymorphism (RFLP), are among the most frequently used genetic markers in population genetic studies (Goswami et al. 2017; Sharma et al. 2014, 2017; Rai et al. 2016b; Kashyap et al. 2015a, b, 2016a, b; Singh et al. 2014; Kumar et al. 2013; Christopher et al. 2013; Madhavan et al. 2010). Despite importance of anthracnose, there have only been a few studies concerning the diversity of *Colletotrichum* species associated with pepper anthracnose. The random amplified polymorphic DNA (RAPD) technique has been widely used to differentiate between *Colletotrichum* species infecting different hosts. Shin et al. (2000) used RAPD-PCR to detect variation among *C. gloeosporioides*, *C. coccodes*, *C. dematium* and *C. acutatum* isolated from Korea and China. Sharma et al. (2005) found a variable population of *C. capsici* causing fruit rot/die back or anthracnose of chilli in the Northwestern region of India based on differential inoculation tests and RAPD analysis. Using similar molecular technique, Ratanacherdchai et al. (2007) analysed eighteen isolates of two species, *C. gloeosporioides* and *C. capsici*, isolated from three varieties of chilli viz., Chilli pepper (*C. annuum*), long cayenne pepper (*C. annuum* var. *acuminatum*) and Bird'seye chilli (*C. frutescens*) and reported clear difference between *C. gloeosporioides* and *C. capsici*. Later on, by using inter simple sequence repeat (ISSR), they obtained two distinct groups of *C. gloeosporioides* and *C. capsici* populations associated with chilli in Thailand (Ratanacherdchai et al. 2010). Genetic diversity was correlated with geographic

distribution, but, there was no clear relationship between genetic diversity and pathogenic variability among isolates of *C. gloeosporioides* and *C. capsici*. Sangdee et al. (2011) analysed the variability among *C. capsici* population from Northern Thailand by cultural morphology, sensitivity to carbendazim, virulence pattern and RAPD analysis and reported huge variation among the isolates. Saxena et al. (2014) also analysed genetic variability and population structure of *Colletotrichum* species (*C. capsici* and *C. acutatum*) in the North-eastern region of India using RAPD and ISSR markers. Interestingly, RAPD marker system efficiently separated the isolates at species level, while ISSR marker system vaguely grouped the isolates on the basis of their geographic origin. Ranathunge et al. (2009) developed and optimized twenty seven sequence-tagged microsatellite site (STMS) markers for *C. capsici* isolates collected from India, Sri Lanka and Thailand. In contrast, examination of *C. acutatum* from a limited collection of isolates from chilli anthracnose epidemic areas in Brazil, Korea, Taiwan region and the US indicated that they belonged to a single group based on mtDNA-RFLP analysis (Correll et al. 2007). They also found that most of the examined isolates belonged to a single vegetative compatibility group (VCG). As the sexual stages of *C. acutatum* have never been found in nature (Wharton and Diéguez-Uribeondo 2004), the asexual reproduction mode of *C. acutatum* is likely to be an important role in its population structure. Than et al. (2008) differentiated isolates of chilli anthracnose from Thailand into three species: *C. acutatum*, *C. capsici* and *C. gloeosporioides*, based on morphological characterization, sequencing based on rDNA-ITS region and partial beta tubulin gene and pathogenicity testing. Hong and Kim (2007) reported that Korean isolates of *C. acutatum* were phylogenetically separated from the global groups of *C. acutatum* A1 to A8 based on the partial beta-tubulin 2 (exons 3–6) gene sequence. Restriction fragment length polymorphisms (RFLP) of ITS region by *AluI*, *RsaI* and *BamHI* have also been employed to differentiate *Colletotrichum* species causing chilli anthracnose in Taiwan region (Sheu et al. 2007). Four species of *Colletotrichum* were identified by ITS-RFLP fingerprinting and observation of undistinguishable isolates of *Colletotrichum* from their studies indicated the various inter- and intra-species variations in *Colletotrichum* species. Recently, Katoch et al. (2016) performed metageographic population analysis of *C. truncatum* associated with chilli fruit rot and other hosts using ITS region nucleotide sequences. Study suggested that *C. truncatum* may have expanded its distribution in the past with the co-migration of the host plants as one of the haplotypes found predominant across the host and geographical regions. Moreover, phylogenetic study suggested that *C. truncatum* is a single species with no distinct lineage as all the isolates originating from 23 countries clustered together in a single clade. These results clearly indicate a high level of gene flow among the geographically distinct populations of the pathogen. Besides this, Median joining network analysis of various *C. truncatum* isolates revealed that some of the haplotypes were population specific and unique whereas some of the populations suggests their haplotype specific evolution (H1 and H7)) and high gene flow among populations. The haplotypes H2-H4, H5-H6 and H8-H9, from North India, South India and Mexico seem to have originated from the predominant haplotype H1 as

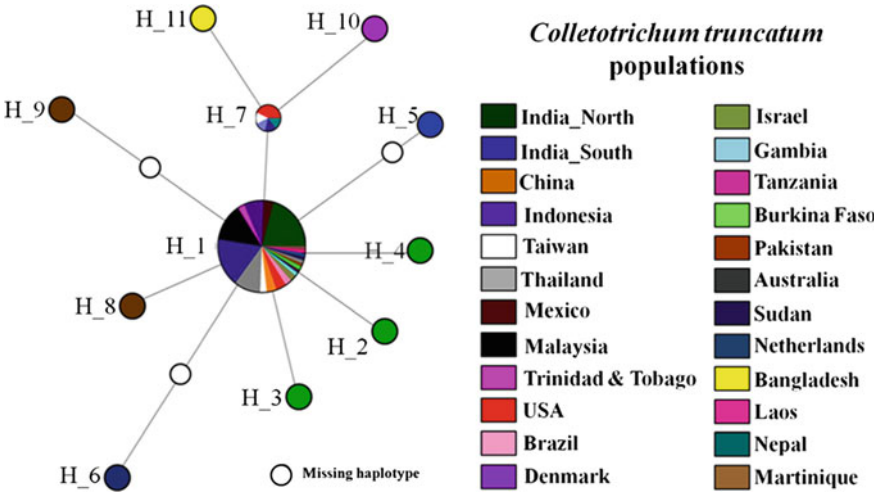

Fig. 2 Median joining network of rDNA haplotypes of *C. truncatum* populations. Size of the circle is related with frequency of haplotypes (*Source* Katoch et al. 2016)

the same was also detected in *C. truncatum* populations of these regions. The haplotypes H10 and H11 are specific to the populations from Trinidad and Tobago and Bangladesh, respectively. These populations might have originated from haplotype H7 representing *C. truncatum* isolates from Nepal, South India, Thailand, Taiwan and the USA. These results suggest that haplotype H11 comprising of Bangladesh isolates most likely were introduced from South India, Nepal or Thailand than from Taiwan and USA due to geographic proximity of the these regions to Bangladesh. By contrast, the invasion of *C. truncatum* in Trinidad and Tobago seems to be more likely from USA than the other countries harboring H7 haplotype (Fig. 2). These findings were further supported by Rampersad et al. (2016) while analysing the diversity and phylogenetic relationships of *C. truncatum* infecting pepper by comparing ITS and β-tubulin gene sequences. AMOVA and STRUCTURE analyses suggested significant genetic variation within populations relative to among populations. A consensus Maximum Likelihood tree based on β-TUB gene sequences revealed earlier divergence of Trinidad isolates than populations collected from India, USA, Malaysia and Thailand, providing clues of directional migration.

3 Pathogen Ecology and Epidemiology

Colletotrichum species can survive in and on seeds as acervuli and micro-sclerotia (Pernezny et al. 2003). Survival of mycelia and stroma in colonized chilli seeds had also been reported (Manandhar et al. 1995). It has been shown that the pathogen

readily colonizes the seed coat and peripheral layers of the endosperm even in moderately infected seeds. Heavily colonized seeds had abundant inter- and intracellular mycelia and acervuli in the seed coat endosperm and embryo, showing disintegration of parenchymatous layers of the seed coat and depletion of food material in endosperm and embryo (Chitkara et al. 1990).

Fungi can overwinter on alternative hosts such as other solanaceous or legume crops, plant debris and rotten fruits in the field (Pring et al. 1995). *Colletotrichum* species naturally produce micro-sclerotia to allow dormancy in the soil during the winter or when subjected to stressful conditions and these micro-sclerotia can survive for several years (Pring et al. 1995). During warm and wet periods, conidia from acervuli and micro-sclerotia are splashed by rain or irrigation water from diseased to healthy fruit and foliage. Diseased fruit acts as a source of inoculum, allowing the disease to spread from plant to plant within the field (Roberts et al. 2001).

Development of chill anthracnose can be influenced by the aggressiveness of pathogenic races, inoculum density in soil and environmental conditions. Environmental conditions play a crucial role in the development of disease epidemics. The rainfall intensity, duration and crop geometry and the dispersal of inoculum possibly lead to different levels of disease severity (Than et al. 2008; Saxena et al. 2016). The effects of temperature often interact with other factors, such as leaf surface wetness, humidity, and competitive microbiota (Royle and Butler 1986). The duration of the surface wetness, however, appears to have the most direct influence on the germination, infection and growth of the pathogen on the host. Usually, warm and humid environmental conditions support the infection and spread of the disease. Temperature around 27 °C with high relative humidity (>80%) have reported to be the most optimum conditions for successful establishment of the disease (Roberts et al. 2001). Besides this, the development of the disease also depends on the resistance level of host cultivar.

Association of high relative humidity or rainfall frequency and high temperature with anthracnose epidemics on chilli plants has been recognized, but relative humidity was found to be the most important climatic parameter related to anthracnose development of chilli (Kim and Park 1988; Saxena et al. 2016). Inocula for epidemics can come from various sources such as residues of previous crops or soil and surrounding alternate hosts. Therefore, the degree of anthracnose incidence could vary according to seasonal condition.

4 Infection Process and Disease Cycle

Colletotrichum employs different strategies for causing infection to the host plant which initiate from the intracellular hemibiotrophic mode to the intramural necrotrophic mode of nutrition (Bailey and Jeger 1992). Liao et al. (2012) has reported an intermediate stage showing partial endophytic life style of the pathogen before adapting to the necrotrophic mode of nutrition in the host plant. Different species of

this genus exhibit distinct mechanism of infection depending on the host infected. For instance, Peres et al. (2005) reported the epiphytic or endophytic mode of survival of *C. acutatum* in an orchard infected with the bitter rot of apple. Also, intramural necrotrophy by *C. capsici* was reported by Pring et al. (1995) while infecting cowpea leading to subsequent necrosis caused due to dissolution of cell wall structures. The biotrophic phase of infection by *C. capsici* is also well studied in the infection caused to broad bean or lentil characterized by the presence of large multilobed, multi septate, vesicular primary hyphae (Latunde-Dada and Lucas 2007).

Preliminary infection starts with the attachment of the conidia to the host surface preceded with its germination and production of adhesive appressoria followed with its penetration into the host epidermis (O'Connell et al. 2000; Perfect et al. 1999) (Fig. 3). This is further accompanied by the growth and colonization of plant tissue by the fungus, resulting in the formation of specific symptom (Prusky et al. 2000; Prusky 2011). The pathogen sometimes remains in quiescent state in the form of appressorial structures in tissues of unripe fruits and cause infection after the fruits ripe or mature (Than et al. 2008). A dendroid structure composed of multiple, thick-walled hyphal branches with swollen or sharp ends from the penetration pore of the appressorium has been reported to be an intermediate structure which penetrates the host cuticle layer and infect the epidermal cells during *C. acutatum* infestation in chilli (Liao et al. 2012).

Though the pre-penetration mechanisms exhibited by *Colletotrichum* species appear somewhat related to each other, the post penetration events such as spore adhesion, melanization and cutinization hold certain disparity. Based on the previous studies, four kinds of infection strategies with varied hosts have been observed in *C. acutatum* host pathosystem (Peres et al. 2005). First is the biotrophic growth of the pathogen, where the formation of appressoria from the conidia is followed by the formation of secondary conidia which further infects and spreads the pathogen inside the host leaves. The second is the subcuticular intramural necrotrophy with the development of wide and swollen hyphae in the anticlinal and periclinal walls of host epidermal cells. The third strategy is the hemibiotrophic mode of infection where the pathogenic hyphae interact with the infection vesicles within the host cells. The fourth type of interaction is the combination of hypertrophic and subcuticular intra and intracellular development of the pathogen generally observed during infestation of almond leaves and fruits.

As far as studies related to infection and colonization by *Colletotrichum* species i.e., *C. gloeosporioides* on susceptible chilli cultivars is considered, infection vesicle has been found during the infection (Kim et al. 2004). An increased number of small vacuole with the condensed cytoplasm in the epidermal cells followed with cell destruction extending to the sub epidermal cells of the plant due to the action of pathogen enzyme has been noticed during the early stages of infection in chilli plant. During the later stages, inter and intra cellular colonization of the pathogen occurs indicating the governance of necrotrophic mode of fungal growth. The pictorial representation of different stages of infection of *Colletotrichum* spp. in chilli and disease cycle has been shown in Figs. 3 and 4.

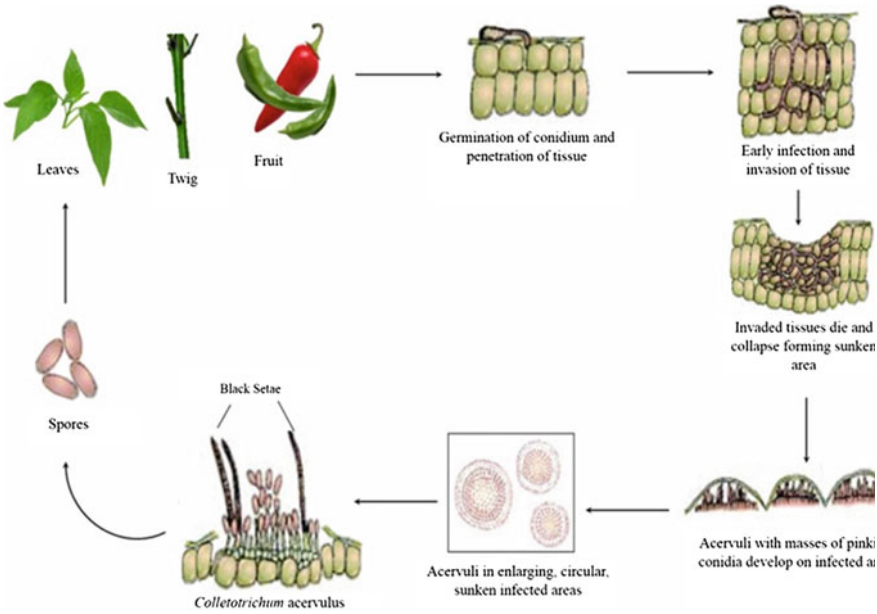

Fig. 3 Cycle of the anthracnose disease of chilli (*Capsicum annuum* L.) caused by *Colletotrichum* spp. (*Source* Saxena et al. 2016)

In case of *C. capsici* infections on chilli two different pathways has been followed: (i) invasion through the seed coat and (ii) invasion through the openings of the testa (Than et al. 2008; Saxena et al. 2016). Jewsakun (1978) mentioned that *C. capsici* caused root rot of seedlings; however, whether chilli anthracnose can be seed-transmitted, and the role of seed infection and seedling infection in pre- and post-emergence damage of chilli plants are still doubtful. It is also observed that *C. acutatum* infect chilli seeds either by reducing the germination rates or by causing damping off of seedlings. However, very little is known about the disease cycle of the pathogens that cause anthracnose in chilli. There are still several questions to be answered.

5 Management

Effective management of chilli anthracnose have been relied on the exclusion of the pathogen as well as by reducing the amount and/or efficiency of the initial inoculum. Disease control measures for such aim include: (i) use of pathogen-free seeds; (ii) site selection to avoid sowing into high risk soils; (iii) reduction or elimination of inoculum in soil; (iv) use of resistant cultivars; (v) protection of healthy seeds from resident inoculum by means of seed treatment with fungicides or biocontrol

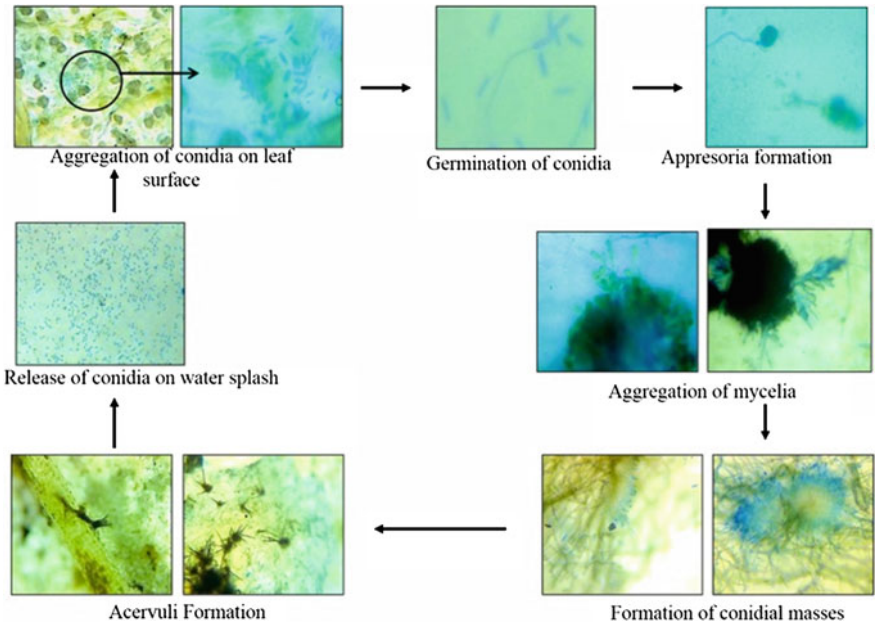

Fig. 4 Different stages of infection by the *Colletotrichum* spp. on chilli leaf as seen under microscope (*Source* Saxena et al. 2016)

agents; and (vi) choice of cropping practices to avoid conditions favoring infection of the plant by the pathogen. The disease has been successfully managed if these disease control measures are used within an integrated manner whereby their use is combined either simultaneously or in a sequence (Than et al. 2008; Kashyap and Dhiman 2010; Sahitya et al. 2014; Saxena et al. 2016, Oo and Oh 2016).

5.1 Disease Diagnosis

Early and exact diagnosis is a first step to ensure efficient management of chilli anthracnose. Traditionally, *Colletotrichum* species have been identified by a range of cultural and morphological characteristics, such as conidial morphology, presence or absence of setae, fungicide sensitivity, colony colour and growth rate (Saxena et al. 2016; Masoodi et al. 2013; Srinivasan et al. 2014; Kumar et al. 2015a, b). Although valuable, these criteria alone are not always adequate as morphological characteristics may vary under different environmental conditions (Cannon et al. 2000). Standard blotter test and pathogenicity tests are often used for detection of *C. capsici* in chilli seed samples (ISTA 2005). However, these methods are time consuming, laborious and require extensive taxonomic knowledge.

In recent years, polymerase chain reaction (PCR)-based methods have emerged as major tools for the diagnosis of plant diseases, although the role of the traditional assay continues to be an important method for plant disease diagnosis (Kashyap et al. 2016c, 2017b; Sharma et al. 2017). Torres-Calzada et al. (2011) designed a primer set based on the sequences of the ribosomal internal transcribed spacer (ITS1 and ITS2) regions of *C. capsici* and standardized the detection of *C. capsici* in infected plant tissues using PCR assay. The sensitivity of detection with this PCR method was 10 pg of genomic DNA from the pathogen. Srinivasan et al. (2014) developed conventional and real-time PCR protocols for specific and sensitive detection of *C. capsici* in chilli using sequence characterized amplified region (SCAR) marker. The SCAR primers successfully amplified ~250 bp amplicon from DNA derived from *C. capsici*-infected chilli fruits. The sensitivity of developed assay was 1 pg of purified *C. capsici* DNA template and 25 ng of DNA from *C. capsici*-infected chilli fruits. The developed assay may be highly useful in detection of this important pathogen in chilli seeds and fruits in plant quarantine laboratories.

5.2 Cultural Practices

Several cultural practices have been reported to manage chilli anthracnose due to the special etiology of the pathogen. These precautionary measures are implemented to reduce the rate of infection and minimise the inocula pressure even before fruits are mature and harvested. Than et al. (2008) and Ali et al. (2016) in their review reported that, chilli seeds free of pathogen should be planted and weeds eliminated. They also reported that rotating crops which are not alternative hosts to *Colletotrichum* spp. after every 2–3 years is very effective for controlling chilli anthracnose. Other clean crop and sanitation practices including the provision of good drainage systems on the field to channel out waste water during irrigation regimes, on-farm fruit disinfection such as fruit washing at packing houses and finally removal of plant debris which may serve as source of inoculum (Than et al. 2008). If there was history of disease in a particular field, then other crops should be rotated in isolation from other solanaceous plant for at least alternate years (Roberts et al. 2001). By doing so, the life cycle of the pathogen on the field to begin an infection process is disrupted and the chances of infection are reduced. At the end of the growing season, it is also recommended to deep plough to completely cover diseases plants or removing infected plant debris from the field (Sharma and Kulshrestha 2015). Early planting of chilli or planting cultivars that bear fruit within a short ripening period to allow the fruit to escape fungal infection is also recommended. Other alternative sanitation practices such as weeding, removal of infected or wounded fruits should be carried out regularly to prevent the pathogens from using such wounds as sites of infection.

5.3 Plant Extracts

Antimicrobial or metabolic compounds synthesized by plants are one of the best options to controlling plant diseases. In chilli, several workers have shown the efficacy of plant extracts against *Colletotrichum* spp. (Begum et al. 2007; Nduagu et al. 2008; Johnny et al. 2011; Saravanakumar et al. 2011; Ajith et al. 2012; Rashid et al. 2015). Ajith et al. (2012) have shown that botanicals or plant extracts from *Catharanthus roseus*, *Coleus aromaticus*, *Manilkara zapota* and *Azadirachta indica* confer antifungal effects on the radial mycelial growth of *C. capsici*. Further, Singh and Khirbat (2014) reported the efficacy of aqueous extract of three wild plants viz., *Albizza lebbeck*, *Acacia Arabica* and *Clerodendrum infortunatum* to control chilli fruit rot. Recently, Alves et al. (2015) reported the efficacy of 1% aqueous or 20% ethanol plant extracts to control bell pepper anthracnose caused by *C. acutatum*. In this study, 6% aqueous garlic, mallow and ginger extracts reduced disease severity by more than 97%. Even though recent research suggests the use of these plant extracts as bio-fungicides, but still more studies on their efficacy in the controlling of chilli anthracnose need to be performed under field conditions.

5.4 Essential Oils and Polymers

Essentials oils are basically concentrated hydrophobic liquid containing a mixture of volatile aroma compounds resulting from plant secondary metabolism and have long been known to provide effective control over phytopathogens. Several studies have suggested that the use of essential oil to control *Colletotrichum* spp. in several crops (Bakkali et al. 2008; Ali et al. 2016). A study by Ali et al. (2014a) revealed that cinnamon oil (0.1%) in combination with Brazilian green propolis (5%) was fungicidal (100% inhibition) on the mycelial growth and conidial germination of *C. capsici*. Further, Ali et al. (2014b) also assessed the efficacy of only ethanol extracted propolis (EEP) on *C. capsici* and observed that mycelial growth inhibition was positively related to the EEP concentration and significantly delayed anthracnose development (Ali et al. 2014b). Application of lemongrass essential oil (0.5%) with chitosan (1.0%) as edible coating, significantly maintained fruit quality of *C. annuum*. However chitosan alone was more effective in extending the shelf life (Kashyap et al. 2015a, b; Ali et al. 2015). It has been proposed that, cinnamon oil which contains cinnamaldehyde penetrates into the appressoria of fungus to inhibit its growth thereby slowing down the anthracnose (Maqbool et al. 2010). Citral aldehyde or citral present in lemongrass oil is attributed to cause irreversible cell membrane disruption through cross-linkage reaction leading to leakage of electrolytes and subsequent depletion of amino acids and sugars (Inouye et al. 2000), whiles other oil may selectively be inserted into the lipid rich portion of the cell membrane, thereby disturbing membrane function (Regnier et al. 2008; Ali et al. 2016).

In chilli, chitosan (0.8%) was effective as seed treatment in reducing *Colletotrichum* spp. infection and improving seedling growth of chilli cv. 'Jinda' (Photchanachai et al. 2006), since the fungus is seed-borne and infected seeds had reduce germination and their seedlings had poor vigor. Recently, chitosan degraded by gamma irradiation to obtain irradiated oligochitosan significantly inhibited the mycelial growth of *C. gloeosporioides* isolated from chilli (Kewsuwan et al. 2014). Edirisinghe et al. (2012) reported that chitosan (1.5%) decreased anthracnose severity in green bell pepper (*C. annuum* cv. 'Meno') caused by *C. capsici* by 76% after 28 days of storage. It has been proposed that the chitosan coating act as a barrier, limiting the penetration of the fungus and stimulates structural defense reactions such as the formation of hemispherical protuberances along host cell walls, cell wall thickening and occlusion of many intercellular spaces with a fibrillar material in bell pepper (Ghaouth et al. 1997), or acts as a stimulus to induce host defense responses such as activation of defense related enzymes (Bautista-Banos et al. 2006). Other possible mechanism of antifungal action of chitosan includes interaction between positively charged chitosan molecules due to ammonium groups and negatively charged residues on the fungal cell wall surface due to phospholipids head groups, which lead to increase in plasma membrane permeability (Ali et al. 2016).

5.5 Biological Control

The potential for biological control of *Colletotrichum* species had been suggested as early as in 1976 by Lenné and Parbery (1976). Jeger and Jeffries (1988) also stressed the possibilities of biological control of post-harvest fruit diseases by using *Pseudomonas fluorescens*. Antagonistic bacterial strains (DGg13 and BB133) were found to effectively control *C. capsici* (Intanoo and Chamswarng 2007). It is also believed that *Trichoderma* species are able to effectively compete for surface area, thereby reducing pathogen infection success (Kashyap et al. 2017a; Rai et al. 2016b; Maymon et al. 2004; Jeffries and Koomen 1992). *Trichoderma* species have been applied to control *Colletotrichum* species in chilli (Boonratkwang et al. 2007), strawberries (Freeman et al. 2001), and citrus in Belize (Moretto et al. 2001) with concomitant disease reduction. It has been reported that antifungal metabolites (100 mg L^{-1}) secreted from *Trichoderma harzianum* Rifai strain number T-156co5 significantly controlled *C. capsici* isolated from *C. annuum* L. (Warin et al. 2009). Other biological control agents that have been tested for efficacy against *C. acutatum* include *Bacillus subtilis* and *Candida oleophila* (Wharton and Diéguez-Uribeondo 2004) and *Pseudomonas aeruginosa* FP6 (Sasirekha and Srividya 2016). Chanchaichaovivat et al. (2007), reported a significant effect of *Pichia guilliermondii* Wick strain R13 in reducing the disease incidence on *C. capsici* infected chilli fruit to as low as 6.5%. It has been proposed that *P. guilliermondii* strain R13 and other yeasts suppresses *Colletotrichum* spp. through multiple modes of action including nutrient competition, their tight

attachment to host plant resulting in competition for space between biological antagonist and the pathogen fungus, production of killer toxin, possibly induction of plant resistance and production of hydrolytic enzyme (Janisiewicz et al. 2000; Chanchaichaovivat et al. 2008; Nantawanit et al. 2010; Ali et al. 2016). Some studies have also shown that these antagonists secretes an antifungal metabolite (isoharziandione) which inhibits spore germination and germ tube growth of certain plant pathogens (Warin et al. 2009). Recently, biological control of anthracnose of chilli with rhizosphere and rhizoplane fungal isolates from perennial grasses has been reported (Vasanthakumari and Shivanna 2013). Among them, three fungal species *C. globosum*, *T. harzianum* and *F. oxysporum* decreased disease incidence and severity in seedlings and mature plants, and promoted plant growth and increased yield in the greenhouse and field. Besides this, some entomopathogenic fungi, such as *Cordyceps sobolifera*, have been reported for use as a biocontrol agent against *C. gloeosporioides* (Imtiaj and Lee 2007; Jaihan et al. 2016).

5.6 Chemical Control

Use of chemicals is the most common disease management strategy and a practical method to control anthracnose disease. However, fungicide resistance often arises quickly, if a single compound is relied upon too heavily (Staub 1991). A fungicide widely recommended for anthracnose management in chilli is manganese ethylene bisdithiocarbamate (Maneb) (Smith 2000). Chakravarthy and Anil Kumar (1975) recommended that soaking of chilli seeds for 12 h in 0.2% thiram is best way to control seed borne *Colletotrichum* species. The strobilurin fungicides azoxystrobin (Quadris), trifloxystrobin (Flint), and pyraclostrobin (Cabrio) have recently been recommended for the control of chilli anthracnose (Than et al. 2008; Anand et al. 2010). Moreover, various fungicides have been found to be effective, including 0.2% mancozeb, 0.1% ziram, Blitox 50, 0.1% bavistin and 0.5 or 1% bordeaux mixture; benlate, delsene M and azoxystrobin are used for seed dressings (Yu et al. 2009). Additionally, systemic fungicides such as propiconazole, difenoconazole, benomyl (Gopinath et al. 2006; Boonyapipat 2013) have been used in the pre- as well as post-harvest management of chilli anthracnose. It is imperative to note that benomyl and its associated fungicides carbendazim and thiophanate methyl (both of which registered) has raised major health concerns such as causing eye defects, and other birth related effects by disrupting the process of cell division making their use unacceptable and dangerous (APVMA 2009). Additionally, the emergence of resistant strains of *C. capsici* isolates in chilli fruit against benomyl, which were cross-resistant to thiophanate methyl and carbendazim was reported in Malaysia (Sariah 1989). Recently, resistance of *C. truncatum* to benomyl and strobilurin-fungicides (azoxystrobin and kresoxim-methyl) has also been reported in Trinidad (Ramdial et al. 2016; Inada et al. 2008; Hu et al. 2015). Under such circumstances, combined application of *Pseudomonas fluorescens* along with half of the recommended dose of azoxystrobin fungicide has been found effective and

viable option to control fruit rot (Anand et al. 2010), since application of fungicide alone requires three to four sprays for effective control of these diseases with a period of 45–60 days in the field.

5.7 Resistant Cultivars

Resistant varieties are the most practical and cost efficient disease control measure for management of chilli anthracnose. Moreover, use of resistant cultivars would enhance the efficacy of other disease control measures in an integrated management strategy. However, the challenging task of resistant breeding is exceptionally difficult in *Colletotrichum*-chilli pathosystem due to the association of more than one species of the pathogen with the disease along with the differential ability of the pathogenic virulence (Sharma et al. 2005; Than et al. 2008; Montri et al. 2009; Saxena et al. 2014; Suwor et al. 2015). Most of the commercially important chilli cultivars are considered to be anthracnose-susceptible species. Broad spectrum resistance sources have been identified in *C. baccatum* L. (PBC80, PBC81, PI594137, PI497985-1 and PI260550) and *C. chinense* Jacq. (PBC932) (Montri et al. 2009; Park et al. 2009). PBC80 (VI046804) and PBC81 (VI046805) were resistant to most of the isolates, while PBC932 showed resistance to few isolates of *C. acutatum*, *C. capsici* and *C. gloeosporioides* (Benz.) Penz. and Sacc. (Kim et al. 2008b; Than et al. 2008; Park et al. 2009). At AVRDC—The World Vegetable Center (AVRDC), Taiwan, resistance from *C. chinense* PBC932 was successfully transferred to *C. annuum* progressive lines through conventional backcrossing. In South Korea, resistance from *C. baccatum* PBC81 was introgressed into *C. annuum* by overcoming the interspecific hybridization barrier using embryo rescue (Yoon et al. 2006). At Maejo University, Thailand, putative anthracnose resistant *C. annuum* progressive lines were developed using *C. baccatum* PBC80 through embryo rescue (introgressed into susceptible lines PBC398 and PBC758). Depending on resistance sources, pathotypes and fruit maturity stages, inheritance of anthracnose resistance is reported to be polygenic (Voorrips et al. 2004; Mishra et al. 2017), monogenic recessive (Pakdeevaraporn et al. 2005; Kim et al. 2008c) and duplicate genes (Lin et al. 2007). Recently, Garg and colleagues (2014) identified nine resistant varieties (BS-35, BS-20, BS-28, Punjab Lal, Bhut Jolokia, Taiwan-2, IC-383072, Pant C-1, and Lankamura Collection) of *Capsicum* spp. which could be employed for developing successful resistant cultivars through breeding programs. The information on the resistance varieties against *Colletotrichum* spp. may also be utilized for studying the inheritance of the resistance and also to locate and study the quantitative trait loci (QTLs) maps for resistance (Mahasuk et al. 2016). Lee et al. (2010) identified two QTLs that are associated with anthracnose resistance in chilli pepper. A major dominant cluster of QTLs have been identified on Chromosome 5 of *C. annuum* with effective resistance against *C. acutatum*. Some of these QTLs have been used for the development of sequence tagged site (STS) and sequenced characterized amplified region

(SCAR) markers towards identification of resistant genotypes against the *Colletotrichum* spp. (Suwor et al. 2015). Recently, Sun et al. (2015) reported on the inheritance of resistance to *C. acutatum* from a *C. chinense* accession (PBC932) in a BC1 population derived from hybrid with *C. annuum* line 77013 (susceptible) using QTL analyses. Identification of recombinant individuals suggested that resistance in green versus red fruit may be controlled by distinct genes within the QTL interval on P5 (Sun et al. 2015). Further studies need to be undertaken to investigate the importance of these distinct genes in the management of chilli anthracnose. Nevertheless, the genetic mechanism associated with chilli resistance to anthracnose is still poorly understood mainly due to lack of information on the defence signalling modules governing the resistance mechanism.

6 Conclusion

Anthracnose disease is a major constraint to chilli production in the tropical and subtropical regions of the world. Development of the disease is favored by the long survival of the pathogen in seed and soil and the occurrence of different pathotypes in its populations. These pathotypes differ in virulence on chilli genotypes as well as in aggressiveness on susceptible cultivars, the latter being correlated with the amount of inoculum and environmental conditions required for disease epdiemic. Thus, more studies are required for acquiring in-depth information regarding various modes of infection by the pathogen. An insight into the pathogen's lifestyle would provide valuable information required to develop targets for developing resistant varieties of chilli against the pathogen. Also, modifications in conventionally recommended cultural practices suiting to a particular agro-climatic region will prove helpful in better management of the disease. The overall knowledge about the key aspects of a disease triangle will enable better prediction and management of the disease. Combining the use of resistance cultivars with other pre-panting disease control measures (including pathogen-free seed, sanitation to reduce inoculum in soil, choice of sowing site and time to reduce disease potential, and protection of healthy seeds with fungicides or biocontrol agents) would enhance efficiency in the integrated management of chilli anthracnose. The pre-planting decision-taking process for efficient integrated disease management requires skillful assistance to famers and involvement of well-trained professional plant pathologists. Moreover, more research is required to find better alternatives to control chilli anthracnose.

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Nematode Parasites of Grapevines

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Abstract Nematodes parasites on grapevines are serious concern for viticulturists because nematodes divert nutrients from normal vine growth to supply their own needs, which in turn results in lack of vine vigour and reduced crop yield. They are widely distributed throughout the grape growing areas of the world. *Xiphinema americanum* is found in 85% of western Oregon, 10% areas of California, 12.5% areas of southern Spain and three-quarters of the vineyards in France. Similarly, *Meloidogyne arenaria*, *M. incognita*, and *M. javanica* are serious problem of most grapevine growing areas in California, the Mediterranean Basin and South Africa; *M. hapla* in Southern Australia; *M. ethiopica* in Chile and *M. chitwoodi* in California. This chapter reviews diversity, distribution, economic losses and management of nematodes parasitic on grapevines. The worldwide annual loss due to plant parasitic nematodes on grapes is 12.5%. In Australia, yield loss due to root-knot nematode *Meloidogyne* spp. has been estimated at 7% while in California it is 20%. The nematodes have diverse nature of attack. *Meloidogyne* are sedentary endoparasites that form galls on grapevine roots; *Pratylenchus* are migratory endoparasites which cause lesion on roots; *Tylenchulus semipenetrans* are

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E. Lichtfouse (ed.), *Sustainable Agriculture Reviews 31*, Sustainable Agriculture Reviews 31, https://doi.org/10.1007/978-3-319-94232-2_7

semi-endoparasites that attack the roots and their young females can be observed in groups clinging to rootlets. *Criconemoides*, *Tylenchorhynchus* feed ectoparasitically on roots. Other nematodes such as *Xiphinema* spp., *Longidorus* spp. and *Trichodorus* spp., besides feeding ectoparasitically on roots, also act as vector, transmitting the viruses to plants. The synergistic effects of nematode and virus cause drastic reduction in fruit set, which may go up to 80% and may even lead to death of the grape vines. Crop rotation, soil solarization, soil amendments, application of chemicals, use of certified root stocks and planting of nematode resistant root stocks are some management practices which need to be included in an effective manner in integrated nematode management of grapevines.

Keywords Grapevine · Nematode · Diversity · Distribution · Yield loss

1 Introduction

Grapes (*Vitis* spp.) belonging to family Vitaceae is a commercially important fruit crop. It is native to Asia Minor and the Caucasus region, was distributed throughout Europe, and is now extensively grown in the Mediterranean Basin, the subtropical regions of Australia, Southern Africa, and North and South America (Brown et al. 1993). Most grapes come from cultivars of *Vitis vinifera*, the European grapevine native to the Mediterranean and Central Asia. *V. labrusca* (native to eastern United States and Canada), *V. riparia* (native to eastern United States and north Quebec), *V. amurensis* (Asia) and *V. rotundifolia* (southeastern United States from Delaware to the Gulf of Mexico) are other species which contribute minor amounts of fruit and wine (https://en.wikipedia.org/wiki/Grape). Grapes are cultivated in 75,866 km^2 of the world; 71% world grape production is used for wine, 27% as fresh fruit and 2% as dried fruit (https://en.wikipedia.org/wiki/Grape). The major grape producing countries are China, USA, Italy, France, Spain and Turkey. During the year 2005–06, the highest percentage of world grape production was shared by Italy (12.6%), followed by USA (10.5%), France (10.0%) and China (9.7%), however India ranked first in productivity (25.4 ton/ha), followed by USA (18. 7 ton/ha) (Kumar 2007). As a result of human migration and settlement, grapevines have been moved between countries and continents, imported and cultivated in numerous countries that resulted in increased events of pests and diseases on this crop (Esmenjaud and Bouquet 2009).

Almost all the plants of economic importance are attacked by nematodes. These plant parasitic nematodes are microscopic, ungemented, bilaterally symmetrical, pseudocoelomate, vermiform, hyaline animal that tapers towards both ends and circular in cross section. They can be divided into three major groups: (i) the tylenchs (comprise tylenchids and aphelenchids) (ii) the longidorids and (iii) the trichodorids (Luc et al. 1990). The length of these nematodes may vary; tylenchs range from 0.2 to 1 mm, longidorids from 0.9 to 12 mm and trichodorids from 0.5 to 1.1 mm (Luc et al. 1990). Their body width vary from 0.01 to 0.05 mm (Jonathan 2010), however in few genera of tylenchs, the females on maturity loses

the vermiform shape and assumes pear (*Meloidogyne*), globular (*Globodera*), lemon (*Heterodera*), reniform (*Rotylenchulus reniformis*) or saccate and irregular (*Tylenchulus semipenetrans*) shape. They feed on roots, buds, stems, crown, leaves and developing seeds (Parvatha Reddy 2008). Globally, the annual losses due to plant parasitic nematodes on 40 crops of agricultural importance are 13.5%, while in monetary terms for 37 crops it has been estimated US$358 billion (Abd-Elgawad and Askary 2015). The extent of damage caused to plants by nematodes varies with the genetic resistance of the plant, the nematode type and the nematode population size (Pietsch and Burne 2008; Askary et al. 2012a).

Grapes are subject to attack by numerous plant parasitic nematodes, the prominent among them are dagger nematode, *Xiphinema* spp., root-knot nematode, *Meloidogyne* spp., root-lesion nematode, *Pratylenchus* spp., needle nematode, *Longidorus* spp., citrus nematode, *Tylenchulus semipenetrans*, ring nematode, *Criconemoides xenoplax*, stunt nematode, *Tylenchorhynchus* spp. and stubby root nematode, *Trichodorus* spp. The annual losses in the yield of grapes due to plant parasitic nematodes have been estimated 12.5% (Sasser and Freckman 1987). The nematode causes injury to the vine roots that leads to their decay and prevent the development of new roots. Such infected roots are unable to meet above ground demand of nutrients and water, especially during peak demand periods, the symptoms of which in the earlier stage are exhibited by plant in the form of nitrogen or water deficiency (McKenry and Bettiga 2013). As a result of nematode infection there is slight yellowing of the leaves and decline in the plant health and vine vigour. Moreover, the injuries thus caused by nematodes on plant root tissues are predisposed for further infection by other pathogens present in the soil such as bacteria and fungi. The cumulative impact on plants intensifies over years which may be seen ultimately in the form of reduced quality and quantity of crop (De Kierk and Loubser 1988). Unfortunately, the aboveground symptoms on plants caused due to plant parasitic nematodes are in most cases not very much specific and often confused with other problems such as water stress, nutritional disorders, fungal or bacterial infection and by the time the disease is diagnosed the nematode has already caused significant damage to plants (Jonathan 2010; Askary 2017). Among the nematodes described above, *Xiphinema*, *Longidorus* and *Trichodorus* not only feed on vine roots but also transmits virus to the plant. Approximately, 4000 species of plant parasitic nematodes have been described and out of which only 30 species i.e. less than 1% acts as virus vectors (Brown et al. 2004). *X. index* during feeding on grapevine roots transmit grapevine fanleaf virus. The combined effect due to feeding by *X. index* and its association with grapevine fanleaf virus may kill grapevines (Nicholas et al. 2007). Another species, *X. americanum* transmits tomato ringspot virus that causes yellow vein virus disease in grapes (McKenry and Bettiga 2013). Therefore, with an aim to improve the quality and yield of grape, management of these plant parasitic nematodes is essential but in such a way that are eco-friendly and economical.

This chapter provides comprehensive and updated information of plant parasitic nematodes associated with grape cultivation. The efforts undertaken to manage the nematodes are also described in the present context.

2 Nematodes Associated with Grape Cultivation

Plant parasitic nematodes pose serious threat to many fruit and horticultural trees (Askary and Haidar 2010). Nearly, 2500 species of plant parasitic nematodes have been reported in the world, of which around 1600 are associated with grapevines. Among these, some are of great economic significance, whilst others cause no known economic damage. However, their presence in the rhizosphere of grapevine possibly may have some role in the soil ecosystem. Recently, in India Askary et al. (2018) isolated four plant parasitic nematodes viz., lesion nematode (*Pratylenchus* spp.), stunt nematode (*Tylenchorhynchus* spp.), reniform nematode (*Rotylenchulus* spp.) and *Tylenchus* spp. from the rhizosphere of grape rootstocks planted in polyhouses (unpublished data). A brief description of few economically important nematodes associated with grape cultivation has been presented.

2.1 Dagger Nematode

2.1.1 Brief Description

Dagger nematodes (*Xiphinema* Cobb, 1913) are root ectoparasites of grapevines. The genus *Xiphinema* was first described by Thorne in 1939. They are migratory ectoparasites armed with long stylets which they insert deep into root tissues normally at the growing tips of the fine feeder roots. Direct feeding often result in a swollen root tip which are often confused with the galls caused by root-knot nematodes. The galls are produced only at the root tip when infected by *Xiphinema*. The roots become necrotic and their growth stops. The distal part of a root dies by girdling, the part that remains alive usually develops branches without any stimulation by the parasite. Suppressed or clubbed roots from which fine feeder roots arise, gives the appearance of a "witches broom", the resulting abnormalities in heavily parasitized roots. However, feeding by *Xiphinema americanum* do not form galls on root system.

2.1.2 Economic Importance

Xiphinema is reported to cause yield losses in grapes. Van Gundy et al. (1965) noted a reduction of 38% in root weight of grapevine caused by *X. index*. Other workers also reported significant reduction in top and root weight, inflorescence number and fruit size of grapes when this nematode species was applied at the rate of 500/pot (Krikpatrick et al. 1965; Boubals et al. 1971).

2.1.3 Distribution

Xiphinema is present in all major grapevine-growing regions of the world like USA, South Africa, mid-Atlantic region and Australia. On the basis of genetic analysis of a wide range of *X. index* population, isolated from grapevine vineyards of different regions of the world, it was concluded that *X. index* originated from the Middle East from where it spread and was introduced into the grapevine countries in the Western Hemisphere (Esmenjaud et al. 2014). Though, these workers further suggested that their hypothesis need confirmation by including new locations in the study.

X. americanum, *X. diversicaudatum* and *X. index* are common in South African vineyards (Malan 1995) and are found in a variety of soils (Shurtleff and Averre III 2000). In USA, *X. americanum* is reported from 85% grapevine growing areas surveyed in western Oregon; *X. pachtaicum*, and/or *X. americanum* from 74% of eastern Washington vineyards. The weak vineyards of California (Ferris and McKenry 1975) and Michigan (Ramsdell et al. 1996) are supposed to suffer indirect damage from *X. americanum*. *X. index* is reported to occur in about 10% of the grape-growing areas of California, predominantly in the north coast and north San Joaquin Valley regions. This nematode species is also reported from Kern County (McKenry et al. 2004). *X. rivesi* has been found associated with grape vines in western Australia (Sharma et al. 2003). About three-quarters of the vineyards in France have *X. index* populations. On the basis of a survey conducted in southern Spain, *X. index* and *X. italiae* was reported 12.5 and 10.9%, respectively from the soil and root samples collected from commercial vineyards (Téliz et al. 2007). *X. index* can transmit grapevine fanleaf virus (Bird et al. 1994) that may reduce the lifespan of vineyards by about 12–20 years (Raski 1988). In USA, the nematode has been found to spread grapevine fanleaf virus causing significant economic damage in California vineyards. A report says that 5% of California acreage is infested with grapevine fanleaf virus (McKenry and Bettiga 2013). *X. americanum* is the specific vector of tomato ringspot virus, the causal organism of yellow vein virus disease in grapes (McKenry and Bettiga 2013).

Systematic Position

Phylum	Nematoda
Class	Adenophorea
Order	Dorylaimida
Suborder	Dorylaimina
Superfamily	Dorylaimoidea
Family	Longidoridae
Subfamily	Xiphineminae
Genus	*Xiphinema*

2.1.4 Diagnostic Characters

Body long and slender. Lip region continuous or slightly to markedly set off from body. Spear very long (60–250 µm), anterior is odontostyle which is needle like with a forked base whereas posterior is odontophore with three prominent basal flanges; guiding ring located at base of odontostyle; Oesophagus 2 part cylindrical, anterior part long and coiled. *Female*: Monodelphic or pseudo-monodelphic or amphidelphic; ovaries reflexed, one reduced and extending anteriorly, the other posteriorly; vulva usually at 40–50% but may be more anterior; uterus short; tail short rounded to filiform, tail of *X. index* has terminal peg situated ventrally which is a distinct characteristic of the species. *Male*: Two opposed outstretched testes, spicules arcuate, strong with lateral guiding pieces. Bursa absent (Siddiqi 1979; Luc and Cohn 1982; Swarup et al. 1989; Jairajpuri and Ahmad 1992; Luc et al. 1990; Ali 1995; Shurtleff and Averre III 2000; Walia and Bajaj 2003; Loof and Luc 1990).

2.1.5 Biology and Life Cycle

Males are very rare; reproduction takes place parthenogenetically and female lays eggs singly in the soil. However, the first moult occurs outside the egg and emergence of adults takes place after three more successive moults (Swarup et al. 1989). All the four juvenile stages and adults are infective and parasitic on plant roots.

Among all the plant parasitic nematodes, *Xiphinema* are characterized with longest life cycle, however the duration of life cycle (egg to egg) is highly variable among species. *X. americanum* (Cobb, 1913) requires a year to complete its life cycle, whereas *X. diversicaudatum* (Micoletzky, 1927) Thorne 1939, takes up to three years (Flegg 1968; Jaffee et al. 1987; Bridge and Starr 2007). *X. brevicolle* (Lordello and da Costa, 1961) completes its life cycle on grapevine in 4–7 months at 20–23 °C, whereas *X. index* (Thorne and Allen 1950) requires 21–27 days at 24 °C on the same crop (Swarup et al. 1989). Geographical conditions are also responsible for variable length of life cycle. In California, USA, *X. index* completes its life cycle in 22–27 days at 24 °C, while in Israel the nematode completes its life cycle in 7–9 months at 20–23 °C (Chitambar 2015). *X. index* is reported to survive in moist sterile soil without food for 9–10 months, but in field soil left with grapevine roots (without top growth), it survived for 4.5 years (Raski and Hewitt 1960; Taylor and Raski 1964; Radewald and Raski 1962). Other studies indicate survival of *X. index* in field soil for at least 4 years (Esmenjaud et al. 2014); Demangeat et al. 2005).

2.1.6 *Xiphinema*-Virus Interactions

Xiphinema received considerable attention when Hewitt et al. (1958) discovered that the nematode acts as vector and is capable of transmitting virus causing fanleaf virus disease in grapevine. The leaves of such infected plant exhibit widely open petiolar sinuses and abnormally gathered primary veins causing a fan-like shape, so the virus named as 'Grape fanleaf virus' (Pearson and Goheen 1988). The deformities on leaf and shoot appear early in the season but as the time advance it get fade (Hewitt 1954). Vine shoots can also be malformed, showing abnormal branching, double nodes, short internodes and zigzag growth (Raski et al. 1983). Yellow mosaic develops on leaves of affected vines. Specks vary from a few scattered spots to total yellowing. Bunches are fewer and smaller than usual with shot berries and irregular ripening (Pearson and Goheen 1988). The disease can cause up to 80% reduction in fruit set. Symptoms can be confused with herbicide damage and mite injury (Nicholas et al. 2007). Affected vines show yellow vein banding along the main veins of mature leaves. Discoloured leaves show little malformation (Pearson and Goheen 1988). This symptom has been shown to be the result of cross infection with yellow speckle viroid (Szychowski et al. 1995).

The presence of *Xiphinema* alone may not be a problem, but its interaction with virus is a matter of concern in grape cultivation. The nematode playing the role of a vector spreads the virus from plant to plant in the field. Thus, the spread of the virus in a field reflects the distribution of the nematode in the ground (Villate et al. 2008). Grapevine fanleaf virus is ring spot virus which is called "NEPO" derived from Nematode transmitted polyhedral shaped particles. The release of virus particles into plant cell takes place with the help of oesophagus. The nematodes possess modified bottle shaped oesophagus with glands connected by short ducts directly to the lumen of the oesophagus. This actually helps in the transmission of viruses (Jonathan 2010). All stages of *X. index* have the ability to transmit virus (McKenry and Roberts 1985). It is evident that nematode transmitted viruses do not persist through a nematode molt and do not pass through nematode eggs. The virus is acquired and transmitted by nematodes by feeding which takes nearly one day. Once acquired, the virus persists in nematode body for a longer period. The virus is retained in the cuticle lining of spear extension and oesophagus and the retention is due to close biological association between virus and the vector (*Xiphinema*). Earlier, it was reported that the nematode can transmit the virus for up to 4–8 weeks when feeding on virus-free plants, and that the virus can persist in starving *X. index* for at least 30 days (Taylor and Raski 1964; Raski and Hewitt 1960). However, recent report by Esmenjaud et al. (2014) stated that the virus may survive in the adult nematode for at least 4 years and slightly less in fourth stage larvae, thereby indicating that elimination of the virus from soil mainly depends on the possibility of eliminating the nematode vector than on grape residues in infected field soils.

2.1.7 Management

A. Cultural

i. There should be a gap of at least one year between vine removal and replanting. During this period nematodes will die due to starvation and their population will get reduce.
ii. Weeding should be done in the area of grape cultivation. A good weeding may prove helpful in preventing virus introduction and/or spread.
iii. Crop rotation for a period of three years is sufficient (Raski 1955) but other studies suggested that the sites infested with *X. index* should be left fallow or rotated to crops other than grapes or figs for at least 10 years (McKenry 2000). During this period of time the old roots will get decompose and nematode population will decrease. A fallow land also gives a control on weeds, which in many cases are good host of plant parasitic nematodes. However, grapevine fanleaf virus can still be detected in nematodes kept in soil without roots for four years (Demangeat et al. 2005).
iv. Certified rootstock and/or scion should only be used that have been tested and found free of all known viruses. However, even in healthy grapevines, there are reports of infection with grapevine fanleaf virus transmitted by *X. index* from soil within three years after planting (Hewitt et al. 1962).
v. The nurseries or vineyards if infested with *X. index*, the rootings/bench grafts or rootstocks of such areas when later moved for planting may spread the nematode in new non-infested areas. It is therefore recommended that planting material should be given hot water (52 °C) agitated soak for five minutes (Nicholas et al. 2007).
vi. Heat therapy and meristem culture can also prove helpful in making the plant materials free from viruses (Torres-Viflals et al. 2004). Heat therapy is meant for viruses that readily invade plant meristems such as nepoviruses, whereas meristem culture is effective in eliminating phloem limited viruses (Gambino et al. 2009). Buds from a candidate vine of unknown virus status can be grafted onto a nurse plant and heat-treated in a growth chamber at 37 °C for two to three months. Now the buds are forced to grow and the resulting shoots are checked for the presence of virus by indexing or PCR-based testing. At high temperature RNA based viruses degrade and are eliminated before plant cells can be damaged. Though, this method is not very much effective but was widely used in the past (Gifford and Hewitt 1961).
vii. Cover crop: Barley, Columbia, Sudangrass, SS-222, Vetch, Cahaba White are antagonistic to *X. americanum* and non host of *X. index*; Brome and Blando are poor host of *X. index*.

B. Chemical

Soil fumigation is one of the best methods to control plant parasitic nematodes. However, this method can eradicate nematodes generally up to one meter depth

and therefore not very much effective for those perennial crops which have deep root system (Lear et al. 1981). It is advised that prior to the application of nematicides and fumigants, soil must be ripped and cleared of as many old roots as possible and dried to as great a depth as possible (Nicholas et al. 2007). This will make the fumigants to work properly in an effective manner. Fumigation such as metham sodium and dichloropropene can be used in grapevine yards, however they are expensive, require special equipment to apply. Other concerns are technical expertise and safety issues.

Application of non-fumigant nematicides to established vineyards can be done by using soil drenches or through the drip irrigation system. But they have a negative impact on human health as they may leave residues in or on fruit (Nicholas et al. 2007). Due to this reason their use are being highly restricted in many vineyards of the world (Bouquet et al. 2000).

C. Host plant resistance

Growers have been using rootstocks to protect grapevine against soil pests for the last 150 years. Prior to the selection of rootstocks, the important points to be taken into consideration are their easily propagation, graft compatibility with scion varieties and adaptability to a range of soil conditions (Reisch et al. 2012). As far as reaction of grapevine rootstocks against nematode is concerned they differ in susceptibility. They may be tolerant or resistant to one species of nematode, while susceptible to other species. The use of resistant root stocks can be helpful in solving the grapevine malady caused due to *X. index*-Fanleaf virus interaction. Nemadex Alain Bouquet, a rootstock developed in France, is resistant to *X. index*. A rootstock O39-16 has been found tolerant to *X. index* (McKenry et al. 2004). O39-16 is a hybrid between *Vitis vinifera* and *Muscadinia rotundifolia*. The latter is responsible for causing resistance against *X. index*. GRN1-5 developed from native grapes are resistant to *X. index*. *V. arizonica*, *V. solonis*, *V. rufotomentosa* and *V. mustangensis* are resistant to *X. index* (Harris 1983; Walker and Jin 1998; Meredith et al. 1982).

D. Somatic embryogenesis

Somatic embryogenesis has been found to eliminate several phloem-limited viruses from grapevine material (Goussard et al. 1991). This technique is useful to eliminate grapevine fanleaf virus from grapevine tissue in combination with heat therapy of the explants (Goussard and Wiid 1992). In an experiment somatic embryogenesis was used without applying heat therapy to eliminate grapevine fanleaf virus from plantlets. The success rate of the experiment is approximately 100%. The virus was however detected in all tested anthers and ovaries by using 'Reverse transcription polymerase chain reaction' technique, but not in the regenerated plantlets two years after transfer to greenhouse conditions (Gambino et al. 2009).

E. Inheritance and mapping of DNA markers for resistance to *Xiphinema index*

The report of the first grapevine genome sequences (Jaillon et al. 2007; Velasco et al. 2007) provided a new generation of molecular tools in grapevine breeding. It was earlier reported that *V. arizonica* was resistant to *X. index*

(Kunde et al. 1968) and resistance was inherited as a single heterozygous gene (Meredith et al. 1982). Now the 9621 population has been mapped with highly informative and co-dominant simple sequence repeat (SSR) markers, further positioning resistance to *X. faslidiosa* and placing a major quantitative trait locus (QTL) for *X. index* resistance *(XiR1)* on chromosome 19 (Xu et al. 2008). QTL and linkage disequilibrium-based mapping were implemented in order to get a better understanding of the genetic structure of grapevines, though success achieved was only for a small portion of the genetic diversity of grapes (van Zyl et al. 2011). The grape genome sequence, in addition to rapidly developing technologies, can play a significant role in improving the existing grape cultivars by incorporating specific traits and disease resistance (Martinez-Zapater et al. 2009; Myles et al. 2011). Genetic mapping efforts found that the RAPD (Random Amplffied Polymorphic DNA) marker OPA-12 (Operon) was tightly linked to *X. index* resistance (Walker and Jin 1998).

2.2 Root-Knot Nematode

2.2.1 Description

Root-knot nematodes, *Meloidogyne* (Goeldi, 1987) are sedentary endoparasites embedded within the root. The total number of described species is nearly 100 but the four most common species i.e. *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, *M. javanica* (Treub, 1885) Chitwood, 1949, *M. arenaria* (Neal, 1889) Chitwood, 1949 and *M. hapla* (Chitwood, 1949) constitute around 95% population, have wide host range and these four if considered combined, are parasitic on almost all crop plants of agricultural importance. As far as grapevine is concerned, more than 50 species of *Meloidogyne* have been found associated with it, however the above mentioned four species are most prominent that cause economic damage to grapevine.

2.2.2 Economic Importance

The threshold population density of *Meloidogyne* spp. that can cause yield losses in grapevine is 50 eggs and J_2/100 cm^3 of soil (Anwar and Van Gundy 1989). In Australia, yield loss due to root-knot nematodes was estimated by 7% (Stirling et al. 1992) while in California the production loss was 20% (Brown et al. 1993).

2.2.3 Distribution

M. arenaria, *M. incognita*, and *M. javanica* are serious problem for most grapevine grown in sandy soils under the mild temperatures in California, the Mediterranean Basin, and South Africa (Lider 1960; Raski et al. 1973; Brown et al. 1993; Nicol et al. 1999; Anwar et al. 2000; Quader et al. 2001). *M. hapla* is predominant in grapevine growing cooler areas of Southern Australia (Stirling and Cirami 1984), cooler regions north of San Francisco, southern California (Bettiga 2013), eastern Washington (Howland et al. 2014) and Hungary (Jenser et al. 1991); also recovered from 10% Oregon vineyards (Pinkerton et al. 1999). *M. ethiopica* infect grapevine in Chile (Carneiro et al. 2004), *M. nataliei* in Michigan (Bird et al. 1994), *M. chitwoodi* in California (McKenry and Bettiga 2013) and *M. hispanica* in South Africa (Kleynhans 1993). In Australia, yield loss due to root-knot nematodes was estimated by 7% (Stirling et al. 1992) while in California the production loss was 20% (Brown et al. 1993).

Systematic Position

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Suborder	Tylenchina
Superfamily	Tylenchoidea
Family	Heteroderidae
Subfamily	Meloidogyninae
Genus	*Meloidogyne*

2.2.4 Diagnostic Characters

Mature female: Sedentary, round to pear shaped (pyriform) with elongated neck at the anterior end, cuticle whitish; vulva subterminal and very close to anus; cuticular striation concentrated around perineal area. Phasmids dot-like, slightly above and on either side of anus. Stylet cylinder, short with small basal knobs; oesophagus well developed, median bulb large, isthmus short; ovaries paired, prodelphic, convoluted; rectal glands are large, six in number which secrete gelatinous matrix wherein eggs are deposited; eggs not retained in body. *Male*: Vermiform, free living in soil, cuticle strongly annulated; stylet robust with large knobs; tail short, hemispherical; testis single or paired; spicules slender, gubernaculum simple, bursa absent. *Second stage juvenile* (J_2): Vermiform, migratory and only infective stage; cephalic region with coarse annules; stylet slender with rounded basal knobs; oesophageal glands overlap intestine ventrally; median bulb with large oval valve plates; tail elongate conoid with hyaline region starting near the tail tip, tail tip is narrow (Sasser and Carter 1985; Jepson 1987; Swarup et al. 1989; Luc et al. 1990; Ali 1995; Ahmad 1996; Walia and Bajaj 2003; Fig. 1).

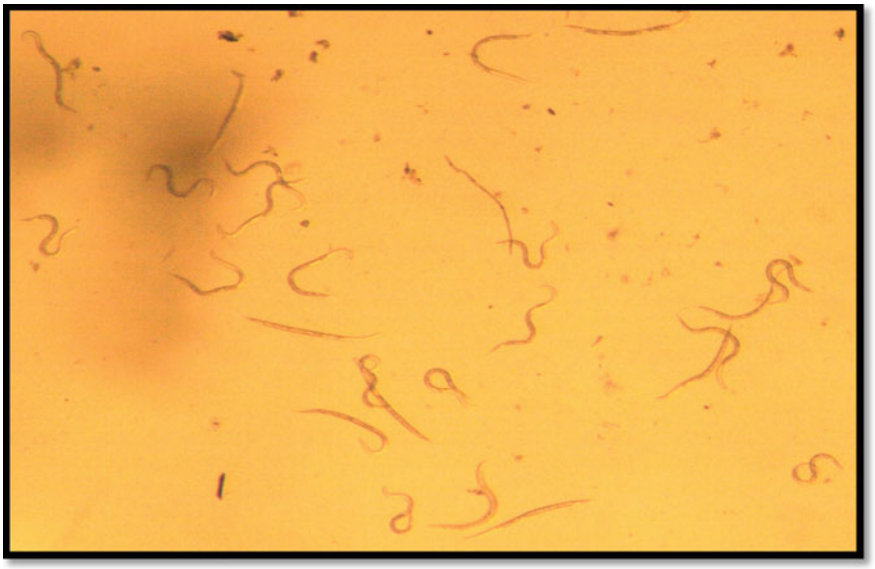

Fig. 1 Freshly hatched second stage juveniles of root-knot nematode, *Meloidogyne hapla*

2.2.5 Biology and Life Cycle

Life cycle consists of six stages which include egg, four juvenile stages (J_1, J_2, J_3 and J_4) and adult. The second stage juvenile (J_2) is the only infective stage, with root tips of plant being the primary infection court; J_2 migrates through cortical tissues, establish permanent feeding site in the pericycle region and induce formation of giant cells (nutrient sink). After fourth or final moult, the adult female develops into pear shape and become sedentary. Gall formation takes place within 48 h after infection. Adjacent galls coalesce and more than one nematode may be embedded in the same gall (Askary 2017). Gall formation takes place on the root due to cortical hypertrophy, pericycle hyperplasia and may be due to formation of giant cells, the feeding site of nematode. The rectal glands of fertilized female secrete gelatinous matrix on the posterior portion wherein the eggs are deposited in the form of egg mass. On an average 200–500 eggs are laid in a single egg mass. Egg masses are generally found on the surface of the galled root. Reproduction takes place parthenogenetically, males not required.

Lifecycle is completed between 27 and 32 days (in case of *M. incognita* and *M. javanica*) at an optimum temperature of 25–30 °C and more than one generation is completed by nematode in a single cropping season.

2.2.6 Management

A. Host plant resistance
There is no report of a single rootstock resistant to all root-knot nematodes however, rootstocks such as *Vitis aestivalis*, *V. champinii*, *V. cinerea*, *V. mustangensis* (syn. *V. candicans*), *V. rupestris* and *Muscadinia rotundifolia* are sources of resistance to *M. incognita* (Bloodworth et al. 1980; Walker et al. 1994; Cousins et al. 2003; Lider 1954); 101-14, 110R, 3309C, 420A, Dog Ridge, Free-dom, Harmony, Ramsey, Riparia Gloire, and St. George are resistant to *M. hapla* (Moyer 2016); Dog Ridge, Free-dom, Harmony, Ramsey did not support penetration of *M. arenaria* juveniles after 24 h exposure (Ferris and Hunt 1979). RS 2 and RS 9 have shown resistance to root-knot nematodes and are rated as medium vigor, based on evaluations in California's San Joaquin Valley. Matador and Kingfisher, released in 2010 are just now becoming available from commercial nurseries. Five-nematode-resistant selections (GRN1-5) were developed during a grape-breeding program at University of California. All were found resistant to root-knot, though they vary in resistance to other nematode species.

B. Cultural

i. The planting material should be selected from nematode free nurseries.
ii. Crop rotation with non-host crops or resistant crops in case of high nematode population.
iii. Use of trap crops and antagonistic crops such as planting *Tagetes erecta* (African marigold) and *Crotolaria spectabilis* in nematode infested soil. This will provide an effective management against the root-knot nematode.
iv. Cover crops, that include mustards, arugula, sudangrass when planted in the area where vines are to be planted may help in lowering down the population of nematodes in that area. However, prior to planting of cover crop, it is important to correctly identify the target nematode species because cover crops vary in effectiveness against different nematode species. Barley, Columbia, Strawberry clover, Salina are poor host of *M. incognita*; Strawberry clover and Salina are poor hosts of *M. javanica* and non host of *M.* arenaria; Sudangrass, SS-222 is poor host of *M. hapla*.

C. Soil Solarization
This method involves mulching of moist field soil with transparent polyethylene plastic sheet for 4–6 weeks in hot summer. This will cause short wave radiation from sun to easily penetrate the polythene sheet and heat the soil but the long wave radiation leaving the soil unable to do so, hence retained under the sheet. The temperature will increase in the upper soil layer by about 5–10 °C over normal for several hours daily thereby killing or causing metabolic exhaustion of many nematodes. Soil solarization is effective against root-knot nematodes all over tropical and subtropical parts of the world. The nematode population can be reduced drastically by this method but cannot be totally eradicated.

D. Soil amendments
Application of neem, castor cake, compost or manures as soil amendments can improve vine vigour and frequently reduce the effect of nematode infestations. Proper irrigation and fertilizer application in vineyard lessen the stress on vines which ultimately reduce the adverse effect of nematodes especially root-knot nematode species.

E. Biological
Fungal biocontrol agents such as *Paecilomyces lilacinus*, *Pochonia chlamydosporia*, *Trichoderma harzianum* and *Aspergillus niger* have been found effective against different species of root-knot nematodes. These fungal biocontrol agents are eco-friendly, economically feasible, commercialized in various countries of the world under different trade names and are used by crop growers for the management of root-knot nematodes (Askary 2015a). Though, still there is a need to educate the growers via extension services for proper handling and application of these biocontrol agents to achieve satisfactory results (Abd-Elgawad 2016).

F. Chemicals

i. Application of carbofuran 3G @ 50 g/vine and watering.
ii. Nematode infested vineyards when soil drenched with DBCP (Dibromo chloro propane) was found effective in reducing the population of nematodes.
iii. Prior to plantation of grapevines, soil fumigation with Vapam (metam-sodium), Telone II (1,3-Dichloropropene) and Basamid G (dazomet) has been found effective in reducing the population of almost plant parasitic nematodes. Soil borne plant pathogens and viable weed seeds are also reduced in soil due to these fumigants.

G. Biofumigation
In this method toxic substances of plants are exploited to suppress nematodes present in soil. Cruciferous plants are used for biofumigation as they contain glucosinolates which is at its highest concentration when the plant is at flowering stage. At this stage the above ground growth is chaffed as finely as possible to break down all the plant cells and thereby release the maximum amount of glucosinolates. The fresh plant mass is immediately incorporated into the soil at a depth of 15–20 cm. The glucosinates are converted enzymatically into isothiocyanates, the actual active ingredients. It is better to seal the soil after incorporating the plant mass into soil by gentle rolling or light irrigation as this will preserve the active substance in soil for some longer period. The succeeding crop is planted at least two weeks after the start of biofumigation. This will help preventing any damage to the main crop by nematodes.

2.3 *Root-Lesion Nematode*

2.3.1 Brief Description

The root-lesion nematode, *Pratylenchus* (Filipjev, 1936) are migratory endoparasites that move within the root both intra and inter-cellular, feeds on cortical cells that results in cell death and breakdown. Main species attacking grapevines are *Pratylenchus vulnus* (Allen and Jensen, 1951) and *P. zeae* (Graham, 1951).

2.3.2 Distribution

Pratylenchus spp. are commonly found in the vineyards of eastern Washington (Howland et al. 2014). It was recovered from more than 85% of the Oregon vineyards (Pinkerton et al. 1999). *P. vulnus* are commonly distributed in California vineyards, causing serious problem and affecting the grape yield (Lider 1960; Raski et al. 1973).

Systematic Position

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Suborder	Tylenchina
Superfamily	Tylenchoidea
Family	Pratylenchidae
Subfamily	Pratylenchinae
Genus	*Pratylenchus*

2.3.3 Diagnostic Characters

Vermiform, body gradually tapering posteriorly. No sexual dimorphism in the anterior part of the body. Lip region is low, flattened, continuous with body and heavily sclerotized. Deirids absent. Phasmids near middle of tail. Stylet strong with well developed rounded, anteriorly flat or indented basal knobs; Oesophageal glands extending over intestine ventrally. Median bulb oval to round, very muscular; oesophago-intestinal valve not well developed. *Female*: Vulva is present typically on the posterior fourth of the body (75–80%). Post-vulval uterine sac may or may not have rudiments of posterior ovary. Mono-prodelphic ovary with only anterior ovary functional. Spermatheca is large, rounded usually axial; tail subcylindrical to conoid, usually about 2–3 anal body width long, terminus smooth or annulated (Fig. 2). *Male*: Spicules slender, arcuate. Gubernaculum simple. Bursa encloses tail terminus (Loof 1978; Handoo and Golden 1989; Café Filho and Huang 1989; Luc et al. 1990; Ali 1995; Ahmad 1996; Walia and Bajaj 2003; Jonathan 2010).

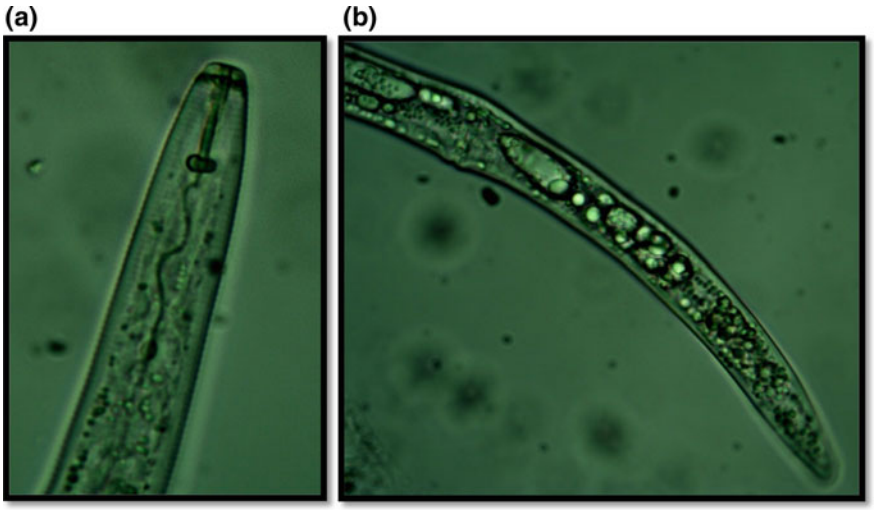

Fig. 2 Adult female of *Pratylenchus*: **a** Anterior portion showing flattened lip, short stylet with rounded basal knobs. **b** Posterior portion showing vulva just above tail on the posterior fourth of the body

2.3.4 Biology and Life Cycle

The nematode completes its entire life cycle inside the root. Reproduction may be sexual or parthenogenetic and the female lays eggs singly into the cortical cavities of infected roots (Swarup et al. 1989). Four moulting takes place. The first moult occurs within the egg while the three moults occur outside (Askary et al. 2012a). All the life stages, except the first stage juvenile (J_1) are parasitic. The nematodes move intercellular and intracellular within the cortical tissues, feed on there, which result in cell death and breakdown. Due to this migration and feeding, dark brown necrotic lesions appear on the epidermal, cortical, and endodermal cells of roots. The lesion first appears as a tiny, elongate, water-soaked spot. The necrotic spots get coalesce with the passage of time, causing necrosis in the entire root (Askary 2017). The entire root system may be destroyed when the nematode infection is severe. The extent of damage depends on the phenolic content of the cells as the oxidation of the phenol is responsible for browning of the lesion (Walia 1986). The lesions produced on the root surface open avenues for other pathogens particularly fungi to invade such root tissues. Stunted growth, yellow to yellowish brown leaves and wilting of plants during hot sun hours are the aerial symptoms observed on plants (Askary et al. 2012a). Under suitable environmental conditions the life cycle is completed in about one month.

Pratylenchus is a migratory endoparasitic nematode and therefore its population densities are typically much greater in plant roots than in the rhizosphere (Bridge and Starr 2007). However, initially the population of nematodes is more in soil but during the vegetative stage of the crop, the nematode population builds up

tremendously and is much greater than soil population. At the time of crop maturity, the nematode leaves the root and moves into the soil, thus again increasing the soil population (Walia and Bajaj 2003).

2.3.5 Management

i. Host plant resistance: UCD GRN1 and VR O39-16, both with *M. rotundifolia* parentage, are poor hosts of *Pratylenchus vulnus* (Ferris et al. 2012).
ii. Cover crop: Barley, Columbia, Brome, Blando, Sudangrass, SS-222 are non host of *P. vulnus*.
iii. Sanitation: It is advised to use certified rootstock and/or scion that have been tested and found free of nematodes. In nursery, hot water treatment of dormant planting stock is recommended as it can minimize the spread of nematodes. An effective nematode control was achieved at 5 min immersion of rootstock in each of three water tanks—preheating at 30 °C (86 °F), hot water at 52.8 °C (127 °F), and cooling at 23 °C (73.5 °F) (McKenry and Bettiga 2013). The equipments used in vineyard should be cleaned with water to remove the clinging soil particles and roots that may harbour plant parasitic nematodes.

2.4 Needle Nematode

2.4.1 Brief Description

Needle nematodes, *Longidorus* (Micoletzky, 1922) are migratory ectoparasites that cause serious damage to many plant species (Cohn, 1974). *Longidorus africanus, L. elongatus, L. goodeyi, L. magnus* and *L. diadecturus* are some important species pathogenic on grapevines. They possess long hollow stylet that is used by them to feed deeply within root-tips. The nematode penetrate root cells with the help of odontostyle, the anterior part of the stylet, while the posterior part odontophore helps to discriminate between sites deep within plant roots (Robertson and Taylor 1975). The nematodes cluster at the tip of the roots, and feed exclusively at, or just behind root tips that results in cessation of meristematic activity and root elongation. The root systems are greatly reduced; lateral and sometimes tap roots are severely stunted. An overall stubby root appearance is created. The feeding leads to swellings or galls on the root tips. These galls have large cells with dense cytoplasm (Brown et al. 2004). A relatively short period of feeding can be enough to initiate these symptoms (Cohn 1970, 1974; Szczygiel 1975). Some species of *Longidorus* are vectors of nepoviruses (Brown and Trudgill 1989; Taylor and Brown 1997).

2.4.2 Economic Importance

The direct feeding on grapevine roots by *Longidorus* results in yield reduction. The damage is caused mostly in seedlings and young cuttings. However, when the nematodes transmit viruses to the grapevine roots the synergistic effect of nematode and virus lead to severe damage causing substantial reduction in yield.

2.4.3 Distribution

Longidorus are widespread reported from different warmer and temperate regions of the world (MacGowan 1982). *L. pisi* (syn. L. siddiqii) are reported from Israel causing mild decline of grapevine (Cohn 1970). *L. diadecturus* is widely distributed in central USA (Robbins and Brown1991). Kumari and Decraemer (2007) reported three species viz., *L. elongates*, *L. euonymus* and *L. leptocephalus* from the grape vineyards of Bohemia and South Moravia in Czech Republic.

Systematic Position

Phylum	Nematoda
Class	Adenophorea
Order	Dorylaimida
Suborder	Dorylaimina
Superfamily	Dorylaimoidea
Family	Longidoridae
Subfamily	Longidorinae
Genus	*Longidorus*

2.4.4 Diagnostic Characters

Body slender and narrow, size varies from 2 to 12 mm in length. Amphids large, pouch like, unlobed or bilobed at base which opens via a minute inconspicuous pore. Odontostyle/odontophore junction not forked. Odontophore lacks basal flanges. Oesophagus 2 part cylindrical, anterior part long and coiled. Guiding ring located in anterior half of odontostyle. Female: reproductive system amphidelphic, vulva near mid-body. Male: spicules arcuate and caudal alae absent. Tail short, conoid and similar in both sexes (Jairajpuri and Ahmad 1992; Luc et al. 1990; Walia and Bajaj 2003; Bridge and Starr 2007).

2.4.5 Biology and Life Cycle

Males are rare and presumably unnecessary for reproduction (Raski 1988), although amphimictic populations also take place (Ye and Robbins 2004). The first stage juvenile emerges from the egg, undergoes three successive moults to develop into adult. Life cycle varies among different species and may be completed in few months to 2 years. Cohn and Mordechai (1969) reported that *Longidorus africanus* parasitic on grapevines completed their life cycle in 3–4 months.

2.4.6 *Longidorus*-Virus Interactions

Longidorus is reported to transmit viruses which cause substantial damage to a wide range of crops in different parts of the world (Brown and Trudgill 1989; Taylor and Brown 1997). *L. diadecturus* transmit the peach rosette mosaic virus (PRMV) to grape (Allen et al. 1982), causing serious damage to the crop (Brown, et al. 1993; Stace-Smith and Ramsdell 1987).

2.4.7 Management

A gap of at least one year between vine removal and replanting will help in reducing the nematode population as during this period nematodes will die due to starvation. The field where grapes are to be planted should be weeded properly as this can prevent introduction or spread of viruses. Certified rootstock/scion that are free from all known viruses should be used for planting.

2.5 Citrus Nematode

2.5.1 Brief Description

Citrus nematodes, *Tylenchulus semipenetrans* (Cobb, 1913) are semi-endoparasitic nematodes that are most prevalent and damaging in medium-textured soils i.e. sandy loam to clay loam and in those areas where grapes are cultivated in association with citrus, or in old citrus orchard sites where vineyards are established. Their host range is limited and they are mainly parasitic on citrus, olive and grapevine. The nematodes cause stunting of grapevines and deterioration of their roots. Verdejo-Lucas et al. (1997) reported that the three biotypes citrus, mediterranean and *Poncirus* (Inserra et al. 1980, 1994) of this nematode can reproduce in grapevines in Spain.

Systematic Position

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Suborder	Tylenchina
Superfamily	Criconematoidea
Family	Tylenchulidae
Subfamily	Tylenchulinae
Genus	*Tylenchulus*

2.5.2 Diagnostic Characters

Immature female: Vermiform, free living in soil; head region rounded, continuous with body contour. Stylet long with rounded knobs. Oesophagus with strong medium bulb which is not well separated from procorpus. Vulva too much posterior in position. Ovary single, immature with few oocytes, anteriorly outstretched. Excretory pore posteriorly situated but slightly anterior to vulva. Tail conical. Anus and rectum absent. *Mature female*: Body variably saccate, widest at excretory pore, narrowing abruptly at vulva and ending in a digitate terminus. Neck elongate. Stylet long, well developed with rounded knobs; isthmus elongate, basal bulb offset from intestine; ovary single, coiled, extending to oesophageal region; vulva prominent at the posterior end; excretory pore just anterior to vulva. Excretory cell well developed and produce gelatinous matrix. Genital tract convoluted with several eggs; anus and rectum invisible, tail tapering, short and blunt. *Male*: Vermiform, short and slender, free living in soil. Stylet and oesophagus reduced; spicules slightly curved. Bursa absent. Tail conical, pointed. *Second stage juvenile* (J_2): Vermiform; head region rounded continuous with body contour; stylet with rounded basal knobs. Oesophagus with strong medium bulb which is not well separated from procorpus; glands forming a basal bulb. Deirids present just behind nerve ring. Excretory pore at 50–60% of body length; tail elongate-conical (Luc et al. 1990; Ahmad 1996; Walia and Bajaj 2003; Jonathan 2010).

2.5.3 Biology and Life Cycle

The juveniles upon hatching from the eggs are of two different sizes i.e. short and long. The short juveniles develop as males without feeding whereas the long juveniles which are destined to be females parasitize the roots (Swarup et al. 1989). Initially, the nematodes are ectoparasites, feed on the epidermal cells of the root but later on second stage female juveniles (J_2) insert their head and neck into the root cortex with their posterior body region outside the root surface. A series of feeding or nurse cells are initiated around the head of the nematode. The developments take

place while they are attached with the roots and the nematodes develop as young females which are observed in groups clinging to rootlets. At maturity, the exposed part of the female swells into a characteristic asymmetrical shape. The mature females secrete gelatinous matrix through the excretory pore. Eggs are deposited in gelatinous matrix.

Parthenogenetic reproduction, males not required. The nematode completes its life cycle in 6–8 weeks at 25 °C (Jonathan 2010); several generations occurs in a year.

2.5.4 Management

Host plant resistance: Some grapevine rootstocks such as Richter 110, SO4 are resistant to *T. semipenetrans* (Aballay and Navarro 2005; McKenry and Anwar 2006). UCD-GRN1, UCD GRN5 and RS-3 are also poor hosts of this nematode (Ferris et al. 2012).

2.6 Ring Nematode

2.6.1 Brief Description

Ring nematode *Mesocriconema xenoplax* (Raski 1962; Luc and Raski 1981) *Criconemella* (De Grisse and Loof 1965) are sedentary ectoparasitic nematodes. They are also known as ring nematodes due to coarsely striated cuticle that gives a segmented appearance, like a series of rings on their body.

2.6.2 Distribution

Mesocriconema xenoplax are commonly distributed in the vineyards of eastern Washington (Howland et al. 2014). They are reported to cause moderate yield loss of grapes in California (Lider 1960; Raski et al. 1973). In Oregon, this nematode was recovered from more than 85% vineyards (Pinkerton et al. 1999). In commercial grape vineyards of southern Spain, *M. xenoplax* is predominant nematode species and detected in 34.4% soil and root samples (Téliz et al. 2007).

Systematic Position

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida

Suborder	Tylenchina
Superfamily	Criconematoidea
Family	Criconematidae
Subfamily	Criconematinae
Genus	*Criconema*

2.6.3 Diagnostic Characters

Female: Body typically short, wide with large annules. Stylet strong, basal knobs anchor shaped. Oesophagus with medium bulb and a narrow posterior bulb. Vulva located near posterior part of body. Monodelphic, single ovary extending anteriorly. Spermatheca present. Tail bluntly rounded to pointed. *Male*: Body slender and short with much smaller annules, anterior end rounded. Stylet absent. Oesophagus reduced. Spicule short, slightly curved; bursa weak; tail pointed. *Juveniles*: Resembles female. Body annules smooth to crenate, no row of scales (Raski and Luc 1987; Luc et al. 1990; Ahmad 1996).

2.6.4 Biology and Life Cycle

The nematodes feed on the cortical cells of plant roots causing pits or lesions, leading the vines debilitated. Due to feeding, there is rapid darkening and destruction of root tissues. The root systems are stunted with a few feeder roots (Santo and Bolander 1977). Such infested grape plants show stunted growth, chlorosis and poor vigour and are possibly more prone to winter injury.

Female lays eggs singly in the soil; first moult occurs inside the egg and second stage juvenile (J_2) hatches out, locate the root and feed ectoparasitically.

Parthenogenesis reproduction takes place and life cycle is completed in 25–34 days (Walia and Bajaj 2003).

2.6.5 Management

i. Host plant resistance

In western Washington, the rootstocks, 420A and 101-14 are highly resistant, while 110R is moderately resistant to the ring nematode. Since, ring nematode has a wide host range therefore it is difficult to eliminate this nematode by means of crop rotation i.e. growing other crop before planting a vineyard. Under such condition it will be important to continue to screen candidate rootstock material against several populations of these nematodes. This will aid to find out the broadest possible resistant rootstock material. *Muscadinia rotundifolia* cv Cowart, and its offspring

UCD GRN1, have been reported sources of resistance to ring nematode (Ferris et al. 2012).

ii. Cover crops: Sudangrass, SS-222 are antagonistic to *Mesocriconema xenoplax*.

2.7 Stubby Root Nematode

2.7.1 Brief Description

The feeding by stubby root nematodes (*Trichodorus* Cobb, 1913) can cause a stunted or stubby appearing root system and that's why these nematodes are commonly called 'stubby-root' nematodes. They feed on root hairs and on epidermal cells adjacent to the zone of elongation at root tips. *Trichodorus* are economically important plant parasites that also act as vector in transmitting virus to plants. They belong to the order Triplonchida which is characterized by having six layered cuticle (body covering). Their feeding apparatus is unique in the sense that they have an onchiostyle, a curved, solid stylet or spear which is different from all other plant-parasitic nematodes having straight, hollow stylets. *Trichodorus*, described by Cobb in 1913 was the only genus in its family but later in 1974, Siddiqi split the genus into two genera, *Trichodorus* and *Paratrichodorus*.

2.7.2 Distribution

Trichodorus and *Paratrichodorus* species have been found associated with grape cultivation in different regions of the world. *T. variopapillatus* and *T. viruliferus* reported from the rhizosphere of grapevine in Italy (Roca and Lamberti 1984), *T. orientalis* from Iran (De Waele and Hashim 1984), *T. californicus* from California (Al-banna and Gardner 1996), *T. viruliferus* from England, Holland and Germany (Decker 1989), *Trichodorus* sp. from Spain (Téliz et al. 2007) and *Paratrichodorus tansaniensis* and *P. tunisiensis* from Italy (Roca and Lamberti 1984).

Systematic Position

Phylum	Nematoda
Class	Enoplea
Order	Triplonchida
Suborder	Diphtherophorina
Superfamily	Diphtherophoroidea
Family	Trichodoridae
Genus	*Trichodorus*

2.7.3 Diagnostic Characters

Body cigar shaped. Onchiostyle (= stylet) thin, bristle like, solid, typically curved dorsally, without knobs. Oesophagus composed of two parts, an anterior slender tube and posterior with typical pyriform basal bulb which encloses oesophageal glands and their orifices. *Female:* Amphidelphic, ovaries paired, reflexed; spermatheca usually present. Vulva near middle of the body; vagina strongly muscular with prominent cuticularisation; tail very short, bluntly rounded; anus terminal. *Male*: Monorchic, with an extended testis; spicules arcuate, usually irregular tapering; gubernaculum always thin and relatively long; tail bluntly rounded; bursa absent or very small if present (Decraemer 1980; Luc et al. 1990; Jairajpuri and Ahmad 1992; Walia and Bajaj 2003; Jonathan 2010).

2.7.4 Biology and Life Cycle

Trichodorus are migratory ectoparasites that are mobile during each stage of their life cycle (Stark and Love 2003). The adult females lay eggs that remain in soil until they hatch as second-stage juveniles. Upon locating a root the juveniles aggregate around the growing root tips and starts feeding, moults three times to finally become an egg laying adult. The feeding by nematodes ceases the root elongation. Generally, the nematode completes its life cycle in 6–8 weeks.

The feeding on plant roots by *Trichodorus* consists of five phases: (i) exploration (ii) puncturing of the cell wall (iii) injection of glandular secretions (iv) ingestion and (v) withdrawal from the cell (Brown et al. 2004). They use their onchiostyle to puncture holes in plant cells. There it secretes from its mouth (stoma) salivary material into the punctured cell. The salivary material hardens rapidly into a feeding tube which functions as a straw enabling the nematode to withdraw and ingest the cell contents through the tube (Wyss 1981, 1987). After feeding on an individual cell, the nematode move on to feed on other cells, leaving old feeding tubes behind and forming new ones in each cell that it feeds from (Crow 2004). The feeding is normally restricted to epidermal cells and root hairs but when gregarious, feeding may extend up to cortical cells (Swarup et al. 1989). This lead to discolouration, superficial cracks on roots, shortened or stubby roots and stunted growth of the plant. The infected roots are less capable of supplying the plant with adequate water and nutrients from soil. The ultimate results can be seen in the form of reduced quality and quantity of crops.

2.7.5 Management

As described above under *Longidorus*.

2.8 *Stunt Nematode*

2.8.1 Brief Description

Stunt nematodes, *Tylenchorhynchus* (Cobb, 1913) are soil dwelling ectoparasites of a large number of crop plants of economic importance that also include grapes. They are found in a variety of soil but generally prefer sandy loam or loamy sand soil for their activity.

Systematic Position

Phylum	Nematoda
Class	Secernentea
Order	Tylenchida
Suborder	Tylenchina
Superfamily	Tylenchoidea
Family	Belonolaimidae
Subfamily	Telotylenchinae
Genus	*Tylenchorhynchus*

2.8.2 Diagnostic Characters

Small nematode, vermiform in shape. No sexual dimorphism in the anterior part of the body. Head region rounded, continuous with or offset from body. Cephalic framework light to moderately sclerotized. Stylet moderately sclerotized with rounded, backwardly sloping knobs. Lateral lines 2–5. Median bulb round to oval with distinct valve plates. Basal bulb offset from intestine or with base slightly extending over intestine (Fig. 3). Phasmids prominent, deirids often absent. *Female*: Vulva is almost near the middle of the body (Fig. 4). Spermatheca round, axial. Ovaries paired, outstretched, one directed anteriorly, one posteriorly. Tail variable from conoid with bluntly rounded terminus to cylindrical or clavate with rounded terminus (Fig. 5). *Male*: Spicules slightly curved. Gubernaculum well developed about half as long as spicules, generally rod-like, protrusible. Tail elongate, conoid and completely enveloped with bursa. (Luc et al. 1990; Ali 1995; Ahmad 1996; Siddiqi 2000).

2.8.3 Biology and Life Cycle

They are migratory ectoparasites, feeding on the epidermal and outer cortical cells often at the root tips. The female lays eggs singly in the soil and each stage have to feed on a suitable host in order to develop to the next stage (Bridge and Starr 2007). The general symptoms exhibited by plant are stunted growth, leaf chlorosis and retarded root system.

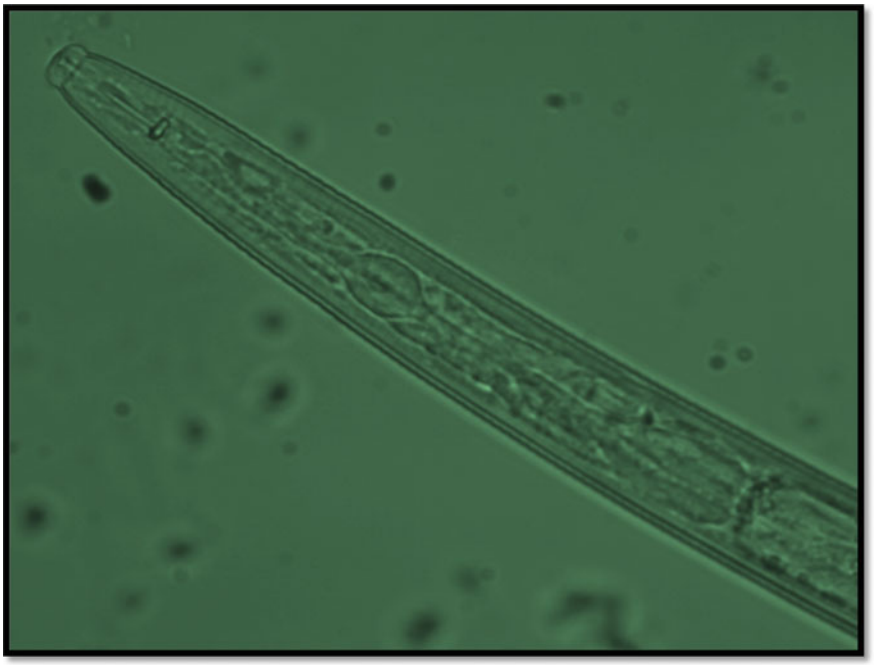

Fig. 3 Anterior portion of *Tylenchorhynchus*-Lip region offset from the body, stylet with large basal knobs, tripartite oesophagous with basal bulb slightly extending over intestine

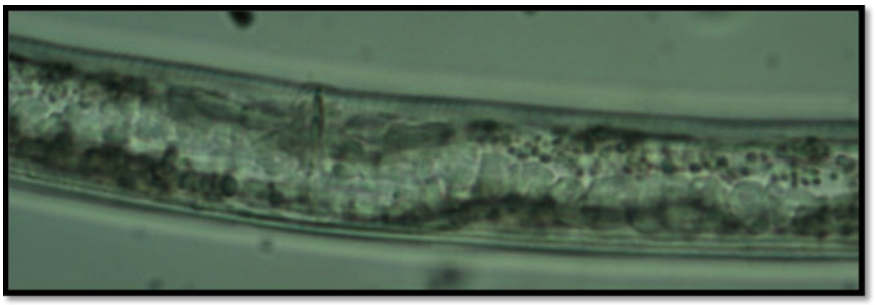

Fig. 4 Vulval slit in the middle portion of the body of adult female of *Tylenchorhynchus*

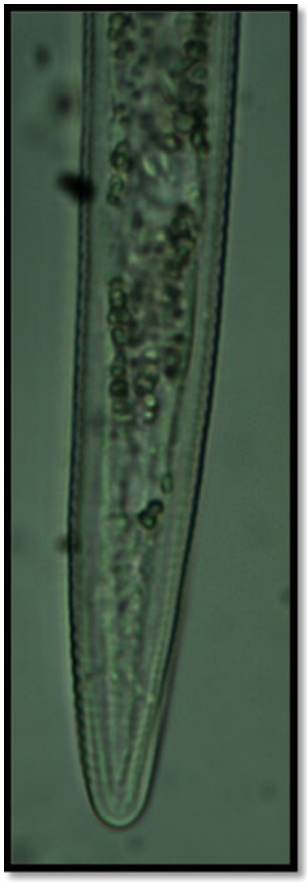

Fig. 5 Tail of *Tylenchorhynchus* showing conoid portion at the end

Life cycle is completed in 25–38 days depending upon the nematode species (Swarup et al. 1989).

2.8.4 Management

i. Use of certified rootstocks that are free from nematodes.
ii. Crop rotation with non-host crops in case of high nematode population.
iii. Application of neem, castor cake, compost or manures as soil amendments prior to planting can be effective in reducing the soil population of nematodes.

3 Conclusion

In the present chapter we have described the importance of natural infection by plant parasitic nematodes on grapevine rootstocks. Once a nematode problem develops it is difficult to correct. Chemical nematicides are considered one of the quick and best options but their application may disrupt the food web and cause negative impacts on beneficial organisms. Crop rotation or fallowing the land for a long period is not always justified for viticulturists. On the other hand, use of biocontrol agents alone may not prove successful particularly under field conditions and therefore its application requires conjugation with other management practices (Askary 2015a, b; Askary and Martinelli 2015), but they too may not provide full protection to grapevines against nematodes. Under these circumstances, developing of nematode resistance rootstocks seems a ray of hope and in most of the cases has been found successful in protecting plants against nematodes, thus a best option in the direction of quality and cost competitive grape production.

The diagnostic characters of some nematodes parasitic on grapevines are only a brief introduction in this chapter. The correct identification of species needs expertise. The correct identification coupled with analysis of rootstock responses to infection of particular nematode species would facilitate the selection of nematode tolerant/resistant cultivars which in turn would minimize the dependence on nematicides. The rootstocks that possess a degree of tolerance or resistance to some species of plant parasitic nematodes particularly *Xiphinema*, *Meloidogyne*, *Pratylenchus*, *Tylenchulus* and *Mesocriconema* must be evaluated by conducting multilocational trials so that their response to locally occurring nematode species or isolates may be confirmed, before being considered tolerant/resistant and recommended for planting in new areas. In fact, the aim of researchers should be to develop rootstocks with broad and durable nematode resistance. Simultaneously, a database needs to be prepared that would aid selection of rootstocks based on nematodes present in proposed vineyards. In the past few years research workers have developed and released some resistant rootstocks USDA 10-17A, USDA 10-23B, USDA 6-19B, RS-3 and RS-9, which exhibit resistance to more than one nematode species (Anwar et al. 2002; Gu and Ramming 2005).

In order to identify potentially damaging nematode species, extensive survey of vineyards should be conducted. Additionally, the relationship between nematode density and vine damage need to be characterized. Resistant grapevine rootstocks are used to limit damage caused by nematodes but the long-term use of the resistant rootstocks can impose strong selection pressure on a nematode population. Thus, nematodes able to reproduce and adapt to the roots of a resistant or moderately resistant rootstock will give rise to new pathotypes, making that rootstock useless for control of the nematode (McKenry 1987). Hence, nematode population of different sexes and stages, relative virulence of a particular species/pathotype, its host specificity and tolerance level in host, needs further investigation for assessing the role of these parameters in the management of plant parasitic nematodes (Askary et al. 2012b, 2014).

Acknowledgements This work was supported in part by MIDH Project entitled "Revival of grape culture through demonstration of improved propagation and canopy management practice techniques" running in the Division of Fruit Sciences, SKUAST-K, Srinagar, Jammu and Kashmir, India.

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Behavioural Ecology of Entomopathogenic Nematodes, *Steinernema* and *Heterorhabditis* for Insect Biocontrol

Tarique Hassan Askary, M. Jamal Ahmad, A. R. Wani, Sajad Mohiddin and Muneer Ahmad Sofi

Abstract Entomopathogenic nematodes *Steinernema* and *Heterorhabditis* are excellent biocontrol agents of crop insect pests. These beneficial nematodes decrease the reliance on synthetic pesticides and assist in maintaining a desirable ecological balance. They adapt to a wide range of behavioural strategies, and their degree of efficacy varies with species and the ecological niche where nematode-insect interactions take place. Occurrence and distribution of entomopathogenic nematodes are related to certain insect groups that are considered their suitable hosts. Soil texture and presence of a host affect the nematode's direction of movement. The specific behaviours and cues used by infective juveniles of different nematode species vary while searching and finding the hosts. The process of infection is governed by several factors such as host recognition behaviour, acceptance behaviour and infection behaviour. Cues such as feces or cuticle associated with living insects affect a nematode's foraging behaviour which varies with respect to nematode species. Therefore, correct selection of nematode species is of foremost importance in the insect pest management strategies. Apart from nematodes' habitat preference and infection behaviour, survival strategy and reproductive behaviour are other important parameters that need attention.

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E. Lichtfouse (ed.), *Sustainable Agriculture Reviews 31*, Sustainable Agriculture Reviews 31, https://doi.org/10.1007/978-3-319-94232-2_8

Keywords Entomopathogenic nematodes · *Steinernema* · *Heterorhabditis* Foraging behaviour · Infection behaviour · Reproductive behaviour

1 Introduction

Entomopathogenic nematodes belonging to the families steinernematids and heterorhabditids are widespread soil nematodes that are parasitic on various insect pests. These nematodes show different behaviour which is a set of activities and responses translated by nervous system in response to internal and external stimuli and are assessed in terms of locomotion, movement, feeding, mating, migration and penetration (Gaugler and Bilgrami 2004). While searching host these nematodes behave differently (Askary 2010), as some are cruise foragers such as *Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema cubanum*, *S. longicaudum* and *S. kraussei* which move actively through soil using distant volatile cues that assist them in finding the host whereas others are ambush foragers such as *S. carpocapsae*, *S. scapterisci* and *S. siamkayai* that utilize sit and wait foraging strategy, remain near the soil surface, lifting their body into the air in order to attach the passing insects. However, there are also few species that employ intermediate foraging behaviour such as *S. glaseri* and *S. feltiae* which act both as cruiser and ambusher (Campbell and Gaugler 1997; Csontos 2002).

The functional role of nematodes is generally determined by metabolic and behavioural activities (Askary and Abd-Elgawad 2017). The third stage juveniles of *Steinernema* and *Heterorhabditis* which are called infective juveniles are the only free living form found in soil. They are motile, virulent, possessing chemoreceptors, have high reproductive potential and the ability to seek out hosts, besides having the potential for long-term establishment through recycling and compatibility with other management strategies, such as chemical pesticides (Sankaranarayanan and Askary 2017). Infective juveniles emerging from a single cadaver may differ in behaviour. Males of *S. glaseri* emerge first from hosts and show responsiveness to volatile cues from an unidentified host in comparison to females. Contrary to this, males of *S. carpocapsae* and *S. feltiae* emerge later than females. The behaviour of some *Steinernema* species in relation to the soil habitat and in the presence or absence of the insect host showed that the survival of infective juveniles was for much longer periods in aerated water than in moist sand at a given set of temperature (Molyneux 1983). *Steinernema* have the ability to parasitize insects over a wide range of temperature but the temperature range of infectivity for insects differs between the species isolated from same geographical area. Therefore, in order to develop a quality entomopathogenic product, prior considerations should be the nematode host finding ability, virulence potential and reproductive ability in the target pest which demands a sound knowledge on behavioural ecology of nematodes (Askary and Ahmad 2017).

The present effort is aimed to provide comprehensive information on the behaviour of entomopathogenic nematodes in relation to their foraging strategy,

associated insect hosts, habitats, bacterial symbionts and reproductive ability so that these beneficial nematodes may be exploited efficiently when included into integrated insect pest management programme.

2 Foraging Behaviour

Foraging behaviour of nematodes is one of the most important considerations during a biological control programme as the recommendations of nematode species for the control of specific target insect pests are done on this basis. Infective juveniles of entomopathogenic nematodes find their host generally by employing three behaviours (i) Ambushing (ii) Cruising and (iii) Intermediate behaviour.

i. Ambushing behaviour: Ambushers adopt 'sit and wait' behaviour to infect insects when the later come in contact with the nematode on the surface of the soil. The nematodes acting as ambusher nictate during foraging i.e. they overcome meniscus forces, standing vertically out from the substrate and attach to a passing insect. Nictation has various forms that range from straight motionless behaviour to partial lifting from the substrate and waving back and forth. In fact, when the nictating anterior does not contact another surface projection, it bends in a loop, contacting a moist point on the posteriad body. The force generated by the subsequent opposing body wave abruptly breaks the anterior end free of surface tension at the point of contact. This can fling the anterior end upward with sufficient momentum to break the surface tension holding the posterior end to the substrate and jettison the entire body through the air (Reed and Wallace 1965; Campbell and Kaya 1999a, b; Gaugler and Bilgrami 2004). Ambushers do not respond to host released cues and they are considered suitable for mobile hosts. They are generally engage in "ranging search" on a smooth surface (Lewis et al. 1993). Species of *Steinernema* such as *S. carpocapsae* and *S. scapterisci* show ambushing behaviour i.e. standing on their tails and infect passing insect hosts easily by jumping on them.

Though, the standing and jumping behaviours of *S. carpocapsae* are suited for attaching to mobile hosts at the soil surface (Campbell and Gaugler 1993; Campbell and Kaya 2002), but this should not preclude them from use against subterranean pests. For jumping, nematodes form a loop with bodies, with the head held to the side of the body by the surface tension. Infective juveniles can leap distances of nine body lengths in the presence of a host (Campbell and Kaya 1999a, b). The frequency of jumping varies among species and increase by mechanical context, air movement and volatile host cues. Campbell and Kaya (2002) assessed the standing and jumping behaviour of the nematodes belonging to families Heterorhabditidae and Steinernematidae. Eleven species of *Steinernema* showed jumping behaviour, however jumping rate varied among the nematode species. Variation in the duration of crawling bouts, the tendency to body wave, the rate of standing, the duration of standing bouts, and the tendency to jump after standing contributed to the differences in frequency of jumping among species. The nematodes showing jumping behaviour within a species were similar in size, infectivity and sex ratio in

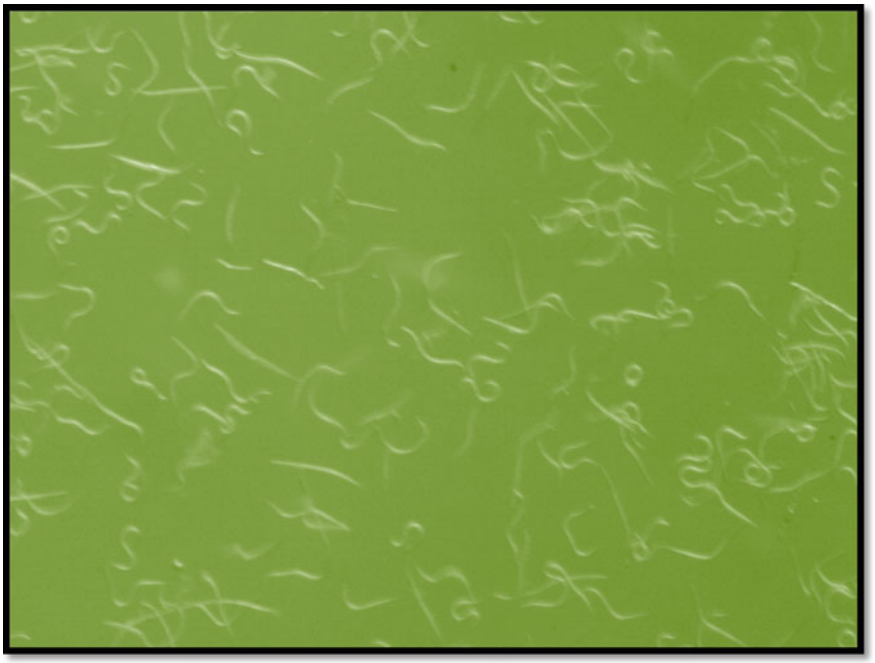

Fig. 1 Infective juveniles of entomopathogenic nematode, *Heterorhabditis bacteriophora*

comparison to those that did not jump. The jumpers were more infective and were found to shed their second stage cuticle.

ii. Cruising behaviour: Cruisers search their host by moving actively in soil. These nematodes are distributed throughout the soil profile and are best suited for immobile hosts, such as those which are deep in soil or in other cryptic habitats (Gaugler et al. 1997). They never nictate but respond to carbon dioxide released by insects as cues (Lewis et al. 1993). *Heterorhabditis* are generally cruisers, move actively in soil in search of their suitable host (Fig. 1). *S. glaseri* and *S. kraussei* also exhibit cruising behaviour.

iii. Intermediate behaviour: Some nematodes exhibit intermediate foraging behaviour, i.e. employing both ambush and cruise strategies to attack the mobile as well as sedentary insects present on the surface or deep in the soil. *Steinernema* are ambushers though their some species such as *S. feltiae*, *S. glaseri* and *S. riobrave* show intermediate behaviour i.e. cruising and also ambushing (raising themselves on substrate) for a short while (Grewal et al. 1994; Campbell and Gaugler 1997; Campbell and Kaya 2002; Griffin et al. 2005; Askary 2010).

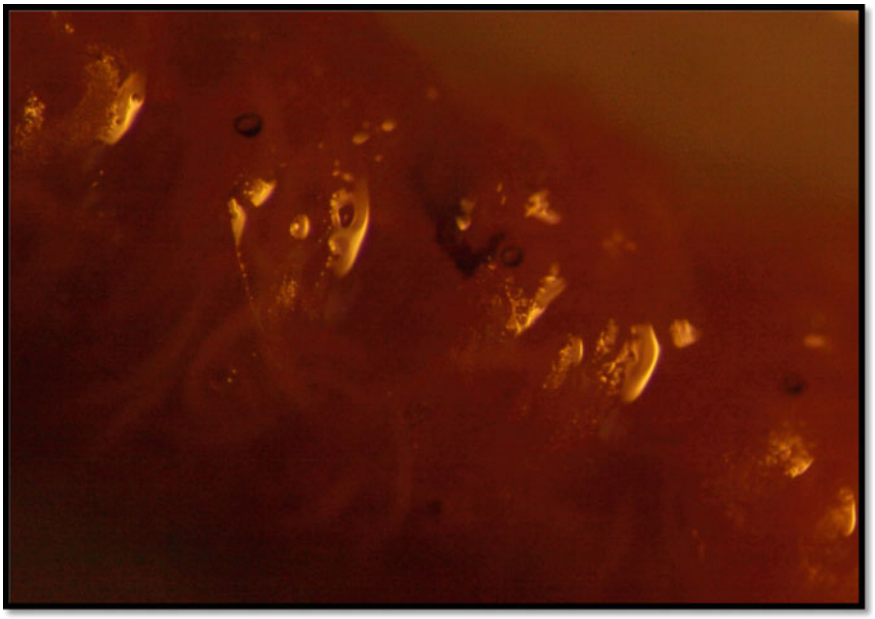

Fig. 2 Infective juveniles of *Heterorhabditis bacteriophora* inside the cadaver portion of rice case worm *Corcyra cephalonica*

3 Infection Behaviour

An important aspect which needs attention is recognition of host by infective juveniles and then entry into it. Infective juveniles of *Steinernema* and *Heterorhabditis* after entering into insect host develop there (Fig. 2). Host recognition can be measured by logging change in several types of behaviour in response to host related mortality (Ali et al. 2005). The movement parameters of entomopathogenic nematode that play an important role in host searching are duration of crawling, body waving, head waving and head thrusting. Though, infective juveniles have the ability to encounter both uninfected and infected hosts however they differ in responding. The quality of host also depends on the stage of the infection. Ramos-Rodriguez et al. (2007a) hypothesized that nematode response to infected hosts gets change over the course of an infection. They tested the attraction response of three different species of nematode i.e. *Steinernema carpocapsae*, *S. glaseri* and *S. riobrave* with different foraging strategies to infected and uninfected insects (*Galleria mellonella* and *Tenebrio molitor*). The test was performed at 24 h intervals from start of infection to emergence of infective juveniles from depleted host. *S. carpocapsae* was not very responsive to hosts but *S. glaseri* was highly responsive and *S. riobrave* was intermediate. Generally, the level of attraction did not change with time after infection and was similar between infected and

uninfected hosts. However, *T. molitor* infected with *S. glaseri* tended to be less attractive to *S. glaseri* than uninfected hosts.

The lipid reserves of infective juveniles decline with age which in turn has a negative effect in causing infection in a susceptible host. When the two foraging abilities of nematodes were compared, cruisers were found with high metabolic rate as compared to ambushers. However, the foraging ability declines with age. The ambushing behaviour (ability to stand on tail and wave the body in order to attach to a passing host) of *S. carpocapsae* has been observed decline with age (Lewis et al. 1997). The metabolic rate of *S. glaseri* is intermediate between cruisers and ambushers but they are hypothesized to contain more energy stores and are reported to survive up to 32 weeks. This is perhaps due to their larger size, approximately eight times the volume of the other species. Lewis et al. conducted a study on the three parameters i.e. locomotion, infectivity and lipid reserves of entomopathogenic nematodes, *Heterorhabditis bacteriophora*, *S. carpocapsae* and *S. glaseri.* They concluded that there was a significant effect of nematode ageing on these parameters. Locomotion, infectivity and lipid reserves declined as the age of nematodes increased. There was also a decrease in symbiotic bacteria with the increase in nematode age. Some species of *Heterorhbditis* such as *H. indica* and *H. pakistanensis* when stored in aqueous suspension, the nematodes settle and form a precipitate on the bottom of the container. An agglomerate is formed, leaving the nematodes under stress situation in an environment of low oxygen content, which may accelerate the loss of energy reserves, such as lipids (Gaugler et al. 2002). So they get close and arrange in rosette form. A technique employed to reduce these aggregates is the use of a sodium bicarbonate solution ($NaHCO_3$), which aids in breaking the rosettes without causing other effects on the nematodes (Woodring and Kaya 1988).

The infection behaviour also varies among nematode species. When the infection behaviour of three nematode species, *S. feltiae*, *S. riobrave* and *S. carpocapsae* was studied, a significant decrease in infection rate was observed at 6–9 h after injecting a host with conspecifics (Glazer 1997). Grewal et al. (1997) found that infective juveniles of *S. carpocapsae* were repelled from insect hosts infected for at least 4 h with heterospecific nematodes. Infective juveniles can make their entry into a host that has already been occupied by conspecifics, even to the point of overcrowding (Lewis et al. 2006). Christen et al. (2007) reported that though the number of invading infective juveniles of *S. riobrave* declined over time, but they continued to enter the host for at least 72 h since first infection and that may be presumably after the death of the host. Dillon et al. (2006) on the basis of an experiment reported that infective juveniles of *S. carpocapsae* were repelled from host that was infected with *S. glaseri.* It is assumed that the subsequent invasion is prevented due to an inhibitory substance diffused from the host (Selvan et al. 1993). On the other hand, infective juveniles of *S. glaseri* respond no differently to a host infected with *S. carpocapsae* than to a non-infected host. Overcrowding of infective juveniles in a host which has already harbouring many competitors appears maladaptive because overcrowding results in lower reproductive output per founder (Koppenhöfer and Kaya 1995; Ryder and Griffin 2002). It is suggested that such

behaviour may be due to few alternative hosts available, in which case it is better to invade a suboptimal host than to reject it and die of starvation (Christen et al. 2007). Also infective juveniles may have limited ability to assess the quality of the host, and this may be due to lack of suitable cues from the host or suitable sensory abilities of the infective juveniles. Kunkel et al. (2006) reported that waste products from host late stage of infection repel nematodes. According to Lewis et al. (2006) there are mixed evidence available on infective juveniles, whether or not they respond differently to conspecific and heterospecific infected hosts and most studies have looked at a limited number of time-points post-infection. It has been observed that 4 days old infective juveniles of *S. glaseri* were more attracted to parasitized hosts at 4–6 h after their exposure (Lewis and Gaugler 1994) in comparison to unparasitized hosts. *S. glaseri* is reported to invade hosts that were killed 10 days previously while *H. indica* entered hosts that had been dead for three days (San-Blas and Gowen 2008). These workers have the opinion that entomopathogenic nematodes should be considered facultative scavengers rather than obligate parasites. A phenomenon known as 'phased activation' wherein a proportion of infective juveniles that emerged from a host cadaver are infectious at any time has also been studied by some workers. It has been noticed that there is significant variability among individuals emerging from the same host cadaver in the timing of their maximum infectivity (Campbell et al. 1999; Perez et al. 2003).

There are certain host species to which entomopathogenic nematodes cannot kill but have the ability to reproduce in them (Puza and Mracek 2010). It has also been revealed that infective juveniles of most entomopathogenic nematode species can enter the hosts killed by some other causes (e.g. freezing) (San-Blas and Gowen 2008; Puza and Mracek 2010). This has a great significance for the persistence of natural populations of entomopathogenic nematode. The recycling of applied entomopathogenic nematodes is enhanced, particularly when applied during integrated pest management programs where hosts killed by other biological agents or chemical methods may be available and suitable for development. The report of entomopathogenic nematodes developing in hosts killed by insecticides has been confirmed by several workers (Hara and Kaya 1983; Koppenhöfer et al. 2003).

Dillon et al. (2006) reported that under field condition the rate of parasitization declined as the depth of the insects in soil increased. However, the rate of decline was similar for all entomopathogenic nematode species tested, irrespective of their behaviour i.e., ambush, cruise or intermediate foragers. *S. carpocapsae* parasitized larvae and pupae of the large pine weevil (*Hylobius abietis*) which live under the bark of tree roots. When application of *S. carpocapsae* was done at the soil surface, they parasitized these insects within the roots at depths of up to 40 cm in the soil. Acid pH is reported to reduce the efficacy of *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* when exposed to diapausing larvae of spruce webworm, *Cephalcia abietis* (Jaworska 1993), however at pH 6.9 and 8.0, mortality of cotton leaf worm, *Spodoptera littoralis* by *S. carpocapsae* and *H. bacteriophora* was higher and more rapid than at pH 5.6 (Ghally 1995).

4 Foraging Strategy-Host Interactions

The process of infection is governed by host recognition behaviour, acceptance behaviour and infection behaviour. Infective juveniles assess their hosts for entry by responding to cues such as feces or cuticle associated with living insects (Grewal et al. 1993; Lewis et al. 1996). The existence of a host and its chemical cues are likely to affect a nematode's foraging behaviour. The behavioural response of *S. carpocapsae* to various host species is correlated with the level of reproduction supported by the hosts (Lewis et al. 1996). The foraging strategy of nematodes while interacting with hosts is correlated with other aspects such as parasite ecology, behaviour, physiology and anatomy (Campbell and Lewis 2002). While selecting host, entomopathogenic nematodes have to follow a series of steps that include search for host habitat, host finding, host acceptance, and host suitability (Doutt 1964). The specific behaviours and cues used by infective juveniles of different nematode species vary while searching and finding the hosts. Ambushers use 'sit and wait' foraging strategy and respond to volatile chemical cues from the host when in a standing posture to enhance contact with a passing insect (Campbell and Kaya 2000), but they are not attracted to host volatile cues while crawling on the substrate, unless they have already contacted a host (Lewis et al. 1995). Cruisers readily respond to volatile chemicals cues encountered by crawling toward the source of the cues (Lewis et al. 1992, 1993). Factors affecting infective juveniles behaviour include CO_2, temperature gradients, and host feces (Gaugler et al. 1980; Byers and Poinar 1982; Grewal et al. 1993; Lewis et al. 1993). Species of *Steinernema* which show nictation and jumping behaviour are stimulated by CO_2. Campbell and Lewis (2002) found *S. feltiae* showing significant infection preference for a host that was parasitized 24 h before in comparison to unparasitized host. The actual reason for these changed behaviour are unknown, however differences in CO_2 production between infected and uninfected insects have been reported (Ramos-Rodriguez et al. 2007b). Ramos-Rodriguez et al. (2007a) are of the opinion that the level of attraction would change as the quality of the host as a resource changes over time from initial infection. It is suggested that attraction of infective juveniles to host would be greatest at the time-points when the CO_2 production from infected hosts is at distinct peaks. Since the distribution of entomopathogenic nematodes in soil is often in clump (Stuart and Gaugler 1994; Glazer et al. 1996; Campbell et al. 1998), therefore, infective juveniles have the potential to encounter the hosts already infected. Infective juveniles infecting a host that has already been infected, particularly at time-points late in the infection process, may lead to increased competition for diminished resources or even the lack of sufficient nutrients in a host cadaver to complete development (Selvan et al. 1993). Besides infecting hosts by conspecifics, nematodes may also encounter hosts infected by heterospecific individuals. This ability will ease to distinguish between infected or uninfected hosts, and among hosts at different time-points after infection (Griffin 2012).

Entomopathogenic nematodes are also able to detect the presence of other pathogens of insect. Barbercheck and Kaya (1991) found accumulation of *S.*

carpocapsae and *H. bacteriophora* more around the healthy insect host than a host infected with the fungus *Beauveria bassiana.* Grewal et al. (1994) tested the foraging strategies of eight entomopathogenic nematode species for their response to host volatile cues and dispersal behaviour on 2-dimensional substrates. *H. bacteriophora*, *H. megidis*, *S. anomali* and *S. glaseri* showed positive directional response to chemical cues and travelled similar distances on smooth (agar) or nictation substrates (agar overlaid with sand grains) which suggest their cruising approach to find hosts. *S. carpocapsae* and *S. scapterisci* did not showed any directional response and travelled less distance on nictation substrate, than on smooth agar. Thus, these two nematode species can be considered to have ambushing mode of foraging. *S. feltiae* and *Steinernema s*p. were intermediary in the search continuum sharing both the characteristics of ambush and cruise foragers. They responded directionally to host volatiles, but travelled less distance on the nictation substrate than on smooth agar, however neither of the two nictate. The cruisers located their hosts more effectively in the sand columns, whereas the ambushers were more effective at finding hosts on filter paper. Both *S. feltiae* and *Steinernema* sp. performed equally on filter paper as well as in the sand column. Ennis et al. (2010) reported in the case of pine weevil larvae that roots facilitated the movement of *S. carpocapsae* through media (sand or sand/peat mix), and this movement was enhanced by physical and/or chemical stimuli from weevils feeding on the roots. Susurluk (2008) compared the vertical movement of Turkish isolates of *S. feltiae* (TUR-S3) and *H. bacteriophora* (TUR-H2) at different temperatures in the presence and absence of greater wax moth, *Galleria mellonella* larvae. When the larva was placed on the bottom of the column, both the nematode species moved faster towards there. The vertical dispersal ability was found greater in *S. feltiae* as compared to *H. bacteriophora*. There was a direct relationship between nematode movement and temperature. As the temperature increased, the vertical movement of both the nematode species increased. Lower temperature depressed the movement of *H. bacteriophora* more than *S. feltiae*. The pathogenic potential of nematodes that had migrated different distances were compared with *G. mellonella.* The positive correlation between the distance travelled and infectivity indicated that there was a link between host-searching behaviour and infection behaviour in *S. feltiae* and to a lesser extent, also in *H. bacteriophora* (Susurluk 2008). *S. carpocapsae* proved much better than *H. bacteriophora* and *S. glaseri* in host finding ability, in case when the hosts were present at the soil surface but was less effective when hosts were below the surface (Alatorre-Rosas and Kaya 1990; Koppenhöfer et al. 1996).

5 Habitat Preference

Steinernematids and heterorhabditids are widespread soil nematodes which occur in various ecosystems and habitats. Their occurrence and distribution are related to certain insect groups that are considered their suitable hosts (Griffin 2012). They are

frequently available in insect rich habitats, such as tree (especially deciduous tree) habitats. Mracek et al. (1999) recovered highest numbers of entomopathogenic nematodes from apple and cherry verges along roadsides, lime and poplar hedgerows, and oak forests. The nematodes were recorded from 67% of habitats with high or moderate insect abundance. *S. kraussei* can be found in higher altitude in more spruce forests above 1000 m altitude. They were located at 2530 m in the Swiss Alps (Steiner 1996); 1290 m from subalpine meadows in Vitosha Mts in Bulgaria and from mountain forests at altitudes ranging from 700 to 1300 m (Shishiniova et al. 1997). Soil texture and presence of a host affect the nematode's direction of movement (Gaugler and Kaya 1993). Kruitbos et al. (2010) are of the view that *S. carpocapsae* instead of an ambush forager, is a habitat specialist, adapted to organic media such as peat or leaf litter. Their experiment showed that infective juveniles moved towards hosts more readily in peat than in sand. They suggested that *S. carpocapsae* remain near the surface because it does not move well through the mineral soils or the pure sands frequently used in experiments. The body waving characteristic of *S. carpocapsae* infective juveniles is an adaptation for bridging large pore spaces rather than attaching to passing insects at the soil surface. Natural association of *S. carpocapsae* with organic soils (e.g. peat) or soil horizons (e.g. litter layers) would support this hypothesis, however according to Griffin (2012) any such association has not been reported to date. In a study, infective juveniles of different species were observed to move into different directions when placed in the middle of the soil column. Most *H. bacteriophora* and *S. carpocapsae* move upwards rather than downwards, whereas most of the *S. glaseri* move downwards rather than upwards (Gaugler and Kaya 1990). Morton and García-Del-Pino (2009) isolated *S. feltiae* and *H. bacteriophora* from stone-fruit orchards in two Mediterranean regions of Spain. The activities of these nematodes such as tolerance to environmental heat, desiccation and hypoxia, the effect of temperature on infectivity and reproduction and nematode migration in sand columns were compared among isolates and also with a strain of *S. carpocapsae*. The results showed differences among species and a great variability within species too. Koppenhöfer et al. (1997) hypothesized that survival of entomopathogenic nematode in dry soil increased when contained within an insect cadaver. *H. bacteriophora* causing infection produced a gummy consistency in host cadaver that is supposed to help in retaining moisture.

6 Bacterial Symbionts and Nematode Behaviour

The bacterial symbionts (*Photorhabdus*, *Xenorhabdus*) of entomopathogenic nematodes play a significant role in the death of the host and nutritional requirements of nematodes (Han and Ehlers 2000; Ciche et al. 2006). These bacteria have an effective variety of toxins and antibiotics (Askary and Abd-Elgawad 2017). The behaviour of nematodes is also affected by bacterial symbionts and most of the effects on nematode behaviour occur during the growth phase of the bacteria within

the host. Few workers reported that *S. carpocapsae* infective juveniles without symbiont survived longer than those with them, and this was presumed to be due to the energetic cost of maintaining the bacteria (Mitani et al. 2004; Emelianoff et al. 2007). Also, infective juveniles of S. *carpocapsae* harbouring few bacteria survived for longer period than those with more bacteria however, the former had a lower reproductive rate upon entering into a host (Emelianoff et al. 2008). The impact of bacterial symbiont on behaviour of would-be scavengers of the insect cadaver is also reported. Some workers have reported cadaver repellent to ants due to bacterial products from both *Xenorhabdus* and *Photorhabdus* (Baur et al. 1998; Zhou et al. 2002). European robin, *Erithacus rubecula* which is an avian predator, was not attracted to the cadavers infected with *Heterorhabditis bacteriophora* due to the red colour reinforced by unpalatable taste (Fenton et al. 2011).

7 Reproductive Behaviour

Lewis et al. (2006) have the opinion that infective juveniles decide whether the reproduction is to take place within insect cadavers. The choice of invading the host by infective juveniles also affects the pool of mating partners (and competitors) available to them when mature. Pheromones have been implicated in the sexual attraction of over 30 species of nematode, but relatively few genera (Lee 2002). The females of *S. carpocapsae* produce a pheromone that has the characteristic to attract males (Neves et al. 1998). It has been reported that male *S. carpocapsae* were attracted to virgin females, but not to mated ones (Lewis et al. 2002). Thus, it may be suggested that once after mating the females do not secrete pheromone or the females do not mate repeatedly. An ethogram was developed to show that males of *S. carpocapsae* respond strongly to virgin females when present in their immediate proximity. A high percentage (80%) of males crawled to the females and among them 56.25% approached the female middle section where the vulva was located, 37.5% approached the female head while the rest i.e. 6.25% approached the tail (Lewis et al. 2002). While mating, the male of *Steinernema* coils around the female at the vulval region (Strauch et al. 1994; Lewis et al. 2002), however the male of *Heterorhabditis* has been observed to align its body approximately parallel to the female without coiling around her; male head is pointed in the opposite direction from that of the female, with much of its body away from the female (Huettel 2004), thus making copulation in liquid culture impossible (Strauch et al. 1994). The morphological reasons for the difference in the mating positions are still not clear. Ebssa et al. (2008) reported that besides attraction, the diffusible substances that originate by the females of *Steinernema* may have organizational effects on males. Presence of female nematode in close proximity is supposed to develop a male nematode for maturation. The males of *S. longicaudum* that developed alone did not mature sexually and were incapable of fertilizing eggs and no sperm were visible in their reproductive tract, but when exposed to conspecific females, though separated by a barrier allowed males to mature. This has implications for mass production as

unproductive amphimictic adults that are produced by the first generation hermaphrodites compete for resources with the hermaphrodites and the infective juveniles that develop in them (Ehlers 2001). In case of *heterorhabditis* sperm competition may occur i.e. competition between males and hermaphrodites to fertilize a given set of ova. *H. bacteriophora* hermaphrodites have been found to prefer male sperm (Dix et al. 1994). Mating or copulatory plugs are deposited over the vulva by male *Heterorhabditis* spp. (Dix et al. 1994; Strauch et al. 1994). Such plugs are assumed to have evolved in response to male-male competition, so that mating from a second male can be prevented (Barker 1994). However, plugs are not always effective (Timmermeyer et al. 2010). It is yet to be tested whether remating of *Heterorhabditis* spp. females or hermaphrodites are prevented by copulatory plug.

The juveniles of *Heterorhabditis* developing within the mother become exclusively infective juveniles, while eggs laid into the cadaver may continue to develop as adult, so it is likely that the "sacrifice" of its soma to infective juveniles is an adaptive response designed to optimize the use of a limited and ephemeral cadaver. In case of *Heterorhabditis*, only those juveniles that develop within the mother are colonized by bacteria. It is suggested that intra-uterine development is an adaptation for symbiont transmission (Ciche et al. 2008). However, this phenomenon has evolved to optimize female's reproductive success in response to stressful or starvation conditions, and may be seen as a form of parental behaviour (Griffin 2012).

8 Conclusion

In the foregone review it is very clear that there are variations in every trait of entomopathogenic nematode. These variations may be due to differences in environmental and internal factors, including insect host, soil texture, age of infective juveniles, the conditions under which they developed, and the bacterial load carried by the infective juveniles (Griffin 2012). A better understanding of nematode behaviour and physiology will certainly lead to further development of perfect formulations and ultimately their exploitation in farmers' field (Coupland et al. 2017). In the past two decades, the advances or breakthrough in the areas like isolation, formulation and their application against numerous economically important insect pests entail that there is much relevant information for research practitioners working in different laboratories of the world (Abd-Elgawad et al. 2017). The host-finding abilities of *Steinernema* and *Heterorhabditis* have still not been clearly understood though their some species have excellent pathogenic potential but have a narrow host range which needs special attention. Since they are applied inundatively, they may be tailored by breeding or transformation, to their intended purpose and to ecological incompetence, so as to improve their efficacy and ecological safety (Downes and Griffin 1996). It is difficult to understand precisely the functional significance either for entomopathogenic nematode fitness or biocontrol utility, since most behavioural studies are necessarily conducted in simplified conditions, that differs from natural environment where the situation is

often complex. Therefore, extensive studies are required because there are differences to what is known in the laboratory to the real lives of entomopathogenic nematodes in soil, whether as natural populations or applied for biological control. Researchers should pay attention to isolate indigenous nematodes in order to expand the genetic base of indigenous nematode strain, knowing their adaptability to exploit them in a tactful manner (Askary et al. 2017). A detail study is needed on habitat preference, host finding ability, host specificity and infection ability of entomopathogenic nematodes in soil. Another aspect that has got less attention by research workers is the reproductive behaviour of entomopathogenic nematodes. Though, as reviewed above, sex pheromones, copulation behaviour, sperm competition and sexual maturation have all received some attention but still enhanced knowledge on the subject is required so as to obtain maximum benefits from these beneficial microorganisms in sustainable agriculture.

Acknowledgements This work was supported in part by All India Coordinated Research Project (AICRP), ICAR on Biological Control of Crop Pests and Weeds running in the Biological Control Laboratory, Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Srinagar, Jammu and Kashmir, India.

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Beneficial Soil Microbiome for Sustainable Agriculture Production

Carmine Crecchio, Tanja Mimmo, Davide Bulgarelli, Ilaria Pertot, Youry Pii, Michele Perazzolli, Marina Scagliola and Stefano Cesco

Abstract The projected increase in world population and the need to reduce the reliance on non-renewable inputs, such as synthetic agrochemicals, are challenging the current vision of agriculture. In particular, to achieve a fair and sustainable global food security, disruptive changes in crop production are unavoidable. A promising strategy proposes to exploit the metabolic capabilities of soil microbial communities, i.e., the microbiome, to conjugate stable yield with reduced impact on the agroecosystem. In this chapter, we introduce the microbiome populating the

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E. Lichtfouse (ed.), *Sustainable Agriculture Reviews 31*, Sustainable Agriculture Reviews 31, https://doi.org/10.1007/978-3-319-94232-2_9

root-soil interface from an evolutionary perspective. Next, we discuss the molecular bases of plant-microbe interactions in soil and how these interactions impact plant growth, development and health. We illustrate how plant-probiotic members of the microbiome can be isolated from soil and further characterized for their biological activities, a key pre-requisite for translational applications. In addition, we focus on paradigmatic examples of soil microbes turned into inoculants for agriculture, their fate on soil, their impact on the native microbiome and the beneficial effects exerted on crop production.

Keywords Beneficial soil microbiome · PGPR · Plant-microbiome interactions Bio-fertilizers · Bio-fungicides · Bio-herbicides · Dynamic soil microbiota

1 Introduction

The soil microbiome defines the community of microorganisms, and their encoded functions, inhabiting the soil environment. The soil microbiome acts as a main source of inoculum for the rhizosphere, the thin layer of soil adhering to and influenced by plant roots. Bacteria, fungi and, in specific environments, protozoa and algae too, coexist in the rhizosphere, bacteria being the most abundant (Tate 1995). Such a microbial diversity within the rhizosphere mirrors a functional diversification in the interactions with plants. For instance, some rhizosphere microorganisms, pathogens, can have detrimental effects on their host whereas others may exert beneficial effects, ranging from stimulating plant growth to reducing the damage from soil-borne pathogens.

Therefore, understanding the structure and function of the rhizosphere microbiome has a great potential for discoveries both in basic science and translational agriculture. Studies conducted with both model and cultivated plants revealed that the recruitment and maintenance of the microbiome is affected by different factors. Plant morphology creates different habitats that give rise to distinct microbial composition in the vicinity of and associated with plant roots (Bulgarelli et al. 2013; Edwards et al. 2015). Likewise, plant rhizodeposition, i.e. the release of organic compounds serving in general as nutrients for soil microorganisms, is a key factor that discriminates among microbial groups and species, thus directly influencing microbiome proliferation and diversity (Rosier et al. 2016). Considering the relatively high concentration of nutrients, particularly carbon sources, the rhizosphere microbiome is mainly composed of copiotroph microorganisms, respect to oligotrophs, which survive in much lower carbon concentrations, such as non-amended mineral bulk soils. The main soil characteristics, such as pH, micro and macronutrients content and availability, redox and water potential, as well as environmental factors do contribute to shape microbiome composition and activity, too.

Due also to human activities, environmental health is worldwide declining causing a decrease in nutrient availability, a loss of productivity and desertification. This situation is further exacerbated by the continuously increasing amount of pollutants released into the environment. Besides traditional solutions, the use of beneficial microorganisms as bio-fertilizers and biocontrol agents is promising to achieve sustainable agricultural production. Nevertheless, how these microbes beneficially affect plants and how they interact as a community and between each other is still not clearly understood (Fig. 1).

The aim of this review is to comprehensively describe (i) the structure and the functioning of the microbiome thriving at root-soil interface and (ii) the mechanisms by which plant and microbes interact; (iii) the methods to estimate active bacteria in soil and how they can be screened and isolated; (iv) agricultural uses of beneficial soil bacteria, focusing on current as well as potential applications; (v) other bio-simulants alternative or cooperating with beneficial bacteria.

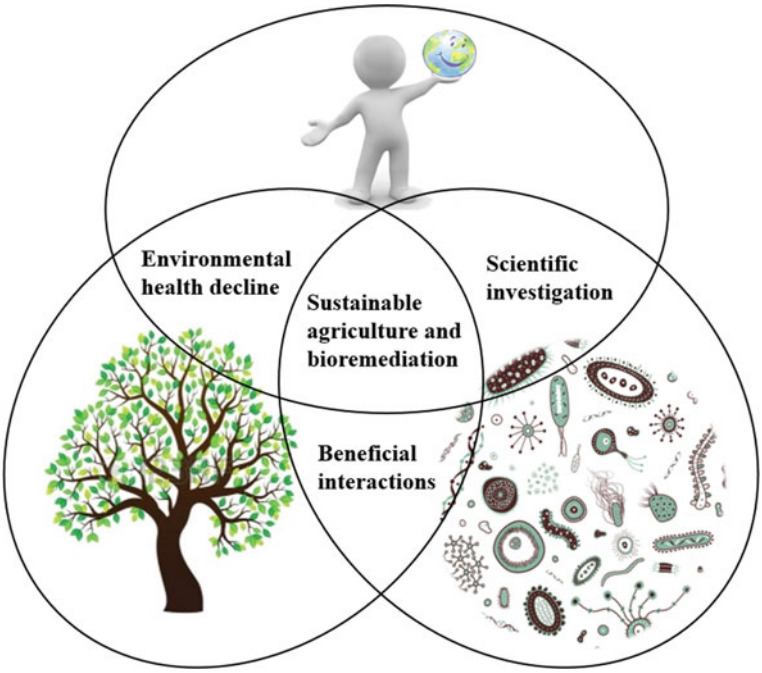

Fig. 1 Relevance of scientific investigations to study beneficial soil microbiome and their applications for a sustainable agriculture and to contrast negative effects of human activities on environmental health. Unsustainable agricultural practices have negatively affected environmental health, causing the degradation of soil properties. Soil microbiome plays a key role in preserving soil chemical and biological balance, through beneficial interaction with plants. On these basis, scientists aim is to investigate the mechanisms underlying the plant-microbiome interactions to achieve sustainable agriculture and bioremediation

2 Structure and Functioning of Plant-Associated Soil Bacteria

Since the inception of the transition from the aquatic to the terrestrial environment, early land plants became exposed to a wide variety of microorganisms, including bacteria, fungi and protists (Kenrick and Crane 1997). The establishment of interactions with this 'pristine' soil biota represented a hallmark for plants' adaptation to the new ecosystem: fossil evidence indicates that plants engaged in symbiosis with arbuscular mycorrhizal fungi, microorganisms capable of enhancing plant's mineral uptake form soil, as early as ~400 million years before present (Lambers et al. 2009). Likewise, phylogenetic investigations support the hypothesis that a single evolutionary innovation, likely occurred ~100 million years before present, has granted to certain angiosperms the capacity to develop specialized symbiotic relationships with nitrogen-fixing bacteria (Werner et al. 2014). In the last decade, advances in experimental and computational approaches have allowed scientists to unlock and catalogue the diversity of microbes interacting with plant roots at an unprecedented depth (Lebeis et al. 2012). These investigations revealed that plants host complex microbial communities, *the plant microbiota*, of which arbuscular mycorrhizal fungi and nitrogen-fixing bacteria can be considered 'extreme forms' of a continuum of symbiotic associations that comprises mutualism, commensalism and parasitism (Schlaeppi and Bulgarelli 2015). Indeed, the plant microbiota and their encoded genes, *the plant microbiome*, represent a still untapped resource of plant probiotic functions, such as enhanced mineral acquisition from soil and indirect pathogen protection (Bulgarelli et al. 2013).

Studies conducted with both model plants (Bulgarelli et al. 2012; Lundberg et al. 2012) and crops (Peiffer et al. 2013; Edwards et al. 2015) indicated that the soil type is a major determinant of the structural and functional composition of the plant microbiota. A prediction of this observation is that a major source of inoculum for the plant microbiota is the soil and, in turn, its characteristics modulate microbiota's recruitment. Comparative studies indicate that a taxonomically congruent group of bacteria, namely members of the phyla Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria, largely dominate the microbial assemblages associated with both monocotyledonous and dicotyledonous plant species (Hacquard et al. 2015). Intriguingly, this observation is in striking contrast with the broad taxonomic diversity of the soil biota (Fierer et al. 2012). Therefore, the plant microbiota emerges as a distinct community not randomly assembled from the soil biota but rather, as the outcome of specific recruitment cues modulating the proliferation of bacteria thriving in association with plant roots. A survey of the available literature indicates that this selection is operated, at least in part, by the host plant itself. In particular, a multilayered process triggered by a substrate-driven selection, i.e. the plethora of organic compounds released through rhizodeposition, represents an initial recruitment step for the plant microbiota, whose composition is ultimately fine-tuned by other or additional host-molecular mechanisms (Bulgarelli et al. 2013; Hacquard et al. 2015).

Recent breakthrough discoveries brought the host immune system (Jones and Dangl 2006) at center stage in the recruitment and maintenance of the plant microbiome. For instance, studies conducted with the model plant *Arabidopsis thaliana* indicate that a fully-functional immune system is required for the assembly of the endogenous, non-pathogenic, root microbiota (Lebeis et al. 2015; Hiruma et al. 2016). These observations are further strengthened by the fact that microbial components capable of 'dialing' into the plant immune system, such as the Type-III secretion system (Guttman et al. 2014), represent a distinctive feature enriched in and discriminating between plant-associated and unplanted soil microbiome (Ofek-Lalzar et al. 2014; Bulgarelli et al. 2015). From an evolutionary perspective, crop plants are the result of man-driven on-going selection processes, designated domestication and diversification, which progressively differentiated modern cultivated varieties from their wild ancestors (Abbo et al. 2014). In a relatively narrow time scale, i.e., since the dawn of agriculture ~10,000 years before the present, these selection processes markedly impacted on the genetic and phenotypic characteristics of crop plants (Doebley et al. 2006). Perhaps not surprisingly, a critical appraisal of the current literature suggests that domestication and diversification impacted also on the root-associated microbial communities (Perez-Jaramillo et al. 2016). Therefore, humans should be considered as a determinant of the plant

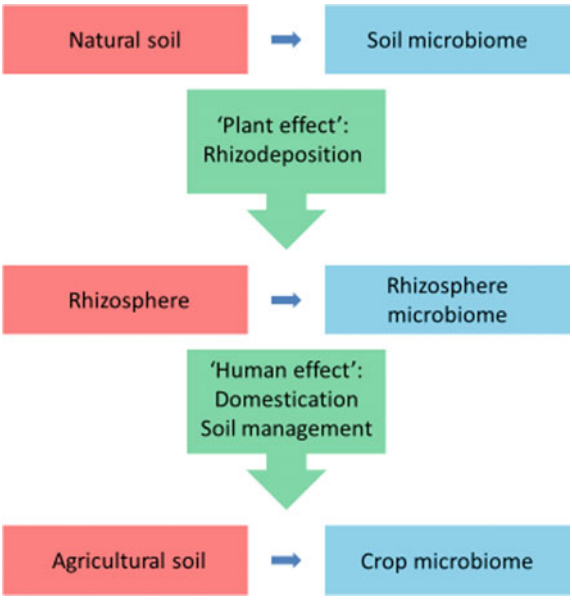

Fig. 2 Progressive differentiation of the agricultural microbiome from the native soil biota. Soil characteristics drive the composition of the soil microbiome. Plant colonization, through multiple processes including, but not limited to, rhizodeposition sculpts the microbiome thriving at the root-soil interface. Human intervention can contribute to the process of microbiome differentiation directly, through agricultural practices modifying soil properties, and indirectly, through the cultivation of defined plant genotypes derived from domestication and breeding selection

microbiome. For this reason, a detailed understanding of plant-microbe interactions taking place at the root-soil interface, both in model and domesticated plants will be paramount to unlock the potential of the microbiome for agriculture (Fig. 2). In the next section, we will critically discuss the recent insights into this research field and we will highlight promising avenues for future investigations.

3 Mechanisms of Plant-Bacteria Interactions

Within the microbiome associated with plant roots, plant growth-promoting rhizobacteria (PGPR) is the group of rhizosphere bacteria, which can exert positive effects on the growth of plants, basically through two kinds of mechanisms. The direct mechanisms include those modes of action by which PGPR can promote the growth of plants regardless of the presence of pathogens (Lugtenberg and Kamilova 2009), as in the case of the improvement of mineral nutrients acquisition (Pii et al. 2015a; Alegria Terrazas et al. 2016) and by the modulation of hormones levels (i.e. auxins and ethylene) in the root tissue (Glick 2012). The indirect mechanisms encompass all those actions that are aimed at protecting plants from the attack of pathogenic microorganisms (Lugtenberg and Kamilova 2009), by activating the induced systemic resistance (ISR) and by inducing the synthesis of stress related compounds (Parray et al. 2016) (Fig. 3).

3.1 Direct Mechanisms

Among the direct mechanisms, the enhancement of plant mineral nutrition is one of the best studied, since plant growth and productivity strongly depend on the bioavailability of mineral nutrients in the rhizosphere (Pii et al. 2015a). PGPR can facilitate the acquisition of mineral resources in different ways, e.g. biological nitrogen fixation, phosphorus solubilization, production of siderophores and manipulating the biochemical and molecular pathways devoted to nutrient acquisition (Pii et al. 2015a, 2016; Alegria Terrazas et al. 2016).

(a) *Biological nitrogen fixation*

The biological nitrogen fixation (BNF) is the conversion of molecular nitrogen (N_2) to ammonia (NH_3) catalyzed by nitrogenase enzyme (Kim and Rees 1994), and it can be carried out by both symbiotic N_2-fixing bacteria (i.e. rhizobiaceae and *Frankia*) and free-living diazotrophs that are capable of establishing non-obligate relationships with host plants, such as *Cyanobacteria*, *Azospirillum*, *Azotobacter*, and *Azoarcus* (Bhattacharyya and Jha 2012; Bhat et al. 2015). Nevertheless, it has been estimated that the free-living diazotrophs can contribute only with a small amount of bioavailable N to the plants, whilst the most efficient N_2 fixation process

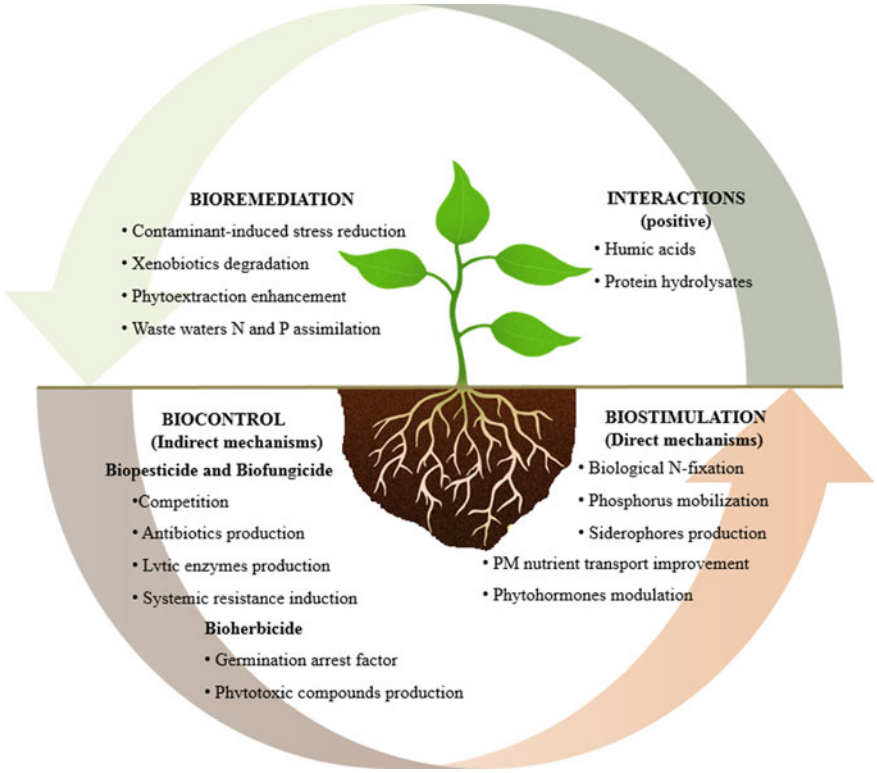

Fig. 3 Schematic overview of the plant beneficial microbial functions on plants and environment. PGPR can positively affect plant growth and development by several activities, directly as well as indirectly. The direct mechanisms enhance plant nutrient acquisition, while indirect mechanisms protect plants against pests. Likewise, these beneficial microorganisms are considered an interesting resource in bioremediation, enhancing plants in extraction, degradation and elimination of several soil contaminants. PGPR would seem to have a positive interaction with other bio-stimulants, such as humic substances and protein hydrolysates, increasing their beneficial effects on plants

occurs within specialized organs, the root nodules, that are developed following the establishment of a symbiotic relationship, i.e. between legume plants and rhizobia (Jones et al. 2007).

(b) *Phosphorus solubilization*

Soil can contain large amount of phosphorus (P), both organic and inorganic, however it is estimated that less than 1% of P is readily available for plants (Bhattacharyya and Jha 2012; Alegria Terrazas et al. 2016). The available P form for plants occurs as monobasic phosphate ($H_2PO_4^-$), which displays an approximate 10 μM concentration in the soil solution (Bieleski 1973; Marschner 2011); it is therefore clear that immobilized P forms have to be made available for plants,

through solubilization processes in the case of inorganic forms or by enzymatic mineralization in the case of organic P (Giles and Cade-Menun 2014; Gerke 2015).

Concerning the inorganic P, mainly bound to iron (Fe), aluminum (Al) and calcium (Ca) in soil (Igual et al. 2001; Gyaneshwar et al. 2002), the release of organic acids (formic, gluconic, 2-ketogluconic and shikimic acid) represents the major process through which phosphate-solubilizing microorganisms (e.g. *Pseudomonas, Burkholderia, Enterobacter, Bacillus, Penicillium and Aspergillus*) are able to enhance P bioavailability, through direct chelation of the counter ions (Fe, Al, Ca) (Hinsinger 2001; Hunter et al. 2014); in addition, the exudation process is often coupled with the cotransport of protons across the plasma membrane causing an acidification of the external media that further induce the acid dissolution of the immobilized P sources (Alegria Terrazas et al. 2016). With regard to the organic sources, it has been demonstrated that phosphatase activity exerted by microorganisms can mineralize the different P-containing molecules, present in the soli, and release orthophosphate groups (Rodríguez et al. 2006). According to a recent survey, the microbial genera featuring the ability of utilizing P from organic sources encompass for instance *Bacillus, Enterobacter, Klebsiella, Lactobacillus, Penicillium and Pseudomonas* (Azeem et al. 2015).

(c) *Siderophores production*

Fe is an essential micronutrient for all the living organisms and it can occur in two oxidation states, i.e. Fe(II) and Fe(III); however, in aerobic environments Fe is mainly present as Fe(III), which forms insoluble (oxy)hydroxides that are sources of Fe not available for both plants and microorganisms (Colombo et al. 2014; Mimmo et al. 2014). Commonly, microorganisms acquire Fe from the external environment by synthesizing and releasing low molecular weight organic compounds, generally termed microbial siderophores (MSs) (Lemanceau et al. 2009), which display a very high affinity for Fe(III), with an affinity constant ranging from 10^{23} to 10^{52}. MSs form very stable complexes with Fe(III), which are then transported into microbial cells through specific transporters (Neilands 1981; Guerinot 1994; Hider and Kong 2010). It has been suggested that plants can exploit Fe mobilized by MSs. Such hypothesis has been tested by using different experimental approaches; for instance, the use of radiolabelled Fe-MS complexes and natural Fe-pyoverdine complexes have allowed to demonstrate that plants are able to acquire Fe from MSs-chelated form (Crowley et al. 1988; Duijff et al. 1994a, b; Walter et al. 1994; Yehuda et al. 1996; Siebner-Freibach et al. 2003; Jin et al. 2006; Vansuyt et al. 2007; Robin et al. 2008).

However, the relative contribution of this mechanism to the whole amount of Fe acquired by plants is not quantified yet. In addition, with respect to grasses (monocots non graminaceous) it should be also considered that due to the high stability of the Fe(III)-MSs complexes, the exchange reactions between Fe(III)-MSs and phytosiderophores (PSs) could be very limited (Colombo et al. 2014). Nonetheless, it is suggested that monocots are able to use Fe(III)-MSs as Fe sources through an indirect mechanisms based on MSs degradation by microorganisms

(Duijff et al. 1994b; Robin et al. 2008). More recently, several pieces of evidence have showed that the inoculation of Fe-deficient plants, grown in either artificial or calcareous soils, with siderophores-producing PGPR can alleviate the symptoms of Fe deficiency, demonstrating a role for these microorganisms in favoring the root acquisition of the nutrient via also, at least in part, an effect in its soil availability (Sharma et al. 2003; de Santiago et al. 2009, 2013; Pii et al. 2015b).

(d) *Effects on biochemical/molecular mechanisms*

The improvement in mineral nutrition, achieved through the inoculation of plants with PGPR, can be indeed due to the increase bioavailability of key nutrients at the rhizosphere (as described above), but also to an enhanced ability of the plants themselves to take up nutrients. Evidence concerning these aspects has emerged following the observations that the PGPR *A. brasilense* could affect the efflux of protons from wheat roots, which was restored also in plants pretreated with orthovanadate. These results corroborate the idea that the PGPR could act directly on plasma membrane (PM) H^+-ATPases (Bashan et al. 1989; Bashan 1990). Several experiences, carried out with different model plants and different PGPRs, have further strengthened these observations (Bertrand et al. 2000; Canellas et al. 2002, 2013). In the case of mineral nutrition, PM H^+-ATPases generate and maintain a transmembrane H^+ electrochemical gradients, which is required for the transport of several nutrients (e.g. $H_2PO_4^-$, sulfate SO_4^{2-} and nitrate, NO_3^-) across the plasma membrane (White 2003). Therefore, the enhanced H^+ extrusion induced by PGPR inoculation might be suggested as a further mechanism through which bacteria could improve the uptake of mineral nutrients.

Similarly, the inoculation of seed rape with the soil-isolate *Achromobacter* induced an increased accumulation of NO_3^- in plant tissues, which was ascribed to a putative activity of the PGPR on the constitutive high-affinity transport system (cHATS) for NO_3^- (Bertrand et al. 2000; Nacry et al. 2013). Still concerning the N nutrition, *Phyllobacterium* STM196 was shown to induce in *A. thaliana* plants the upregulation of *NRT2.5* and *NRT2.6* genes, which was hypothesized to function as transceptors for a systemic signal generated by the inoculation with the PGPR (Mantelin et al. 2006; Kechid et al. 2013).

Also in the case of Fe, it was observed that the fungus *Trichoderma asperellum* could induce an increased Fe uptake in Fe sufficient cucumber and *Lupinus albus* by enhancing the activity of the root Fe-chelate reductase, the enzyme devoted to the reduction of Fe(III) to Fe(II) prior the uptake via IRT1 transporter (de Santiago et al. 2013; Zhao et al. 2014). Furthermore, it has been demonstrated that *Bacillus subtilis* GB03 could stimulate Fe sufficient *A. thaliana* plants to induce the molecular mechanisms (e.g. Fe reduction, H^+ extrusion and genes expression) correlated with the response to the lack of Fe (Zhang et al. 2009). More recently, Pii et al. (2016) showed that the influence of *A. brasilense* on the Fe acquisition mechanisms in cucumber plants was independent on the Fe nutritional status, suggesting that the PGPR might differently affect the different actors (Fe-chelate

reductase, FeII transporter, PM H^+-pump) of the mechanism underlying Fe acquisition by roots.

(e) *Modulation of phytohormones*

Plant hormones play an important role in plants growth, development and in the response to environmental stimuli (Taiz and Zeiger 2006; Glick et al. 2007). An important aspect of phytohormones activity in plants is related to the plasticity of the root system, which shows a surprisingly high adaptability depending on the availability of the nutrient sources (Kloepper et al. 2007). Furthermore, it has been demonstrated that also PGPR-derived phytohormones (i.e. auxin, gibberellin, cytokinin) can have a role in altering the root architecture (Vacheron et al. 2013). In spite of the fact that many PGPR can produce gibberellins, cytokinins or both and that they can affect plants growth, a detailed understanding of the role played by these bacterially-synthesized hormones in plants growth promotion is still lacking (Glick 2012). Several PGPR, as well as pathogenic bacteria, symbiotic and free-living rhizobacteria, have been reported to synthesize auxin (indole-3-acetic acid, IAA) as secondary metabolite and to release it in the external medium (Scagliola et al. 2016).

In plants IAA, also in conjunction with other hormones, controls cell division, extension and differentiation, vascular bundles development, the tropic responses to light and gravity, and initiate lateral and adventitious roots formation (Sachdev et al. 2009; Overvoorde et al. 2010). The PGPR-secreted IAA can affect the aforementioned plant developmental processes, since the endogenous pool of auxin can be integrated by the external amount absorbed (Spaepen et al. 2007; Glick 2012). In addition, bacterial IAA can contribute in increasing root surface and length, thus providing plants with a better access to the nutrient sources in soli (Xie et al. 1996; Mayak et al. 1999). Furthermore, IAA plays also a fundamental role as reciprocal signaling molecule, inducing the expression in both plants and microorganisms of key genes that are required for a proficient establishment of the interaction between plants and PGPR (Spaepen and Vanderleyden 2011).

With a completely opposite mechanism, PGPR expressing the 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which cleaves the ethylene precursor ACC into 2-oxobutanoate and ammonia (Arshad et al. 2007), can facilitate and improve plant growth by modulating the levels of ethylene (Glick 2014). The hormone ethylene is found in all higher plants and it is involved in key developmental processes as well as in the responses to a wide variety of environmental stresses (Abeles et al. 1992). The so-called “stress ethylene” occurs in two subsequent peaks that differ in magnitude, being the first one much smaller than the second one; whilst the first peak of ethylene is believed to induce the activation of defense and/or protection mechanisms, the second peak is generally originated from the de novo synthesis of ACC and has detrimental effects on plant growth, inducing senescence, chlorosis and leaves abscission, thus further exacerbating the environmental stress (Glick 2014).

PGPR exhibiting ACC deaminase activity, belonging to the genera *Acinetobacter*, *Achromobacter*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia* and *Rhizobium* (Parray et al. 2016), are thought to function as sink for ACC, thus mitigating the effect of the massive production of ethylene, and, as a consequence, its negative effects on plants growth (Glick 2014). Therefore, plants inoculated with ACC deaminase rhizobacteria feature an enhanced root and shoot growth, an improved ability to establish symbiotic relationships with both rhizobia and mycorrhiza and a better N, P and potassium (K) uptake (Nadeem et al. 2007, 2009; Shaharoona et al. 2008).

3.2 Indirect Mechanisms

The indirect mechanisms of interaction are mainly involved in biocontrol and they may be distinguished in three main groups, namely *Competition, Production of antibiotics and lytic enzymes, Induced Systemic Resistance,* based on the features of the mechanism itself. It is worth noting that for long time they have been raising the interest of the scientific community for the idea of using bacteria expressing these phenotypic traits in place of chemical pesticides in the pest defense programs.

(a) *Competition*

PGPR can out-compete pathogenic microorganisms on two different levels, (i) competition for niches and (ii) competition for essential nutrients. Despite being it not clearly demonstrated, some pieces of evidence indicate that the direct competition between PGPR and pathogens can reduce the severity and the incidence of the diseases. For example, Innerebner et al. (2011) showed that the treatment with *Sphingomonas* sp. induced a strong reduction of the pathogens load in *A. thaliana* plants infected by *Pseudomonas syringae* pv. tomato, also preventing the development of the disease symptoms. As mentioned above, in Fe limiting conditions, PGPR can synthesize microbial siderophores (MSs) to solubilize the micronutrient from sparingly available sources. In this case, the production of MSs can represent also an effective biocontrol mechanism, since they can prevent phytopathogens from acquiring sufficient amount of Fe, thus limiting their ability to proliferate (Lugtenberg and Kamilova 2009); this is mainly due to MSs having generally higher affinity for Fe than the siderophores produced by pathogenic fungi (Schippers et al. 1987).

(b) *Production of antibiotics and lytic enzymes*

The production of a wide range of antibiotic compounds (e.g. 2,4-diacetylphloroglucinol, phenazine, pyrrolnitrin, tensin, zwittermicin A, xanthobaccin) is another phonotypical trait of PGPR associated with biocontrol, especially against the proliferation of pathogenic fungi (Whipps 2001; Haas and Keel 2003; Compant et al. 2005; Mazurier et al. 2009). The biosynthesis of

antibiotics is dependent from the general metabolic state of the bacterial cells that is determined, for example, by nutrient availability and external stimuli (Duffy and Défago 2000). Indeed, it is worth highlighting that each PGPR strain might produce more than one antibiotic compounds, whose synthesis might be induced by different environmental conditions (Duffy and Défago 1999).

For these reasons, some of the isolated biocontrol PGPR strains have been commercialized to replace the common chemical pesticides; nevertheless, some phytopathogenes can still develop resistance against specific antibiotics. Besides antibiotics, several biocontrol PGPR strains have been shown to produce lytic enzymes, including chitinases, cellulases, glucanases, proteases and lipases, that can compromise the integrity of the cell walls of many phytopathogenic fungi. Several pieces of research have demonstrated that PGPR producing these enzymes are effective in preventing the infection of a wide range of fungi, among which *Botrytis cinerea*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *Phytophthora* spp., *Rhizoctonia solani* and *Pythium ultimum* (Singh et al. 1999; Frankowski et al. 2001; Kim et al. 2008).

(c) *Induced Systemic Resistance*

The triggering of the so-called plant induced systemic resistance (ISR) is defined as a physiological state of enhanced defensive capacity that can be elicited by either a non-pathogenic organism or specific environmental stimuli; in this status, the plant's innate defenses are "primed", therefore they result potentiated and react faster against subsequent pathogens attack (van Loon et al. 1998). In addition, this induced resistance is systemic because the defensive capacity is enhanced not only in the site of the primary infection, but it is spread all over the plant organism; this is mainly due to the involvement of a jasmonate- and/or an ethylene-based signaling, which stimulates the plant defense mechanisms toward a wide range of pathogens (Verhagen et al. 2004). The signaling inside plants is in turn stimulated by bacterial determinants, as for instance the flagellar proteins, chitin, β-glucans and cyclic lipopeptide surfactants (Annapurna et al. 2013).

4 Characterization of Beneficial Soil Bacteria

A key step to use beneficial microbiome for sustainable agricultural practices is the screening and the isolation of beneficial species from different soil environments. Independently of the plant growth promotion traits researched, the first approach in this quest is the collection of rhizosphere soil that, after serial dilutions, is plated on agarized medium. If the nutrient agar is non-selective for any plant growth promoting activity (enriched of nutrients), the higher is the number of the colony forming units, the higher is the probability to isolate bacteria with potential plant growth promoting traits. Alternatively, the growth medium can be selective for one of the main plant growth promoting traits, such as P-solubilizing activity,

siderophore and indole-3-acetic acid production, therefore reducing the number of isolates but, on the other hand, pre-selecting bacterial strains on the basis of the desired phenotypical trait. Besides a microbiological, morphological and biochemical characterization of potential beneficial bacteria, as well as 16S rDNA sequencing and phylogenetic analysis, quantitative assays for the main activities should be performed for an in vitro evaluation of the potentiality of isolated strains. Two research papers (Ahmad et al. 2008; Dastager et al. 2010), among many others, described in detail the analytical procedures to determine production and release of indoleacetic acid, NH_3, HCN and siderophores, phosphate and Zn oxides solubilization, ACC-deaminase activity, all potential beneficial activities. Another key feature that should be evaluated before considering the possibility of using beneficial soil bacteria in agriculture is the persistence of microbe in the soil, where competition for nutrients with autochthonous microbial populations very likely occurs. For an easy detection in soil, bacteria can be transformed by a plasmid carrying a green fluorescent protein (Miller and Lindow 1997). Many other molecular tools are also available to follow the fate and the persistence of a bacterial inoculum in a fresh soil. PCR-based approaches, consisting of DNA extraction from soil, amplification by PCR/qRT-PCR of a selected region targeting specifically the inoculating bacterium have been routinely performed in the last two decades. Since many factors affect the persistence, including genetic and metabolic characteristics of the inoculum, density and frequency of the inoculum, physical-chemical soil properties, autochthonous microbial community structure and functioning, genetic and biochemical properties of the plant to be grown and the related agricultural practices, environmental aspects, each case should be accurately evaluated at the laboratory scale before being used at the field one.

Since only active microorganisms interact with plant roots and, more in general, drive biogeochemical processes in soil, it is fundamental to understand which part of the total microbiome may have, actually or potentially, a role for sustainable agricultural production. Cells are considered active when actually involved in biochemical transformations and ready to respond to substrate input; differently, potentially active microorganisms can switch in few minutes to hours and contribute to ongoing processes, responding to changes in substrates availability or environmental conditions (De Nobili et al. 2001). Since it is reasonable that beneficial soil bacteria may enhance plant growth and health once they are added to soil, instead of or in cooperation with chemical fertilizers, it is important to understand the transitions (active/potentially active/dormant) they can undergo after their inoculum, as well as the transitions eventually occurring in the already existing microbial community.

Many approaches, recently reviewed by Blagodatskaya and Kuzyakov (2013), allow the estimation of different physiological states in soil, including plate counts, fluorescent microscopy combined to complementary staining, biochemical determinations (ATP and PFLA content, enzymes activities) and molecular methods such as microarray and real time-PCR, by using RNA instead of DNA.

5 Agricultural Use of Beneficial Soil Bacteria

5.1 Fate in Soil of Microbial Inoculants

Beneficial microbial inoculants can be used to treat seeds in drum priming (Bennett and Whipps 2008) or before sowing (Walker et al. 2002), plant roots before transplanting or directly the soil by drenching or incorporating the biocontrol agents into it (Weller 1988). In spite of the increasing interest on the use of beneficial soil bacteria in agricultural soil, scarce information is available on their survival after application. Most of the available data refer to few specific strains, mainly studied as model microorganisms or developed as commercial products. Although some general conclusions on the fate of the introduced inoculant in soil can be drawn, the precise values of survival rates should refer to the specific strain and conditions tested.

The persistence of the microbial inoculum in the soil or in the rhizosphere is a key factor for efficient biocontrol activity, therefore understanding the conditions that optimize the survival rate could be crucial to guarantee a successful practical application. On the other hand, the presence of high concentrations of an introduced strain may imply a disturbance of the native microbial communities; therefore, the survival may have an indirect relevance also on the risk assessment of the application of inoculants to soil (Weller 1988).

When the inoculants are introduced in soil, they are exposed to soil environmental conditions and the competition with native microorganisms, which in general results in a decrease of their populations (Raaijmakers et al. 2009). In addition, the oligotrophic soil environment quite often does not suit the nutritional requirement of the introduced inoculum. Therefore, a basic distinction between survival in the rhizosphere and in the bulk soil should be made when studying the fate of an introduced microorganism in the soil. When considering the bulk soil, besides the intrinsic characteristics of the strain, the specific conditions of the soil play a relevant role on its survival (i.e. physical, chemical and biological characteristics, temperature, relative humidity, pH). In fact, the survival under different soil condition can result in very different patterns. On the other hand, besides the soil composition, the rhizosphere is deeply influenced by the root exudates, which in turn depend on the plant genotype, its physiological/nutritional status, but also on the cross talk with the microbial inhabitants (Dutta and Podile 2010). The residing microflora and microfauna in the bulk soil or rhizosphere may also deeply directly influence the survival of the introduced microorganism. In fact, a microbial population reacts to the introduction of an external strain with a complex pool of responses (Perazzolli et al. 2016). Nevertheless, it should be noted that the role of the natural microbial antagonisms on the survival in soil of the added microorganisms is more an assumption than a proven evidence. In fact, although it is commonly accepted that introduced inoculants are greatly influenced by the native soil microbial communities, not much is known regarding these interactions and the specific effect on the survival of the inoculant (Raaijmakers et al. 2009).

The lack of data on the survival of microbial inoculants in soil is mainly due to the difficulties of monitoring their fate. Before the advent of the non-culturable techniques based on the detection of the DNA of the specific introduced microorganism, most of the studies of the fate of an introduced microorganism relied on the retrieval of the cells (colony forming units, CFUs) of the introduced inoculants on selective media. In most cases, this implied the selection of antibiotic-resistant mutants in the colonies of the target microorganism (Troxler et al. 1997). Although most of the studies demonstrated the absence of biological and physiological difference compared to the wild types, the bias introduced by the resistance to antibiotics might not theoretically excluded. In addition the counting of CFUs tend to underestimate the real numbers of viable bacterial cells when they tend to stick together (Gamalero et al. 2004) or when they lose their colony-forming ability under stress soil conditions, for example in the case of some gram-negative bacteria as *Pseudomonas fluorescens*. The Kogure's direct viable count can partially solve the latter problem (Troxler et al. 1997). The introduction of the green fluorescent protein (GFP) gene to mark strains is a powerful tool that allowed the in situ visualization of the patterns of colonization of the selected strain especially in the rhizosphere (Gamalero et al. 2004; Chen et al. 2005; Poonguzhali et al. 2008). The specific detection and/or quantification by PCR approaches improved dramatically out possibility to monitor the fate of the introduced inoculants, making it a routine analysis especially for the registration requirements (Segarra et al. 2016). PCR-based detections methods however are not exempt from biases: for example underestimation due to a low efficiency of the DNA extraction from soil matrix, overestimation of viability, in fact the detected DNA can originate from dead cells, etc. (Savazzini et al. 2008) (Table 1).

Table 1 Approaches to investigate the survival of microbial inoculants

Techniques		Advantages	Disadvantages
Culturable	Plate counting	Simple and cheap	• Antibiotic resistant mutants utilization • Underestimation of real number
	Green fluorescent protein labelling	In situ localization	Mutants utilization
Un-culturable	DNA approaches (PCR/qRT-PCR, microarray, etc.)	Rapid and simple	• Underestimation of real number (low efficiency of DNA extraction) • Overestimation of viability (dead cells, etc.)
	RNA approaches (PCR/qRT-PCR, microarray, etc.)	Targeting viable cells	Low and un-reproducible RNA extraction yields (RNA degradation)

In general, the population of introduced inocula declines gradually after field application, because of exposure to abiotic stress and antagonism with indigenous resident microbiota. However, some specific patters can be recognized according to the type or strain of the microorganism applied.

(a) *Pseudomonas* spp.

Once introduced in soils, *Pseudomonas* spp. tend to face an initial sharp decline in the number of culturable cells and then establish themselves at a basal stable number (Troxler et al. 1997; Fischer et al. 2010; Gao et al. 2012). In general, *Pseudomonas* spp. survives better in the rhizosphere, than in the bulk soil (Hase et al. 2000; Fischer et al. 2010), indicating a general good rhizosphere competence of strains belonging to this genus. However, once the viable-but-non-culturable cells are compared to the culturable ones of *P. protegens* CHA0 (previously *P. fluorescens*), it appears that the survival of the microorganism is indeed declining over time, but less sharply than expected from the mere CFUs counting (Troxler et al. 1997). Differences between total cells and CFUs counts are reported also for other strains, for example *P. fluorescens* A6RI (Gamalero et al. 2004). In the bulk soil, the soil type has a relevant influence on the survival rate over time, and especially on the occurrence of the viable-but-non-culturable *P. protegens* CHA0 cells (Hase et al. 2000). Although the organic amendments and substrate composition and origin are known to strongly influence the survival of biocontrol agents in soil (Hoitink and Boehm 1999), the cause of this non-culturable status of the cells is not the deprivation of a single nutrient or multiple nutrients (Hase et al. 1999). The non-culturable status is not a physiological strategy to improve survival under adverse conditions, but it is more a reaction to stress conditions, in particular oxygen limitation combined with reducing conditions (Mascher et al. 2000; Troxler et al. 2012) and soil pH (Mascher et al. 2014).

Root colonization is related to the concentration of root exudates, which vary along the root. In fact *P. fluorescens* A6RI densities during time were found to vary according the zoot zone, with a fast decrease in the parts, which include apex, elongation and young hairy zone and a more stable concentration pattern in the older part of the root, supporting the hypothesis of a relation between root exudates concentration and proportion of culturable *Pseudomonas* cells (Gamalero et al. 2004). Interestingly the biocontrol agent *P. fluorescens* 32 was reported to show a wave-like oscillations along wheat roots, most probably linked to cell growth and death cycles, with a more pronounced oscillation in conventionally managed than in organically managed soils (van Bruggen et al. 2008).

When *P. protegens* CHA0 cells are applied on the soil surface, they spread in the entire soil profile, with some differences between covered (ley) and uncovered soil. The presence of ley guarantees a better survival in the top layer in comparison to uncovered soil and relatively high concentration up to a depth of 150 cm, below which it markedly decreases. In the uncovered soil, the highest numbers of cultivable cells concentrate in the soil immediately above the plow pan, followed by a sharp decrease and a gradual increase. Just after the release on the soil, the

P. protegens CHA0 cells spread along the walls of the macropores, but then, during time, they also colonize the micropores. The water movement into soil is most probably the main factor causing the spread of cells in the vertical profile. However, the cells concentration and/or transportation may depend also on other factors related to the microhabitat. For example the presence of roots, relative humidity, physical barriers (e.g. the plow pan), the activity of earthworms can positively influence the survival pattern in the vertical soil profile (Troxler et al. 2012).

(b) *Bacillus* spp.

Similarly to *Pseudomonas* spp., *Bacillus* cells persist better on the rhizoplane and rhizosphere than bulk soil (Cao et al. 2011). Regarding root colonization patterns after application, *B. subtilis* SQR initially colonizes the root tip and elongation zone of the primary roots and then spread on the elongation and differentiation zones of the plant primary roots and on the lateral root junctions (Cao et al. 2011). *Bacillus megaterium* C4 also colonizes firstly the primary and lateral roots and then in the lateral root junctions, however without being found on the root tip (Liu et al. 2006). Many *Bacillus* strain can penetrate the root, most probably from the crack formed at the lateral root junction, and establish within the root tissues (Cao et al. 2011; Liu et al. 2006).

Similarly to *Pseudomonas* strains or other bacterial inoculants (Larkin 2016; Segarra et al. 2016), the long-term survival of vegetative cells *Bacillus* strains in bulk soil is also very limited. For example *B. subtilis* GB03 cells cannot be detected in bulk soil at the end of the growing season (Larkin 2016). However, the origin of the strain seems to be relevant in the successful colonization of bulk soil. For instance the survival in the rhizosphere of *B. cereus* B11, a strain isolated originally from non-rhizosphere soil, was not improved by the presence of the roots (Young et al. 1995). The initial rapid decline of the *B. subtilis* vegetative cells in bulk soil is mainly due to nutritional starvation, but also to the antagonistic effect of indigenous microorganisms (Podile 1994; Tokuda et al. 1995). *Bacillus* strains may need repeated applications and some time to functionally adapt to the soil abiotic and biotic conditions (Jeong et al. 2013). Although vegetative cells decline, *Bacillus* strains can stabilize and survive for long time thanks to the formation of spores (Tokuda et al. 1995). Spores are known to be more resistant to the adverse effect of soil biotic and abiotic stress: for example, when *B. cereus* B11 are introduced as spores its survival was much higher compared to the introduction as vegetative cells (Young et al. 1995).

As in the case of *Pseudomonas*, soil abiotic conditions can affect the survival of *Bacillus* strains. For example montmorillonite and high soil matric potentials enhance the survival (Lee and Stotzky 1999), but, differently from *Pseudomonas*, soil temperature is irrelevant (Schmidt et al. 2004). Specific inoculum carriers, as for example biochar, may support a better survival (Sun et al. 2015).

5.2 Impact of Inoculants on Native Microbial Communities

While the survival of bacterial inoculants in the rhizosphere and bulk soil have received a lot of attention because of its relation to the desired effect of the inoculum, the impact on the native soil microbial populations attracted the interest of researchers only in recent times thanks to the recent advances in metagenomics technologies (Maron et al. 2011). Biocontrol inoculants had negligible impacts on species diversity and abundance of cultivable (Girlanda et al. 2001; Moenne-Loccoz et al. 2001; Thomas and Sekhar 2016) and uncultivable soil microorganisms (Felici et al. 2008; Kim et al. 2010; Piromyou et al. 2011; Gao et al. 2012; Guo et al. 2012; Chowdhury et al. 2013; Jeong et al. 2013; Yin et al. 2013; Krober et al. 2014; Kong et al. 2016). Effects of biocontrol inoculants (*e.g.* *Bacillus* spp., *Brevibacillus* spp., *Klebsiella* spp., *Pseudomonas* spp. and *Serratia* spp.) on native soil microbial communities are related to the inoculant establishment and survival in soil (Ambrosini et al. 2016), but are commonly limited in terms of intensity (Björklöf et al. 2003; Correa et al. 2009; Piromyou et al. 2011; Chowdhury et al. 2013; Jeong et al. 2013; Krober et al. 2014; Kong et al. 2016; Larkin 2016), time (Scherwinski et al. 2008; Gao et al. 2012; Guo et al. 2012; Yin et al. 2013; Krober et al. 2014; Thomas and Sekhar 2016) or space (Moenne-Loccoz et al. 2001). Biocontrol inoculants decreased the abundance of soil plant pathogens, such as *Ralstonia solanacearum* in *B. amyloliquefaciens* ZM9-inoculated soil (Wu et al. 2016), and attenuated the impact of phytopathogenic fungus *R. solani* on the rhizosphere and phyllosphere lettuce microbiome (Erlacher et al. 2014), Particularly, it has been shown that *Firmicutes* abundance increased in *Bacillus* spp.-inoculated soils, while *Proteobacteria* and *Gammaproteobacteria* abundances slightly decreased (Jeong et al. 2013; Krober et al. 2014). Changes in the abundance of *Cyanobacterium* spp., *Beta-proteobacteria* spp., *Staphylococcus* spp., and *Bacillus* spp. were detected at early stages after *P. fluorescens* 2P24 soil inoculation (Yin et al. 2013), while *Arthrobacter* spp. and *Nocardioides* spp. were specifically identified in soils inoculated with the biocontrol strain *B. subtilis* B579 (Chen et al. 2013). Interestingly, putative PGPRs belonging to *Acinetobacter* spp., *Azospirillum* spp., *Bacillus* spp., *Bradyrhizobium* spp., *Burkholderia* spp., *Dyella* spp., *Mesorhizobium* spp., *Pseudomonas* spp., *Psychrobacter* spp., *Rhizobium* spp. and *Stenotrophomonas* spp. increased in soils inoculated with *B. amyloliquefaciens* ZM9 (Wu et al. 2016) or *B. subtilis* Tpb55 (You et al. 2016), suggesting that biocontrol inoculants might increase the proportion of beneficial microorganisms in the plant microbiota. However, microbiome changes elicited by biocontrol bacteria are generally smaller than those caused by plant growth (Girlanda et al. 2001; Piromyou et al. 2011; Krober et al. 2014) and environmental conditions (Scherwinski et al. 2008; Chen et al. 2013). Impacts of biocontrol bacteria were also related to the soil chemical and biological properties (Schreiter et al. 2014; Li et al. 2015) and, according to the ecological theory, soil microbial communities with a greater diversity are less susceptible to disequilibria caused by the invading microorganisms (Fliessbach et al. 2009). Thus, the soil

microbiome is resilient to the perturbation that may be caused by biocontrol inoculants (Moenne-Loccoz et al. 2001), indicating that biocontrol agents should possibly compete with indigenous communities to occupy niches and display antagonistic properties against the phytopathogens (Perazzolli et al. 2016).

5.3 Plant Bio-stimulation by Inoculants

The term bio-stimulants indicates those substances that are promoting the plant growth without being nutrients, soil amendments or pesticides, and being applied in little amounts. However, the lack of legal and regulatory definition at world level prevents an accurate classification and listing of all the substances and microorganisms covering the concept of bio-stimulants (du Jardin 2015). Nevertheless, some class of compounds and microorganisms (e.g. humic and fulvic acids, protein hydrolysates and N-containing compounds, seaweeds extracts, chitosan and biopolymers, and PGPR) are widely recognized as bio-stimulants by the scientific community, the regulators and the stakeholders (Calvo et al. 2014; Halpern et al. 2015). For the proposes of the present chapter we will review experimental evidence about the biostimulants properties only in the case of PGPR applied to horticultural and fruit crops. In the case of horticultural crops, the majority of the experiments have been carried out under controlled conditions (e.g. growth cabinet, greenhouse) and only few in open field. In this latter case, both broccoli and lettuce, inoculated with *Brevibacillus reuszeri/Rhizobium rubi* and *R. leguminosarum* bv. *phaseoli* strain P31, respectively, showed a promotion in the growth of the root system, an increased yield and an enhanced macro- and micronutrients uptake, highlighting the efficacy of the candidate PGPR strains (Chabot et al. 1996; Yildirim et al. 2011). In contrast, the growth promotion experiments carried out by inoculating PGPR in fruit crops (e.g. apple, apricot, banana, cherry) have been predominantly carried out in open filed conditions, also adopting different inoculation strategies as compared to horticultural crops (foliar spray vs. root dipping) (Esitken et al. 2002, 2003, 2006; Kavino et al. 2010; Ryu et al. 2011). The PGPR used for fruit crops inoculation belonged to the genus *Pseudomonas* and *Bacillus* and, in particular, *Bacillus* sp. OSU-142 was able to increase the production, the weight and the quality parameters in the aforementioned fruits (Esitken et al. 2002, 2003, 2006; Kavino et al. 2010; Ryu et al. 2011). In spite of these encouraging data, the main concerns in using PGPR inoculants in open field conditions for horticultural and fruits production are i) the persistence of bacterial strains in soil after the application and their interaction with the autochthonous microflora, as well as ii) the survival of PGPR in the bio-stimulants commercial formulation during the storage. Apple trees inoculated with *Pseudomonas* spp. showed approximately 2-fold increase in the fruit yield after two years of cultivation, suggesting that the PGPR strains were effectively persistent and active in the orchard (Aslantas et al. 2007). However, this specific evidence was obtained in freshly planted pre-inoculated material, so that PGPR could efficiently colonize roots without being

out-competed by soil microflora; on the other hand, such practice is not applicable, for instance, in already established orchard. Furthermore, the persistence of the introduced bacterial strains in the rhizosphere is monitored within very few studies and this is due to the difficulties in specifically recovering the inoculated bacteria (Von Felten et al. 2010). Moreover, the inoculation with fresh bacterial cultures is hardly feasible from an agronomical point of view, therefore the research is orienting towards the development of dry inoculation methods (Bashan et al. 2014; Ruzzi and Aroca 2015). At present, the most popular techniques for the delivery of PGPR into the field is the encapsulated formulation, wherein bacteria are immobilized within a polymeric matrix, as for instance calcium alginate that might occasionally contain also other substances like humic acids, skimmed milk, starch or bentonite (Young et al. 2006; Minaxi and Saxena 2011; Wu et al. 2012).

5.4 *Beneficial Plant-Associated Microorganisms as Bio-fungicides*

The discovery of the antagonistic properties against phytopathogens of several plant-associated microorganism stimulated, already long time ago, the idea of their possible implementation as biological fungicides (Henis and Chet 1975; Stutz et al. 1986) (Fig. 3). Most of the strains used as bio-fungicides, were isolated from suppressive-soils (Chet and Baker 1981) or identified as effective antagonists of phytopathogens in dual-culture plates (Tjamos et al. 2004). These screening approaches may explain why most of the existing active ingredients of bio-fungicides are acting mainly by the production of lytic enzymes, siderophores and antibiotics or as hyperparasites (Markovich and Kononova 2003; Bennett et al. 2006; Vinale et al. 2008; Santoyo et al. 2012; Atanasova et al. 2013). Only recently the induction of resistance in plants by beneficial plant-associated microorganisms received increasing attention, due to the promising results obtained under controlled conditions (Perazzolli et al. 2012). However its tangible efficacy under field condition is still debated (Dagostin et al. 2011). In spite a wide number of species and strains have been shown to possess antagonistic properties against numerous phytopathogens, the active ingredients of the most part of the existing bio-fungicides are mainly few bacterial strains, belonging to the genera *Bacillus* and *Pseudomonas* (Jacobsen et al. 2004; Weller 2007) and fungal strains, belonging to the genera *Trichoderma* and *Coniothyrium minitans* (Whipps and Gerlagh 1992; Vinale et al. 2008).

Once the suitable strain is identified, the beneficial microorganism is commonly produced in industrial fermenters (submerged or solid state fermentation), formulated and applied in large quantities to soil (inundated biocontrol) (Montesinos 2003). One of the most important roles of formulation is guaranteeing a sufficient shelf-life of the plant protection product, in order to be compatible with the business model of the distributing company and the practical needs of the growers

(Segarra et al. 2015). Fungicides based on microbial stains are subject to the same authorization protocol as chemical ones, meaning that they must be proved to be not toxic for humans, animals and the environment. In several countries (e.g. European Union), the bio-fungicide must also be proved to be sufficiently effective against the target disease. In addition, to maximize it efficacy under field conditions, the mechanism of action, the best timing and conditions of application must be identified. Therefore, before placing a bio-fungicide on the market a large body of evidences must be collected and it is not surprising that the most studied strains in literature are indeed the active ingredients of the main commercial products (Velivelli et al. 2014). The depiction of the existing biofungicides is out of the scope of this review and we refer to existing literature (Whipps and Gerlagh 1992; Druzhinina et al. 2011; Santoyo et al. 2012) and we concentrate the discussion mainly on the most important traits for the success and the weaknesses of the existing strains.

Easiness of fermentation at industrial scale, production of spores, which guarantees a good shelf life of the biofungicide, wide spectrum of activity against several bacterial and fungal pathogens and the multiple modes of action, makes *Bacillus* strains the most commercially successful bacterial active ingredients of fungicides. Currently available biofungicides are based on strains belonging to *B. subtilis*, *B. amyloliquefaciens* and *B. pumilus.* These strains antagonize pathogens by direct antibiosis (Stein 2005), but can also induce resistance on plants (Santoyo et al. 2012). Efficacy not only depends on the successful root colonization, but also on the method of application, the plant species and its physiological status (Szczech and Shoda 2006; Pertot et al. 2013).

Similarly to *Bacillus*, *Pseudomonas* strains can colonize rhizosphere and directly (antibiosis) and indirectly control (ISR) a wide range of plant pathogens (Weller 2007). Existing commercial biofungicides are based on *P. fluorescens* species complex (Garrido-Sanz et al. 2016). They can also be efficiently produced in fermenters, however, differently from the *Bacillus* biocontrol strains, they do not form spores (Weller 2007). Therefore, the formulation of Gram negative bacteria is relatively complex compared to spore-forming Gram positive bacteria (Segarra et al. 2015). Difficulties in the formulation, the short shelf-life and the precise conditions of storage may explain why, in spite of relative good efficacy under field conditions, *Pseudomonas* strains have been less attractive for industrial development of biofungicides, compared to *Bacillus*.

5.5 *Beneficial Plant-Associated Microorganisms as Bio-herbicides*

Weeds constitute a serious issue in agricultural production, since they compete with crops and they are thus associated with a limitation in crops productivity and yield (Harding and Raizada 2015). In this context, the agricultural management has been

changed by the introduction of selective herbicides, which enable controlling weeds without affecting non target crops, or at least reducing the effect on them, due to differences in plants at biochemical levels (Mithila et al. 2011). However the use of a limited variety of herbicides, impacting on the same biochemical mechanisms, has led to the development of resistant weeds population in response to the selective pressure, thus exacerbating the need of finding new weeds control methods to preserve the agricultural productivity (Green and Owen 2011; Mithila et al. 2011; Darmency 2013). In this context, the biological control, i.e. the introduction of organisms in the ecosystem to prevent the development of not desired species, has received a great attention in the last decades, mainly focusing on the possible use of bacteria and fungi as controlling organisms (Charudattan 2001; Li et al. 2003; Bailey et al. 2011). Within the biological control of weeds, two main approaches can be distinguished: (i) the classical methods, which is based on the release of natural predators and/or pathogens of the undesired species with the awareness that they will persist in the environment, hindering pests growth throughout the whole ecosystem, and (ii) the bioherbicide strategy (also known as inundative biological control), wherein fungal spores or bacteria are propagated and then released only within the managed area at a concentration that would not naturally occurs in the ecosystem (Dane and Shaw 1996; TeBeest 1996; Auld et al. 2003; Caldwell et al. 2011) (Fig. 3).

A wide number of bacteria have been investigated for the possibility to be used as bioherbicies. *Pseudomonas fluorescence* is generally characterized for its ability to promote the growth of plants (Gamalero et al. 2005), however several strains showed inhibitory effects on germination and growth of plants. The strain D7 was isolated from winter wheat (*Triticum aestivum*) and was shown to feature an inhibitory activity on the germination and growth of downy brome (*Bromus tectorum*) (Gealy et al. 1996), most likely exerted by a combination of extracellular peptides and a lipopolysaccharide (Gurusiddaiah et al. 1994). To date, the most thoroughly studied is the strain WH6, which was shown to impair the germination of 21 monocot and 8 dicots species, with the exception of the modern maize hybrids (Banowetz et al. 2008). The germination inhibition is mediated by the Germination Arrest Factor (GAF), which was characterized as 4-formilaminooxy-L-vinylglycine, synthesized from the amino acid homoserine (Banowetz et al. 2008; McPhail et al. 2010; Halgren et al. 2013). This class of compounds are known to interfere with pyridoxal phosphate-dependent enzymes, including those involved in the ethylene biosynthetic pathway (Halgren et al. 2013). *Xanthomonas campestris* is another bacterial species that has been studied as potential candidate as bioherbicide; several strains (e.g. JT-P482, LVA-987) have been shown to control grasses as annual bluegrass (*Poa annua*) and horseweed (*Conyza canadensis*), however, to date, the effector phytotoxic compounds have not been identified yet (Imaizumi et al. 1997; Boyette and Hoagland 2015).

Besides bacteria, fungi have been also investigated as potential bioherbicides and a survey of the scientific literature about this topic highlighted that mainly three fungal genera have been studied for this aspect, i.e. *Colletotrichum*, *Phoma* and *Sclerotinia*.

Several species belonging to the genus *Colletotrichum* are able to control weeds like hemp sesbania (*Sesbania exaltata*) and spiny cocklebur (*Xanthium spinosum*) (Auld et al. 1988, 1990; Schisler et al. 1991); the mechanisms used by these fungi to inhibit plants growth has not been clarified yet, however the analysis of the genome revealed the presence of sequences putatively associated with pathogenesis, as for instance cell wall degrading enzymes (Gan et al. 2013). In addition, *Colletotrichum* spp. encode also for the biochemical pathway for the biosynthesis of indole-3-acetic acid, whose analogues and derivatives are well established herbicides (Grossmann 2010; Gan et al. 2013).

Among the genus *Phoma*, the species *P. macrostoma* has been thoroughly investigated since it was observed to specifically inhibit the growth of dicot plants (Bailey et al. 2011, 2013; Smith et al. 2015). The strategy adopted by *P. macrostoma* aiming at contrasting the growth of plants consists in the production and release of macrocidins, a member of the tetramic acid family, that impair the photosynthesis especially in the new leaves, suggesting that this effector molecule could be transported within plants via phloematic stream (Graupner et al. 2003; Bailey et al. 2011). Nevertheless, the exact mechanism of action of macrocidin remains to be elucidated. In addition, *P. macrostoma* has been shown to produce also anthraquinone pigment (Quereshi et al. 2011); similar pigments have been isolated from other fungi and can provoke necrosis on the leaf of both wheat and legumes in a light dependent manner (Bouras and Strelkov 2008; Andolfi et al. 2013). However, also in these cases the exact mode of action of the active principle still remains elusive.

Two species of the genus *Sclerotinia*, *S. minor* and *S. sclerotinium*, have been investigated for they proved herbicidal actions against dandelion and creepy thistle (*Cirsium arvense*) (Abu-Dieyeh and Watson 2007; Skipp et al. 2013) and it was observed that the production of oxalate is required so that fungi can express their virulence towards target plants (Magro et al. 1984; Briere et al. 2000). The production of oxalic acid can be stimulated by the addition of succinate to the growth media, thus inducing a higher virulence of the fungi; oxalate is thought to acidify the cell wall, which will enable the degradation through acid hydrolase, and to interfere with the activity of polyphenol oxidase, which play a role in plant defense (Cessna et al. 2000).

In conclusion, considering the need of continuously producing new herbicides to meet the challenge of controlling the development of resistant weeds, the use of bioherbicides could offer higher advantages as compared to traditional herbicides. The most claimed benefits of bioherbicides is the environmental compatibility, which is basically due to (i) the target specificity (Auld and Morin 1995), (ii) the rapid degradation of the effector molecules (Li et al. 2003) and (iii) the inability of the bioherbicide species to freely propagate in the environment (Johnson et al. 1996; Hoagland et al. 2007). In addition, the costs associated with the development and production of a bioherbicide are reported to be generally lower as compared to those required for synthetic herbicides (Auld and Morin 1995; Li et al. 2003). At present, several microbial species have been considered for this role in the

agriculture; nevertheless, to further implement this strategy of weeds control, it will be necessary to develop techniques that allow obtaining the same efficacy observed in small-scale conditions also at the field scale.

5.6 Other Environmental Applications of PGPR Inoculants

Besides the above-described uses of beneficial bacteria in crop production, they have been applied in distinct areas of bioremediation. A comprehensive evaluation of these aspects has been reviewed by de-Bashan et al. (2012). There are clear pieces of evidence that the association of PGPR, arbuscular mycorrhizal (AM) fungi and rhizobia helps restoring vegetation in semiarid areas, undergoing desertification, of southern Europe (Requena et al. 1997, 2001) and tropical regions (Founone et al. 2002) although, so far, no long-term field trial has been reported (Fig. 3).

Inoculation with PGPR enhances the capacity of plants to contain, degrade or eliminate different contaminants from soils, such as pesticides, heavy metals, crude oil (Glick 2003; Mendez and Maier 2008). In general, beneficial bacteria assist plants to overcome contaminant-induced stress, i.e. by ACC-deaminase activity (Huang et al. 2004) or enhance their growth by IAA hormone production (Khan et al. 2009); sometimes rhizosphere microorganisms living in association with plant roots contribute to the decontamination through xenobiotic degradation.

The removal of metals from the environment by concentrating them within the biomass of the plant (phytoextraction) is improved by PGPR and AM fungi by enhancing general growth of plant (the larger the plant biomass, the higher the removal) or by increasing metal mobilization by microbial metabolites (Reichman 2007; Lebeau et al. 2008). Mine tailings, lacking plant cover and soil structure, are a relevant source of metal pollution and pose a long-term health hazard to nearby urban areas. Phytostabilization, that is the use of plants as ground cover, is limited by the weak capacity of most plant species to grow under high metal concentration, low pH, lack of water-retaining clays and essential minerals. Several studies demonstrated the feasibility for phytostabilization of the use of PGPR such as *Azobacterium chroococcus, B. megaterium* and *Arthrobacter* spp. (previously isolated from tailings), in combination (often) with reduced doses of compost or chemical fertilizers (Petrisor et al. 2004; Grandlic et al. 2009).

The bacteria inoculation effects in marginal ecosystems (desertified lands, tailings, highly contaminated soils) is usually more evident than in agricultural for the (generally) low content of competing heterotrophic bacteria in the first (Mendez and Maier 2008). Therefore, the replacement of autochthonous community occurs more easily and lasts longer. Finally, *A. brasilense* has been used to enhance the capacity of microalgae (a kind of "unicellular plant" that may respond to beneficial bacteria as do "eukaryotic plant") to reduce nitrogen and phosphorus in municipal wastewaters (de-Bashan and Bashan 2008).

6 Other Bio-stimulants and Their Interaction with Beneficial Bacteria

Humic substances (humic and fulvic acids, humins) have been shown beneficial to plant growth, yield and nutrition. Calvo et al. (2014) summarized structural characteristics of humic substances, how their activity is related to the source of organic matter and the time of its transformation (Berbera and Garcia 2014) and present a comprehensive summary of publications, reporting effects of humic and fulvic acids on morphology, growth and physiology of various crops (Pinton et al. 1999).

Although the aims of this review do not deal with these aspects, it is worth noting that most of the effects induced by humic substances on plants are similar to those induced by PGPR, (for instance increased uptake of nutrients, enhanced tolerance to abiotic stresses, changing in root structure). Accordingly, a study by Befrozfar et al. (2013) demonstrated that a combination of PGPR and humic acids increased yield in basil plants.

Protein hydrolysates, obtained from both animals and plants, have been reported to enhance nutrient uptake and yields in plants, to stimulate carbon and nitrogen metabolism, as well as defenses to biotic and abiotic stresses. Despite the initial concerns about the safety of protein-based products, Corte et al. (2014) clearly demonstrated that hydrolysates showed no toxic effects on soil microbiota and yeasts, opening a new frontier in the field of plant bio-stimulants (Fig. 3).

7 Conclusions and Future Challenges

The comprehension of structure and functioning of whole soil microbiome and the isolation of new beneficial soil microorganisms could surely contribute to the urgent need to improve crop production (to meet the challenge of feeding the growing population) and to protect plants from biotic and abiotic stresses with always more environmentally-friendly approaches (to meet the challenge of saving non-renewable resources). At the same time, these pieces of information are undoubtedly future goals necessary to sustain the growing market of bio-stimulants. Therefore, efforts should be spent at the scientific level in order to make more efficient PGPR available for long-term field trials. A promising research avenue aims at moving beyond the one-microbe-at-a-time approach, i.e., where only individual strains are deployed for a given crop/conditions. Combining the metabolic potential of two or more microbes, for instance plant growth promotion with pathogen protection, will be likely a key towards more consistent results. Therefore, the study and the application of microbial consortia and synthetic microbiotas is gaining momentum in this research field. Concurrently fundamental research should focus on the biochemical and molecular processes affected in plants by these microorganisms. Akin to the role envisaged for the human microbiota in personalized medicine, an increased knowledge of plant-microbiota interactions will allow

scientists to rationally design crop- and soil-specific solutions. Together, projected outcomes of these investigations, combined with advancements in other research fields, such as plant breeding and agronomy, hold the key to ensure a fair and sustainable future for the planet.

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Chemotaxis Behavior of Pseudomonas Species and Biodegradation of Pollutants

Amina Meliani and Ahmed Bensoltane

Abstract Chemotaxis in Pseudomonas species is one of the most diversified and best-understood signal transduction network. Most strains possess one or two sets of the chemotaxis genes. They have the ability to sense changes in the concentration of chemicals in the environment and respond by altering their pattern of motility using the two chemotaxis pathways. The flagella-mediated pathway and the pili-mediated system are involved in bioremediation, pathogenesis and plant protection. Although a large number of Pseudomonas species have chemotaxis abilities, only seven species have had their chemotaxis abilities screened. *P. aeruginosa* is now considered as a model organism for the study of chemotaxis systems. This chapter reviews chemotaxis and biodegradation in Pseudomonas species.

Keywords Chemotaxis · Biodegradation · Pollutants · Pseudomonas

1 Introduction

Several studies have reported the isolation and characterization of bacteria responding chemotactically to a wide variety of hazardous environmental pollutants, including toluene, trinitrotoluene, atrazine and a variety of nitroaromatic compounds (Parales and Harwood 2002). Parales et al. (2000) reported that the soil bacteria can sense and swim towards the toxic compounds toluene, benzene, trichloroethylene, and related chemicals suggests that the introduction of chemotactic bacteria into selected polluted sites may accelerate bioremediation processes. To our knowledge, two mechanisms for bacterial chemotaxis towards xenobiotic compounds are studied metabolism dependent or independent (Pandey and Jain 2002).

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E. Lichtfouse (ed.), *Sustainable Agriculture Reviews 31*, Sustainable Agriculture Reviews 31, https://doi.org/10.1007/978-3-319-94232-2_10

Thus, a question remains open in the present chapter: how about the diversity of chemotactic responses in a versatile bacterial genus such as the *Pseudomonas*? Given this diversity, it is not surprising that each species differ in many respects of motility, including their capacity to find optimum conditions for their growth and with a coordinate chemosensory system.

Notably, many members of this genus play significant roles in their environment, such as in the degradation of organic hydrocarbons, in plant growth promotion, and in nitrogen fixation (Qian et al. 2013). There are over 200 species of *Pseudomonas* and countless strains; however, only seven *Pseudomonas* species have had their chemotaxis abilities screened and published: *P. aeruginosa, P. fluorescens, Pseudomonas pseudoalcaligenes, P. stutzeri, P. syringae, P. entomophila, and P. putida* (Sampedro et al. 2014).

Chemotaxis of pollutant-degrading bacteria is a trait important for bioremediation, especially in situ bioremediation. Furthermore, microbial chemotaxis has recently been proposed as a widespread phenomenon among motile bacteria towards several distinct xenobiotic compounds and it may therefore be advantageous to use such bacteria in bioremediation (Gordillo et al. 2007).

It has been recognized that microbial chemotaxis towards the pollutant can also be a major determinant (Parales and Harwood 2002). The migration of pollutant degraders to pollutants is expected to speed the biodegradation process because it should bring the cells into contact with pollutants (Parales and Harwood 2002). Furthermore, it is suggested that chemotaxis can enhance biodegradation by effectively improving pollutant bioavailability and/or by promoting the formation of microbial consortia with diverse microorganisms harboring complementary degradation capabilities (Law and Aitken 2003; Pandey and Jain 2002).

Bacterial chemotaxis, movement under the influence of a chemical gradient, either toward (positive chemotaxis) or away (negative chemotaxis) from the gradient helps bacteria to find optimum conditions for their growth and survival (Pandey and Jain 2002).

Interestingly, genome analysis reveals that a large number of environmental motile bacteria possess several genes involved in chemosensing and chemotatic signal transduction. Motile bacteria sense changes in the concentration of chemicals in their environment and respond in a behavioral manner (Adler 1966). A cell capable of chemotaxis must be able to (i) detect ('sense') changes in the concentration of a chemoeffector, (ii) transmit this information to locomotory organelle(s), and (iii) make the appropriate response; it must also be able to adapt by resuming the unbiased mode of motility when in a uniform concentration of chemoeffector, regardless of the actual concentration (Singleton and Sainsbury 2006).

Furthermore, chemotaxis is broadly divided into two categories on the basis of signal transduction strategies. One kind of chemotaxis is independent of the metabolism of the chemoeffector molecule, whereas metabolism of the signaling molecule is a prerequisite for the other kind of chemotaxis (Alexander and Zhulin 2001).

In metabolism-independent chemotaxis: (i) essentially non metabolizable analogues of metabolizable attractants are also attractants, (ii) mutations in the

metabolism of a chemical attractant do not affect chemotaxis, and (iii) a chemoattractant attracts bacteria even in the presence of metabolizable compounds (Pandey and Jain 2002). Metabolism-independent chemosensing occurs through transmembrane chemoreceptors (chemotaxis transducers) that transmit information to flagella via two-component regulatory systems that direct the cells to move in preferential directions (Bren and Eisenbach 2000). Metabolism-independent chemical sensing is found in several bacterial species, including *E. coli*, *Salmonella* sp., *Bacillus subtilis*, and *Pseudomonas* sp. toward a number of chemicals (Berg 1975).

As its name indicates, the second kind of chemotaxis require metabolism of the chemoeffector molecule. Metabolism-dependent chemotaxis is best studied in an α *proteobacterium*, *Azospirillum brasilense*, where (i) nonmetabolizable analogues of metabolizable attractants are not attractants, (ii) inhibition of the metabolism of a chemical attractant completely abolishes chemotaxis to that particular attractant, and (iii) presence of another metabolizable chemical prevents chemotaxis to all chemoattractants studied (Pandey and Jain 2002). We describe here recent discoveries in *Pseudomonas* chemotaxis toward pollutants and how they may be explored and exploited for bioremediation applications.

2 Methyl-Accepting Chemotaxis Proteins

Bacterial chemotaxis is a biased movement towards higher concentrations of life-sustaining nutrients and lower concentrations of toxins. It involves sensing a gradient of chemicals as small as a few molecules (Wadhams and Armitage 2004). Many aspects of chemotaxis are now understood, at least superficially, but many questions remain. The best-characterized mechanism of adaptation involves methylation of specific residues on membrane-bound chemoreceptors called methyl-accepting chemotaxis proteins (MCPs) (Hazelbauer et al. 2008; Sourjik and Armitage 2010). Analysis of bacterial genomes reveals that motile bacteria differ enormously in the number of MCPs that is in most cases significantly larger than that of *E. coli*. The range of MCP abundance in a bacterial genome ranges from 64 MCPs in *Magnetospirillum magnetotacticum* (Alexander and Zhulin 2001) to various strains that have a single MCP (Sampedro et al. 2014).

The specificity of a chemotactic response is determined by the ligand-binding region (LBR), a periplasmic component of methyl-accepting chemotaxis proteins (MCPs), with the exception of aerotaxis, where the LBR is cytosolic (Ferrandez et al. 2002).

In order to learn bacterial chemotaxis in response to the pollutants, it is important to present a simple focus on one class of receptors called methyl-accepting chemotaxis proteins (MCPs). The methyl-accepting chemotaxis proteins (MCPs) are the principal sensory receptors of the bacterial chemotaxis system. They have a structural organization, membrane topology, and mode of function that is typical of type I receptors in all cells (Stock and Surette 1996). They occur in the cytoplasmic

membrane (CM) with functional regions in both the periplasm and cytoplasm. There are different types of MCP, each type recognizing its own range of chemoeffectors.

MCPs are membrane-spanning homo dimers. Typical structural features of MCPs areas follows: appositively charged N terminus followed by a hydrophobic membrane-spanning region, a hydrophilic periplasmic domain, a second hydrophobic membrane-spanning region and a hydrophilic cytoplasmic domain (Rehm Bernd 2008).

Chemoreceptors have been initially described in the context of chemotactic signaling. However, more recent studies reveal that chemoreceptors are also involved in the regulation of different cellular processes, such as the synthesis of second messengers (Hickman et al. 2005). Chemotactic ligands are detected by cell surface chemoreceptors (Schmidt et al. 2011). Typically, chemoreceptors are composed of a ligand-binding region (LBR) and a signaling domain. The LBRs can be classified into two clusters, which are base-line resolved. Cluster I LBRs are characterized by a size between 120 and 210 amino acids with an average size of 156 ± 11 amino acids. Cluster II proteins have an LBR with a size between 220 and 299 amino acids that is centred at 262 ± 18 amino acids. For the totality of bacterial and archaea sequences, cluster I proteins represent the most abundant group (54% of the analysed proteins), whereas cluster II proteins account for 39%. Only 7% of the proteins have an LBR outside the limits of cluster I and II with sizes either below 120 or above 299 amino acids (Lacal et al. 2010).

Signal recognition by the ligand-binding region creates a molecular stimulus that is conveyed to the signaling domain, which forms a complex with *Che*A and *Che*W (Pineda-Molina et al. 2012). This molecular stimulus modulates *Che*A autophosphorylation and, sub-sequently, transphosphorylation toward the response regulator (Hazelbauer et al. 2008).

These ligands bind to periplasmic domains of MCPs which are considerated as transmembrane chemosensory proteins for environmental stimuli and their binding initiates chemotaxis signaling. The diverse ligand specificities among MCPs reflect amino acid sequence diversities of periplasmic domains of MCPs (Rehm Bernd 2008). Furthermore, several homologous transmembrane receptors (MCPs) sense extracellular stimuli and produce signals that are transmitted to their cytoplasmic domains (Schmidt et al. 2011). The cytosolic signaling domains of MCPs show a high degree of sequence conservation, and the presence of a methyl-accepting (MA) domain is the standard criterion for annotation of proteins as MCPs (Alexander and Zhulin 2007).

In Bacteria, the structure of the chemosensory array is universal across all species imaged to date (Briegel et al. 2009). It is even conserved between membrane-bound and cytoplasmic arrays (Briegel et al. 2014). Historically, the enteric bacterium *Escherichia coli* has been the model organism for chemotaxis studies. In *E. coli*, the chemotaxis system consists of methyl-accepting chemotaxis proteins (MCPs), six cytoplasmic chemotaxis proteins (*Che* proteins) and flagella. It has also been reported that *E. coli* has four transmembrane chemoreceptors MCPs, each of which binds a set of chemicals directly or in complex with specific

periplasmic binding proteins (Liu et al. 2009). *Che*A and *Che*Y are a histidine protein kinase and a response regulator of a two-component regulatory system, respectively (Rehm Bernd 2008). MCPs send signals to the flagellar motor via a complex signal transduction system that is composed of six soluble chemotaxis proteins, through which the bacterium modifies its swimming behavior based on the signal(s) received (Falke et al. 1997) (Fig. 1).

Additionally, there are two sets of flagellar stators in *Pseudomonas* spp. Compared to one set for *E. Coli* and *Salmonella enterica* serovar *Typhimurium* (Doyle et al. 2004). Likewise, while *E. Coli* is equipped with four MCPs and one MCP-like energy taxis receptor metabolically (Parkinson et al. 2005), versatile soil bacteria such as *Pseudomonas* species contain ≥ 25 MCP-like proteins (Parales et al. 2004). *Pseudomonas putida* strains KT2440 and F1 share 25 out of 27 MCP-like proteins (Parales et al. 2013). To date, the specific functions of relatively few *Pseudomonas* MCPs have been characterized (Sampedro et al. 2014). In addition, genome sequence analyses have revealed that *Pseudomonas* strains have numerous putative MCP genes. For example, the genome of *Pseudomonas*

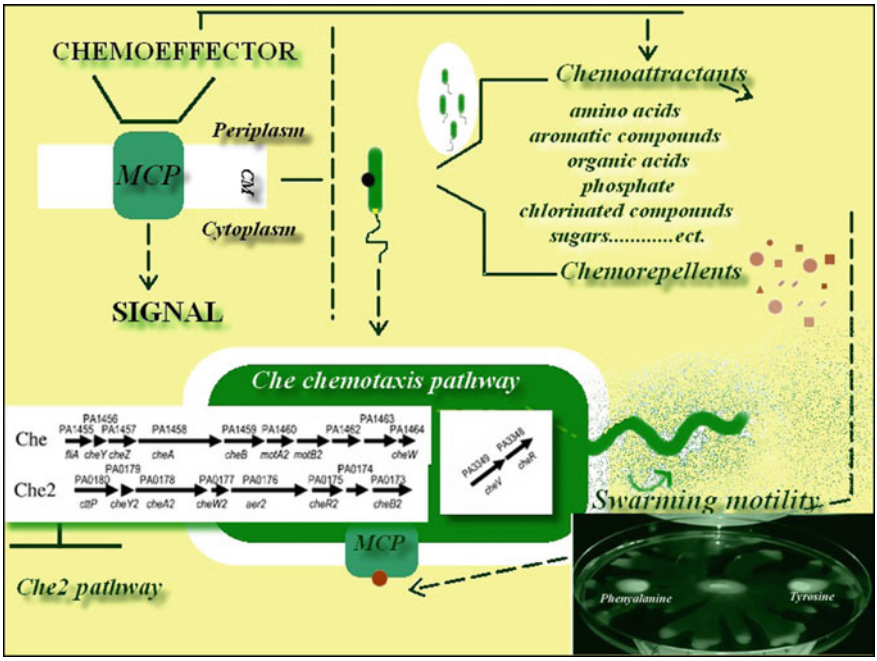

Fig. 1 Chemotactic pathway in *P. aeruginosa,* cell motility are a consequence of flagella rotation, exhibiting different motility pattern (swarming, swimming, twitching and sliding) as presented for a swarming motility of a *P. aeruginosa* strain, in the presence of amino acid (phenyalanine and tyrosine). Note the importance of the *che* patway to induce motility and signal or interaction between MCP (methyl-accepting chemotaxis proteins) and chemoattractants

aeruginosa PAO1 (Stover et al. 2000) encodes 26 MCP-like proteins, *Pseudomonas putida* KT 2440 (Nelson et al. 2002).

It is interesting to point out that a large number of MCPs are present in pseudomonads, relatively few have been functionally characterized (Liu et al. 2009; Oku et al. 2012). Sequence analysis reveals that these *Pseudomonas* species possess genes encoding MCPs and homologs of six *Che* proteins. Astriking feature of the genomes of *Pseudomonas* species is that the number of putative chemotaxis genes is quite large (Rehm Bernd 2008). *Pseudomonas* species have >25 MCPs encoded in their genomes, some of which have been functionally characterized (Parales et al. 2004). For example, 13 of the 26 MCP-like proteins in the genome *P. aeruginosa* have been functionally characterized (Kato et al. 2008).

Moreover, functional redundancy of MCPs has been reported previously, particularly in *Pseudomonas* species. For example, *P. aeruginosa* and *P. fluorescens* each have three receptors with overlapping specificity for amino acids (Oku et al. 2012).

2.1 Methyl-Accepting Chemotaxis Proteins in Pseudomonas

In many cases taxis is based on the recognition of the environmental signal by methyl accepting chemotaxis proteins (MCPs). These receptors contain typically an N-terminal ligand binding region (LBR), and a C-terminal signalling region containing a methyl-accepting (MA) domain. The latter region interacts with the *Che*A senor kinase and the *Che*W adaptor protein (Lacal et al. 2010). The signaled LBR creates a molecular stimulus that is transmitted to the signalling domain, which in turn alters *Che*A autophosphorylation activity and consequently transphosphorylation of the CheY response regulator. *Che*Y interacts with the flagellar motor and taxis is based on a differential modulation of motor activity by *Che*Y and *Che*Y-P (Szurmant and Ordal 2004).

It is interesting to note that MCPs differ largely in their topology and four major topologies have been identified of which some can be further subdivided (Wuichet and Zhulin 2003). The signalling domain is in all cases cytosolic. The sensing domain can either be located in the periplasm or in the cytosol. Most of the MCPs possess transmembrane (TM) regions but others are entirely cytosolic. Some MCPs were found to lack an LBR. However, there is no information available on the abundance of each of these topologies in bacteria and archaea (Lacal et al. 2010).

Pseudomonas, *Burkholderia* or *Ralstonia*, which could be classified either as metabolically versatile, inhabitants of complex and diverse environments (water, soil, rhizo-sphere), or capable of establishing complex interactions with plant roots. However, all of these microbes are characterized by an elevated number of MCPs what may be related to their ability to adapt to multiple and changing environments (Lacal et al. 2010). However, it is also important to note that *Pseudomonas* species have >25 MCPs encoded in their genomes, some of which have been functionally

characterized (Parales et al. 2004). For example, 13 of the 26 MCP-like proteins in the genome *P. aeruginosa* have been functionally characterized (Kato et al. 2008).

The LBRs of *Pseudomonas* MCPs studied so far have been found to bind ligands directly, and no evidence has been obtained for the participation of ligand-binding proteins. The size of the LBRs differentiates the chemoreceptors. The cluster I category has c.150 amino acids, while the cluster II category has c.250 amino acids (Sampedro et al. 2014).

Studing MCP-LBRs in *P. aeruginosa* PAO1 and *P. putida*, revealed that both species have approximately the same number of MCP-like genes. The *P. aeruginosa* PAO1 genome encodes 26 MCPs (Kato et al. 2008). Thirteen of its 26 MCP-like proteins have been functionally characterized, 10 of which have been shown to mediate positive responses to oxygen (Aer and Aer-2), inorganic phosphate (CtpH and CtpL), malate (PA2652), aminoacids and gamma aminobutyrate (GABA) (PctABC), ethylene (TlpQ), and chloroethylenes (McpA; Taguchi et al. 1997; Kim et al. 2007; Alvarez-Ortega and Harwood 2007). According to Nichols and Harwood (1999), the MCP Aer, a cluster I LBR, is the energy taxis receptor in *P. putida* PRS 2000. However, Aer-2 mediated *P. putida* KT 2440 response toward oxygen, phenols, and methylphenols (Sarand et al. 2008). A previous study revealed that an ∼60 kDa protein in *P. putida* PRS2000 was methylated in response to aromatic attractants, suggesting that an MCP was involved in the chemotactic response (Harwood 1989).

Thus, it is not surprising that other *Pseudomonas* differ enormously in the number of MCPs.49 putative MCPs in *Pseudomonas syringae* DC3000 (Parales et al. 2004), 32 putative MCPs in *Pseudomonas entomophila* (Muñoz-Martinez et al. 2012) and 18 putative MCPs in *P. putida* F1 genome encodes (Parales et al. 2013). A study conducted by Luu et al. (2015) identified a single MCP in *P. putida* F1 that is responsible for the detection of multiple aromatic and hydroaromatic chemicals that feed into the β-KA pathway. β-KA (β-ketoadipate) pathway serves as a major funneling pathway for the aerobic degradation of a wide variety of aromatic compounds (Harwood and Parales 1996). This MCP also senses several non metabolizable aromatic compounds (Luu et al. 2015). In another study, Oku et al. (2012) reported that the *P. fluorescens* proteins CtaA, CtaB, and CtaC (homologues of *P. aeruginosa* PctABC) are the major chemosensory proteins for *P. fluorescens* Pf0-1 chemotaxis toward amino acids. Additionally, NbaY, a cluster I LBR, is the chemoreceptor for 2-nitrobenzoate in *P. fluorescens* KU-7 (Iwaki et al. 2007).

It is also noteworthy that chemoreceptor genes have also been identified on a number of plasmids isolated from *Pseudomonas* strains, such as the hydrocarbon-responsive McpT chemoreceptor from plasmid pGRT1 (*P. putida* DOT-1E; Molina et al. 2011). This plasmid has also been found in other *Pseudomonas* species, including *Pseudomonas* stutzeri AN10 (Brunet-Galmes et al. 2012) and *Pseudomonas resinovorans* (Maeda et al. 2003). The NahY LBR for naphthalene is also found on a plasmid, in this case, pNAH7 in *P. putida* G7 (Grimm and Harwood 1997). Therefore, *P. putida* is an efficient model for bioremediation.

3 Pseudomonas Chemotaxis

Pseudomonas is a large genus within the γ subclass of Proteobacteria known for its ubiquity in the environment, utilization of a striking variety of organic compounds as energy sources (Lessie and Phibbs 1984). The number of species in this genus is increasing every year (Mulet et al. 2010). *Pseudomonas* is one of the more diverse genera, and its taxonomy has undergone many changes since earlier descriptions (Palleroni 1984). The members of the genus *Pseudomonas* (sensu stricto) belong to Palleroni's RNA group I, in the Gammaproteobacteria (Palleroni 1984). The *Pseudomonas* genus have been divided by multilocus sequence analysis (MLSA) into nine major groups: *P. fluorescens*, *P. syringae*, *P. Lutea*, *P. putida*, *P. anguilliseptica*, *P. straminea*, *P. aeruginosa*, *P. oleovorans* and *P. stutzeri* (Mulet et al. 2010).

Each species function as key components of ecological processes that suppress plant diseases in agricultural and natural environments (Weller et al. 2002), and several strains are used commercially to manage plant diseases in agriculture (Stockwell and Stack 2007) including bioremediation.

Since a long time, mechanisms of motility and chemotaxis remain unclear. Current evidence suggests that the chemosensory system and flagellar apparatus arrangement in the strains belonging to this genus are more complex than those of other bacterial species. Therefore, *Pseudomonas* species that have complex chemotaxis systems with several sets of *che* gene clusters and a number of MCP genes are much better model microorganisms for investigation of ecological aspects of chemotaxis in environmental bacteria than the enteric bacteria of which the chemotaxis system is relatively simple (Rehm Bernd 2008).

Consequently, elucidating the motility and chemotactic mechanisms for *Pseudomonas* spp. can be beneficial in many studies extending to bioremediation and host-pathogen interactions (Qian et al. 2013). Manson et al. (1998) reported that chemosensing in pseudomonads is not well known but it is known that like *Escherichia coli* they also utilize methyl-accepting chemotaxis proteins as transducers. In the case of *Pseudomonas aeruginosa*, five gene clusters studied and are involved in chemotaxis, with 26 methyl-accepting chemotaxis proteins (MCPs) and 20 chemotaxis (che) genes, compared to *E. coli*, which has one gene cluster, with four MCPs and six *che* genes (Kato et al. 2008).

The chemotaxis system integrates environmental cues into a behavioral response by using a dedicated signal transduction pathway. This pathway is composed of chemotaxis transducers, histidine kinase protein *Che* A coupled to the chemotaxis transducers via docking protein *Che*W, response regulator *Che*Y, and adaptation proteins *Che*B and *Che*R. Homologous chemotaxis systems have been identified for distantly related bacteria and archaea (Zhulin 2001).

Furthermore, *Pseudomonas aeruginosa*, have multiple chemotaxis-like operons that have provided new insight into their diverse functions. *P. aeruginosa* has four major *che* clusters; two are involved in chemotaxis with different suggested roles, a third that regulates type IV pili motility and biosynthesis, and the fourth is involved

in biofilm formation (Hickman et al. 2005). Moreover and unlike *E. coli*, which has only one set of chemotaxis (*che*) genes in a single gene cluster, *Pseudomonas* species have multiple *che* gene homologs organized in several unlinked gene clusters (Parales et al. 2004). *Che*A and *Che*Y/*Che*B belong to a family of two-component, stimulus-response regulators (Hoch and Silhavy 1995). Besides motility, processes regulated by these proteins in diverse bacteria include sporulation, virulence, membrane transport, and intermediary metabolism (*Burkart* et al. 1998). Wadhams and Armitage (2004) reported that the basis for the prokaryotic chemosensory signal transduction process centres around a two component system comprising a sensory histidine kinase (*Che*A) and a response regulator (*Che*Y). *Che*A receives input signals from specific chemoreceptor proteins, and transmits signals to *Che*Y by transferring a phosphoryl group. *Che*Y-P interacts with the flagellar machinery to modulate rotation of the flagellar motor and thus change cell behavior and movement. A third group of proteins allows cells to adapt to current conditions by modulating the activity of *Che*A (Roberts et al. 2010).

3.1 Chemotatic Signal Transduction

Chemotaxis in microbiology refers to the migration of cells toward attractant chemicals or away from repellents. Currently, chemosensory systems are functionally classified into those regulating flagellar motility, type IV pili (TFP)-based motility, and alternative cellular functions (ACF) (Wuichet and Zhulin 2010; Briegel et al. 2015). The ACF pathways regulate diverse processes such as cell development, biofilm formation (He and Bauer 2014).

Chemotaxis systems are commonly classified on the basis of their signaling kinase, *Che*A (Wuichet and Zhulin 2010). The specificity of bacterial chemotaxis systems resides in the chemoreceptors, which include MCPs and periplasmic ligand-binding proteins (Parales et al. 2013)

All bacteria share a conserved set of six different regulatory proteins that serve to direct cell motion toward favorable environmental conditions. Essentially the same regulatory system operates irrespective of whether motility involves one or several flagella, or whether it occurs by a mechanism such as gliding motility that does not involve flagella (Willey et al. 2008).

In Bacteria, one such pathway, the chemotaxis system, relays information on the chemical environment to the flagellar motor to bias swimming direction. Roughly half of all bacteria are chemotactic (Wuichet and Zhulin 2010).

Chemotaxis allows bacteria to swim toward attractants, such as nutrients, and away from repellants by controlling the rotational direction of the flagellar motor (Willey et al. 2008). Flagella can rotate in a clockwise or counterclockwise direction. Sensory proteins called methyl-accepting chemotaxis proteins (MCPs) control the directional switch in the flagellar motor. Clockwise rotation causes a cell to tumble (stop), counterclockwise rotation causes a cell to run (go) (Willey et al. 2008). The chemotaxis machinery allows the organism to move toward attractants

and away from repellents by a biased random walk (Rehm Bernd 2008). A major driving force in the evolution of chemotaxis is the capacity to sense and approach compounds that function as carbon or nitrogen sources (such as sugars, amino acids, or compounds that function as electron acceptors like oxygen, nitrate, fumarate, in the bacterial metabolism (Schweinitzer and Josenhans 2010).

One striking example in bacteria is the localization of proteins involved in chemotaxis. To date, two different paradigms for the spatial organization of chemotaxis proteins have been reported: polar localization (Lybarger and Maddock 2000) and both polar and cytosolic localization (Harrison et al. 1999).

Since the Pseudomonads include important plant and animal pathogens and potential agents of geochemical cycles, biocontrol, bioremediation, and chemotaxis is thought to play an important role in microbe host and microbe substrate interactions, ecological aspects of chemotaxis have been intensively investigated in *Pseudomonas* species (Rehm Bernd 2008).

Pseudomonads can sense chemical gradients and respond to the musing flagella or pili coupled to a chemosensory system with multiple copies of chemosensory genes (Sampedro et al. 2014). Unsurprisingly, studies have shown that *Pseudomonas putida* strains are capable of chemotaxis towards a wide range of growth substrates, including aromatic compounds, amino acids and the tricarboxylic acid (*TCA*) cycle intermediates (Parales et al. 2000, 2004). Therefore, there has been much research on chemotaxis of Pseudomonads toward environmental pollutants.Thus, benzoate-degrading *P. putida* PRS2000 is attracted by aromatic acids including benzoate, p-hydroxybenzoate, toluates, salicylate and chlorobenzoates (Rehm Bernd 2008).

3.2 Motility Patterns and Chemotaxis

Another line of research is devoted to understanding the link between motility patterns and chemotaxis towards pollulants. The extracellular filamentous appendages produced by motile microorganisms are responsible for the attachment process and interact with surface in a different manner.

Till date, flagella and pili had been the subject of intense study mainly for two reasons. First, their responsibility in behavior motility. Second, because of their consideration as one of the three major matrix components (Meliani 2015). In response to gradients in temperature, chemicals, or electric fields, bacteria are able to alternate their motility to locate favorable niches and avoid dangerous locations (Taktikos et al. 2013). Furthermore, cells displaying chemotaxis can sense chemicals such as those adsorbed to soil particles in a particular niche and swim toward them; hence, the mass-transfer limitations that impede the bioremediation process can be overcome (Al-Awadhi et al. 2003).

Pseudomonads range from 1 to 5 μm in length and can be propelled by polar flagella, monotrichous for *Pseudomonas aeruginosa* and lophotrichous for *Pseudomonas putida*, polar pili [1–25] for *P. aeruginosa*, or as part of a social

response via swarming or sliding motility (case of *P. aeruginosa*) (Sampedro et al. 2014).

Flagella are very fine threads of the protein flagellin with a helical structure extending out from the cytoplasm through the cell wall. Flagella may have a diameter between 0.01 and 0.02 mm, and a length of up to 10 mm (Meliani 2015). The bacterial flagellum consists essentially of three parts: (i) a protein filament which projects from the cell surface and which, by rotating, provides the thrust for flagellar motility; (ii) a curved protein structure, the hook, contiguous with the proximal end of the filament, and (iii) the flagellar motor (basal body)—a structure which anchors the hook in the cell envelope and incorporates the energy-converting apparatus (Singleton and Sainsbury 2006). The bacterial flagellar motor is a nanotechnological marvel, no more than 50 nm in diameter, built from about 20 different kinds of parts. It spins clockwise (CW) or counterclockwise (CCW) at speeds on the order of 100 Hz, driving long thin helical filaments that enable cells to swim. Thus, this important part is the output organelle of a remarkable sensory system, the components of which have been honed to perfection by billions of years of evolution (Berg 2003).

Most sequenced bacterial genomes include homologues of genes that are known to encode components of flagella and chemosensory pathways, which indicate that motility is widespread and probably provides a selective advantage, especially in non-homogeneous, nutrient-limiting environments (Wadhams and Armitage 2004). These authors signaled that a decreased concentration of attractant results in decreased attractant binding to the MCPs, which stimulates *Che*A trans-autophosphorylation. This result in an increase in the concentration of *Che*Y–P. *Che*Y–P then binds to the flagellar motor and causes it to switch to clockwise rotation, which results in cell tumbling and direction change. The *Che*Y–P signal is terminated by the phosphatase *Che*Z. *Che*B is also phosphorylated by *Che*A–P, which results

In an increased methylesterase activity and an increased demethylation of the MCPs. Demethylated MCPs have a reduced ability to induce *Che*A autophosphorylation (even in the presence of a low concentration of attractant), so the rate of *Che*A autophosphorylation and therefore the rate of direction changing returns to the prestimulus level. The system has now adapted and is primed to sense any subsequent increases or decreases in ligand binding.

On the other hand, the latter apparent chemosensory pathway senses an environmental signal through a classic methyl-accepting chemotaxis protein (MCP)-like receptor that controls the phosphorylation of the chemotaxis protein (*Che*)A. Thus, one of the four *Pseudomonas aeruginosa che* operons seems to be essential for the expression of pathogenicity genes, and one operon in *Pseudomonas fluorescens* has been proposed to be involved in cellulose biosynthesis (Wadhams and Armitage 2004).

The genes controlling chemotaxis in *E. coli* are homologous to the genes controlling chemotaxis in many other bacterial organisms, including *Pseudomonas aeruginosa*, *Rhodobacter sphaeroides*, and *Bacillus subtilis* (Porter et al. 2011).

Furthermore and unlike *E. coli*, *P. aeruginosa* has only a single polar flagellum. Therefore, clockwise (CW) flagellar rotation will result in *P. aeruginosa* being pulled backwards in a straight trajectory (Taylor and Koshland 1974), not tumbling as for *E. coli*. A recent study on tethered *P. aeruginosa* revealed a run-reverse-turn motility pattern (Qian et al. 2013). However, how *P. aeruginosa* regulates motility to achieve chemotaxis in a heterogeneous environment is not well understood (Cai et al. 2016). The same authors found that *P. aeruginosa* efficiently chemotaxed, despite very limited angular changes in orientation, by decreasing the likelihood of changing direction when it was going up a favorable gradient and increasing the likelihood of changing direction when it was going down the same gradient.

Interestingly, *P. aeruginosa* seems to regulate the length of time between switches in rotation direction but left the relative probability of clockwise (CW) and counterclockwise (CCW) flagellar rotation unchanged. This symmetric motor regulation indicates that the flagellum can push or pull the *P. aeruginosa* cell toward the attractant (Qian et al. 2013).

As was recently pointed out by Cai et al. (2016), the physiological difference between multiflagellated *E. coli* and singly flagellated *P. aeruginosa* implies that biased motor regulation should prevent *P. aeruginosa* populations from chemotaxing efficiently. With the analytical modeling, and simulations, these authors demonstrated that *P. aeruginosa* uses unbiased, symmetric regulation of the flagellar motor to maximize its chemotaxis efficiency. According to Cai et al. (2016) results, this mode of chemotaxis was not previously known and demonstrates a new variant of a paradigmatic signaling system in an important human pathogen.

The same findings have shown that another way of achieving chemotaxis was reached, by adjustment of the flagellar motor switching complex, and imply that even though the switching complex protein is conserved across bacterial species, it likely has evolved variations to be compatible with different types of swimming motility (Cai et al. 2016).

However, bacteria moving in homogeneous environments often have a very distinct motility pattern, which is defined by the phenotype of the cell. It remains unclear how different motility patterns of bacteria can affect their ability to perform chemotaxis (Taktikos et al. 2013). Many flagellated bacteria have more than one mode of locomotion, moving independently in bulk liquid (swimming) or moving in association with other cells in a thin film of liquid over a moist surface (swarming). Both modes use the same mechanism of propulsion, with thrust generated by rotating helical flagella (Darnton et al. 2007).

3.3 Pseudomonas Surface Translocation

Six different types of surface translocation have been recognized so far: (i) swarming, dependent on excessive development of flagella and partly on cell to cell interaction; (ii) swimming, dependent on flagella and fluid; (iii) gliding, dependent on intrinsic motive forces and partly on cell to cell interaction;

(iv) twitching, dependent on intrinsic motive forces (and fimbriae?); (v) sliding, dependent on growth and reduced friction (i.e., spreading by expansion); and (vi) darting (Henrichsen 1972). Motile populations, such as bacteria, can rapidly reach novel niches, which they can colonize; this provides ecological advantages to the bacteria (Rather 2005).

In the present section, we made an attempt to explore the wonderful swarming—swimming and twitching motility in *Pseudomonas* species. Swarming as fantastic dendritic fractal-like patterns motility is widespread in many genera of Gram-negative and Gram-positive flagellated bacteria and from which the bacteria are thought to extract water and nutrients (Verstraeten et al. 2008). Swarming motility seems to be narrowly conserved in the bacterial domain and is currently restricted to three families (Fig. 2) (Kearns 2010). Bacterial motility has been classified into discrete types, based on structural surface appendages or internal structures involved, and bacterial species may employ more than one type for translocation and colonization (Jarrell and McBride 2008).

While flagella are required for swimming and swarming motility, type IV pili display twitching motility. Motility often plays a dual role in biofilm formation, being required for biofilm formation and the development of the three-dimensional architecture, but also for biofilm dissolution (Rehm Bernd 2008). As was pointed out by (O'Toole and Kolter 1998) the presence of functional type IV pili and flagella ensures motility, which is also required for the development of mature and elaborate biofilm structures.

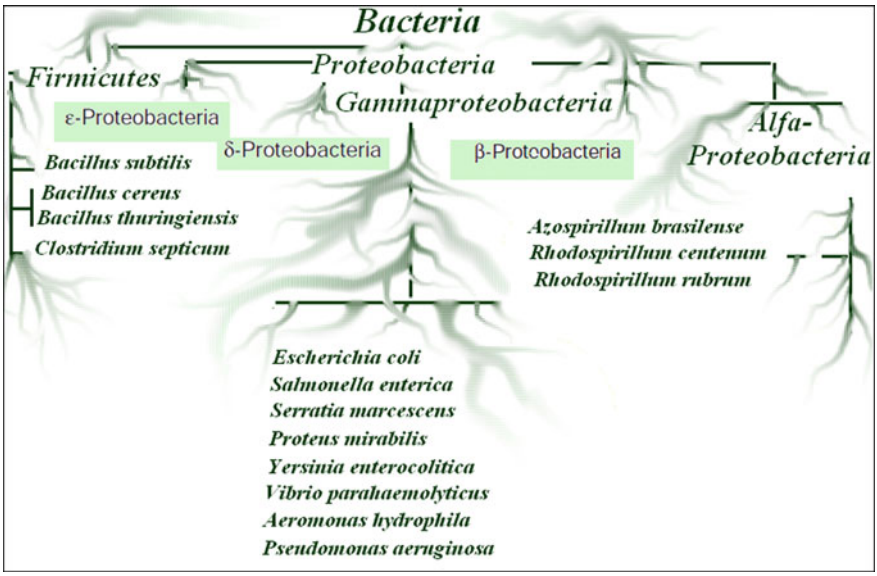

Fig. 2 Main species with swarming ability. Classes of *Betaproteobacteria Deltaproteobacteria* and *Epsilonproteobacteria* are not included

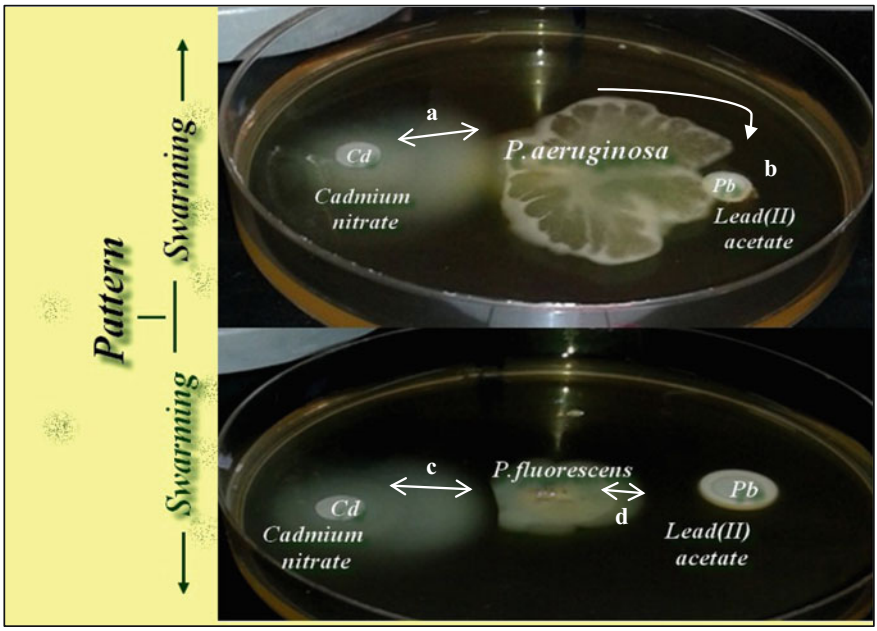

Fig. 3 As studied with *P. aeruginosa* and *P. fluorescens*, cadmium nitrate Cd $(NO_3)_2$ present a good activity of a chemoattractant (**a**, **c**), and induce a swarming and swimming motility on Luria-Bertani (LB) broth. Additionally, lead acetate (Pb $(C_2H_3O_2)_2$) presented no visible effect on swarming motility of *P. fluorescens*. The fact that two bacterial strains sensed Cd $(NO_3)_2$ as chemoattractant cannot be disregarded. Note the formation of dendritic fractal-like patterns formed by migrating swarms moving around the disc of lead acetate for *P. aeruginosa*

Furthermore, *Pseudomonas* swim by rotating flagella, generating a force that moves the cell body forward. Flagella-mediated swimming follows a counter-clockwise—clockwise—pause-counterclockwise (CCW-CW-P-CCW) flagella rotation pattern (Taylor and Koshland 1974). When responding positively to a chemical gradient, the frequency of clockwise rotations deceases (Sampedro et al. 2014).

Chemoattractants that stimulate flagella-mediated motility include amino acids, aromatic compounds, organic acids, phosphate, chlorinated compounds, and sugars, among others. For example, *P. aeruginosa* shows positive chemotaxis toward all 20 amino acids (Taguchi et al. 1997), ethylene (Kim et al. 2007), malate (Alvarez-Ortega and Harwood 2007), and chloroform (Kato et al. 2001). In addition, Parales et al. (2000) signaled that *P. putida* strains have been shown to be attracted to aromatic hydrocarbons such as toluene and naphthalene. Furthermore, *P. aeruginosa* PA01 can twitch toward dilauroyl-phosphatidylethanolamine (Kearns 2010). In our own ongoing work on serial *P. aeruginosa* and *P. fluorescens* isolates from different ecosystems, we discovered a diversified pattern of motility towards heavy metals. The *P. aeruginosa* exhibited a positive

attraction via swarming motility to lead acetate although Cadmium nitrate seems to be the best attractant. However, for *P. fluorescens* we signaled a swimming behavior vis a vis cadmium nitrate (Figs. 3 and 4). For instance, when a toxic compound is present in the environment, bacteria can detect it and swim away as presented in Fig. 3d. In contrast, if a new carbon source is available, chemotaxis induces bacteria to swim towards it. Not only carbon sources may act as inductors, nitrogen compounds, phosphate, oxygen or even some metals can behave in the same way (Harwood et al. 1984).

This versatility also shows up in an extensive phenotypic diversity towards heavy metals. Within this stimulus theses species exhibited different pattern of motility as detailed in the following sections.

Microorganisms display a wide range of social behaviors, such as swarming motility (Czárán and Hoekstra 2009). Among the different forms of cellular motility, swarming is probably the most complex one, representing a coordinated multicellular process that is influenced by various factors (Tremblay et al. 2007). Swarming is a complex type of motility usually defined as a rapid and coordinated translocation of a bacterial population across a semi-solid surface (Fraser and Hughes 1999). This motility can be distinguished from swimming motility in that swarming is required to move across a hydrated, viscous semisolid surface, while swimming allows movement through a relatively low-viscosity liquid environment (Rehm Bernd 2008). Swarming motility is characterized by the formation of elaborate dendritic patterns by the swarming colony (Murray and Kazmierczak 2008) (Fig. 4). Thus, motile populations, such as swarming bacteria, can rapidly reach novel niches, which they can colonize; this provides ecological advantages to the bacteria (Rather 2005).

Of these motility mechanisms, the least investigated is sliding motility, which Henrichsen (1972) defined as surface translocation produced by expansive forces in the growing colony combined with special surface properties to lower the friction between the cells and substrate (Fall et al. 2006) as shown in Fig. 4.

Contrary to surface colonization that requires active appendages, sliding is defined as a passive bacterial translocation created by expansive forces accelerated by surfactants that reduce surface tension (Kearns 2010).

Moreover, most bacteria that swarm have a peritrichous arrangement of flagella, in which multiple flagella are distributed randomly on the cell surface (Kearns and Losick 2003). *Pseudomonas aeruginosa* is a short, rod-shaped bacterium that also makes a polar flagellum. During swarming, *P. aeruginosa* retains its polar flagella but synthesizes an alternative motor that is specifically required to propel movement on surfaces and through viscous environments (Toutain et al. 2005). Thus the expression of alternative motors is at least one way to facilitate swarming motility besides the use of peritrichous flagella (Kearns 2010). According to this author, when cells transition from swimming to swarming, the number of flagella on the cell surface increases. Organisms with alternative flagellar systems become hyperflagellate in the transition from expression of a single polar flagellum to expression of multiple peritrichous flagella.

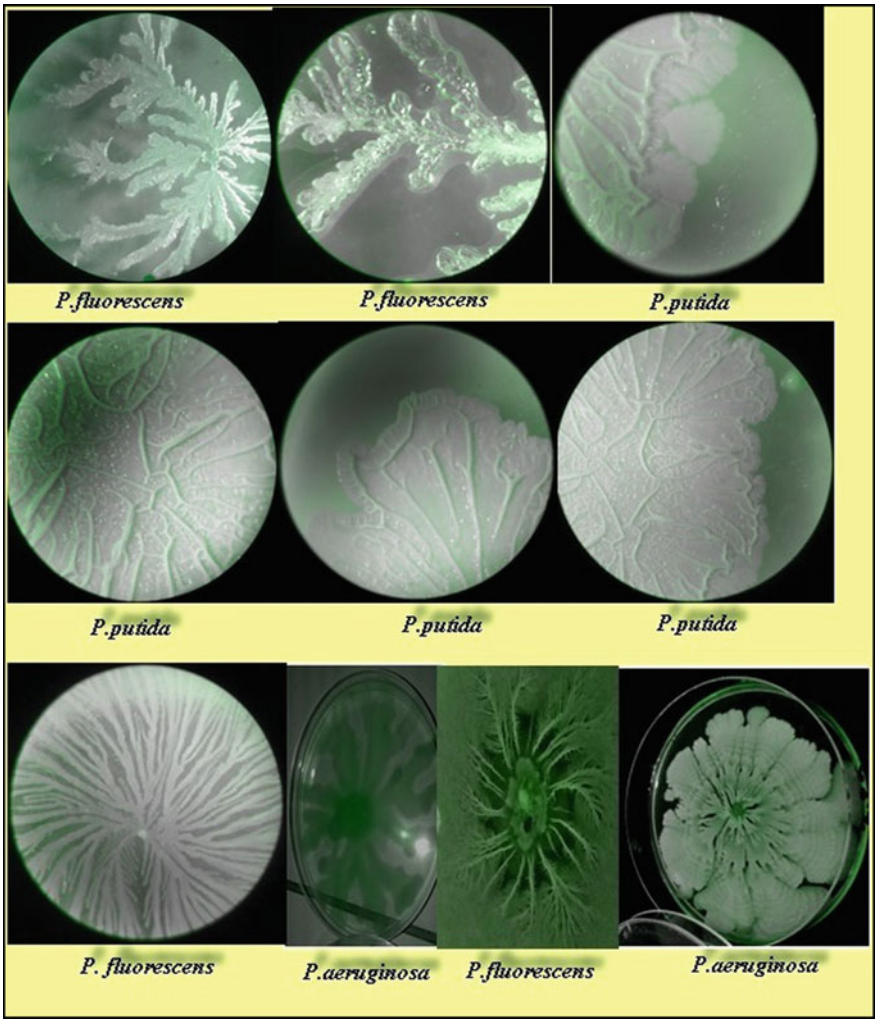

Fig. 4 Swarming phenotypes of Pseudomonas strains isolated from environmental niches. Swarming motility on TSA medium (1.5% agar) for 72 h at 30 °C. After a central spot of 5 ml of an overnight bacterial culture in Tryptic soy broth (TSB). The formation of dendritic fractal-like patterns formed by migrating swarms moving away from an initial location, primary and secondary tendrils are seen to develop at the swarm edge. Top view of an expanding sliding colony (**a**, **b**) (left)

Swarming motility is a process by which bacteria can rapidly (several μms^{-1}) advance on moist surfaces in a coordinated manner. It requires functional flagella and is coupled to the production of a viscous slime layer. The slime layer is thought to extract water from the agar and keeps the cells in a moist environment. Swarming is a group behavior that requires the cells to reach a certain cell number before the

process is initiated. Furthermore, swarmers are often elongated as a result of the suppression of cell division (Verstraeten et al. 2008).

Swarming motility appears to require motile flagella (Kohler et al. 2000). This bacterial motility has been shown to be important to biofilm formation (Caiazza et al. 2007), where cells act not as individuals, but as coordinated groups to move across surfaces; often within a thin-liquid film (Kearns 2010). It was reported that flagella are required for robust biofilm formation (Klausen et al. 2003), and likely contribute to persistent colonization. type IV pili, which power twitching motility across solid surfaces (Mattick 2002).

It is important to note that *Pseudomonas aeruginosa* for example exhibits three types of motility–flagellum-mediated swimming, flagellum and type IV pilus-mediated swarming, and type IV pilus-mediated twitching (Rehm Bernd 2008).

To our knowledge and in addition to flagella, swarming of *P. aeruginosa* requires the release of two exoproducts, rhamnolipids (RLs) and 3-(3-hydroxyalkanoyloxy) alkanoic acids (HAAs), which act as wetting agents and chemotactic-like stimuli (Kohler et al. 2000). Similarly, swarming motility is promoted by the production of bacterial wetting agents, such as HAAs and mono- and di-rhamnolipids (Deziel et al. 2003; Tremblay et al. 2007). The role of rhamnolipids in surface motility, and most notably swarming motility, is well documented. This biosurfactant is required for a swarming motility called, where it functions as a wetting agent and reduces surface tension (Deziel et al. 2003).

Swarming motility generally requires an energy-rich, solid medium, but the specific conditions that support swarming depends on the organism concerned (Kearns 2010). It is interesting to point out that flagella are the most important requirement for swarming motility, along with an increase in flagellar biosynthesis. According to this author this type of movement also requires an increase in cell–cell interactions and the presence of a surfactant (Meliani 2015).

During swimming motility, peritrichous flagella on one cell coalesce into a bundle and rotate to propel the bacterium in an approximately straight run. A swimming cell tumbles when one or several of these flagella change their direction of rotation (Kearns 2010). However, twitching motility is a surface motility powered by the extension and retraction of type IV pili, which confers slow cell movement, often with a jerky or 'twitchy' appearance (Mattick 2002). These appendages function as molecular grasping devices through their ability to extend, attach to a solid substrate, and then retract, effectively tugging the cell across a solid surface (Eisenbach et al. 2004).

To our knowledge, and like other kinds of pili and fimbriae, type IV pili (T4P) provide the ability to adhere to chemically diverse surfaces from glass and stainless steel to mammalian cells and promote bacterial cell aggregation involved in microcolony formation and virulence (Burrows 2012). However, T4P are unique in mediating flagellum-independent motility (Ottow 1975). Twitching occurs on moist surfaces of moderate viscosity, equivalent to that of 1% agar. Although individual cells are capable of movement, it is common to see them moving in rafts, groups of cells preferentially aligned along their long axes (Semmler et al. 1999). Individual

cells often snap into alignment with the group when they encounter a raft, contributing to the jerky appearance of twitching motility.

The exact distance traveled by twitching cells depends on both extrinsic and intrinsic factors. Extrinsic factors include medium (nutrient) composition (Huang et al. 2003), viscosity, and hydrophobicity of the surface (Semmler et al. 1999), and intrinsic factors include the amount of pili produced and their retraction rates, and the production of surface-tension-reducing surfactants (Burrows 2012). T4P are so thin (<8 nm in diameter), they cannot be visualized readily by light microscopy, and thus it was initially difficult to conclusively link pilus retraction to motility (Burrows 2012).

Henrichsen (1972) defined this motility as a kind of surface translocation that produced spreading zones and a fagellar-independent surface motility, on solid media, in which the colony edge exhibited varying micromorphological patterns, with cell movement being 'intermittent and jerky' and 'predominantly singly, although smaller moving aggregates occur'. This author also concluded that twitching motility on agar is dependent on a surface film of liquid (and accompanying humidity), as well as the presence of polar fimbriae, later termed type IV fimbriae (or pili). These one are filaments of about 6 nm in diameter which range up to several micrometres in length (Semmler et al. 1999). They occur at one or both poles of the bacterium and are primarily composed of a small structural subunit (pilin or PilA in *P. aeruginosa*) with a characteristic highly conserved and highly hydrophobic amino-terminal region, which forms the core of the helical structure, whose outer face is comprised of the more hydrophilic and more variable domains of the subunit (Parge et al. 1995).

Furthermore, twitching motility is achieved by type IV pili, which extend and retract to pull the cell forward. The assembly of functional type IV pili on the bacterial pole requires approximately 40 gene products (Rehm Bernd 2008). It has been shown that type IV pili are required for a form of surface-associated movement known as twitching motility. Twitching motility is thought to be a consequence of the extension and retraction of type IV pili, which propels the bacteria across a surface by an undescribed mechanism (O'Toole and Kolter 1998). Twitching motility has been shown to be induced in *P. aeruginosa* biofilms by iron limitation (Singh et al. 2002).

Mobility (swimming, swarming and twitching) serves the planktonic organism in seeking nutrients, avoiding toxins and finding a suitable surface for aggregation (Chávez et al. 2005). For the purpose of this review, we would, however, like to present some chemotaxis signal toward some environmental pollutants in certain species of the mentioned genus. It has been reported that the Wu et al. (2003) that a motile byphenyl-degrading bacteria (*Pseudomonas putida* P106) showed significant positive chemotactic response toward biphenyl. It is also noteworthy in Gordillo et al. (2007) study that this result of chemotaxis toward biphenyl was described in a motil *Pseudomonas* sp. B4 with it swarming pattern.

Furthermore, and in another report, because of nutritional properties benzoate and its chloroderivatives (CBAs) compound can elicit different chemotactic response. As studied for Pseudomonas sp. B4 (Gordillo et al. 2007) and *P. putida*

PRS2000 (Harwood et al. 1990). In accordance with this author, 3-and 4-chlorobenzoate are attractants to *P. putida* PRS2000 previously grown on benzoate or 4-hydroxybenzoate.

Moreover, chemotaxis by *P. putida* strain G7 was shown to enhance the degradation of naphthalene diffusing not only from a naphthalene-saturated aqueous buffer contained in a capillary (Chávez et al. 2005).

Previous studies have shown that *Pseudomonas* sp. have been shown to be chemotactically attracted toward different aromatic hydrocarbons (Harwood et al. 1984). Parales et al. (2000) reported toluene as a chemoattractant for three toluene-degrading bacteria (*P. putida* F1, *Ralstonia pickettii* POK01, and *Burkholderia cepacia* G4). *P. putida* F1 was also reported to be chemotactic toward seven other organic pollutants, some of which served as sources of carbon and energy, but almost every compound was found to be a substrate for toluene dioxygenase. Furthermore, *P. putida* PRS2000 was also found to be attracted toward 3- and 4-chlorobenzoate, although these compounds are not metabolized by this organism ((Harwood et al. 1984). Thus, we underline through these data that the list of chemotactic species toward pollutants is not-exhaustive and the possibility of applying chemotactis Pseudomonas in xenobiotic degradation deserves further investigation.

4 Conclusion

The studies reviewed here demonstrate the importance of chemotaxis signal in maintaining contact of bacteria with different ecosystems. Exploring *Pseudomonas* chemotaxis by figuring out their possible relationships with bioremediation of xenobiotic compounds has started a new and fascinating area of investigations in the chemesensory signal research. It is important to note that future endeavors are needed to elucidate how this motility pattern is coordinated with this wonderful specific phenotype toward each pollutant. We believe that this information will help us to provide insight into the role of these species in multifarious role in many fields right from bioremediation to biomedical applications.

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E. Lichtfouse (ed.), *Sustainable Agriculture Reviews 31*, Sustainable Agriculture Reviews 31, https://doi.org/10.1007/978-3-319-94232-2