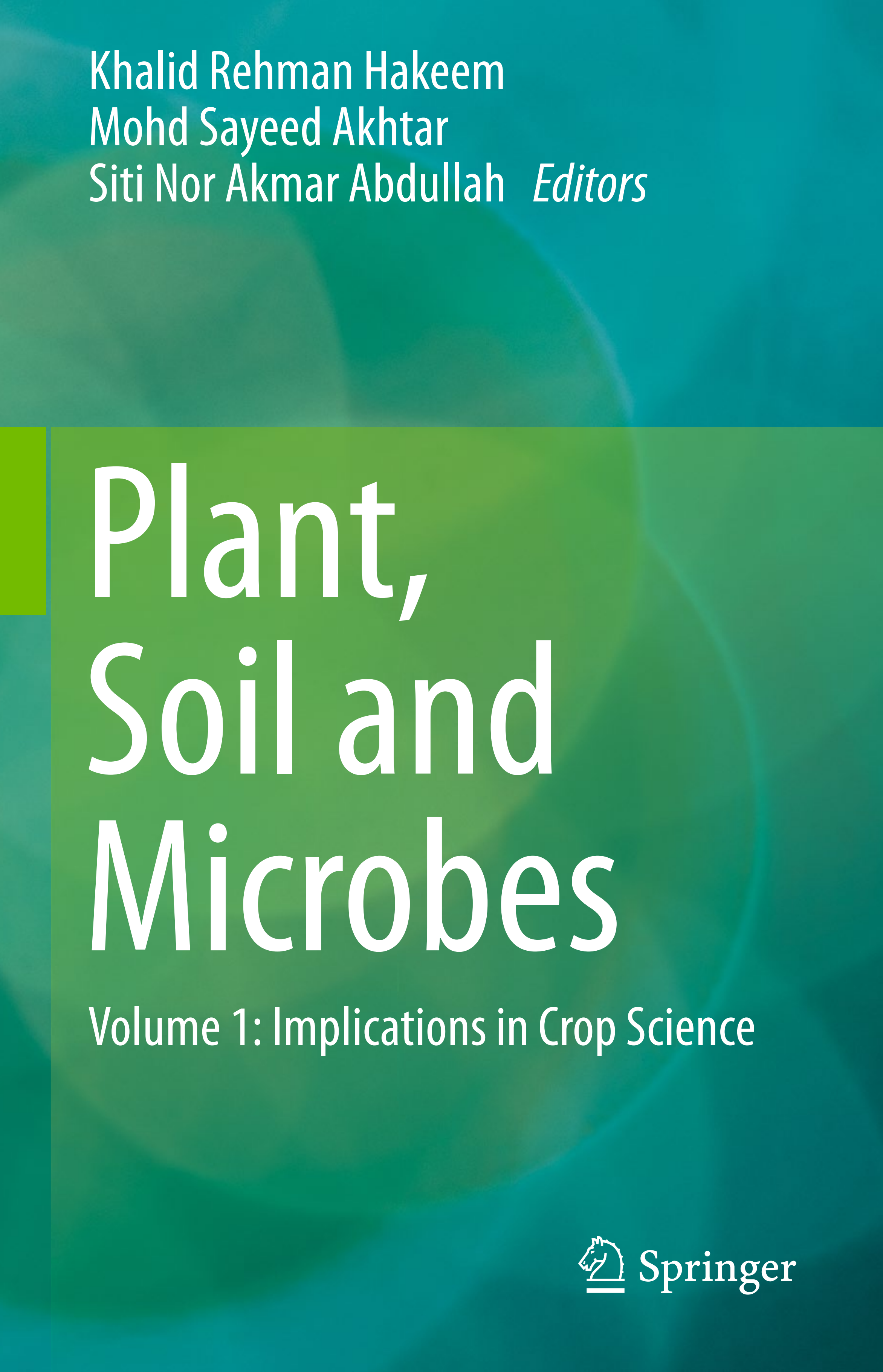

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Mohd Sayeed Akhtar
Siti Nor Akmar Abdullah *Editors*

Plant, Soil and Microbes

Volume 1: Implications in Crop Science

Springer

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Editors

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Foreword

The flowering plants were said to have originated approximately 125 million years ago. All through the past centuries, the number of species and diversity increased steadily. Currently they outnumber other plant groups, because more than 250,000 species of flowering plants have been described. Every year a large number of new species are added, because we are passing through an "age of angiosperms." Their influence on the evolution of animals has been profound, and domestication is now accepted as the main factor responsible for the rise of human civilizations nearly 10,000 years ago. They are regarded as indispensable for the survival of living beings in general. All humans on our planet earth depend on crop species like wheat, rice, maize, and a few others for their survival. They also provide us with vegetables, fruits, oils, fibers, spices, drugs, and fodder, in addition to other uses, and are therefore of great importance. Their importance to humans is responsible for the great past and present interest in studying the plant world.

Keeping in view that global population has crossed the level of 6.5 billion, a major part of it being underfed or undernourished, it is of prime importance to improve the crop plants using eco-friendly and sustainable approaches. The demographic developments make it clear that improving human nutrition throughout the world must involve efficient crop production. Efforts to improve crop productivity mainly rely on the soils.

The soils are influenced by parent materials, organic matter content, pH, texture, age, drainage, vegetation, and microbial, anthropogenic, and natural processes involving the amounts of nutrients. The latter are relatively high in soils on a global basis, but their deficiencies are frequently reported in several crops all over the world due to increase in nutrient demands from intensive cropping practices and adaptation of high-yielding cultivars with high nutrient demands; production of crops on marginal soils low in nutrients; the use of high amounts of fertilizers with low nutrients; decrease in the use of animal dung, composts, and crop residues; and finally involvement of natural and anthropogenic factors limiting adequate plant availability and breaking nutrient balance. Nearly four billion ha of the earth's ice-free land is subject to mineral stresses for plants. The increased crop yields, loss of nutrients through different ways, liming and misuse of manures, and the use of chemical fertilizers with high purity are leading to an accelerated loss of available soil nutrients. The uptake of nutrients is a process controlled by soil, plant, microbial, and environmental factors. Plant factors such as root and root hair morphology (length, density, surface area), root-induced changes, root exudation of organic acids, sugars, nonproteinogenic amino acids, secretion of enzymes, plant demand, plant species/cultivars, and microbial associations also have an impact on plant growth and development on the soil.

In addition to the facts cited above, the microbial populations in the soil improve growth by enhancing atmospheric nitrogen fixation, producing phytohormones, enhancing root surface areas to facilitate uptake of nutrients, as well as mobilizing and solubilizing unavailable organic and inorganic nutrients. Many microorganisms produce siderophores which strongly and specifically bind nutritional elements. The microorganisms in the rhizosphere are associated with differences among cultivars in their effectiveness to grow with low mineral levels. A mycorrhizal colonization of roots increases root surface areas to enhance root exploration of large soil volumes compared to uninfected roots and increase nutrient uptake and tolerance in plants, toxics, and drought. Mycorrhizal associations particularly increase acquisition of some nutrients in the plants grown under deficiency conditions and decrease uptake of others which are in excessive amounts. They also undertake the biological control of root pathogens and in nutrient cycling. Microbial interactions may also influence nutrient mobility. Several microbes involved in redox reactions in soil have been identified. Noninfecting rhizosphere microorganisms do enhance plant nutrition by improving growth and morphology of roots, physiology and development of plants, and nutrient uptake processes by roots.

Plant, Soil and Microbes: Implications in Crop Science, with 17 contributions from Australia, Brazil, Egypt, France, India, Italy, Malaysia, Pakistan, Portugal, Bangladesh, and Saudi Arabia, contains an inclusive information and latest research results in understanding the interactions and implications between plant, soil, and microbes. This information could be useful for researchers, educators, graduate students, and industry persons for understanding and developing crop improvement strategies. In this edited volume an attempt has been made to highlight the current status of crop productivity. Emphasis has been laid on different topics related to the key principles influencing crop improvement together with an elucidation of the nature of new approaches in improvement. I am sure that a new generation of researchers will be benefitted much from this book and share the respect for the crop plants we all live by and concern for the maintenance of diversity.

Izmer, Turkey Münir Öztürk

Preface

Soil provides an excellent and diverse ecological niche for the rhizospheric microorganisms and seems to be a hotspot for most of the microbial interactions taking place below ground level. Among the various rhizospheric microorganisms, mycorrhizal fungi, plant growth-promoting bacteria and root nodule bacteria are of prime importance, because they have the unique ability to interrelate with the soil and host plant and are also responsible for the assorted underground interactions. These microorganisms also play a vital role in nutrient uptake, water relation, ecosystem establishment, and plant diversity and also had an adverse effect on the pathogen in a diverse range of pathosystems. Besides these functions, microorganisms also influence the water use efficiency and biological function during plant–soil interactions, directly or indirectly by altering the microbial balance in the rhizosphere and suppressing the broad spectrum of plant pathogens.

Considering the importance of these ground rhizospheric microorganisms in the plant disease protection, it came into highlight from research that the combined application of these microorganisms is more beneficial than the use of a single agent and provides a better management against the soilborne plant pathogens. The interaction of these microorganisms also provides an overview about the biological functions of soil and its interaction with the plant–microbe system, nutrient management, biogeochemical cycling, various water environmental conditions in response to biotic and abiotic stresses, signaling of molecules during host–pathogen interaction, role of phytohormones against the environmental stresses, and the major challenges in the formulation of microorganisms for the biocontrol products. The molecular approach of these microorganisms is also the basis for understanding the mechanism involved in disease suppression by these hidden underground beneficial microbes.

This volume with 17 Chapters describes the basis of nutrient exchange between the microorganisms and the host plants, mechanism of disease protection, and recent molecular techniques involved in understanding these multi-tropic interactions. We hope that the book will be helpful to graduate students, teachers, researchers, and industry persons, who are interested in soil microbiology, plant pathology, ecology, environmental sciences, and agronomy.

We are highly grateful to all our contributors for readily accepting our invitation for not only sharing their knowledge and research but for venerably integrating their expertise in dispersed information from diverse fields in composing the chapters and enduring editorial suggestions to finally produce this venture. We greatly appreciate their commitment. We are also thankful to Prof. Munir Ozturk for his suggestions and writing the foreword for this volume.

We thank the Springer International team for their generous cooperation at every stage of the book production.

Selangor, Malaysia

Khalid Rehman Hakeem
Mohd Sayeed Akhtar
Siti Nor Akmar Abdullah

Contents

About the Book

The interactions between the plant, soil, and microbes are very complex in nature and may be antagonistic, mutualistic, or synergistic, depending upon the types of microorganisms and their association with the plant and soil. The multitrophic tactics are involved in these types of interactions to nourish the plants in various habitats and conditions. Understanding the mechanisms of these interactions is highly desired to utilize the knowledge in such an eco-friendly and sustainable way, which may not only resolve the upcoming food security issues but also make the environment green by reducing the chemical inputs. *Plant, Soil and Microbes: Implications in Crop Science* provides an inclusive information on the applied, efficient, and determined efficacy of plant, soil, and microbes. The book reviews the implications of plant, soil, and microbes for the improvement in crop yield and protection against various diseases caused by phytopathogens and nutrient deficiencies.

About the Editors

Khalid Rehman Hakeem is working as a fellow researcher at the Faculty of Forestry, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia, and also a visiting professor at Fatih University, Istanbul, Turkey. He obtained his M.Sc. (Environmental Botany) as well as Ph.D. (Botany) from Jamia Hamdard, New Delhi, India, in 2006 and 2011, respectively. He completed his postdoctorate in the fields of forest dynamics and biotechnological studies from Universiti Putra Malaysia from 2012 to 2013. Dr. Hakeem has more than 9 years of teaching and research experience in plant ecophysiology, biotechnology and molecular biology, plant–microbe–soil interactions, as well as in environmental sciences. Recipient of several fellowships at both national and international levels, Dr. Hakeem has so far edited and authored more than sixteen books with international publishers. He has also to his credit more than 70 research publications in peer-reviewed international journals, including 25 book chapters with International publishers. He is also the editorial board member and reviewer of several high-impact international journals. Dr. Hakeem is currently engaged in studying the plant processes at ecophysiological as well as proteomic levels.

Mohd Sayeed Akhtar is working as a fellow researcher in the Institute of Tropical Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. He received his Ph.D. degree from Aligarh Muslim University (AMU), India, in 2008. He conducted his postdoctoral research at the Botanical Institute, University of Basel (BIB), Switzerland (2008–2010), and Chonbuk National University (CBNU), Republic of Korea, in 2011, respectively, and also worked as assistant professor at the Department of Biology, College of Natural Sciences, Jimma University, Jimma, Ethiopia, from 2011 to 2014. Dr. Akhtar has more than 12 years of research and teaching experience in soil microbiology, applied microbiology, environmental microbiology, molecular biology, plant pathology, and plant nanobiotechnology. Dr. Akhtar has received several prestigious fellowships at national and international levels. His promising approach and dedication stand him in the row of foremost scientist in the field of plant–microbe interaction and plant nanobiotechnology. He is an author and coauthor of about 45 research articles in peer-reviewed journals, contributed 12 book chapters in the books published by Springer-Verlag, and also edited three books with international publishers. He is serving the scientific community as editorial board member and reviewer of several high-impact international journals. His current research is focused on the rhizospheric plant–microbe interactions and their molecular biotechnology, bioremediation, biomineralization, nanofertilizers, and nanobiotechnology.

Siti Nor Akmar Abdullah is currently the head of the Laboratory of Plantation Crops at the Institute of Tropical Agriculture and a professor in plant molecular biology at the Faculty of Agriculture, Universiti Putra Malaysia (UPM). Her Ph.D. research was mainly conducted at the John Innes Centre in UK. Her current research focuses on the transcriptional regulation of oil and vitamin E biosynthesis, fruit ripening process, host plant–pathogen interaction, and nutrient uptake mechanism in oil palm. She has led several research projects at the national level and secured research grants from oil palm industry members. She has published more than 50 papers in citation-indexed journals and also five patents granted in Malaysia and the USA particularly on oil palm tissue-specific promoters. Twenty Ph.D. students from Malaysia and other countries have graduated under her as the main or co-supervisor. She has delivered several talks as an invited speaker in various countries like China, India, and Indonesia and also served as a reviewer for many international peer-reviewed journals. She has led national and international conferences and workshops on plant biotechnology jointly organized by UPM and Wageningen University and the University of Nottingham. She has established the *Arabidopsis thaliana* laboratory in MPOB, Malaysia, and transgenic greenhouse (certified as Biosafety Level 2) in UPM.

Plant Growth-Promoting Microorganisms: Interaction with Plants and Soil

Vankayalapati Vijaya Kumar

Contents

Abstract Soil acts as a medium for a wide variety of microorganisms such as bacteria, fungi, actinomycetes, algae, and protozoa. Soil also gives the necessary support for anchorage to plant roots. The complex interactions between soil, plants, and microorganisms lead to different associations in the rhizosphere. These interactions are useful in (a) more nutrient recycling by nitrogen fixation, phosphate and potash solubilization; (b) disease suppression in crop plants; and (c) bioremediation in contaminated soils. Plant roots secrete different inorganic and organic compounds which encourage the growth of microorganisms; in turn the chemicals secreted by

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microorganisms release the bound minerals from the organic materials in the soil, which are absorbed by plant roots. This chapter reviews bacteria, fungi and their associations and interactions with plants and soil for beneficial effects on crop plants such as mineral nutrition, disease suppression, bioremediation, etc.

Keywords Bacteria • Bioremediation • Fungi • Nutrient recycling • Disease suppression

1 Introduction

Microorganisms in the rhizosphere soil play a key role in maintaining the soil fertility (Yadav et al. 2015), which is key for successful crop production to meet the increasing global food demand. Soil is a mixture of minerals, organic matter, gases, liquids, and many other organisms that are supporting the plant life. Soil acts as a reservoir of air, water, and nutrients that is essential for plant growth. Only a few grams of soil contain hundred millions to billion microorganisms. Bacteria are the most abundant microbes in soil followed by fungi; however, the actinomycetes are ranges in between bacteria and fungi. The fungi and actinomycetes degrade various plants and animal residues that reach soil, such as complex carbohydrates, simple sugars, starch, cellulose, hemicelluloses, pectins, lignins, proteins, fats, oils, waxes, resins, and other products. Bacteria finish the degradation by eating the digestible materials. Other microbes that are found in smaller numbers are algae, cyanobacteria, protozoa, and nematodes. Soil gives the necessary support for anchorage to plant roots (Yadav et al. 2012; Bhaduri et al. 2015). The metabolism of microorganisms and root growth in the soil add to texture and fertility. The association of microorganisms with plants leads to different associations such as mutualism (both plants and microorganisms are benefited), commensalism (one partner is benefited and others remain unaffected), and parasitism (one partner benefited and the other harmed).

The microorganisms present in the rhizosphere that colonize plant roots are termed as plant growth-promoting microorganisms. The roots provide anchorage to plant, increase the uptake of water and mineral nutrients, and secrete a variety of compounds as root exudates. The secretion of the chemical compounds alters the physical and chemical properties of soil, and also regulates the microbial community in the vicinity of the rhizosphere (Yadav et al. 2012). This may help in attracting active microorganisms to metabolize the secreted compounds in the rhizosphere. Some of the root exudates attract microorganisms, while others act as repellents against a wide range of microorganisms. This chapter highlights the plant growth-promoting microorganism types, their associations and interactions with plants and soil.

2 Plant Growth-Promoting Microorganisms (PGPM)

Plant growth-promoting microorganisms are defined by three intrinsic characteristics: (1) they must be able to colonize the root/rhizosphere, (2) they must survive and multiply in micro-habitats associated with the root surface, in competition with other microbiota, at least for the time needed to express their plant promotion/protection activities, and (3) they must promote plant growth. The PGPM are either bacteria or fungi that are living in the soil. The bacteria are either free living or possess a symbiotic association with plant roots. Plant growth-promoting bacteria enhance the growth of plants by their microbial processes such as nitrogen fixation, phosphate solubilization, potash mobilization, zinc solubilization, micronutrient mobilization, and also phytohormone secretion (auxin, cytokinin, gibberellins like substances), desirable for the growth and development of crop plants (Akhtar and Siddiqui 2010; Akhtar et al. 2010). Similarly, arbuscular mycorrhizal fungi (AMF) increase P nutrition, micronutrient mobilization (Akhtar and Siddiqui 2008; Akhtar and Panwar 2011). The AM fungal hypae extend into the soil beyond roots and transport nutrients to the plant and increase the surface area of roots (Akhtar et al. 2011). This may help mycorrhizal plants to withstand several abiotic and biotic stresses. However, the secretion of glomalin by AM fungi increases soil quality and porosity, which may assist plant roots in aeration.

2.1 Plant Growth-Promoting Rhizobacteria (PGPR)

The term PGPR was first coined by Kloepper and Schroth (1978) to describe the beneficial rhizospheric bacterial populations that may colonize plant roots and exhibit growth promotion attributes. In general, the PGPR are free-living bacteria, colonize roots, and promote plant growth directly by nitrogen fixation, phosphate solubilization, production of phytohormones and siderophores or indirectly by their biocontrol properties such as antibiotic production, production of lytic enzymes, competition with phytopathogens for nutrients and colonizing sites, and induced systemic resistance (Akhtar et al. 2011).

The application of a mixture of PGPRs (*Azospirillum lipoferum*, *Azotobacter chroococcum*, *Pseudomonas fluorescens*, and *Bacillus megaterium*) has increased germination rate, shoot and root length and dry weight, chlorophyll and nutrient (NPK) content compared to control or individual application in *Catharanthus roseus* (Lenin and Jayanthi 2012). Similarly, improvement in growth parameters (fresh and dry weight, stem diameter, seedling height, chlorophyll content, and leaf area) was reported in cabbage seedlings by the application of PGPR strains (*B. megaterium*, *B. subtilis* and *Pantoea agglomerans*) compared to control (Turan et al. 2014). Sen and Chandrasekhar (2014) reported the significant increase in plant height, and dry weight by the inoculation of *P. fluorescens* in rice seedlings under salt stress conditions. Furthermore, the application of different PGPR (*Bacillus*, *Pseudomonas*, and *Serratia*) species has improved germination percentage, seedling height, root length, chlorophyll content, dry weight of maize seedlings (Almaghrabi et al. 2014). Moreover, Elekhtyar (2015) reported that *P. fluorescens* has increased plant growth attributes (seedling germination, seed vigor index, shoot and root ratio, fresh and dry weight, leaf area), chlorophyll concentration in leaves, and nutrient uptake in rice.

PGPR are also advantageous in bioremediation of contaminated sites with pollutants like hydrocarbons, chloralkali, effluents from the distillery and textiles, heavy metals, etc. Among the rhizosphere bacteria *Azospirillum*, *Azotobacter*, and *Rhizobium* are the major N_2-fixing bacteria, while the *Bacillus* spp. (*B. megaterium* and *B. subtilis*) and *Pseudomonas* spp. are P solubilizers and are used as both biofertilizers and PGPR. Moreover, some other bacteria (*Burkholderia*, *Enterobacter*, *Erwinia*, and *Mycobacterium*) could also be recognized as PGPR. The continuous application of NPK nutrients for crop plants leads to changes in soil pH, soil fertility, and soil microbial communities, leading to decreased yields. The PGPR enhances the growth directly by increasing the nutrient acquisition by nitrogen fixation, phosphate solubilization, potassium mobilization, and zinc solubilization. *Bacillus* isolated from different agroclimatic zones has shown good phosphate-solubilizing ability, and are positive for gelatin liquefaction, catalase test, indole production test (Patil et al. 2013). Akram et al. (2013) reported that *B. subtilis* and *B. fortis* effectively controlled *Fusarium* wilt in a split root experiment on tomato under greenhouse condition and increased the level of phenolics (55.45–67.15 %), peroxidase (56.70 %), polyphenol oxidase (41.56 %), and phenyl ammonia lyase (57.57 %). Apart form this, they also secrete vitamins, amino acids, hormones (IAA and gibberellic acid), and are now widely used in the biocontrol of a wide range of phytopathogenic fungi. Similarly, *Pseudomonas* is a good P solubilizer, and also produced significant levels of plant IAA and showed increased HCN production, siderophore production compared to control (Deshwal and Kumar 2013). The high level of chitinase activity is responsible for the degradation of the fungal cell walls of pathogenic fungi such as *Colletotrichum gloeosporioides* (58.3 %), *Alternaria brassicola* (50 %), *A. brassicae* (12.5 %), *A. alternata* (16.66 %), *Fusarium oxysporum* (14.28 %), *Rhizoctonia solani* (50 %), and *Phytophthora* (15 %) (Ramyasmruthi et al. 2012). *Burkholderia* sp. isolated from the rhizosphere of *Rhododendron arboreum* has shown good P solubilization, and IAA and siderophore production. *Burkholderia sp.* isolated from the rhizosphere of *Rhododendron arboretum* has shown good P solubilization, IAA and siderophore production. The germination percentage of treated seed was high (54.18 %) compared to control (38.12 %) (Nailwal et al. 2014).

2.1.1 Nitrogen Fixation

Azospirillum is an associative symbiotic nitrogen-fixing bacterium found in many grasses and cereals. It lives either on the root surface or inside roots. It fixes the atmospheric nitrogen by the enzyme nitrogenase in nonleguminous crops. It could also be used as a biofertilizer because of its ability to produce plant growth-promoting substances. Inoculation of different isolates of *Azospirillum* in tomato has increased the yield from 34.9–92.4 % compared to control. Apart from yield improvement in growth parameters such as plant height, root length, and fresh weight, number of fruits/plant, fruit diameter, fruit weight, yield per plant were also recorded. Tripathi et al. (2013) reported that the leaf nitrogen content was significantly improved in *Azospirillum*-treated plots. The study conducted by Faruq et al. (2015) on maize with *Azospirillum* in the presence and absence of nitrogen has improved all test parameters such as shoot length, root length, root number, and biomass compared to control. The *Azospirillum*-treated seedlings have shown higher numbers of lateral and tertiary roots in the presence and absence of nitrogen compared to control. However, inoculation of *Azospirillum* has increased leaf area, shoot dry matter, and yield compared to control plots in maize (Marini et al. 2015).

Similarly, *Azotobacter* is an aerobic, free-living, heterotrophic nitrogen fixing soil bacterium. It fixes atmospheric nitrogen through the enzyme nitrogenase in nonleguminous crops. It secretes hormones such as IAA, gibberellins and vitamins, and also produces antifungal metabolites. *Azotobacter* evolved a special defensive mechanism for nitrogen fixation, which may reduce the concentration of oxygen in cells. It is found beneficial in a wide range of crops covering cereals, millets, vegetables, cotton, and sugarcane (Tabar 2013). Inoculation of *Azotobacter* in *Ocimum* increased plant growth, number of leaves (Shanmugapriya et al. 2013) in the potted plant in pot experiment, and grain yield of wheat (Soleimanzadeh and Gooshchi 2013) compared control treatments. *Rhizobium* is a symbiotic bacterium associated with leguminous crops and fixes atmospheric nitrogen. Trabelsi et al. (2011) reported that inoculation of *R. gallicum* and *Ensifer meliloti* increased nodule number, shoot dry weight, and grain yield in *Phaseolus vulgaris* compared to the uninoculated controls. The inoculation of *Rhizobia* in *Vigna radiata* has increased plant length and dry weight compared to control plants under pot experiments and an increase in IAA, HCN, ammonia, siderophore, and phosphate solubilization activity has also been observed (Rajpoot and Panwar 2013). In another study, Patra et al. (2012) concluded that inoculation of different strains of *Rhizobia* increased the growth and yield of soybean under field condition.

2.1.2 Phytohormones Production

Indole acetic acid (IAA) is the most common natural auxin having a positive effect on root growth. Most of the rhizobacteria colonizing the seed coat or root surface is proposed to act in conjunction with endogenous IAA in stimulating cell proliferation and uptake of minerals and nutrients from the soil (Akhtar and Siddiqui 2009). IAA affects plant cell division, extension, and differentiation; stimulates seed and tuber germination; increases the rate of xylem and root development; controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light, gravity, and florescence; affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions. Tryptophan commonly found in root exudates is identified as the precursor for the production of IAA by the rhizobacteria.

Several PGPR (*Azotobacter* sp., *B. subtilis*, *Pantoea agglomerans*, *Paenibacillus polymyxa*, *P. fluorescens*, *Rhizobium* sp., and *Rhodospirillum rubrum*) have the ability to produce cytokinins or gibberellins during growth and development events. Similarly, ethylene can also affect plant growth and development in a number of ways, including root initiation, inhibiting root elongation, fruit ripening, lowering wilt, stimulating seed germination, promoting leaf abscission, and activating the synthesis of other plant hormones.

Due to the secretion of auxins under the root exudate deficit conditions, plant roots sense the auxin molecules and lateral roots, root hairs are emitted which are the sources of root exudates to maintain the bacterial population in the root zone. Most of the *P. fluorescens* species isolated from paddy soils produced IAA (Noori and Saud 2012). However, IAA production was also reported by *Azotobacter* sp., in wheat. The IAA-producing PGPR strains lead to vigorous root growth resulting in more surface area and thus enabling access to more nutrients in the soil (Singh et al. 2013). Moreover, the production of IAA by bacteria and fungi on Pikovskaya broth under in vitro condition was reported by Priya et al. (2013). They reported that out of 28 isolates, only 18 produced IAA by using tryptophan in the growth medium. However, the IAA production was achieved in JNFb liquid medium at 100 μg/ml tryptophan concentration (Srivatsava 2013).

2.1.3 Siderophore Production

Iron is one of the most important essential nutrients for growth of microorganisms in diverse environments. It is required for various cellular, metabolic, and biosynthetic processes, including DNA synthesis, electron transport system, formation of heme, cofactor for enzymes, oxygen transport, synthesis of ATP, and nitrite reduction in the nitrogen cycle. Although it is abundant in nature, it is not easily available in the preferred state. In the presence of oxygen and neutral pH it undergoes rapid oxidation from Fe^{2+} to Fe^{3+} and finally forms insoluble ferric-oxyhydroxide, which is almost unavailable for acquisition by microbes. The siderophores are relatively low molecular weight (400–1500 Da) iron-chelating compounds produced by many bacteria and fungi under iron-starved conditions. Generally, siderophores can be classified into three categories depending upon the moiety that donates oxygen ligands for Fe^{3+} coordination: (a) catecholates (or phenolates) (b) hydroxymates (or carboxylate), and (c) mixed types. Siderophores mainly scavenge iron and also form complexes with other elements (i.e., Mo, Mn, Co, and Ni) from the surrounding environment and make them available to host microbial cells. They promote plant growth by creating an antagonistic impact on phytopathogens. In soya bean, seed germination, shoot and root length were increased by the application of siderophore-producing *Bacillus* spp. GN-01 isolated from groundnut soil (Afreen and Chavan 2014). Siderophore production was observed in iron-limited King's B medium by *P. fluorescens* isolated from the rhizosphere soil of faba bean (Alemu 2013).

Siderophore-producing *Bacillus* spp., isolated from rice, chili, ragi, and beans controlled *Fusarium oxysporum*. Maximum siderophore secretion was recorded on second and third day, and thereafter a decline was observed.

Siderophores directly stimulate the synthesis of antimicrobial compounds by increasing the availability of minerals and suppressing the growth of pathogenic organisms (Sobha and Kumudini 2012). Siderophores produced by *Arthrobacter luteolus* isolated from the rare earth environment from Kerala, India reported the accumulation of rare earth metals Samarium and Scandium (Emmanuel et al. 2012). The nodule-forming bacteria isolated from the root nodules of leguminous plants such as *Rhizobium* spp., *Bradyrhizobium* spp. and *Sinorhizobium* spp. also produced siderophores (Deshwal et al. 2013). Verma et al. (2012) reported that *R. meliloti* has the ability to produce siderophores.

2.1.4 Phosphate Solubilization

Phosphorus (P) is the most important element in the nutrition of plants, next to nitrogen (N). It plays a key role in all major metabolic process, including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis, respiration, and nitrogen fixation. Phosphorus is abundant in soils in both inorganic and organic forms. Inorganic phosphorus occurs in soil mostly in insoluble complexes, some of them appearing after frequent application of chemical fertilizers. Organic matter is also an important reservoir of immobilized P in soils. It has been suggested that the accumulated P in agricultural soils would be sufficient to sustain maximum crop yields worldwide for more than 100 years. Several bacterial and fungal species have been described for their phosphate-solubilizing abilities. *Bacillus* and *Pseudomonas* are the predominant bacteria, while *Aspergillus* and *Penicillium* are the predominant fungi. The other important P-solubilizing bacteria include *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Gordonia*, *Phyllobacterium*, *Azotobacter*, *Xanthomonas*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Vibrio*, *and Xanthobacter*. Nodule-forming *Rhizobium* has also shown P-solubilizing activity. Moreover, some species of *Trichoderma* and *Rhizoctonia* also have P-solubilizing potential. Apart from bacteria and fungi, actinomycete genera *Streptomyces* and *Micromonospora*, algae such as cyanobacteria, and arbuscular mycorrhizal fungi and *Entrophospora colombiana* have shown P-solubilizing ability. *Azotobacter* spp. isolated from slightly alkaline soils have shown a good P-solubilizing ability along with nitrogen fixation under in vitro conditions (Nosrati et al. 2014). Garg and Sharma (2013) reported that 16 rhizobial isolates from root nodules *Trigonella* and *Tephrosia* were found positive for P solubilization and 14 isolates were found positive for IAA production. *Klebsiella oxytoca* isolated from heavy metal contaminated soil has shown P solubilizing activity in the presence of various C & N sources. Highest P solubilization was recorded with glucose (460 μg/ml) followed by fructose (444 μg/ml) and galactose (435 μg/ml) in the medium. Very poor P solubilization was recorded when lactose (141 μg/ml) was used as C source. Among different N sources $(NH_4)_2SO_4$ (460 μg/ml)was best for growth and P solubilization of *K. oxytoca*, where as yeast resulted in poor growth and P solubilization (215 μg/ml) (Walpola et al. 2014). Co-inoculation of tomato plants with *P. agglomerans* and *Burkholderia anthina* has shown P solubilization under lab conditions and increase in plant growth parameters under field conditions (Walpola and Yoon 2013).

2.2 Plant Growth-Promoting Fungi (PGPF)

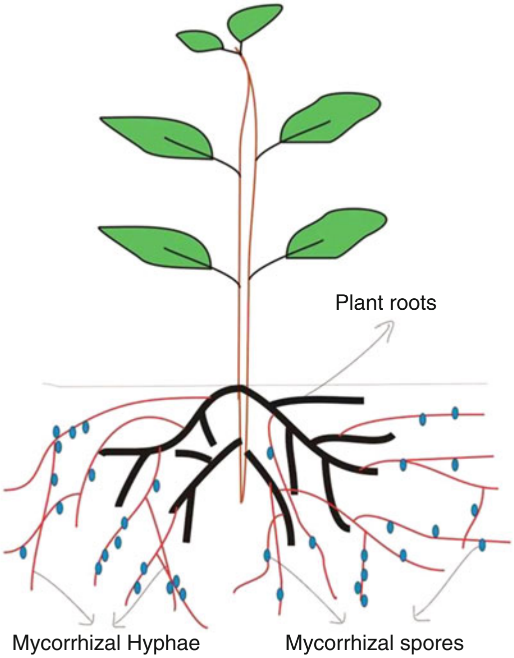
The arbuscular mycorrhizal fungi (AMF) and *Trichoderma* spp. are well recognized as plant growth-promoting fungi. AMF promote growth of crop plants by supplying various nutrients (mainly phosphorus). Through its hyphae nutrients are released into plant roots through arbuscules. The vesicles formed in the cortical region store the excess phosphorus in the form of polyphosphate, and this polyphosphate is again converted into phosphate through enzymatic action and utilized by the plant under phosphate-deficient condition, whereas *Trichoderma* promotes growth of crop plants by controlling the disease-causing fungi and also through biofertilization potential. *Trichoderma* decomposes the organic materials through the secretion of enzymes, cellulases, hemicellulases, etc., thus releasing the nutrients that are readily available to plants.

2.2.1 Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular mycorrhizal fungi have unique characteristic structures known as “arbuscules” formed in the cortical region. The arbuscules are dichotomously branched structures and are the main sites for nutrient exchange between plant roots and the fungus (Akhtar et al. 2011). The host plant absorbs nutrients through the hyphae and in return the fungus obtains sugars and other essential nutrients from the plant (Akhtar and Siddiqui 2008). In the intercellular spaces the hyphae forms oval to globose structures called vesicles. These vesicles store P in the form of polyphosphate granules. Under phosphate-deficient conditions the polyphosphate from the vesicles is released by enzymatic action into the roots. Some species of AM fungi form spores within the roots known as intraradical spores (Fig. 2). The hyphae inside and outside the roots form abundant spores. AM fungi stimulate plant growth by nutrient acquisition and P uptake. AM fungi take up P from the same pool of soluble ions and act as an extension of the root system. There is also evidence that phosphatase activity is higher in mycorrhizal roots compared to non-mycorrhizal roots. AM fungi also improves the uptake of zinc, iron, calcium, copper, magnesium, and manganese and the hyphae travels beyond the nutrient depletion zone and transport nutrients to roots (Fig. 1).

AM colonization on roots increases the root surface area of the host plant. Mycorrhizal plants will withstand biotic and abiotic stresses such as heavy metal toxicity, salinity, alkalinity, pathogens and pests, etc. Mechanisms of heavy metal tolerance in fungi include reduction of metal uptake and/or increased efflux, metal

Fig. 1 Diagrammatic representation of mycorrhizal associations in plants



immobilization (e.g., cell-wall adsorption), extracellular metal sequestration, e.g., exo-polysaccharides or other extracellular metabolites, intracellular chelation, e.g., metallothioneins or phytochelatins, and metal localization/sequestration within vacuoles. Accumulation of metal ions on the cell wall has been shown to be an important mechanism leading to metal immobilization by AM fungi. AM fungi produce glomalin, a strong and irreversible sequester of Cu, Cd, and Zn. When it is applied to the plants growing in nursery in pots and polybags, the seedling survival improved on transplantation. This is due to the faster generation of new roots in nursery plants on transplantation. Mycorrhiza secrete a substance called glomalin, a glycoprotein which binds the soil particles together forming aggregates, giving way for good aeration and water-holding capacity, organic matter accumulation, and root penetration.

Significant growth enhancement was observed in bamboo seedlings inoculated with four AM fungi strains (*G. intraradices*, BEG 193 and 141; *G. mosseae*, BEG 167; and *G. etunicatum*, BEG 168). The results showed that the bamboo seedlings inoculated with all the strains of AM fungi significantly increased shoot number and diameter, leaf number, leaf area in different growth stages compared to control (Jiang et al. 2013). Similarly, Abohatem et al. (2011) reported that application of AM fungal consortium (*Acaulospora* sp., *Glomus* sp., *Sclerocystis* sp., and *Scutellospora* sp.) improved growth of seedlings in date palm and also reduced the incidence of pathogens by the stimulation of the secretion of defense-related enzymes. Najjar et al. (2012) found that use of *Glomus* sp. and *P. fluorescens* along with rock phosphate increased dry matter yield and nutrient uptake (NPK and Mg) in maize and faba bean.

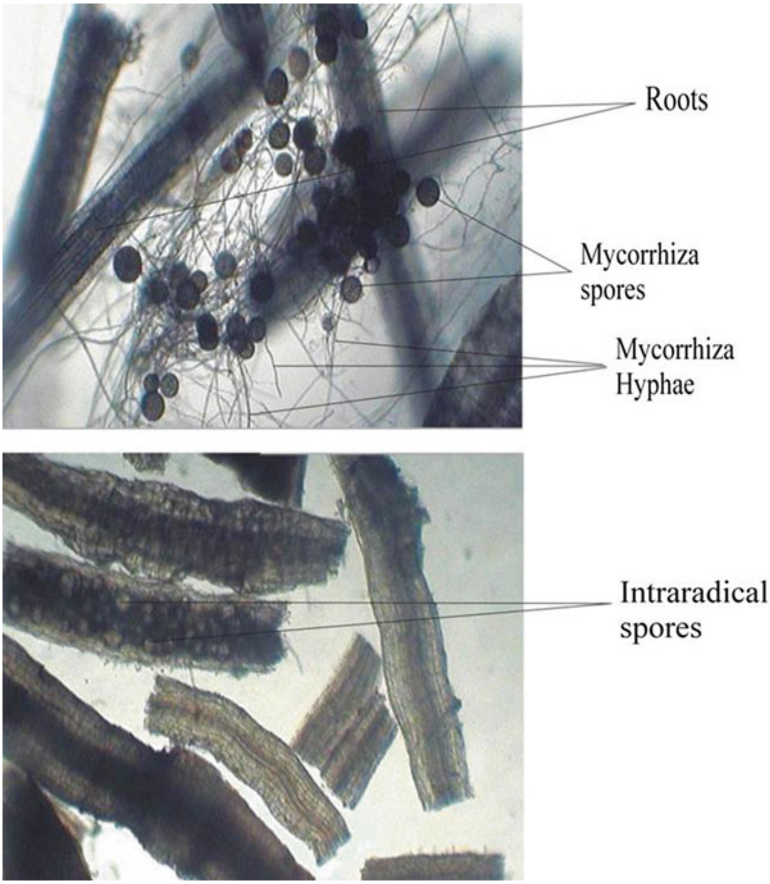


Fig. 2 Microscopic view of root showing intraradical mycorrhizal spores and hyphae

2.2.2 Trichoderma

Trichoderma is a filamentous fungus isolated from soil, dead wood, and organic materials. Currently, different species of *Trichoderma* such as *T. harzianum*, *T. viride*, and *T. virens* have proven their worth to be used as biocontrol agent against a wide range of pathogenic fungi (*Rhizoctonia* spp., *Pythium* spp., *Botrytis cinerea*, and *Fusarium* spp. *Phytophthora palmivora*). *Trichoderma* also has a unique ability to produce siderophores. The production of siderophores chelate the available iron from the environment and this iron starvation causes the death of phytopathogens. Srivastava et al. (2013) reported that all the three tested *Trichoderma* strains (MPPLUNS1, MPPLUNS2, and MPPLUNS3) had the ability to produce siderophores, but MPPLUNS1 was found best among all the tested strains. Likewise, antibiosis is another mechanism of disease protection, where the metabolites are secreted by underground parts of plants, soil microorganisms, plant residues, etc.

and resulted in the production of lytic enzymes, volatile and toxic compounds. A wide range of antibiotics such as tropolone, gliotoxin, gliovirin, viridin, viridol, trichodermin, trichozianin, pyrones, and terpenes is produced by *Trichoderma* sp. These antibiotics may play a significant role in the biocontrol of plant pathogens. Qualhato et al. (2013) concluded that the *Trichoderma* species grown in liquid cultures challenged with fungal pathogens secreted various cell wall-degrading enzymes, viz., β-1,3-glucanase, *N*-acetyl-b-D-glucosaminidase, chitinase, acid phosphatase, acid proteases, and alginate lyase.

Mycoparasitism involves sequential events such as recognition of host, attack and subsequent penetration, and subsequent killing of pathogens. The cell wall surface of the host and non-hosts contains D-galactose and *N*-acetyl D-glucosamine residues as lectin binding sites. With the help of lectins present on cell wall, *Trichoderma* recognizes the suitable sites (residues of lectins) and binds the hyphae. After attachment with pathogens, it makes a coil around the pathogens and secretes the cell wall-degrading enzymes to digest the cell wall and enters into the lumen of the host. Akrami et al. (2012) reported that use of *T. harzianum* and *T. asperellum* alone or in combination reduced Fusarium rot on *Phaseolus vulgaris* by the action of mycoparasitism. Similarly, Gajera et al. (2012) found that different species of *Trichoderma* controlled the growth of *Macrophomina phaseolina* by the action of cell wall-degrading enzymes (chitinase, β-1,3-glucanase, protease, and cellulase) under in vitro conditions. Muriungi et al. (2013) reported that the inoculation of *T. viride* and *T. koningii* effectively controlled the growth of *F. oxysporum* under in vitro conditions. Leelavathi et al. (2014) concluded that the extract of *T. harzianum* at a concentration of 100–150 μl/ml controlled growth of *Aspergillus*, *Cladosporium*, *Rhizopus*, and *Fusarium*. Moreover, root colonization of *Trichoderma* sp. directly influenced plant growth and productivity, production of growth regulators like zeatin and gibberellin. It may also increase the uptake of nutrients and resistance against abiotic stresses.

3 Bioremediation

Bioremediation is a waste management technique that uses the organisms to remove or neutralize pollutants from contaminated sites. Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or the levels below concentration limits established by regulatory authorities. By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. Bioremediation may occur on its own (natural attenuation or intrinsic bioremediation) or may only effectively occur through the addition of fertilizers, oxygen, etc., that help encourage the growth of the pollution-eating microbes within the medium (biostimulation). Bioremediation is classified as in situ when the pollutant is treated at the site or ex situ when the pollutant is taken elsewhere and treated. Some examples of bioremediation are bioventing, bioleaching, landfarming, bioreactor, composting, bioaugmentation, and rhizofiltration. Bioremediation is becoming a very useful tool in many industries to prevent environmental pollution. The microorganisms applied to the contaminated sites secrete enzymes that degrade various chemical pollutants. Many microorganisms have been isolated from the polluted site, indicating that they have the ability to tolerate pollutants (Akhtar et al. 2013).

Bioremediation has been demonstrated and is being used as an effective means of mitigating hydrocarbons, halogenated organic solvents and compounds, nonchlorinated pesticides and herbicides, nitrogen compounds, metals, and radionuclides. *P. aeruginosa*, isolated from the crude oil contaminated sites in the Mangala oil field, Barmer district, Rajasthan, could be used for the bioremediation of oil spills because it has the potential to utilize crude oil as a sole carbon source (Prakash and Irfan 2011).

In an interesting experiment Ajao et al. (2011) found that the inoculation of immobilized culture of *P. aeruginosa* and *B. subtilis* into a bioreactor fitted with air sparger containing textile effluent reduced COD from 1200 to 200 mg/l, BOD from 750 to 23 mg/l in 15 days. The other parameters in textile effluent such as total solids, suspended solids, dissolved solids, heavy metals, nitrate, sulfate, phosphate also have been reduced significantly and copper disappeared within this period from the textile effluent. Similarly, use of *Trichoderma* species culture based on diffusible and volatile metabolites under in vitro conditions reduced the growth of *Pythium* in tomato Patil et al. 2012). The volatile metabolites exhibited broad-spectrum inhibition of *Pythium* compared to diffusible metabolites.

4 Interaction of PGPM with Plants and Soil

Plant roots under nutrient-deficient condition releases certain nutrients like carbohydrates, amino acids, and vitamins into the soil to attract microorganisms. The microorganisms utilize the root exudates for their growth and multiplication. The microbes produce hormones like auxins (IAA, IBA), cytokinins, GA3, abscisic acid (ABA). The plant roots sense these auxins and start emitting lateral roots and root hairs which absorb water, minerals from the rhizosphere. The uptake of minerals is increased due to the increased surface area of the roots. Some microorganisms may colonize in the rhizosphere soil (e.g., *Azotobacter*, and phosphate-solubilizing bacteria (PSB), some (e.g., *Rhizobium*, *Azospirillum*, and AM fungi) colonize either on the surface or inside the roots.

The interaction of microorganisms with plants and soil helps in the improvement of nutrient (NPK) uptake by various mechanisms. The combined application of *Azospirillum* and *Azotobacter* in maize has resulted in the increase in plant growth, yield, and hormone (IAA, GA3, cytokinin) production (Naseri et al. 2013). Mehran et al. (2011) reported that increase in yield was not significant between treated (bacteria inoculated) and control plots, whereas a significant increase in yield was observed in manure applied treatment. Rafi et al. (2012) found that the co-inoculation of *Azospirillum* and PSB together resulted in increased shoot and root dry weight, panicle weight, and 1000 seed weight compared to control or individual inoculation in foxtail millet. It has been also reported that inoculation of *Azotobacter* and *Rhizobium* together resulted in increase water and nutrient uptake in faba bean (Dashadi et al. 2011). However, inoculation of *Rhizobium* with PGPR alone or in combination significantly increased the nodule number, nodule fresh and dry weights, grain yield compared to control under salt stress conditions in mung bean and also improved K/Na ratio in grains by decreasing the Na content compared to control (Aamir et al. 2013). Similarly, inoculation of *Azotobacter* and *Azospirillum* in maize significantly increased grains weight and yield when bacteria was rapped with seeds before sowing compared to control (Amiri and Rafiee 2013).

5 Conclusions and Future Prospects

The interaction of PGPM with plants and soil is very important in improving crop productivity. Soil is fortified with various root exudates of plants attracting microorganisms, which in turn help plants through nitrogen fixation, phosphate solubilization, and nutrient mobilization. Different bacteria and fungi are used as biofertilizers due to their ability in producing phytohormones, siderophores, antimicrobial compounds, developing induced systemic resistance and bioremediation. Microorganisms could be applied alone or in combination in various crops, but it is advisable to apply nitrogen-fixing bacteria along with phosphate-solubilizing bacteria because P requirement during nitrogen fixation is met by phosphate-solubilizing bacteria. The selection of microorganisms is crucial for a field application for maintaining the quality of crops. By adopting the biofertilizer strategies in sustainable agriculture practices the adverse effects of chemical fertilizers and pesticides could be easily nullified. Moreover, the isolation of microorganisms from the contaminated sites and their potential application in bioremediation is quite a permissible approach. The future research will be more focussed on the revolutionization of a consortium of microorganisms for agricultural inputs and bioremediation of contaminated sites by the efficient application of microorganisms and their interaction with the plant and soil in various ecological niches.

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Managing the Soil Mycorrhizal Infectivity to Improve the Agronomic Efficiency of Key Processes from Natural Ecosystems Integrated in Agricultural Management Systems

S. Wahbi, H. Sanguin, E. Baudoin, E. Tournier, T. Maghraoui, Y. Prin, M. Hafidi, and R. Duponnois

Contents

Abstract Phosphorus (P) is a major nutrient limiting plant growth in many soils. To reduce P deficiencies and ensure plant productivity, large quantities of soluble forms of P fertilizers are applied worldwide every year. However up to 80 % of P chemical fertilizer amendments are lost as it is easily precipitated into insoluble

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forms (CaHPO4, $Ca_3(PO_4)_2$, $FePO_4$, and $AlPO_4$) and becomes unavailable for plant uptake. Some soil microorganisms are known to be involved in the solubilization of insoluble phosphate by excreting organic acids, phenolic compounds, protons, and siderophores. Among phosphate-solubilizing microorganisms, it has been reported that mycorrhizal fungi have the ability to actively mobilize and translocate nutrients from minerals and soil organic matter, directly to their host plant. Mycorrhizal fungi constitute a key functional group of soil biota that greatly contribute to productivity and sustainability of terrestrial ecosystems. These are ubiquitous components of most of the ecosystems throughout the world and considered key ecological factors in governing the cycles of major plant nutrients and in sustaining the vegetation cover. It has been suggested that the integration of key processes from natural ecosystems (plant-plant facilitation, positive plant soil feedback) in agricultural management systems could resolve increasing agricultural problems. Since these natural processes are frequently connected with arbuscular mycorrhizas, it is necessary to apply mycorrhizal inoculation technologies or to manage native arbuscular mycorrhizal fungus communities to replace or reinforce the mycorrhizal potential in these degraded areas. This chapter aims to describe the influence of some cultural practices (rotation, intercropping, mycorrhizal inoculation) that mimic these natural processes in agrosystems, on soil microbiota (i.e.: soil mycorrhizal infectivity) leading to a sustainable microbial complex with high efficiency against phosphorus mobilization and transferring phosphorus from the soil organic matter or from soil minerals to the host plant.

Keywords Facilitation • Plant soil feedback • Intercropping • Rotation • Mycorrhizal symbiosis • Mycorrhizosphere • P availability

1 Introduction

The Green Revolution has been designed from a series of researches, development, and technology transfer programs, performed between the 1940s and the late 1960s, in order to enhance agricultural production worldwide with a focus on the developing countries. These initiatives have begun more markedly in the late 1960s. This technological revolution was based on a package of modern inputs based on three main cultural practices such as irrigation, improved seeds, and use of fertilizers and pesticides. The Green Revolution had primary ambition to transform agricultural systems in developing countries to significantly enhance the productivity of cultivated areas and thus to ensure sustainable food security for the populations of these regions (Freebairn 1995).

Despite significant positive results in the fight against food insecurity, the intensive farming practices, ignoring the ecological characteristics of the environment, have led to a dramatic impact on the environment. Indeed, the recommended cultural practices have generated widespread soil pollution resulting from the intensive use of pesticides and chemical fertilizers, and an overall loss of biodiversity and agro-biodiversity. They also altered soil quality due to a worsening of water and wind erosion. The social consequences were also recorded through an increased rural–urban migration and the emergence of slums on the outskirts of megacities.

In order to minimize the negative impacts of the expansion and intensification of modern agriculture, the development of sustainable farming practices that sustain yields and optimize the use of localized resources has been proposed. It has led to the emergence of new concepts such as sustainable agriculture in France, Ecoagriculture of IUCN (International Union for Conservation of Nature), and Agroecology. Hence the new concept of "doubly green revolution" has been suggested that combines the objectives of the Green Revolution and the maintenance of biological diversity and ecosystem resilience.

Agroecology is usually defined as the science that uses ecological theory to study, edify, manage, and evaluate agricultural systems in a context of a sustainable agricultural production (Wezel and Soldat 2009). This innovative approach considers all the interactions between the main components of farming systems (biophysical, technical, and socioeconomic components). In particular, it requires agricultural practice innovations to put agroecological technologies into practice.

The integration in agricultural cropping systems of some biological mechanisms governing the spatial and temporal evolution, productivity, and resilience of natural ecosystems has been suggested as models for the design of sustainable systems of land use. These long-term evolving natural processes result in ecosystems that are highly productive, resilient (rate of recovery after disturbance), and stable (the ability of an ecosystem to maintain a steady state) (Webster et al. 1975; Leps et al. 1982; Ewel 1999). These natural processes (i.e.: plant-plant facilitation, positive plant soil feedback) that improve plant stress resistance and plant mineral nutrition are usually connected to arbuscular mycorrhiza (AM) associations between plant roots and fungi belonging to the small fungal phylum Glomeromycota (Schüßler et al. 2001). AM symbiosis is an ubiquitous symbiotic process in all the terrestrial systems and on most plant species (80 % of plant families). Although enhanced plant phosphorus uptake is considered as the main benefit of AM fungi (AMF) to plants, this fungal symbiosis may also provide "non-nutritional" effects with improvements in soil structure and soil microbiology and in plant stress resistance. The potential of AMF to improve food security results from the ability of all globally important food crops to form mycorrhizal symbiosis in natural conditions. It is now well known that the magnitude of the benefits expected to the plant growth by appropriate application of AMF is mainly dependent to the AM propagule abundance, the AMF community structure, the composition of the cropping systems, the soil, and climate context (Burrows and Pfleger 2002). Hence managing AM soil infectivity in agrosystems could follow two different schemes that could be combined in practice: (1) the "reductionist" scheme and (2) the "holistic" scheme (Fig. 1). The reductionist pattern aims to improve plant performance in disturbed soils by adding specialized AMF inocula adapted to the environmental conditions and to the target crop. The objectives of the holistic pattern are rather at preserving and restoring the composition

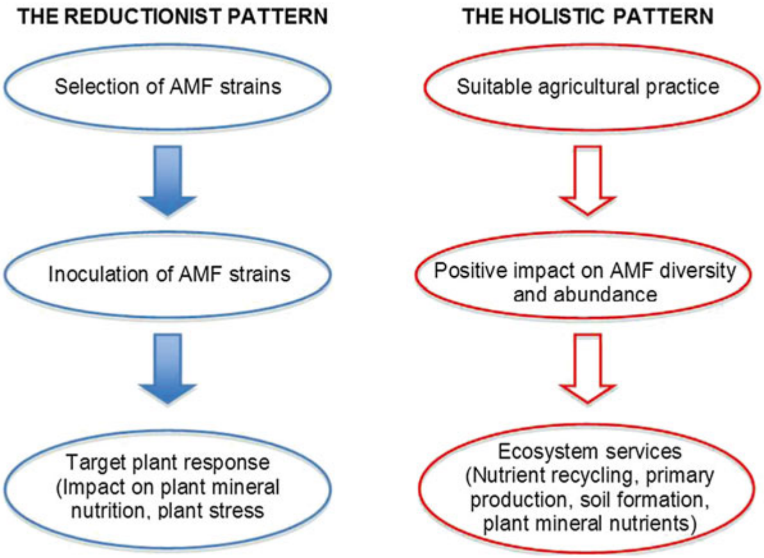

Fig. 1 Different patterns to manage the AM soil infectivity in agrosystems. The reductionist pattern aims to improve crop productivity by inoculating selected AM fungal strains whereas the holistic pattern allows to improve AM fungal diversity and abundance for ensuring AMF-dependent ecosystem services (adapted from Fester and Sawers 2011)

of native AMF communities. To present the benefits expected from the integration of AMF in agricultural practices through the combination of the "reductionist" and "holistic" approaches, we review (1) the importance of AMF in plant mineral nutrition, especially for the plant phosphorus nutrition, (2) the benefits to important staple crops resulting from the AMF inoculation (wheat, maize, vegetables, etc.), and (3) the potentialities of multispecies plant-cropping systems to sustainably manage the composition of AMF communities and their expected impact on plant growth.

2 Ecological Importance of the Arbuscular Mycorrhizal Symbiosis in the Plant Phosphorus Acquisition

Phosphorus (P) is an essential macronutrient for plant growth and its uptake from soil is effective almost exclusively in the form of soluble phosphate anions (Schachtman et al. 1998). Many studies have reported the significance of AMF for growth of crop species through different impacts on the nutrient supply and changes in plant physiology and morphology (Pellegrino and Bedini 2014). In general, the reported effect of mycorrhiza on plant P uptake is not always consistent because of the complexity and interactions between the involved components of the system (host plant genotype, AMF, environment conditions) (Baum et al. 2015). In particular, it has been shown that the AM symbiosis promotes the inflow of slowly mobile nutrients to plant roots, predominantly P (Antunes et al. 2007). Koide and Kabir (2000) showed that extraradical hyphae of the AM fungus, *Glomus intraradices*, can hydrolize organic P (i.e., phytate) and that the resultant inorganic P can be taken up and transferred to host root. It has also been reported that mycorrhized plants respond positively to the soil amendment with insoluble forms of inorganic phosphorus such as rock phosphates (RPs) (Cabello et al. 2005; Duponnois et al. 2005). However the mechanisms involved in this plant response remain unclear although it has been hypothesized that AMF hyphae could excrete some chelating agents that could actively mobilize soluble P from the phosphate inorganic forms. Antunes et al. (2007) showed that the mechanisms underlying increased P uptake by the AM symbiosis establishment did not result from the fungal release of H+ ions alone or in combination with organic acid anions. It suggested that the positive AM effect on P uptake from RP was the result of interactions between AM symbionts and the soil microflora (Jayachandran et al. 1989). It is well known that extraradical hyphae of AMF provide an important area for interactions with soil microbes and a large pathway for the translocation of energy-rich plant assimilates to the soil (Johansson et al. 2004). The AM associations impact the composition of soil microflora leading to a zone influenced by both the mycorrhizal fungus and the host plant, commonly named the *mycorrhizosphere*, and a more specific zone resulting from the impacts of individual fungal hyphae, the *hyphosphere* (Linderman 1988; Johansson et al. 2004). Inside these two compartments occurred diverse multitrophic interactions that influence the effects of the mycorrhizal symbiosis on the plant growth resulting from a direct positive effect (nutritional mechanisms) and an indirect positive effect via a selective pressure on microbial communities (Fig. 2) (Frey-Klett et al. 2005). Numerous studies have shown that some phosphate-solubilizing bacteria can interact synergistically with mycorrhizal fungi and facilitate phosphorus uptake by the plants (Muthukumar et al. 2001; Caravaca et al. 2004; Cabello et al. 2005). In field conditions, it has been reported that the abundance of phosphate-solubilizing bacteria belonging to the fluorescent pseudomonad group was correlated to the level of plant mycorrhizal colonization (Duponnois et al. 2011). The roles of the mycorrhizosphere microorganisms have to be considered in order to ensure the productivity and stability of agrosystems in the context of a sustainable agriculture.

3 Application of AMF to Increase the Overall Yield of Important Staple Crops

It is well known that mycorrhizal fungi are already present in all agricultural soils but AMF community structure is highly dependant to the environmental conditions (i.e., soil characteristics) and the cultural practices (Smith and Read 2008). Hence the implementation of AMF inoculation is particularly important when mycorrhizal

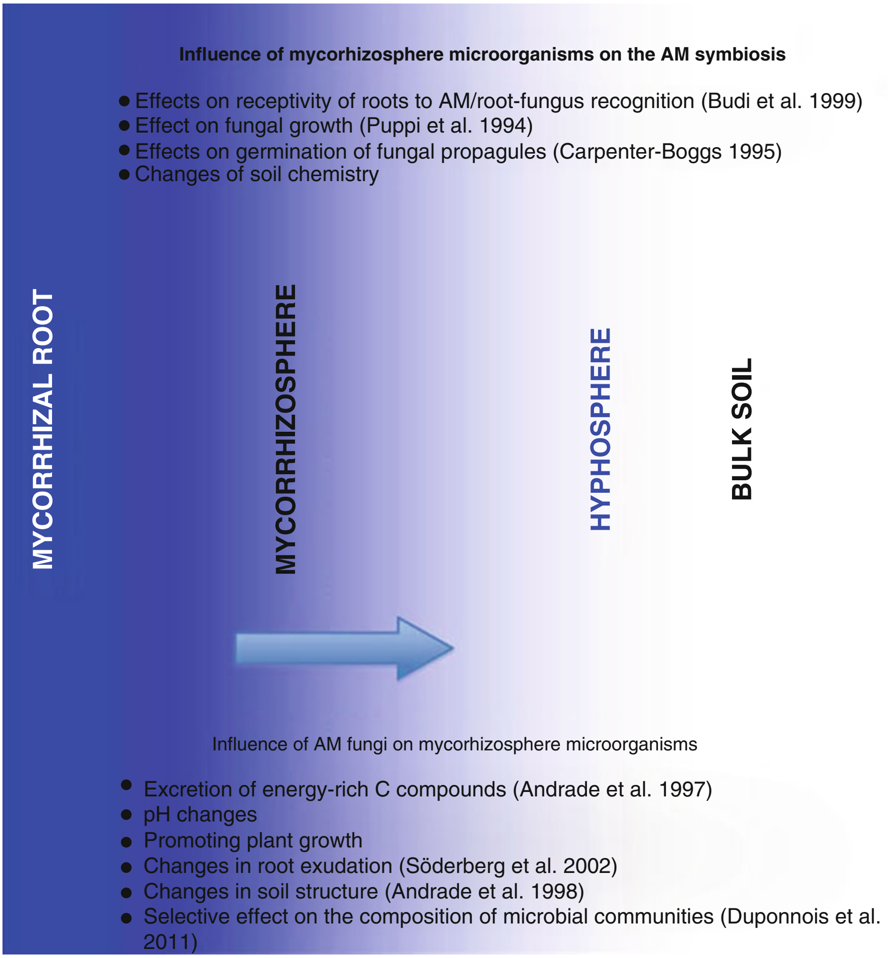

Fig. 2 Interactions between the AM symbiosis and the mycorrhizosphere/hyphosphere microbial communities (modified from Johansson et al. 2004)

potential of native soil is quantitatively and qualitatively inadequate (Requena et al. 1996; Koide and Mosse 2004). A meta-analysis of 38 published field trials has been conducted to determine the impacts of AMF inoculation on P, N, and Zn uptake, growth, and grain yield of wheat (Pellegrino et al. 2015). The results showed that AMF inoculation promoted aboveground biomass, grain yield, aboveground biomass, P content and concentration of aerial parts, straw P content, N content and concentration of aerial parts, grain N content, and grain Zn concentration. The positive impact of AMF inoculation on wheat growth was dependent on organic matter concentration, pH, total N and available P concentration, texture of soil, climate, and the AMF species inoculated. Lekberg and Koide (2005) reported the benefits on yield, biomass, and phosphorus concentrations expected from AMF inoculation by conducting a meta-analyses of 290 published field and glasshouse trials. They concluded that the impacts of AMF inoculation were highly dependent on the levels of soil P and indigenous mycorrhizal soil infectivity. Most AMF inoculation field trials have been conducted in temperate agroecosystems where soil fertility (especially bioavailable phosphorus content) is higher that that frequently recorded in tropical and Mediterranean soils (Friesen et al. 1997). This lack of knowledge on the AMF potentialities to increase crop production is surprising since it is in these deficient soils that AMF application provides the strongest effect on the plant growth (Rodriguez and Sanders 2015). Some controlled mycorrhization field experiments performed in arid and/or tropical areas on field-grown wheat are listed in Table 1. However one important food plant in the tropics, Cassava (*Manihot esculenta* Crantz), has been widely studied for its response to AMF application. It has been demonstrated that Cassava was highly mycorrhizal dependent for its growth and mineral nutrition with a mycorrhizal dependency of 95 % (Sieverding and Howeler 1985; Howeler et al. 1987). In another experiment, the effect of inoculation with Glomus clarum on the growth of the cassava cultivar TMS 30572 has been investigated in field conditions in a low-nutrient tropical soil (Fagbola et al. 1998). The fungal inoculation provided an increase of the fresh tuber yield.

Table 1 Effect of AMF inoculation with arbuscular fungi on the growth and nutrient uptake of the productivity of field-grown wheat

Wheat cultivars	Country	AMF species	Biomass yield (%)	Grain yield (%)	References
Wheat cv. TAM-105	Iran	*Glomus etunicatum* *Glomus mosseae*	+21.6 +6.2	+41.4 0	Al-Karaki et al. (2004)
Wheat cv. Steardy	Iran	*Glomus etunicatum* *Glomus mosseae*	+31.6 0	+41.8 0	Al-Karaki et al. (2004)
Wheat cv. Tetra	Mali	*Glomus intraradices*	+22.1	+22.4	Babana and Antoun (2006)
Unknown	India	*Glomus fasciculatum*	+77.9	+55.5	Khan and Zaidi (2007)
Wheat cv. UP-2338	India	Natural mycorrhiza consortium	nd	+81.0	Mader et al. (2011)
Wheat cv. HD 2643	India	*Glomus fasciculatum*	nd	+21.2	Mahanta et al. (2014)
Wheat cv. WH 147 × WH 533	India	*Glomus fasciculatum*	+8.7	+18.1	Singh et al. (2004)
Wheat cv. WH 533 × Raj 3077	India	*Glomus fasciculatum*	+5.5	+12.3	Singh et al. (2004)
Unknown	India	*Glomus mosseae*	+15.7	+12.6	Suri et al. (2011)
Unknown	India	*Glomus intraradices*	+15.4	+13.4	Suri et al. (2011)

nd not determined

4 Potentialities of Multispecies Plant-Cropping Systems to Sustainably Manage the Composition of AMF Communities

Alterations in plant diversity are known to impact aboveground ecosystem functioning (Cardinale et al. 2007; Hector and Bagchi 2007). Numerous studies studying the impact of plant diversity on the composition of soil microflora reported either positive (Milcu et al. 2008) or no influences (Habekost et al. 2008). Hence multispecies cropping systems may often be considered as a practical application of ecological principles based on biodiversity, plant interactions, and other natural regulation mechanisms (plant facilitation, positive soil feedback). Some studies have reported positive links between the composition of the cover plants and different ecological processes (i.e., primary productivity, soil nutrient content, and resilience capacity) (Erskine et al. 2006). In agroecosystems, multispecies plant-cropping systems can reduce pests and diseases resulting from an improvement of biological control or direct control of pests (Gurr et al. 2003). Different cropping systems can be designed according to their composition and their management (Table 2). In multispecies plant-cropping systems, the use of legume plants is fundamental to maintain soil fertility, mainly resulting from the tripartite symbiotic interaction between legumes, rhizobia, and AMF that positively influences P and N crop mineral nutrition (Scheublin et al. 2007) but also to maintain the mycorrhizal soil infectivity (Azcon-Aguilar et al. 1986).

Table 2 Forms of species mixture in annual crop agricultural systems (from Malézieux et al. 2009)

Type of system	Species number	Number of strata	Example/location
Combination (intraspecific mixture)	1	1	Cereals
Relay cropping (time overlap only during one part of the life cycle of each species)—crops or crop and service plant	2	1 or 2	Maize/beans, groundnut/cotton (Africa)
Row intercropping (growing two or more species in rows)—crops with crops or crops with service plant	2	1 or 2	Cereals/herbaceous legumes and grasses, e.g., rice/arachis pintoi (Europe, South America)
Mixed intercropping (no distinct row management)	$2-n$	1	Two species (maize-sorghum, maize cassava, etc.) to n species (tropical garden, e.g., rice, maize, tomato, cassava) (humid tropics), annual grassland (Europe)

5 Conclusion and Future Prospects

Although numerous studies focussed on the potentialities of AMF application to sustainably improve crop productivity, this technical approach has not been widely integrated into modern agriculture practices. It has been frequently argued that the lack of high-quality mass produced AMF inoculum. However, it could also be noted that the AMF application remains too simplistic without taking into account the basic ecological concepts. Hence researches have to perform in order to explain (1) the biological determinants of the introduced AMF inoculant (soil receptivity to the AMF inoculation), and (2) the impacts of the AMF inoculation to the soil functioning and the composition of the soil microflora. Finally, the AMF potentialities have to integrate the design of multispecies agricultural systems in order to optimize the productivity of these innovative cultural practices.

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Plant and Necrotrophic Fungal Pathogen Interaction: Mechanism and Mode of Action

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Abstract Necrotrophic fungal pathogens cause major losses to fruit, vegetable, and cereal crops annually and the economic impact is more than that of diseases caused by biotrophic pathogens. These pathogens are devastating because they kill as they colonize through production of cell wall-degrading enzymes and phytotoxins, obtaining nutrients for growth and reproduction from the dead plant cells. They explore a wide variety of virulence strategies and based on these the pathogens are classified into host-specific and broad host-range necrotrophs. Plants are equipped with an immune system as a defense mechanism while the necrotrophic fungal pathogenic arsenal suppresses the immune responses for disease manifestation. Plant defense response involves the interplay of signaling molecules which include various phytohormones like jasmonic acid, ethylene, salicylic and abscisic acid which also serve as regulators of the immune response. Coordination at the transcriptional level of genes for the production of defense molecules including antimicrobial phytoalexins and pathogenesis-related proteins by transcription factors such as WRKY33 and ERF which are responsive to the signaling molecules has been observed. The roles for several important transcription factors already unveiled through studies of mutants in the model plant, *Arabidopsis thaliana* and some of the information translatable to crop plants. The present chapter shows the interconnection between cell wall integrity and the action of signaling molecules in the expression of defense-related genes. Moreover, the epigenetic mechanism through DNA and histone modification is also discussed.

Keywords Necrotrophic fungi • Phytohormones • Pathogenesis-related proteins • Signaling molecules • Transcription factors • Epigenetic

1 Introduction

Infectious microbes found in the environment pose continuous threats to plant survival and productivity, leading to significant economic losses to major crops (Wilkinson et al. 2011). Plant microbial pathogens obtain nutrients from their host cells for growth and reproduction and the devastation caused by them relates to their feeding modes. They are divided into three classes: necrotrophs, biotrophs, and hemibiotrophs based on their methods of nutrient acquisition. The necrotrophs are the most damaging as they kill the host while colonizing and live on the contents of the dead cells. Biotrophs, on the other hand, maintain the viability of their host as they thrive on the nutrients from living cells. Hemibiotrophs display both modes starting at the early phase as biotrophs then switching to the necrotroph mode of nutrition. There is great variability in the duration of the biotrophic and necrotrophic phases among hemibiotrophs (Meinhardt et al. 2014).

The lifestyle and infection strategy differ between necrotrophs and biotrophs. The necrotrophic microbial pathogens which include bacterial, fungal, and oomycete species have notorious and aggressive pathogenesis strategies leading to extensive necrosis, tissue maceration, and rotting symptoms on the plant. Prior to or during colonization, the pathogens secrete disease causing agents which include phytotoxins and cell wall degrading enzymes (CWDEs) into host tissues. These toxic molecules and lytic enzymes kill the host cells and the decomposed plant tissues are consumed by the pathogens. In contrast, biotrophic pathogens, in general, do not produce toxins and secrete very limited amount of CWDEs (Oliver and Ipcho 2004). The present chapter shows the interconnection between cell wall integrity and the action of signaling molecules in the expression of defense-related genes. Moreover, the epigenetic mechanism through DNA and histone modification is also discussed.

2 Classification of Necrotrophs

2.1 Host-Specific Necrotrophs (HSNs)

Necrotrophs are classified into host-specific necrotrophs (HSNs) and broad host-range necrotrophs (BHNs). The HSN infects a single or few related plant species by producing a host-selective toxin (HST). The HST is a strain-specific effector required for pathogenicity and virulence on the natural hosts (Friesen et al. 2009). *Cochliobolus* and *Alternaria* spp. are examples of HSN that produce HSTs. *C. victoriae* produces victorin, which is responsible for the Victoria blight disease in oats (Wolpert et al. 2002). Similarly, *A. alternata* and *A. brassicae* also produce HSTs that define their host range (Thomma 2003; Mamgain et al. 2013).

2.2 Broad Host-Range Necrotrophs (BHNs)

The broad host range necrotrophs (BHNs) include fungal species belonging to the genera *Monilia*, *Sclerotinia*, and *Botrytis*. For instance, *Botrytis cinerea* can infect more than 200 hosts of dicotyledonous and monocotyledonous plant species widely distributed in diverse geographical locations. These include vegetables, ornamental plants, and important fruit species like kiwi-fruit and strawberry (Elad et al. 2004). Most BHNs infect aerial tissues, while some species from the genera *Rhizoctonia*, *Fusarium*, and *Colletotrichum* are soil borne necrotrophs that gain entry through roots. Their success is attributed to the extensive array of phytotoxic metabolites, CWDEs, and cell death elicitors which alone or together can interfere with the structural and biological functions found in common in the different plant families (Okubara and Paulitz 2005).

3 Fungal Necrotrophs Infection of Host Plant

Infection of host plant by fungal necrotrophs in general follows a sequence of events as follows: conidial attachment, germination, penetration into the host, formation of primary lesion, lesion enlargement, and tissue maceration followed by sporulation (Fig. 1). Penetration may be achieved through either active or

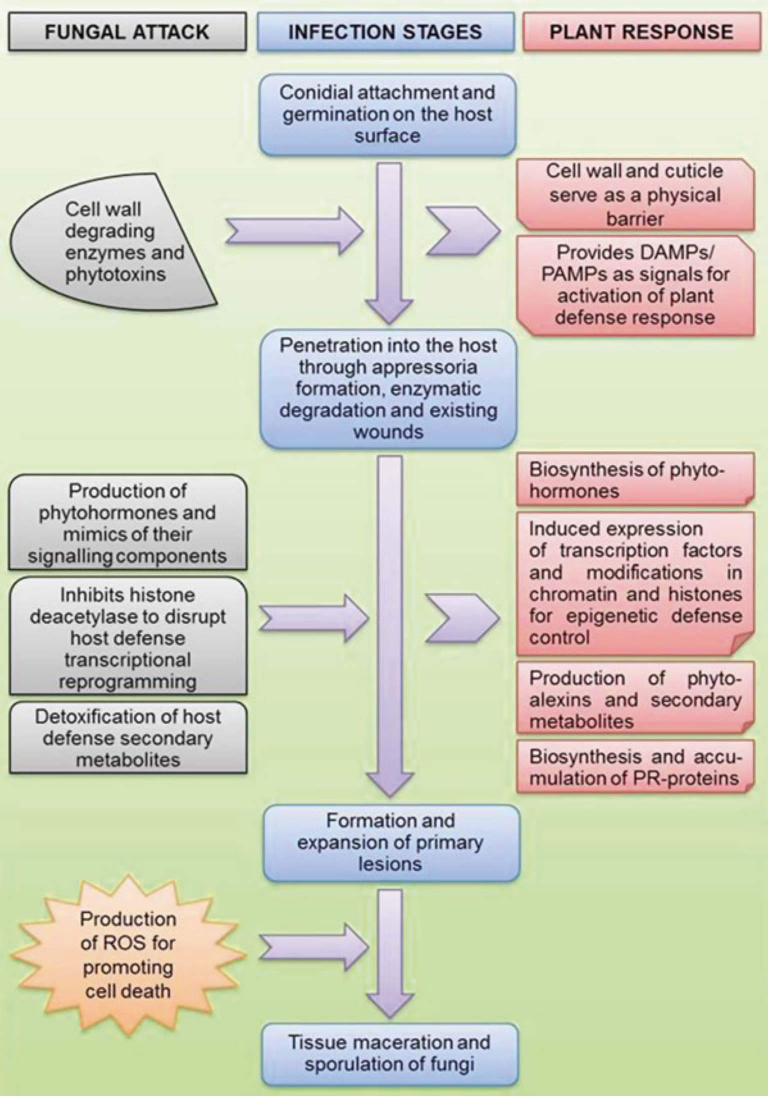

Fig. 1 Schematic diagram of plant response to pathogenic necrotrophic fungal infection

passive mechanisms. The active mechanisms include appressoria formation and enzymatic degradation. Passive mechanisms occur through an existing wound or infection sites as well as stomata. Lytic enzymes employed for initial penetration as well as toxic levels of reactive oxygen species (ROS) are involved in cellular dismantling to decompose the tissues. To ensure the progression of the disease, these fungi actively manipulate to suppress the host cell defense machinery (Prins et al. 2000). Among the necrotrophic fungal plant pathogens, the gray mold *B. cinerea* has been studied in great detail. Targeted mutagenesis of the genome sequence revealed the roles played by a variety of *B. cinerea* genes in the infection process. These include the genes required for signaling, penetration, killing the host cells, and decomposition of the plant tissues (van Kan 2006). The information generated is essential for developing knowledge-based strategies for disease control.

4 Molecular Mechanisms in Necrotrophic Fungi and Plant Interaction

The attack by the different types of microbial pathogens will trigger the plant immune response. This is a complex and multifaceted cellular reaction involving histological, physiological, biochemical, and molecular events, which work in concert to limit the pathogen spread and disease manifestation. Plant response in the early stages of necrotrophic fungal infection involves cell death, production of antimicrobial secondary metabolites, signaling molecules, including phytohormones and reactive oxygen species (ROS), callose deposition and the various cell wall modifications. Plants employ the same set of response to the different pathogens; however, the magnitude and rate of production of the different compounds vary in specific host–pathogen interactions and some of the responses may contribute to resistance. The necrotrophs employ various strategies for virulence and pathogenicity by counteracting the different processes and steps in the plant immune response pathways (Mengiste 2012). They may manipulate host phytohormone levels or synthesize their own hormone to disrupt defense signaling in the infected plants (Kazan and Lyons 2014). Some necrotrophs have mechanisms to detoxify host metabolites that interfere with virulence (Morrissey and Osbourn 1999). An effective plant immune system neutralizes the pathogenic arsenal of the infectious necrotroph or weakens its effect, curbing spread and development of disease symptom. Resistance traits against host-specific necrotrophs (HSNs) may be inherited; however, resistance to BHNs involves many contributing genes and pathways. Host genetic components and the processes at cellular and molecular levels that mediate and contribute to plant immune responses to necrotrophs are an important area of study for developing strategies for early diagnosis and disease management.

4.1 Pathogen-Triggered Immunity (PTI)

Pathogen associated molecular patterns (PAMPs) are signature-pattern molecules released from pathogens such as fragments of chitins from fungal cell wall when they infect plants. The microbial activity releases degradation products from constituents of plant cells such as fragments of plant cell wall release from the fungal CWDEs activities referred to as damage associated molecular patterns (DAMPs). Pathogen-triggered immunity (PTI) is a form of quantitative resistance that is activated upon recognition of PAMPs and DAMPs by pattern recognition receptors (PRRs). Membrane localized receptor-like kinases (RLKs), peptide receptors (Yamaguchi et al. 2010), and wall-associated kinase 1 (*WAK1*) as receptor of oligogalacturonides (OG) are examples of PRRs. Basal resistance and PTI responses to the corresponding pathogens diminished in the absence of PRRs (Mengiste 2012).

4.2 Roles of Reactive Oxygen Species and Nitric Oxide in Plant–Fungal Interaction

Attacks by biotrophs and necrotrophs trigger the production of ROS through the plant immune response pathways. The generation of ROS for regulating cell death is essential for resistance to biotrophs and hemibiotrophs through the hypersensitive response mechanism. It appears that the continuous generation of ROS facilitates cell death and promotes susceptibility to necrotrophs. However, early stimulation of ROS production could induce resistance mechanisms. Involvement of ROS in the activation of various immune responses plays a role in the plant resistance mechanism to both necrotrophs and biotrophs. In this context, the kinetics of the oxidative burst are more important than the absolute levels of ROS being produced as an indicator of resistance in plant microbial pathogen interaction (Mengiste 2012).

The ROS serve as the virulence factor for some necrotrophs. The pathogen induces generation of ROS once infection is established, resulting in plant cell death, which offers significant advantages to the pathogen as this increases susceptibility of the plant to the fungal attack (Govrin and Levine 2000). The fungal secreted enzyme superoxide dismutase may play a role in stimulating oxidative burst by *B. cinerae* from the time of cuticle penetration. Deletion of the gene encoding the enzyme reduced the virulence of the fungal pathogen on its different host plants (van Kan 2006). Over-accumulation of ROS resulting from interference with the breakdown of chlorophyll led to increased susceptibility to some necrotrophs (Kariola et al. 2005). Disease progression is promoted through a large disturbance of the redox status in the infected tissues (Lyon et al. 2004). Furthermore, the pathogenicities of *B. cinerea* and *S. sclerotiorum* correlated with the level of hydroxyl (OH^-) radicals and hydrogen peroxide (H_2O_2) produced. At advanced stages of infection, it was found that *Botrytis* could trigger significant changes in the antioxidant system of the peroxisomes, causing the collapse of the protective mechanism (Kuzniak and Sklodowska 2005). The type 3 metallothionein, *MT3-A* and *MT3-B* expression was induced in leaf tissues of oil palm artificially inoculated with the necrotrophic fungi, *Ganoderma boninense*. Since one of the important roles of metallothionein is in scavenging ROS, *MT3-A* and *MT3-B* may be produced in response to the generation of ROS in the infected oil palm tissues. Thus, the oil palm type 3 metallothioneins could potentially be suitable biomarkers for the oxidative stress due to *G. boninense* infection (Fahimeh et al. 2011).

Nitric oxide is produced in response to both biotic and abiotic stresses and evidence is accumulating to suggest its significant role in the plant–pathogen interaction and signaling (Crawford and Guo 2005; Mur et al. 2013). Nitric oxide concentration influences the synthesis of cellulose an essential component of plant cell wall (Correa-Aragunde et al. 2008), alleviate cadmium and aluminum stress through alteration of cell wall composition (Xiong et al. 2009; Zhang et al. 2011). Plant hormones, including auxins and cytokinins, are involved in regulating the production and signaling by nitric oxide. Auxins work synergistically with nitric oxide in various developmental and morphological plant responses, whereas nitric oxide's interaction with cytokinins is complex and the outcome appeared to be dependent on tissue type and plant species (Freschi 2013; Nafisi et al. 2015).

4.3 Fungal Toxins and Phytotoxic Proteins as Necrotrophic Effectors

Toxins and phytotoxic proteins produce by necrotrophs are critical for their virulence strategies. Together they are referred to as necrotrophic effectors. Phytotoxins such as HC-toxins, victorin, AM-toxin, ergotamine, and sirodesmin PL are produced by plant-pathogenic fungi through the activity of polyketide synthases (PKSs) and non-ribosomal peptide synthetases (NRPSs) (Vleeshouwers and Oliver 2014). The effectors target specific host proteins and cellular processes. For example, Fumonisin B1 and AAL toxin inhibit ceramide synthesis in the plant host that consequently interferes with sphingolipid metabolism (Abbas et al. 1994). However, the interaction of the necrotrophic effectors may not directly bind to the dominant host susceptible gene, even though *Hmr* interacted with the necrotrophic effector. It was the first disease resistance gene cloned (Walton 2006).

HSTs are secreted as virulence factors by many necrotrophs. Specific host target proteins conferred susceptibility to the pathogen. *Periconia circinata* produces HST causing milo disease in sorghum. Susceptibility to the pathogen is conditioned by the *Pc* gene which encodes the nucleotide-binding site leucine-rich repeat (NBS-LRR) protein (Nagy et al. 2007). The wheat tan spot caused by *Pyrenophora tritici-repentis* is mediated by ToxA toxins and *Tsn 1*, the wheat toxin sensitivity gene that encodes the R-like protein (Faris et al. 2010). However, it was suggested that the toxin does not physically interact with the R-protein, but rather the protein functions indirectly for toxin sensitivity and pathogen susceptibility. As effectors, the HSTs suppress host defenses while, serving as determinants of host responses. Resistance to HSN emulates effector-triggered immunity (ETI). It involves a single gene-encoded protein not affected by the toxin or can detoxify the HSTs (Wolpert et al. 2002). For example, HC-toxin can be detoxified by the carbonyl reductase encoded by the maize *HM1* gene. This blocked the inhibition of histone deacetylases (HDACs) by the toxin, thus conferring race-specific resistance (Johal and Briggs 1992).

BHNs produce nonspecific toxins that most probably target host proteins. BHNs secrete necrosis and ethylene-inducing proteins (NEPs) causing cell death in dicotyledonous (Staats et al. 2007). NEPs serve as the virulence factors for the BHNs. The *Fusarium oxysporum* secreted necrosis and ethylene-inducing protein (*NEP1*) that cause plant cell death. *NEP1*-like fungal proteins (NLPs) are small conserved molecules that induce HR-like cell death. NLPs only exhibit conserved toxicity to dicot plants across a wide range of taxa (Ottmann et al. 2009). The response triggered by both HSTs and NEPs mimics the plant immune response (Qutob et al. 2006; Wolpert et al. 2002). The toxins behave like virulence promoting cytolytic toxins that function by interfering with integrity of the plasma membrane (Ottmann et al. 2009). The disruption once detected by the host plants leads to the activation of defenses.

4.4 Downstream Defense Responses

4.4.1 Pathogenesis-Related Proteins

The biosynthesis and accumulation of inducible pathogenesis-related (PR) proteins is one of the main biochemical defense responses of plants to infection by pathogens. These proteins are of low molecular mass in the range of 6–43 kDa and strongly resistant to proteolytic cleavage and low pH values which enables survival in the harsh environment such as the vacuolar compartment or the intercellular spaces (van Loon 1985). PRs are found in almost all organs, including roots, flowers, and stems with maximum abundance levels in the leaves (van Loon and Van Strien 1999). To date, there are 17 families of structurally and functionally unrelated PR-proteins where they are numbered sequentially based on the time of discovery (van Loon et al. 2006). The PR-proteins are strongly induced by infections. Their expression and accumulation is strongly linked with the type of pathogen and the type of plant tissues being infected. They are produced at the infection site, surrounding tissues and systemic tissues further away from the infected site. Production in uninfected parts of the plants will protect the affected plants from further infection. Acidic PR proteins are found in the intercellular space, whereas the basic forms are transported to the vacuole by C-terminal signals (van Loon et al. 2006). Proteins which have sequence homology with PR proteins, but are constitutively expressed in some tissues or during specific developmental stages are referred to as PR-like proteins (Linthorst et al. 1990).

Several families of PR proteins, including PR-1, PR-2 proteins (β-1,3-glucanases), PR-3 and PR-4 proteins (chitinases), thaumatin-like proteins and osmotins (PR-5), peroxidases (PR-9), defensins (PR-12), and thionin (PR-13) exhibit antifungal activities which were mostly demonstrated through in vitro studies suggesting their involvement in plant defense. Since both β-1,3-glucans and chitins are the main constituents of fungal cell wall, co-induction for a coordinated expression of *β-1,3-glucanase* and *chitinase* following infection has been reported in many plant species. Thaumatin-like proteins and osmotins are believed to be involved in creating transmembrane pores, while, during resistance responses, peroxidases have been linked with accumulation of phenolics in plant cell walls. Induction of peroxidase activities in infected plants is associated with phenolics-mediated resistance against necrotrophs (van Loon et al. 2006).

Functional characterization to demonstrate the antifungal properties of PR-proteins, either using overexpressed recombinant proteins or transgenic systems has been done. For example, rice overexpressing thaumatin-like protein demonstrated increased resistance to *Rhizoctonia solani* (Datta et al. 1999), while thaumatin-like protein, chitinase, and glucanase were stably expressed in wheat for resistance against the devastating pathogen, *Fusarium graminearum* (Anand et al. 2003). There is interest in using PR-proteins as natural substances to protect plants from pathogen infection; however, it should be realized that some PR-proteins cause allergic reactions in humans (Hoffmann-Sommergruber 2002). The PR-proteins are also being used to develop biomarkers for defense response of specific plant pathogen and tissue interaction.

4.4.2 Phytoalexins

Phytoalexins are antimicrobial secondary metabolites that are of low molecular weight, synthesized and accumulated in plants when exposed to microorganisms. For example, in response to necrotrophic fungal pathogens, camalexin is produced in *Arabidopsis*, zealexin in maize, scopoletin in tobacco, and resveratrol in grapevine (Ahuja et al. 2012). Phytoalexins play a role in disease resistance (Dixon 2001) and most of the evidence has been obtained based on the studies done on camalexin, an indole derivative of tryptophan which is one of the most widely studied phytoalexins. It was initially isolated from the *Camelina sativa* leaves infected by *Alternaria brassica* (Browne et al. 1991). In *Arabidopsis*, it is a major phytoalexin, where *pad2*, *pad3*, *bos2*, *bos4*, and *esa1* mutants which are defective in camalexin synthesis and accumulation showed susceptibility towards the different pathogens (Glazebrook et al. 1997; Veronese et al. 2004). Induced synthesis of camalexin occurs at the site of infection by necrotrophs. It exhibits antimicrobial activity similar to the systemic fungicide thiabedazole. High concentrations of camalexin induce ion leakage and inhibit proline uptake resulting in the disruption of pathogen membrane (Pedras et al. 2006).

The necrotrophic fungus *Alternaria alternata* causes major damages in *Nicotiana* species. It infects mature tobacco leaves; however, young leaves are highly resistant to the pathogen. Scopoletin, a phenolic coumarin, which produces strong blue fluorescence under ultraviolet light, is synthesized through the phenylpropanoid pathway (Kai et al. 2008). These secondary metabolites can be isolated from *Nicotiana* and several other plant species (Gnonlonfin et al. 2012). In both in vitro and in vivo conditions in tobacco, scopoletin exhibited strong antifungal activity against *A. alternata* and it is recognized as an important phytoalexins against this pathogen. *A. alternata* induced blue fluorescence in *N. attenuata* leaves due to production of scopoletin and scopolin. The young wild tobacco leaves produces more scopoletin in response to attack by *A. alternata* and the synthesis was dependent on jasmonic acid signaling. Thus, the higher level of accumulated scopoletin was the main contribution to the resistance against the *A. alternata* in young leaves (Sun et al. 2014). Similarly, in the oil palm it was observed that the tolerant progenies produced higher percentages of defense-related secondary metabolites such as sterols and tocopherols than the susceptible progenies when treated with the necrotrophic fungal pathogen, *G. boninense* (Nusaibah et al. 2011).

Grapevine is susceptible to *B. cinerea* and produces resveratrol (an antifungal phytoalexins) as the result of its defense machinery. Zheng et al. (2011) showed that resveratrol impedes germination of the fungal conidium, thus preventing it from penetrating the host cell wall. The researchers also carried out gene-expression profiling using LongSAGE (long serial analysis of gene expression) libraries constructed from *B. cinerea* conidia treated in vitro with resveratrol in comparison to non-treated germlings in order to determine the influence of the phytoalexins on transcriptional regulation in *B. cinerea* germlings. Functional categorization of differentially expressed genes demonstrated that primary metabolism of the germinating conidia was strongly affected by resveratrol treatment, while concomitant induction of putative metabolic pathway genes for secretion of virulence-effector that disrupt the plant barriers and detoxify the resveratrol phytoalexin was observed. It was believed that the huge amount of virulence-effector being produced is the key to the eventual success of the pathogen.

4.5 Roles of Phytohormones

Plant hormone biosynthesis and signaling are important in modulating plant response to necrotrophic pathogens. The interactive roles of jasmonic acid (JA), ethylene (ET), and salicylic acids (SA) in defense signaling and plant immune response are well established, however, in recent years, the important contributions of other phytohormones such as abscisic acid (ABA), gibberellic acid (GA), auxins, cytokinins, and brassinosteroids in regulating plant defense response either alone or in conjunction with JA, ET, or SA have been discovered (Robert-Seilaniantz et al. 2011). Resistance and susceptibility to pathogens are affected by pathological conditions created through changes in plant hormonal homeostasis. JA and ET works in synergy in defense against necrotrophs, in direct antagonism to SA-mediated defense.

Infection with certain pathogens or wounding, induce the synthesis of JA and ET followed by activation of their signaling pathways. Necrotrophic fungal infection promotes ET production in the host plant. Ethylene biosynthesis involves conversion of S-adenosyl-L-methionine to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase and ACC is converted to ET by ACC oxidase. There are overwhelming data implicating JA and ET in resistance to necrotrophs. For example, *Arabidopsis* mutants affecting genes involved in JA and ET biosynthesis or signaling such as *fad3/fad7/fad8*, *ein2*, *ein3*, and *eil1* mutants are more susceptible to infection by necrotrophs (Stintzi et al. 2001; Pre et al. 2008). The molecular components of ET response pathways and factors involved in their regulation contribute to plant immune responses to necrotrophic infection. Many components of the ET-response pathway such as EIN3 and EIL1 are known to have a defense function against necrotrophs where loss of function mutations led to disease manifestations (Alonso et al. 2003; van Wees et al. 2003).

The *fad3/fad7/fad8* triple mutant lacking in JA which showed enhanced susceptibility to *Pythium mastophorum* (Vijayan et al. 1998) led to the discovery of the functional role of JA in defense response. A common occurrence in plants following injury is peroxidation of free linolenic acid catalyzed by 13-lipoxygenases, then allene oxide synthase-mediated epoxide formation followed by cyclization by allene oxide cyclase (AOC), and finally three rounds of β-oxidation to produce JA (Wasternack and Hause 2013). JA and its derivatives are well accepted as key regulators of defense mechanism against fungal pathogens. CORONATINE INSENSITIVE1 (COI1), an F-box protein is the JA-receptor essential for most JA-dependent responses. It was implicated in *B. cinerea* and *A. brassicicola* induced gene expression and resistance to necrotrophic pathogens (van Wees et al. 2003; AbuQamar et al. 2006). The strong resistance to *B. cinerea* and *A. brassicicola* displayed by *Arabidopsis rst1* mutant was dependent on COI1 (Mang et al. 2009). The increase in the levels of JA-biosynthesis and JA responsive gene expression and elevated cuticular lipids were the phenotypes displayed by *rst1*.

The extensive interactions among the plant hormones enable fine-tuning of host immune responses towards specific pathogen. The *Arabidopsis dde2/ein2/pad4/sid2* quadruple mutant with defects affecting essential components of the different phytohormone signaling pathways was studied in order to evaluate the contributions of JA, ET and SA to immunity against *A. brassicicola*. Interestingly, it was found that JA, ET, and SA signaling components contribute positively to immunity against *A. brassicicola* (Tsuda et al. 2009). Such information on the complex interaction is valuable and it could not be derived from single-mutant analysis.

4.5.1 Intervention of Phytohormone Balance and Function by Necrotrophs

Manipulation of host phytohormone biosynthesis and/or signaling pathways for defense is often employed by most pathogens. Many of these pathogens can synthesize the different phytohormones affecting the balance and functions of the endogenous plant hormones. Some pathogens manipulate the phytohormone signaling pathways essential for physiological and/or developmental features, for example stomatal opening and senescence in order to ease their entry and disease symptom development in the hosts (Melotto et al. 2008). Molecules that resemble phytohormones or phytohormone signaling components in structure, function, or both, referred to as "phytohormone mimics," are also used by pathogens to trick the hosts.

SA and JA modulate downstream defenses when attack by pathogens. To counter the effects, the pathogens often manipulate phytohormone cross talk to promote disease development. It is well known that the pathogens exploit the SA and JA cross talk when infecting plants (Thaler et al. 2012). For example, *B. cinerea* secretes β-(1,3)(1,6)-D-glucan as an exopolysaccharide (EPS) effector molecule which contributes to disease development by exploiting the SA-JA antagonism. Non-expressor of pathogenesis-related gene1 (*NPR1*) is a master regulator of SA signaling. Enhanced susceptibility to *B. cinerea* was observed in tomato plants pretreated with the EPS. It was suggested that EPS acts by activation of the SA pathway through *NRP1*, JA signaling was then suppressed (El Oirdi et al. 2011).

4.5.2 Cell Wall, Cuticles, and Phytohormone Interplay

The plant cell wall and cuticle serve as a physical barrier for inhibiting infection by necrotrophs. The severity of disease in the host is closely related to the susceptibility of the cell wall to degradation by CWDEs. Cellulose and pectins are attacked by the fungal enzymes to facilitate penetration. Disruption of cell wall integrity upregulates the biosynthetic machinery of the cell wall to maintain its integrity. It provides PAMPs/DAMPs and signals for recognition by the host. The cell wall is equipped with an integrity sensory system for activating the intracellular signaling cascades to induce the multitude of host defense responses. Phytohormones influence cell wall composition and structure. This affects the ability of necrotrophs to digest the cell wall and the cell wall capacity in generating DAMPs which will be detected by the cell wall integrity sensory systems. When the host sensed the degradation of cell walls by the invading pathogen through DAMP, the signaling cascades involving phytohormones cross talks leading to induced or increased expression of defense related genes are activated.

Cuticle serves as a platform for activating immune responses. It facilitates the host in sensing and signaling early irritations by the invading necrotrophic pathogen. Altered cuticle development and composition involving cuticular lipid and cutin polymer profile can enhance resistance to *B. cinerea* through faster recognition of fungal elicitors, better diffusion of defense signals to the infected site, and faster oxidative burst to counteract the pathogen virulence (L'Haridon et al. 2011; Mang et al. 2009).

Short fragments of OGs are released through the degradation of pectin homogalacturonan in plant cell wall by the polygalacturonase enzyme secreted by necrotrophs during infection. Mechanical damage can also induce the expression of endogenous polygalacturonases (PG) that is involved in the production of OGs (Orozco-Cardenas and Ryan 1999). OGs are DAMPs effective in eliciting defense responses (Cervone et al. 1989). A multitude of defense responses in plants, including accumulation of antimicrobial secondary metabolites or phytoalexins (Davis et al. 1986), expression of PR genes, including chitinase and glucanase (Broekaert and Pneumas 1988), deposition of callose, generation of ROS (Ridley et al. 2001; Denoux et al. 2008) and nitric oxide are induced by OGs. The extent of pectin methylation effects PG hydrolysis and the length of OGs produced and this influence host defense (De Lorenzo et al. 2001; Lionetti et al. 2007). It was found that OGs with a degree of polymerization between 10 and 15 are optimal for inducing the plant defense responses (Ferrari et al. 2013).

The cell wall-phytohormone homeostasis is a highly integrated system that is important in plant–necrotrophic pathogen interaction (Nafisi et al. 2014). Resistance can be produced through mutation of the member of the CesA family of cellulose synthase subunits. It was shown that the mutation led to an increase in the level of endogenous ABA and expression of ABA-responsive genes (Hernandez-Blanco et al. 2007). Further, *cev1* mutant, which is cellulose deficient elicits ET and JA signaling which results in improved resistance to pathogens (Ellis et al. 2002a, b). Auxin has an antagonistic effect on OGs and vice versa. It was found that auxin can abolish the effects of improved resistance of tobacco plants to *B. cinerea* conferred by a fungal endo-PG, which most probably through the constitutive production of OGs (Ferrari et al. 2008). The catalytic activity of fungal PGs can be inhibited by polygalacturonase inhibiting proteins (PGIPs) expressed in plants (Sicilia et al. 2005). PGIPs can limit the degradation of homogalacturonan by fungal PG, hence reducing the generation of the elicitor-active OGs (Ferrari et al. 2013). Transgenic tobacco overexpressing a grapevine PGIP (*Vvpgip1*) had a higher level of IAA and higher content of lignins and a decreased level of xyloglucan endotransglycosylase activity (Alexandersson et al. 2011). It was revealed through detailed cell wall analysis that overexpression of *Vvpgip1* leads to constitutive compositional changes. Thus, suggesting that PGIP-induced initial changes in the cell wall. This leads to altered auxin accumulation and stress responses, resulting in additional cell wall structural and compositional changes.

4.6 Transcription Factors

The outcome of plant–pathogen interactions is strongly influenced by the transcriptional control of gene expression by the specific transcription factors. The transcription factors specifically bind to *cis*-acting elements in the promoters of genes involved in immune response pathways of plants, enabling coordinated and precise timing of their expression in response to infection by necrotrophs. These include genes involved in stress signaling, cell death, cell wall dynamics, and biosynthesis of phytohormones and secondary metabolites. Since resistance and susceptibility of host are dependent on the speed and level of expression of the immune response pathway genes, transcription factors have great impact on plant defense. Certain families of plant transcription factors are involved in plant defense to microbial pathogens; for example, different transcription factor families, including ERFs, WRKY, MYB, zinc-finger, and HD-ZIP, are induced in response to *B. cinerea* infection (AbuQamar et al. 2006). WRKY33, MYB, ERF1 and ERF104, ZFAR1, AS1, and HD-ZIP homeodomain (Smith et al. 2014) are important for resistance to necrotrophic fungi.

The WRKYs are plant specific transcription factors believed to be the intermediate signaling components of the various PTI responses (Asai et al. 2002). WRKY33 and WRKY70 are required for responses to *B. cinerea*, suggesting a potential role in plant resistance (AbuQamar et al. 2006; Zheng et al. 2006). *Arabidopsis* WRKY33 was shown to be a major regulator of immune responses to necrotrophic fungi based on analysis of loss-of-function mutants and interactions with other PTI pathway components. It is involved in regulating expression of camalexin (Qiu et al. 2008; Mao et al. 2011), an antimicrobial secondary metabolite secreted by the plants at the early stage of infection and the autophagy gene *ATG18a* (Lai et al. 2011a). *Arabidopsis* AtWRKY33 interacts with the Sigma Factor interacting proteins 1 and 2 (SIB1 and SIB2) whose production are induced by the necrotrophic fungus *B. cinerea*. Deletion mutants (*SIB1* and *SIB2*) showed a decrease in plant resistance supporting their role as AtWRKY33 activators (Lai et al. 2011b). From 16 canola (*Brassica napus*) *BnWRKY* genes assayed, 13 *BnWRKY* were responsive to hormonal treatment as well as *Sclerotinia sclerotiorum* and *Alternaria brassicae*, the fungal pathogen causing stem rot and *Alternaria* black spot, respectively. This suggests that in response to hormonal stimuli and fungal pathogen, a large proportion of *BnWRKY* proteins play a role in the transcriptional regulation of defense-related genes (Yang et al. 2009).

Many transcription factors regulate transcription of genes in the JA and ET pathways and affecting resistance to necrotrophs. Selective binding of the MYB-related gene *ASYMMETRIC LEAVES 1* (*AS1*) to the promoters of JA-regulated genes (Nurmberg et al. 2007) suppressed inducible resistance against *B. cinerea* while the loss of *AS1* function, enhanced resistance in *Arabidopsis* to *B. cinerea*. MYB46 suppresses resistance to *B. cinerea* through transcriptional reprogramming of genes for cell wall proteins and enzymes (Ramirez et al. 2011). Many MYB proteins regulate the expression of genes involved in the production of secondary metabolites. For example, MYB51 regulates the transcriptional activation of genes for the biosynthesis of indole glucosinolate (Clay et al. 2009), a tryptophan-derived secondary metabolite that contributes to resistance to necrotrophic fungi (Bednarek et al. 2009; Kliebenstein et al. 2005; Sanchez-Vallet et al. 2010). *BOTRYTIS SUSCEPTIBLE 1* (*BOS1*) that encodes an R2R3 MYB was shown to restrict necrosis caused by necrotrophic pathogen. *BOS1* and *BOTYRYTIS SUSCEPTIBL E1 INTERACTOR* (*BOI*), a ligase that restricts cell death may integrate plant response to various signals caused by stress factors.

The necrotrophic fungus *Gaeumannomyces graminis* var. *tritici* (Ggt) causes a devastating root disease in wheat. At least six wheat defense-related genes showed significant upregulated expression in the TiMYB2R-1 transgenic wheat lines. It was shown that enhanced resistance to the disease which is known as take-all was achieved in the *TiMYB2R-1*-overexpressing wheat lines (Liu et al. 2013).

ERF belongs to AP2/ERF superfamily of transcription factors which is responsive to ET. ERF binds to the ethylene-responsive element (ERE), also known as the GCC-box (AGCCGCC) a common promoter element found in ethylene-inducible defense genes. In *Arabidopsis*, a subgroup known as octadecanoid-responsive *Arabidopsis* AP2/ERF (ORA) is rapidly induced by JA. Many of the ORA transcription factors play important roles in disease resistance involving JA and ethylene signaling pathways. Infection with certain pathogens or wounding induces the synthesis of JA and ethylene followed by activation of their signaling pathways. The expression of ORAs is activated by JA and or related oxylipins via COI1 which serves as the central regulator of all JA-dependent responses (Garcia-Marcos et al. 2013)

ORA59 is involved in increasing expression of several defense genes such as *PDF1.2* and *ChiB* and *β-glucosidase 2* (*BGL2*) and its overexpression results in increased resistance against the necrotrophic fungus *B. cinerea*. The ORA37 transcription factor is different from the other ORAs because it contains an ERF-associated amphiphilic repression (EAR) motif at its C-terminal region that functions as a transcriptional repressor (Otha et al. 2001). The induction of a subset of JA- and ethylene-responsive genes was significantly lower in plants overexpressing *ORA37* including the defense genes *PDF1.2*, *HEL*, and *ChiB* when treated with JA or ET (Atallah 2005).

Rhizoctonia solani is a fungal pathogen that has led to significant economic losses to diverse range of crops including cereals, canola, and legumes. Root specific expression of a *Medicago truncatula ERF* transcription factor *MtERF1-1* in Medicago enhanced resistance to *R. solani* and *Phytophthora medicaginis* without adversely affecting symbiosis with *Rhizobia* (Anderson et al. 2010). The defense regulatory mechanism of ERF transcription factors may involve interaction with other transcription factors, for example, the interaction observed between bZIP transcription factor and ERF72 for regulating expression of a PR-1 type protein from tobacco (Büttner and Singh 1997; Alves et al. 2013). The MYC2 has been well characterized where in Arabidopsis it was shown to differentially regulate the expression of JA-responsive pathogen defense and wound response (*VSP*) genes (Dombrecht et al. 2007). Expression of *PDF1.2*, *ChiB*, and *HEL* are regulated by JA and ET-signaling pathways during necrotrophic fungal infection. Their induced expression is negatively regulated by AtMYC2 indirectly via negative regulation of expression of ERF1 transcription factors. We have summarized the various transcription factors involved in regulating defense response in tabular form (Table 1).

Table 1 Functions and roles of transcription factors in plant defense response against necrotrophic pathogens

Plant	Pathogen	Transcription factor	Role	References
Arabidopsis	*Botrytis cinerea*	ORA37	Contains an ERF-associated amphiphilic repression (EAR) motif at C-terminal end that functions as a transcriptional repressor	Otha et al. (2001)
Arabidopsis	*B. cinerea*	WRKY70	Potential role in plant resistance	
Arabidopsis	*B. cinerea*	ORA37	Overexpression of ORA37 resulted in significantly lower induction of a subset of jasmonic acid and ethylene responsive genes including the defense genes (*PDF1.2*, *HEL*, and *ChiB*)	Atallah (2005)
Arabidopsis	*Plectosphaerella cucumerina*, *B. cinerea*, *Fusarium oxysporum*, and *Pseudomonas syringae*	MYC2	Differentially regulate the expression of jasmonic acid responsive pathogen defense and wound response (*VSP*) genes	Dombrecht et al. (2007)
Arabidopsis	*B. cinerea*	WRKY33	Regulates the expression of camalexin and interacts with the sigma factor interacting proteins SIB1 and SIB2	Qiu et al. (2008)
Arabidopsis	*B. cinerea*	ORA59	Increased the expression of defense related genes (*PDF1.2*, *ChiB*, and β-*BGL2*), and also integrate in the ethylene and jasmonic acid signaling pathways	Pre et al. (2008)

(continued)

Table 1 (continued)

Plant	Pathogen	Transcription factor	Role	References
Arabidopsis	Microbial	MYB51	Involved in the transcriptional activation of indole glucosinolate biosynthetic genes	Clay et al. (2009)
Brassica napus	*Alternaria brassicae* *Sclerotinia sclerotiorum*	BnWRKY	Takes part in the transcriptional regulation of defense-related genes	Yang et al. (2009)
Medicago truncatula	*Rhizoctonia solani*	MtERF1-1	Enhanced the resistance towards *R. solani*	Anderson et al. (2010)
Arabidopsis	*B. cinerea*	MYB46	Suppressed resistance towards *B. cinerea* by selective transcriptional reprogramming of genes encoding the cell wall proteins and enzymes	Ramirez et al. (2011)
Arabidopsis	*B. cinerea*	WRKY33	Regulator of immune response against the necrotrophic fungi	Mao et al. (2011)
Triticum spp.	*Gaeumannomyces graminis* var. *tritici*	TiMYB2R-1	Enhanced resistance against take-all disease in wheat transgenic lines overexpressing *TiMYB2R-1*	Liu et al. (2013)

4.7 Chromatin and Histone Modifications

Alterations in the expression of genes due to processes not associated with changes in the underlying DNA sequence are referred to as epigenetics. The processes include DNA methylation, histone modification, and nucleosome remodeling which alters the dynamics of the chromatin, a highly compacted organized complex of DNA and histone proteins. Histone modifications alter the net charge of nucleosomes and these affect DNA histone and histone–histone interactions at the inter- or intra-nucleosome level.

The chromatin states in eukaryotic nuclei are influenced by the different combinations of posttranslational modifications of the histone proteins. The lysine residues of the histones in a eukaryotic chromatin can be covalently modified through acetylation, methylation, and mono-ubiquitination, resulting in an epigenome which plays an important role in the fine-tuning of transcription process. Acetylation of the lysine residues on histone 3 and histone 4 is often linked with active transcription. Histone acetyltransferases (HATs) catalyze the addition of an acetyl group to multiple lysine residues and this is a dynamic process where changes occur over time. The opposing action is catalyzed by HDACs. The antagonistic action between the two families of enzymes maintained the homeostatic balance of histone acetylation for proper cellular function and differentiation (Jeon et al. 2014).

4.7.1 Fungal Toxins as HDAC Inhibitors

The toxins produced by the necrotrophic fungal pathogens *C. carbonum* and *A. brassicicola* inhibit HDACs. Thus, the toxins are targeting chromatin modifying enzymes possibly to interfere with the transcriptional activity of immune response genes. The HC-toxin is an HST secreted by *C. carbonum*. The toxin specifically inhibits maize HDACs, but does not have an effect on HATs. It was proposed that the HC-toxin helps the pathogen to establish compatibility with maize via the interference of HDACs (Brosch et al. 1995). *A. brassicicola* produces depudecin as a virulence factor which is also a HDAC inhibitor (Kwon et al. 1998; Matsumoto et al. 1992). Induced *HDAC19* expression in *A. thaliana* infected with *A. brassicicola* correlates with changes in expression of JA and ET-regulated genes (Zhou et al. 2005). Thus, it is possible that plant HDACs are conserved regulators of defense-related genes in plants and inhibition of their activities enable the pathogens to disrupt the defense response transcriptional programs of host plants (Jeon et al. 2014).

4.7.2 Epigenetic Control of Defense Priming

It has been hypothesized that chromatin modification primes the defense genes for more rapid and robust activation (Bruce et al. 2007). This led to the concept of epigenetic control of defense priming. SET DOMAIN GROUP 8 (SDG8)-mediated methylation of histone H3 lysine 6 in the promoters of JA-inducible defense genes accompanied the induction of expression of these genes in response to fungal infection (Berr et al. 2010). This enables a long-lasting priming of JA-dependent defense genes against future infection by the necrotrophs. Priming of the WRKY transcription factor 29 gene (*WRKY29*) with benzothiadiazole is associated with the trimethylation and acetylation of specific lysine of the histone proteins in the *WRKY29* gene promoter (Jaskiewicz et al. 2011). This allows the activation of the expression of the *WRKY29* gene when the plants were confronted with further stress stimulus. Thus, defense priming essentially involves chromatin modification of promoters of defense genes before true activation of these genes. The modifications in the chromatin could loosen histone–DNA interaction to allow access to the open chromatin structure of the various transcription co-activators, effector proteins, and chromatin remodeling factors (Alvarez-Venegas 2013).

5 Conclusions and Future Prospects

The mechanisms of infection, including secretion of the phytotoxins and cell wall degrading enzymes and strategies in mimicking components of plant defense by pathogen and the series of plant immune response pathways to counteract the effects are well studied for model crops involving certain pathogens. The information needs to be translated to other crops of economic importance and peculiarities of specific pathogen plant interaction must be understood. It is important to have an early intervention strategy to protect the plant following initial plant–pathogen interaction due to the notorious nature of the necrotrophic fungal pathogens. Often the control measures taken were too late to save the plant as when the physical symptoms are recognized, the toxins and reactive oxygen species had killed the cells in the important tissues and organs essential for the plants survival. Thus, work on predisposing factors and strategies for early disease diagnosis before the appearance of symptoms needs to be developed. For these purposes early inducible defense response, including components of the signaling cascades that have been discussed and early defense response secondary metabolites such as the phytoalexins are valuable targets and their roles in inhibiting fungal ingress is a key area of research to focus on for producing disease resistant plants. It is clear that the plant immune response is complex, involving the interplay of various phytohormones, cell wall components, and production of different antimicrobial molecules playing their roles at the different infection phases from pathogen entry to disease development. This is the result of the transcriptional reprogramming that occurs in response to the different signaling molecules. Mechanisms of transcriptional controls addressed through systems approach via omics platform which had enabled identification of the key pathways of pathogenic attack and plant defense response should be emphasized. This would lead to the identification of different compounds and proteins with functional and regulatory roles covering both transcriptional and translational processes. Chromatin modifications that fine-tune transcriptional regulation of immune responses through histone modification are also being targeted by the pathogen to overcome the plant's immune response. Epigenetic control of defense priming enables the plants to be more prepared for recurrent attack and should be an important area of research for a holistic and effective approach in sustaining crop survival and productivity.

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Nitrogen Fixing Symbiosis in a Sustainable Agriculture

Isabel Videira e Castro, Paula Fareleira, and Eugénio Ferreira

Contents

Abstract In most agricultural systems, the primary source of biologically fixed N_2 takes place through the symbiotic interactions of legumes and rhizobia bacteria. As a collective name, rhizobia cover several genera in the alpha- and beta-Proteobacteria. As a rule, all species of rhizobia form nodules with a range of hosts, determined by their nodulation genes. Nodulation and N_2 fixation in these symbioses need that host and microorganisms are compatible, and also that the soil environment is appropriate for the exchange of signals that precede infection. Thus, soil abiotic factors, such as pH, temperature, salinity, and heavy metals, are also critical in the ecology of rhizobia. Besides nitrogen fixation, rhizobia may also benefit plants by other processes, being naturally associated with nonlegume plants and affecting positively

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their growth through one or several mechanisms independent of symbiotic nitrogen fixation. In this chapter, the focal point is related with some aspects of the *Rhizobium*-legume symbiosis taking into account that the improvement in molecular biology methods contributed significantly to a major advance in the knowledge of rhizobial diversity. We highlight the importance of certain abiotic stress conditions and the emerging knowledge of the potential of rhizobia as plant growth-promoting bacteria. Special emphasis is given to legumes in natural and sown pasture, namely a particular case on the Mediterranean area exemplifying a long-term sustainable agrosilvopastoral ecosystem.

Keywords Abiotic stress Rhizobia • Nitrogen fixation • Diversity • PGPR • Pastures

1 Introduction

Agriculture in developed countries has become increasingly dependent on chemical fertilizers and pesticides in order to achieve high crop yields. In response to the needs, the industrial production of fertilizers has increased from 3.5 million tons in 1950 to 80 million tons in 1989 (Hardy 1993). As FAO estimates (FAO 2011) this number will reach 120 million tons in 2015 (Fig. **1**), and it is expected to be about 137.4 million tons by 2030 (Tenkorang and Lowenberg-Deboer 2008). Adverse effects of these chemicals on the population, environment, and food safety have confirmed the requirement for modifying the agricultural production methods toward sustainable economic production, preservation of natural resources, and reduction in the employment of synthetic chemicals. Harwood (1990) defined sustainable agriculture as “an agriculture that can evolve indefinitely toward greater human utility, greater efficiency of resource use, and a balance with the environment that is favorable both to humans and to most other species (includes animals, plants and microorganisms).”

Biological nitrogen fixation (BNF) is considered a key process on earth, and the upgrading of agricultural sustainability will need its utilization as a main source of nitrogen for crop plants. BNF also offers an economically attractive and ecological approach of decreased external nitrogen inputs, improving the quality and quantity of internal resources, therefore being ideal for sustainable agriculture (Graham and Vance 2003; Rashid et al. 2015). It is generally accepted that the total N_2 fixation is 175 million metric tons (Tg) annually (Burns and Hardy 1975) and is estimated that the contribution of legumes (grain, pasture, and natural ecosystems) ranges from 25 to 90 Tg (Smil 1999). On the other hand, Kipe-Nolt and Giller (1993) suggested that 10 % of legume N may be left in the root at harvest, with additional N passing directly into the soil during the senescence of nodules and fine roots.

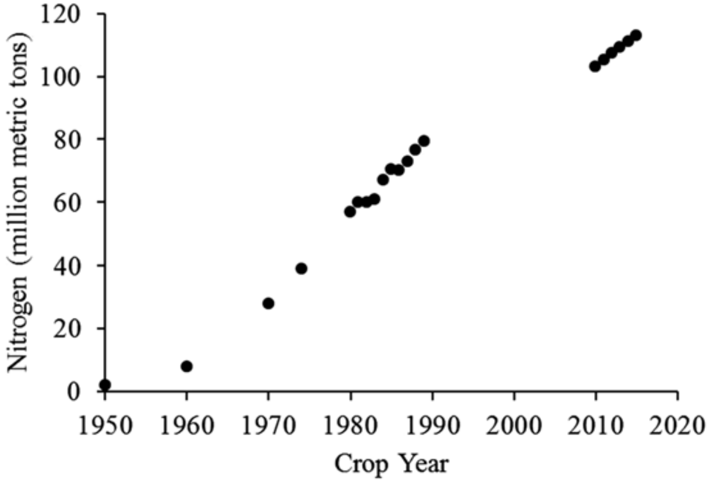

Fig. 1 World fertilizer consumption of nitrogen totals, 1950–1989 and 2008–2015. Adapted from *Fertilizer Facts and Figures*, 1990, The Fertilizer Institute, Washington, D.C. (Hardy 1993) and from FAO (2011)

Generally, in agricultural systems, the primary source of biologically fixed N_2 takes place through the symbiotic interactions of legumes and soil bacteria called rhizobia. Nodulation and N_2 fixation in this symbiosis need that host and microorganisms are well matched, but also that the soil environment is appropriate for the exchange of signals that precede infection (Zhang et al. 2002; Hirsch et al. 2003). The soil abiotic features on rhizobia ecology are also critical. In fact, N_2-fixing legumes are so vulnerable to environmental stresses than those plants depend on mineral nitrogen, and in some cases they are even more negatively affected. For example, extremes of temperature, pH, high salinity, and high content of heavy metals adversely affect the formation and function of root nodules (Fentahun et al. 2013). The range of genetic diversity to tolerate these stresses is very important for both symbionts. Unfortunately, synthetic agricultural inputs applied to soil, such as herbicides, nitrogenous fertilizers, or pesticides, can also affect rhizobial populations and legume nodulation with negative effects, mainly in the reduction in rhizobial cells and nodulation (Caballero-Mellado and Martínez-Romero 1999; Depret et al. 2004).

The present chapter focuses on some aspects of the *Rhizobium*-legume symbiosis taking into account that the improvement in molecular biology methods contributed significantly to a major advance in the knowledge of rhizobial diversity and also highlighted the importance of certain abiotic stress conditions and the emerging knowledge of rhizobia potential as plant growth-promoting bacteria. Moreover, special emphasis is given to natural and sown pastures, namely a particular case of the Mediterranean area exemplifying a long-term sustainable agrosilvopastoral ecosystem.

2 Influence of Legume Host on Diversity and Symbiotic Function of Rhizobial Populations

2.1 Taxonomy

The *Fabacea* (*Leguminosae*), commonly known as legumes, is a large family of plants, with about 19,000 described species, and has a broad geographical distribution (Sprent 1995). The most important legume species have defined places of origin and these coincide with the diversification centers for their "specific" symbiotic bacteria. It is accepted that the *Fabacea* origin is tropical and subtropical.

Legumes have the capacity to establish symbiotic interactions with soil bacteria named rhizobia, which allows that these plants can be autonomous in relation to the levels of nitrogen available in the soil. Rhizobia (or root- and stem-nodule bacteria) are able to form nodules on plants of the *Fabacea* family (Rashid et al. 2015). Inside the nodules, the rhizobia fix atmospheric nitrogen and carry it to their hosts in the form of amino acids in the presence of suitable host, and the success of legumes can be partially recognized by their capacity to colonize environments with low soil nitrogen because of their symbiotic association with N_2-fixing rhizobia (Sprent 1994).

As a collective name, rhizobia cover several genera in the alpha-Proteobacteria and in the beta-Proteobacteria. As a rule, all species of rhizobia form nodules with a range of hosts, determined by their nodulation genes. Traditionally, restricted geographical allocation of some rhizobial species has been observed, such as strains of *S. fredii* from Chinese soils, which nodulated soybean (Camacho et al. 2002), and strains *of Bradyrhizobium* which nodulated also soybean and did not exist in natural fields of Brazil (Barcellos et al. 2007). The early classification of rhizobia was based on the cross-inoculation group, whereby rhizobia were grouped based on their capacity to specifically nodulate and fix nitrogen with a certain group of legumes (Fred et al. 1932). This classification would be realistic if rhizobia host ranges reflected legume taxonomy, but since many rhizobia form symbioses across taxonomic divisions, it cannot be used alone in the taxonomic classification of rhizobia. However, the symbiotic phenotype has been always considered as an essential feature for understanding the biology and classification of rhizobia (Graham et al. 1991).

The discord of this host-dependent approach terminated in an alteration to a more polyphasic taxonomy (Vandamme et al. 1996; Graham 2008) including morphological and biochemical characteristics, genetic fingerprinting (Versalovic et al. 1994), fatty acid methylester analysis (Jarvis and Tighe 1994), and 16S rRNA gene sequence analysis (Martinez-Romero et al. 2000). This polyphasic strategy has led to the report of other genera and species of root-nodule bacteria, and also to the finding of a variety of behaviors different from those visualized by previous researchers in this area, such as plant growth-promoting activities and capacity to degrade pollutants.

In the past 20 years the taxonomy of rhizobia has been altered deeply being the original genus *Rhizobium*, presently separated into different genera. Currently, the existence of 16 genera and about 100 species of root-nodule bacteria has been recognized (Lindström and Young 2011; Vinuesa 2013), although additions occur frequently. In fact, the study of new geographically disseminated host plants has been a source of many new species and it is expected that many others arise. The other reason for the increase in the number of genera and species is in the progress of taxonomic research. The development of new methodologies to study cell DNA and RNA has led to a more exhaustive characterization, resulting in phylogenetic and polyphasic classifications. Presently, a large number of bacterial genome is becoming accessible, which will surely have a great impact on future bacterial taxonomy. Traditionally rhizobia have been divided into six genera, namely *Rhizobium*, *Ensifer* (formerly *Sinorhizobium*), *Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium*, and *Allorhizobium* (Table 1).

Table 1 Genus and species of root- and stem-nodule bacteria of the family *Rhizobiaceae* and their legume hosts (Martinez-Romero et al. 2000; Willems 2006; Graham 2008; Lindström and Young 2011; Vinuesa 2013)

Genera	Species	Host legumes
Rhizobium	*alkalisoli*	*Caragana intermedia, Caragana microphylla, Phaseolus vulgaris, Vigna radiata*
	azibense	*Phaseolus vulgaris*
	calliandrae	*Calliandra grandiflora*
	daejeonense	*Medicago* sp.
	endophyticum	*Phaseolus vulgaris*
	etli	*Phaseolus vulgaris, Mimosa affinis*
	freirei	*Phaseolus vulgaris*
	galegae	*Galega orientalis, Galega officinalis*
	giardini	*Phaseolus vulgaris, Leucaena, Macroptilium, Desmanthus*
	grahamii	*Phaseolus vulgaris, Macroptilium atropurpureum, Vigna unguiculata, Mimosa pudica*
	hainanense	*Stylosanthes, Centrosema, Desmodium, Tephrosia*
	huautlense	*Sesbania herbacea*
	indigoferae	*Indigofera* spp.
	jaguaris	*Calliandra grandiflora*
	laguerreae	*Vicia faba*
	leguminosarum	
	bv. *trifolii*	*Trifolium* spp.
	bv. *viciae*	*Pisum, Vicia, Lathyrus, Lens*
	bv. *phaseoli*	*Phaseolus vulgaris*
	leucaenae	*Leucaena*
	loessense	*Astragalus, Lespedeza*
	lusitanum	*Phaseolus vulgaris*
	mayense	*Calliandra grandiflora*
	mesoamericanum	*Phaseolus vulgaris, Macroptilium atropurpureum, Vigna unguiculata, Mimosa pudica*

(continued)

Table 1 (continued)

Genera	Species	Host legumes
	mesosinicum	*Albizia julibrissin, Kummerowia stipulacea, Kummerowia striata, Dalbergia* spp.
	miluonense	*Lespedeza*
	paranaense	*Phaseolus vulgaris*
	pisi	*Pisum sativum, Trifolium repens, Phaseolus vulgaris*
	phaseoli	*Phaseolus vulgaris, Trifolium repens*
	sphaerophysae	*Sphaerophysa salsula*
	sullae	*Hedysarum coronarium*
	taibaishanense	*Kummerowia striata*
	tibeticum	*Trigonella archiducis-nicolai, Medicago lupulina, Medicago sativa, Melilotus officinalis, Phaseolus vulgaris, Trigonella foenum-graecum*
	tubonense	*Oxytropis glabra*
	tropici	*Phaseolus vulgaris, Leucaena, Macroptilium, Dalea, Onobrychis*
	yanglingense	*Amphicarpaea, Coronilla, Gueldenstaedtia*
Ensifer (*Sinorhizobium*)[a]	*abri*	*Abrus precatorius*
	adhaerens	*Sesbania grandiflora, Medicago sativa, Leucaena leucocephala, Pithecellobium dulce, Lotus arabicus*
	americanus	*Acacia* spp.
	arboris	*Acacia senegal, Prosopis chilensis*
	fredii	*Glycine max*
	garamanticus	*Argyrolobium uniflorum, Medicago sativa*
	indiaense	*Sesbania rostrata*
	kostiense	*Acacia senegal, Prosopis chilensis*
	kummerowiae	*Kummerowia stipulacea*
	medicae	*Medicago truncatula, Medicago polymorpha, Medicago orbicularis*
	meliloti	*Medicago, Melilotus, Trigonella*
	mexicanus	*Acacia angustissima*
	morelense	*Leucaena leucocephala*
	numidicus	*Argyrolobium uniflorum, Lotus creticus*
	saheli	*Sesbania, Acacia*
	terangae	*Sesbania, Acacia*

Table 1 (continued)

Genera	Species	Host legumes
Mesorhizobium	*abyssinicae*	*Acacia abyssinica*
	alamii	*Medicago ruthenica*
	albiziae	*Albizia kalkora*
	alhagi	*Alhagi sparsifolia, Sophora alopecuroides, Glycyrrhiza inflata, Medicago sativa, Indigofera endecaphylla, Vicia cracca, Sophora flavescens*
	amorphae	*Amorpha fruticosa*
	australicum	*Biserrula pelecinus, Astragalus membranaceus, Macroptilium atropurpureum*
	camelthorni	*Alhagi sparsifolia, Sophora alopecuroides, Glycyrrhiza inflata, Medicago sativa*
	caraganae	*Caragana* spp.
	chacoense	*Prosopis alba*
	ciceri	*Cicer arietinum*
	gobiense	*Glycyrrhiza uralensis, Lotus corniculatus, Oxytropis glabra, Robinia pseudoacacia*
	hawassense	*Acacia abyssinica, Acacia tortilis*
	huakuii	*Astragalus sinicus, Acacia*
	loti	*Lotus corniculatus*
	mediterraneum	*Cicer arietinum*
	metallidurans	*Anthyllis vulneraria*
	opportunistum	*Biserrula pelecinus, Astragalus adsurgens, Astragalus membranaceus, Lotus peregrinus, Macroptilium atropurpureum*
	plurifarium	*Acacia senegal, Leucaena, Prosopis juliflora*
	qingshengii	*Astragalus sinicus*
	robiniae	*Robinia pseudoacacia*
	sangaii	*Astragalus luteolus, Astragalus ernestii*
	septentrionale	*Astragalus adsurgens*
	shangrilense	*Caragana microphylla, Caragana intermedia, Glycyrrhiza uralensis, Astragalus adsurgens, Vigna unguiculata, Vigna radiata, Phaseolus vulgaris*
	silamurunense	*Astragalus* spp.
	tamadayense	*Anagyris latifolia, Lotus berthelotii*
	tarimense	*Glycyrrhiza uralensis, Lotus corniculatus, Oxytropis glabra, Robinia pseudoacacia*
	temperatum	*Astragalus adsurgens*
	tianshanense	*Glycyrrhiza, Sophora, Glycine, Caragana*

(continued)

Table 1 (continued)

Genera	Species	Host legumes
Bradyrhizobium	*canariense*	*Chamaecytisus, Lupinus*
	cytisi	*Cytisus villosus*
	daqingense	*Glycine max*
	elkanii	*Glycine max*
	ganzhouense	*Acacia melanoxylon*
	huanghuaihaiense	*Glycine max*
	icense	*Phaseolus lunatus*
	iriomotense	*Entada koshunensi, Macroptilium atropurpureum*
	japonicum	*Glycine max*
	jicamae	*Pachyrhizus erosus, Lespedeza* spp.
	lablabi	*Lablab purpureus, Arachis hypogaea*
	liaoningense	*Glycine max*
	manausense	*Vigna unguiculata*
	ottawaense	*Glycine max*
	pachyrhizi	*Pachyrhizus erosus*
	paxllaeri	*Phaseolus lunatus*
	retamae	*Retama sphaerocarpa, Retama monosperma*
	rifense	*Cytisus villosus*
	yuanmimgense	*Lespedeza, Medicago, Melilotus*
Allorhizobium	*undicola*	*Neptunia natans, Acacia, Lotus, Faidherbia*
Azorhizobium	*caulinodans*	*Sesbania rostrata*
	doebereinerae	*Sesbania virgata*

[a] In 2008, the Judicial Commission (Opinion 84) confirmed the earlier synonym, *Ensifer*, as the correct name for the genus better known as *Sinorhizobium*

Young et al. (2001) proposed the genus *Agrobacterium* to be incorporated in the genus *Rhizobium*, but had not obtained a general agreement. In fact, from the 16S rDNA phylogeny, it is comprehensible that *Rhizobium* and *Agrobacterium* are highly related and their species are interconnected. With this proposal, the genus *Rhizobium* will be a large, widely defined, and phylogenetically more diverse group than the other genera in its phylogenetic vicinity (Willems 2006). To make a decision about the polyphyletic place of the genus *Agrobacterium* (which does not include symbiotic N_2 fixers), the International Committee on Systematics of Prokaryotes/Subcommittee on the taxonomy of *Agrobacterium* and *Rhizobium* (Lindström and Young 2011) decided to transfer one of the most distantly related species called biovar 2 of *Agrobacterium* into the genus *Rhizobium* (Keane et al. 1970). Consequently, *Rhizobium rhizogenes* is now a legitimate name for biovar 2 (Costechareyre et al. 2010).

Recently, the bacteria isolated from legume nodules were phylogenetically placed out of the conventional groups of rhizobia, which include *Aminobacter* (*A. Anthyllidis*, Maynaud et al. 2012), *Devosia* (*D. neptuniae, D. yakushimensis,* Rivas et al. 2003; Bautista et al. 2010), *Methylobacterium* (*M. nodulans*, Sy et al. 2001; Jourand et al. 2004), *Microvirga (M. lotononidis, M. lupini, M. zambiensis,* Ardley et al. 2012), *Ochrobactrum* (*O. ciceri,* Imran et al. 2010; *O. cytisi*, Zurdo-Piñeiro et al. 2007; *O. lupini*, Trujillo et al. 2005), *Phyllobacterium* (*P. endophyticum*, Flores-Félix et al. 2013; *P. loti*, Sanchez et al. 2014; *P. trifolii*, Valverde et al. 2005), and *Shinella* (*S. kummerowiae,* Lin et al. 2008) in the alpha-Proteobacteria, and also bacteria belonging to different genera in the beta-Proteobacteria. These genera of bacteria include *Burkholderia* (e.g., *B. phymatum*, which nodulates the roots of tropical legumes, Vandamme et al. 2002, *B. diazotrophica* and *B. symbiotica*, which were isolated from root nodules of *Mimosa* spp., Sheu et al. 2012), *Cupriavidus* (formerly *Ralstonia*) (*C. taiwanensis*, Chen et al. 2001), and *Herbaspirillum* (*H. lusitanum,* Valverde et al. 2003). These novel root nodule bacteria are phylogenetically different from rhizobia, but do carry *nod* genes analogous to those of rhizobia. The *nod* genes were most probably obtained during lateral gene transfer (Rivas et al. 2009; Rashid et al. 2015). However, if the collective name "rhizobia" will be extensive to comprise the species of beta-Proteobacteria above remains to be determined (Graham 2008).

2.2 *Rhizobia Diversity*

Rhizobia are considered highly competent and heterotrophic bacteria, which can stay alive for a long time in the absence of host legumes (Giller and Wilson 1991). However, the existence of a compatible host legume gives protection to the microsymbionts against environmental stresses (Andrade et al. 2002). On the other hand, a greater diversity of rhizobial populations in soil enlarges the series of legume hosts that can be nodulated in such soils. So, there is a reciprocal advantage between aboveground (legume) and belowground (rhizobia) biodiversities. There are signals that the rhizobia nodulating legumes in areas outside the hosts' center of diversity may be microsymbionts of local legumes, and can also nodulate the introduced species (Martinez-Romero and Caballero-Mellado 1996; Bala et al. 2003). So, promiscuity in host range shows to be the rule for tropical legumes and rhizobia (Giller and Wilson 1991). This feature appears to be determined by the enormous diversity of rhizobial population in tropical soils, opposite to the idea of a homogenous and promiscuous group of rhizobia of the 'cowpea miscellany' in these soils (Singleton et al. 1992). Several researchers have shown that tropical rhizobia are diverse, with subgroups of different symbiotic specificity and effectiveness (Mpepereki et al. 1996; Rashid et al. 2015).

For studying diversity among rhizobia, several techniques based on phenotype were used comprising serology (Bohlool and Schmidt 1980; Castro and Sequeira 1989), intrinsic antibiotic resistance (Beynon and Josey 1980; Stein et al. 1982), plasmid contents (Hirsch et al. 1980; Hynes and O'Connell 1990; Castro et al. 1997), phage typing (Bromfield et al. 1986), and electrophoresis of whole cell proteins (Noel and Brill 1980). Later, the multilocus enzyme electrophoresis (MLEE) was extensively used in order to study the genetic difference within rhizobia species and to evaluate the genetic structure (Martinez-Romero and Caballero-Mellado

1996). However, the DNA-based molecular techniques have been used for studying the diversity of rhizobia natural populations. DNA-typing methods have been widely employed in these studies, offering greater discrimination and reproducibility for strain characterization than the usual phenotyping approaches. This is the case for methods based on the polymerase chain reaction (PCR) which use randomly generated primers (RAPD) to amplify DNA polymorphisms (Harrison et al. 1992) or primers to amplify conserved repetitive DNA sequences such as Enterobacterial Repetitive Intergenic Consensus (ERIC) or Repetitive Extragenic Palindromic (REP) sequences (Castro and Ferreira 2006) to evaluate genetic diversity of rhizobial population. Also, another method, the M13-PCR fingerprinting, which uses a single primer specific to a particular sequence of the gene encoding the core protein of wild-type phage M13 and has been applied to genomic profiling of different microorganisms, was successfully used for rhizobia genotyping (Kure et al. 2002; Dourado et al. 2009).

Restriction fragment length polymorphism (RFLP) methodology used in combination with a variety of DNA probes has been frequently employed to evaluate the genetic diversity and variation within the population of rhizobia species (Young and Wexler 1988; Bromfield et al. 1998). Analysis of the finger printings obtained by PCR-RFLP of the 16S rRNA gene or by symbiotic genes (*nod* and *nif*) has been used to discriminate between rhizobia at the species level and to deduce phylogenetic relationships (Lorite et al. 2012). Also, PCR-RFLP analysis of the intergenic spacer region (IGS) between the 16S and 23S rRNA genes has been used with success to detect genetic variation within a particular rhizobial species (Bala et al. 2002). In addition, genotypic methods, including DNA sequencing of the 16S or 23S rRNA genes, are valuable for assessing evolutionary relationships between rhizobia (van Berkum and Eardly 1998). Normand et al. (2007) pointed out that the diversity of symbiotic bacteria is connected with the distribution and evolutionary history. In the meantime, horizontal transfer of symbiotic genes between strains belonging to several rhizobial species, even different genera, has been noticed in nature and it is assumed to be a significant mechanism for the evolution of new symbiotic species. Also, vertical transfer is one more and widespread mechanism in rhizobia to preserve their nodulation skill (Vandamme et al. 2002; Moulin et al. 2004; Wang et al. 2009).

2.3 Symbiotic Effectiveness

Symbiotic relationships involving rhizobia depend on chemical signals between the two organisms. These signals define the “rhizobia–host specificity” in the relationship. Selecting for the optimal combination of *Rhizobium* and the host legume usually results in more effective symbiosis and better growth of the host legume plant. The host range is determined by the production of lipo-chito-oligosaccharide nodulation factors (*Nod* factors) by the rhizobia. These factors play a crucial role in the molecular signal exchange, infection, and induction of symbiotic responses in legumes, leading to the formation and development of nodules and finally to the differentiation of the bacteria into bacteroids surrounded by plant membrane. It is now more obvious that within these symbiosomes, rhizobia fix atmospheric nitrogen and supply it to their respective host plant, thereby promoting plant growth independently of the available soil nitrogen (Broughton et al. 2000; Perret et al. 2000).

There are two types of mature nodules, called determinate or indeterminate. The first ones are typical of some tropical and subtropical legumes (e.g., soybean and bean) and are characterized by a round-shaped appearance, beginning of nodule primordia in the outer cortex, and meristematic activity that disappears early after nodule initiation. Oval-shaped, indeterminate nodules generally form on roots of temperate legumes (e.g., pea, alfalfa, and vetch), nodule primordia initiate in the inner cortex, the meristematic activity is constant, and the central tissue consists of different zones (Crespi and Gálvez 2000). *Medicago truncatula* (Bell et al. 2001) and *Lotus japonicus* (Kawasaki and Murakami 2000) are currently considered the best model legumes. Their genomes sequences will contribute to efficiently determine plant responses, taking place during all phases of nodule development.

Despite, the great numbers of rhizobial strains that nodulated host legumes and the high level of genetic diversity among them (Bala et al. 2003), only few strains are symbiotically effective on their hosts (Gibson et al. 1975; Vincent 1981). The tolerance of the hosts may not guarantee effectiveness in N_2 fixation and may lead to the development of ineffective nodules. For example, Nutman and Ross (1970) estimated that 15 % of rhizobia isolated from a Rothamsted soil were ineffective or poorly effective in N_2 fixation. Similar results were obtained by Ferreira and Marques (1992) with rhizobial isolates of subterranean clover, collected from several Portuguese soils. An equilibrium is consequently required between the need for diversity of gene pools and the existence of effective microsymbionts for any particular legume host, when nodulated by some competitive strains, which are not greatly evolved to fix N_2 with these legumes (Andrade et al. 2002). An illustrative example of the natural rhizobia population was isolated from the nodules of different *Medicago sativa* from the southern Portugal (Rodrigues et al. 2010). This genus is an important forage legume that comprises perennial and annual species, such as *M. sativa* L. and *M. polymorpha* L., respectively. *M. sativa* and its symbiont *E. meliloti* have a long history of coexistence and coevolution. In a region where *M. sativa* has been cultivated for a long time, the authoctone nodulating population of *E. meliloti* has a very important role in satisfying the nitrogen needs of the plants. However, autochthonous populations of rhizobia isolated from *M. sativa* could be related to *E. medicae*. On the other hand, *M. polymorpha* is native to the Mediterranean basin (including Portugal), where it grows spontaneously, being natural and effective nodulation restricted to *E. medicae* (Garau et al. 2005). High genetic diversity was verified among the rhizobial population, being 73 % of the isolates identified as *E. medicae* and only 18 % as *E. meliloti.* Symbiotic effectiveness showed differences among *Ensifer* sp., strains tested. Strains of *E. medicae* were more effective with *M. polymorpha* than with *M. sativa*, although these strains had been isolated from the last host. The experimental results showed that *E. meliloti* strains had lower values of symbiotic effectiveness with both *Medicago* species compared to *E. medicae.*

The rhizobia population in the legumes cultivated soils varied from <10 to 10^7 per gram of soil (Ferreira et al. 2010). This population may also be phenotypically or genotypically varied, and correspond to a precious resource for the selection of bacteria for use as inoculants intended to capitalize legume crop productivity. In this regard, a global research effort has decided on the selection or development of bacterial inoculant strains that have high N_2-fixing efficiency in association with the host plant (Herridge et al. 2002; Simon et al. 2014). An additional central feature that has received much consideration relates to the capacity of inoculant strains to fight for infection of the host legume with rhizobia resident in soils. It is known that even when legumes are inoculated with an inoculant strain selected for high N_2-fixing ability, they may still become infected by resident soil strains (Sadowsky and Graham 1998; Graham 2008; Yates et al. 2011). The high quality of strains to eventually inhabit a nodule is influenced by the host plant, the strain of rhizobia, the environment, and the interactions between them. Also, management can influence nodule occupancy (McInnes and Haq 2003; Howieson and Ballard 2004). On the other hand, it is known that a variety of genes which affect motility (Mellor et al. 1987), nodulation efficiency (Boundy-Mills et al. 1994), cell surface characteristics and exopolysaccharide synthesis (Laguerre et al. 1992), and toxin production (Triplett and Sadowsky 1992) can affect the nodulation outcome. The great number of rhizobial genomes that are currently being sequenced could be particularly useful to comprehend the process of selective nodulation. Recently, several researches have shown that many legumes had the ability to be infected by various rhizobial species when they grew in different regions (Han et al. 2008; Man et al. 2008; Akhtar et al. 2010; Fentahun et al. 2013), and in some cases, clear correlations have been observed between the rhizobial species and ecological regions (Gu et al. 2007; Tian et al. 2007; Jaramillo et al. 2013).

3 Soil Environmental Factors Affecting Biological Nitrogen Fixation (BNF)

The impact of climate changes on biota has recently gained prominence, given the significant concern toward global warming and the consequent increase of environmental stress conditions. With the predicted increasing temperature and rainfall distribution, it is expected that this problem will be not only intensified but also dispersed worldwide in a very near future. Therefore, increasing the contribution of BNF will be a significant challenge toward a more sustainable agriculture. Legume fixing nitrogen is no less vulnerable to environmental stresses than plants depending on mineral nitrogen, and sometimes they can be even more negatively affected. Several studies have found clear correlations between rhizobial species and ecological regions, indicating that symbioses result from the interaction between legumes, rhizobia, and environmental factors (Fierer and Jackson 2006). Environmental factors affect the genetic and phenotypic characteristics of the rhizobial population present in soil and can influence all aspects of nodulation and symbiotic N_2 fixation, in some cases reducing rhizobial survival and diversity in soil; in other cases environmental factors may affect nodulation, nitrogen fixation, or even the growth of host plants. The range of genetic diversity for tolerating the environmental stresses is poorly known for both the plants and microsymbionts. Therefore, the search for stress tolerant combinations of rhizobia and leguminous plants is an issue of major importance.

Any environmental factor that adversely affects the growth of rhizobia or the host plant may have a strong impact on symbiotic nitrogen fixation. The most significant abiotic factors include soil pH, temperature, salinity, and heavy metals. However, other factors such as the soil water content, desiccation (drought), and soil mineral contents are also very important and have great influence on the symbiosis, as well as on the individual growth and survival of the partners (Graham and Vance 2000; Sadowsky 2005; Arrese-Igor et al. 2011). Moreover, plants and bacteria adopt distinct strategies to cope with these stress factors. In the case of rhizobia, the induction and repression of nodulation genes are tightly regulated and are a major factor influencing the response to environmental factors, as well as host specificity (Spaink et al. 1987; Talbi et al. 2012).

3.1 Soil pH

The influence of soil pH on nodulation has been extensively investigated, partly because of the wide occurrence of acidic soils all over the world (Hungria and Vargas 2000; Ayalew, 2011; Fentahun et al. 2013). Soil acidity restricts agricultural production over more than 1.5 Gha worldwide, and it is expected that the problem will increase as a result of acid rain and long-term nitrogen fertilization (Graham and Vance 2000). Thus, it can be considered that as much as 25 % of arable lands on the planet are affected by problems associated with soil acidity (Munns 1986).

Low soil pH is commonly recognized as an indicator of conditions under which some other soil properties may limit crop growth, rather than as the primary reason for poor growth. Besides the direct effects of soil acidity, growth of legumes may be indirectly diminished through the impairment of nodulation and nitrogen fixation. In fact, legumes and their rhizobia exhibit diverse response to soil acidity. Most legumes require a neutral or slightly acidic soil for growth, especially when depending on symbiotic nitrogen fixation (Bordeleau and Prevost 1994; Fentahun et al. 2013). However, differences in acid soil tolerance by legumes have been reported and found that *Medicago sativa* are extremely sensitive to acidity (Rice et al. 1977). On the other hand, rhizobia may tolerate soil acidity differently from their host partners and may even be more sensitive to low pH (Hungria and Vargas 2000). In these conditions, the nodules formed by rhizobia may not fix nitrogen or have an inadequate fixation rate. Indeed, in many instances, it is the incapacity of the microsymbiont to thrive under acidic conditions that diminishes the symbiotic effectiveness. Brockwell et al. (1991) observed a large decrease in the number of *E. meliloti* in soils with pH below 6.0 (with only 37 cells g^{-1} soil) when compared to those with pH above 7.0 (with 8.9×10^3 cells g^{-1} soil). Also, it has been shown that competitive interactions may be influenced by soil pH. In general, strains of *Bradyrhizobium* are more acid tolerant than those of *Rhizobium* (Date and Halliday 1979; Sadowsky and Graham 1998). Therefore, soil acidity may be considered one of the most relevant environmental factors limiting the establishment of the rhizobia-legume symbiosis, mainly because acidity limits rhizobia survival and persistence in soil, leading to reduced or inexistent nodulation (Zahran 1999). Consequently, the selection of rhizobial strains tolerant to acidity may improve the tolerance of legumes to low pH, by allowing an efficient symbiotic nitrogen fixation under acid conditions. However, the relationship between soil acidity, rhizobia competitiveness, and the ability to survive in acid soils is not always straightforward (Sadowsky 2005). For instance, many rhizobia from acid soils were found to be sensitive to acidity (Richardson and Simpson 1989), and it was suggested that soil microniches could protect these rhizobia from extreme values of soil pH. Thus, the simple isolation of rhizobial strains from root nodules of plants grown in acid soils is not a guarantee that these strains will be acid resistant (Graham 1992).

Acidic soils are often associated with increased aluminum and manganese toxicity since these metals become more soluble at lower pH; this can also contribute to the inhibition of growth and persistence of rhizobia in acid soils (Coventry and Evans 1989; Hungria and Vargas 2000). Moreover, the levels of calcium and phosphorus in the soil are also influenced by soil pH and may also affect the growth and survival of rhizobia in acid soils (Bell et al. 1989). Several studies point out that the rhizobial tolerance to acidity is often correlated with the ability of each strain to maintain internal pH approaching neutrality (pH 7.2–7.5) (Chen et al. 1993; Graham et al. 1994). It has been concluded that the acid tolerance capability could be due to several traits like acid-shock responses (Bhagwat and Apte 1989), exopolysaccharide production (Cunningham and Munns 1984), and glutathione production (Riccillo et al. 2000; Muglia et al. 2007). Rhizobia employ various mechanisms for maintaining the intracellular pH, including (1) decreased membrane permeability, (2) internal buffering, (3) amelioration of external pH, (4) proton extrusion/uptake, and (5) prevention of metal ion toxicity (Dilworth and Glenn 1999). Using proteomics approaches, several researchers have shown that approximately 20–50 rhizobial genes are predicted to be involved in acid tolerance, out of which about 15–20 may be essential for growth at low pH including *actA*, *actP*, *actR/S*, *exoH*, and *exoR* (Reeve et al. 2002; Vinuesa et al. 2003; Reeve et al. 2006; de Lucena et al. 2010).

3.2 Soil Temperature

Symbiotic nitrogen fixation is strongly affected by temperature (Hungria and Franco 1993) and nodulated legumes grow optimally within narrower temperature ranges than those receiving mineral nitrogen (Lie 1981). High temperature is a major factor limiting nitrogen fixation in tropical, and even in some subtropical regions. High temperature can be considered a major cause for the failure of nodulation, limiting rhizobial growth and survival in soil and affecting all stages of symbiosis (Piha and

Munns 1987; Hernández-Armenta et al. 1989; Hungria and Vargas 2000). High temperature may also contribute to undesirable change in rhizobia, including plasmid deletions, genomic rearrangements, and decreased diversity (Hungria and Vargas 2000). Moreover, temperature has a strong influence on the survival and persistence of rhizobia in soils. For example, rhizobia from a dry soil of West Africa grow at 37 °C, and more than 90 % of the strains isolated from the same region could grow well up to 40 °C (Eaglesham et al. 1991).

The influence of temperature on rhizobia appears to be strain dependent. For example, *Bradyrhizobium* sp., was less susceptible than *R. leguminosarum* bv. *trifolii* to high soil temperatures. Also, Hungria and Franco (1993) demonstrated that high temperatures (35 °C or above) can affect the symbiosis of *P. vulgaris*-*R. leguminosarum* bv. *phaseoli* and the strains tested were not able to fix N_2 under stress conditions. Soil temperature also strongly affects competition for nodulation (Triplett and Sadowsky 1992) and has great influence on specific strain and cultivar interactions (Arayankoon et al. 1990). It seems that each legume-rhizobia combination has an optimum temperature, which is about 30 °C for clover and pea, between 35 and 40 °C for soybean, peanut, and cowpea, and between 25 and 30 °C for common bean (Long 2001). High soil temperatures may result in the death of many rhizobia originating from temperate climates. However, strains from tropical regions generally survive better at high soil temperatures. Additionally, temperature-tolerant rhizobial strains can be selected either naturally (Zahran et al. 1994) or artificially (Hartel and Alexander 1984; Karanja and Wood 1988). However, it has been shown that excessive temperature shock can cure plasmids in fast-growing strains, and that some strains isolated from high-temperature environments have a Fix^- phenotype (Moawad and Beck 1991; Hungria and Franco 1993).

3.3 Soil Salinity

Soil salinity affects over 800 Mha of arable lands throughout the world (Munns and Tester 2008), and this area is increasing. Salinity impacts agricultural production in arid and semi-arid regions, where rainfall is limited and insufficient to ensure the transport of salts from the plant root zone (Tester and Davenport 2003). Both legumes and the process of nodule initiation are more sensitive to salinity and osmotic stress than the rhizobia (Graham 1992). For instance, in free-living conditions, some strains of rhizobia, such as chickpea rhizobia, tolerate 500 mM NaCl but others cannot grow even when the NaCl concentration is lowered to 100 mM NaCl (Elsheikh and Wood 1990; Zucuk and Kivanc 2008). However, the nodulation and nitrogen fixation in some symbioses can be inhibited between 50 and 100 mM salt (Delgado et al. 1994).

Legumes are considered as salt-sensitive crop species (Lauchli 1984). Their production is particularly affected by salt stress since these plants depend on symbiotic nitrogen fixation to meet their nitrogen needs (Elsheikh and Wood 1995). This reduction in productivity is associated with minor growth of host plants, poor symbiotic performance of root-nodule bacteria (Georgiev and Atkins 1993), and consequently, a reduction in the nitrogen fixing capacity (Delgado et al. 1993; Zahran 1999). Besides these physiological responses, salt stress speeds up the greening of nodules and reduces the leghemoglobin content (Delgado et al. 1993), which is regarded as an indicator of nodule senescence. On the other hand, rhizobia exposed to salt stress may undergo morphological changes, leading to modifications in cell morphology and size or alterations in the pattern of exopolysaccharides (EPS). The latter responses may have an impact on the symbiotic interaction because EPS and LPS (lipopolysaccharides) are necessary to establish symbiosis.

The ways in which bacteria and plants are affected by salinity are (1) the induction of ionic stress due to the high concentration of ions; and (2) the induction of osmotic stress through the changes in the solute concentration around the cells, producing a water deficit and desiccation (Demir and Kocacaliskan 2002). Response and adaptation to salinity stress is a complex phenomenon involving many physiological and biochemical processes which probably reflect changes in gene expression. Several genes were identified as being involved in rhizobial salt stress responses, contributing to a better knowledge of the mechanisms that influence rhizobia salt tolerance (Brígido et al. 2012; Dong et al. 2013). In recent years, strategies to reduce the effect of salt stress on the production of legumes have been based on the selection of host genotypes tolerant to salinity (Cordovilla et al. 1995). Therefore, the use of rhizobia with increased tolerance to salinity might constitute another approach to improve the productivity of plants under symbiosis.

3.4 Soil Metal Contamination

Contaminated soils are increasing gradually and the pollution due to heavy metals and metalloids has received a great attention all over the world. The most important sources of heavy metals pollution are mining, industries, and use of metal-containing pesticides, fertilizers, and sewage sludge. Heavy metals such as Zn, Cu, Ni, and Cr are essential or beneficial micronutrients for plants, animals, and microorganisms and others such as Cd, Hg, and Pb have unknown biological and/or physiological functions, being toxic even in small concentrations (Castro and Ferreira 2006). In polluted soils the presence of these toxic inorganic compounds has an important impact on the resident microflora, which seems to be much less varied in these areas. So, pollution can lead to a reduction in microbial diversity due to the disappearance of species that do not have sufficient tolerance to the stress imposed, and on the other hand, may increase the population of other species that grow well under stress (Denison and Kiers 2011). Microorganisms are the first biota affected directly and indirectly by heavy metals that are in available forms in soil solution or weakly adsorbed on soil colloids.

Numerous past studies have revealed that metals negatively influence microorganisms affecting their growth, diversity, morphology, and activities (Khan and Scullion 1999; Oliveira and Pampulha 2006), including symbiotic N_2 fixation (Castro et al. 1997; Lakzian et al. 2002; Zornoza et al. 2002; Pastor et al. 2003; Castro and Ferreira 2006; Pereira et al. 2007). However, microorganisms within the species of the same genus or within strains of the same species can be different in their sensitivity toward the metals (Castro et al. 2003). For example, Giller et al. (1993) verified that *E. meliloti* was less sensitive, in terms of growth, to Cd than *R. leguminosarum* and *M. loti*. Similar results were reported by Carrasco et al. (2005) and Pajuelo et al. (2011) with alfalfa plants, being reduced only the total number of nodules, probably due to a decrease in the number of rhizobial infections. Nevertheless, the N_2 fixation was not affected by As and heavy metals in soil. Analogous results have been obtained with *Bradyrhizobium* strains isolated from *Lotus uliginosus* growing in a polluted soil (mainly with Hg and As) from an industrial area of Portugal, which showed great diversity and high symbiotic effectiveness with their host legume (Castro et al. 2008; Castro and Ferreira 2010). The levels of tolerance of *Bradyrhizobium* strains toward the As and Hg could be comparable to those reported by previous researchers (Pereira et al. 2007; Oliveira et al. 2008). The existence of heavy metal multiple tolerance in rhizobia has been shown by Pereira et al. (2007) and stated that the *Rhizobium* population isolated from the deactivated lead mine soil had high tolerance to Pb and also to other metals (As, Cu, Ni, and Co). This fact suggests that the selective pressure induced by Pb made possible the development of resistance mechanisms that confer tolerance to different metal stresses as corroborated by Diaz-Ravina et al. (1994) and Nies (2003).

Interactions with metals depend not only on the particular element but also on its chemical speciation (Valls and Lorenzo 2002). For example, As is not essential for plants, though growth is stimulated when supplied at low concentrations (Carbonell et al. 1998). In the case of microorganisms they have a specific or a set of resistance mechanisms for each metal, which are encoded by chromosomal genes (Bruins et al. 2000; Nies 2003). Also, microbial metal uptake in contaminated soils must be well regulated so as to avoid any toxic effects to the cells. The efficiency of these mechanisms depends on several parameters, such as the metal itself, the species studied, time, temperature, pH, existence of certain plants, as well as the interactions of the metal with other compounds. One of the metal resistance mechanisms is commonly associated with the transport-related membrane proteins that mediate bacterium's direct metabolic interactions with the soil harsh conditions. In the past years, several researchers demonstrated the existence in rhizobia of heavy metal resistance and reported the detection of gene clusters in *Rhizobium* strains that are regulated by heavy metals (Yang et al. 2005; Sá-Pereira et al. 2009). Similarly, the function of *ABC* transporters in the resistance genetic systems in rhizobia, namely *M. loti* and *E. meliloti* strains, seems to involve translocation of lead through a pore formed by two integral membrane protein domains (Sá-Pereira et al. 2009). In fact, efflux systems pump the toxic ions out of the cell, avoiding their increase that could prevent the growth, or cause cell death. This mechanism is seen as the most widespread of heavy-metal resistance (Silver 1998). On the other hand, when studying arsenic resistance in *E. meliloti*, Yang et al. (2005) showed that the bacteria reduce arsenate to arsenite and turn arsenite out the cell. This trait is the way that some microorganisms have to resist to the arsenate attack, developing or acquiring genes that consent the cell to neutralize the toxic effects of arsenic through the elimination of arsenic from the cells. In addition, Sá-Pereira et al. (2007) identified two arsenic resistance genes, *arsA* and *arsC,* in *M. loti* strains isolated from nodules of legumes growing in contaminated soils. Therefore, these strains have the genetic information that lets them stay alive in these harsh habitats.

However, rhizobial population isolated from nodules of legumes growing in contaminated soils, especially those tolerant to the highest concentration of heavy metals and metalloids, could be a very promising approach for bioremediation experiments in contaminated soils using wild legume plants, since it could have a lower ecologic impact on the zone (Pajuelo et al. 2008; Castro and Ferreira 2010; Mahieu et al. 2011; Ruiz-Díez et al. 2012). This allows the leguminous plant to provide nitrogen to the soil, since nodulation is still efficient. So, these symbioses could have a great patrimonial interest in the biology of conservation and restoration and could contribute to the rehabilitation of contaminated soils helping its fertility. This evidence can also open the possibility of using leguminous plants together with other plants already used in phytoremediation in long-term actions (Lasat 2000).

4 Rhizobia as Plant Growth-Promoting Bacteria

For more than a century, rhizobia have been primarily known for being efficient suppliers of nitrogen to their legume hosts. However, multiple evidences obtained in recent decades have shown that rhizobia may also benefit plants by other processes. It has been shown that rhizobia can naturally associate with nonlegume plants and positively affect plant growth through mechanisms independent of symbiotic nitrogen fixation, acting as plant growth promoters (Vargas et al. 2010). Plant growth-promoting activities have been reported in members of many other groups of soil bacteria, including the genera *Azospirillum*, *Azotobacter*, *Bacillus*, *Gluconacetobacter*, *Klebsiella*, *Paenibacillus*, and *Pseudomonas*, among others. These bacteria, which have the ability to colonize plant roots and to stimulate plant growth, are designated as plant growth-promoting rhizobacteria (PGPR). Most PGPR are rhizosphere colonizers, although some genera also include endophytic species (Antoun and Prévost 2006). The main strategies used by PGPR to stimulate plant growth include the production of phytohormones, inhibition of ethylene synthesis by plants, improved availability of nutrients, stimulation of mechanisms conferring disease resistance, and protection against soil-borne phytopathogens (Pérez-Montaño et al. 2014).

4.1 Association of Rhizobia with Nonlegumes

It has recently been proposed that the rhizobial lipo-chito-oligosaccharide Nod factors, the key molecules for the establishment of rhizobia-legume symbioses, can be recognized not only by legumes, but also by plants in general, via a mechanism that results in the suppression of the innate immune response (Liang et al. 2013). In fact, the association of rhizobia with nonlegume plants has been extensively observed, particularly in situations involving either crop rotation or the simultaneous occurrence with legumes. Endophytic strains of *R. leguminosarum* bv. *trifolii* were found associated with the roots of rice in the Nile delta, where this crop has been grown in rotation with berseem clover for over seven centuries (Yanni et al. 1997). Photosynthetic *Bradyrhizobia* were isolated from the roots of African wetland wild rice, a species that often cohabits with an aquatic legume associated with photosynthetic bradyrhizobia (Chaintreuil et al. 2000). Endophytic strains of *R. etli* bv. *phaseoli* were recovered from roots of maize traditionally cultivated in association with common bean in Mesoamerica (Gutiérrez-Zamora and Martínez-Romero 2001). Webster et al. (1997) demonstrated the intercellular colonization of rice and wheat roots by a strain of *Azorhizobium caulinodans*. Other reports describe the association of endophytic rhizobia with the roots of sweet corn and cotton (McInroy and Kloepper 1995), as well as barley, wheat, and canola (Lupwayi et al. 2004). Recently, strains of *R. miluonense* were isolated from the rhizosphere and surface-disinfected roots of annual ryegrass grown in soil samples collected from natural pastures in central Portugal (Castanheira et al. 2014).

4.2 Production of Phytohormones and Other Plant Growth Regulators

Phytohormones are low molecular mass, organic molecules that regulate plant growth and development. These compounds act at very low concentrations and their biosynthesis in plants is extremely regulated. Several plant-associated bacteria, including rhizobia, are able to produce and export phytohormones. By doing so, these bacteria can disturb the hormone balance of the plants and interfere with their development, with either beneficial or harmful consequences (Dobbelaere et al. 2003). Five major groups of phytohormones are recognized: auxins, gibberellins, cytokinins, abscisic acid, and ethylene. Auxins are the best known plant growth-promoting hormones, particularly indole-3-acetic acid (IAA), which is synthesized from the amino acid tryptophan present in root exudates (Spaepen and Vanderleyden 2010). Bacterial production of auxins may induce changes in root architecture, increasing the total root surface, enhancing nutrient uptake and augmenting root exudation (Dobbelaere et al. 2003).

The capability of rhizobia to promote the growth of plants by the production of phytohormones has been thoroughly evidenced. Inoculation of rice with phytohormone-producing strains of *Rhizobium* sp. and *R. leguminosarum* bv. *trifolii* has resulted in increased growth and yield. The observed beneficial effects include enhanced seedling emergence, radical elongation, root and leaf areas, plant dry mass, photosynthetic rate, grain and straw yields, nutrient uptake, and harvest index (Yanni et al. 2001; Peng et al. 2002). Increases in shoot growth, root length, and nutrient concentration in root tissues upon inoculation with IAA-producing *E. meliloti*, *R. leguminosarum* bv. *viciae*, and *B. japonicum* were also recorded with sorghum (Matiru et al. 2005). Inoculation of canola and lettuce with *R. leguminosarum* improved early seedling root growth; this effect was related with the bacterial production of IAA, and possibly cytokinin (Noel et al. 1996). It was also shown that *R. leguminosarum* strains producing IAA could increase the yield and the seedling and fruit quality of tomato and pepper plants (García-Fraile et al. 2012). Growth enhancement and flowering induction in mango trees inoculated with *Burkholderia caribensis* and *Rhizobium* sp. were attributed to rhizobial production of phytohormones (Santos-Villalobos et al. 2013). Increased root growth and uptake of nutrients were also observed upon inoculation of cotton with *B. japonicum* producing IAA (Hafeez et al. 2004). Likewise, the stimulation of barley early growth by a non-phytopathogenic strain of *R. radiobacter* (formerly *Agrobacterium radiobacter*, Lindström and Young 2011) was correlated with the production of gibberellic acid (Humphry et al. 2007).

Other molecules produced by rhizobia may influence seedling development and plant growth (Mehboob et al. 2009). For instance, it was shown that lipo-chitooligosaccharide Nod factors could favor the germination of seeds, the mass and length of roots, the production of photosynthates, and grain yield in various nonleguminous plants (Smith et al. 2002; Miransari and Smith 2009). Rhizobial Nod factors may also promote root colonization by arbuscular mycorrhizal fungi, and thus stimulate the establishment of mycorrhizal symbioses (Xie et al. 1995). Rhizobia also produce riboflavin, which is readily transformed into the signaling molecule lumichrome either through enzymatic or photochemical processes. Several evidences have shown that lumichrome may act as a plant growth promoter, increasing root respiration and whole-plant carbon assimilation in alfalfa (Phillips et al. 1999), or stimulating seedling development and plant growth in legumes and cereals (Dakora et al. 2002; Matiru and Dakora 2005). Rhizobial production of EPS may also be related with plant growth promotion, as suggested by the increase in the growth of water stressed sunflower plants inoculated with *Rhizobium* sp. producing EPS (Alami et al. 2000).

4.3 ACC-Deaminase Activity

Several rhizobacteria synthesize 1-aminocyclopropane-1-carboxylate (ACC) deaminase, the enzyme that converts ACC, the direct biosynthetic precursor of ethylene in plants, into ammonia and α-ketobutyrate (Nascimento et al. 2014). Ethylene is a strong regulator of plant growth and development; however, if present in high concentrations, ethylene may inhibit root elongation. By lowering the levels of ethylene, ACC-deaminase activity by rhizobacteria may prevent the inhibitory effects on root elongation and facilitate plant growth (Glick et al. 1998). Ethylene also inhibits nodulation in several legumes, and hence rhizobia having ACC-deaminase activity may represent an additional advantage for the success of nodulation; indeed, it was shown that rhizobial ACC-deaminase modulates the levels of ethylene during the early stages of nodule development and promotes nodulation (Ma et al. 2003, 2004).

4.4 Solubilization of Inorganic Phosphate

Most phosphorus in soil or applied to the soil as fertilizer is immobilized as insoluble or very poorly soluble forms, and thus is unavailable to plants. Phosphate solubilizing bacteria can transform the soil insoluble mineral phosphates into soluble forms, mainly through the production of organic acids and acidification of the surrounding soil, increasing the availability of phosphorus to plants (Dobbelaere et al. 2003). The capability of rhizobia to solubilize inorganic phosphate and the growth-promoting effects on various plants has been extensively reported (Antoun et al. 1998; Alikhani et al. 2006; Sridevi and Mallaiah 2009; Vargas et al. 2009). For instance, inoculation of lettuce and maize with phosphate-solubilizing *R. leguminosarum* bv. *phaseoli* resulted in increased plant dry matter (Chabot et al. 1996; 1998). Also, inoculation of barley and chickpea with a strain of *M. Mediterranean* able to mobilize insoluble phosphate resulted in substantial increments in plant growth and phosphorus content (Peix et al. 2001). These and other studies came to show that, besides providing symbiotically fixed nitrogen to their host legumes, rhizobia may also play a relevant role in the nutrient balance of both legumes and nonlegumes by increasing phosphorus availability, especially in phosphorus-deficient soils where the lack of this element may be a major restricting factor for plant growth.

4.5 Production of Siderophores and Antagonism Against Plant Pathogens

Iron is essential for both microbial and plant growth. However, although total iron in soils is normally quite abundant, it occurs predominantly in the form of crystalline and amorphous iron oxides and other iron minerals, hence being relatively unavailable for direct bio-assimilation. Soil microorganisms may circumvent this problem by releasing low molecular mass, high-affinity iron-chelating molecules, known as siderophores. These molecules scavenge the iron trapped in soil minerals and form soluble Fe^{3+}-complexes, which can be transported into the cell cytoplasm where the iron becomes available for microbial growth (Neilands 1981; Hider 1984). By increasing the mobility of soil iron in the rhizosphere, siderophores may enhance its availability for plants, although there is some controversy regarding the direct use by plants of iron bound to microbial siderophores (Dobbelaere et al. 2003). On the other hand, the removal of most iron from the rhizosphere may inhibit the proliferation of plant pathogens due to iron starvation, ultimately resulting in their exclusion from the root vicinity. Thus, when associated with siderophore-producing microorganisms, plants may become better skilled against soil-borne phytopathogens.

Microbial siderophores may be classified into three major types: catecholates, hydroxamates, and carboxylates. The ability to produce siderophores is widespread among soil bacteria, including rhizobia (Dilworth et al. 1998; Chandra et al. 2007;

Vargas et al. 2009), and inhibition of plant pathogens by rhizobial strains producing siderophores was demonstrated in some studies. For instance, a strain of *M. loti* secreting a hydroxamate-type siderophore was shown to have a strong antagonistic effect against *Sclerotinia sclerotiorum*, a white rot pathogen of *Brassica campestris* (Chandra et al. 2007). Likewise, strains of *E. meliloti* producing siderophores were able to inhibit *Macrophomina phaseolina*, the widely occurring plant pathogen causing charcoal rot in groundnut (Arora et al. 2001).

Many other reports describe the role of rhizobia as biocontrol agents in the suppression of plant diseases, although without establishing a direct link with the production of siderophores. The antagonistic activity of *E. meliloti* and *B. japonicum* toward *M. phaseolina*, *R. solani*, and *Fusarium* spp. with reduction of infections in sunflower, okra, soybean, and mungbean was reported by Haque and Ghaffar (1993). Other examples are the biological control of *Phytium* damping-off of sugar beet and pea by *R. leguminosarum* bv. *viciae* (Bardain et al. 2004), and the protection of chickpea from *M. phaseolina* root rot by *Rhizobium* sp. (Akhtar and Siddiqui 2008). In the latter case, the reduction of fungal infection was attributed to colonization of the growing hyphae by the rhizobial cells, preventing the contact between the pathogen and the host root tissue. Rhizobia may also reduce plant diseases by activating plant resistance mechanisms (induced systemic resistance) in similar fashion like other PGPR (Siddiqui 2006). An example is the *Rhizobium*-mediated induction of phenolic compounds in rice, particularly in *Rhizoctonia solani* infected plants; such compounds are known to mediate induced systemic resistance and provide protection against infection by pathogens (Mishra et al. 2006).

5 Nitrogen Fixation in Natural and Sown Pastures

BNF plays a major role in the improvement of agricultural sustainability, particularly with regard to the contribution of pasture legumes (Sequeira 2008). In fact, pasture legumes are among the most efficient leguminous plants in terms of nitrogen fixation and, depending on adequate management and on the establishment of effective symbioses with adequate rhizobia, they may contribute to high input rates of fixed nitrogen into the soil (Materon 1988). A successful example of the BNF contribution in pastures is given by a particular agrosilvopastoral system in the Mediterranean area of the Southern Iberian Peninsula. The system, which is designated "montado" in Portugal or "dehesa" in Spain, represents the most extended agroforestry system in Europe, covering more than 3.5 million hectares over the west, south-west, and central parts of the Iberian Peninsula (Olea and San Miguel-Ayanz 2006). The "montado" has been developed for a long time on poor or non-agricultural land, based on extensive livestock production associated with the exploitation of cork and holm oaks. Both natural and sown pastures are implemented among scattered oak trees and support the direct grazing by cattle and sheep. The Mediterranean climate is characterized by hot and dry summers, usually lasting for several months, and cool winters with irregular, often scarce rainfall; the same type of climate is found at middle latitudes in all continents, including large areas of West Asia, Australia, and North Africa (Saxena 1988). Desertification is a common situation, particularly in regions where the precipitation regime is more inconsistent, resulting in progressive degradation of the vegetation cover and erosion of surface soil. As a consequence, soils in the "montado" ecosystem are generally poor, deficient in phosphorus and calcium, and contain low levels of organic matter, making arable and intensive farming unsustainable.

A recent study investigated the role of BNF on a range of long-term natural pastures in the "montado" ecosystem of Southern Portugal, covering different edapho-climatic environments (Ferreira and Castro 2011). Legume yields and BNF in field conditions were evaluated in 36 sites, using the isotopic ^{15}N-dilution technique to access the amount and percentage of nitrogen derived from BNF. Although the amounts of fixed nitrogen were highly variable among sites, the results showed that nitrogen fixation was closely linked to the legume biomass production (Table 2). On average, nitrogen fixation contributed with 25 kg of nitrogen per 1000 kg of shoot dry biomass, a value that is similar to other field measurements undertaken at Mediterranean-type environments in Australia (Peoples et al. 2001; Baldock and Ballard 2004). The percentages of nitrogen derived from BFN were generally high (87–89 %) and similar between sites, despite the large diversity of native legumes

Table 2 Legume yields and amounts of fixed nitrogen in long-term natural pastures in the "montado" ecosystem of southern Portugal

Soil		Site	Shoot yield (kg/ha)	Amount of fixed N (kg/ha)	Soil		Site	Shoot yield (kg/ha)	Amount of fixed N (kg/ha)
Schist	1	Castro Verde	42	1.0	Granite	1	Alter	90	2.5
	2		724	16.6		2		30	0.8
	3		720	24.1		3		68	1.8
	4		107	3.1		4	Crato	42	1.2
	5		211	6.4		5		125	2.7
	6		14	0.4		6		561	12.8
	7	Crato	42	1.2		7		1003	26.4
	8		125	2.7		8		1632	33.5
	9	Nisa	281	7.7		9	Monforte	178	5.3
	10		252	6.7		10		712	22.5
	11		242	6.6		11		302	6.4
	12	Ourique	0	0.0		12	Portalegre	732	21.9
	13		7	0.1		13		100	2.7
	14		139	2.6		14		772	17.3
	15	Serpa	1346	28.6		15		71	1.6
	16		319	6.8		16		296	6.9
	17		548	16.4		17		919	20.4
	18	Sousel	6	0.1					
	19		88	2.5					

and the differences in edaphoclimatic conditions. It was concluded that BNF in these natural ecosystems provides almost all the nitrogen present in legumes, indicating that the natural symbioses are well adapted to these environments. Nevertheless, the study also confirmed that the legume productivity in these natural pastures is very low, as the result of poor natural flora. In this context, the introduction of improved legumes with higher yield potential and previously inoculated with specific and highly effective rhizobia strains represents an efficient way of increasing productivity.

A model for improving pastures in the "montado" ecosystem started to be developed in Portugal in the late 1960s and has been largely diffused since then, spreading throughout similar Mediterranean environments in southern Europe. The strategy is based on the establishment of biodiverse permanent pastures rich in legumes, by sowing a diversity of selected and improved species, in which inoculated legumes are preponderant. These biodiverse legumes-rich mixtures provide better productivity than the natural flora and are able to renew themselves on a permanent basis (Crespo 2006). At least 30 % of the sown mixtures are made up of hard seed legumes, including a range of annual clovers and annual medics, yellow serradella and biserrula. Inoculation of the legume seeds with specific and effective rhizobia ensures enhanced symbiotic nitrogen fixation in the pasture. This approach has demonstrated marked improvements on soil fertility and rapid build-up of soil organic matter through carbon sequestration, offering superior pasture productivity and animal carrying capacity. Nowadays, it is considered as a powerful management tool for improving pastures yield in the "montado" ecosystem, in which legumes are important components of the strategy for increasing productivity and sustainability, using symbiotic nitrogen fixation as a major process of providing nitrogen to the soils. Therefore, by using a strategy founded on the efficiency and diversification of structures, the "montado" represents a rational form of land use in these environments, taking advantage of every natural resource with minimum inputs of energy and materials (Joffre et al. 1988; Olea and San Miguel-Ayanz 2006). When properly implemented, this multifunctional and versatile system ensures the optimization of available energetic resources through biomass production, circulation of nutrients, conservation of soil and water, and preservation of biodiversity, also contributing to climate bio-regulation or microclimate stability (Trujillo and Mata 2001).

6 Conclusions and Future Prospects

The dependence of agricultural crops and pastures on nitrogen fertilizers inputs, as well as the respective environmental costs, underscores the importance of BNF by rhizobia-legume symbiosis, mainly due to the benefit of being environmentally friendly and perfect for sustainable agriculture. Several concerns are important for understanding the cause and mechanisms of BNF, which made it possible for the practical application through rigorous effort. The knowledge about the ecology of different groups of rhizobia will help to envisage their environmental responses, bringing a more practical sense to rhizobial diversity and also to their taxonomy. Moreover, soil populations of rhizobia have a high genetic diversity and a certain set of features such as plant growth-promoting activities, tolerance to soil acidity or salinity, or the ability to degrade pollutant compounds that can be easily operated by selection or genetic manipulation in order to optimize the productivity of legume crops. Another important issue concerns the legume breeding programs, which must give greater emphasis to the symbiosis between hosts and rhizobia. Practical approaches to enhanced nitrogen fixation and improved tolerance to edaphic constraints would also permit lower costs and a more sustainable form of agriculture. Despite decades of advances in knowledge, with the acquisition of a great quantity of information, the physiological and molecular basis for tolerance of *Rhizobium*-legume symbiosis to environmental stress is still partially unknown.

Finally, a better understanding of the diversity and dynamics of native rhizobial populations, and how they influence the establishment of newly introduced strains is essential for the development of highly competitive commercial inoculants. Besides the introduction of inoculants, also agricultural practices may have a major impact on rhizobial diversity, and more efforts should be invested in this issue. In conclusion, the supply of essential nutrients such as nitrogen may be achieved by the combination of a biotechnological approach, using microbial fertilizer, with a low-input technology and is therefore regarded as a sustainable practice.

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Soil Microbial Community Interactions Under Tillage Systems in Australia

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Contents

Abstract For several years suboptimal land management, such as tillage and stubble burning, led to accelerated soil deterioration in Australia. The excessive use of tillage affected crop productivity and decreased soil health. Tillage can alter water and oxygen flow, soil structure, temperature and aggregate formation that directly or indirectly affect soil microbiomes. Microbial communities are involved in the different biogeochemical cycles and soil formation. Alterations to this habitat may compromise the productivity of soils. This chapter shows the impact of tillage on soil physicochemical properties and how these changes can affect the function and diversity of soil microbial communities. A better understanding of the soil properties interaction will help to improve land management and protect our soils from further deterioration.

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1 Introduction

Tillage practices in Australia have been divided into three main practices: no-tillage (NT) or zero tillage, reduced or minimum tillage and conventional or frequent, aggressive tillage. In recent years, the tendency among growers has been towards practising strict minimum disturbance and complete stubble retention in Australia, providing effective protection against soil erosion, improving soil health and crop yields (Holland et al. 1987). Zero or no-tillage practices have reduced seeding times, decreased the risk of soil erosion and increased retention of organic matter (D'Emden et al. 2008). However, the adoption of this system has grown at a slow pace, even though there has been a number of research, development and extension investments to promote and demonstrate its benefits. There is still a lack of confidence in the adoption of such a rigid system due to (1) build-up of stubble-borne diseases; (2) hard-to-kill weeds; (3) weed resistance to herbicides; (4) nutrient stratification and (5) concerns on the effect of the use of herbicides on- and off-site (Thomas et al. 2007). Furthermore, the increase of annual rainfall during recent years in the north-eastern Australia's grains growing region proliferates the appearance of diseases and weed in no-till farming systems and some conventional systems; thus growers will have to adopt new strategies to battle against them. However, the main concern among growers is that tillage will cause serious negative long-term impacts to their system (Crawford et al. 2014; Dang et al. 2015).

Tillage in Australia has evolved from the knowledge of the first European settlers, who had more expertise on fertile soils and high-precipitation weather conditions. History showed that this knowledge was not suited to the Australian environment where precipitation is low and unreliable and evaporation often exceeds rainfall (Russell and Isbell 1986). By the end of the nineteenth century, the consequence of land mismanagement was severe soil erosion; therefore farmers were called to take action. *Sand Drift Act 1923* was created to give power to landowners to take control over their neighbours' land if there was any drifting sand problems (Chisholm and Dumsday 1987). In the 1930s soil erosion declined due to wheat/fallow rotation. As a result, the agricultural ministers with the Council for Scientific and Industrial Research (currently known as CSIRO) formed a committee to make improvements in the use of soils for all states in Australia. The *Soil Conservation Act 1939* was then created with a focus on wind and water erosion, and enforced the preservation of trees on roadsides to protect groundcover, as well as the acquisition of land for creation of reserves for soil conservation. Nine years later, the act was amended and emphasised more on soil conservation education and investigation. Moreover, soil conservation boards were created to contribute with reports and proposals on soil erosion and soil conservation (Chisholm and Dumsday 1987; Davis and Gale 2013).

The long-term use of conventional tillage caused deterioration in soil landscapes, mainly due to inappropriate and excessive tillage, decline in organic matter content, sodicity, erosion and compaction by wheel traffic. As a result, crop water use efficiency decreased and crop yields were affected. During the 1960s, the Queensland Government started a conservation service to decelerate soil erosion. The program aimed at increasing the retention of soil residues and paired field management with its capacity. Throughout this period, zero and no-tillage practices were introduced as a demonstration for soil conservation and enhanced productivity (Thomas et al. 2007). This chapter shows the impact of tillage on soil physico-chemical and biological properties and their impacts on the function and diversity of soil microbial communities.

2 Effect of Tillage System on the Physico-Chemical and Biological Properties of Soil

Tillage and cropping systems clearly have an influence on the soil chemical, physical and biological properties (Fig. 1). The degree of soil disturbance and the use of crop residues influence moisture, temperature, aeration and the availability of

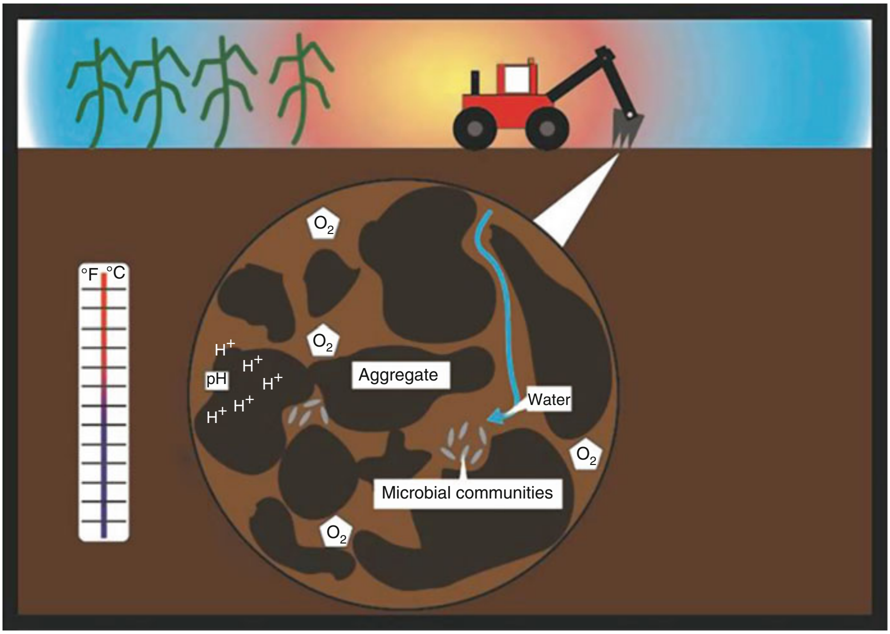

Fig. 1 Impact of tillage on soil physical and chemical properties can alter microbial community interactions. Depending on the type of tillage used, for example it can disrupt soil aggregates and affect porosity, change localised oxygen levels, reduce water-holding capacity and lead to a reduction in microbial biomass

organic compounds and minerals for the soil biota. This conglomerate of elements can affect population, diversity and activity of the different soil organisms. Besides, soil communities also have an impact on soil physical and chemical conditions. From macrofauna to microfauna, all parts interact and therefore play a role in nutrient cycling and organic matter decomposition. Here, we explain how soil porosity, soil moisture and texture, temperature and pH are affected by tillage and its repercussion on microbial communities

2.1 Soil Texture and Soil Structure

Soil texture plays an important role in nutrient management. For instance, finer texture soils tend to have greater capability to store soil nutrients. Soil structure is given by the arrangement of soil particles, which can strongly bind to each other and form aggregates. Soil microorganisms, roots and mineral particles help to form aggregates in soil. They can excrete substances that help to cement soil particles. Roots also excrete sugars that help to bind minerals. Stable aggregates are valuable for productive soils; however, the stability of the aggregate will depend on the minerals present in the soil (Brown 2014).

Soil texture influences the distribution of minerals, organic matter retention, microbial biomass and other soil properties. For instance, the highest contents of soil organic carbon (SOC) and total nitrogen are found in fine-textured soils, whereas the lowest were found on coarse-textured soils (Silver et al. 2000). Matus et al. (2007) observed that soil organic carbon tends to associate with finer fractions of soils like clay-rich soils. Meanwhile, fine-textured soils have more stable aggregates that contain greater amounts of carbon and nitrogen (Raiesi 2006). In comparison to clay soils, sandy soils retain water and nutrients for a shorter period of time (Hamarashid et al. 2010). As well, soil types influence the structure of microbial communities. Fine-textured soils support a higher microbial biomass than coarse-textured soils (Carney and Matson 2005). The distribution of soil microorganisms in soils with different texture and aggregate arrangement stability may be explained by soil moisture and nutrient availability.

2.2 Soil Porosity and Soil Moisture

The use of different tillage practices can change soil physical properties, such as water-holding capacity, pore size distribution and aggregation. The available habitats are demarcated by the topography of the soil pore network. The interaction between organisms will be defined by these delimited spaces and connections between the pores. Tillage evidently affects the size, distribution and topography of pore networks, consequently influencing access to oxygen, substrates and water for the different organisms. For instance, comparing no-till systems over conventional tillage it is found that the former result in a reduction of macroporosity in no-till soils. Besides, the formation of new pores might decrease water and gas movement (Drees et al. 1994; Shipitalo and Protz 1987). Water films can restrict the entrance of oxygen and other gases into the pores, which impact biological processes. Moreover, water can act as a corridor to easily connect long points, which are far from each other, being therefore essential for organism migration. The physical protection of the pore can affect the interaction and availability of substrates. This is the case of organic matter and clay particles, which can seal organic matter into pores, restricting access of some organisms (Adu and Oades 1978; Beare et al. 1994). When soils are tilled, new rearrangements of pore networks are created, which allows newly exposed substrates to be used until a new balance is reached (Young and Ritz 2000).

Tillage causes new pore structures to interfere with the translocation and transportation of gases that are vital for survival and functioning of microorganisms. If oxygen flow decreases, aerobic activity will cease and in the presence of nitrate denitrification will occur. Waters films play an important role in the diffusion of these gasses; therefore, moisture in pores is vital to control diffusion rates in any point of the aggregates. Good moisture distribution is important to complete several processes like mineralisation and denitrification (Young and Ritz 2000). Any changes in aeration and soil compaction can induce changes in composition and activity of microbial communities; consequently, crop sustainability and soil fertility can be affected (Ceja-Navarro et al. 2010).

Soil water management is essential to soil health and crop production. Soil moisture depends mainly on soil properties and appropriate tillage treatment. For instance, no-tillage systems result in higher water contents compared to conventionally tilled soils due to a decrease of evaporation (Blevins et al. 1971; Mitchell et al. 2012). Water content in soils influences microbial communities indirectly and directly through impacts on nutrient availability and oxygen concentrations. Elevated water flows reduce oxygen and facultative and anaerobic microorganisms take over, whereas low water content results in lower microbial activity and selects for spore formers and fungi (Sylvia et al. 2005). Previous studies on agricultural soils showed that there is a strong correlation between soil, water and organic carbon availability as determinants of microbial community composition (Drenovsky et al. 2004; Williams and Rice 2007).

2.3 Soil Temperature and Soil pH

Other soil properties including temperature and pH can alter interactions within soil communities. Tillage practices can reduce soil temperature at different depths. This can be attributed to heat flux changes and total heat input on the soil profile (Johnson and Lowery 1985). Furthermore, residues left on the surface can affect soil temperatures, changing the radiant energy balance of the system. Radiation balance is influenced by the reflection of the radiation by surface residues, water evaporation and heating of soil and air (van Doren and Allmaras 1978). Zogg et al. (1997) suggested that microbial community composition can be affected by soil temperature through changes in the kinetics of microbial respiration. Temperature can affect microbial activity directly or indirectly by changing microbial biomass and enzymatic activity (Insam 1990; McClaugherty and Linkins 1990). Furthermore, research on seasonal temperature variation has shown that low microbial biomass can be associated with high temperatures and low-moisture conditions. Both of these environmental variables have been pointed out to be so closely related that it is difficult to isolate the effect of these variables individually on soil microbial communities (Gunapala and Scow 1998).

Dick (1983) compared three different tillage treatments: no-tillage, minimum tillage and conventional tillage. No tillage decreased pH levels compared to minimum tillage and conventional tillage through the top 30 cm soil depth. The study concluded that continuous tillage could change the distribution of C, N and P and pH on soil profiles. As a result, soil microbial communities can be strongly affected by these changes in soil pH. For instance, fungal respiration increases under acidic conditions while bacterial respiration decreases (Bewley and Parkinson 1985; Anderson and Domsch 1973; Blagodatskaya and Anderson 1998). Both abiotic and biotic factors are influenced by soil pH. These abiotic factors include carbon availability, nutrient availability (Kemmitt et al. 2006) and solubility of metals (Flis et al. 1993). Microbial biomass and enzymatic activity are examples of those pH-affected biotic factors (Dick et al. 2000; Fierer and Jackson 2006).

2.4 Soil Organic Carbon

Soil organic carbon is the largest active carbon pool in the terrestrial ecosystems. More than two-thirds of SOC is contained in soil organic matter (OM) which directly supports microbial, plant and animal life. In turn, soil microbes contribute to the formation of SOC (McGill et al. 1975; Bellamy et al. 2005). Soil OM plays important roles in biological, chemical and physical processes within the soil. It acts as both a source and sink of atmospheric CO_2, and influences climate change through greenhouse gas emissions (Trumbore and Czimczik 2008).

In general, tillage has been shown to result in a decline of SOC through fragmentation of macro-aggregates, thereby improving microbial access to aggregate-protected soil carbon (Six et al. 2004) and/or by stimulating decomposition of soil carbon (Fontaine et al. 2007). The decline in SOC due to tillage is likely to result from both short-term and long-term effects. Short-term effects of tillage result from the physical soil disturbance and aggregate disruption occurring during tillage, while long-term effects include changes in physical, chemical and biological soil properties after several years of tillage (Oorts et al. 2007). On the other hand, tillage incorporates aboveground fresh organic matter into soil, which provides nutrients and energy for microbial growth and therefore stimulates the decomposition of soil carbon (Luo et al. 2010).

A number of studies have reported higher concentrations of SOC in NT systems as compared to CT systems; however, these differences were restricted to the surface soil (<0.10 m soil depth). In general, labile SOC is considered to be more easily decomposed by soil microbes and lost due to tillage than humic SOC (Grandy et al. 2006). These differences in SOC concentration between two systems can be attributed to a greater input of carbon via plant residues and/or decreased decomposition of SOC in the NT systems (Page et al. 2013).

3 Conclusions and Future Prospects

It is clear that soil microbial communities are responsive to disturbances on soils. Consequently, changes in nutrient input, climate change and soil management have the potential to indirectly or directly alter community diversity and potentially impact a range of soil functions. Recent studies have demonstrated that changes in soil communities across space are strongly related with changes in soil chemistry (Frey et al. 2004; Nilsson et al. 2007; Lauber et al. 2009). It is of paramount importance to conduct comprehensive studies, which takes into account a number of different variables (biotic and abiotic) that affect soil microbial communities for a better understanding of processes occurring in the soils. In that way, sustainable alternatives of soil management can then be proposed.

Australia will face a big challenge as climate change keeps affecting cropping systems. It will require adaptation and mitigation procedures according to each regional condition. According to the Food and Agriculture Organization, food production needs to increase 70 % by 2050 (FAO 2009). The challenge is to increase production and be sustainable. Identifying the biotic and abiotic factors that determine microbial community composition will provide a better understanding of microbial processes. Insight into microbial community composition and the factors that determine them may provide insights into improved biogeochemical and biodegradation processes, food web dynamics and overall soil quality.

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Sustainable Crop Production System

Sameen Ruqia Imadi, Kanwal Shazadi, Alvina Gul, and Khalid Rehman Hakeem

Contents

Abstract Sustainable crop production refers to agricultural production in such a way that does not impose any harm to environment, biodiversity, and quality of agricultural crops. Producing crops sustainably increases the ability of the system to maintain stable levels of food production and quality for long term without increasing the demand and requirements of agricultural chemical inputs to control

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the system. Sustainable crop production deals with keeping the soil alive with organic matter, integrated pest management and reduction in usage of pesticides, protecting biodiversity, ensuring food safety and food quality, improving nutrient quality, and fertilizing the soil with organic fertilizers. Sustainable agricultural production leads to lowering of greenhouse gas emission and carbon footprint of overall world. Sustainably produced crops and food are more beneficial to consume by humans as compared to commercial crops. Sustainable usage of resources ensures the pollution-free environment for our future generations. This chapter deals with all the aspects of sustainable crop production and the methods to ensure sustainable production of crops. The chapter also intents to discuss the harmful effects of non-sustainably produced crops and finally this chapter looks forward towards the future protection by sustainable usage of resources and future prospects of sustainability.

Keywords Crop system • Agricultural sustainability • Pest management • Organic farming

1 Introduction

It is a known fact that agriculture has changed dramatically after green revolution. Agricultural productivity after the green revolution increased due to mechanization and the inclusion of new technologies in it. The major benefits behind this mechanization were the reduction in labor and increase in yields of crops. Many positive effects have been achieved with the indulgence of technology in agriculture. It has not only reduced the labor, but also reduced the cost of crops by generating huge yield. Food security is also achieved after green revolution. But green revolution is associated with a number of cons. The ultimate results of the green revolution are observed as depletion of topsoil, contamination of ground water, increased production cost, and decline of family farms (Pimentel et al. 2005). Since the past few decades, a concept of sustainable agriculture has been started. Sustainable agriculture is considered to be the methods of farming which is based on principles of ecology. It is an integrated system in which plant production practices have a site-specific application which has long-term benefits. Sustainable crop production not only satisfies human food and fiber need but also enhances quality of environment and quality of natural resources (Gold 2009).

Organic agriculture is considered to be an approach that will benefit the environment through sustainability but in a long term. This approach of agriculture will be an important contributor in food security. A gradual shift from conventional agriculture to organic agriculture is needed to enhance sustainable production systems (Azadi et al. 2011). Farmers are required to grow crops in a sequence for development of sustainable agriculture production system in which internal resources can be used. Sustainable system will be achieved once internal resources like crop synergism, nutrient cycling, and soil water have more influence on final yield as compared to external resources like weather. Yield and quality of agricultural crops can be enhanced by taking the advantages of synergism and avoiding the antagonisms that may occur between planted crops and harvested crop residues (Tanaka et al. 2007).

Crop allelopathy is an approach which can be used to minimize serious problems in agricultural production. Allelopathic crops can be used as mulch, cover crops, green manures, smother crops, and rotational sequences. The major benefits which can be achieved using allelopathic crops are reduction in weeds and plant pathogens. They also improve the quality of soil and yield of crops. Allelopathic crops contain chemicals which are known as allelochemicals. These chemicals have their role in development of biological pesticides and herbicides. These crops are considered to be the target crops to be used for developing sustainable system for agriculture (Khanh et al. 2005). Applications of sustainable agriculture can be found in improvements of crops, agroforestry, conservation of soil, conservation of agriculture, integrated pest management, horticulture, development of livestock and fodder crops, and aquaculture (Pretty et al. 2011). Sustainable agriculture system is mentioned in Fig. 1.

Nowadays much effort is focused on the phenomenon more crop per drop. This is due to the fact of depleting freshwater reservoirs from environment. Water can be preserved in a sustainable way. Improvement in water productivity will further contribute to sustainable agriculture systems (Morison et al. 2008). Options to secure ecological sustainability and economic viability may differ considerably in different cases. But, the general mechanisms and protocols always remain the same (Khan et al. 2006). Evergreen agriculture is an agricultural system which refers to the integration of particular tree species into annual food crop systems. The trees which are

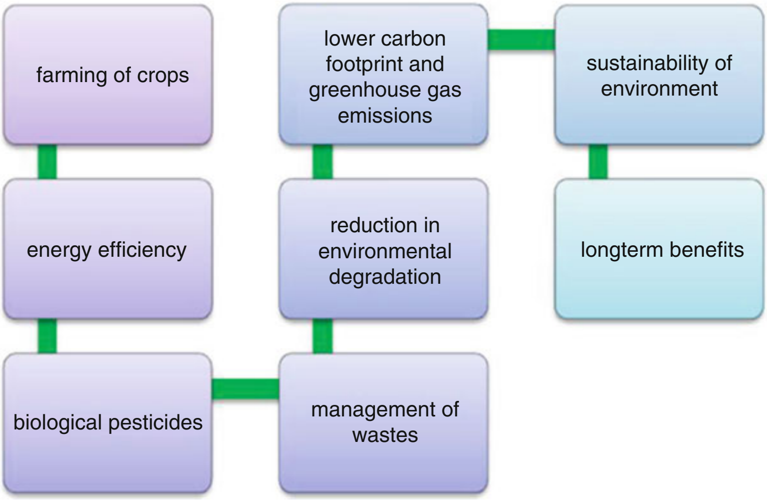

Fig. 1 Sustainable agriculture production system

intercropped are meant to provide a green cover on land which will maintain vegetative soil cover, bolster nutrient supply through biological nitrogen fixation, enhance quantity of organic matter in soil, increase production of food, and enhance carbon storage. Evergreen agriculture is a sustainable agriculture approach through which maximum beneficial output can be gained with low input (Garrity et al. 2010).

Crop diversification techniques can be applied in order to avoid the negative consequences on environmental changes like extreme climate changes. This is a cost-effective method and it also helps in getting rid of pests and diseases. It enhances the resilience of plants by suppressing pest outbreaks and pathogen transmissions. Crop diversification can be done in a variety of forms following various methods. This technique also provides an opportunity to farmers to choose a strategy of farming that is providing resilience and cost efficiency (Lin 2011).

2 Sustainable Agriculture and Natural Resources

Sustainable approaches should be designed to use agricultural residues of food crops and feed crops for production of biofuel and biodiesel. The goal of sustainable agriculture can be attained with the use of 50 % leftover biological residues of food crops and fodder crops in biofuel production. This will not only reduce the quantity of land covered by cash crops, but also perform a two-in-one function (Fischer et al. 2010).

2.1 Pest Management

Looking back into the history, it was for the thousands of years that tillage and agriculture were considered the same thing. It was never in practice to grow crops before tilling for weeds in the soil. As the technology grew, it gifted green revolution which provided farmers with an opportunity to cultivate crops without tilling. It was due to the advent of herbicides and weedicides. Every new invention has some benefits and some associated disadvantages. Same goes for herbicides in particular and pesticides in general. It was the use of pesticides that led to environmental pollution and degrading of soil (Triplett and Dick 2008). To avoid the potential harmful effects of conventional pesticides, integrated pest management approaches can be implied which not only control the pests but also reduce the amount of pesticides used. It is an economical as well as ecologically sustainable and applicable method to get rid of pests (Oerke 2006).

Diversified landscapes hold more potential for conservation of biodiversity and sustaining of pest control function (Bianchi et al. 2006). Integrated pest management has an objective to manage the population of pests below the economic threshold so that they are unable to destroy crop yield. Intercropping and trap cropping techniques are considered to be very favorable for maintaining natural arthropod populations and eradicating pest populations (Deguine et al. 2008). Excessive use of agrochemicals in conventional farming and crop management methods resulted in serious health and environmental concerns. It is a major factor behind the loss of biodiversity. Plants which are grown under nutrient-rich soil are more prone to pests and insects as compared to plants that are grown in nutrient-deficient soils. Methods of crop rotation can be applied in order to get rid of pests and insects. During crop rotation, plants which are less susceptible to that particular pest should be grown which will increase the natural mortality rates of pesticides and decrease the overall population (Ghorbani et al. 2008). Push-pull strategies can be used to manage excessive pests in an integrated manner. These strategies include manipulation of behavior of insect pests and their natural enemies. The strategies use a stimulus that acts to be unattractive for pests (push) and a stimulus which is attractive to pests so that pests attract towards that stimuli (pull) from where they are removed (Cook et al. 2007).

2.2 Biodiversity and Living Soil

Biodiversity is a fundamental principle for proper functioning of the food web (Savary et al. 2012). It is expected from living soil resources to increase the production of crops, produce large quantities of ligno-cellulosic biomass, enhance biodiversity, and improve the environment and carbon sequestration, developing the system which has a low risk for pollution and contamination and preservation of species (Lal 2008). Soil acidification is a natural process which is further enhanced by conventional farming methods. Acidity is attributed to growth-limiting factors for plants by complex interactions which include physical, chemical, and biological properties of soil. Calcium, phosphorus, and magnesium are deficient in acidic soil (Fageria and Baligar 2008). Agricultural biodiversity is a valued source which can be used to increase productivity of crops. It is due to agricultural biodiversity that plants can cope up with changes in climate and environment. Sustainable and secured food production is attributed to agricultural biodiversity (Frison et al. 2011).

Most of the healthy plants which are propagated in the natural environment are colonized by communities of endophytic bacteria. This is a cause of large level of biodiversity associated with agriculture. The bacteria form a nonpathogenic relationship with plants which can be beneficial, detrimental, or neutral. These bacteria provide nitrogen in nonleguminous plants, which plays a role in reduction of requirement of nitrogen fertilizers. Certain plants favor association with growth-promoting bacterial endophytic population. It results in maintenance of fertile and disease-suppressive soil. This provides a sustainable system for growth of plants and enhancement of biodiversity (Sturz et al. 2000). One of the best techniques which can be applied for sustainability of soil is conservation agriculture. Conservation agriculture is a term which is defined as minimal soil disturbance and permanent soil cover through mulch which is combined with crop rotations. It is observed that this technique not only improves agriculture through water infiltration and reduction in erosion, but also improves soil surface aggregates and reduces compactness of soil. Conservation agriculture also promotes biological tilling approaches, levels of surface soil organic matter, and carbon content. It is also observed to be associated with a decrease in quantity of weeds (Hobbs 2007).

2.3 *Fertility of Soil and Fertilizers*

Soil infertility is the major constraint which limits the yield of crop worldwide. Maintenance of the quality of soil can also reduce many problems like degradation of land. Major components of soil are minerals, organic matter, and microorganisms. Physical, chemical, and biological properties of soil largely depend on these three components. Bio-fertilizers are a natural and sustainable way to enhance fertility of soil. These fertilizers are composed of cells of different types of beneficial microorganisms. These fertilizers act as a source of nitrogen, potassium, and phosphorus. They not only increase productivity of soil, but also enhance its sustainability. Low input and low cost are required to add bio-fertilizers in soil which can result in high output and enhanced yield of crops (Mohammadi and Sohrabi 2012). Negative effects of depletion of soil fertility on food security are of immense economic importance. These effects are further enhanced due to changes in climatic conditions and rise in prices of global fertilizers. For this purpose, efforts are being made to develop options of gaining soil fertility without harmful effects. Nitrogen-fixing plants and fertilizer trees can act as the best option in this regard. It is observed that fertilizer trees add large amounts of nitrogen in soil through biological nitrogen fixation. The nutrients which contribute to soil by fertilizer tree biomass reduce the requirement of mineral nitrogen fertilizer to as low as 75 %. These trees also enhance the growth and yield of crops. Besides all these advantages, fertilizer trees are also observed to be profitable and economical (Akinnifesi et al. 2011).

For sustainable production of crops like wheat, maintenance of nitrogen levels in soil is very important. For this purpose, crop rotation approach is applied in which wheat plants were rotated with mung beans. It was observed that during the period of growth of mung beans, the concentration of nitrogen in soil was increased which was utilized by wheat during its growth. Application of organic fertilizers further increased the quantity of nitrogen in soil. Soils which are low in nitrogen can be treated with crop rotation technique to enhance nitrogen concentrations in a sustainable system (Bakht et al. 2009). Organic waste is considered to be organic treasure because it can be recycled into organic fertilizers through composting. One extra benefit of organic waste is that it can be used to produce biogas. Crop rotation with legumes is a significant method to enhance nutrient efficiency of soil (Yang 2006). In time and space, nitrogen supply should match the demands of nitrogen. This rule should not only be implied on single crop plants, but also for rotating crops as an integrated system. This will lead to higher agronomic nitrogen use efficiency (Spiertz 2010). Efficient management practices can lower the use of nutrients (Tilman et al. 2011).

3 Methods of Sustainable Agriculture

Conventional system of agriculture can lead to degradation of the environment, economic problems, as well as social conflicts. Amount of organic matter in soil usually depends on input of organic material. Concentration of organic matter in the soil makes it living. If the decomposition rate of organic matter is low and input of materials is high, then the organic matter is high in soil. For cropping systems, equilibrium levels of organic matter of clay soil will be higher as compared to sandy soil (Johnston et al. 2009). Conventional pesticides are heavily used in conventional and industrial agriculture approaches. These pesticides are observed to be linked with endocrinal disorders as well as different types of cancers (Horrigan et al. 2002).

Methods of crop rotation can be applied in order to get rid of pests and insects. During crop rotation, plants which are less susceptible to that particular pest should be grown which will increase the natural mortality rates of pesticides and decrease the overall population (Ghorbani et al. 2008). Push-pull strategies can be used to manage excessive pests in an integrated manner. These strategies include manipulation of behavior of insect pests and their natural enemies. The strategies use stimuli that act being unattractive for pests (push) and stimuli which are attractive to pests so that pests attract towards that stimuli (pull) from where they are removed (Cook et al. 2007). It is very essential to study interactions of plants with arbuscular mycorrhizal fungi and other organisms for development of sustainable agriculture systems (Johannson et al. 2004).

Conservation agriculture is considered to be the most sustainable agriculture production system for the future. In the next decade, agriculture is required to produce more food through less land and low resources with more efficient usage of nutrients and approaches. This will have a minimal impact on the environment and will also meet the demands of the ever-growing populations (Hobbs et al. 2008). Various plant species which are cultivated are found to be agents of bioremediation. These plants are involved in the sustainable reclamation of calcareous as well as saline sodic soils. These plants can be grown in saline and saline sodic soils to remove excess chemicals and to make these soils reasonable for the growth of different plant species. This is an eco-friendly as well as environmentally sustainable technique (Qadir and Oster 2004).

Intercropping is a technique which is one of the oldest to be used in agriculture. It is a sustainable technique in which multiple crops are planted in a sequence which not only protect the plants from diseases and pests but also help in reducing environmental degradation. This technique uses available growth resources and produces a high yield of mixture of crops. It also improves the fertility of soil by biological nitrogen fixation (Lithourgidis et al. 2011).

4 Organic Farming: A Sustainable Approach

The desire for sustainable agriculture system is universal (Rigby and Cáceres 2001). Organic farming is a sustainable farming system and is considered to be a potent solution for loss of biodiversity and degradation of the environment (Hole et al. 2005). Organic farming is observed to offer a number of characteristics that increase resilience in plants (Milestad and Darnhofer 2003). Symbiotic relationships with mycorrhizal fungi and leguminous bacteria act as a support in an organic farming system. In this phenomenon, the fungi or bacteria penetrate into the roots of plants and benefit them with an increase in yield and quality of crops. It is due to symbiotic relationships that nutrition of the soil is enhanced and soil structure gets improved. Pest and disease control is also one of the aspects of symbiotic relationships during organic farming (Gosling et al. 2006).

Organic farming is perceived to be more environmentally friendly as compared to conventional farming techniques. Most of the consumers nowadays are attracted towards organic food because this food is without any chemicals (Darnhofer et al. 2010). Organic farming is associated with an increase in richness of species in soil. It is observed that the species biodiversity in organic farms is 30 % more than that in conventional farms. Birds, insects, and plants are usually healthier in organic farming. The yield of the crops is enhanced. On an average living beings are 50 % more in organic farming which leads to the development of sustainable systems (Bengtsson et al. 2005). It is also observed that organic farming of crops is associated with an increase in the richness of butterfly species (Rundlöf and Smith 2006). It has a positive impact on the environment; hence, it is expected that conversion to organic farming will lead a way to overcome shortcomings of current conventional methods of agriculture (Darnhofer 2005).

Organic farming has a greater impact for long-term sustainability. It is also observed that organic farming emits a low number of greenhouse gases. Organic farming is a technique which can reduce total water, energy, and greenhouse gases involved in food production (Wood et al. 2006). In an experiment in which organic farming and conventional farming were compared on 293 examples, it was observed that organic farming has a potential to contribute substantially to global food supply as well as it reduces detrimental impacts on the environment (Badgley et al. 2007).

5 Greenhouse Gas Emissions

Nowadays agriculture system accounts for almost 15 % of total anthropogenic emissions, which basically include methane and nitrous oxide (Popp et al. 2010). Conventional agriculture is a source of three primary greenhouse gases. These include carbon dioxide, methane, and nitrous oxide. Sustainable agriculture act as a sink for carbon dioxide because it sequesters it and uses it as organic matter in soil. It is sustainable agriculture approaches which lead to a decrease in greenhouse gas emissions. Conservation practices that are employed in sustainable system of agriculture decrease soil erosion and enhance carbon methane consumption in soil. Manure management is a way to get rid of the excess nitrous oxide and methane. Agricultural environment footprint can be significantly reduced using these methods (Johnson et al. 2007). Agriculture is rightly recognized as a source of greenhouse gas emissions and carbon footprint. Organic agriculture tends to improve yield of crops due to which low input is provided. Due to low amount of input, carbon number and greenhouse gas emissions can be reduced (Burney et al. 2010). Agriculture land occupies almost 37 % of earth's land surface. 52 % of global anthropogenic methane and 84 % of global anthropogenic nitrous oxide emissions are a gift of agriculture. Improvement in crop land, restoration of degraded land, cultivated organic soils, and management of grazing land lower the potential concentration of emitted greenhouse gases (Smith et al. 2007).

6 Benefits of Sustainable Crop Production System

Sustainable agricultural practices ensure the conservation as well as enhancement of agro-biodiversity for maintaining the security of food. It is the need of time to adopt an agro-ecosystem which is sustainable in nature and focuses on genetic resource conservation (Thrupp 2000). Sustainable agriculture is associated with a decrease in rate of soil and nutrient loss, increase in levels of microorganisms in the soil, and reduction of amount of chemicals which leach into water table (Carolan 2006). Features of sustainable crop production system are given in Fig. 2.

6.1 Social Desirability

One of the most important advantages of sustainable agriculture is that it is accepted by a large number of population. Since it is a technique in which organic fertilizers are used and pests are controlled biologically, moreover labor is also used; hence there are very low chances of contamination of the final product with toxic

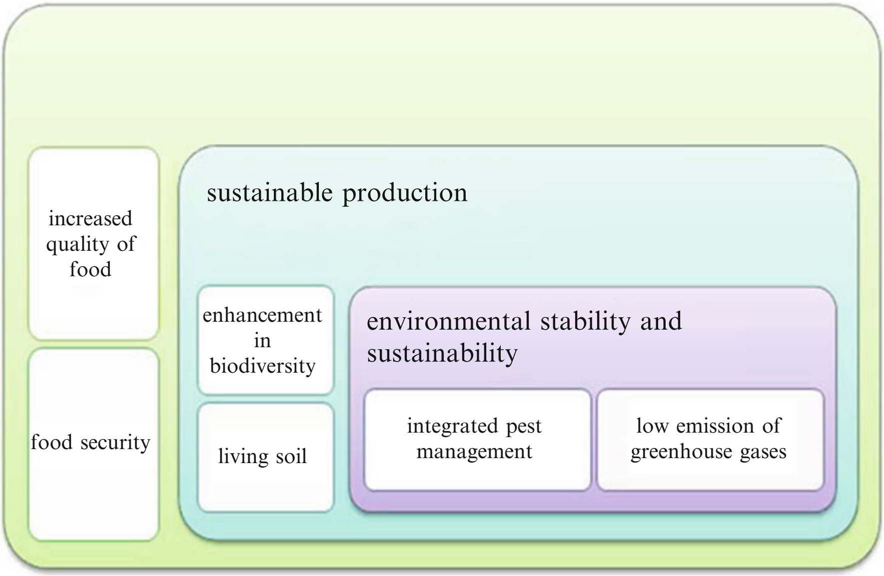

Fig. 2 Advantages of sustainable crop production system

substances used in pesticides and insecticide sprays. It is due to this fact that it is highly recommendable. This type of agriculture approach not only involves low levels of chemicals, but also reduces the degradation of environment and soil which makes it the best approach for agriculture. It is socially desirable because it is as impactful for small farms as for the large farm lands. Conservation tillage techniques are used in sustainable agriculture due to which natural resources are also conserved (Fowler and Rockstrom 2001).

6.2 Economic Feasibility

Sustainable agriculture approaches are economical in nature. These techniques cost less because they use low number of chemicals and produce high yields. Though these approaches can only be employed to smaller farms yet these farms that follow sustainable agricultural practices produce high yield, use less water resources, are nitrogen efficient and do not use chemicals. All these factors make a sustainable agriculture system as a very affordable type of agriculture. It not only contributes to the economy, but also has a huge function in the prevention of environmental degradation and climatic changes. Usage of biological pesticides and multiple cropping techniques further reduces the cost of sustainable agriculture (Vermeir and Verbeke 2006). Sustainable agriculture is a vital form of agriculture in today's world. It uses special techniques of farming in which environmental resources are fully utilized which also ensure no harm to soil and the environment. Thus, it can be said that this technique is environmental friendly and provides safe and healthy crops (Singh et al. 2011).

6.3 Ecological Viability

The best features of sustainable agriculture are that this approach of agriculture is ecologically viable. It is an environmental friendly approach, which not only preserves biodiversity, but also reduces the use of agrochemicals that result into the sustainability of environment. The world is moving towards sustainable agriculture and that is only due to the reason for saving the environment. Environmental degradation is also reduced with the use of sustainable agriculture approaches (Fowler and Rockstrom 2001).

7 Conclusion and Future Prospects

Since the very beginning, conventional farming methods based on green revolution have been questioned. The sustainability of conventional farming methods cannot be achieved because of the high production costs, reliance on nonrenewable resources, reduction in biodiversity, contamination of water, chemicals in food, degradation of soil, and health risk to farmers due to exposure with chemicals. It is known that the system of organic farming is less efficient and produces half the yield of conventional farming methods (Reganold et al. 2001). In regard of this integrated farming approach should be enhanced in which organic farming can be combined with conventional farming methods which can form a sustainable system. Integrated systems not only produce high yield, but also enhance the quality of soil and lower the negative impact of the environment. But before implementing an integrated system of agriculture, research should be performed for quality of fruits and yield produced.

In future biodiesel can be produced through agricultural nonfood crops in a sustainable system. The large amount of biodiesel can be produced following sustainable agriculture approaches as crops are produced in bulk and thus yield is high (Ahmad et al. 2011). Second-generation biofuels and biodiesel can be prepared as a by-product from food crops. It is a sustainable process through which high cost output can be gained in low input. It is observed that biotechnological fuel development does not harm the environment (Imadi and Kazi 2015). It is the need of time to include crop rotation, multiple cropping, conservation agriculture, and cropping intensification approaches in daily agriculture to attain sustainable agriculture production systems (Yang 2006). One of the major goals in sustainable agriculture is to use fossil fuels efficiently for crop production (Hoeppner et al. 2006).

Carbon neutral biofuels which are renewable in nature can be a sustainable substitute to fossil fuels. These fuels can be produced by oilseed crops (Patil et al. 2008). In future, public sector should be involved in the support of sustainable agriculture. Agricultural policy should be designed so that it may support production and encourage environmental benefits to ensure sustainable future (Pretty et al. 2011). Long-term sustainability will require agricultural diversification which will basically involve a slow transition from intensive synthetic nitrogen inputs to crop rotations based on legumes (Mulvaney et al. 2009). Developing sustainable agricultural production systems requires research on different scales from single crops to diverse crops and different farming systems. It is the need of time to develop an environmental friendly agriculture system which is possible only after quantitative system research, development of best practices, and enhancement of those practices and legislations by government and organizations (Spiertz 2010).

Conservation agriculture is considered to be the most sustainable agriculture production system for future. In the next decade, agriculture is required to produce more food through less land and low resources with more efficient usage of nutrients and approaches. This will have a minimal impact on environment and will also meet the demands of ever-growing population (Hobbs et al. 2008). With the advent of omic technologies, the goal of sustainable agriculture system seems to be achieved. Proteomics can be used to study the nutritional value of crops, yield of the crop, and adverse effects of environmental conditions on crops. Proteomics, metabolomics, and transcriptomics should be integrated into agricultural production systems to generate a sustainable long-lasting system that provides benefits in long term (Salekdeh and Komatsu 2007).

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Role of Biopesticides in the Management of Nematodes and Associated Diseases in Horticultural Crops

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Abstract Technological advancements in crop protection have immensely contributed to the success of green revolution in enhancing the production of food, fiber, and fodder. To feed the ever growing global population, predicted to reach 9.1 billion by 2050, an increase in food production by 70 % is anticipated to meet the global demands. Agricultural intensification and protected cultivation technologies have resulted in the excessive use of synthetic chemical pesticides for reducing the estimated 45 % crop loss due to pests, diseases, and nematodes amounting to Rs. 290 billion per annum. However, increasing public awareness on organic food owing to the hazardous effects of these chemicals on human health and environment like pollution, pesticide residues, pest resistance and resurgence have forced the researchers and pesticide industries to shift their focus to more reliable, sustainable, and environmental friendly biocontrol agents. Biopesticides are the formulated form of active ingredients based on microorganisms such as bacteria, viruses, fungi, nematodes or naturally occurring substances, including plant extracts and semiochemicals as insect pheromones.

Biopesticides have overwhelming advantages of high selectivity to target pests; safety to humans and non-target organisms; amenability to individual applications and integrated pest management and suitability for organic niche products including export oriented commodities. There are about 1400 biopesticide products being sold worldwide which is expected to produce a global market of $3.2 billion by 2014. Despite several advantages in using biopesticides, the rate of their consumption is not to the optimum level in comparison with the chemical pesticides mainly due to their shorter shelf life, susceptibility to environmental conditions, expensive production systems, and efficacy problems. This alarming situation can be intervened through formulation improvements which can offer longer shelf life, minimum contamination, ease in handling and application, convenience in storage and transport, easy quality control, and export potential. This chapter deals with biopesticides, current scenario of biopesticide industry, their formulations and scope in crop management against major nematode pests and associated disease complexes in horticultural crops especially in India.

Keywords Biopesticides • Nematodes • Disease complex • Management • Horticultural crops

1 Introduction

Plant parasitic nematodes continue to threaten horticultural crops throughout the world, particularly in tropical and subtropical regions. Primarily as pathogens by themselves, and secondarily as predisposing agents for easy entry of pathogenic fungi and bacteria from soil into the host plants, nematodes cause huge economic losses quantitatively and qualitatively. Overall average annual yield loss of the world's major horticultural crops due to damage by plant parasitic nematodes is estimated at 13.54 %. For the seven life-sustaining horticultural crops, viz., banana, cassava, coconut, field bean, potato, sugar beet, and sweet potato, an estimated annual yield loss of 12.77 % is reported due to nematodes. The 16 economically important horticultural crops (cocoa, citrus, coffee, cowpea, eggplant, grapevine, guava, melons, okra, ornamentals, papaya, pepper, pineapple, tea, tomato, and yam) having food or export value are reported to have an estimated annual yield loss of 14.31 % and monetary loss of US $ 19.37 billion owing to damage by nematodes (Reddy 2012).

To contain the nematode damage in horticultural crops, farmers resort to indiscriminate use of chemical nematicides posing serious hazards to the environment and biotic life. Since the horticultural produce especially fruits and vegetables are consumed afresh, consumers expect residue-free produce both for internal and export markets. This brings into focus the importance of managing the nematode pests with minimal hazard to the ecosystem and ensuring absolute safety to human beings. Therefore, intensive research efforts for exploring alternate eco-friendly nematode management strategies have become imperative. There is a great need for environmentally friendly microbial technologies in agriculture. There is a need for restructuring the crop rhizospheres for improving and sustaining the nutrient supply in the soils and enhancing the health and yield of crops through sustainable practices based on microbial technologies as "biopesticides." These are the formulated forms of active ingredients based on microorganisms such as bacteria, viruses, fungi, nematodes or naturally occurring substances. They also include plant extracts and semiochemicals as insect pheromones (Gasic and Tanovic 2013).

2 What Are Biopesticides

According to the US Environmental Protection Agency (EPA 2015), biopesticides are certain types of pesticides derived from natural materials such as animals, plants, bacteria, and certain minerals. Biopesticides are classified into three major categories:

1. *Microbial pesticides*: consist of a microorganism (e.g., a bacterium, fungus, virus, or protozoan) as the active ingredient.
2. *Plant-Incorporated-Protectants* (PIPs): pesticidal substances that plants produce from genetic material that has been added to the plant.
3. *Biochemical pesticides*: naturally occurring substances (such as insect sex pheromones) that control pests by nontoxic mechanisms.

Microbial biopesticides include the principles of microbial ecology, which encompass inoculation of crops with beneficial microorganisms and the use of cultural practices that enrich indigenous beneficial microorganisms in individual agricultural fields.

3 Biopesticide Industry: Global and Indian Scenario

In 2005, the share of biopesticides in total pesticide market accounted for 2.5 %, which was merely 0.2 % in 2000. This share was expected to grow to about 4.2 % by 2010 and 10 % in the next 5 years (Sinha and Biswas 2008). There are about 1400 biopesticide products sold worldwide, which is expected to produce a global market of US$ 3.2 billion by 2014. The USA consumes maximum biopesticides (40 %) of the global production followed by Europe and Oceanian countries (20 %) (NAAS 2013). In India, 16 biopesticides have been registered under the Insecticides Act, 1968 (CIB and RC 2014) which represent only 2.89 % of the overall pesticide market and is expected to grow annually by 2.3 % in the coming years (Thakore 2006).

In India, biopesticide production is currently dominated by antagonistic fungi and bacteria such as *Trichoderma* spp. and *Pseudomonas fluorescens*, but the production of nucleopolyhedrosis viruses (NPV), granuloviruses (GV), and entomopathogenic fungi is also expanding (Rabindra 2005; Singh et al. 2012). At least 410 biopesticide production units have been established in India, 130 in the private sector and the rest at IPM centers under Ministry of Agriculture and Biocontrol Laboratories under State Departments, State Agricultural Universities and Indian Council of Agricultural Research (ICAR) Institutions (Rabindra 2005). ICAR-Indian Institute of Horticultural Research, Bengaluru, India is the pioneering Institute in India for its frontier research and commercialization of biopesticides all over India. It has registered five biopesticide products, namely *Paecilomyces lilacinus* 1 % W.P., *Pochonia (=Verticillium) chlamydosporia* 1 % W.P., *Trichoderma viride* 1.5 %, *Trichoderma harzianum* 1 % W.P., and *Pseudomonas fluorescens* 1 % W.P. with the Central Insecticides Bureau & Registration Committee, New Delhi under section 9(3b) of the Insecticides Act, 1968 for managing nematode problems and associated disease complexes in horticultural crops in the country. These technologies are transferred so far to 360 industries/licensees all over India licensed for commercial production of biopesticides. Newer biopesticide products of *Pseudomonas putida, Bacillus pumilus*, *B. subtilis*, and *B. amyloliquefaciens* also hold promise against nematode disease complexes and are in the pipeline for registration.

4 Use of Biopesticides Against Nematodes in Major Horticultural Crops

*4.1 Banana (*Musa *spp.)*

4.1.1 Burrowing Nematode (*Radopholus similis*)

The burrowing nematode is a serious problem in banana and causes “rhizome rot” or “toppling” or “black head” disease of banana. *R. similis* is responsible for 30.76–41 % yield loss in banana (Fig. 1). Rhizosphere treatment with *P. fluorescens* Pf1 at 20 g/plant along with soil application with 100 g/rhizome of talc powder based formulation gave 56.5 % reduction in nematode population (Johnson and Devarajan 2004). Senthilkumar et al. (2008) tested several strains of *P. fluorescens* and reported that field application of *P. fluorescens* (isolate PfB 13) at 10 g/plant significantly reduced *R. similis* population in soil and roots and root lesion index; and increased plant height, pseudo stem girth, leaf area, number of leaves, and fruit yield.

Fig. 1 Damage caused by burrowing nematode damage in banana roots

Table 1 Effect of combination of *Bacillus subtilis* and *P. lilacinus* against *Meloidogyne incognita* and *Radopholus similis* in banana under field conditions

Treatment	Nematode population (in 10 g root)		Yield (per plant)	% increase in fruit yield
	M. incognita	*R. similis*		
BS + FYM	14.5 (73.5)	11.1 (69.1)	28.9	16.5
PL + FYM	13.6 (75.2)	10.6 (70.5)	29.4	18.5
BS + PL + FYM	6.9 (87.4)	5.3 (85.2)	31.6	27.4
FYM alone	38.9 (29.0)	29.6 (17.5)	26.4	6.5
Chemical	15.6 (71.5)	9.8 (72.7)	29.1	17.3
Control	54.8	35.9	24.8	–
CD ($P = 0.05$)	5.32	2.3	2.14	–

Figures in parentheses are percent decrease in nematode population
BS = *Bacillus subtilis* 1 % A.S. (10^9 cfu/g); PL = *Paecilomyces lilacinus* (10^6 cfu/g); FYM = Farmyard manure alone; Chemical = Furadon at 40 g/plant (biocontrol agents were enriched in FYM and applied at 2 kg per plant at the time of planting and at an interval of 6 months)

Application of 2 kg farmyard manure enriched with liquid formulation of *Bacillus subtilis* 1 % A.S. (10^9 cfu/g) and *Paecilomyces lilacinus* (10^6 cfu/g) per plant at the time of planting and at an interval of 6 months was found effective in reducing infestation of lesion nematode, *Radopholus similis* (85.2 %) and root-knot nematode, *Meloidogyne incognita* (87.4 %) and increasing fruit yield (27.4 %) in banana (Rao and Umamaheswari 2015a) (Table 1) (Figs. 2 and 3).

Fig. 2 Application of biopesticides enriched FYM to banana

Fig. 3 Biopesticide treated banana plants

4.1.2 Root-Knot Nematodes (*Meloidogyne* spp.)

The root-knot nematodes, *M. incognita* and *M. javanica* attack bananas. *M. incognita* causes 30.90 % loss in fruit yield of banana (Reddy 2012). The root-knot and the burrowing nematode on banana were effectively managed by integration of neem cake at 500 g and FYM enriched with *T. harzianum* at 2 kg/plant. The above treatment increased the fruit yield to 45 kg/plant and bunches came to harvest 65–75 days earlier (Reddy 2012).

4.1.3 Lesion Nematode (*Pratylenchus coffeae*)

Poornima et al. (2004) reported that application of *Paecilomyces lilacinus* at 10 g along with FYM at 500 g or neem cake at 250 g/plant effectively suppressed the lesion nematode population by 60 and 63 %, respectively. Also, application of *Trichoderma viride* at 20 g/plant at the time of planting and repeated 3 months after planting was effective in controlling *P. coffeae* as well as reducing the incidence of Panama wilt in banana (Reddy 2012).

*4.2 Citrus (*Citrus *spp.)*

4.2.1 Citrus Nematode (*Tylenchulus semipenetrans*)

The nematode causes "slow decline" and is considered to be one of the factors responsible for die-back of citrus trees in India. *T. semipenetrans* was responsible for 69 %, 29 %, and 19 % loss in fruit yield of sweet orange, lemon, and sweet lime, respectively.

Paecilomyces lilacinus is a potential biocontrol agent for controlling citrus nematode (Mani et al. 1989). It was observed that fruit diameter of the orange cv. 'Valencia' treated with *P. lilacinus* was significantly greater than that from trees treated with temik, vydate and recorded significantly lower number of nematodes in roots and soil around citrus trees (Jatala 1986). Application of *P. lilacinus* in the form of spore suspension checked the multiplication of *T. semipenetrans* to 71.7–97.8 % (Mani and Murthy 1989). In citrus, a monthly rate of 1.1 kg a.i. per ha of avermectins (*Streptomyces avermitilis*) for 7 months gave maximum increase in yield and reduction of *T. semipenetrans* population (Garabedian and Van Gundy 1983). Application of *P. fluorescens* at 20 g/tree three times in a year at 15 cm depth and 50 cm away from tree trunk helps in reducing the nematode population. Also, integration of neem cake with *P. fluorescens* gave maximum reduction in citrus nematode population both in soil and roots and increased plant growth of acid lime (Reddy et al. 2000) (Table 2).

*4.3 Papaya (*Carica papaya*)*

4.3.1 Root-Knot Nematodes (*Meloidogyne incognita* and *M. javanica*)

Root-knot nematodes are reported to cause 10–20 % reduction in papaya fruit yield (Fig. 4). In papaya under nursery conditions, Rao (2007a) reported that seed treatment with *P. fluorescens* (10^8 spores/g) combined with soil application of *T. harzianum*

Table 2 Effect of *P. fluorescens* with oil cakes on plant growth and population of *T. semipenetrans* on acid lime

Treatment	Dose (g)/ plant	Plant weight (g)	CFU/ g root	Nematode population	
				In soil (100 ml)	In root (10 g)
P. fluorescens	4×10^9 spores	21.14	2.0×10^7	9116	2418
Castor cake	50 g	23.00	–	9012	2264
Karanj cake	50 g	22.09	–	9228	2426
Neem cake	50 g	23.40	–	8804	2310
P. fluorescens + Castor cake	½ dose each	25.97	12.4×10^7	6398	1218
P. fluorescens + Karanj cake	½ dose each	24.42	10.2×10^7	6548	1298
P. fluorescens + Neem cake	½ dose each	28.97	18.6×10^7	6034	1010
Control	–	17.60	–	13,456	6142
CD ($P = 0.05$)	–	1.76	–	234.64	212.34

Fig. 4 Root-knot nematode infected papaya seedling

(10^6 spores/g) and *P. fluorescens* (10^8 spores/g) each at 5 g/kg soil gave significant reduction in *M. incognita* population both in soil and roots, number of eggs per egg mass and increased root colonization by both the bioagents in soil and roots. Nursery bed treatment with *T. harzianum* and *P. lilacinus* each at 5 or 10 g/kg soil resulted in production of highly vigorous papaya seedlings whose roots were colonized with both the bioagents. There was significant reduction in root galling in the combination treatments (2.9–3.2) compared to control (8.9) (Rao and Naik 2003).

Application of 2 kg farmyard manure enriched with liquid formulations of *Bacillus subtilis* 1 % A.S. (10^9 cfu/g) and *Paecilomyces lilacinus* (10^6 cfu/g) per plant at the time of planting and at an interval of 6 months reduced reniform nematode *R. reniformis* by 77.6 %, *M. incognita* by 79.1 % and increased the fruit yield by 23.8 %. Mortality due to *Pythium aphanidermatum* was also recorded least in this treatment (15.6 % compared to control—41.3 %) (Rao and Umamaheswari 2015a) (Fig. 5; Table 3).

Fig. 5 Biopesticide treated papaya plants

Table 3 Effect of combination of *Bacillus subtilis* and *P. lilacinus* against disease complex caused by *M. incognita* and *Pythium aphanidermatum* in papaya under field conditions

Treatment	Nematode population (in 100 cc soil)		Root-knot index (1–10)	Mortality (%) due to wilt disease complex	% Increase in fruit yield per
	M. incognita	*R. reniformis*			
BS+FYM	76.2 (66.70)	88.1 (65.60)	4.8	20.5	11.5
PL+FYM	82.3 (64.10)	91.4 (64.50)	4.1	18.8	13.5
BS+PL+FYM	51.3 (77.60)	53.4 (79.10)	2.1	15.6	23.8
FYM alone	167.6 (26.80)	176.0 (31.25)	7.4	40.5	5.6
Chemical	64.1 (72.00)	76.1 (70.3)	2.5	20.1	7.2
Control	228.9	256.0	8.1	41.3	–
CD ($P=0.05$)	7.82	6.66	0.41	2.19	–

Figures in parentheses are percent decrease in nematode population

BS=*Bacillus subtilis*; PL=*Paecilomyces lilacinus*; FYM=Farmyard manure alone; Chemical=Furadon at 1 kg a.i./ha; (biocontrol agents were applied as seed treatment at 20 g/ka; substrate treatment at 10 g/kg substrate in polybags; and soil application after enrichment with FYM at 2 kg per plant at the time of planting and at an interval of 6 months)

*4.4 Grapevine (*Vitis vinifera*)*

4.4.1 Root-Knot Nematodes (*Meloidogyne incognita* and *M. javanica*)

M. incognita caused 55 % loss in fruit yield of grapes, while *M. javanica* caused 53 % loss in yield (Reddy 2012). Sundarababu et al. (1999) observed that *P. fluorescens* reduced root-knot nematode population effectively and enhanced yield in grapevine. Santhi et al. (1998) reported that pruning (during July) and soil application of 4 g talc formulation of *P. fluorescens* (containing 15×10^8 cfu/g)/vine around root-knot infested grapevine at 15 cm depth in the basin significantly reduced root galling due to *M. incognita* (39 %), number of egg masses (250 %) and increased fruit yield (166 %).

4.4.2 Wilt Disease Complex in Grapevine (*Meloidogyne incognita* and *Fusarium moniliforme*)

Application of *P. fluorescens* at 100 g and FYM at 20 kg/vine effectively managed *M. incognita* + *F. moniliforme* disease complex and improved the plant stand by reducing the final soil nematode population (56.9 %), root gall index (1.8), and disease incidence (15.67 %) and increasing the number and weight of fruit bunches (17.83 and 155.40 %, respectively) and fruit quality (more TSS—13.53 Brix, TSS–acid ratio—14.87, lower acidity–0.91 %) (Senthilkumar and Rajendran 2004).

*4.5 Pomegranate (*Punica granatum*)*

4.5.1 Root-Knot Nematode (*Meloidogyne incognita*)

The root-knot nematodes were responsible for 24.64–27.45 % loss in fruit yield of pomegranate (Fig. 6). Pawar et al. (2013) reported that *P. fluorescens* at 20 kg/ha was significantly effective in reducing the root-knot nematode population, *M. incognita* race 2 (31.28 %) and number of root galls/5 g roots (29.28 %) and increasing the fruit yield (18.99 %) with benefit–cost ratio of 2.37 (Table 4).

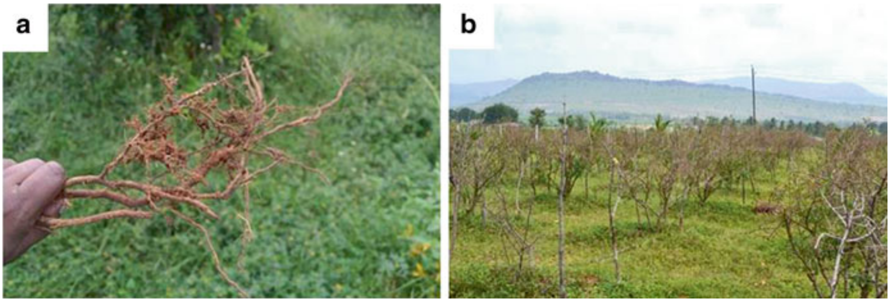

Fig. 6 Infestation of root-knot nematodes on pomegranate (**a**) Damage of pomegranate root by root-knot nematode; (**b**) Pomegranate orchard affected by root-knot nematode

Table 4 Effect of bioagents for the management of root-knot nematodes infecting pomegranate

Treatment	Decline in nematode populations (%)	Decline in root galling (%)	Yield (MT/ha)	Benefit–cost ratio
P. fluorescens at 20 g/m^2	31.2	29.2	18.4	2.37
T. viride at 20 g/m^2	28.3	23.9	18.0	2.33
Cartap hydrochloride at 0.3 g a.i./m^2	27.2	20.4	17.6	2.27
Carbofuran at 0.3 g a.i./m^2	29.8	25.1	17.8	2.27
Untreated control	–	–	15.4	–
CD ($P = 0.05$)	2.99	2.30	1.17	–

4.6 *Potato (*Solanum tuberosum*)*

4.6.1 Cyst Nematodes (*Globodera rostochiensis* and *G. pallida*)

An average loss of about 9 % of global potato is accounted to the cyst nematodes amounting to about 45 million tonnes. Application of talc based formulation of *P. fluorescens* (15×10^8 cfu/g) at 10 kg/ha reduced the penetration of roots by the juveniles of potato cyst nematodes at 45 days after planting by 63.4 %. Significant reduction in cyst count and egg production and significant increase in potato tuber yield by 76.2 % was observed with the above bioagent (Seenivasan et al. 2007).

Plant health promoting rhizobacteria, namely *Agrobacterium radiobacter* and *Bacillus sphaericus*, were reported to reduce the penetration of *Globodera pallida* by 41 % in potato roots and improving yield (Racke and Sikora 1992). Reitz et al. (2000) showed that the lipopolysaccharides of *Rhizobium etli* G12 induce the systemic resistance to infection by the cyst nematode *G. pallida* in potato roots. Among several biopesticides tested, *P. fluorescens* Pf1 recorded significantly higher growth parameters and yield (16.03 t/ha) coupled with lower cyst population of *G. rostochiensis* and *G. pallida* in soil and roots and nematode reproduction (Rf: 1.38) (Umamaheswari et al. 2012).

4.7 *Tomato (*Lycopersicon esculentum*)*

4.7.1 Root-Knot Nematodes (*Meloidogyne incognita* and *M. javanica*)

M. incognita causes 30.57–46.92 % loss in tomato yield, while *M. javanica* causes 77.5 % loss in yield (Fig. 7). The inoculants Equity (contains 47 strains of bacilli in a liquid formulation), BioYield (contains *Bacillus subtilis* strain GB03 and *B. amyloliquefaciens* strain GB99 in a chitosan carrier), and FZB42 (a strain of *B. amyloliquefaciens*) induced significant reduction in nematode galls per plant on tomato (Cadenaa et al. 2008). AgBlend, containing microbial metabolites, reduced number of galls. Treatment with each of the inoculants also increased root weight.

The effects of six isolates of Plant Growht Promoting Rhizobacteria, *Pseudomonas putida*, *P. fluorescens*, *Serratia marcescens*, *Bacillus amyloliquefaciens*, *B. subtilis*

Fig. 7 Tomato root infected by root-knot nematode showing galls

and *B. cereus*, were studied on tomato plant growth and root-knot nematode reproduction. The highest shoot dry weight (43.00 g), fruit yield (319.6) and the lowest numbers of J_2/10 g of soil (78), galls/root (24.33), egg masses/root (12.66), and eggs/egg masses (280.66) were observed in the plants treated with *S. marcescens* (Almaghrabi et al. 2013).

Application of *P. fluorescens* at 10 g/m^2 in nursery beds gave good control of root-knot nematodes. The level of infestation of root-knot nematode *M. incognita* on tomato was reduced with fewer galls and egg masses in the soil following root dipping with *P. fluorescens* strain Pf1 (Santhi and Sivakumar 1995). Similarly, application of the bacterium, *P. chitinolytica* reduced the root-knot nematode infection in tomato crop (Spiegel et al. 1991).

Consortial formulation of biocontrol agents viz., *P. fluorescens* Pf 128 and *B. subtilis* Bbv 57 recorded the highest defense enzymatic activity (peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase) and lowest nematode population in tomato roots compared to other strains either alone or in combination (Sankarimeena et al. 2012). Gautam et al. (1995) reported that combined application of *B. subtilis* and *P. lilacinus* suppressed root-knot nematode population by application of bioagents individually. Biocontrol agents suppressed the numbers of root galls, females, eggs, and second stage juveniles.

4.7.2 Reniform Nematode (*Rotylenchulus reniformis*)

R. reniformis is responsible for 42.25–49.02 % loss in fruit yield of tomato. Nursery bed treatment with *P. fluorescens* (with 1×10^9 spores/g) and *P. chlamydosporia* (with 1×10^6 spores/g) each at 20 g/m^2 and field application of five tonnes of enriched

Table 5 Effect of application of *P. fluorescens* enriched neem cake or vermicompost on root population densities of *M. incognita* and *R. reniformis*, fruit yield, and root colonization by the bioagent in tomato

Treatments	No. of *M. incognita*/ (in 10 g root)	No. of *R. reniformis*/ (in 10 g root)	Increase in fruit yield (%)	Root colonization by *P. fluorescens* (cfu/g × 10^6)
T1	24.03	21.23	8	3.0
T2	15.58	14.62	10	6.3
T3	17.80	17.05	15	4.2
T4	12.46	21.52	26	8.3
T5	16.47	16.10	21	5.3
T6	23.35	21.23	6	0.0
T7	29.37	26.10	4	0.0
T8	20.47	18.79	12	3.6
T9	21.36	19.84	10	4.5
T10	44.50	34.80	–	0.0
CD ($P = 0.05$)	3.46	2.84	–	0.76

*Values in each column are the mean of five replicates

FYM with the above bioagents each at 5 kg significantly reduced reniform nematodes in tomato by 72 % over control. The yield increase was up to 21.7 % with cost–benefit ratio of 1:4.9 (Reddy 2012).

The efficacy of neem cake along with *P. fluorescens*, against root-knot and reniform nematodes (*M. incognita* and *R. reniformis*) were evaluated on tomato. Tomato seedling treatment with *P. fluorescens* and application of neem cake enriched with *P. fluorescens* was found to be significantly effective in reducing the population of nematodes. This treatment reduced root population of *R. reniformis* by 64 % and *M. incognita* by 72 %. There was significant increase in the yield of tomato by 26 % (Rao 2011) (Table 5).

Application of *Bacillus thuringiensis* caused 95 % mortality of *M. javanica* juveniles due to β-exotoxin production and suppressed gall formation, egg mass production, and nematode population in soil. Field application of a nematicidal *B. thuringiensis* strain to tomato in Puerto Rico reduced galling in roots due to *M. incognita* and soil population of *Rotylenchulus reniformis* and increased yield significantly (Zuckerman et al. 1993).

4.7.3 Damping off Disease Complex in Tomato (*Meloidogyne* spp., and *Rhizoctonia solani*)

Damping-off of tomato is more severe in soil infested with both *M. javanica* and *R. solani* than with the fungus alone. *M. javanica* increased the extent of damage by pre- and post-emergence phases of damping off caused initially by *R. solani* in tomato. Combined application of *Pseudomonas aeruginosa* and *Paecilomyces* *lilacinus* significantly suppressed soil-borne root-infecting pathogens such as *R. solani* and *M. javanica* (Siddiqui et al. 2000). *P. aeruginosa–Bacillus subtilis* treatment was the most effective in the suppression of root-rot disease complex with enhancement of plant growth (Siddiqui and Haque 2001).

4.7.4 Bacterial Wilt Disease Complex in Tomato (*Meloidogyne incognita* and *Ralstonia solancearum*)

Wilt disease development occurred earlier and with a higher mortality rate in both wilt-resistant and susceptible tomato cultivars grown in *R. solanacearum* and *M. incognita*-infested soil. Treatment of nursery bed with *P. fluorescens* (10^9 cfu/g) and *T. harzianum* (10^6 cfu/g) each at the rate of 20 g/m^2 and subsequent application of 5 MT of farmyard manure enriched with 5 kg each of *P. fluorescens* (10^9 cfu/g) and *P. lilacinus* (10^6 cfu/g) per hectare, significantly reduced *M. incognita* in tomato roots by 70 %; reduced the incidence of bacterial wilt; and increased the yield by 24.2 %. Benefit–cost ratio (calculated for the additional cost of the biopesticides and additional returns accrued by the application of the biopesticide) was 4.4 (Rao et al. 2009).

*4.8 Brinjal (*Solanum melongena*)*

4.8.1 Root-Knot Nematodes (*Meloidogyne* spp.)

M. incognita causes 27.30–48.55 % loss in fruit yield of brinjal. Nursery bed treatment with *Bacillus macerans* at 25 g/m^2 + soil drench (2 % solution) 10 days after sowing gave maximum reduction in nematode population and maximum yield of 3.825 t/ha (Sheela and Nisha 2004). *Azotobacter chroococcum* caused significant inhibition in hatching of egg masses of *M. incognita*, reduction in penetration of juveniles in brinjal roots and reduction in soil and root population of root-knot nematodes and root galling. Among different strains of *A. chroococcum* tested, strain 23 proved better than other strains (Chahal and Chahal 1986, 1988, 1999). However, the treatment of *P. fluorescens* at 10 kg/ha + neem cake at 1 t/ha was found effective in reducing *M. incognita* population (49.28 %), number of root galls/egg masses (68.45 %) and gall index (58.5 %) and increasing the yield (30.66 %) of brinjal with 4.64 as benefit–cost ratio (Zore et al. 2013).

Borkakaty (1993) observed that inoculation of *P. lilacinus* at 4 g/kg of soil in combination with mustard oil cake at 0.5 and 1.0 t/ha increased plant growth with corresponding decrease in number of galls, egg masses and eggs/egg mass of *M. incognita* on brinjal. Application of castor cake extract based formulation of *T. harzianum* (at 500 ml/m^2 containing 9.9×10^3 spores/ml) to nursery beds of brinjal was effective in producing vigorous seedlings (with maximum seedling weight) with least root galling. The above treatment also increased root colonization and parasitization of *M. incognita* females by *T. harzianum* (Rao et al. 1998).

4.8.2 Bacterial Wilt Disease Complex in Brinjal (*Meloidogyne incognita* and *Ralstonia solanacearum*)

Eggplant is prone to many soil-borne diseases among which the bacterial wilt (*R. solanacearum*) in combination with root-knot nematode (*M. incognita*) takes heavy toll every year all over the world. Barua and Bora (2008) observed that *P. fluorescens* at 5 g/kg soil significantly reduced the final soil nematode population and wilt disease incidence in brinjal. Further, combined application of wheat bran formulations of *P. fluorescens* significantly reduced root galling, egg mass production, and final population of *M. incognita* and *R. solanacearum* in soil and increased population of the antagonists in soil (Barua and Bora 2009).

Dhawan et al. (2008) found that root dip of brinjal seedlings in *P. fluorescens* (5 g/l of water for 30 min) gave maximum suppression of root-knot nematode population in soil followed by *P. fluorescens* (soil application at 10 g/plant + root dip at 5 g/l of water for 30 min) and *P. fluorescens* as soil application (10 g/plant). Root gall index (RGI) and wilt disease incidence due to *Ralstonia solanacearum* (WDI) was minimum in combination treatment of *P. fluorescens* + *P. chlamydosporia* followed by *T. harzianum* + *P. fluorescens* where the RGI was 1.81 and 1.93, respectively, and that of WDI was 25.13 % and 27.28 %, respectively (Naik 2004).

*4.9 Bell Pepper (*Capsicum annuum*)*

4.9.1 Bell Pepper Root-Knot Nematode (*Meloidogyne incognita*)

M. incognita was responsible for 24.54–28.00 % loss in fruit yield of bell pepper. Capsicum seedling treatment with *P. fluorescens* and soil application of neem cake enriched with *P. fluorescens* was found to be significantly effective in reducing *R. reniformis* (69 %) and *M. incognita* (68 %) and increasing the yield of capsicum by 19 %. In general the growth of capsicum plants treated with *P. fluorescens* was better (Rao 2011) (Fig. 8).

Fig. 8 Biopesticide treated (*right*) and untreated (*left*) capsicum seedlings

Integrated management of *M. incognita* infecting capsicum was achieved by seed treatment with *P. fluorescens* at 50 g/kg seed combined with nursery bed treatment with *P. chlamydosporia* at 50 g/m^2. The above treatment was significantly effective in increasing the seedling growth and root colonization with bioagents and in reducing the root galling. These seedlings when transplanted in field, significantly reduced nematode population both in soil and roots, root galling and increased root colonization by bioagents, propagule density in soil, parasitization of eggs, and fruit yield (Rao et al. 2004).

In capsicum grown under protected conditions, plots treated with *Bacillus subtilis*, *Trichoderma harzianum*, and *Paecilomyces lilacinus* as seed treatment at 20 g/kg seeds, substrate treatment at 10 g/kg cocopeat and soil application with FYM enriched with 2 kg each of the biopesticides at 1 kg/m^2 recorded maximum yield (56.66 t/ha) and minimum nematode population (15 per 5 g root; 91 per 100 g soil) (Rao and Umamaheswari 2015a) (Fig. 9).

4.9.2 Foot and Root-Rot Disease Complex in Bell Pepper (*Meloidogyne incognita* and *Phytophthora capsici*)

The disease incidence was increased up to 54 % when Capsicum was inoculated with *Meloidogyne* sp. and *Phytophthora* sp. Capsicum seed treatment with consortia of *P. fluorescens* (three strains) at the rate of 10 g/kg and subsequent application of consortia formulation of *P. fluorescens* at the rate of 15 g/m^2, significantly reduced *M. incognita* in capsicum roots by 74 %; reduced the incidence of *Phytophtora* foot and root rot and increased the yield by 26 %. Cost–benefit ratio (calculated for the additional cost of the biopesticides and additional returns accrued by the application of the biopesticide) was 1:3.8 (Rao et al. 2009).

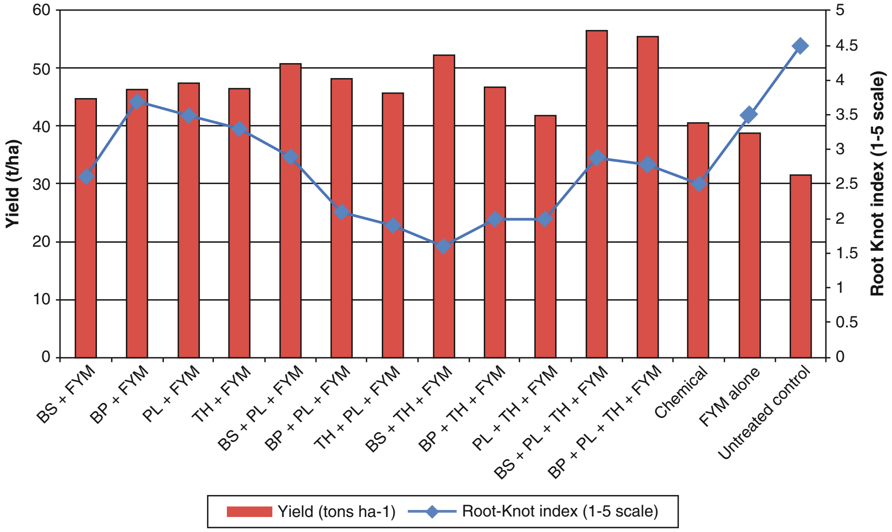

Fig. 9 Efficacy of biopesticides on yield and nematode population in capsicum under protected conditions

4.9.3 Bacterial Wilt Disease Complex in Bell Pepper (*Meloidogyne incognita* and *Ralstonia solanacearum*)

Combined application of neem based formulations of *P. fluorescens* and *P. chlamydosporia/T. harzianum* at 40 g/m^2 in nursery beds and transplanting these seedlings in the main field resulted in significant reduction in disease index and root-knot index in capsicum to the tune of 70 % and increased the crop yield by 37 % (Rao et al. 2002) (Table 6).

4.9.4 Wilt Disease Complex in Bell Pepper (*Meloidogyne javanica* and *Fusarium oxysporum*)

Kumar et al. (2009) reported that integration of *P. fluorescens* at 50 kg/ha with neem seed powder at 250 kg/ha in nursery beds was found to be effective in increasing the number of germinated seedlings (81/0.5), fresh weight of seedlings (136.7 g) and reducing root galling due to *M. incognita* (0.7 RKI) and percent root infection by *F. solani* (5.5 %) in nursery beds of chilli. Perveen et al. (1998) reported that *Pseudomonas aeruginosa* and *Paecilomyces lilacinus* when used alone or together significantly increased plant height and reduced infection of *M. javanica* and root infecting fungi, viz., *Macrophomina phaseolina, Rhizoctonia solani*, *F. solani*, and *F. oxysporum* on chilli. *P. aeruginosa* was more effective than *P. lilacinus* in reducing the *M. javanica* infection (Table 7).

Table 6 Effect of integration of neem-based bioagents on the growth of transplants and management of disease complex and yield of capsicum

Treatment	Seedling weight (g)	Root-knot index (1–10)	Disease index (1–9)	Yield in kg/4 m^2
Seed treatment with *P. fluorescens*	421	5.6	6.4	4.3
Seed treatment with neem-based *P. fluorescens*	428	5.2	6.7	4.7
Nursery treatment with *P. fluorescens*	435	4.6	5.4	4.8
Nursery treatment with *P. fluorescens* + *P. chlamydosporia*	463	4.1	5.2	4.0
Nursery treatment with *P. fluorescens* + *T. harzianum*	493	3.8	3.5	5.1
Control	340	8.7	8.2	2.6
CD ($P=0.05$)	27.20	0.49	0.38	0.25

Table 7 Effect of *Pseudomonas aeruginosa* and *Paecilomyces lilacinus* on plant height and control of root-rot disease complex in capsicum

Treatment	Plant height (cm)	Root-knot index	Infection %			
			R. solani	*F. solani*	*M. phaseolina*	*F. oxysporum*
Control	10.5	3.3	19	75	31	37
P. lilacinus	14.5	2.9	0	56	19	44
P. aeruginosa	16.5	2.5	6	75	12	21
P. lilacinus + *P. aeruginosa*	14.7	2.1	25	69	12	12
CD ($P=0.05$)	2.2	0.34	6.1	6.1	6.1	6.1

*4.10 Onion (***Allium cepa***) and Garlic (***Allium sativum***)*

Seed treatment with *P. fluorescens* (10^9 cfu/g) at 10 g/kg and subsequent soil application of 5 t of FYM enriched with 5 kg each of *P. fluorescens* (10^9 cfu/g) and *Pochonia chlamydosporia* (10^6 cfu/g) per ha significantly reduced *M. incognita* population in roots by 69 % and increased bulb yield by 21 % (Anon 2012).

*4.11 Cabbage (***Brassica oleracea** *var.* **capitata***) and Cauliflower (***Brassica oleracea** *var.* **botrytis)**

4.11.1 Club Rot Disease Complex in Cabbage (*Meloidogyne incognita* and *Plasmodiophora brassicae*)

PGPR strains (*Pseudomonas fluorescens*, *Bacillus subtilis*) combined with fungal biocontrol agents (*Trichoderma viride*, *T. harzianum*) were found to be effective in reducing the nematode-fungal disease complex in cabbage (Loganathan et al. 2001). The consortia mixture of *P. fluorescens*, *T. viride*, and chitin effectively reduced the disease complex in cabbage and cauliflower (Table 8).

*4.12 Okra (***Abelmoschus esculentus***)*

4.12.1 Root-Knot Nematodes (*Meloidogyne* spp.)

M. incognita was responsible for 28.08 % loss in fruit yield of okra, while *M. javanica* caused 20.20–41.20 % loss in yield.

Table 8 Efficacy of bioformulation mixtures against root-knot nematode–club root disease complex in cabbage

Treatment	Club root index	Nematode incidence	
		Population	Root-knot index
Trichoderma viride	25.99 (30.65)	129	2.66
Pseudomonas fluorescens	28.20 (32.07)	112	2.33
T. viride + *P. fluorescens*	25.33 (30.22)	114	2.33
T. viride + Chitin	25.44 (30.29)	108	2.33
P. fluorescens + Chitin	25.66 (30.43)	111	2.00
T. viride + *P. fluorescens* + Chitin	22.22 (28.12)	108	2.00
Chitin alone	31.70 (34.26)	139	3.00
Carbendazim	19.90 (26.49)	264	4.66
Carbofuran	39.90 (39.17)	106	1.66
Carbendazim + Carbofuran	15.00 (22.79)	103	1.66
Plasmodiophora brassicae alone	48.90 (44.37)	0.033	0.133
Meloidogyne incognita alone	0.03 (0.60)	280	5.00

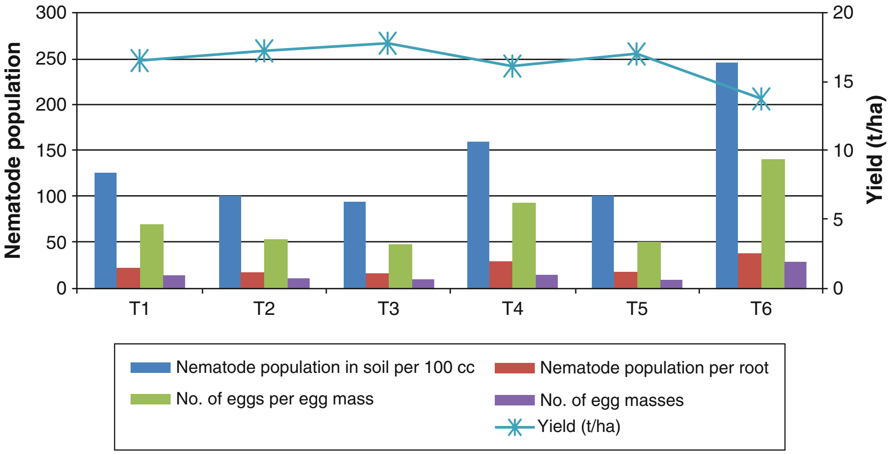

Fig. 10 Effect of *P. putida* on *M. incognita* infected okra

4.12.2. Wilt Disease Complex in Okra (*Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *vasinfectum*)

Soil application of 25 g/m^2 of deoiled neem cake enriched with *P. fluorescens* (2×10^6 cfu/g) proved to be an effective treatment in combating the damage caused by *M. incognita* and *F. oxysporum* f. sp. *vasinfectum* to the tune of 68 %. This treatment also increased the yield of okra fruits by 24 % under field conditions (Chaaya et al. 2010).

In okra, seed treatment with *Pseudomonas putida* 1 % A.S at 10 ml/kg seed and application of 5 t of FYM enriched with *P. putida* at 5 l/ha significantly suppressed the hatching of root-knot nematode eggs (65.53 %) and final nematode population (61.95 %) compared to control. It also significantly increased the yield by 29.61 % (Rao and Umamaheswari 2015b) (Fig. 10). Also, seed treatment of okra with *Bacillus pumilus* 1 % A.S at 10 ml/kg seed and application of 5 t of FYM enriched with *B. pumilus* at 5 l/ha recorded significantly lower *M. incognita* population (64.16 %) and higher yield (30.36 %) (Fig. 11). It was on par with seed treatment with *B. pumilus* 1 % A.S at 10 ml/kg seed and application of 2.5 t of FYM enriched with *B. pumilus* at 2.5 l/ha in reducing the final nematode population (60.21 %) (Rao and Umamaheswari 2015b).

*4.13 French Bean (*Phaseolus vulgaris*)*

4.13.1 Root-Knot Nematodes (*Meloidogyne* spp.)

Yield reductions due to high populations of root-knot nematodes may range from 45 to 90 % in French bean. The combination of *Bacillus subtilis* (isolate K194) inoculum and cow manure led to a 54 % reduction in numbers of root-knot nematodes, compared to the untreated control (Wepuhkhulu et al. 2011).

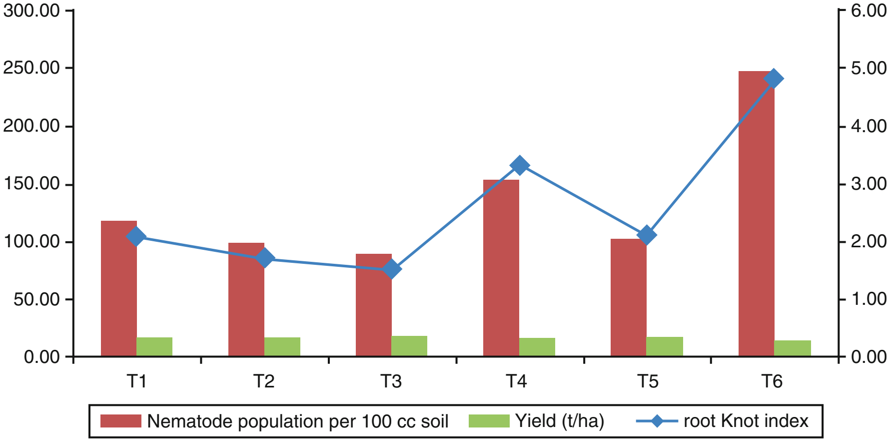

Fig. 11 Effect of *B. pumilus* on *M. incognita* infected okra

*4.14 Pumpkin (*Cucurbita moschata*)*

4.14.1 Root-Rot and Wilt Disease Complex in Pumpkin (*Meloidogyne javanica* and Root *Macrophomina phaseolina/ Fusarium oxysporum/F. solani*)

P. aeruginosa and *P. lilacinus* used alone or together significantly reduced infection of root-knot nematode *M. javanica* and root infecting fungi viz., *M. phaseolina*, *R. solani*, *F. solani*, and *F. oxysporum* on pumpkin. *P. aeruginosa* was more effective than *P. lilacinus* in reducing *M. javanica* infection. Combined use of *P. lilacinus* and *P. aeruginosa* was more effective in reducing the infection of *M. phaseolina* and *F. oxysporum* on pumpkin than either used alone (Perveen et al. 1998).

*4.15 Cucumber (*Cucumis sativus*)*

4.15.1 Root-Knot Nematodes (*Meloidogyne* spp.)

Combined inoculation of arbuscular mycorrhizal fungi and *P. fluorescens* had positive effect on root-knot nematode control in cucumber (Jakobsen 1999).

*4.16 Carrot (*Daucus carota*)*

4.16.1 Root-Knot Nematodes (*Meloidogyne* spp.)

M. incognita was responsible for 56.64 % loss in yield of carrots (Fig. 12). Seed treatment with *Pseudomonas putida* (10^9 cfu/g) at 10 g/kg and subsequent application of 5 t of FYM enriched with 5 kg each of *P. putida* (10^9 cfu/g) and *T. harzianum*

Fig. 12 Root-knot nematode affected carrot

(10^6 cfu/g) per ha significantly reduced reniform and root-knot nematode population in roots by 70 and 77 %, respectively. Significant increase in yield (24 %) was also observed. Benefit–cost ratio calculated for marginal cost of biopesticides and returns accrued by application of biopesticides was 5.3 (Anon 2012).

4.16.2 Wilt Disease Complex in Carrot (*Meloidogyne incognita* and *Erwinia carotovora* s. sp. *carotovora*)

The neem cake enriched with *Pseudomonas fluorescens* applied at 10 g/m^2 increased the root colonization of the bioagent and reduced the incidence of *M. incognita* and *E. carotovora* s. sp. *carotovora* by 68 and 56 %, respectively. There was also a significant increase in the yield of carrot to the tune of 23 % (Sowmya et al. 2010).

*4.17 Carnation (***Dianthus caryophyllus***)*

The root-knot nematode, *M incognita* is one of the serious limiting factors in commercial cultivation of carnation under polyhouse conditions. *M incognita* was responsible for 27 % loss in flower yield of carnation. In carnation, application of *Pseudomonas* sp. strain WCS 417r protected plants systemically against *Fusarium* wilt disease (Anusuya and Vadivelu 2002). Combined inoculation of AMF and *P. fluorescens* had positive effect on root-knot nematode control on carnation (Anusuya and Vadivelu 2002).

Fig. 13 Root-knot nematode infected gerbera

*4.18 Gerbera (*Gerbera jamesonii*)*

Root-knot nematode, *M. incognita* causes 20–30 % yield loss in gerbera (Fig. 13).

4.18.1 Root-Rot Disease Complex in Gerbera (*Meloidogyne incognita* and *Phytophthora cryptogea*)

Seedling treatment with *P. fluorescens* + soil application with neem cake enriched with *P. fluorescens* proved significantly effective in the management of disease complex caused by *M. incognita* and *P. cryptogea* on gerbera under field conditions. There was a significant reduction in the root-knot index of *M. incognita* and percentage of disease incidence by *P. cryptogea.* The flowers harvested from the above treatment showed an increased vase life compared to control (Manoj Kumar and Rao 2011) (Table 9).

Combined application of neem cake enriched with *P. fluorescens* [mixing 50 g *P. fluorescens* (2×10^8 cfu/g) in 1 kg of neem cake] applied at 25 g/m^2 was found effective for the management of disease complex and increased the flower yield by 26 % in gerbera cv. Debora (Manoj Kumar et al. 2010).

*4.19 Gladiolus (*Gladiolus *spp.)*

4.19.1 Root-Knot Nematodes (*Meloidogyne* spp.)

Application of 5 MT of FYM per ha enriched with *P. fluorescens* (with 1×10^9 cfu/g) significantly reduced *M. incognita* by 61–76 %, *R. reniformis* by 65–70 % in the roots of gladiolus and increased flower yield by 19–22 % (Reddy 2012) (Fig. 14).

Table 9 Effect of bioagents and botanicals on the root-knot and root-rot disease complex

Treatments	Plant height (cm)	Root galling (1–10 scale)	Disease incidence (%)	% increase in yield	Root colonization by *P. fluorescens* (CFU/g × 10^6)
T1	15.0	7.7 (18.94)	38.06 (24.67)	15.12	1.03
T2	23.2	6.3 (3.68)	20.04 (60.34)	21.97	1.35
T3	19.2	7.1 (25.26)	29.93 (40.76)	20.36	1.31
T4	27.2	4.1 (56.84)	18.16 (64.06)	28.52	2.10
T5	24.6	5.3 (44.21)	25.77 (49.00)	26.92	1.97
T6	19.4	8.5 (10.52)	45.40 (10.15)	10.78	0.00
T7	18.2	9.3 (2.10)	49.49 ()2.05	9.63	0.00
T8	22.1	6.4 (32.63)	35.67 (29.40)	16.22	1.78
T9	19.3	7.4 (22.10)	35.61 (29.52)	15.97	1.65
T10	11.6	9.5	50.53	0.00	0.00
CD ($P = 0.05$)	2.26	0.44	–	–	0.74

Values in parentheses are % decrease over control

Fig. 14 *M. incognita* infested gladiolus

*4.20 Crossandra (*Crossandra undulaefolia*)*

Treatment of the nursery bed with the formulations of *P. chlamydosporia* and *P. fluorescens* each at 50 g/m^2 and seed treatment with latter was significantly effective in reducing number of nematodes in roots and soil, increasing the percent parasitization or percent suppression of eggs by biocontrol agents and also flower yield of crossandra. The seedlings were colonized by both the bioagents and when transplanted in the field, the bioagents reached the field soil as they were recovered from root and soil samples at harvest of the crop. Individual effect of bioagents was maximized when both these organisms were integrated in the nursery bed stage. This could be due to the combined effect of both organisms on root-knot nematode. Combined use of *P. fluorescens* and *P. chlamydosporia* did not affect the colonization of each other on root (Rao 2007b).

5 Biopesticide Formulations in India

Currently, biopesticides are formulated mainly in solid carriers like talc, peat, kaolinite, vermiculite, and lignite in India. Vidhyasekaran and Muthamilan (1995) demonstrated the carrier based formulations of fluorescent pseudomonads by mixing the fermentor biomass with different carrier materials (talc/peat/kaolinite/lignite/vermiculite) and stickers. Krishnamurthy and Gnanamanickam (1998) developed talc based formulation of *P. fluorescens* in which methyl cellulose and talc were mixed at 1:4 ratio and blended with an equal volume of bacterial suspension at a concentration of 10^{10} cfu/ml. Nandakumar et al. (2001) developed talc based strain mixture formulation of fluorescent pseudomonads by mixing equal volume of individual strains and blended with talc as per Vidhyasekaran and Muthamilan (1995). Talc based strain mixtures were effective against diseases and increased plant yield under field conditions than the application of individual strains.

However, these solid formulations suffer from major setbacks like shorter shelf life, high contamination, and low field performance (Hegde 2002). In addition, when talc formulations are used for management of plant diseases in horticultural and plantation crops through micro-irrigation techniques, concerns are raised regarding the blockage of nozzles and distribution of bioinoculants. Moreover, the process of biohardening in horticultural plantlets, tissue culture plants, and hydroponics systems necessitates development of liquid-based bioformulations (Manikandan et al. 2010).

Liquid formulations offer longer shelf life (up to 2 years), with high purity, carrier-free activity, ease in handling and application, convenience in storage and transport, easy quality control, and enhanced export potential. In addition, they are also compatible with machinery on large farms and are preferred by farmers and industries (Pindi and Satyanarayana 2012). Liquid biopesticide formulations are microbial cultures or suspensions amended with substances to improve stickiness, stabilization, surfactant and dispersal abilities (Singleton et al. 2002). These formulations use broth culture or liquid suspension mainly in water, and also in mineral or organic oils. They include nutrients, cell protectants and inducers responsible for cell/spore/cyst formation to improve efficacy (Bashan et al. 2014). Comparison of liquid *B. subtilis* 1 % A.S. formulation and talc-based solid formulation of *B. subtilis* 1 % W.P. against root-knot disease complex of tomato revealed better performance of liquid over solid formulations in reducing the root-knot nematode population (55.5 %) and disease incidence (20.33 %) and increasing the yield (7.3 %) (Tables 10 and 11) (Rao et al. 2015).

Table 10 Bio-efficacy of *B. subtilis* 1 % A.S. (liquid formulation) against disease complex of tomato (2013–2014)

Treatments	Root-knot index (1–5)		No. of *M. incognita*/ 10 g of root		Disease incidence (%)		Yield/plot of 4×2.5 m (kg)		% increase in yield[a]		Root colonization by *B. subtilis* (CFU/g root)×10^6	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
T1	4.2	4.4	24	27	29.6	31.6	21.9	23.8	02.3	01.7	1.3	1.5
T2	3.7	3.4	20	22	26.9	27.8	22.4	24.3	04.6	03.4	2.4	2.6
T3	3.0	2.9	17	16	21.7	23.3	24.2	27.2	13.1	15.8	3.8	3.5
T4	2.3	2.5	08	09	12.2	14.5	25.4	28.0	18.7	19.2	4.2	4.0
T5	4.5	4.4	27	29	30.3	31.6	22.3	24.2	04.2	03.1	0	0
T6	2.6	2.8	12	16	19.7	22.5	23.7	26.3	10.7	12.6	0	0
T7	4.7	4.9	30	33	31.2	33.6	21.4	23.5	0	0	0	0
CD (*P*=0.05)	0.23	0.27	2.16	2.41	2.68	2.92	1.29	1.25	2.43	2.76	0.27	0.31

[a] CD was computed using angular transformed values. [T1—Seed treatment with *B. subtilis* 1 % A.S.—20 ml/kg of seed; T2—T1+ nursery bed treatment with 50 ml *B. subtilis* 1% A.S.; T3—T2+2 t Vermicompost enriched with 2.5 lit *B. subtilis* 1 % A.S./ha; T4—T2+2 t Vermicompost enriched with 5.0 lit *B. subtilis* 1% A.S./ha; T5—2 t Vermicompost/ha; T6—Chemical treatment (Carbofuran 1.0 kg a.i./ha+streptocycline 1 kg/ha); T7—Control]

Table 11 Bio-efficacy of *B. subtilis* 1 % W.P (talc formulation) against disease complex of tomato (2013–2014)

Treatments	Root-knot index (1–5)		No. of *M. incognita*/ 10 g of root		Disease incidence (%)		Yield/plot of 4×2.5 m (kg)		% increase in yield[a]		Root colonization of *B. subtilis* (CFU/g of root)×10^6	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
T1	4.3	4.6	25	24	31.5	30.2	25.0	23.0	01.8	01.5	1.5	1.7
T2	4.0	3.9	23	22	28.3	29.1	25.1	23.2	02.4	02.2	2.1	2.2
T3	3.3	3.2	19	16	24.2	23.2	27.1	25.9	10.2	11.4	3.2	3.0
T4	2.6	2.9	12	14	17.5	18.2	28.3	26.1	15.2	14.9	3.5	3.3
T5	4.4	4.6	25	26	29.3	30.1	25.7	23.6	04.4	04.2	0	0
T6	2.7	2.8	14	13	23.4	22.8	27.6	25.3	12.3	11.7	0	0
T7	4.7	4.6	34	29	33.9	35.6	24.6	22.7	0	0	0	0
CD (P=0.05)	0.21	0.24	1.95	2.24	2.74	2.31	1.73	1.96	2.79	2.61	0.45	0.36

[a] CD was computed using angular transformed values. [T_1—Seed treatment with *B. subtilis* 1% A.S.—20 ml/kg of seed; T_2—T_1 + nursery bed treatment with 50 ml *B. subtilis* 1% A.S.; T_3—T2+2 t Vermicompost enriched with 2.5 lit *B. subtilis* 1% A.S./ha; T_4—T_2+2 t Vermicompost enriched with 5.0 lit *B. subtilis* 1% A.S./ha; T_5—2 t Vermicompost/ha; T_6—Chemical treatment (Carbofuran 1.0 kg a.i./ha+streptocycline 1 kg/ha); T_7—Control]

6 Conclusions and Future Prospects

Biopesticides play an important role in maintaining crop and soil health through versatile mechanisms: nutrient cycling and uptake, suppression of plant pathogens, induction of resistance in plant host, and direct stimulation of plant growth. Thus, microbial biopesticides have great potential to provide holistic health and sustainable crop yields. Future progress in better understanding of microbial diversity, colonization ability, mechanisms of action, formulation, and application could facilitate their development and are reliable components in the management of sustainable agricultural systems.

The major goal for evergreen revolution in India is to develop local sourcing of biopesticides as a means of ensuring availability at a low cost to benefit poorer farmers, and as a base for expanding an Indian biotechnology industry. Further improvement of techniques and multidisciplinary research of plant protection scientists and agricultural engineers are likely to provide good, safe, effective, and inexpensive biopesticide formulations for plant protection. Measures in terms of technology, government support, subsidies, and constructive awareness among the agrarians are emphasized.

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Impact of Drought on Soil and Microbial Diversity in Different Agroecosystems of the Semiarid Zones

Seema B. Sharma and Thivakaran A. Gobi

Contents

Abstract Drought is a complex natural hazard affecting the world agricultural production and is projected to worsen with anticipated climate change due to global warming. The ever-increasing demand placed upon agriculture to supply food is one of the major challenges of agrarian communities. Therefore, a concerted effort focussed on the soil biology and agroecosystem is the need of the time. Soil quality is pivotal to agricultural sustainability. For an ecosystem to flourish, maintaining the quality of the soil becomes a critical factor. All parts of our environment and communities are directly or indirectly affected by drought or related conditions. The impact of drought can be broadly classified into three categories, namely economic,

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environmental, and social. This chapter focuses on the environmental aspects of the droughts, which directly or indirectly affect the social and economic aspects. Agricultural activities in the calamity-prone semiarid tropics face a scarcity of resources and abiotic stresses as droughts aggravate the problems. Thus, the agricultural systems need to be managed in such a way so that they are more resilient to the impacts of drought.

Keywords Drought • Microbiological properties • Organic-amendments • Semiarid tropics • Soil quality

1 Introduction

Drought is one of the atrocious natural phenomena. Drought can be defined as a prolonged deficiency of precipitation usually for a season or more (Trenberth et al. 2014). This imbalance leads to water scarcity which is detrimental to agricultural production. Climatic factors such as soaring mercury levels, high wind velocity, and suboptimal relative humidity are precursors of drought. Its impact results from interaction between a natural event and excessive demands on the existing water supply, and more often than not it gets exacerbated by human interventions. Drought is a complicated phenomenon, and can be hard to define. The empirical evidences from drought conditions have exposed the fragility of human societies to this natural hazard. It is often referred to as a "creeping phenomenon" and its impacts vary from region to region (Wilhite et al. 2014). Therefore, it is very hard for the people to understand and define the drought. For example, in countries like Libya (annual rainfall is less than 180 mm), 6 days without rain would not be considered as drought (NDMC 2015). Thus, the drought means different things in different regions. "Drought" is not a set number or equation. It is defined depending on the average amount of precipitation an area is accustomed to receiving. Any deviation in atmospheric circulation patterns can obstruct storm tracks for a significant period of time, which may considerably affect the quantum of precipitation a region normally receives. This imbalance may result in drought or floods. The amount of moisture a region can absorb is also influenced by changes in wind pattern.

The relevance of climate extreme events such as droughts and its impacts is well recognized. Climate change modeling studies have shown that the tropics of Asia and Africa could experience a significant change in the frequency of occurrence and the intensity of droughts. It is assumed that global warming may lead to more sustained droughts in semiarid areas and the desert belts, possibly connected with a climatic pattern of less but heavier rainfall events (IPCC 2007). If this scenario materializes, it could lead to more frequent crop failures, accelerated desertification, and loss of arable land, ultimately forcing people to abandon the desert belts. The following migrations may become a challenge for more developed countries as well.

In order to cope with such a development, or to prevent it, it is discussed whether a reduction of human pressure can compensate for the increased climatic forcing, and how land use can better adapt to changing conditions (Lucke et al. 2008).

It has been verified that there exists a link between certain climate patterns and drought (NDMC 2015). El Nino is a weather event where the surface water in the Pacific Ocean along the central South American coast rises in temperature. These warmer waters alter storm patterns and are associated with droughts in Indonesia, Indian subcontinent, Australia, and north-eastern South America. El Nino events are not predictable and can occur every 2–7 years. La Nina is the counterpart to El Nino, when the surface water in the Pacific Ocean along the coast of South America decreases in temperature. The cooler waters affect storm patterns by contributing to drier-than-normal conditions in parts of North and South America. While El Nino usually lasts less than a year, La Nina can extend for 1–3 years. During this, less heating leads to colder sea waters off the western South American Coast, thus making it a high-pressure zone. This pushes moist sea winds towards the Indian Ocean and increases the chance of normal or excessive rainfall in the Indian subcontinents (Aalst and Maarten 2006).

Various simulating models have been developed over the past years to predict droughts based on a phenomenon like the El Nino and La Nina and various other climatic and topographic features, but these prior warnings cannot circumvent the severity of the droughts in the regions affected by it because the deficiency of precious natural resource “water” cannot be mitigated by any artificial source. Hereafter, the only alternative left is to readily accept the occurrence of a drought and prior mitigation measures to deal with it in a holistic way. With an emphasis on agricultural systems such measures would include gearing our management systems in a direction that could possibly help in mitigation of the impacts on soil fertility. This chapter focus on the environmental aspects of the droughts having direct or indirect effect on social and economic aspects. Moreover, the agricultural activities in the calamity-prone semiarid tropics facing a scarcity of resources and abiotic stresses is also addressed.

2 Drought and Its Impact on the Farming Practices

Amongst all the natural hazards, drought affects the maximum number of people globally causing devastating impacts. It is a reality that drought results in sets of socioeconomic impacts, including crop yield, failure, unemployment, erosion of assets, income decrease, poor nutrition, and decreasing risk-absorptive capacity, thereby increasing the vulnerability of the community as a whole. Drought adversely affects both the flora and fauna of the ecosystem. It hampers the food supply which can severely damage their habitat. The impact of drought can be short lived or it can be perpetual. Losses of soil biodiversity and soil erosion are the two main dreadful impacts of the drought, which are a consequence of water scarcity. A sustained drought or different rainfall pattern may lead to a reduction of organic matter, loss of aggregate stability, and long-term degradation of soil structure and nutrient availability. The multidimensional effects of drought are shown in Fig. 1.

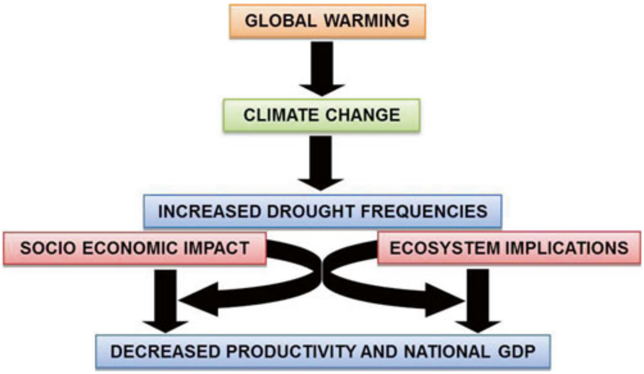

Fig. 1 Schematic representation of multifaceted impact of drought on various components of ecosystem

2.1 Effect of Drought on the Fertility of Soil

Drought stress affects not only the physical and chemical parameters of soil fertility, but also the microbiological parameters and only those soil systems that can endure such stress are able to withstand their fertility in the long term (Rousk et al. 2013). Drought stress is the biggest impediment to the agricultural productivity. One of the possible ways to minimize the drought impact is to develop plant tolerance by effective management of plant nutrients. The primary impact of drought on a crop is a reduction in yield as well as the quality of produce, which occurs due to decline in available macro- and micronutrients and associated microbial activities (Hu et al. 2007). Nitrogen is an important nutrient, mostly present in the volatile or mobile forms in the soil (Moore 2005). However, phosphorous and potassium are more stable in the soil compared to nitrogen. A fertilizer that is not used by the crop, due to lower yield caused by drought, will most likely be available the next season for use by the crop. Soil characteristics in semiarid tropics are conducive for P fixation as they are rich in aluminum and iron oxide. It is generally accepted that the uptake of P by crop plants is reduced in dry-soil conditions (Sanchez-Rodriguez et al. 2010; Heidari and Karami 2014).

Drought adversely affects the plant health by impairing the uptake of nutrients through root system due to decrease in the permeability of cell membranes. The depletion in moisture content further suppresses the rate of diffusion of the nutrients in the soil to the root surface (Marschner 2012). Losses in crop yield caused by drought are comparatively higher than the losses due to any other factor because both the severities and the duration of the drought are decisive factors in deciding the impact of the calamity. Considering that droughts are frequent in semiarid zones, it is essential to validate how this type of stress affects the nutrient uptake and assimilation of these in the crops grown.

2.2 Effect of Droughts on the Activities of Soil Microorganisms

The extreme moisture deficiency that is observed with the prolonged drought has dire consequences on the soil microorganisms and their activities. Through evolution, microorganisms have developed many indirect and direct mechanisms that may protect them against the various environmental stresses. There are different survival mechanisms adopted by the microbial community (Kremer 2012). In the soil, the microbial cells may be coated with clay minerals or organic substances that provide indirect protection of cellular structures and contents. Some bacteria (*Bacillus* spp., and Actinobacteria) form heat-resistant, dormant spores that withstand in dry conditions and high temperatures. On the reversal of optimum condition they subsequently germinate and produce active bacterial cells. Similarly, many fungi also produce various types of resting structures that could survive in high temperature. Moreover, some microorganisms form the gummy substances or slime layers that encase the individual or clusters of cells that congregate together to form slime layers or *biofilms* that adhere to inorganic (rocks, soil pore walls, water conduits) or organic (roots) surfaces and also insulates the entire microbial community against the effect of high temperature.

Many microorganisms fail to cope up with the severe drought conditions due to heat stress or lack of potential survival mechanism. Viewing microbes from this perspective improves our understanding to know how they withstand or perish under harsh condition including drought. The excessive heat under severe drought conditions leads to removal of water from living cells; this drying process is called as desiccation. It is responsible for the irreversible damages to cellular contents such as protein and nucleic acids, which may impair the microbial growth. In worst cases, the membranes and cell walls are ruptured and the cellular contents spill out, effectively killing the microbe. Different microbes are affected differently under heat stress and the overall microbial diversity in soil changes. However, if a component of the microbial community is highly sensitive to drought stresses, then the system becomes imbalanced due to loss of the function carried out by the sensitive microbes. For example, activities of enzymes that cycle nitrogen from proteins and urea, phosphorus from phospholipids and nucleic acids, and carbon from cellulose are reduced by 80 %. These processes are important for plant growth, but it is not known how quickly these can recover to benefit plants when soil moisture is replenished. Soil moisture deficit not only adversely affects microbial diversity; it also affects the availability of organic substances for microbial metabolism. Organic substances must be in a dissolved or soluble form of movement to microbial cells and then transferred across cellular membranes for breakdown into nutrients and energy. Extreme soil temperatures and moisture deficits profoundly affect soil biological processes by reducing, if not inhibiting, availability of organic sources to microbes. The degradation of organic compounds (both natural sources or synthetic compounds) by microorganisms is the most important process for complete transformation into mineral components. The detrimental effects of drought can effectively slow or even stop the biodegradation process by eliminating available soil water (Kremer 2012).

Recently, several investigations have been carried on the impact of water stress on the abundance, composition, and metabolized production by microorganisms (Manzoni et al. 2011; Davidson et al. 2012; Moyano et al. 2013). They concluded that the intensification of drought stress weakens the microbial activity in the soil. Moreover, the soil organic C/N ratio also declined with the intensification of drought (Li and Sarah 2003a, b). Whilst the effect of drought on plants has been studied in depth, little has been done to understand the implications of water stress on microbial soil ecosystems. This has primarily been due to the inability to cultivate many soil microbes in lab conditions, or to readily and cheaply process and sequence genetic information from soil samples. Due to recent advances in sequencing technology, it has now become feasible to mass sequence and analyze vast amounts of genetic data from environmental samples. This has delivered the tools to analyze soil for taxonomic and phylogenetic perspectives of microbial ecology, providing a means to compare soil ecology between sites.

Soil microorganisms are resilient and eventually return to pre-stress population levels on the reversal of optimum condition. However, it is not known whether all processes associated with a healthy ecosystem will be immediately and fully operational. Some processes such as phosphate solubilization and nitrogen fixation are associated with specialist microbial groups that do not make up a large segment of the microbial community, and may be highly susceptible to stress relative to other microbial groups (Kremer 2012). For example, nitrogen-fixing bacteria are sensitive to high temperatures. This may suggest that nitrogen transformation in soils could be disrupted because fixed nitrogen that feeds into the nitrogen cycle is reduced. Losses of many microbes due to heat stress could also lead to losses in genetic traits involved in alternate (and perhaps unknown) biological processes that might promote efficiency of nutrient cycling, biological control of pathogens, or plant growth enhancement. As we can only culture 0.1 to 1.0 % of the microorganisms in soil to identify their roles in biological and ecological soil processes, the unknown losses to drought stress is magnified.Moreover, the genetic potential lost among sensitive microbes destroyed by drought is immeasurable (Kremer 2012).

3 Management of Agricultural Systems Towards Drought

Drought stress is the one of the major limitations of agricultural productivity worldwide (Gornall et al. 2010). Plant nutrient management geared towards better and timely availability of both macro- and micronutrients in conjugation with the environmental sustainability is the key to effective drought management. Organic agrosystems have a higher potential to endure the drought stress due to the reason that added organic matter improves soil structure, increases water-holding content, and provides dissolved or soluble C necessary for microbial activity. Research findings advocate the fact that content of organic matter (OM) in the soil has directly affected the water-holding capacity of soil. Compost and manures applied in organic agri-management systems are rich in OM in different forms, which are useful as a source of nutrients for soil microorganisms and subsequently also for plants (Fischer and Glaser 2012). Therefore, the application of compost contributes to the development of microbial activity and thus to the development of soil organic mineral complex, which allows better uptake and utilization of soil water. The beneficial effect of compost application on microbial activity in soil, content of OM, and its biophysical properties has been confirmed by Lee et al. (2004).

Agricultural management activities involving higher inputs of microbe-based fertilizers are an emerging option for meeting agricultural challenges imposed by the still-growing demand for food, especially in the drought-prone semiarid tropics, where the use of chemical-based fertilizers and pesticides has deteriorated the soil quality (Malhotra et al. 2015). Thus, obtaining high yields, keeping in mind the environmental sustainability, is the main challenge for agriculture. In the past both the producers and consumers have increasingly focused on the health and quality of foods, as well as on their organoleptic and nutritional properties (Torjusen et al. 2001). Application of biofertilizers is a sustainable solution, especially in the semiarid tropics which face scarcity of resources. However, before biofertilizers can contribute to such benefits, scientists must learn more about them and explore ways and means for their better utilization in the farmers' fields. Future research should focus on managing plant–microbe interactions, particularly with respect to their mode of actions and adaptability to conditions under extreme environments for the benefit of plants. Furthermore, scientists need to address certain issues, like how to improve the efficacy of biofertilizers, what should be an ideal and universal delivery system, how to stabilize these microbes in soil systems, and how nutritional and root exudation aspects could be controlled in order to get maximum benefits from the application (Khalid et al. 2009). In brief, biofertilizer application provides an excellent opportunity to develop environment-friendly supplements and/or alternatives to chemical fertilizers (Sharma et al. 2013) as a primary drought mitigation measure in the calamity-prone semiarid tropics.

4 Mitigation of Drought in the Semiarid Tropics (Kachchh Scenario)

Kachchh is a very unique ecological terrain of Western India with harsh climatic conditions and abiotic stresses like salinity, erratic rainfall, and frequent droughts. Owing to its unique ecological and geomorphological setting, Kachchh is classified as biogeographic zone "3A" experiencing tropical arid climate (GEC 2011). Kachchh is the second-largest district in India and sprawls over 24 % of the total area of the Gujarat. It is coordinately situated in between 22°41′11 ″ to 24°41′47″ N latitude and 68°09′46″ to 71°54′ 47″ E longitude with an average area of 45,612 sq km. With a coastline of 406 km boasting nine ports, this area in northwest Gujarat is located in the state's arid tract and seven of its nine talukas are rated drought prone. The frequency of droughts in Kachchh is said to be once in every 2.5 years. The unique topography of Kachchh compounded with erratic rainfall makes agricultural practices a challenging issue and the availability of both major and micronutrients is often a limiting factor. The Kachchh region is named so due to its topographic resemblance to the back of a tortoise (*kachbo* in Gujarati), with a central portion (near Bhuj) elevated, and the land gently sloping downwards from there in all four directions. Due to this sharp gradient all the rivers and streams of Kachchh are non-perennial and have a high runoff rate; therefore, agriculture practices in this area are highly rain dependent, with some exceptions where underground bores are used for irrigation (GEC 2011).

Soil of Kachchh displays varied characters with respect to their depth, morphological features, and physicochemical properties. The soil is generally shallow to deep, light colored, calcareous, and salt rich in nature. It is generally presumed that the soil of Kachchh has developed under the paleoclimatic regime, because of xerophytic vegetation, arid climate, and unique topography. The agroclimate of the Kachchh district is characterized by very dry climate and scanty and uncertain rainfall even during the southwest monsoon season. The cold season from December to February is followed by the summer season from March to about middle of June. Mid-June to mid-September constitute the southwest monsoon season. October and November form the post-monsoon transition period between the rainy and cold season. Hydrological cycle in Kachchh is short. Analysis of rainfall data showed that the average annual rainfall of the district is 340 mm and varies from 420 mm (Mundra taluka) in the south to 225 mm (*Pachchham* island) in the north. About 94 % of the annual rainfall in the district is received during the southwest monsoon season. The frequency of annual rainfall in the district is shown in Table 1. It also reflects that about 95 % of the rain falls within the monsoon season and July is the month in which district receives maximum rainfall. On an average, there are 15

Table 1 Average rainy days and rainfall in different parts of Gujarat, India (GEC 2011)

	South Gujarat		North Gujarat		Saurashtra		Kachchh	
Months	Rainy days	Rainfall (mm)	Rainy days	Rainfall (mm)	Rainy days	Rainfall (mm)	Rainy days	Rainfall (mm)
January to April	0.5	4.0	0.4	5.0	0.1	1.0	0.5	5.5
May	0.5	11.0	0.4	8.7	0.4	17.0	0.1	1.0
June	7.5	243.0	3.0	92.0	4.4	99.0	2.0	61.0
July	17.0	539.0	11.0	281.0	9.0	196.0	5.0	118.0
August	15.0	365.0	9.8	221.0	6.3	153.0	4.0	85.0
September	9.0	213.0	5.4	176.0	5.0	105.0	2.4	45.0
October	1.0	17.0	0.3	5.0	1.0	17.0	0.5	9.0
November	0.6	13.0	0.3	8.0	0.5	4.7	0.5	5.5
December	0.2	3.3	0.1	2.0	0.1	1.1	–	–
Total	51.0	1448.0	31.0	805.0	27.0	594.0	15.0	330.0

rainy days (i.e., days with rainfall of 2.5 mm or more) in a year in the district. There are great year-to-year fluctuations in the rainfall with almost one drought year for every 4–5 years. Due to its aridity, Kachchh district naturally falls under permanent agricultural drought category (Table 1).

The low rainfall in different talukas of Kachchh, however, shows variation in the rainfall pattern. It is therefore necessary to understand drought in Kachchh, which also happens to be the most drought-affected district of Gujarat. Analysis of rainfall data in Kachchh district spanning the last 120 years shows that severe droughts have been on the increase in recent years, while in earlier decades such severe droughts had not been recorded.

Water resources of Kachchh district are mainly dependent on rainfall, which itself is very erratic and irregular (GEC 2011). All the streams are monsoon torrents retaining water only for a few days after rainfall. The water resource potential of Kachchh includes surface and groundwater resources. The surface water resources of the district have been developed in the form of medium irrigation dams, minor irrigation dam, check dams for lift irrigation, percolation tanks, as well as village tanks.

4.1 Forms of Agriculture

Kachchh has high diversity in different forms of agriculture, which ranges from absolute rain-fed farming to having fruit orchards like those of South Gujarat. This is mainly due to variation in geological conditions, especially the water-bearing strata. Some farming systems are extensive in nature, like rain-fed farming, while some are highly resource intensive with irrigation through tube well for field crops, e.g., summer groundnut. The various forms of agriculture can be divided on the basis of source of irrigation and its quality. This can broadly be divided into rain-fed and irrigated farming categories. Irrigated agriculture can broadly be divided into two categories, i.e., irrigation through surface storage and irrigation through groundwater.

4.2 Rain-Fed Agriculture

About two to three decades ago, Kachchh was known for the rain-fed farming system. Currently, about 80 % of the total agricultural areas are under rain-fed conditions, wherein the success or failure of a crop depends directly on rainfall and the area sown and the production varies directly with rainfall in Kachchh. The principal crops grown under rain-fed conditions are bajra, jowar, moong, guar moth, groundnut, sesame, and castor. In case of sufficient and timely onset of monsoon, groundnut is the preferred cash crop in dry farming condition. Erratic rainfall pattern and very less rainy days (average 12–15 rainy days in a year) greatly hamper the agriculture activities dependent on rainfall in the district.

4.3 Irrigated Agriculture Based on Surface Storage

Several moderate and minor irrigation schemes have been constructed to increase the irrigation potential through surface storage. Kachchh district has 20 moderate and 162 minor irrigation schemes. All the reservoirs of the schemes fall under the Panchayat (local administrative body). Irrigation water from reservoirs is mainly supplied during *Rabi* season (winter crop) directly through surface flow where diesel engines are used to lift water from a canal or tanks. However, this form of irrigation is also used in case of poor rainfall in the latter part of the *kharif* season (summer crop). Water from these reservoirs serves the farmer on a per acre charge basis. Generally, farmers follow the check basin method for irrigation of *Rabi* crops like wheat, cumin, and mustard. *Kharif* crops like cotton and groundnut and *Rabi* crops like wheat, cumin, and mustard are grown through surface storage.

4.4 Irrigated Agriculture Based on Groundwater

Kachchh has wide variation in the depth of groundwater availability and its quality. There are areas in Kachchh where there is no groundwater or water with high salinity, while the other areas have good quality of water. Water quality and water table fluctuation are highly influenced by the rainfall. In some areas, the groundwater is found at the depth of 250–450 ft and the main sources of lifting devices (submersible pumps) run on the subsidized electricity supply. In such cases, where the water quality ranges from good to poor, the availability of groundwater throughout the year resulted in increase in the yield of *kharif*, *Rabi*, and *zaid* crops. However, such type of agriculture is not sustainable and reduces the level of groundwater and tends towards the salinity problem. It may gradually deposit salt in the soil and finally reduce soil quality.

With the advent of Gujarat Green Revolution Company (GGRC) and its scheme of subsidy and advance, farmers are rapidly adopting Micro Irrigation System (MIS). This scheme encouraged farmers to grow mango, papaya, and pomegranate, where good quality of water is available, and where the water is saline they have chosen date palm and sapota crops. However, the field crops that are being increasingly covered under micro-irrigation system are cotton and castor. This farming system represents the green revolution face of Kachchh where water-intensive farming is taking shape. Despite the less area under cultivation in this form, well-established support systems like electricity supply, extension support, agro-services, as well as institutional setup have been established. The various source of irrigation areawise in different talukas of Kachchh district has been shown in tabular form (Table 2).

Table 2 Various sources of irrigation and irrigation area (ha) in different talukas of Gujarat (Statistical Department, Kachchh District Panchayat, 2009)

Taluka	Net irrigated area (ha)	Total sourcewise irrigated area (ha)			Area (ha) irrigated more than once	Total irrigated area (ha)
		Canal	Dugwell	Tubewell		
Abadasa	16,072	1410	20,346	366	650	22,122
Anjar	16,743	750	19,184	0	3191	19,934
Bhachau	18,638	509	20,324	0	2195	20,833
Bhuj	36,775	1197	37,201	3057	4680	41,455
Gandhidham	242	0	242	0	0	242
Lakhpat	4448	852	337	4171	872	5360
Mandvi	22,228	4528	25,995	385	8680	30,908
Mundra	14,191	2530	14,961	2850	6150	20,341
Nakhatrana	32,233	387	30,107	3931	2192	34,425
Rapar	16,419	1046	25,614	0	10,241	26,660
Total	178,029	13,209	194,311	14,760	44,251	222,280

Table 3 Cropping pattern in rain-fed and irrigated agriculture

Agriculture type	Kharif	Rabi
Rain-fed agriculture	Mix cropping of pearl millet, sorghum, castor, sesame, cotton, groundnut, greengram, clusterbean	Castor and cotton grown in kharif are continued
Irrigated agriculture—field crops	Pearl millet, castor hybrid and Bt. cotton, groundnut, sorghum, and lucerne	Castor and cotton grown in kharif are continued. Isabgul, cumin, wheat, mustard, and vegetables
Irrigated agriculture—fruit crops	Good-quality water—mango, papaya, banana Poor-quality water—date palm, sapota, and amla	

4.5 Cropping Patterns

Due to the presence of different sources of water with varying quality, and high diversity in the cropping pattern, the Kachchh district is characterized as an extensive rain-fed agricultural area. However, the irrigation systems like the "Narmada Canal Project" have boosted the confidence of farmers in venturing into irrigation-based crops over the past few years. Various preferred crops depending upon the employed farming system have been summarized in Table 3.

Traditional knowledge based on database and practical innovations of indigenous communities has developed over the centuries and is continuously transferred verbally from generation to generation. In India there is an enormous wealth of traditional ecological knowledge, but it is being lost or surviving only in bits and pieces. These traditional practices, if supported by scientific knowledge, could sustain the agroecosystems for the long term. The beneficial existing practices are being lost rapidly in a changing world. Thus, there is a need to protect these cultural practices (Singh and Sureja 2008). An example is organic agriculture, which is a blend of traditional and modern farming systems (Sharma et al. 2013). The studies conducted have shown that on farm preparations like the 'Jivamrit' (It is a fermented concoction of cow dung, cow urine, jaggery, gram flour and soil and is a natural soil fertility enhancer.) using locally available agricultural waste like cow-dung, farm yard manure (In Kachchh most of the farming communities rear cattle on the farm) and natural pesticides not only help in agri-waste management but have promising micro- flora which serve as cost efficient biofertilisers (Sharma et al. 2014). Potent microbes developed into cost effective consortia in conjugation with technical know-how of the lost traditional knowledge adapted to local needs can help sustain the agriculture and relieve the socio-economic drudgery of the farmers. Thus, the drought mitigation scenario in Kachchh district is a vivid example of how the semiarid tropics in spite of the harsh climatic conditions could have a good agricultural resource through proper management of water resources and drought enduring agri-management practices using the traditional knowledge. The Kachchh "Keri" (native mango variety), pomegranate, and dates being exported to many European countries stand a testimony to this.

5 Conclusions and Future Prospects

Drought is an inevitable natural phenomenon which affects the farming systems. Mitigation of the impacts of this calamity through soil and water management strategies is the need of the time. The capacity of soil to retain and release water depends on a wide range of factors such as soil texture, depth, soil-building capacity, moisture content, organic matter content, and biological activity. The moisture content of soil is affected by various factors and understanding these factors could help in managing the drought-resistant soil. The consequence of the higher infiltration rate in conjugation with a higher organic matter contents increases water storage capacity of soil. Therefore, an increase in soil organic matter content is a key to drought-resistant soil. Organic matter intimately mixed with mineral soil particles has considerable influence in increasing water-holding capacity. Thus, organic management practices to moderate the effects of drought could be applicable for most agro-ecosystem practices not only for improving the crop and forage growth and development but also as a preventive measure against the effects of drought. Amendment of organic matter influences the physical, chemical, and biological properties together to maintain high soil quality, which seems to withstand the effects of drought. However, the development of drought-resistant varieties is another approach to combat with the ill effect of drought. Molecular breeding approaches such as marker-assisted and genome-wide selection have been suggested to develop the drought-tolerant cultivars which will also enhance the food security in the context of a changing or variable climate. Moreover, human activities could also influence the drought through many unusual farming activities. The new irrigation techniques have increased the land area for farming, but simultaneously also increased the farmers' dependency on water.

Traditional farming practices allow the land to “rest” by rotating crops each season and alternating areas where livestock graze. Nowadays, with many areas in the world struggling with overpopulation and a shortage of farmland, there is often not enough arable land to support these sustainable farming practices. Over-farming and over-grazing can lead to soil being compacted and unable to hold water. As the soil becomes drier, it is vulnerable to the deleterious effects of drought. Traditional farming techniques including practices like mulching with organic waste help reduce moisture evaporation from the soil. Agricultural systems need to be managed in such a way so that they are more resilient to the impacts of drought, maintain a modicum of production in hard times, and recover quickly afterwards, without further degrading landscape condition.

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Biofertilizer Use for Sustainable Agricultural Production

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Abstract The economic and environmental costs of the indiscriminate use of chemical nitrogenous fertilisers in agriculture are a global concern. Sustainability considerations mandate that alternatives to nitrogen fertilisers must be urgently sought. Biological nitrogen fixation (BNF), a microbiological process which converts atmospheric nitrogen into a plant-usable form, offers this alternative. Nitrogen-fixing systems offer an economically attractive and ecologically sound means of reducing external inputs and improving internal resources. Symbiotic systems such as that of legumes and rhizobium can be a major source of nitrogen in most cropping systems and that of *Azolla* and *A*nabaena can be of particular value to flooded rice crop. Nitrogen fixation by associative and free-living microorganisms can also be important. Bio-fertilisers generate plant nutrients like nitrogen and phosphorous through their activities in the soil or rhizosphere and make available to plants in a gradual manner. Bio-fertilisers are gaining momentum recently due to the increasing emphasis on maintenance of soil health, minimise environmental pollution and cut down on the use of chemicals in agriculture that will lead to sustainable agricultural production.

Keywords Agricultural productivity • Bio-fertilisers • Nitrogen fixation • Sustainable agriculture

1 Introduction

Root nodules of the plants (primarily belonging to the family Fabaceae) associate with bacteria that fix atmospheric nitrogen and form a symbiotic relationship. During this symbiotic association, nitrogen gas of the atmosphere is converted into ammonia and is assimilated by the plant in amino acids and other cellular constituents. The members of the legume family (Fabaceae) reduce the use of chemical fertilisers as they fix atmospheric nitrogen and make them ideal agricultural organisms. Nodule formation on the stem also acts as a growth stimulant (Burdass 2002).

Nitrogen fixation in the root nodule is an oxygen-dependent process. The root nodules harbour an iron-containing protein called leghaemoglobin, closely related to animal myoglobin, to facilitate the conversion of nitrogen gas to ammonia.

Currently, the main problem facing agriculture is contamination of soil with excessive use of chemicals, including pesticides (Bushby and Marshall 1977). Continuous use of agrochemicals degrades soil and reduces soil fertility. These chemicals cause damage to soil health and create many ecological and economic problems. Thus, symbiotic nitrogen fixation technology is better to maintain the soil condition for sustainable agricultural production (Parr et al. 1990).

1.1 Observation of a Cut Root Nodule

Root nodule is pink or red inside when it is active (Fig. 1). The colour is due to the presence of leghaemoglobin. More active nodule has the redder colour. Root nodules which are younger and do not fix atmospheric nitrogen are white or grey in colour. Nodules not fixing nitrogen are discarded by the plant, because of the poor plant nutrition or inefficient rhizobium strain. Rhizobium is the most important bacterial group in nitrogen fixation (Burdass 2002)

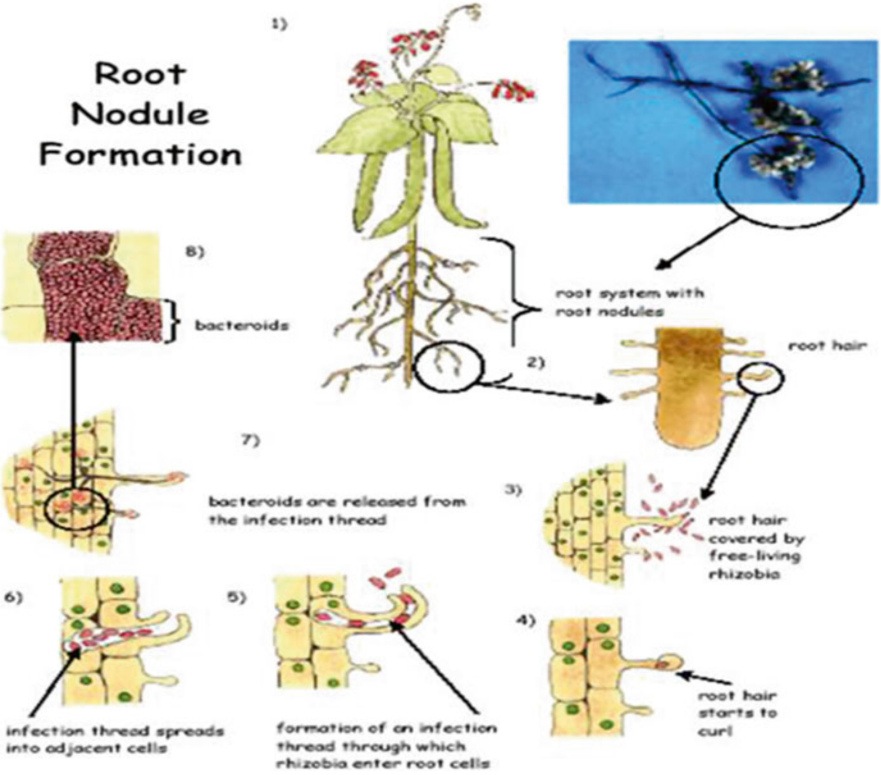

Fig. 1 Observation of a cut root nodule (Source: Society for General Microbiology 2002)

1.2 Nitrogen Fixation and Agriculture: The Way Forward

Nitrogen fixation by rhizobia is of great importance in agriculture. Legumes such as peas, beans, lentils, soybeans, alfalfa and clover help to feed the meat-producing animals of the world as well as humans. Rhizobium provides less nitrogen fixation and greater pest extermination (Dean 2009). In nodulated plants, crop production is increased; legumes are used in poor soils to support plant growth and yield. Soybeans have a symbiotic relationship with nitrogen-fixing rhizobia (De Moraes 2004). Globally, the input of nitrogen by legumes is 2.95 million tonnes per year of pulses and 18.5 million tonnes for oilseed legumes (Herridge et al. 2008). Roots of legumes can be decayed in soil as they avail nitrogen for new generation. Rotation of leguminous crops with nonleguminous crops is a source of natural fertilisation (Burdass 2002).

The use of natural fertilisers reduces the use of chemical fertilisers, thus saving money and also preventing the pollution caused by chemical fertilisers which causes eutrophication of waterbodies and growth of nonfood crops in agricultural land. Thus, the use of natural fertilisers leads to sustainable agricultural production.

2 Brief Description of Bio-fertilisers Used

2.1 Azotobacter *Bio-fertiliser*

The genus *Azotobacter* has been extensively studied since its discovery by Beijerinck in 1901 (shown in Fig. 2). It was thought that a study of the effect on nitrogen fixed by Azotobacter when grown in association with other soil microorganism would be

Fig. 2 *Azotobacter*

interesting since this aspect has not been yet thoroughly investigated (Gomare et al. 2013). For the first time, Beijerinck isolated and described *Azotobacter chroococcum* and *A. agilis*. Later on *A. agilis* was renamed as *Azomonas*.

Azotobacter grow in the rhizosphere; they are free-living microorganisms and fix atmospheric nitrogen non-symbiotically equivalent to 25–30 kg of nitrogen per hectare. In addition, these help in enhancing the plant growth and yield. *Azotobacter* produces hormones like indoleacetic acid (IAA) and gibberellins. Vitamins like biotin, folic acid and different B-group are also produced. The application of *Azotobacter*, supported by judicious use of organic matter, ensures good seed germination, increasing productivity. It is a heterotrophic bacterium that derives energy by degrading plant residues. Besides, strict anaerobic nitrogen-fixing species are the genera: *Chlorobium*, *Clostridium*, *Chromatium* and *Desulfovibrio*. Species of *Azotobacter* are found in slightly acidic (*A. beijerinckii*) to neutral and alkaline (*A. chroococcum*) soils. *Azotobacter* fixes 20–40 kg nitrogen per hectare per annum. It also produces IAA, gibberellic acids, vitamins, etc. Hence, it is recommended as bio-fertiliser for rice, wheat, millets, cotton, etc.

Chroococcum is a soil and rhizosphere inhabitant, while *A. agilis* is waterborne species. Thereafter, several other species were described such as *A. beijerinckii*, *A. insignis*, *A. paspali*, *A. macrocytogenes*, *A. vinelandii*, etc. The species of *Azotobacter* are placed in the family *Azotobacteraceae*.

- *Azotobacter* is gram-negative, aerobic bacteria. It is usually motile, oval or spherical in shape.
- They are typically polymorphic, i.e. of different sizes and shapes. The size of cells ranges from 2 to 10 μm long and 1–2 μm wide.
- *Azotobacter* is found on alkaline soils, aquatic environments, plant rhizosphere and phyllosphere. In Indian soils, the population of *Azotobacter* is not more than 10,000–1,00,000 per gram of soil.
- *Azotobacter* naturally fixes atmospheric nitrogen.
- All plants get benefited by *Azotobacter* which fixes atmospheric nitrogen symbiotically.

2.1.1 Use as Bio-fertiliser

Azotobacter is used as fertiliser as it fixes atmospheric nitrogen.

2.2 Azospirillum *Bio-fertiliser*

Beijerinck (1925) for the first time described a nitrogen-fixing bacterium found in the root of digit grass in Brazil and named . In 1956, the Russian investigators also reported some of the nitrogen-fixing *spirilla*. During 1963, its nitrogen-fixing ability could be proven by several workers. Tarrand et al. 1978 renamed *Spirillum* as *Azospirillum* (nitrogen-fixing *Spirillum*) (shown in Fig. 3). During the 1970s,

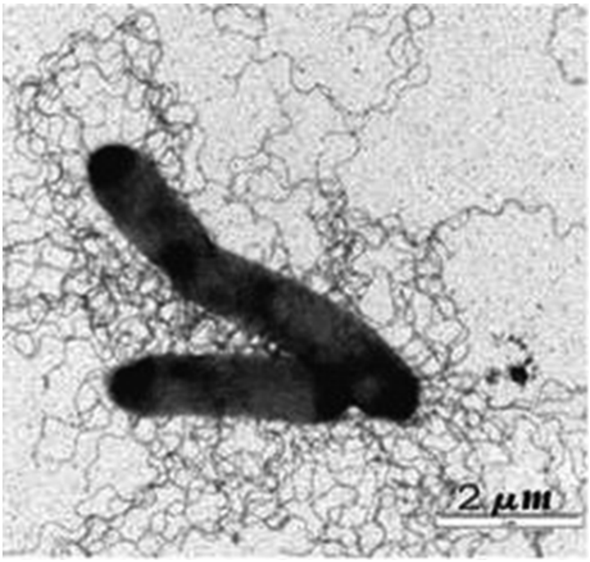

Fig. 3 *Azospirillum*

this bacterium could also be isolated from Indian soils and rhizosphere regions of many plants. Soil pH governs the distribution of *Azospirillum*.

They help plants to produce growth-promoting hormones (like auxins, gibberellins and cytokinin) and vitamins. It is estimated that almost 10–15 % of the required nitrogen can be met by *Azospirillum* bio-fertilisers.

Soil pH between 5.6 and 7.2 promotes nitrogenase activity, whereas pH below 5–6 does not encourage nitrogenase activity in soil. By using semi-solid sodium malate enrichment medium, *Azospirillum* could be isolated from *Digitaria decumbens*. The essential requirements for its isolation are the surface sterilisation of the root by 70 % ethanol and creation of microaerophilic condition in the medium.

It is an associative symbionts because it effectively colonises the root and infects the cortex also. It is present inside and outside the roots without developing any apparent structure on roots. It has been found to occur in xylem vessels of black gram and sugarcane. Moreover, it is capable of fixing 20–40 kg nitrogen under micro-aerobic conditions. So far there are five species of *Azospirillum*: *A. lipoferum*, *A.brasilense*, *A. amazonense*, *A. halopraeferns* and *A. trakense*. The first two species are most commonly used as bio-fertiliser. *Azospirillum* and *Azotobacter* can be used for most perennial nonleguminous crops.

Advantages:

- Nitrogen fixation by nitrogenase enzyme in plant rhizosphere.
- It fixes 20–40 kg N/ha and the yield is increased by 15–30 %.
- Helps in water and mineral uptake.
- Promotes vegetative growth and root development and increases seed germination.
- They exhibit antifungal activities also.

Fig. 4 *Bacillus subtilis*

2.3 Bacillus subtilis

This is used as a biocontrol agent; its foliar application controls leaf diseases of many crops. It produces many enzymes which degrade natural substrates thus help in nutrient cycling (Alexander 1977). It is the common saprophyte that contributes to nutrient cycling (Fig. 4). Normally it grows in aerobic conditions, but in complex media in the presence of nitrate, anaerobic growth can occur (Claus and Berkeley 1986). Under unfavourable conditions, it produces endospore to resist heat and desiccation (Claus and Berkeley 1986).

2.3.1 Mode of Action

The bacterium colonises the developing leaf and root system of the plant and thus competes with and thereby suppresses plant diseases.

2.3.2 Advantage

- They have antagonistic interactions with various soilborne pathogens.
- They protect plants from root and seed diseases.

2.4 Blue-Green Algal Bio-fertilisers

Blue-green algae are the simplest living autotrophs (Fig. 5). They are responsible for water blooms in stagnant waterbodies. They adopt in unfavourable weather conditions like snow and hot springs. Blue-green algae (BGA) photosynthetic

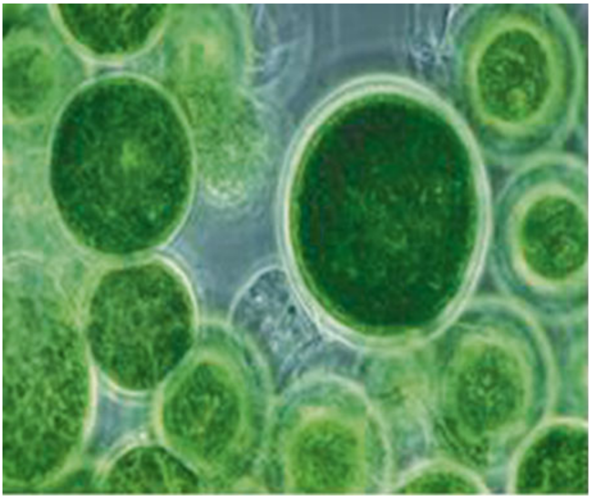

Fig. 5 Blue-green algae

prokaryotes grow frequently in rice fields and fix atmospheric nitrogen and convert insoluble phosphorus into soluble form (Irisarri et al. 2001).

They are of immense economic value. Many barren lands have been reclaimed and made productive by the use of BGA. They are economically attractive and ecologically sound means for increasing productivity; especially in rice, cultivation is offered by *Cyanobacteria*. Nitrogen fixation by free-living cyanobacteria in a wetland rice ecosystem also significantly supplements soil nitrogen (Watanabe et al. 1951).

2.4.1 Where Are They Suitable?

- Blue-green algae mostly grow in wet places. Hot and moist weather regions are congenial for algae growth.
- Blue-green algae, besides fixing nitrogen, carry out photosynthesis and secrete certain growth hormones (vitamin B12, auxins and ascorbic acid) which are beneficial to rice plants.
- The use of blue-green algae is confined only to rice crop. Various experiments have proved that the use of algae increases the rice yield by 10–15 %.
- It is estimated that in general blue-green algae supply about 30–40 kg of nitrogen per hectare to paddy crop.
- Blue-green algae have also shown its effectiveness in improving saline and alkaline soils.

2.5 Anabaena

Anabaena is found as plankton and is a genus of blue-green algae (Fig. 6). It fixes nitrogen and forms symbiotic association with certain plants like mosquito fern. They belong to one of the genera of cyanobacteria. They produce neurotoxins. They are one

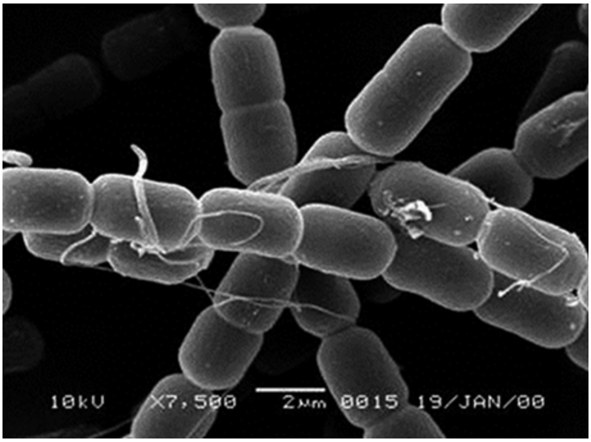

Fig. 6 *Anabaena*

of the genera of cyanobacteria and produce neurotoxins. *Anabaena* grows inside pockets of the tissues of the water fern *Azolla* to be found at the front bench. Pteridophyte *Azolla* is an excellent bio-fertiliser and green manure having global distribution. The ability of *Azolla-Anabaena* system to fix atmospheric nitrogen at faster rates makes it an outstanding agronomic choice for the cultivation of rice under tropical conditions. Nitrogen fixation potential of the *Azolla-Anabaena* system has been estimated to be 1.1 kg N ha^{-1} day^{-1}, and one crop of *Azolla* provided 20–40 kg N ha^{-1} to the rice crop in about 20–25 days (Watanabe et al. 1977). The ability of nitrogen fixation is due to the presence of heterocysts (Moore 1969). *Azolla* has multifaceted uses and has gained considerable importance in the recent times as bio-fertiliser, green manure and poultry feed and cattle fodder (Singh and Subudhi 1978).

The application of cyanobacteria is effective in increasing crop productivity and in maintaining soil fertility. However, the application of herbicides to increase crop productivity is not only harmful to weeds but to bio-fertiliser strains of cyanobacteria (Watanabe et al. 1977).

2.6 Nostoc

Nostoc is a genus of freshwater cyanobacteria and forms colonies of moniliform cell in a gelatinous sheath. When formed on soil, they are not clearly seen, but when it rains, these swell up into conspicuous jelly-like mass. Once it was thought that they have fallen from the sky hence named as fallen star and star jelly. It is a member of the family Nostocaceae. *Nostoc* is an excellent natural fertiliser on account of nitrogen-fixing ability in rice fields. In dry season, *Nostoc* is collected in the form of dry balls and is used in rainy season to enhance nitrogen supply in rice fields. Algalisation benefits crop plants through excreting part of the biologically fixed nitrogen, secreting growth-promoting substances and different types of secondary metabolites, adding appreciable amounts of organic matter into the soil, solubilising insoluble phosphates and improving the physico-chemical nature of the soil (VenKataraman 1993).

2.7 Azolla

Azolla is a species of aquatic fern. It is a free-floating water fern. *Azolla* is a free-floating water fern that floats in water. It is regarded as the “soybean plants in rice field” because it can fix assimilated nitrogen owing to association to cyanobacteria *Anabaena*. *Azolla* is found in ponds, ditches and wetland of tropical and temperate regions of the world. *Azolla* plant is triangular or polygonal in shape and floats in water surface individually or in mats. The plant has alternately arranged branches and adventitious roots remaining hanging in standing water. *Azolla* multiples vegetatively and sexually by producing spores. Vegetatively reproducing reproduction is most common and continues at all times. Presently, six species of *Azolla* have been recognised worldwide. These are *Azolla filiculoides*, *A. caroliniana*, *A. mexicana*, *A. microphylla*, *A. pinnata* and *A. nilotica*. Singh and Singh (1990) found that the application of *Azolla* increases total nitrogen, organic carbon and available phosphorus in soil. Van Hove (1989) found the application of *Azolla* improves soil structure and supplies large quantities of organic matter.

2.7.1 Importance of *Azolla*

- Rice-growing areas of many countries have been showing great interest in the use of *Azolla* as an alternative to commercial nitrogen fertilisers. Potentialities of *Azolla* as alternate green manure can contribute 40–60 kg N/ha per rice crop with significant increase in yield and productivity. Moreover, due to its rapid decomposition nature, *Azolla* can add considerable amount of organic matter in the soil subsequently releasing other essential nutrients like K, Ca and Mg for better utilisation by rice crop. *Azolla* grows well in soils with less clay and more sand with an optimum pH ranging between 5 and 7 and temperature range of 20–300 °C. *Azolla* can be dried and preserved for later use as manure 300 kg dry *Azolla* is equivalent to 5 t of green *Azolla*.

2.7.2 Some Multiple Benefits of *Azolla*

It acts as biological herbicide depressing germination of weeds.

- Accumulate nutrients from flood water and release to rice crop after decomposition.
- Due to its high protein content, it can be used as organic feed substitute for livestock.
- Due to its rapid decomposing nature, *Azolla* can be used as manure for dry land crops, vegetable and ornamental plants.
- When it dies and decays in the soil, nitrogen becomes available to plant.
- In 3–4 weeks, 5–10 t of biomass can be obtained from one hectare providing at least 30 kg nitrogen.

- It contains 45 % (N), 2.6 % (P) and 0.9 % (K).
- It is a secondary source of nutrients such as calcium, magnesium and sulphur.
- The use of *Azolla* in rice field at the rate of 200 g per square metre area can increase rice yield by 12–38 %.
- The application of 10 t of fresh *Azolla* biomass in one hectare adds as much as 100 kg nitrogen.

2.8 Phosphate-Solubilising Bio-fertilisers

Phosphorus is the important nutrient required by plants. Many organisms solubilise the phosphorus from rocks. Phosphorus solubilises bacteria in soil like species of *Pseudomonas* and *Bacillus*. Fungi like *Aspergillus* and *Penicillium* solubilise bound phosphate to make it available for plants. Inoculation of microbes fulfils 20–25 % phosphorus requirement of plants. Fixed phosphorus of soil is also solubilised by phosphate-solubilising bacteria (PSB). Therefore, its use in agriculture not only reduces manufacturing costs of phosphate fertilisers but also mobilises insoluble fertilisers in soil (Chang and Yang 2009; Banerjee et al. 2010). The presence of phosphorus-solubilising microorganisms in rhizosphere dates back to 1903 (Khan et al. 2007). For the solubilising of phosphorus, bacteria are more effective than fungi (Alam et al. 2002). Phosphate-solubilising bacteria have 1–50 %, while phosphate-solubilising fungi have 0.1–0.5 % phosphorus solubilisation potential (Chen et al. 2006).

There are several phosphate-solubilising microorganisms present in soil, for example, the species of *Pseudomonas*, *Bacillus*, *Micrococcus*, *Flavobacterium*, *Aspergillus*, *Penicillium*, *Fusarium*, *Sclerotium*, etc. They can utilise tricalcium phosphate, apatite, rock phosphate and $ALPO_4$ as sole phosphate source present in the medium. The indication of utilisation is that they produce clearing zones aroundww each colony. They secrete organic acids. Consequently, bound form of phosphates is solubilised, and charged molecules of phosphorus ($PO^{-3}{}_4$) are absorbed by the plants.

These bio-fertilisers play a significant role in solubilising insoluble phosphate. About 95–99 % phosphorus in soil is not directly available to the plants; phosphate solubilisers convert insoluble phosphate to soluble form.

2.8.1 *Pseudomonas*

They are rod-shaped gram-negative bacteria and produce growth-promoting substances.

Important microorganisms:

- *Bacillus polymyxa*
- *Aspergillus awamori*
- *Penicillium digitatum*

Phosphate-solubilising bio-fertilisers (PSB) can be used for all crops, and vegetable methods of application include seed treatment, seed dipping and application on soil.

2.8.2 Advantages

- Make the phosphorus available in soil and exhibit antifungal activities.
- Increase yield by 10–15 %.
- By hydrolytic activities of organic acids, insoluble phosphorus is made available in soil and enrichment of soil with bacterial metabolites, which help in solubilisation and uptake of native and applied phosphorus.

2.9 Vesicular Arbuscular Mycorrhiza (VAM)

Symbiotic association of plant roots and soil fungus helps in inducing the plant growth. Mycorrhizae provide resistance to the plants against nematodes and soilborne pathogens. Introduction of organisms like *Rhizobium*, *Azospirillum*, *Azotobacter* and phosphate-solubilising bacteria around roots of plants exerts synergistic effect on plant growth.

Mycorrhizal fungi form a bridge between plant roots and soil, thus helping in gathering of nutrients from soil (Fig. 7). Mycorrhizae are of two types, viz. ectomycorrhizal fungi and endomycorrhizal fungi; both types penetrate the roots, but ectomycorrhizae spread hyphae between root cells, and endomycorrhizae hyphae penetrate root cells.

Endomycorrhizae are most commonly found in grasses, shrubs and many other plants and trees. Ectomycorrhizae include members of pine, oak and beech families.

Food for fungi and bacteria secreted by plant roots attracts nematodes, and nematodes secrete nitrogen, phosphorus and sulphur in plant-usable form. Ectomycorrhizae assist in picking up water away from roots and block access of pests to plant roots (Peters 2002).

2.10 Plant Growth-Promoting Rhizobacteria (PGPR)

Rhizobacteria colonise the rhizosphere roots. The bacteria which colonise the rhizosphere of the root are commonly known as rhizobacteria. Rhizobacteria is a group of rhizosphere bacteria that have a beneficial effect on plant growth and are known as plant growth-promoting rhizobacteria (Schroth and Hancock 1981). PGPR belong to genera of *Azotobacter*, *Agrobacterium*, *Bacillus*, *Rhizobium*, *Pseudomonas*, *Xanthomonas*, etc. (Weller 1988). The PGPR which have been discovered by most of them are fluorescent pseudomonas. The other types are nonfluorescent pseudomonas, e.g. *Serratia* and *Arthrobacter*. The most common

Fig. 7 Vesicular arbuscular mycorrhiza (VAM)

species of *Bacillus* are *B. polymyxa*, *B. circulans* and *B. macerans*. PGPR reach root surfaces by active motility guided by chemotactic responses which implies that PGPR competence highly depends on their ability to take advantage from the specific environment or their ability to adopt to changing environment or plant species (Nihorimbere et al. 2011).

These bacteria increase the growth of host plants. The increase in plant growth is due to:

- Changes in balance of rhizosphere microflora producing an indirect effect on the crop
- Control of pathogens and other harmful microorganisms in the rhizosphere
- Production of growth hormones like gibberellins and indoleacetic acid
- Release of nutrients from soil
- Possible production of vitamins or conversion of materials to a usable form by the host
- Possible nitrogen fixation by rhizobacteria

*2.11 Sulphur-Solubilising Microbes (*Thiobacillus*)*

They obtain nutrients by oxidation of sulphur and iron. They possess iron oxidase, which helps in metabolising metal ions.

3 Application and Uses of Biofertilizers

- They fix atmospheric nitrogen in root nodules of legumes and soil.
- They help in solubilising the phosphate like aluminium phosphates, iron and tricalcium.

- They produce growth-promoting hormones.
- They help in mineralisation and decomposition of organic matter in soil.
- They improve yield by 10–25 %, when applied to seed or soil by increasing the availability of nutrients without harming the soil and environment.

Some of the bio-fertilisers are crop specific, while some can be used for all crops. Different methods of application are adopted depending on the bio-fertilisers used and the crop grown. The performance of bio-fertilisers is more when used with organic manures. In rain-fed farming, moisture is limiting, and moisture conservation practices can be best adopted along with bio-fertilisers. Peoples et al. (2009) proposed the following changes for traditional cropping systems: delaying the cultivation of legume phases from autumn to spring, intercropping of cereals with legumes and including nonleguminous species in legume-based pastures.

4 Tips for Improving Effectiveness of Bio-fertilisers

- Bio-fertilisers should be stored at room temperature for longer shelf life.
- During storage avoid direct contact with chemical fertilisers, pesticides and insecticides.
- Avoid direct contact with sunlight.
- Inoculants should be used before expiry.
- Apply fungicides before insecticides and bio-fertilisers should be applied after all other treatments. When fungicides and insecticides are to be used, apply fungicides before insecticide application.
- Specific inoculants should be used for specific crop.

5 Advantages of Bio-fertilisers

5.1 Cheap Source of Nutrients

Bio-fertiliser can be called as poor man’s technology. Bio-fertilisers are cheaper than chemical fertilisers. Twenty five kg of nitrogen chemical fertilisers costs Rs. 360 per hectare, whereas the BGA costs Rs. 60 per hectare to produce 10 kg BGA culture (http://www.karmayog.org/agri/agri_15322.htm).

5.2 Suppliers of Micronutrients

Bio-fertiliser not only supplies nitrogen and phosphorous but also some micronutrients essential for plant growth. Sometimes the yield which is limited by micronutrients and application of nitrogenous, phosphatic and potassic fertilisers does not improve the yield significantly. In this situation, the application of bulky bio-fertilisers like blue-green algae and *Azolla* increases the yield due to greater supply of micronutrients.

5.3 Supplier of Organic Matter

Organic matter is the essential component of the soil. It provides energy and nutrients for plants and microorganisms. *Azolla* and BGA produce an average 8–10 t of biomass per hectare which adds to the organic matter pool of soil.

6 Counteracting Negative Impact of Chemical Fertilisers

When chemical fertilisers are excessively and continuously used for a few years, they may create acidity or alkalinity, thus reducing the soil quality. The soil also becomes unresponsive to further use of similar fertilisers. The application of bio-fertilisers can avoid this problem to a great extent. Besides, the large amount of organic matter supplied by the bio-fertilisers imparts tolerance power (buffering capacity) to the soil against acidity or alkalinity. It also withholds metallic elements from entering the plant roots, thereby reducing harmful effects of pesticide (http://www.karmayog.org/agri/agri_15322.htm).

6.1 Secretion of Growth Hormones

Plants also need for their growth and development some natural complex chemical compounds called hormones. Though growing plants do not themselves synthesise hormones in adequate amounts, *Azotobacter*, blue-green algae and *Azolla* have been found to synthesise growth hormones (e.g. indelicate acid and vitamin B) which benefit the main crop. Sometimes, bio-fertiliser application gives significant response even if the soil is already rich in plant nutrients. This occurs due to the supply of growth hormones by bio-fertilisers to the main crop.

6.2 Production

Organic fertilisers are made by natural processes, and chemical fertilisers are man-made.

6.3 Effect on Biological Activity

Organic fertilisers boost microbial activity in soil while as chemical fertilisers kill the microbes. These microbes help in degrading complex compounds present in fertilisers.

6.4 Effect on Soil

Excessive use of fertilisers is not good for overall composition of soil or plant. Overuse of organic fertilisers may also have a problem, but slow-releasing nutrients will any way take time to get absorbed.

6.5 Effect on the Environment

They are easily available in nature and have no adverse effect on the environment. But chemical fertilisers have negative impact on the environment like contamination of waterbodies.

6.6 Cost

Organic fertilisers are cheaper as compared to chemical fertilisers. Organic fertilisers are easily available in nature and only require packaging, but chemical fertilisers need expensive procedures for their production.

7 Conclusion and Future Prospective

Bio-fertilisers help in increasing the crop productivity and increase availability and uptake of nutrients. Bio-fertilisers have replaced the chemical fertilisers and reduce the environmental pollution and economic costs. It will help in achieving sustainability in agricultural production.

After green revolution, the main aim was to produce high-yielding varieties of crops and to increase productivity. Excessive use of chemical fertilisers has deteriorated the soil quality and proved harmful to humans and animals (Gupta and Singh 2006). Chemical fertilisers have also affected microflora and fauna (Gupta and Singh 2008). Thus to obtain sustainable agriculture, the use of bio-fertilisers has assured great promise to meet out nutrient demand. The role of bio-fertilisers assumes great significance in the present context of expensive chemical fertilisers. It will help farmers to achieve goal of increased productivity. Keeping in mind the environment safety, food security and availability of resources, it becomes obligatory to harness the full potential of the available bio-fertilisers. Thus, the role of bio-fertilisers in sustainable agricultural production assumes special significance.

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Plant Growth-Promoting Rhizobacteria: An Eco-friendly Approach for Sustainable Agroecosystem

Sohel S. Shaikh, Riyaz Z. Sayyed, and M.S. Reddy

Contents

Abstract Soil-borne diseases caused by fungal and bacterial pathogens are a major threat to crop and its yield. These diseases significantly reduce the crop yield and lead to the production of micronutrient deficient staple crops. Consumption of these staple crops or food has been the main cause of many micronutrient deficiency disorders in human beings, for instance, iron deficiency anemia (IDA) caused due to iron deficiency. Frequent use of chemical fungicides to control these diseases and use of chemical fertilizers to promote plant growth and crop yield have widely affected the agroecosystem and have various detrimental effects and numerous side effects. Sustainable control of such plant diseases has been an important challenge to agricultural field. Continuous search for eco-friendly management of plant disease and promotion of plant growth and

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crop yield has headed toward the use of plant growth-promoting rhizobacteria (PGPR) as an effective and eco-friendly means of controlling soil-borne phytopathogens and simultaneously promoting the plant growth. This chapter focuses on various aspects and mechanisms of biocontrol of plant pathogens; plant growth promotion, commercialization of their wide applications, and future prospects of PGPR are also discussed.

Keywords Plant diseases • Biocontrol • Plant growth promotion • Plant growth-promoting rhizobacteria

1 Introduction

The rhizosphere represents a highly dynamic site for interactions between roots, pathogenic and beneficial soil microbes, invertebrates, and other competitors of root (Yadav et al. 2015). Associations among the rhizospheric have been categorized as positive, neutral, or negative associations. Positive associations, which contribute to plant growth promotion and biological control of plant diseases, include symbiotic associations with epiphytic microbes, mycorrhizal fungi, and root colonization by biocontrol agents (BCAs). Negative associations include competition or antagonism (Pliego et al. 2011). All those organisms present in rhizosphere play very important role in plant health promotion or demotion. The rhizobacteria that exert a beneficial effect on plant growth are called as plant growth-promoting rhizobacteria (PGPR), while those which caused detrimental effect are referred to as plant deleterious rhizobacteria (PDB) (Kloepper et al. 1980). PGPR are also termed plant health-promoting rhizobacteria (PHPR), yield-increasing bacteria (YIB), or nodule-promoting rhizobacteria (NPR) (Kloepper et al. 1989; Hayat et al. 2010).

Since the last few decades, the world had paid attention to the PGPR due to their promising nature to the environment. The most essential factor for obtaining highest yield in all agriculture systems has been the use of chemical fertilizers; however, chemical fertilizers have adversely affected the soil health. Thus, the use of PGPR as an alternative to chemical fertilizers and pesticides has good potential in increasing the productivity of crops; its supplication will reduce the use of agrochemical and therefore will help in eco-friendly agricultural practices. Increase in crop yield through the use of PGPR can be achieved directly or indirectly (Glick 1995; Akhtar and Siddiqui 2010). Some PGPR promote the plant growth by direct mechanisms (Vessey 2003; Ahemad and Kibret 2014) and act as biofertilizers when pathogen's pressure is absent (Lugtenberg and Kamilova 2009). Indirect mechanism includes the inhibition of phytopathogens by several biocontrol mechanisms (Labuschagne et al. 2010). The various ways of functioning of PGPR include synthesis of phytohormones, acceleration of uptake of certain soil nutrients, and prevention of plant diseases (Hayat et al. 2010).

PGPR also improve root development, mineral nutrition, seed germination, and water uptake (Akhtar and Siddiqui 2010; Akhtar et al. 2010; Akhtar and Azam 2014). Large number of reviews and chapters have been published recently focusing the various aspects of PGPR (Akhtar and Siddiqui 2010; Heydari and Pessarakli. 2010; Khan et al. 2010; Labuschagne et al. 2010; Hayat et al. 2010, 2012; Saharan and Nehra 2011; Saraf et al. 2011; Simova et al. 2011; Beneduzi et al. 2012; Glick 2012; Sayyed et al. 2012, 2013; Ashraf et al. 2013; Junaid et al. 2013; Sharma et al. 2013a, b; Ahemad and Kibret 2014; Behera et al. 2014; Mabood et al. 2014; Shaikh and Sayyed 2015). However, in-depth studies on different aspects of plant growth promotion and various mechanisms of biocontrol of plant pathogens are needed to be focused. Commercial aspects of their wide applications and future prospects of PGPR are also discussed in this chapter.

2 PGPR as Plant Growth Promoters

Plant growth promotion is the most important eco-friendly and sustainable aspect of PGPR. A large number of bacterial species including *Alcaligenes*, *Azospirillum*, *Arthrobacter*, *Acinetobacter*, *Bradyrhizobium*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, and *Rhizobium* associated with the rhizosphere of plant and are able to exert many beneficial effects on plant growth (Saharan and Nehra 2011; Ahemad and Kibret 2014). There are several mechanism by which PGPR can increase the plant growth; they are also known as direct mechanisms of plant growth promotion, and these include nitrogen fixation, production of phytohormones, lowering of ethylene concentration by producing ACC deaminase, and solubilization of phosphorous and various other minerals. These mechanisms are mentioned in Fig. 1.

2.1 Biological Nitrogen Fixation

Nitrogen is a vital macronutrient for growth and yield of crop. Although nitrogen is abundantly (78 %) available in the atmosphere, it is not available for growing crops (Gupta et al. 2012; Ahemad and Kibret 2014). The process of conversion of atmospheric or insoluble N_2 into soluble form by plants is known as biological N_2 fixation. It is eco-friendly in nature and best substitute for sustainable agriculture (Cheng 2008). Nitrogen fixation changes nitrogen to ammonia by nitrogen-fixing microorganisms by a set of complex metaloenzyme system called nitrogenase (Kim and Rees 1994; Rees and Howard 2000). Enzyme nitrogenase has two components, i.e., Fe protein and iron, molybdenum cofactor (Dixon and Kahn 2004). This enzyme system is under the control of nif genes (Dixon and Kahn 2004; Glick 2012). Nitrogen-fixing organisms are generally categorized as symbiotic and nonsymbiotic nitrogen fixing. Symbiotic N_2 fixers belonging to the family that forms association with leguminous plants include *Azorhizobium*, *Bradyrhizobium*, *Rhizobium*, *Allorhizobium*, *Sinorhizobium*, *Frankia*, and *Mesorhizobium* (Hayat

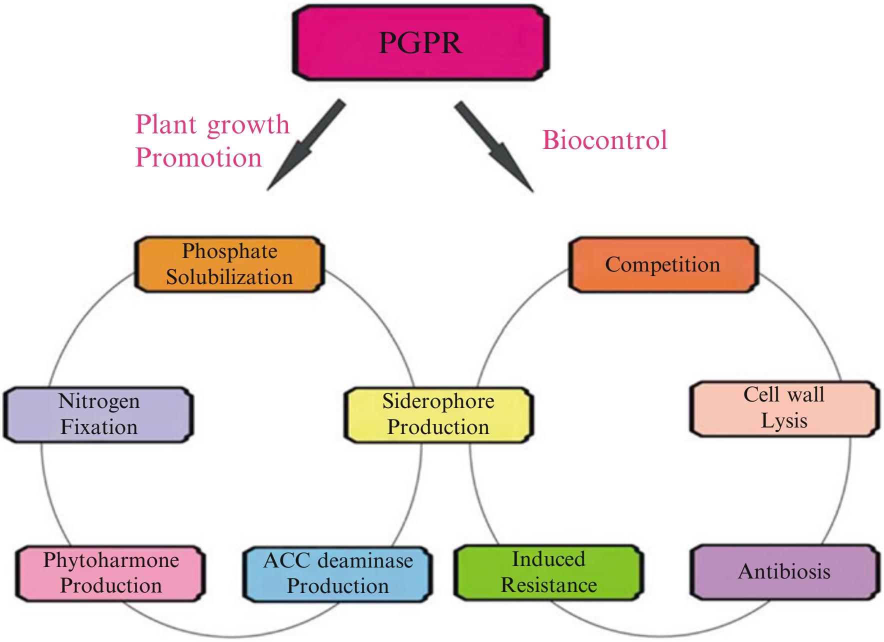

Fig. 1 Showing the schematic view of various mechanisms of plant growth promotion and biocontrol by PGPR

et al. 2012; Rashid et al. 2015). Nonsymbiotic free-living nitrogen-fixing bacteria include *Achromobacter*, *Azotobacter*, *Azospirillum*, *Alcaligenes*, *Acetobacter*, *Arthrobacter*, *Azomonas*, *Beijerinckia*, *Bacillus*, *Corynebacterium*, *Clostridium*, *Derxia*, *Enterobacter*, *Pseudomonas*, *Klebsiella*, *Rhodospirillum*, and *Xanthobacter* (Vessey 2003; Barriuso et al. 2008). Symbiotic rhizobia form relationships with legumes in response to flavonoid molecules released from the legume host (Hayat et al. 2012; Rashid et al. 2015). These plant compounds induce the expression of nodulation genes (nod genes) in rhizobia, which produce lipo-chitooligosaccharide signals that trigger mitotic cell division in roots, leading to the formation of nodules (Matiru and Dakora 2004). This nodule is the site for symbiotic nitrogen fixation and is formed as a result of a series of interactions between leguminous plants and rhizobia (Hayat et al. 2012; Rashid et al. 2015). N_2 fixation is an ATP-consuming process that requires a large amount of ATP; it would be advantageous if rhizobial carbon sources were directed toward oxidative phosphorylation, resulting in the synthesis of ATP (Gamalero and Glick 2011; Glick 2012). Oxygen is both inhibitory to enzyme nitrogenase and is a suppressor of *nif* gene expression, but it is required for rhizobial respiration. Toxicity of oxygen is taken care by leghemoglobin, which binds free oxygen tightly, resulting in an increase in nitrogenase activity (Gamalero and Glick 2011). The plant produces the globin portion of leghemoglobin; more efficient strains of *Rhizobium* spp. may be genetically cloned with bacterial hemoglobin genes (Ramirez et al. 1999).

2.2 Phosphate Solubilization

Phosphorus is the second important key element after nitrogen as a mineral nutrient in terms of quantitative plant requirement (Yadav et al. 2012; Sharma et al. 2013a). It plays a vital role in most of the major metabolic reactions, viz., photosynthesis, signal transduction, energy transfer, biosynthesis of macromolecules, and respiration (Khan et al. 2010; Yadav et al. 2012). Despite this fact, the most of this phosphorus is insoluble which present in organic and inorganic form and therefore not available for plant growth (Glick 2012). This problem can be solved by phosphate-solubilizing bacteria (PSB) which solubilize the insoluble phosphate into a soluble form and make available to the plant for its growth and development, and hence PSB are widely used in biofertilizer preparation. PSB plays an important role in the phosphate nutrition of plants in a more eco-friendly manner. The naturally abundant PSB solubilize insoluble phosphate and convert it into soluble forms for the crop plants (Hayat et al. 2012). Conversion of inorganic unavailable phosphate into available forms, viz., $H_2PO_4^-$ and HPO_4^{2-} for uptake of plant, is a phenomenon known as mineral phosphate solubilization (Yadav et al. 2012; Behera et al. 2014).

The mechanisms of inorganic P solubilization are excretion of H^+; production of organic acid like acetate, lactic acid, oxalic acid, tartaric acid, succinic acid, citric acid, gluconic acid, ketogluconate, and glycolic; and biosynthesis of acid phosphatase (Kim et al. 1997; Rodríguez and Fraga 1999; Lal 2002; Arcand and Schneider 2006). This helps in lowering the pH,or in enhancing chelation of the cations bound to phosphate, by competing with P for adsorption sites on the soil and by forming soluble complexes with metal ions associated with insoluble P (Ca, Al, Fe), and thus P is released (Sharma et al. 2013a). The organic phosphorus is released from the organic compound by phosphatases (phosphohydrolase) that dephosphorylates the phospho-ester bonds, phytases, which releases phytic acid and phosphonatases and C–P lyases, enzymes that perform C–P cleavage of phosphonates (Behera et al. 2014). Many researchers have reported phosphate solubilization and plant growth promotion activity by PGPR (Rodríguez and Fraga 1999; Sayyed et al. 2007; Chuang et al. 2007; Afzal and Bano 2008; Richardson et al. 2009; Zaidi et al. 2009; Collavino et al. 2010; Bashan et al. 2013).

2.3 Phytohormone Production

Phytohormones are also called as plant growth regulators (PGRs) and are important for plant growth and development. Various PGPR are known to produce phytohormone, namely, auxins, cytokinin, and gibberellins (Pliego et al. 2011; Saharan and Nehra 2011; Hayat et al. 2012). When plants encounter growth-limiting conditions, they often attempt to adjust the levels of their endogenous phytohormones in order to decrease the negative effects (Salamone et al. 2005). Phytohormones are organic in nature which promote the growth of plants, even at very low concentrations, and help in tissue development. Plant growth promotion by phytohormone-producing PGPR has been reported by many workers (Joo et al. 2005; Sayyed et al. 2007; Spaepen et al. 2007; Akhtar and Siddiqui 2009; Kang et al. 2009; Ahemad and Khan 2012; Megala and Elango 2013).

Indol acetic acid (IAA) is a most studied phytohormone produced by 80 % rhizospheric bacteria (Patten and Glick 1996; Akhtar and Siddiqui 2009). It affects cell division and germination of tuber germination; increases the rate of xylem and root development; controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light, gravity, and florescence; and affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions (Tsavkelova et al. 2006; Spaepen et al. 2009; Spaepen and Vanderleyden 2011; Ahemad and Kibret 2014).

Production of other phytohormones by PGPR has been identified, but not to the same extent as IAA (Vessey 2003). Another group of phytohormones produced by PGPR includes cytokinin which induces division of plant cells in the presence of auxin; the root or shoot differentiation depends on the balance between the auxin and cytokinin (Pliego et al. 2011). Gibberellins (gibberellic acid) are mainly involved in cell division and elongation of cells within the subapical meristems and hence plays a key role in elongation of internode, seed germination, flowering in plants, and pollen tube growth. Like auxins and cytokinins, gibberellic acids also act in combination with other hormones (Pliego et al. 2011).

2.4 Production of ACC Deaminase

Ethylene, a gaseous phytohormone, causes root growth inhibition (Ma et al. 2014). Synthesis of ethylene is known to occur under stress conditions. Biotic stress conditions include infection by pathogens, and abiotic stress conditions include drought. For this reasons, ethylene is also known as the stress hormone. In the plant, production of ethylene involves the conversion of S-adenosyl methionine (SAM) to 1-ami nocyclopropane-1-carboxylate (ACC) and 50-deoxy-50 methyl thioadenosine (MTA) by ACC synthase (Pliego et al. 2011). Some PGPR have the capability to produce ACC deaminase, an enzyme which cleaves ACC, the immediate precursor in the biosynthetic pathway for ethylene in plants (Glick et al. 1998). So such bacteria which produce ACC deaminase indirectly inhibit the ethylene biosynthesis, thereby promoting the plant root growth and also protecting plant from stress, viz., salination, flooding and organic toxicants, drought, heavy metals, toxic organic compounds, and pathogens (Belimov et al. 2005; Glick 2005; Glick et al. 2007a, b; Hao et al. 2007; Farwell et al. 2007; Rodriguez et al. 2008; Gamalero et al. 2009; Gamalero and Glick 2011). Several bacterial strains have been found to produce ACC deaminase such as *Acinetobacter*, *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia*, *Rhizobium*, etc. (Shaharoona et al. 2007; Nadeem et al. 2007; Zahir et al. 2008, 2009; Kang et al. 2010). Nodulation of legumes and mycorrhizal establishment in the host plant induce increases in ethylene content. In this regard, ACC deaminase-producing bacteria, which lower the ethylene content in the plants, can increase both nodulation and mycorrhizal colonization in pea and cucumber, respectively (Ma et al. 2003; Gamalero et al. 2008). Duan et al. (2009) reported that 12 % of isolated *Rhizobium* sp., possessed this enzyme.

3 PGPR as Biocontrol Agents (BCAs)

The term biocontrol refers to the use of microbial antagonist to suppress the disease or pathogen. Biocontrol of pathogen involves application of beneficial rhizobia or their metabolites that neutralizes the negative effects caused by pathogens and thus promote positive responses by the plant (Junaid et al. 2013). Biological control of plant diseases using antagonist PGPR has emerged in recent years in agriculture as greater step toward sustainability, and public concern about the use of hazardous chemical fungicides and plant disease suppression by PGPR is the best possible alternative of reducing the doses of agrochemicals and its severity in agroecosystem. There are various mechanisms involved in biocontrol of plant diseases caused by PGPR. These mechanisms are generally classified as competition, lysis of cell components, antibiosis, and induction of host resistance. These biocontrol mechanisms indirectly promote the plant growth by reducing severity of diseases, hence, also known as indirect mechanisms of plant growth promotion. Table 1 shows several examples of biological control by PGPR.

3.1 Competition

The PGPR-based BCAs compete for nutrients, space, and essential elements with pathogen, thereby displacing and suppressing the growth of pathogen (Duffy 2001; Sharma et al. 2013b). Both the BCAs and the pathogens compete with one another for the nutrients and space to get established and survive in the environment. In this competition, the one which possesses greater metabolic diversity and competitive potential will survive.

So far as the competition for nutrients is concerned, BCAs compete for the rare but essential micronutrients, such as iron and manganese (Junaid et al. 2013). The best example of this mechanism is iron competition. Iron is abundantly present in the earth, but is not available to the living organisms, due to the aerobic atmosphere of this planet which has converted the surface iron into insoluble form like oxyhydroxide. Maximum of 10^{-18} M of free ferric ion is present in solution at biological pH; however, iron is one of the essential nutrients required by the microorganism for synthesis of ATP, reduction of ribotide precursors of DNA, formation of heme, and a variety of functions (Saraf et al. 2011). This presents a big challenge for microorganisms which require iron at micromolar concentrations for growth. This very less

Table 1 Few examples of biocontrol of plant disease by PGPR

PGPR	Pathogen	Disease	Reference
Fluorescent pseudomonads	*Colletotrichum falcatum*	Red rot of sugarcane	Viswanathan and Samiyappan (2002)
Serratia sp., fluorescent pseudomonad	*Ralstonia solanacearum*	Wilt of tomato	Guo et al. (2004)
P. fluorescens, *Pseudomonas* sp.	*Fusarium culmorum*	Seedling blight, foot rot, and head blight diseases of cereals	Khan et al. 2006
Bacillus sp., *Chromobacterium violaceum*	*F. oxysporum*	Crown rot in sorghum	Idris et al. (2007)
Pseudomonas sp.	*Microdochium nivale*	Seedling blight of wheat	Amein et al. (2008)
Pseudomonas chlororaphis, Fluorescent pseudomonads	*Macrophomina phaseolina*	Charcoal rot of sorghum	Das et al. (2008)
Brevibacterium laterosporus, *Pseudomonas fluorescens*, *Serratia marcescens*	*Pythium ultimum*	Root rot in sorghum	Idris et al. (2008)
Burkholderia cepacia	*Fusarium* sp.	Dry rot of potato	Recep et al. (2009)
Bacillus licheniformis, *Bacillus* sp., *P. aeruginosa*, *Streptomyces fradiae*	Sunflower necrosis virus	Sunflower necrosis	Srinivasan and Mathivanan (2009)
Fluorescent pseudomonad	*Sarocladium oryzae*	Sheath rot	Saravanakumar et al. (2009)
Acinetobacter sp., *Enterobacter* sp.	*Ralstonia solanacearum*	Wilt of tomato	Xue et al. (2009)
Pseudomonas fluorescens, *Enterobacter cloacae*	*Fusarium* sp.	Dry rot of potato	Al-Mughrabi 2010
Bacillus. subtilis	*Monilinia laxa*	Brown rot of nectarine	Casals et al. 2010
Bacillus subtilis, *Burkholderia cepacia*	*F. oxysporum*	Vascular wilt of tomato	Shanmugam and Kanoujia (2011)
Chryseobacterium wanjuense	*Phytophthora capsici*	Phytophthora blight of pepper	Kim et al. (2012)
Bacillus sp.	*F. oxysporum* f. sp. *cucumerinum*	Wilt of cucumber	Li et al. (2012)
Bacillus megaterium, *B. subtilis*, *Pseudomonas* sp.,	*Aspergillus niger*	Root rot of peanut	Yuttavanichakul et al. (2012)
Pseudomonas putida, *Bacillus cereus*	*Ralstonia solanacearum*	Wilt of tomato	Kurabachew and Wydra (2013)

(continued)

Table 1 (continued)

PGPR	Pathogen	Disease	Reference
Bacillus subtilis	*Curtobacterium flaccumfaciens*	Wilt of common bean	Martins et al. (2013)
Pseudomonas chlororaphis subsp. *Aurantiaca*	*F. graminearum*	Head blight on wheat	Hu et al. (2014)
Bacillus. subtilis	*Sporisorium reilianum*	Head smut in corn	Mercado-Flores et al. (2014)
Brevibacterium iodinum	*Stemphylium lycopersici*	Gray leaf spot disease in pepper	Son et al. (2014)
Bacillus thuringiensis, *B. cereus*	*Ralstonia solanacearum*	Wilt of eucalyptus	Santiago et al. (2015)

concentration of ion cannot support the growth of organism (Heydari and Pessarakli 2010). To survive in such environment, certain organism produces ion-binding ligands called siderophore to scavenge iron from the environment (Hider and Kong 2010). Some microorganisms produce siderophore that chelates the available iron and competitively prevents the iron nutrition of phytopathogen (Siddiqui et al. 2007; Akhtar and Siddiqui 2009; Chaiharn et al. 2009; Sayyed and Chincholkar 2009). Siderophore is produced by *Alcaligenes*, *Pseudomonas*, *Bradyrhizobium*, *Bacillus*, *Enterobacter*, and *Rhizobium* (Shaikh et al. 2014; Shaikh and Sayyed 2015; Sayyed and Patel 2011; Sayyed et al. 2013). Many different environmental factors affect the synthesis of siderophores, notably the chemical nature of the organic carbon and energy source (Sayyed et al. 2010), metals (Sayyed and Chincholkar 2010), amino acids (Sayyed et al. 2010, 2011), and organic nitrogen sources (Sayyed et al. 2005, 2010). Any factor influencing siderophore production influences the performance of PGPR in plant growth promotion and phytopathogens suppression (Sharma and Kaur 2010).

BCAs also compete with the pathogen for physical occupation of the site and thereby delay the root colonization by the pathogen and exhaust the limited available substrate (Heydari and Pessarakli 2010). PGPR must be able to compete with the pathogen and efficiently colonize the rhizosphere of the plants to be protected (Akhtar and Siddiqui 2010). Root colonization is widely believed to be an essential aspect for biocontrol (Weller 1983; Parke 1991). Rhizosphere colonization is the first step in the pathogenesis of soil-borne microorganisms and also is crucial in the application of microorganisms for beneficial purposes (Lugtenberg et al. 2001).

3.2 *Induced Resistance*

Some biocontrol agents induce a sustained change in the plant, increasing its tolerance to infection by a pathogen, a phenomenon known as induced resistance. In some cases, it is clear that induced resistance by BCAs involves the same suite of genes and gene products involved in the well-documented plant response known as systemic acquired resistance (SAR) (Handelsman and Stabb 1996). SAR is a state of defense that is activated throughout the plant following the primary infection by pathogens (Ryals et al. 1996). SAR and induced systemic resistance (ISR) are two forms of induced resistance wherein plant defenses are preconditioned by prior infection or treatment that results in resistance against subsequent challenge by a pathogen or parasite (Choudhary et al. 2007). ISR involves salicylic acid, jasmonic acid, and ethylene in the signaling (Niranjan et al. 2005; Pieterse et al. 2014) within the plant, and these hormones stimulate the host plant's defense responses against a variety of plant pathogens, including fungal, bacterial, and viral pathogens, as well as nematodes and insects (Glick 2012; Beneduzi et al. 2012). SAR is characterized by the activation of SAR genes, including genes that encode pathogenesis-related proteins, which are used as markers for the state of induced resistance (Mandal and Ray 2011). PGPR elicit ISR in plants by increasing the physical and mechanical strength of the cell wall as well as changing the physiological and biochemical reactions of the host (Labuschagne et al. 2010). This results in the synthesis of defense chemicals such as peroxidase and pathogenesis-related proteins (Nandakumar et al. 2001; Silva et al. 2004; Labuschagne et al. 2010). Several rhizobacteria trigger the systemic acquired resistance (SAR) pathway by secreting salicylic acid at the root surface, while other rhizobacteria trigger salicylic acid-independent SAR pathway. This salicylic acid-independent pathway which is dependent on jasmonic acid and ethylene signaling has been studied in *Arabidopsis thaliana* (Choudhary et al. 2007). The accumulation of salicylic acid for the expression of SAR was demonstrated by using transgenic NahG plants which express the bacterial salicylate hydroxylase nahG gene, making them incapable of accumulating salicylic acid (Lawton et al. 1996). The following bacterial-derived compounds are also involved in ISR including cell wall components such as flagella, lipopolysaccharides, metabolites like siderophores, cyclic lipopeptides, volatile compounds like acetoin and 2,3-butanediol, antibiotics, phenolic compounds, and quorum-sensing molecules (Vleesschauwer and Hofte 2009; Lugtenberg and Kamilova 2009). Biosurfactants most specifically cyclic lipopeptide act as ISR signaling molecule in plants; cyclic lipopeptides like fengycin, iturin, and surfactin families from *Bacillus* sp. are known to have induced resistance mechanisms in plants (Ongena and Jacques 2008). Also the receptor for bacterial flagellin has been identified as involved in ISR (Gomez-Gomez and Boller 2000). In field treatment, many PGPR strains applied as seed coating or in drenching have shown induction of ISR either protected cucumber plants from anthracnose caused by *Colletotrichum lagenarium*, angular leaf spot caused by *Pseudomonas syringae* pv. *lachrymans*, or bacterial wilt caused by *Erwinia tracheiphila* (Zehnder et al. 2001).

3.3 *Lysis of Cell Components*

Many rhizobacteria release hydrolytic enzymes that hydrolyze a wide variety of polymeric compounds like chitin, proteins, cellulose, hemicellulose, and DNA. Expression and secretion of such enzymes by these rhizobacteria help in inhibiting plant pathogen (Pal and Gardener 2006). Production of cell wall-degrading enzymes such as chitinases, glucanases, cellulases, and proteases is known to cause lysis and degradation of the fungal cell walls and thus help in biocontrol of fungal plant pathogens (Mabood et al. 2014).

Among these enzymes, chitinases are of prime importance; chitinases are produced by rhizobacteria to utilize chitin as a source of carbon and energy. *B. subtilis* BSK17 is known to produce chitinase and β-1,3-glucanase to help in their competence and antagonistic activity (Dubey et al. 2014). Chitinase and β-1,3-glucanase have been reported as major class of lytic enzyme that dissolve the major constituent of fungal cell wall like chitin and laminarin (Kumar et al. 2012). Chitinase-producing *Paenibacillus illinoisensis* provides control of blight in pepper (*Capsicum annuum*) caused by *Phytophthora capsici* (Jung et al. 2005). Jung et al. (2003) also demonstrated the biocontrol potential of chitinase-producing *Paenibacillus illinoisensis* KJA-424 against damping off caused by *Rhizoctonia solani*. Dunne et al. (1997) demonstrated the biocontrol of *Pythium ultimum* in sugar beet by protease-producing *Stenotrophomonas maltophilia*. Chitinase- and protease-producing *Pseudomonas* sp. *Pantoea dispersa* and *Enterobacter ammrenus* strains inhibited the growth of *Fusarium* sp. and *M. phaseolina* (Gohel et al. 2004). β-1, 3-glucanase plays significance role in biocontrol of *Lysobacter enzymogenes* (Palumbo et al. 2005). Chitinase-producing *Bacillus suly* reduced the severity of *Fusarium* infection produced under greenhouse conditions (Hariprasad et al. 2011).

3.4 Antibiosis

Production of antibiotics by PGPR is one of the major mechanisms studied for biocontrol of plant diseases. These antibiotics cause fungistasis, inhibition of germination of fungal spores, lysis of fungal mycelia, or fungicidal effects (Sindhu et al. 2009). A large number of antibiotics, including diacetylphloroglucinol (DAPG), oomycin A, phenazines, pyocyanin, pyrroles, pyoluteorin, pantocin, hydrogen cyanide (HCN), mupirocin, pyrrolnitrin, iturins, bacillomycin, surfactin, zwittermicin A, etc., are produced by PGPR (Nielsen et al. 2002; de Souza et al. 2003; Fernando et al 2005; Sindhu et al 2009; Akhtar and Siddiqui 2010; Ahanger et al. 2014; Mabood et al. 2014; Shaikh and Sayyed 2015). Several BCAs produce multiple antibiotics that inhibit one or more pathogen (Islam et al. 2005; Junaid et al. 2013). Phenazines are the largest family of heterocyclic nitrogen-containing pigment known to have broad-spectrum antibiotic activity (Thomas et al. 2003). The majority of antibiotics is produced by *Bacillus* sp., which are active with both gram-positive and gram-negative bacteria and pathogenic fungi *Alternaria solani*, *Aspergillus flavus*, *Botryosphaeria ribis*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Helminthosporium maydis*, *Phomopsis gossypii*, etc. (Maksimov et al. 2011). Synthesis of antibiotics is affected by many factors like carbon source, pH, temperature, and trace elements (Milner et al. 1996; Duffy and Defago 1997, 1999).

DAPG-producing *Pseudomonas* sp. shows potent in vitro and in vivo antifungal activity against *Fusarium oxysporum* causing wilt of tomato (Kang 2012). Bacillomycin D-producing *Bacillus subtilis* shows antifungal potential against *Aspergillus flavus* (MakMoyne et al. 2001). Koumoutsi et al. (2004) studied bacillomycin D-producing *Bacillus amyloliquefaciens* that inhibited the *Fusarium oxysporum* causing wilt disease. Mycosubtilin-producing *Bacillus* sp. decrease the incidence of damping off disease causing *Pythium aphanidermatum* (Leclere et al. 2005). *Bacillus* sp., producing iturin, inhibits germination of *Penicillium roqueforti* (Chitarra et al. 2003) and *Colletotrichum trifolii* (Duville and Boland 1992). Iturin produced by *Bacillus* sp. also exhibited antifungal activity against *Aspergillus flavus* (Moyne et al. 2001).

4 Merits of PGPR as Bioinoculants

(a) Biocontrol agents give protection to the crop throughout the crop period.
(b) They do not cause toxicity to the plants.
(c) Application of biocontrol agents is safer to the environment and to the person who applies them.
(d) They multiply easily in the soil and leave no residual problem.
(e) Biocontrol agents not only control the disease but also enhance plant growth by exerting beneficial effects on root and other beneficial soil microflora and also increase the crop yield.
(f) Biocontrol agents are very easy to handle and apply to the target.
(g) Biocontrol agent can be combined with biofertilizers.
(h) They are easy to manufacture and amenability for growth on an inexpensive medium in fermenter.
(i) It is harmless to human beings and animals and non-production of secondary metabolites that might be toxic to humans.
(j) High genetic stability.

5 Commercial Aspects of PGPR

Over the last few years, several investigations have been initiated to find BCAs for the suppression and control of plant diseases caused by various fungi, bacteria, and viruses. Despite the numerous reports of successful experiments, there has been limited commercial success because of inconsistent field performance (Sayyed et al. 2005). The demand of fertilizers in India is increased significantly in the last few years. Figure 2 shows the fertilizer consumption (N, P, and K) in India from 1974–1975 to 2010–2011. Intensity of per hectare consumption of fertilizer is more in Northern (91.5 kg/ha avg.) and Southern (85.3 kg/ha avg.) Region vis-à-vis Eastern (44.7 kg/ha avg.) and Western (40.7 kg/ha avg.) region. By 2020, fertilizer's

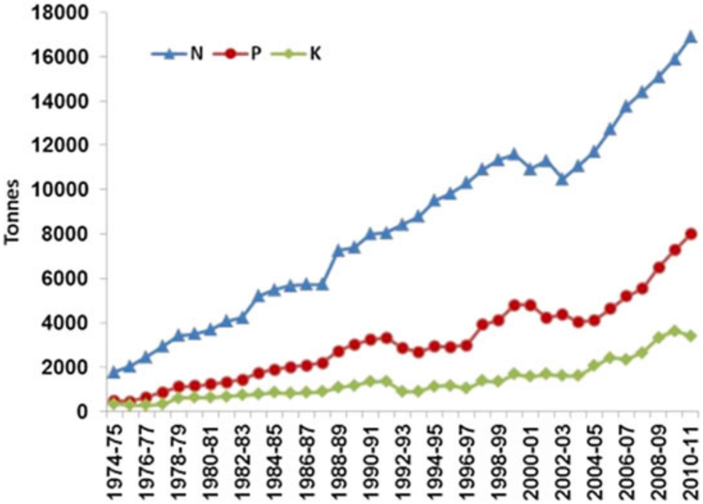

Fig. 2 Shows the fertilizer consumption (N, P, and K) in India from 1974–1975 to 2010–2011

demand in India is projected to increase shoot up to 41.6 million tonnes. If chemical fertilizers are replaced by biofertilizers, the environmental risk of using chemical fertilizers and their negative effects can be substantially reduced. The Government of India has been promoting the use of biofertilizers in agriculture through the National Project on Development and Use of Biofertilizers (NPDB).

Stability of the end products of biofertilizers during storage is high. Biocontrol strains are also resistant to standard fungicides, compatible with other chemical and physical treatments, and safer to the useful soil rhizobia. Under NPDB scheme, the Government provides nonrecurring grants-in-aid up to INR 20,000,00 for setting up of biofertilizer production units of 150 MT capacity. This grant-in-aid is offered to State Departments of Agriculture/cooperatives/public sector undertakings of fertilizers, NGOs, and private agencies provided their proposals are received from respective State Governments.

6 Conclusions and Future Prospects

PGPR improved the nutrient status of plants through various direct or indirect mechanisms and also protect plants against the phytopathogens. Thus, awareness must be brought among the farmers or end users about the positive aspects of PGPR as biofertilizer and BCAs, because it is not only cost effective but also have other positive aspects. This PGPR technology is acceptably applied to the maintained conditions like a laboratory or greenhouse, but field application and root colonization need to be improved. Future challenge is not only to prove the biocontrol of plant diseases but also to improve their efficacy and durability under soil environment. This can be achieved through a better understanding of the biological control mechanisms and interactions between plant and microbes as well as microbial ecology in the soil. The aim should be to enhance the crop yield on a sustainable basis while maintaining the quality and health of soil. To achieve this, the knowledge of PGPR for different crop rhizospheric needs to be improved. Genetic engineering could result in construction of new biocontrol strains with enhanced production of antifungal metabolites, multiple plant growth potential in single stain, improved competence for space or nutrient, wider host range, or enhanced tolerance to biotic and abiotic stress. More efficiency of biocontrol agents can be improved by developing the improved cultural practices and suitable delivery systems, favorable for their establishment in the rhizosphere and efficient root colonization potential.

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Utilization of Biomaterials as Soil Amendments and Crop Protection Agents in Integrated Nematode Management

Mohamed S. Khalil

Contents

Abstract Phytonematodes, or plant-parasitic nematodes, are considered to be among the most important economic pathogens around the world. Certain methods have been developed and used to manage plant-parasitic nematodes, with varying levels of success, but chemical control certainly was and remains the most common approach. However, at the same time, because of the adverse effects of chemical pesticides, it was necessary to find alternative substances that could be used in integrated management programs. One of these alternatives is the use of biomaterials, which have attracted the attention of researchers, who have examined their use as

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soil amendments. Marine organisms, which are plentiful natural sources of chitin and came directly after cellulose, have proved highly effective in fighting phytonematodes under certain conditions. On the other hand, mushrooms are a macro fungus and have been cultivated in China since ancient times and used for therapeutic and nutritional purposes. Furthermore, in the past three decades, many reports have shown that both mushrooms and spent mushrooms have nematicidal effects on various genera of plant-parasitic nematodes. Strategies that, depending on the soil, make use of natural materials, especially chitin, chitinous materials, mushrooms, and spent mushrooms, have been very successful. The application of these materials to soil increases microorganism proliferation and promotes the release of certain compounds that have antagonistic effects on plant nematodes.

Keywords Chitin • Chitinous materials • Mushroom • Spent mushroom • Plant-parasitic nematode

1 Introduction

The technological revolution has contributed significantly to human well-being, with advances continuing at the global level, but we still face problems in the form of unseen underground enemies: the Phytonematodes. Phytonematodes represent a diverse range of populations, but most are root feeders, which means they complete their life cycles in or on root zones, or in shoot zones in some cases (Khalil and Badawy 2012). The prevention and management of such pests will remain one of the most important challenges facing researchers.

Plant-parasitic nematodes are managed using different methods such as chemical compounds, resistant plants, and cultural practices (Barker and Koenning 1998). Among the various known methods, chemical nematicides are the most common method of controlling the population of plant nematodes in field conditions; they are expensive, though economically viable, especially with high-value crops. Meanwhile, environmental pollution with these pesticides is one of the major challenges for modern agricultural practices and human life. Thus, alternative control methods must be found to solve the problem caused by this economically important pest (Noling and Becker 1994). In this regard, significant attention has been devoted to seeking alternative materials in the surrounding environment. Chitin and mushrooms are unique biomaterials that reflect a new trend in phytonematode management.

Chitin is a biodegradable polymer widely spread in nature. Likewise, chitin is mainly of marine origin and has different other sources (Dutta et al. 2004; Mack et al. 2015). The annual estimated quantity of chitin is at least 10^9 tons (Gooday 1990), while the latest figures show that worldwide production reaches levels of up to approximately 10^{12} tons (Gortari and Hours 2013). It is a suitable additive for fertilizers and soil conditioners because it provides nitrogen (Khalil and Badawy

2012). However, mushrooms may be introduced as a special kind of fungus that is edible by humans. These fungi have large bodies, which makes them easy to notice and pick up (Chang and Miles 2004; Wan Rosli et al. 2015). The reputation of mushrooms as a highly nutritive food stuff is well founded. *Agaricus bisporus*, *Pleurotus* spp., and *Lentinula edodes* are the most famous species of mushroom and represent the major sector of global production. Historically, China has been the main source of old types of mushrooms, and scientists discovered that *Lentinus edodes* was the oldest cultivated genus of mushroom in the ancient world, whereas *Agaricus bisporus* was later cultivated in France. In addition, *P. Ostreatus* was grown in the USA in the early twentieth century.

The measured global production of mushrooms in 2007 reached to 33.4 million tons, and biggest producer of mushroom around the world is China (Celik and Peker 2009). The first report about using mushroom as bio-agent against plant-parasitic nematodes was published by Thorne and Barron (1984). Hence, the main purpose of this chapter is to clarify the competence and traits of using some biomaterials as a kind of manipulation of environmental problems that caused by pesticide usage, as well as an alternative strategy to manage plant-parasitic nematodes.

2 Chitin

Chitin $(C_8H_{13}O_5N)_n$ was first discovered in mushrooms in 1811 by Henri Bracannot, a French professor of natural history, who noticed that sulfuric acid did not dissolve some substance found in mushrooms. *Chitine* is the old French name for chitin, which means “tunic” or “coverage” in Greek (Odier 1823; Domard 1996). Chitin is an important biopolymer that is obtained mainly from shrimp and crabs. It was first isolated from insects in 1823 (Odier 1823). Later, Roughet (1859) reported that it could be transformed into water-soluble form by chemical reaction. Later, in 1870, modified chitin was named chitosan (Rinando 2006). Neuberger and Pitt Rivers (1939) described the chemical structure of chitin and stated that pyranose is a ring of *N*-acetylglucosamine. Arthropods and cell walls of fungi are distinguished by chitin, which is the major constituent of their structures, as well as of some algae and yeasts. Organisms that fall in different classification kingdoms also produce chitin. However, vertebrates, plants, and prokaryotes do not contain chitin (Sandford 2004). Kurita (2006) has reported that shrimp cuticle constitutes the main source of chitin, accounting for 30–40 % of it. However, he also indicated that other sources are rich in chitin as well, such as squid pen, krill, and crab cuticle, while fungal cell walls, insect cuticles, and clam shells are considered rare sources of chitin. Recently, advanced research has focused on several species of fungi as new sources of chitin. The obtained amount of chitin from those fungi is dependent on many factors, such as fungus class or species and the conditions of production. Fungi in Class Zygomycetes are candidates as alternative sources of chitin and chitosan because of the richness of their cell walls (Krishnaveni and Ragunathan 2015).

2.1 Production of Chitin

Arthropod shells (e.g., crabs and shrimp) are the most abundantly available waste materials that contain approximately 20–50 % dry weight of chitin. Other potential sources of chitin production exist, such as prawn, krill, squilla, lobster, crayfish, jellyfish, cuttlefish (squid), oysters, clams, insects, and fungi (Cheba 2011). The extraction of chitin from crustaceans is a multistep process, initiated with the dissolution of calcium carbonate and completed with the removal of leftover pigments with a colorless product (Rinando 2006). The steps involved in the production of chitin from crustacean shells are as follows: (1) deproteinization, the removal of proteins from shells by treating them with NaOH or by digestive enzymes; (2) demineralization, which involves the use of hydrochloric acid to separate calcium carbonate and calcium phosphate; (3) decoloration, or the elimination of pigments such as melanin and carotenoids with 0.02 % potassium permanganate at 60 °C or hydrogen peroxide or sodium hypochlorite (Badawy and Rabea 2011; Radwan et al. 2012; Younes and Rinaudo 2015). After obtaining chitin from its sources, its purity must be tested because any remaining impurities might cause problems related to producing products.

2.2 Characteristics of Chitin

Chitin is not soluble in common organic solvents, and it is highly hydrophobic. It dissolves in special solvents like hexafluoroacetone, dimethyl acetamide mixed with 5 % lithium chloride, and hexafluoroisopropanol (Younes and Rinaudo 2015). Saturated methanol with calcium chloride dehydrate is a new solvent system used for dissolving chitin (Muzzarelli et al. 2014). The nitrogen content of chitin may reach up to 8 %, depending on the extent of deacetylation (Dutta et al. 2004). Jayakumar et al. (2006) noted that chitin has attracted commercial interest because it contains 5.5-fold more nitrogen than does cellulose. The crystallographic structures α, β, and γ of chitin were first described by Rudall and Kenchington in 1973. However, the most widespread form is α-chitin (Kurita 2006). Some important properties of chitin that may specify its activity and movements are summarized in what follows.

2.2.1 Biological Properties of Chitin

Biocompatibility

Chitin has no antigenic properties, and so it is compatible with both animal and plant tissues (Younes and Rinaudo 2015).

Biodegradability

Chitinases, which are found around the world, are able to decompose chitin (Younes and Rinaudo 2015).

Bioactivity

Many studies have demonstrated the antibacterial, antifungal, antiviral, antitumor, spermicidal, antigastritis, anti-ulcer, and anti-inflammatory bioactivities of chitin (No et al. 2002; Nagahama et al. 2008).

2.2.2 Physical Chemical Properties of Chitin

Color and Appearance

Chitin is a colorless to off-white, hard, inelastic, nitrogenous polysaccharide (Dutta et al. 2004).

Chemical Stability

Chitin is stable in concentrated alkaline solutions, even at high temperatures (Yen and Mau 2007; Younes and Rinaudo 2015).

Solubility

Chitin is not soluble in water owing to hydrogenous bonds between molecules (No et al. 2007). However, hexafluoroacetone and *N*,*N*-dimethylacetamide are solvents that capable of dissolving chitin (Younes and Rinaudo 2015).

Molecular Weight

Chitin's molecular weight ranges from 1.03×10^6 to 2.5×10^6 Da, on average, but the modification of chitin to chitosan reduces it to 1×10^5 to 5×10^5 (Lee 1975).

Degree of Deacetylation

The degrees of deacetylation in chitin usually range from 5 to 15 % (Kurita 2001).

Crystallinity

Hydrogen bonds form between arranged strands of chitin, and the higher the crystallinity, the more stable will be chitin molecules (Rathke and Hudson 1993).

Chemical Reactivity

Chitin has amino and hydroxyl groups that are easily substituted with other groups.

Processability

Chitin can be easily processed into gels, beads, powders, fibers, membranes, cotton, flakes, sponges, colloids, films, and spins.

2.3 General Applications of Chitin

Among its various potential applications, chitin is used in many industries such as food, agriculture, and pharmaceuticals. Chitin is found in the gastrointestinal tract of mammals and constitutes a component of columns used to obtain lectins and define their structure (Coelho et al. 2009; Ferreira et al. 2011) and to obtain chitinase from fermentation broth (Kao et al. 2009). Chitin and 6-O-carboxymethyl-chitin activate peritoneal macrophages in vivo, decrease the growth of tumor cells in mice, and stimulate nonspecific host resistance against *Escherichia coli* infestation (Krajewska 2004).

Chitin-based materials are also used to minimize pollutants produced by factories and adsorb silver thiosulfate complexes (Songkroah et al. 2004). However, chitin and its derivatives have been developed for medical applications, such as for dressing wounds in the form of films and fibers (Kim et al. 2008; Muzzarelli 2009; Tamura et al. 2011; Younes and Rinaudo 2015), wound healing, especially as nanochitin (Tamura et al. 2011; Azuma et al. 2015), transporting drugs and genes (Jayakumar et al. 2010a; Shang et al. 2014), controlling drug release (Shang et al. 2014; Younes and Rinaudo 2015), fighting cancer (Karagozlu and Kim 2014), and tissue engineering (Jayakumar et al. 2010b; Tamura et al. 2011). Also, several studies have demonstrated the anti-inflammatory properties of chitin and its derivatives (Park and Kim 2010).

Chitin is widely used for applications in the food industry (Aranaz et al. 2009; Xia et al. 2011). Also, in agriculture, chitin is used in seed and fruit coatings, biopesticides, fertilizers, stimulators of plant growth, and inducers of systemic resistance in plants against certain pathogens (Rinando 2006; Hussain et al. 2013a). Chitin has antifungal, antiviral, antibacterial, and nematicidal properties (Ramírez et al. 2010). Chitin and chitosan applied to soybean leaves augment the levels of phenylalanine and tyrosine ammonia lyase, which indirectly induce resistance against pathogens (Rajkumar et al. 2008). Moreover, the use of chitin or chitosan as a foliar spray increases the photosynthesis rate of cultivated plants (Mondal et al. 2012).

2.4 Biological Performance of Chitin Against Plant-Parasitic Nematodes

Nowadays, chitin and its derivatives are used as nematistatic or nematicidal compounds against plant-parasitic nematodes in agriculture and horticulture. The incorporation of effective amounts of chitin or its derivatives in plants enhances growth and yield with no hazardous effects on the environment (Kalaiarasan et al. 2006; Hussain et al. 2013b). Many researchers have documented several effect of chitin-based treatment against plant-parasitic nematodes, especially its impact as a nematicide, on the activity of soil enzymes, levels of microorganism populations in the soil, and the safe quantity of plants (Kalaiarasan et al. 2008; Radwan et al. 2012; Cretoiu et al. 2014). They have proved that chitin can be used to minimize the proliferation of various genera of plant nematodes such as citrus nematode (*Tylenchulus semipenetrans*), root-knot nematodes (*Meloidogyne incognita* and *Meloidogyne chitwoodi*), lesion nematode (*Pratylenchus vulnus*), sugar beet nematode (*Heterodera schachtii*), and soybean cyst nematode (*Heterodera glycines*) in soils.

Furthermore, it has been reported that the addition of chitin at 1 % or more to soil controls root-knot nematode (Rodriguez-Kabana et al. 1984). Spiegel et al. (1989) proved that crustacean chitin (ClandoSan®) suppresses the populations of *Heterodera avenae* and *Tylenchulus semipenetrans* on wheat and orange, respectively. Similarly, Brown et al. (1995) evaluated the effect of soil preincubation with chitin on the populations of soil microbes, especially bacteria, fungi, and phytonematodes in white clover (*Trifolium repens* L.) and perennial rye grass (*Lolium perenne* L.). The utilized concentrations of chitin were 0.5 and 1.0 %, which incubated for 0, 3, 6, and 9 weeks. The obtained results showed that rye grass yield increased with both 0.5 and 1 % concentrations of chitin, while white clover yield decreased, and this may be due to the phytotoxicity of chitin, while soil microbe numbers did not change. The number of plant-parasitic nematodes, *Meloidogyne* spp., and *Heterodera trifolii* were diminished. However, Bélair and Tremblay (1995) found that chitin-urea additions at 0.2 and 0.4 % (v/v) were ineffective at reducing the preplant nematode populations of *Meloidogyne hapla*.

Nevertheless, Hallmann et al. (1999) noted that soil amended with chitin at 1 % (w/w) showed a moderating effect on root-knot nematode in cotton in the first planting, while in the second planting the infestation significantly minified, which confirmed the extended effect of this organic amendment against nematodes. Meanwhile, Chen et al. (1999) documented that chitin alone or in combination with antagonistic fungi and bacteria, in addition to green manure, as soil amendments did not improve lettuce yield or the suppression of *M. hapla*. Chitin from various natural sources such as shrimp shells, escargot shells, and the shells of golden snails at 2, 4, and 6 g, showed nematicidal effects on plant nematode (*Meloidogyne* spp.) in tomatoes in a greenhouse. The nematicidal effects were increased by the incremental amounts of the applied materials (Suganda 1999).

Bell et al. (2000) found that sandy soil amended with chitin significantly suppressed the presence of both *Heterodera trifolii* and *Pratylenchus* spp. in white clover roots and *Paratrichodorus minor* in perennial rye-grass roots. However, admixing chitin at 2, 4, and 8 g/m^2 with soil transplanted with oilseed rape, significantly reduced galls (69.2–75.4 %), egg masses (85–95 %), and females and juveniles (J_2) (94.3–94.7 %) of *M. incognita* (Korayem et al. 2008). Meanwhile, plant indices, such as shoot system length and weight, increased with all chitin treatments. Chitin waste materials, such as crab shells, prawn waste, and fish meal (each at 10 g/pot), were tested in a greenhouse against the root-knot nematode *M. incognita* in tomato plants. Prawn shells showed a maximum decrease of 55.5 % in the root-knot disease incidence, followed by crab shells and fish meal (Jayakumar et al. 2004).

A lower gall index of root-knot nematode, *M. incognita*, was visible on the root of tomato plants grown in chitin-amended soil, and soil enzymes such as chitinase and β-1,3-glucanase showed greater numbers in those soils (De Jin et al. 2005). Also, Kalaiarasan et al. (2006) investigated the effect of organic crab shell chitin at 1 % (w/w) and chitinolytic biocontrol agents (*Pseudomonas fluorescens* at 2.5 kg/ha and *Trichoderma viride* at 2.5 kg/ha) on the growth of groundnut cv. CO_3 infected with *M. arenaria* in greenhouse conditions. The obtained data showed that soil treated with chitin and bioagents improved plant growth, which was reflected in the yield of groundnut at the end of the season. The evaluated treatments not only minified soil nematode populations and galls per plant but also enhanced the levels of microorganisms in the soil by up to 55 %. In pot trials, Saad et al. (2011, 2012) documented that shrimp shell waste powder significantly reduced root-knot nematode (*Meloidogyne* spp.) populations in soil by 68 to 73 % and galling by 58 to 65 %, respectively.

Kalaiarasan et al. (2008) evaluated the effect of crab shell chitin as a soil treatment on the populations of soil microbes and root-knot nematodes (*M. arenaria*) on groundnut under greenhouse conditions. Data revealed that soil amended with chitin suppressed the nematode population and gall numbers. Meanwhile, the performance of soil microbes such as bacteria, fungi, and actinomycetes was enhanced, as was plant growth. Radwan et al. (2012) tested the impact of certain concentrations of chitin and chitosan as a soil amendment with 1, 3, 5, and 10 g/kg soil on tomato plants versus root-knot nematodes (*M. incognita*). Root galls and second-stage juveniles of *M. incognita* in the soil were reduced more significantly by chitosan than chitin, while the dry weight of shoot and root systems showed no remarkable differences. Hussain et al. (2013b) evaluated chitin as a soil amendment using transplant root dip methods against root-knot nematodes (*M. javanica*), which attack chilli in Pakistan. The soil amended with chitin was found to be more effective and safely applicable for controlling root-knot nematodes. Also, a high growth rate of chilli plants was recorded in connection with the soil amendment method vs. the transplant root dip method.

2.5 Mode of Action of Chitin

Despite certain studies, chitin's action mechanism for managing phytonematodes remains unknown. It may depend on two basic facts: the induction of soil microorganisms and released ammonia. It has also been postulated that during the breakdown of chitin, C and N substances are released into the soil and elevate the growth of antagonistic microorganisms such as bacteria, actynomycetes, and limited numbers of fungi (De Jin et al. 2005; Sharp 2013). At the same time, adding chitin to soil stimulates fungal populations, especially those with chitinolytic activity, and the populations of beneficial bacteria against soil-borne diseases including fungi and plant nematodes (Kloepper et al. 1999). On the other hand, toxic levels of ammonia caused by chitin hydrolysis have been found in soils. The breakdown of chitin materials in soil showed an increment of ammonia emissions at toxic concentrations, enough to minimize nematode populations and subsequently reduce their damage to plant roots (Bélair and Tremblay 1995). Free ammonia is very toxic and penetrates more quickly into cellular membranes than ionized ammonia. Chitin and its derivatives contain high levels of N, between approximately 6.1 and 8.3 % (Sharp 2013), and plants can take up this N directly or after its degradation by living organisms (Roberts and Jones 2012).

The stimulation of biochemical defenses in plants by chitin has been studied by many researchers, who found that these defenses were triggered by a release of phytoalexins (Vasyukova et al. 2001), phenolics (El Hassni et al. 2004), terpenes (Croteau et al. 1987), and reactive oxygen species (Kuchitsu et al. 1995). At the same time, formation barriers are considered a kind of physical defense that include the deposition of callose (Bittelli et al. 2001) and lignin (Köhle et al. 1985) and the formation of tyloses (Lafontaine and Benhamou 1996). These physical barriers allow for quick wound formation and sealing while at the same time obstructing plant-parasitic nematode penetration.

3 Mushroom

Mushrooms are macro fungi that include certain edible types (Chang and Buswell 1996). The first cultivated type of mushroom was *Lentinus edodes*, in China. Furthermore, mushrooms have constituted an important food source for hundreds of years in China, Korea, and Japan owing to their health-promoting benefits, in addition to their medicinal and nutritional properties (Sánchez 2004).

3.1 Production of Mushrooms

The main component of mushroom is water, which accounts for around 90% and 10 % dry matter. The nutritional value of mushrooms is contained in protein, which may reach up to 48 %, carbohydrates (less than 60 %), and lipids (between 2 and 8 %)

(Ranzani and Sturion 1998; Morais et al. 2000). The estimated total value of energy from mushrooms is around 1–1.5 J/kg (Oliver and Delmas 1987).

The global production of mushrooms in 2000 was 26 million tons and reached 33.4 million tons in 2007 (Celik and Peker 2009). China is the largest producer of mushrooms, accounting for 42 % of worldwide production, followed by the USA and the Netherlands, with 12 and 8 % of world production, respectively (Celik and Peker 2009). Globally, there are at least 140,000 species of mushrooms, though only 14,000 species have been classified (Lindequist et al. 2005; Hibbett et al. 2007). At the same time, around 2000 species show varying degrees of edibility, and only 10 % are being used for different purposes, such as in food, medical, or pharmaceutical applications (Chang 1999; Erjavec et al. 2012). Twenty species of mushroom are used for industrial purposes, and approximately 35 mushroom species are cultivated for commercial use. The most famous varieties of mushroom around the world are the button mushroom (*Agaricus bisporus*), shiitake (*Lentinus edodes*), oyster mushroom (*Pleurotus* spp.), wood ear mushroom (*Auricula auricular*), winter mushroom (*Flammulina velutipes*), and straw mushroom (*Volvariella volvacea*) (Chang 1999). However, the spent mushroom substrate might be considered a source of environmental contamination owing to its large-scale production. Scientific reports have also demonstrated the activity of the spent mushroom substrate (Machado et al. 2007; Azevedo et al. 2009; Marques et al. 2014). Generally, mushrooms require cellulosic materials, in addition to cereal (wheat, rye, and millet) grains (Poppe 2000), to grow.

3.2 Characteristics of Mushrooms

Mushrooms have unique properties that may play major roles in human life. Certain reports confirm that mushrooms have antihelminthic, antiviral, antibacterial, and antifungal properties (Jasrotia et al. 2012; Wang et al. 2012). The edible mushroom *Xerocomellus chrysenteron* possesses insecticidal properties (Trigueros et al. 2003). Antioxidant and antitumor activities have also been recorded (Ding et al. 2012; Abol Hassan et al. 2015). Currently, many substances are derived from mushrooms and used as dietary supplements (Silva et al. 2010; de Assunção et al. 2012) and to lower cholesterol (Jayakumar et al. 2007; Regina et al. 2008). *Pleurotus ostreatus* is composed of approximately 70–76 g/100 g total carbohydrates, 19–35 g/100 g protein, and 4–20 g/100 g fiber. Analysis of minerals in mushrooms reveals traces of Ca, Cu, Fe, K, Mg, Na, P, and Mn. At the same time, certain investigations have recorded minor levels of fats in mushrooms, in addition to different types of vitamins, such as thiamin (B1), riboflavin (B2), niacin (B3), folic acid (B9), cobalamin (B12), and ascorbic acid (Rabinovich et al. 2007; Wan Rosli et al. 2015).

3.3 General Applications of Mushrooms

Around the world *Agaricus bisporus*, *Pleurotus* spp., and *Lentinula edodes* are considered the main cultivated mushroom species, and they produce millions of cumulative tons of spent substrate. The substrate of spent mushrooms is used for many purposes, such as to remove environmental pollutants from soil, as a biofuel, in animal feed, and as an organic soil amendment in cultivated regions (Palizi et al. 2009; El-Sherbiny and Awd Allah 2014), as well as to improve the physical and chemical properties of soil (Ranganathan and Selvaseelan 1997; Arthur et al. 2012; Adedokun and Orluchukwu 2013). In addition, it was found that the *Agaricus bisporus* spent substrate reduced Colorado potato beetle populations (Stoner et al. 1996; Gent et al. 1998), and the water extracts of *Agaricus bisporus* were effective against apple scab (Yohalem et al. 1996). *Agaricus* spp., *Lentinula edodes*, *Pleurotus* spp., and other genera suppressed infection by different plant pathogens in vitro and in vivo (Chen and Huang 2010) and *Fusarium* wilt of tomato (Raj and Kapoor 1997). The spent substrate and filtrate of *Lentinula edodes* decreased *Rhizoctonia* damping-off of cabbage (Huang and Huang 2000; Chen and Huang 2010), damping-off disease by *Pythium* spp. (Chen and Huang 2010), and chilli leaf and stem necrosis (Upadhyay 2000).

On the other hand, in the field of bioremediation, it was found that *A. bisporus* spent substrate could be used in air purification when mixed with other materials to remove H_2S and volatile organic compounds (Shojaosadati and Siamak 1999). Also, water treated with mushroom spent substrate was found to reduce pollution with radioactive elements and heavy metals (Groudev et al. 1999). Using *A. bisporus* spent substrate as a soil treatment was shown to affect the distribution of zinc (Shuman 1999a, b), cadmium, and lead (Shuman 1998) and to degrade chlorophenols, polycyclic aromatic hydrocarbons, and aromatic monomers (Fermor et al. 2000; De Souza et al. 2011). The spent substrate of *Pleurotus* spp., blended with fish oil, degrades polycyclic aromatic hydrocarbons in contaminated soil (Eggen 1999). The spent substrate of *A. bisporus* was shown to purify soil from α-naphthol produced from carbaryl degradation (Kuo and Regan 1999), as well as to promote the sorption of atrazine and 2,4-D (Baskaran et al. 1996).

Several studies have been conducted on mushroom species such as *Auricularia* spp., *Corilopsis occidentalis*, *Ganoderma lucidum*, and *Schizophyllum commune*, which have long been used in different cultures as a treatment for human diseases such as high blood pressure, intestinal disorders, and pneumonia (Akpaja et al. 2005; Jonathan and Fasidi 2005; Jonathan et al. 2007). It has also been revealed that mushrooms are rich in antioxidants, which may reduce cholesterol, heart disease, and cancer (Wani et al. 2010). Cheung (2010) and Wan Rosli et al. (2015) note that edible mushrooms are used widely in the food industry.

3.4 *Biological Performance of Mushrooms Against Plant-Parasitic Nematodes*

Thorne and Barron (1984) were the first scientists to publish a report about the effectiveness of mushrooms as bioactive agents against nematodes and reported that fungi with the ability to grow on wood can destroy nematodes. They found that *P. ostreatus* had the ability to eliminate nematodes. Later on, several investigations about mushrooms and their biological impacts against plant-parasitic nematodes were published. Barron and Thorne (1987) clarified that the grown hyphae of *Pleurotus* spp. penetrate the body of plant-parasitic nematodes directly, especially in the head region. Data on both in vitro and in vivo experiments documented that *Pleurotus* spp. diminished the population density of cyst nematodes. Moreover, previous researchers proved that the toxin released from *Pleurotus* species strains could paralyze nematodes (Sharma 1994; Thorn et al. 2000).

Sharma (1994) documented that liquid growth media of *Pleurotus sajor-caju* could paralyze *Aphelenchoides camposticola* and reduce nematode populations by up to 90 %, while Xiang and Feng (2000) reported that *P. ostreatus* decreased the population of *M. arenaria* on peanuts in a greenhouse. Kaul and Chhabra (1993) and Verma (1993) confirmed that the population of *M. incognita* was reduced by the presence of *A. bisporus*. In addition, their efficacy against *Pratylenchus penetrans* in potatoes was also proven (Gent et al. 1998, 1999; La Mondia et al. 1999). However, Engler et al. (1998) isolated secondary metabolites from a culture filtrate of a luminescent mushroom, *Omphalotus olearius*, cultured in a yeast malt glucose medium. The mushroom secreted a bioactive compound, omphalotin, that affected the growth and development of *M. incognita* (Anke and Sterner 1997).

The application of culture filtrate and spawn (a mycelium that develops on cereals) of *Neonothopanus nimbi* suppressed root-knot disease incidence and root galling in tomato plants in a greenhouse experiment (Bua-art 2003, 2007). Similarly, Bua-art et al. (2011) reported that the application of the luminescent mushroom *N. nimbi* at varying concentrations (10, 50, 100, and 500 mg/L) showed high toxicity toward juveniles of *M. incognita* at different exposure times under laboratory conditions and recorded a 100 % mortality without adverse effects on beneficial organisms such as entomopathogenic nematodes, *Steinernema carpocapsae*, *Aspergillus* spp., *Trichoderma harzianum*, *Bacillus subtilis*, and *Rhizobium* spp. However, Heydari et al. (2006) revealed that culture filtrates from oyster mushroom (*Pleurotus ostreatus*) demonstrated nematicidal activity and caused mortality in second-stage juveniles of *M. javanica*. Palizi et al. (2009) studied the preying ability of many oyster mushroom species against the cyst nematode *Heterodera schachtii* under in vivo and in vitro conditions. They concluded that all species caused a mortality rate of between 20 and 96 %. Meanwhile, the data showed that 90 % of nematodes were paralyzed by an extract of *P. ostreatus*, while only 50 % of nematodes were paralyzed by *P. eryngii*. In addition, the mushroom compost of *P. ostreatus* and *P. sajor-caju* were mixed at 100 and 200 g per 3 kg/pot reduced the incidence of cysts significantly on sugar beet by over 85 % under greenhouse conditions. The impact of spent composts of both oyster mushrooms and button mushrooms on the productivity of root-knot nematodes was also studied. The results showed that the spent compost of oyster mushroom was more effective than that of button mushroom and was reflected in the suppressed hatchability of eggs and juveniles (J_2). Spent oyster compost was also effective at inhibiting galling and egg mass production on plant roots followed by button compost, thereby enhancing plant growth (Aslam and Saifullah 2013).

Ibrahim et al. (2014) studied the impact of some plant materials, the stems of oyster mushroom (*Pleurotus ostreatus*), the bioagent *Bacillus thuringiensis* (Bt), abamectin, and the nematicide fenamiphos on *Heterodera goldeni* on rice cv. Sakha 101 under greenhouse conditions. Results indicated that orange peels, rubber plant leaves, mushroom stems, and abamectin produced reductions of 80–86 % in second-stage cyst nematode juveniles. At the same time, El-Sherbiny and Awd Allah (2014) compared the effect of preplanting on the waste residues of oyster mushroom, *Pleurotus ostreatus* (applied at 300 g/m^2), and air-dried powders of harvesting residues (leaves) of artichoke, cauliflower, sugar beet, table beet, taro, and turnip on the chemical nematicide fosthiazate 10 % G (Nemathorin®) for managing the root-knot nematode (*M. incognita*) on tomato plants in a field trial. The oyster mushroom showed a minimum reduction for galls of 69.71 %, egg masses of 67.01 %, and nematode population of 67.93 %. All applied treatments recorded increments in plant parameters. Oyster mushroom treatment achieved minified plant parameters and fruit yields.

3.5 Mode of Action of Mushrooms

Few studies have been conducted to evaluate the effect and probable actions of mushrooms against plant-parasitic nematodes, but to date the exact mechanisms remain unknown. Mushrooms are considered an important source of chemicals that could be used in plant protection or as organic compounds with insecticidal properties (Wang et al. 2002). Several researchers have demonstrated that mushrooms and spent mushroom compost contain a number of active compounds, including phenolic compounds, flavonoids, alkaloids, organic acids, flavones, anthocyanins, polyketides, terpenoids, and steroids (Elmastas et al. 2007; Jayakumar et al. 2011; Keleş et al. 2011; Aslam and Saifullah 2013; Vieira et al. 2013; Mondal et al. 2013). Some investigations found that a strain of oyster mushroom (*Pleurotus ostreatus*) released some sort of toxin from hyphae on water agar as tiny droplets, and this toxin reduced the infectious ability of nematodes (Barron and Thorn 1987; Chitwood 2004).

Sterner et al. (1997) reported that the luminescent mushroom is capable of controlling the presence of root-knot nematode in the soil through the release of omphalotin, which is a toxic substance. The chemical structure of omphalotin indicated that several derivatives of cyclopeptides, such as omphalotin A, B, C, and D, suppress the nervous system of the root-knot nematode (Buchel et al. 1998; Meyer et al. 2004). Similarly, lectins are strong proteins found in mushrooms in very low amounts and are provided by other organisms, for example, bacteria, plants, and some animals. It was noted that lectins might also induce plant defenses against plant-parasitic nematodes, insects, and pathogenic fungi (Trigueros et al. 2003;

Karimi et al. 2007; Chen et al. 2009; Alborés et al. 2014). Currently, over 100 lectin derivatives have been isolated and purified from plants, animals, and microorganisms, and around 60 lectin derivatives or products have been developed commercially from *A. bisporus* (Cheung et al. 2012).

Mushrooms are a good supplementary source of organic matter and macroelements such as N, P, and K in the soil. Some substances that are toxic to plant nematodes were released into the soil during the decomposition of organic matter, for example, butyric, acetic, and propionic acids (Aslam and Saifullah 2013). Also, El-Sherbiny and Awd Allah (2014) reported that the estimated C:N ratio of oyster mushroom was 24.7:1, which is reflected in the activity of soil microorganisms, especially antagonistic ones. Recently, certain types of mushrooms demonstrated inhibiting activity with respect to acetylcholinesterase, which is very important for nervous transmission in plant-parasitic nematodes (Orhan and Üstün 2011; Öztürk et al. 2014).

4 Conclusions and Future Prospects

The use of chitin, mushrooms, and their derivatives represents an alternative approach to pest control because these biomaterials have the ability to control certain pathogens such as bacteria, fungi, and plant-parasitic nematodes and so are useful tools in integrated pest management programs. Moreover, chitin, mushrooms, and their derivatives have proven medicinal and pharmacological potentials. Despite their documented role and diverse use, these biomaterials still require some effort in order to be used as real tools in organic and clean agricultures. Therefore, in the future, it will be important to focus on these biomaterials, which could play a pivotal role in pest management, the improvement of soil conditions, the growth of beneficial soil microbes, and plant defense mechanisms. Moreover, these biomaterials are also safer for human health and the environment.

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Enhanced Nutritional Programme: An Innovative Approach to Controlling Plant Diseases in the Tropics

Khairulmazmi Ahmad and Siti Izera Ismail

Contents

Abstract Mineral nutrients are essential for the normal growth and development of both plants and microorganisms and play a crucial role in plant–pathogen interactions, which could be beneficial or detrimental to plant health. Excessive nutrient uptake causes toxicity in plants, so meeting optimal nutrient requirements is very important in the prevention of plant nutrient-deficiency symptoms. In most cases, mineral nutrients represent the primary lines of plant defense against plant pathogens and are directly affected by the plant host, pathogens, and environmental factors. In general, mineral nutrients may affect disease resistance through induced defenses, including by the production of toxins, metabolites, and lignin. The application of an enhanced nutritional programme (ENP) to minimize the deleterious

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effects of phytopathogens has become a hot topic of discussion around the world. Furthermore, various reports from several countries have shown that the application of mineral nutrients such as calcium (Ca), copper (Cu), and zinc (Zn) in a disease-control programme could enhance crop production and quality, suppress various plant diseases, and promote plant growth. Thus, the aim of this chapter is to discuss conventional disease control methods in the tropics with special reference to major diseases in rice, oil palm, and citrus, as well as limitations on their control. Moreover, the potential application of an ENP as part of a disease control programme in plantation crops in Malaysia is also discussed.

Keywords Basal stem rot • Enhanced nutritional programme • Huanglongbing disease • Plant disease • Tropics

1 Introduction

Micronutrients are essential vital elements for growth required in small quantities, and they advance the physiological function of plants in various ways. Micronutrients include iron, cobalt, chromium, copper, manganese, selenium, zinc, and molybdenum. These micronutrients are important because their deficiencies are very common. For example, around 30 % of cultivated soils around the world are deficient in iron. Generally, growers use micronutrients only when deficiency symptoms occur in plants. This leads to crop losses even before the appearance of the deficiency symptoms. For instance, molybdenum is important in the conversion of nitrates into usable forms. Plants having insufficient micronutrient uptake can manifest chlorosis symptoms or chlorophyll formation. If they survive the early growth stages, they may be weak and susceptible to diseases. When whole plant populations suffer from insufficient micronutrients, including a lack of critical minerals, nations cannot fulfill their best potential to produce high-quality plant products. Thus, the Enhanced Nutritional Program (ENP) is a cost-effective nutrition delivery strategy whose scale-up is urgently needed among farmers and other stakeholders. If the nutrition delivery is successful, it will present huge opportunities to improve agricultural production. Well-integrated strategies to address micronutrient delivery to plants at the national level will be critical to reducing micronutrient deficiency symptoms in crops, improving plants' health and economic productivity.

The world's population is expected to grow by 75 million per annum, increasing by 35 % to 9.2 billion by 2050 (FAO 2009). At about the same time, the growth in food demand will increase by as much as 70 % owing to higher incomes and dietary changes in developing countries towards high-quality and healthy food. To date, world cereal production has doubled over levels in the 1960s. Significant strides in cereal yield improvements have been achieved thanks to nitrogen fertilizers, herbicides and pesticides, high-yielding cereal varieties, irrigation, and high-input technologies (Tilman et al. 2002). For these reasons, many developing countries have become major exporters of cereals worldwide. The improvements in global food security brought about by the work of Norman Borlaug need to be maintained in the face of major problems facing the world in connection with climate change and the release of greenhouse gases into the atmosphere resulting from agricultural activities. Therefore, given current trends in population growth, an alternative strategy needs to be implemented in agricultural practices which could make an important contribution to increasing global food production. The National Agricultural Policy (NAP) 2011–2020 was developed to formulate strategic plans with regard to agriculture. It is a long-term programme which aims to address food security and safety issues in Malaysia. The NAP represents a comprehensive focus on improving efficiency in the agrofood industry and strengthening the network linkage with industrial sectors for maximizing the use of natural resources.

It has been estimated that rice (*Oryza sativa* L.) provides almost 27 % of dietary energy and 20 % of dietary proteins in developing countries, especially in South-East Asia as well as Africa (Redoña 2004). According to FAO (2008), around 430 million metrictonnes of rice are produced in Asia. It is the predominant crop in Asia, and approximately 90 % of arable land there is cultivated with rice and (Cantrell and Hettel 2004). In Malaysia, the average rice yield was 3817 kg/ha in 2013, representing a total income of RM 2,053,139, although the self-sufficiency level (SSL) of rice was still relatively quite low (DOA 2014). It is expected that by 2025, about 15–20 million ha of irrigated land planted with rice will experience insufficient water resources (Bouman et al. 2007). To deal with this looming challenge, the International Rice Research Institute has developed an “aerobic rice” technology which has a goal of significantly reducing water usage by up to 70 % within rice-cultivated areas. With the implementation of this technology yields could increase to as much as 7 tonnes/ha, and water usage will be only half that required under standard practice (Cantrell and Hettel 2004). This so-called water-saving rice system could increase crop yields and the response to fertilizers in non-irrigated rice. An aerobic rice system requires the same amount of fertilizer as flooded rice. Another economically important crop in Malaysia is oil palm (*Elaeis guineensis* Jacq.). It is considered a “golden crop” in Malaysia and contributes approximately 39 % to global oil production. Global consumption of palm oil was 52.1 million metrictonnes in 2012 and around 85 % of total worldwide palm oil production was came from just two countries, Malaysia and Indonesia (Ommelna et al. 2012). In 2012, palm oil saw the highest levels of consumption worldwide — by three billion people in 150 countries, including China, India, Indonesia, and countries of the European Union. In Malaysia, five million ha of agricultural land is planted with oil palm and represents around 15 % of total land area (MPOB 2013).

On the other hand, fruit production is less fruitful compared to plantation crops. In 2005, total citrus production in Malaysia was estimated at 33,700 metric tonnes and pomelo accounted for 26 % of total production. Currently in Malaysia, around 4 % of citrus fruit is imported; the Limau mandarin from Thailand meets local consumption demand (FAMA 2008). According to DOA (2010) huanglongbing (HLB) disease is the major cause of the decline in citrus production in Malaysia, accounting for as much as 30 % of that decline, particularly in Citrus Valley, Durian

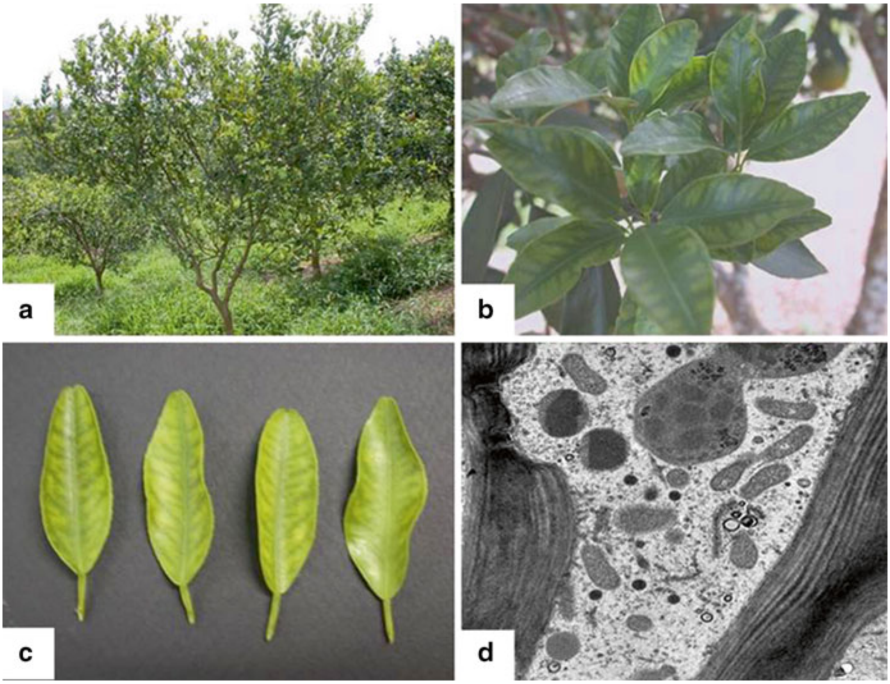

Fig. 1 Field symptoms of HLB disease and *Candidatus* Liberibacter asiaticus bodies on honey mandarin (*Citrus reticulata*): (**a**) sectorial leaf yellowing on infected citrus tree; (**b**) mottling of leaves with green veins; (**c**) interveinal chlorosis of leaves; (**d**) elongated and sperical shapes of *Candidatus* Liberibacter asiaticus bodies

Mentangau, Terengganu. HLB disease is caused by a phloem-limited putative species known as *Candidatus* Liberibacter asiaticus (Fig. 1a–c) (Bové 2006). This pathogenic bacterium can wipe out mature citrus trees within 5 years of planting, and HLB disease incidence can be as high as100 %, even among pathogen-free newly transplanted trees (Yang et al. 2006).

Plant disease management in tropical countries is a major concern for farmers, plant pathologists, and plantation managers because they lack viable management practices. Furthermore, the difficulties in managing diseases in the tropics are also associated with environmental factors such as high humidity and rainfall, warmer climate, and inoculum pressure in the fields (Ploetz 2007). In Malaysia, plant diseases are also controlled using cultural, biological, and chemical methods. A deep understanding of the etiology and epidemiology of a particular plant disease is the only effective way to control the spread of the disease. In the case of HLB disease in citrus, researchers have reported that it is very difficult to detect the pathogen because it is present in very low concentrations, unequally distributed in host tissues, easily spread by vector and vegetative propagation, and impossible to culture in synthetic media (da Graca 1991; Hung et al. 1999). HLB disease has a long incubation period and symptom-less in certain host plants, but infected citrus

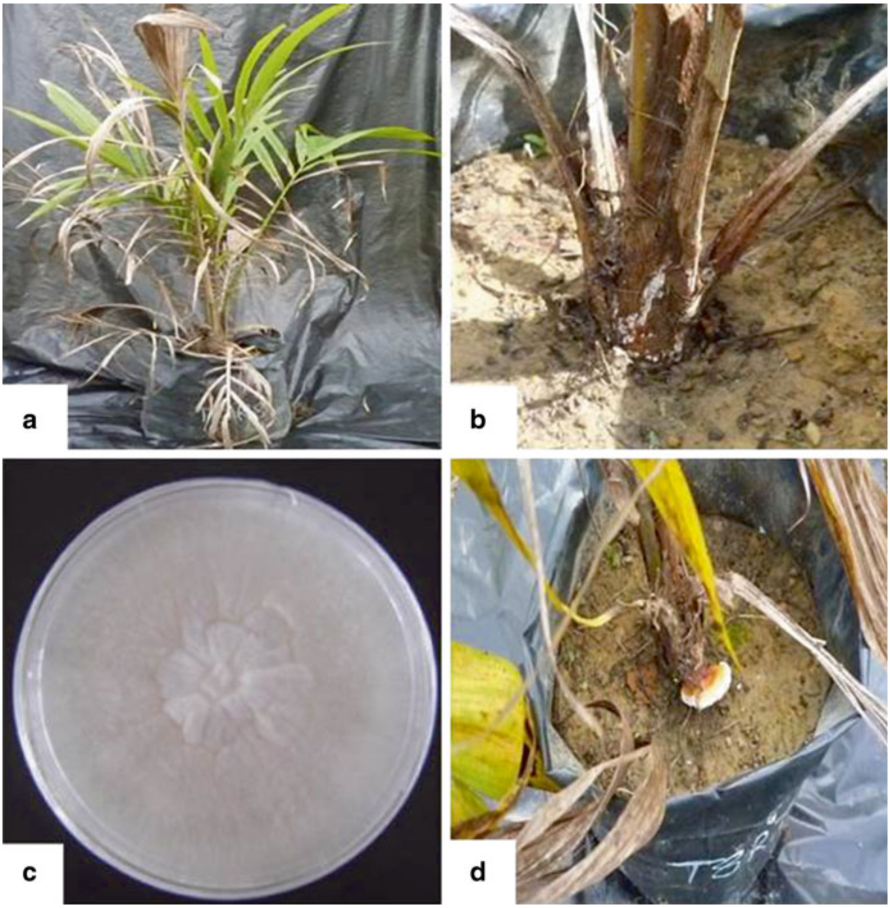

Fig. 2 Signs and symptoms of BSR, caused by *Ganoderma boninense*: (**a**) a lower leaf of infected seedling showing necrotic symptoms and drying; (**b**) white fungal mass appearing at base part of infected seedling; (**c**) pure culture of *G. boninense*; (**d**) fruiting bodies of *G. boninense* on infected seedling

plants occur in fields. Another difficulty is that HLB symptoms resemble symptoms of plant nutrient deficiency or disorders, so it is not easily diagnosed (Weinert et al. 2004).

In Malaysia, the oil palm faces the most severe disease, called basal stem rot (BSR) (Fig. 2a–d), caused by *Ganoderma* spp. (Idris et al. 2000). Adaskaveg and Gilbertson (1986) and Miller (1995) discovered *G. boninense* in Malaysia. However, Idris (1999) reported that another species, *G. zonatum*, existed alongside *G. boninense* on oil palm. Neither species shows any visible symptoms during the early infection stage. Once the infection has progressed 60–70 % in the palm, visible symptoms are noticeable. BSR is characterized by an internal dry rotting of trunk tissues, especially at the junction of the bowl and the trunk portion of the palm.

Ganoderma infection causes progressive devastation of the basal tissues of the oil palm trunk. Infected leaves show external symptoms like wilting and malnutrition after 50–60 % infection by *G. boninense*. Hartley (1988) noted that foliar symptoms can be observed when the fungus has killed half of the basal stem, thus limiting the water supply and nutrients to aerial parts, which eventually leads to further destruction. To date, there is no effective method for controlling BSR and HLB diseases.

Rice cultivation in Malaysia is confronted by many diseases caused by bacteria, fungi, and viruses. Amongst the various diseases in rice, rice blast (*Magnaporthe oryzae*), bacterial leaf blight (BLB) (*Xanthomonas oryzae* pv. *oryzae*), and rice tungro disease (rice tungro virus) cause the most severe damage to this important crop. Outbreaks of these diseases are very serious, and recurrence of the disease is more likely and extremely difficult to control under field conditions. Thus, integrated pest management (IPM) practices are a highly recommended approach to managing rice diseases under field conditions. IPM consists of several major control strategies such as cultural practices, using resistant rice varieties, nutrient management, and chemical control. This chapter deals with the management of major diseases in oil palm, rice, and citrus. Additionally, a discussion on the application of enhanced nutritional programmes (ENPs) for disease suppression and yield improvement, as well as disease control mechanisms, is also presented. ENPs represent an alternative control measure which can be integrated into a long-term disease control programme for an effective approach to managing diseases in the tropics.

2 Conventional Plant Disease Control Practices

2.1 Cultural Method

Cultural practices traditionally have been used for managing and reducing pests and diseases in oil palm, rice, and citrus. Such a control method can be combined with other practices to reduce the risk of crop failure and plant disease losses. For rice, practices that have been found to be beneficial include hand picking, mixed cropping, water table control, mineral nutrient management, and using healthy and certified planting materials. Government subsidies produce many results in rice production. However, subsidies of chemical pesticides and fertilizers offered by the government have made farmers more dependent on inorganic chemicals than organic products. This is an unsustainable practice which can alter the chemical composition of the host. For example, excessive application of N fertilizer will lead to a declining C:N ratio. Nitrogen accelerates plant physiological processes and the production of greater amounts of volatile chemical compounds. These compounds will attract more pests and transmit more diseases. Moreover, excessive application of inorganic fertilizers and pesticides will lead to environmental pollution and increased resistance to those pesticides in pests and vector-borne diseases.

Cultural control of oil palms, which involves digging trenches on diseased palms to prevent mycelial spread of the pathogen to adjacent healthy palms, is the most utilized cultural practice for BSR, but digging trenches is not an effective method (Turner 1981) for oil palm because oil palm roots can still grow underground. However, the approach to BSR control is soil-mounding, a combination of organic and inorganic fertilizers, cultural methods, and chemical treatments. Hasan and Turner (1994) proved that surgery followed by soil-mounding around the base of mature diseased palms can increase the crop yield and vigor of oil palms. Other practices used in Malaysia involve using three replanting techniques: clean clearing, windrowing, and under-planting. However, these technique was not an effective means of reducing disease incidence, and such treatments have not demonstrated curative effects (Singh 1991).

Early identification of symptoms is a cultural method used to control HLB disease in citrus. Early and frequent inspection is extremely important for obtaining updated information regarding the status of HLB disease and its vector on a farm or within localities. The information is very important for growers, who will want to develop contingency plans if HLB disease or its vector is detected. Regular disease and vector inspection (monthly) must be conducted by trained workers using foot- or tractor-pulled platforms (more than two workers for tall trees). It is important that any two inspections be held close enough in time to avoid the development of severe HLB symptoms. Field inspection can be conducted based on field symptoms such as mottle leaf and lopsided fruit. At each inspection, all trees on a farm are inspected one by one to identify symptomatic trees. During inspection the suspected trees are tagged and recorded and symptomatic leaf samples are collected for laboratory analysis. In laboratory analysis, at the very least a conventional PCR method is used to identify the causal pathogen in order to confirm the field symptoms.

Removing infected trees or branches from an orchard or farm could reduce the risk of HLB infection. Field and laboratory data can be used to uproot previously tagged suspected infected trees. Evidence based on a study in Sao Paulo State (SPS) in Brazil revealed that host eradication programmes have a positive impact on HLB management (Belasque et al. 2010). Early symptomatic trees were removed immediately from the cultivation area if they were confirmed positive by PCR protocol. Chainsaws were used to cut the trees approximately 10 cm above ground level. On several farms a front-end loader has also been used. Care should be taken to prevent suckers from growing out of the stumps or roots. Herbicide applications such as glyphosate were applied to stumps to remove the source of inocula remaining on the farm. Based on a study by Belasque Jr. et al. (2010) on the HLB evaluation programme in SPS, Brazil, the following reasons may be cited as factors in the success of the programme:

1. The farms are large, managed commercially (over one million trees), and are less affected by the health of the surrounding citrus trees.
2. The farms are located in regions of low HLB incidence.
3. Trees at maturity (11 years old) have fewer growth flushes than younger trees and are therefore less attractive to psyllids.

4. The farms are located in isolated areas; the distance between the nearest non-HLB-management farm is more than 5 km. Thus, the farms are at a safe distance from infectious psyllids on the non-HLB-management farm.
5. HLB management started early.

The use of healthy and disease-free planting materials is very important. This can be ensured by careful indexing of bud woods collected from mother plants using sensitive diagnostic approaches or obtaining planting materials from accredited nurseries. According to Lim et al. (1990), infected orange seedlings were brought into peninsular Malaysia by immigrants in the early 1900s from infested countries, presumably China or Taiwan. From there the disease started to establish itself in Malaysia, while no attempts were made by government agencies to control the disease at any time. But in 1987, following an epidemic of the disease in several countries which destroyed the most cultivated citrus areas in Malaysia, the mitigation programmes for HLB disease became an important topic for research. After that, Malaysia collaborated with UNDP and FAO on the Citrus Greening Project, and experts declared the presence of HLB disease in Malaysia. Malaysia's experience shows the great difficulty of managing HLB disease. At the farm level, for instance, disease inspection requires workers skilled at HLB and vector monitoring. Moreover, the work is time consuming and laborious and requires special equipment or platforms to monitor the disease, especially in mature citrus orchards. For HLB disease detection and verification, conventional PCR is usually used. Even though PCR is a very fast and precise method, it requires knowledgeable workers, and, moreover, detection per sample is expensive, which puts the method beyond the reach of smallholders.

2.2 *Chemical Treatment*

Chemical treatment is widely used to control pests and diseases in rice cultivation in Malaysia. Many chemical fungicides are available on the market to control fungal diseases, and some of them can also be used to manage bacterial diseases, but they are very expensive for farmers and less effective at dealing with outbreaks, especially during neck blast. For effective fungal disease control, especially in endemic areas, the farmer is advised to treat seed with Pyroquilon 50 WP (1 g/kg), Tricyclazole 75 WP (1 g/kg), or Carbendazim 50 WP (2 g/kg), alone or in combination treatments. Furthermore, when leaf blast symptoms appear in rice fields, farmers should regularly spray with Tricyclazole 75 WP (0.6 g/L), Ediphenphos 50 EC (1 mL/L), Iprobenphos 48 EC (2 g/L), Isoprothiolane 40 EC (1.5 mL/L), Kasugamycin 3 SL (2.5 mL/L), Carpropamid 300 SC (1 mL/L), or Carbendazim 50 WP (1 g/L). However, the control of BLB disease has been hampered by the fact that only a few bactericides are available on the market. Moreover, the use of antibiotics to control BLB is not recommended in Malaysia, and this will limit the choice of disease control methods. *Xanthomonas* sp. is a Gram-negative bacterium which produces a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adheres to an inert or living surface known as biofilms (Costerton et al. 1999). Biofilm formation plays an important role in the early infection of *Xanthomonas* on host leaves. Biofilm formation during the onset and infection stages can cause persistent infections which evade the immune system and resist elimination by antibiotics. This is because biofilms are very recalcitrant towards antimicrobial treatments.

Because of the severe disease incidence in living stands of oil palms, immediate short-term steps to control this disease must be considered. The use of systemic fungicides, combined with an appropriate technique, may provide an effective solution against disease-causing pathogens. In vitro screening of several fungicides—such as cycloheximide and drazoxolon (Ramasamy 1972); benomyl, bilaxazol, triadimefon, triadimenol, caroboxin, carbendazim, methfuroxam, and cycloheximide (Jollands 1983); cyproconazole, hexaconazole, and triadimenol (Khairudin 1990); penconazole, triadimenol, and tridemorph (Lim et al. 1990)—has shown strong inhibitory action against *Ganoderma*. Turner (1981) reported that organic mercury formulations have proven effective against *Ganoderma* under field conditions but became unacceptable for commercial use because of the residues it left. Attempts have been made to control BSR in field conditions using systemic fungicides through trunk injection, soil drenching, or combinations of both and utilizing pressure injection apparatus (Jollands 1983; Khairudin 1990). These techniques showed promising results for the suppression of *Ganoderma* infection in fields where the systemic fungicide bromoconazole was used (Ariffin and Idris 1997). However, to date, no chemical fungicide has been reported to effectively control BSR on plantations worldwide. Immediate short-term strategies for controlling HLB disease must be investigated. Practices similar to those used to control BSR disease could be adopted for HLB management. Antibiotics are amongst the systemic chemicals used in the control of HLB disease on farms. Currently, nearly 40 types or derivatives of antibiotics have been screened for plant disease control, but less than 10 have been used commercially. Of these, only streptomycin and tetracycline have been commonly and effectively used on fruit trees. Recently, the combination of penicillin and streptomycin through root soaking or foliar spraying was effective at eliminating or suppressing *Candidatus* Liberibacter asiaticus (Zhang et al. 2011). The researchers' experimental findings revealed that this technique effectively suppressed the titer of HLB bacterium (70 % regeneration rate) for a much longer period of time using q-PCR. In addition, penicillin g sodium and 2,2-dibromo-3-nitrilopropionamide (DBNPA) were also found to be effective at eliminating or suppressing diseases caused by the HLB bacterium. The application of penicillin g sodium at 50 μg/mL showed that all plants regenerated from infected cuttings having no disease symptoms through the use of nested PCR and q-PCR. In addition, DBNPA was also able to significantly reduce the percentage of *Candidatus* Liberibacter asiaticus-positive plants and the titer of the bacterium at 200 μL/L (Zhang et al. 2010). Even though a large-scale solution to HLB disease has been introduced, the use of streptomycin and tetracycline on plants accounted for only 0.1 % of all antibiotics produced annually in the USA. This might be due to uncertainty regarding long-term side effects. For this reason, antibiotic treatment for plant protection purposes is not recommended in Malaysia, and stringent procedures must be put in place before such treatment is performed .

2.3 Biological Control

The potential control of *Ganoderma* in living stands could be conducted through manipulation of biological agents. Several potential antagonists, namely *Trichoderma* spp. (Shukla and Uniyal 1989; Wijesekera et al. 1996), *Aspergillus* spp. (Shukla and Uniyal 1989), *Penicillium* spp. (Dharmaputra et al. 1989), *Bacillus* spp. and *Actinomycetes* spp. (Abadi 1987), have been investigated and their antagonistic mechanisms against *Ganoderma* have been observed in different culture media. The effectiveness of antagonists in soil can be enhanced under field conditions by fumigation and fertilizer application (Varghese et al. 1975). *Trichoderma koningii* Oud. isolate Marihat (MR14) is one of the most potent antagonists against *Ganoderma* and has been formulated as an active ingredient of biofungicide (Soepena and Purba 1998). The biofungicide contains $5–8 \times 10^6$ conidia and chlamydospores of *T. koningii* per gram of product in a natural medium. Mass production of this antagonist, on oil palm wastes such as oil palm mill effluent and empty fruit bunch, is possible, and this formulation could be applied around the roots of infected oil palms (Singh 1991). Even though much research has been conducted, field application of biological control agents (BCAs) to manage BSR disease is still not a very popular practice amongst planters owing to their lack of effectiveness at controlling disease.

The application of BCAs in rice fields and citrus orchards in Malaysia is considered a new approach. As far as we know, no intensive field application of BCAs to control plant diseases has been reported in Malaysia. However, an effort is under way to describe the microbiomes associated with plants and their impact on plant health and productivity. In rice agro-ecosystems, for example, *Pseudomonas* spp. were reported to produce antimicrobial secondary metabolites such as phenazines (Thomashow and Weller 1988), hydrogen cyanide (Rudrappa et al. 2008), 2,4-diacetylphloroglucinol (Raaijmakers et al. 1997), pyrrolnitrin (Howell and Stipanovic 1979), pyoluteorin (Howell and Stipanovic 1980), cyclic lipopeptides tensin (Nielsen et al. 2000) and viscosinamide (Nielsen et al. 1999), while *Bacillus* spp. were reported to produce antifungal activity compounds such as surfactins (Vitullo et al. 2012) and kurstakins (Bechet et al. 2012). BCAs also help by inducing systemic resistance (ISR) and systemic acquired resistance (SAR) responses through root-to-shoot long-distance intra-plant signaling, priming plants to better resist against pathogen attack (van Loon 2007). In cases of ISR, the response depends on jasmonic acid (JA) and ethylene (ET) compounds. Salicylic acid (SA) signaling is responsible for SAR activity in plant hosts (van der Ent et al. 2009). Both events are triggered by hosts in response to microbial cell components, secretions or volatiles (Ryu et al. 2004) upon being attacked by a pathogen.

2.4 Disease-Resistant Planting Materials

In Malaysia, through the Malaysian Agricultural Research and Development Institute (MARDI), four commercial rice varieties were successfully introduced—MR84, MR185, MR211 and MR219—which are commercially planted in most rice granary areas in peninsular Malaysia. Of these, MR219 is the most successful rice variety because it has high yield potential, up to 10.75 t/ha, and a short maturation period of 105–111 days and it tastes good. This variety was released by MARDI in 2001, and its main characteristic is its resistance to rice blast disease and BLB disease. However, the occurrence of resistance breaking among rice varieties, including MR219, has been reported every year. As a result, research on the development of a new disease-resistant strain of rice is under way, such as the blast-resistant pathotype P7.2 from the F3 population of Pongsu Seribu 2× Mahsuri. This rice line is now being evaluated for phenotypic response against blast disease (Ashkani et al. 2011), and, thanks to advances in science and biotechnology, the process required to develop new disease-resistant varieties can be shortened through the use of molecular markers for rice selection.

Because oil palm is a perennial crop whose culture should last 25–30 years, plant breeders are required to produce good quality planting material by ensuring its authenticity, high yields and resistance to the primary diseases and can advise on the most appropriate material for a given pedoclimatic environment. Thus, there is an urgent need to pursue genetic control of various diseases, focusing on durable resistance, as is the case for vascular wilt, with many years of proven resistance (Cochard et al. 2005). Research on resistant plant material is by far the only effective means of fighting vascular wilt disease of oil palm. Observations in the field and on young plants infected with *Fusarium oxysporum* have indicated significant differences in susceptibility at a very early stage and among different palm strains (Renard et al. 1972), with certain crosses being more tolerant of the disease than other oil palms (Taquet et al. 1985; Renard and Ravisé 1986).

The Quarantine Act of 1976 was developed by the Malaysian government to prevent the introduction of exotic species and new and potentially destructive pathogens into or within the country. However, this act has not yet been implemented with respect to HLB control in Malaysia to limit the movement of infected citrus seedlings. Only strict quarantine measures and restricted movement of citrus bud woods will limit the spread of the HLB pathogen and vector. In addition, under this act, the government can force growers to create buffer zones for their farms, especially in areas where citrus is a major commercial crop. This buffer zone can create a barrier to the movement of HLB vectors. Government, with the help of the Ministry of Agriculture, must develop new procedures, for example, a certification programme at the nursery level. Such a programme would be fundamental to the control of transmissible diseases. When properly implemented, it would provide protection against further spread of graft-transmissible diseases.

3 Enhanced Nutritional Programmes for Disease Suppression and Yield Improvement

Enhanced nutritional programmes (ENPs), which aim to minimize the deleterious effects of HLB disease, have been a hot topic of discussion worldwide (Tian et al. 2014). Gottwald et al. (2012) revealed that ENPs conducted in the US state of Florida do not sustain the tree health, yield, or fruit quality of HLB-infected plants. Similar findings were reported by Razi et al. (2011), who showed no relationship between nutritional deficiency status and HLB incidence in citrus and, in addition, that Zn occurred at significantly higher levels in HLB-infected trees, even though ENPs lead to minor improvements in HLB-infected trees. However, Pustika et al. (2011) showed that the application of foliar fertilizers containing N and minerals leads to a 40 % reduction in the symptom expression of HLB-infected trees. On the other hand, fertilizers applied through soil showed no improvement. This finding demonstrates that foliar application might prolong tree life and reduce yield loss. Studies by Webb (2006) also showed positive effects of ENPs incorporating Zn or Cu ions in combination with Ca; this approach led to a delay in HLB incidence and severity. However, Tian et al. (2014) reported that *Candidatus* Liberib acterasiaticus infection significantly reduced levels of Zn and several other mineral nutrients (Fe, Mn and Cu) of grapefruit trees. This event might be a result of limited remobilization of mineral nutrients in host plants, as observed in the phloem tissues of HLB-affected leaves and stems. Micro-XRF analysis clearly showed a disruption of phloem systems in the stems and leaf petioles of HLB-infected leaves. This study demonstrated a negative correlation between bacterial titers in infected leaves with concentrations of mineral nutrients such as Zn, Fe, Mn and Cu.

In Malaysia, Khairulmazmi et al. (2011) conducted a study on the effect of Ca, Cu and Zn application on plant recovery and severity following infection with HLB disease in Terengganu. In their study, they concluded that the effect of treatments provided minimal protection against the disease. Citrus trees treated with a combination of Ca and Zn at 600 and 10 ppm, respectively, showed a significant increase ($P \leq 0.05$) in terms of fruit production and total soluble solid (TSS) content. This treatment managed to delay disease onset and reduced the area under the disease progress curve value of disease incidence and disease severity, respectively. It also managed to improve the mean leaf lengths, leaf widths and mean leaf areas of treated trees. The improvement observed in the treated trees was probably due to the enhancement of trees' tolerance, as had been reported for other plant-pathogen systems (Oostendorp et al. 2001; Agrios 2005). The application of Ca alone showed no significant protection in these trials, and this application would be considered a complementary treatment in the management of bacterial disease. However, in combination with other micronutrients, such as Zn and Cu, it shows promising results in connection with the suppression of HLB infection. Citrus drip irrigation has been practiced in Sao Paulo, Brazil. This technique allows for the application of fertilizers on the surface of plants through the irrigation system. This system offers advantages such as requiring a lower amount of fertilizer with frequent spray applications and good coverage of fertilizers. This method also increases the efficiency of mineral absorption by plants as a result of the uniform distribution over different phenological stages (Alva et al. 2008). Young citrus trees receive 25–30 fertilizer sprays per year during the first 5 years of citrus cultivation. Quaggio et al. (2006) reported that the efficacy of tropical soils receiving citrus fertilizers increased by up to 25 % with the use of drip irrigation. Therefore, fertigation set up in orchards may offer many benefits by leaching, and it is also recommended for acidic soil, a rather common condition in Malaysia.

Navaratnam and Chee (1965) reported that insufficient K has been associated with increases in upper stem rot (USR) disease incidence in oil palm, although this disease does not have severe outbreaks (Turner 1969). Several field trials were conducted to investigate the association between soil nutrients and BSR disease. Findings showed that the application of K in marine alluvium soil (heavy clay) significantly reduced BSR disease incidence. However, the application of N and P showed no significant effect. However, trials on silty clay mixed with riverine/marine alluvium reported significant reductions in BSR disease incidence with increases in N, while a significant increment of BSR disease incidence was noted when P and K were increased (Singh 1990). Tayeb and Hamdan (1999) reported a decrease in BSR disease incidence when the oil palm is planted on lateritic soil as K and P increase, but no significant effect on N was observed. However, another trial conducted on deep peat soil also reported an increase in BSR disease incidence with increases in N in the soil. Field trials on mineral soils conducted in Sumatera, Indonesia, reported that increases in soil N and P will significantly increase BSR disease incidence, which has been related to a reduction in leaf Cu and Zn but no significant effect when K is increased (Tohiruddin et al. 2010). This study was supported by findings of Nur Sabrina (2011) and Bivi (2013), who showed that a combination of Ca and Cu foliar applications on oil palm seedlings enhanced resistance towards *Ganoderma* infection under glasshouse conditions (Fig. 3).

In rice cultivation, phosphorus (P) is a primary important nutrient for increasing the growth of roots and shoots; it also promotes vigorous seedling growth and affects uniform maturation, thereby increasing yield potential. P fertilizer application is needed to sustain plant growth, including nutrient transport throughout the plant, growth response to the nutrient and its physiological characteristics (Theodorou and Plaxton 1993). The concentration of P is low in the soil owing to fixation, and P is considered the least mobile and available nutrient in soil (Hinsinger 2001). Molybdenum (Mo) fertilizer applied to rice fields can prevent internal molybdenum deficiency symptoms (Kaiser et al. 2005). If the Mo supply in the soil is low, plants become more sensitive to environmental stresses (Cakmak 2000). According to Rahimizadeh et al. (2007), the application of a micronutrient fertilizer in rice fields increases the activities of enzymes in plants. Findings by Liew et al. (2010) showed that the application of K (29.9 kg/ha), Mg (2.4 kg/ha), Zn (4 kg/ha), Cu (4 kg/ha), Mn (3.6 kg/ha) and B (0.25 kg/ha) enhanced rice production by 27 %, from 4.62 to 5.87 t/ha. This field trial was conducted for two seasons at Sawah Sempadan, Tajung Karang, Malaysia. Moreover, the area had been experiencing low rice yields with a mean production of 4.5 t/ha and severe infection of brown

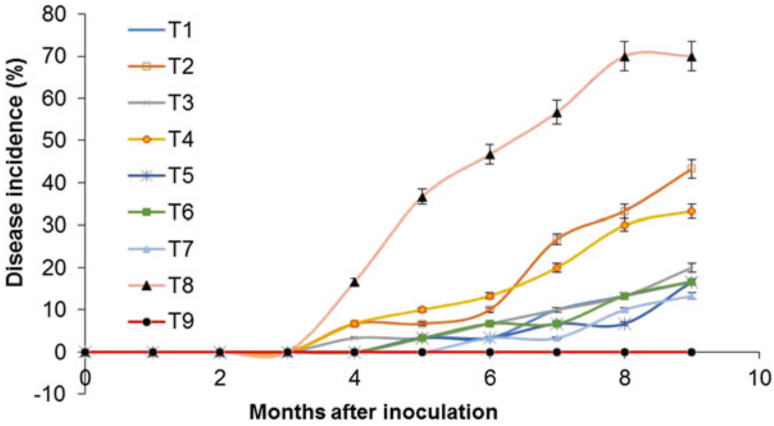

Fig. 3 Effects of Ca^{2+}, Cu^{2+} and SA application on disease development on oil palm seedlings following inoculation with *G. boninense*. Each value is the mean value of ten replicates. *Bars*: standard deviation. T1 (1000 ppm Ca); T2 (50 ppm Cu); T3 (100 ppm SA); T4 (500 ppm Ca/50 ppm Cu); T5 (500 ppm Ca/50 ppm SA); T6 (50 ppm Cu/50 ppm SA); T7 (500 ppm Ca/50 ppm Cu + 50 ppm SA); T8 (positive control); T9 (negative control)

spot and sheath blight diseases. The finding of this study paralleled that of Zayed et al. (2011), in which the application of micronutrients significantly improved the harvest index. A field study conducted at Kelantan, Malaysia, using Zn (5 kg/ha), Cu (4 kg/ha) and Mo (0.5 kg/ha), along with the recommended N, P, K and S at 120 kg/ha, 30 kg/ha, 50 kg/ha and 10 kg/ha, respectively, improved rice plant growth, yield, grain quality and antioxidant activity (Panhwar et al. 2015). The researchers also reported that a combined application of micronutrients, particularly Cu and Zn, would increase phenolic compound and flavonoid content in rice grain by 40 and 71.4 %, respectively. In addition, recent studies have shown the impact of metal ions on the activity of mitochondria cells by inducing oxidative stress on metal binding proteins (Tan et al. 2010).

Silicon (Si) has not been identified as an essential nutrient for rice. However, recent reports have shown that Si could have positive effects on rice, including increased growth and production, improvements in certain morphological characteristics (height, root penetration into the soil, exposure of leaves to light, resistance to lodging), reduced transpiration and resistance to stress, resistance to salinity and toxic metal toxicity, effects on enzyme activity and increased resistance to pathogens (Romero et al. 2011). According to Onodera (1917), supplementation of Si in rice can induce disease resistance to *Magnaporthe grisea*. Ten years later, Kawashima (1927) showed that the application of Si to rice could also increase resistance to *M. grisea*, and the resistance was higher as the concentration of Si applied in the soil increased. A study by Rodrigues et al. (2004) on leaf extracts of rice inoculated with *M. grisea* and fertilized with Si had higher amounts of phytoalexins than rice without Si. These results indicated that a limited production of chitinases could be a defense mechanism of rice plants against *M. grisea*. Plant

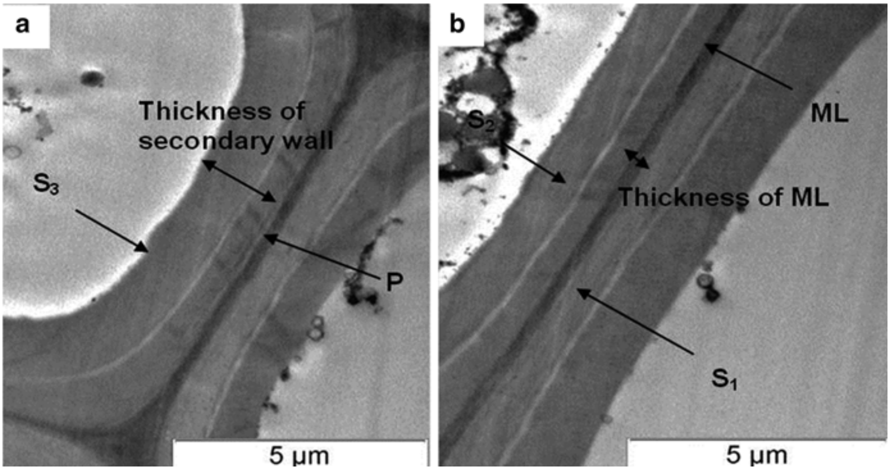

Fig. 4 Transmission electron micrograph of ultra-thin section of oil palm seedling cell wall after being stained with uranyl acetate and lead citrate at a magnification of 6000×. (**a**) Healthy control of oil palm seedling (T9); (**b**) combination of Ca, Cu and SA applied on oil palm seedling. *ML* middle lamellae; *P* primary wall, S_1, S_2 and S_3 secondary wall sublayers

compounds such as phenols and phytoalexins play a crucial role in the defense response, and this finding suggests that Si plays an active role in rice resistance to fungus attack, which is more complex than the formation of a physical barrier in the epidermis of leaves.

3.1 Disease Control Mechanisms of ENPs

3.1.1 Enhanced Lignification

Lignification is a process whereby plant cell walls are sealed by lignin deposition. In this process, lignin deposits accumulate within plant cell walls (Grabber et al. 1998); and this process takes place when the phenolic monomer units polymerize, producing radicals and conjugating them with other monomer radicals. Usually, lignification involves three main phases. Firstly, lignin compounds accumulate around middle lamellae, otherwise known as cellulose microfibrils (CML), and cell corners with deposits of pectate (PEC) and arabinose-galactose rich in hydrocarbons (HCs). Secondly, CMLs and HCs are deposited in secondary cell walls. Lastly, majority of polysaccharides are deposited (Stickler 2008). Lignification provides mechanical strength to the stems, thereby protecting cellulose fibres from chemical injuries and biological degradation (Grabber et al. 1998). According to Bivi (2013), oil palm treated with Ca and Cu can increase the thickness of the secondary cell wall and middle lamellae (Fig. 4a, b) up to 1.64–2.07 µm and 0.21–0.31 µm compared to healthy oil palm, where thicknesses reach 1.47–1.76 µm and 0.15–0.29 µm, respectively. Similarly, Mazza et al. (2000) reported that lignification could restrain the growth of fungus and inhibits fungal proliferation. There are two types of lignin present in plant cell walls, i.e. guaiacyl lignin and guaiacyl–syringl. Guaiacyl lignin is less susceptible to biological degradation and chemical breakdown. On the other hand, guaiacyl–syringl is more resistant to biological degradation.

Lignin, a complex phenylpropanoid-derived polymer, is mainly present in the secondary thickened cell walls of plants and is also the main polymer in middle lamellae between adjacent cell walls (Boerjan et al. 2003). Lignification plays important role as a defense mechanism against fungal attack through plant cell walls. During plant defense responses, lignin or lignin-like phenolic compound accumulation was shown to occur in a variety of plant–microbe interactions (Vance et al. 1980; Nicholson and Hammerschmidt 1992). Lignin, a major component of the cell walls of vascular plants, accumulates in cell wall appositions and surrounding halo areas (Vance et al. 1980; Nicholson and Hammerschmidt 1992; Zeyen et al. 2002) and, thus, is considered a first line plant defense against successful penetration of invasive pathogens.

Copper is an essential nutrient for higher plants as well as fungi and bacteria. However, at high concentrations, Cu is also very toxic to all plant forms. Nevertheless, higher plants, such as crops and ornamental plants, can tolerate much higher Cu levels than microorganisms such as fungi and bacteria. This difference in tolerance enables growers to use Cu as a disease treatment. Copper fertilization has reduced the severity of a wide range of fungal and bacterial diseases. Sandmann and Boger (1980) reported that Cu supplementation produced oxygen reactive species such as H_2O_2 which result in oxidative burst reactions. Moreover, Cu increases the thickness of cuticles and is required for polyphenoloxidase functioning. This event has been observed in treating plants which have been pathogenically attacked and have decreased levels of Cu in their leaves and correspondingly lower levels of lignin in their cell walls (Kopsell and Kopsell 2007). Copper has also been reported to enhance POD in paddy leaves (Fang and Kao 2000) and lignin biosynthesis in soybean roots (Lin et al. 2005).

Calcium is a structural component of cell walls and other plant membranes. It plays a major role in the integrity and function of these structures. A study by Nur Sabrina (2011) provided evidence that Ca and Cu significantly improve lignification in oil palm root. A shortage of Ca results in plant structures which are susceptible to infection by phytopathogens. Cell walls are not only a barrier to infection. When properly functioning, cell walls regulate the passage of sugar and other compounds between cells and other parts of the plant. A low Ca level allows increased transport of sugars from within cells to intercellular spaces in the plant tissue. Higher sugar levels in these areas tend to increase the probabilities of infection and the growth of disease pathogens. A role in the proper structure and function of plant membranes is not the only one played by Ca in reducing the occurrence or severity of diseases. When fungi and bacteria invade plant tissue, they release pectolytic enzymes which dissolve parts of plant tissues. This damages plants and enhances the spread of infection. The activity of these enzymes is suppressed by the calcium ion (Ca^{++}). As the pathogen releases enzymes which dissolve plant tissue, K is lost from the tissue, with the probable loss of the benefits provided by K. Timely spraying of calcium salt have been reported to reduce the incidence of several calcium-related disorders, such as the bitter pit of apples or the internal breakdown of apples (Bangerth 1970), blossom-end rot on tomatoes (Geraldson 1956), the brownheart of escarole, and the blackheart of chicory and celery (Cox et al. 1978). Calcium has also been used to control soil-borne diseases caused by *Pythium*, such as damping-off. The calcium spray has proven effective on several crops including wheat, peanuts, peas, soybeans, peppers, sugar beets, beans, tomatoes, onions and snapdragons (Ko 1988). Researchers in Hawaii reported a reduction in damping-off in cucumbers after amending the soil with calcium and adding alfalfa meal to increase the microbial populations (Ko 1988). In Malaysia, studies have shown that continuous calcium nitrate supplementation can suppress BSR symptoms on oil palm (Sariah et al. 1998; Sariah and Zakaria 2000).

3.1.2 Enhanced Production of Secondary Metabolites

Plants produce the diversity of natural products or secondary metabolites. These compounds play important roles in protecting against predators and microbial pathogens in the communication of plants with other organisms (Schaefer et al. 2009). There are three major groups of secondary metabolites: terpenes, phenolic, and N and S compounds. Phenol is synthesized primarily from the shikimic acid pathway and plays an important defensive role in plants (Rosenthal and Berenbaum 1992). A large number of constitutive plant compounds have been reported to have antifungal activity. Well-known examples include phenols and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides, glucosinolates, 5-alkylated resorcinols and dienes (Schonbeck and Schlosser 1976; Fry and Myers 1981; Bennett and Wallsgrove 1994; Grayer and Harborne 1994; Osbourn 1996; Prusky and Keen 1993).

Copper is associated with enzymes which are involved in redox reactions. Copper is the metal component of three different forms of plant protein, i.e. plastocyanins, involved in electron transfer, peroxidases, which are involved in oxidizing monophenols into diphenols, and, finally, multi-Cu proteins, which serve as oxidases (Sandmann and Boger 1980). Copper-based polyphenoloxidases catalyse oxidation reactions of plant phenols in cell walls involved in the biosynthesis of lignin and formation of brown melanotic substances (Marscher 1995). Moreover, Cu has also been reported to be a precursor for catalysing reactive oxygen species and free radical reactions (Weckx and Clijsters 1996). It also promotes the formation of H_2O_2, which is a very important compound in self-defense mechanisms.

Calcium is a macronutrient for plants, and in a soil environment, exchangeable Ca is the main form of Ca and is easily taken up by roots (Rahman and Punja 2007).

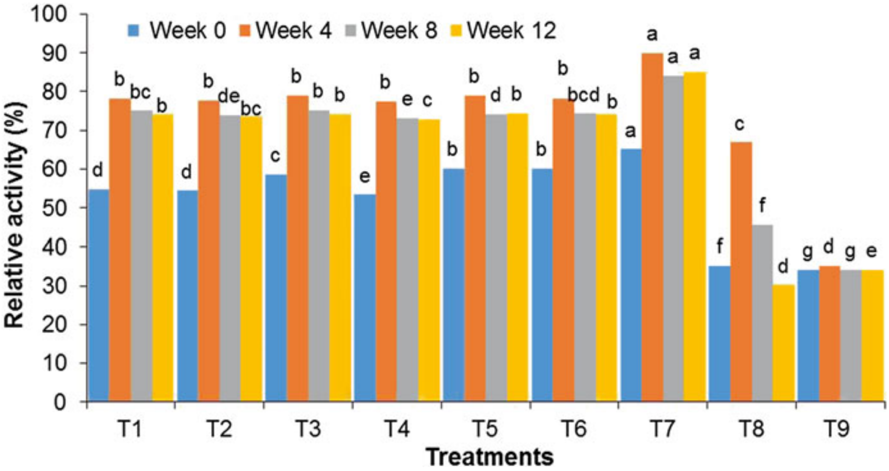

Fig. 5 Results of POD activity in oil palm leaf recorded at 0, 4, 8 and 12 WAI. T1 (1000 ppm Ca); T2 (50 ppm Cu); T3 (100 ppm SA); T4 (500 ppm Ca/50 ppm Cu); T5 (500 ppm Ca/50 ppm SA); T6 (50 ppm Cu/50 ppm SA); T7 (500 ppm Ca/50 ppm Cu/50 ppm SA); T8 (positive control); T9 (negative control). Different *letters* above each *bar* indicate significant differences between means ($P \leq 0.05$) according to Tukey's test

A study by Kolupaev et al. (2005) showed that Ca enhances soluble POD activity. The application of Ca^{2+}, Cu^{2+} significantly increases the production of plant secondary metabolites (Figs. 5 and 6). Studies on the induction of defense mechanisms revealed that a high accumulation of total phenolic content, peroxidase activity, total lignin content and hydrogen peroxide scavenging activity was observed in the leaves of oil palm seedlings compared to the stems and roots (Bivi 2013). These compounds accumulated when the seedlings were treated with Ca and Cu 1 month prior to inoculation with *G. boninense*. A high accumulation of phenolic compounds in the treated seedlings was observed 4 weeks after inoculation (WAI). Similarly, Shraiy and Hegazi (2009) also reported a positive correlation between nutrient application and significant increases in total soluble proteins, phenol, total soluble carbohydrates and sugars in pea (*Pisum sativum* L.) seeds. The direct role of Ca in the biosynthesis of phenols in plants was demonstrated by Castaneda and Perez (1996). In their work, they observed that the foliar application at 10 mM of CaCl increased phenylalanine ammonia-lyase (PAL) activity and the accumulation of phenols, which increased resistance to the fungus *Alternaria alternata* in citrus. Dicko et al. (2006) also reported that Cu increased total phenolic compound (TPC) levels in sorghum by increasing the biosynthesis of phenolic compounds.

Maximum POD activity was observed at high concentrations, especially at the early infection stage, as noted at 4WAI (Bivi 2013). Furthermore, the concentration of POD was also affected by the dosage of mineral nutrients applied, as demonstrated by Nur Sabrina (2011). It is crucial to note that several PODs are linked to the galacturonic domains of pectin in the presence of Ca (Penel and Greppin 1996) and such a link occurs only in pectin chains which are cross-linked to Ca, which are

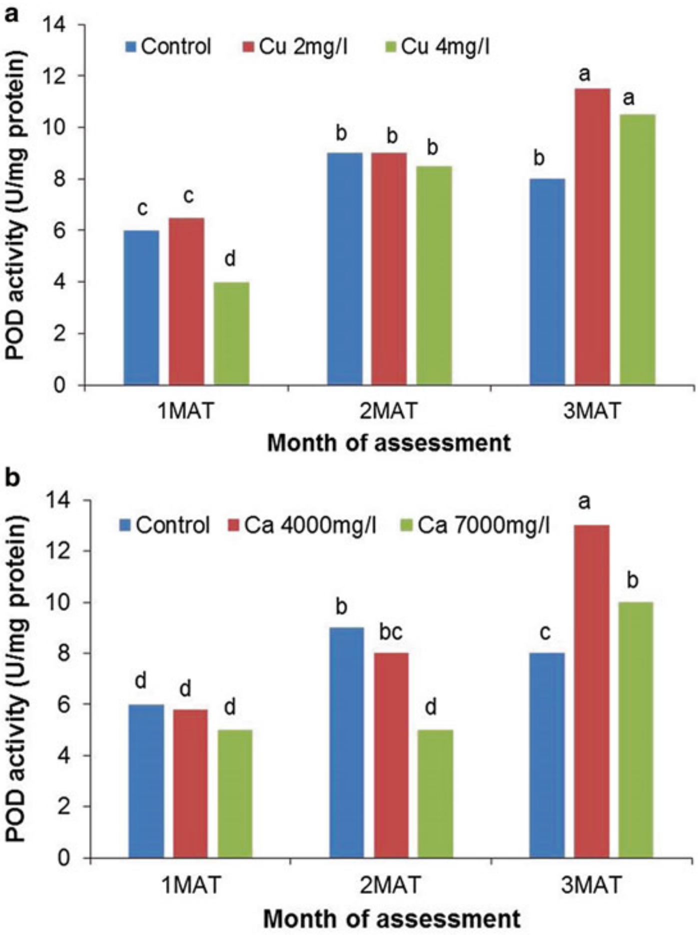

Fig. 6 Effects of Cu (**a**) and Ca (**b**) on activity of POD in oil palm roots

known as Ca-pectates (Carpin et al. 2001). Since the middle lamellae and cell corners are rich in Ca-pectates (Carpita and Gibeaut 1993) and are the first locations to be lignified, PODs linked to these chains of Ca-pectates might act as agents in controlling the spatial deposition of lignin, and changes in the concentrations of Ca would determine the location of these PODs (Carpin et al. 2001). It appears also that Cu has a functional role in terms of POD activity since an amine oxidase-containing Cu that generates H_2O_2 by oxidizing putrescine was co-located with lignin and with POD activity in tracheid elements of xylem in *Arabidopsis* (Møller and McPherson 1998). Plants have developed protective mechanisms to keep these deleterious reactions to a minimum either by anti-oxidative enzymatic defense, which includes peroxidase, polyphenol oxidase, ascorbate peroxidase, catalase and glutathione reductase, or non-enzymatic defense (Chen et al. 1997; Rao 1997).

Table 1 Recommended fertilizer applications for oil palm in South-East Asia

Plant stage	N (kg/ha/year)	P (kg/ha/year)	K (kg/ha/year)	Mg (kg/ha/year)	B (kg/ha/year)	Source
Immature	45	24	108	28	0.6	FAO (2004)
Mature	120	22	286	24	0.6	
Immature	50–120	22–48	54–216	7–24	1.2–3.7	von Uexkull and Fairhurst (1991)
Mature	120–200	30–87	183–581	0–36	2.5–5.6	

4 The Need for ENPs in Long-Term Disease Control Programmes

Mineral nutrition affects the growth and production of crops and causes changes in the pattern of growth, morphology and anatomy, and particularly the chemical composition of plants. Unfortunately, mineral nutrients are not properly managed on plantations. The classic example of a conventional fertilization programme can be observed in oil palm. In mature oil palm fertilizer, the recommendations are based on yearly leaf analyses. In general, fertilizer application, especially NPK, are increased four to five times when the palm reaches full maturity. The dominant fertilizers produced and used on plantations are urea (46 % N); triple superphosphate (TSP, 46 % P_2O_5); rock phosphates (RP, 27–34 % P_2O_5); ammonium sulfate (AS, 21 % N and 24 % S); potassium chloride, also called muriate of potash (KCl or MOP, 60 % K_2O); magnesium sulfate, also called kieserite (17 % Mg, 23 % S); and blended NPK, NP, and PK fertilizers (FAO 2005). In many cases, when it comes to fertilization, programmed application of macronutrients (Table 1) is given top priority compared to micronutrient fertilizers, with the ultimate aim being to maximize production. Due to advancements in new technology and knowledge of the roles of mineral nutrients, conventional fertilization programmes must be revised by focusing on micronutrient fertilization in order to achieve a yield potentialof crop production. In conventional practice, micronutrients like B (100 g/palm/year), $CuSO_4$ (15 g/18 L water) and $ZnSO_4$ (80 g/18 L water) are only recommended when deficiency symptoms are noticed.

Achieving this aim requires a sound understanding of the effects of fertilization on nutrient dosage and nutrient availability in crop tissues. Another important aspect is how the mineral nutrient status might affect pathogen infection, colonization and sporulation. Thus, appropriate modules, workshops and trainings must be developed and implemented by plant pathologists or other responsible agencies to alert plantation managers and farmers about the importance of continuous supplementation of micronutrients (Cu, Zn, B and Si as well as Ca) at appropriate dosages in their fertilization programmes as part of a long-term disease control programme. The existing control measures are not effective at controlling major diseases under field conditions. Furthermore, mineral nutrients have the capacity to induce disease resistance in plants by increasing plant secondary metabolites and enhanced lignication. This approach is also in line with the demands of modern consumers, who are more concerned than ever about food quality and safety. Deep knowledge is needed to develop comprehensive and effective micronutrient application programmes for agricultural crops in the tropics.

5 Conclusions and Future Prospects

The mechanisms of mineral nutrients induce complex and multifaceted changes in disease development as a result of the direct interaction of mineral nutrients with pathogens and plants or indirect effects on plant disease resistance. The effective control of plant diseases requires a holistic approach which encompasses short- and long-term disease control programmes. In most cases, it is rare for diseases in tropical regions to be controlled using a single approach. Future disease control programmes should be regarded as part of an integrated management approach which includes ENPs, water and nutrient management, and cultural, biological and chemical methods. The implementation of ENPs in a long-term control programme is considered an innovative approach because mineral nutrients provide the first line of defense against plant diseases and affect all parts of the disease triangle. Moreover, many researchers around the world have shown that continuous supplementations of Ca, Cu, Si and Zn at the appropriate dosages could enhance crop quality and production and promote effective disease control. In the future, holistic plant disease management must also address the impact of climate change on disease etiology and epidemiology. Changes might occur in many ways, such as in terms of the type and amount of pathogens and diseases of a particular area.

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Effects of Pesticides on Environment

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Contents

Abstract Pesticides are used to kill the pests and insects which attack on crops and harm them. Different kinds of pesticides have been used for crop protection for centuries. Pesticides benefit the crops; however, they also impose a serious negative impact on the environment. Excessive use of pesticides may lead to the destruction of biodiversity. Many birds, aquatic organisms and animals are under the threat of

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harmful pesticides for their survival. Pesticides are a concern for sustainability of environment and global stability. This chapter intends to discuss about pesticides, their types, usefulness and the environmental concerns related to them. Pollution as a result to overuse of pesticides and the long term impact of pesticides on the environment are also discussed in the chapter. Moving towards the end, the chapter discusses the methods to eradicate the use of pesticides and finally it looks forward towards the future impacts of the pesticide use the future of the world after eradicating pesticides.

Keywords Pesticides • Environment • Chronic effects of pesticides • Environmental hazards • Pesticide registration

1 Introduction

A pesticide is a toxic chemical substance or a mixture of substances or biological agents that are intentionally released into the environment in order to avert, deter, control and/or kill and destroy populations of insects, weeds, rodents, fungi or other harmful pests. Pesticides work by attracting, seducing and then destroying or mitigating the pests. Pests can be broadly defined as "*the plants or animals that jeopardize our food*, *health and/or comfort*".

The use of pesticides has increased many folds over the past few decades. According to an estimate, about 5.2 billion pounds of pesticides are used worldwide per year. The use of pesticides for pest mitigation has become a common practice all around the world. Their use is not only restricted to agricultural fields, but they are also employed in homes in the form of sprays, poisons and powders for controlling cockroaches, mosquitoes, rats, fleas, ticks and other harmful bugs. Due to this reason, pesticides are frequently found in our food commodities in addition to their presence in the air (Pesticides n.d.). Pesticides can be natural compounds or they can be synthetically produced. They may belong to any one of the several pesticide classes. Major classes include organochlorines, carbamates, organophosphates, pyrethroids and neonicitinoids to which most of the current and widely used pesticides belong (Pesticides 101-A Primer n.d.). Pesticide formulations contain active ingredients along with inert substances, contaminants and occasionally impurities. Once released into the environment, pesticides break down into substances known as metabolites that are more toxic to active ingredients in some situations (What Is a Pesticide n.d.).

Pesticides promise the effective mitigation of harmful bugs, but unfortunately, the risks associated with their use have surpassed their beneficial effects. Non-selective pesticides kill non-target plants and animals along with the targeted ones. Moreover, with the passage of time, some pests also develop genetic resistance to pesticides. This chapter focuses on the use of pesticides since the ancient times, merits of pesticide usage and most importantly, the harmful impact of pesticides on human health and the environment.

2 Pesticide Use: From Past to Present

The use of pesticides dates back to the times of Ancient Romans where people used to burn sulphur for killing pests and used salts, ashes and bitters for controlling weeds. A Roman naturalist urged the use of arsenic as an insecticide (History of pesticide use 1998).

In 1600s, a mixture of honey and arsenic was used for controlling ants. In late 1800s, farmers in the USA started using certain chemicals such as nicotine sulphate, calcium arsenate and sulphur for field related posts; however; their efforts were unfruitful because of the primitive methods of application (Delaplane 2000). In 1867, an impure form of copper, arsenic was used to control the outbreak of Colorado potato beetle in the USA (History of pesticide use 1998). The major breakthrough in pesticide development occurred in the period around and after World War-II, when several effective and inexpensive pesticides were synthesised and produced. This period is marked by the discovery of Aldrin, dichlorodiphenyltrichloroethane (DDT) in 1939, Dieldrin, β-Benzene Hexachloride (BHC), 2,4-Dichlorophenoxyacetic acid (2,4-D), Chlordane and Endrin (Jabbar and Mallick 1994; Delaplane 2000). A glimpse on the historical account about pesticide use is mentioned in Table 1.

Fungicides, captan and glyodin and organophosphate insecticide Malathion were introduced between 1950 and 1955 followed by the discovery of triazine herbicides in the years 1955–1960 (Jabbar and Mallick 1994). An experimental wartime herbicide named Agent Orange was developed by Monsanto in 1961–1971 and was used during the Vietnam War (History of pesticide use 1998). Moreover, in 1961, the use of pesticides also reached its peak. However, after 1962, there was a marked

Table 1 Historical account of pesticide use

Year	Events
1867	Paris Green (form of copper arsenite) was used to control Colorado potato beetle outbreak
1885	Introduction of a copper mixture by Professor Millardet to control mildew
1892	Potassium dinitro-2-cresylate was produced in Germany
1939	DDT discovered by Swiss chemist Paul Muller; organophosphate insecticides and phenoxyacetic herbicides were discovered
1950s	Fungicides captan and glyodin and insecticide malathion was discovered
1961–1971	Agent Orange was introduced
1972	DDT officially banned
2001	Stockholm Convention

decrease in the development of new pesticides as the public attention was drawn to the environmental hazards associated with indiscriminate pesticide use. In 1962, an American scientist Rachel Carson highlighted in her book, Silent Spring, that spraying DDT in the field causes sudden death of non-target organisms (Jabbar and Mallick 1994; Delaplane 2000) either by direct or indirect toxicity.

Silent Spring resulted in silence in the field of research on pesticide discovery and development. However, in the late 1960s, it opened a new arena in which "integrated pest management" (IPM) was introduced. IPM is a method in which biological predators or parasites are used for controlling the pests. Although the pest population can be reduced to significantly low levels, especially in pest outbreak situations, but unfortunately IPM was not a substitute for chemical pesticides (Delaplane 2000). In 1970–1980s, pyrethroids, sulfonylureas, synthetic fungicides triadimefron and metaxyl were introduced (History of pesticide use 1998). In 1972, DDT was completely banned in the USA followed by the placement of restriction on the use of Endosulfan, Dieldrin and Lindane. The list of banned pesticides has increased ever since. In 2001, 179 nations signed an international treaty known as Stockholm Convention that was intended to completely ban twelve Persistent Organic Pollutants (POP's) including DDT. Later in 2013, the European Union (EU) supported to banning the use of neonicotinoid pesticides (Jacobs n.d.).

It has been observed that the overuse of pesticides on aquatic ecosystems has led to a serious threat to species of fish including salmon. Pesticides are also seen to affect primary producers and macro-invertebrates (Macneale et al. 2010). In Pakistan, before 1980, Plant Protection Department of Government of Pakistan was responsible for the import and distribution of pesticides. Pesticide purchase was on pre-payment basis and there was also subsidy on it. However, in 1980, this responsibility was passed on to the private sector. Since that time, there has been a steady increase in pesticide import and consumption in Pakistan. Registration of a pesticide is renewed sporadically, which ensures the safety of used pesticides (Jabbar and Mallick 1994).

Currently, preference is given to biological control of pests. This is a bioeffector-method of controlling pests using biocontrolling agents including other living organisms. These biocontrolling agents are also known as bio-rational pesticides. An example of bio-rational pesticide is Insect growth regulators (IGRs) which are the hormones that regulate insect growth without affecting non-target organisms (Delaplane 2000).

3 Pesticide Registration and Safety

Registration of a pesticide is a complex, legal and administrative process that takes a considerable amount of time and resources and requires expertise and skills of registration authority as well as pesticide manufacturers. In this process, potential effects associated with the use of pesticide on human health and the environment are assessed (Monaco et al. 2002) in order to ensure the safety of active as well as inert ingredients used in the manufacturing of pesticide.

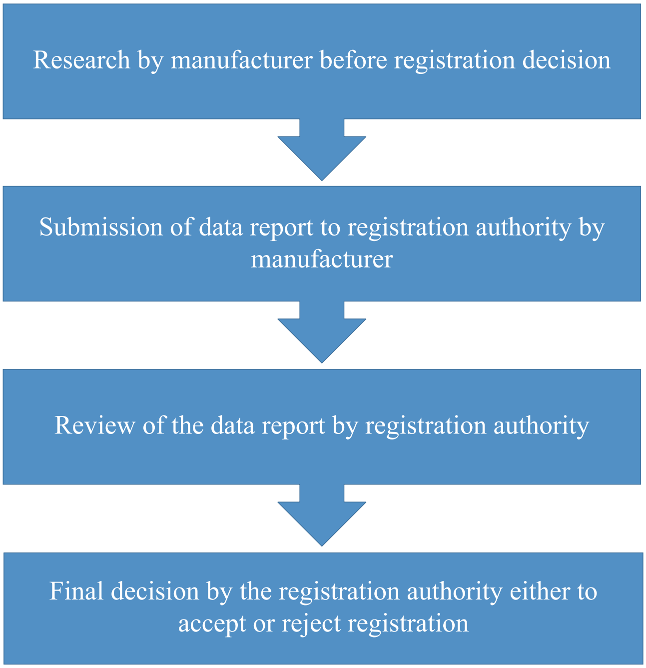


Fig. 1 Pesticide registration process

Registration is an important aspect of pesticide management that ensures that the pesticide product released in the market is authorised and is used only for the intended purpose. It also enables authorities to implement control over quality, price, packaging, labelling, safety as well as advertisement of pesticides to ascertain protection of users' interests (WHO 2010). In the registration process, registrant or the manufacture is required to conduct research and analyse different tests related to product chemistry before submitting the application or data report. These tests gauge the potential pesticide risks on humans, animals and non-target species as well as the fate of the pesticide once it is released in the environment (FAO 2002; WHO 2010). Registration process of pesticides is explained in Fig. 1.

Data report or application of registration include several aspects related to pesticide such as physical and chemical properties of active ingredient as well as formulated product, analytical methods, proposed environmental toxicity and human health hazards, recommended uses and labels, safety data, effectiveness for the intended use, container management, and disposal of waste products. Application is reviewed and analysed by the scientist in registration authority and after environmental, human and biodiversity risks assessment, the authority approves the pesticide as safe to be use or rejects it if it does not meet the standards as set by the regulatory and registration authorities. Furthermore, the registration authority ensures that each registered pesticide continues to meet the highest safety standards. Hence, previously registered pesticides are being reviewed to ensure that they meet current scientific, safety and regulatory standards. This process is called re-registration (Damalas and Eleftherohorinos 2011).

4 Classification of Pesticides

Pesticides are known to be one of the extremely useful and beneficial agents for preventing losses of crops as well as diseases in humans. Based on the action, pesticides can be classified as destroying, repelling and mitigating agents. Insects and pests are getting immune to the commercial pesticides due to over usage. Recently pesticides have been developed which target multiple species (Speck-Planche et al. 2012). Nowadays, chemical pesticides and insecticides are becoming a dominant agent for eliminating pests. When these chemical pesticides are used in a combination of effective natural enemy than that result in enhanced integrated pest management and act as a comprehensive prophylactic and remedial treatment (Gentz et al. 2010).

On the level of population, the effects of pesticides depend on exposure and toxicity, as well as on different factors like life history, characteristics, timing of application, population structure and landscape structure (Schmolke et al. 2010). Nerve targets of insects which are known for development of neuroactive insecticides include acetylcholinesterase for organophosphates and methylcarbamates, nicotinic acetylcholine receptors for neonicotinoids, gamma-aminobutyric acid receptor channel for polychlorocyclohexanes and fiproles and voltage gated sodium channels for pyrethroids and dichlorodiphenyltrichloroethane (Casida and Durkin 2013). It is an observation that the use of neonicotinoid pesticides is increasing. These pesticides are associated with different types of toxicities (Van Djik 2010).

Worldwide pesticides are divided into different categories depending upon their target. Some of these categories include herbicides, insecticides, fungicides, rodenticides, molluscicides, nematicides and plant growth regulators. Non-regulated use of pesticides has led the environment into disastrous consequences. Serious concerns about human health and biodiversity are raising due to overuse of pesticides (Agrawal et al. 2010). Pesticides are considered to be more water soluble, heat stable and polar which makes it very difficult to reduce their lethal nature. Pesticides are not only toxic to people related to agriculture, but they also cause toxicity in industries and public health work places. Depending upon the target species, pesticides can cause toxicities in natural flora, natural fauna and aquatic life (Rashid et al. 2010).

5 Merits of Pesticide Use

Pesticides provide primary as well as secondary benefits. The former ones are obvious after direct usage of pesticides such as the killing of insects that feed on crops. Later are the result of the primary benefits and they are for longer periods.

Worldwide, 40 % of the agricultural produce is lost due to plant diseases, weeds and pests collectively. If there would have been no pesticides, crop losses would have been many folds greater. Moreover, these crop saving substances not only protect the crops from damage rendered by pests, but they also increase the yields of crops considerably (Benefits of Pesticides and Crop Protection Chemicals n.d.). In their study, Webster et al. (1999) indicated that there is a significant increase in crop production due to pesticide usage and stated that economic losses without pesticide use would be much more significant. According to an estimate, yield of bread grains has increased about 10–20 % due to herbicide usage and insect pollinators are responsible for the production of 70 % of the food (What are the benefits n.d.).

As discussed above, crop production would decline if crops are not protected by the disastrous effects of pests. Decline in food production would create food shortage that would ultimately result in increased prices of food commodities (Benefits of Pesticides and Crop Protection Chemicals n.d.). Therefore, pesticides indirectly play a role in keeping the food prices under control.

Many agricultural commodities are vulnerable to attack by aflatoxins and insect control is necessary to prevent the passage of these toxins from insect to plant. Aflatoxin is a carcinogen that can cause liver and other type of cancer in humans, lowers the body’s natural immune response, and can impair growth and development in children. Crop protection chemicals are used to control insect mediated aflatoxin contamination (Benefits of Pesticides and Crop Protection Chemicals n.d.).

Pesticides also prevent disease outbreaks through the control of rodent and insect vectors hence they contribute to improved human health. Deaths of about seven million people all around the world have been prevented through insecticide mediated killing of disease vectors. The most significant example is of malaria control that was responsible for an average of 5000 deaths per day (Ross 2005). Many tick, rodent and insect-borne diseases such as encephalitis, yellow fever, bubonic plague, typhoid fever, typhus, Rocky Mountain spotted fever have been kept in control by the effective use of pesticides (Benefits of Pesticides and Crop Protection Chemicals n.d.; Cunningham n.d.).

Protection of farm and agricultural lands means protection of all forms of life. Pesticides protect forests and other wildlife habitats from invasive species of plants and non-native insects and other pests. Improved agricultural yields help the farmers to produce more food without expanding their agricultural land which consequently protects biodiversity (Benefits of Pesticides and Crop Protection Chemicals n.d.).

Insecticides also improve home sanitary conditions by keeping the population of bugs in control (Delaplane 2000). Moreover, pesticides also preserve the beauty of recreational spots by controlling weeds and also prevent structural damage associated with termite infestations (Benefits of Pesticides and Crop Protection Chemicals n.d.). Moreover, herbicides and insecticides are used to preserve the turf on grounds, pitches and golf course (Aktar et al. 2009).

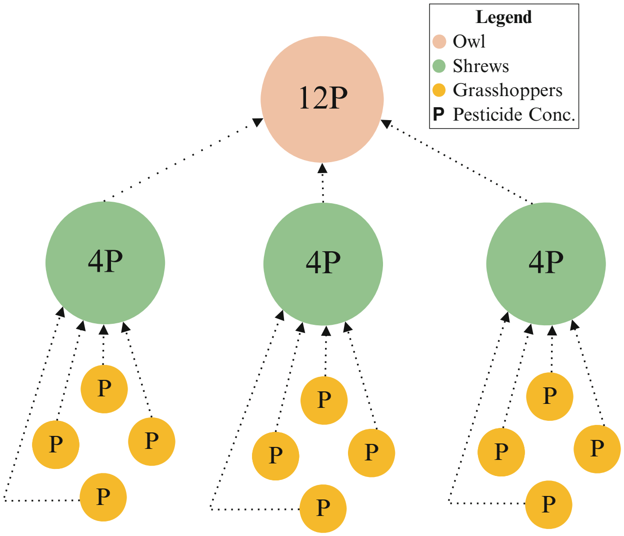
6 Risks Associated with Pesticide Use

Risks associated with pesticide use have surpassed their beneficial effects. Pesticides have drastic effects on non-target species and affect animal and plant biodiversity, aquatic as well as terrestrial food webs and ecosystems. According to Majewski and Capel (1995), about 80–90 % of the applied pesticides can volatilize within a few days of application (Majewski and Capel 1995). It is quite common and likely to take place while using sprayers. The volatilized pesticides evaporate into the air and subsequently may cause harm to non-target organism. A very good example of this is the use of herbicides, which volatilise off the treated plants and the vapours are sufficient to cause severe damage to other plants (Straathoff 1986). Uncontrolled use of pesticides has resulted in reduction of several terrestrial and aquatic animal and plant species. They have also threatened the survival of some rare species such as the bald eagle, peregrine falcon and osprey (Helfrich et al. 2009). Additionally, air, water and soil bodies have also being contaminated with these chemicals to toxic levels.

Among all the categories of pesticides, insecticides are considered to be most toxic whereas fungicides and herbicides are second and third on the toxicity list. Pesticides enter the natural ecosystems by two different means depending upon their solubility. Water soluble pesticides get dissolve in water and enter ground water, streams, rivers and lakes hence causing harm to untargeted species. On the other hand, fat soluble pesticides enter the bodies of animals by a process known as "bioamplification" as shown in Fig. 2. They get absorbed in the fatty tissues of animals hence resulting in persistence of pesticide in food chains for extended periods of time (Warsi n.d.).

The process of bioamplification can be described as follows:

Fig. 2 Bioamplification of pesticide in the environment



1. Small concentration of pesticide enters the bodies of animal that are in low level in the food chain such as grasshopper (primary consumer).
2. Shrews (secondary consumer) eat many grasshoppers and therefore the concentration of pesticide will increase in their bodies.
3. When the high level predator such as owl eats shrews and other prey, the pesticide concentration eventually increases many folds in its body.

Therefore, the higher the trophic level, the greater will be the pesticide concentration which is known as bioamplification. This process disrupts the whole ecosystem as more species in higher trophic levels will die due to greater toxicity in their bodies. This will eventually increase the population of secondary consumers (shrews) and decrease the population of primary consumers (grasshoppers) (Warsi n.d.).

6.1 Threats to Biodiversity

The threats associated with the use of uncontrolled use of these toxins cannot be overlooked. It is the need of the hour to consider the pesticide impact on populations of aquatic and terrestrial plants, animals and birds. Accumulation of pesticides in the food chains is of greatest concern as it directly affects the predators and raptors. But, indirectly, pesticides can also reduce the quantity of weeds, shrubs and insects on which higher orders feed. Spraying of insecticides, herbicides and fungicide have also been linked to declines in the population of rare species of animals and birds. Additionally, their long term and frequent usage lead to bioaccumulation as discussed above (Pesticides reduce biodiversity 2010).

6.1.1 Threats to Aquatic Biodiversity

Pesticides enter the water via drift, by runoff, leaching through the soil or they may be applied directly into surface water in some cases such as for mosquitoes' control. Pesticide-contaminated water poses a great threat to aquatic form of life. It can affect aquatic plants, decrease dissolved oxygen in the water and can cause physiological and behavioural changes in fish populations. In several studies, lawn care pesticides have been found in surface waters and water bodies such as ponds, streams and lakes (How Pesticides Affect the Environment n.d.). Pesticides which are applied to land drift to aquatic ecosystems and there they are toxic to fishes and non-target organisms. These pesticides are not only toxic themselves but also interact with stressors which include harmful algal blooms. With the overuse of pesticides, a decline in populations of different fish species is observed (Scholz et al. 2012).

Aquatic animals are exposed to pesticides in three ways (Helfrich et al. 2009).

- *Dermally*: Direct absorption via skin
- *Breathing*: Uptake via gills during breathing
- *Orally*: Entry via drinking contaminated water

About 80 % of the dissolved oxygen is provided by the aquatic plants and it is necessary for the sustenance of aquatic life. Killing of aquatic plants by the herbicides results in drastically low O_2 levels and ultimately leads to suffocation of fish and reduced fish productivity (Helfrich et al. 2009). Generally, levels of pesticides are much higher in surface waters than groundwater probably because of surface runoff from farmland and contamination by spray drift (Anon 1993). However, pesticides reach underground through seepage of contaminated surface water, improper disposal and accidental spills and leakages (Pesticides in Groundwater 2014).

Aquatic ecosystems are experiencing considerable damage due to drifting of pesticides into the lakes, ponds and rivers. Atrazine is toxic to some fish species and it also indirectly affects the immune system of some amphibians (Forson and Storfer 2006; Rohr et al. 2008). Amphibians are chiefly affected by pesticides contaminated surface waters, in addition to overexploitation and habitat loss (The Asian Amphibian Crisis 2009). Carbaryl has been found toxic for several amphibian species, whereas, herbicide glyphosate is known to cause high mortality of tadpoles and juvenile frogs (Relyea 2005). Small concentrations of malathion have been shown to change the abundance and composition of plankton and periphyton population that consequently affected the growth of frog tadpoles (Relyea and Hoverman 2008). Moreover, chlorpyrifos and endosulfan also cause serious damage to amphibians (Sparling and Feller 2009). Dr. Hayes discovered that 10 % of male frogs raised in atrazine-contaminated water developed into females. Male frogs that were genetically males phenotypically developed ovaries within their testes. They also developed the tendency to mate with other males and lay sustainable eggs (Environmental Impacts n.d.). The reproductive potential of aquatic life forms also reduces due to herbicide spraying near weedy fish nurseries which eventually reduces the amount of shelter that is required by young fish to hide from predators (Helfrich et al. 2009).

6.1.2 Threats to Terrestrial Biodiversity

Pesticide exposure can also cause sub-lethal effects on terrestrial plants in addition to killing non-target plants. Drifting or volatilization of phenoxy herbicides can injure nearby trees and shrubs (Dreistadt et al. 1994). Herbicide glyphosate increases susceptibility of plants to diseases (Brammall and Higgins 1988) and reduces seed quality (Locke et al. 1995). Even low doses of herbicides, sulfonylureas, sulphonamides and imidazolinones have a devastating impact on the productivity of non-target crops, natural plant communities and wildlife (Fletcher et al. 1993).

Pesticides have not even spared the terrestrial animal populations. Populations of beneficial insects such as bees and beetles can significantly decline by the use of broad-spectrum insecticides such as carbamates, organophosphates and pyrethroids. Insect population has also been found to be greater on organic farms compared to non-organic ones. Synergistic effects of pyrethroids and triazole or imidazole fungicides are harmful to honey bees (Pilling and Jepson 2006). Neonicotinoids insecticides such as clothianidin and imidacloprid are toxic to bees. Imidacloprid even at low doses negatively affects bee foraging behaviour (Yang et al. 2008) in addition to reducing learning capacity (Decourtye et al. 2003). The greatest havoc wreaked by neonicotinoids was the sudden disappearing of honey bees at the very start of the twenty-first century. This was a major concern to the food industry as 1/3 of the food production depends on pollination by bees. Honey and wax obtained from commercial hives were reported to contain a mixture of pesticides of which neonicotinoids shared a significant portion. Since 2006, each year, honey bee populations have dropped by 29–36 % (Environmental Impacts n.d.).

Since pre-agricultural times, 20–25 % of the bird populations have declined. One of the major causes of this massive decline is the use of pesticides which was not known before 1962. Pesticide accumulation in the tissues of bird species leads to their death. Bald eagle populations in the USA declined primarily because of exposure to DDT and its metabolites (Liroff 2000). Fungicides can indirectly reduce birds and mammal populations by killing earthworms on which they feed. Granular forms of pesticides are disguised as food grains by birds. Organophosphate insecticides are highly toxic to birds and they are known to have poisoned raptors in the fields. Sublethal quantities of pesticides can affect the nervous system, causing behavioural changes (Pesticides reduce biodiversity 2010).

Pesticides can be applied as liquid sprays on the soil or crop plant, may be incorporated or injected into the soil or applied as granules or as a seed treatment. Once they have reached their target area, pesticides disappear via degradation, dispersion, volatilisation or leaching into surface water and groundwater; they may be taken up by plants or soil organisms or they may stay in the soil (Hayo and Werf 1996). The major concern of pesticide overuse is their leaching into the soil, which affects the microbes residing in it. Soil dwelling microbes help the plants in many different ways, such as nutrient uptake; breakdown of organic matter and increasing soil fertility. But indirectly they are also advantageous to humans as we heavily depend on plants. Unfortunately, pesticide overuse may have drastic consequences and a time may come when we would not have any more of these organisms and soil may degrade.

Several soil microbes are involved in the fixation of atmospheric nitrogen to nitrates. Chlorothalonil and dinitrophenyl fungicides have been shown to disrupt nitrification and de-nitrification bacteria dependent processes (Lang and Cai 2009). The herbicide, triclopyr inhibits soil bacteria involved in the transformation of ammonia into nitrite (Pell et al. 1998). Glyphosate, a non-selective herbicide, reduces the growth and activity of nitrogen-fixing bacteria in soil (Santos and Flores 1995) whereas, 2,4-D inhibits the transformation of ammonia into nitrates carried out by the soil bacteria (Frankenberger et al. 1991).

Herbicides also cause considerable damage to fungal species in soil as pesticides trifluralin and oryzalin both are known to inhibit the growth of symbiotic mycorrhizal fungi (Kelley and South 1978) that help in nutrient uptake. Oxadiazon has been known to reduce the number of fungal spores (Moorman 1989) whereas triclopyr is toxic to certain species of mycorrhizal fungi (Chakravarty and Sidhu 1987).

Earthworms play a significant role in the soil ecosystem by acting as bio-indicators of soil contamination and as models for soil toxicity testing. Earthworms also contribute to soil fertility. Pesticides have not spared earthworms from their toxic effects and the later is exposed to the former mainly via contaminated soil pore water. Schreck et al. (2008) reported that insecticides and/or fungicides produce neurotoxic effects in earthworms and after a long term exposure they are physiologically damaged (Schreck et al. 2008). Glyphosate and chlorpyrifos have deleterious effects on earthworms at the cellular level causing DNA damage. Glyphosates affect feeding activity and viability of earthworms (Casabé et al. 2007). Goulson reviewed the harms of neonicotinoids on environment and animal life. He reported that as neonicotinoids have a tendency to accumulate in the soil, therefore, they can kill earthworms such as *Eisenia foetida* species (Goulson 2013).

7 Pesticide Impact on Human Health

Pesticides have improved the standard of human health by controlling vector-borne diseases, however, their long term and indiscriminate use has resulted in serious health effects. Human beings especially infants and children are highly vulnerable to deleterious effects of pesticides due to the non-specific nature and inadequate application of pesticides. As the pesticide use has increased over the past few decades, the likelihood of exposure to these chemicals has also increased considerably.

According to World Health Organization, each year, about 3,000,000 cases of pesticide poisoning and 220, 000 deaths are reported in developing countries (Lah 2011). About 2.2 million people, mainly belonging to developing countries are at increased risk of exposure to pesticides (Hicks 2013). Besides, some people are more susceptible to the toxic effects of pesticide than others, such as infants, young children, agricultural farm workers and pesticide applicators (Pesticides and Human Health n.d.).

Pesticides enter the human body through ingestion, inhalation or penetration via skin (Spear 1991). But the majority of people get affected via the intake of pesticide contaminated food. After crossing several barriers, they ultimately reach human tissues or storage compartments (Hayo and Werf 1996). Although human bodies have mechanisms for the excretion of toxins, however, in some cases, it retains them through absorption in the circulatory system (Jabbar and Mallick 1994). Toxic effects are produced when the concentration of pesticide in the body increases far more than its initial concentration in the environment (Hayo and Werf 1996).

The effects of pesticides on human health are highly variable. They may appear in days and are immediate in nature or they may take months or years to manifest and hence are called chronic or long-term effects. Acute and chronic effects of pesticide exposure on human health are discussed below.

7.1 Acute Effects of Pesticides

Immediate effects of pesticide exposure include headache, stinging of the eyes and skin, irritation of the nose and throat, skin itching, appearance of the rash and blisters on the skin, dizziness, diarrhoea, abdominal pain, nausea and vomiting, blurred vision, blindness and very rarely death. Acute effects of pesticide exposure are not severe enough for someone to seek medical help (Pesticides and Human Health n.d.).

7.2 Chronic Effects of Pesticides

Chronic effects of pesticides are often lethal and may not appear even for years. These are long term effects that cause damage to multiple body organs. Pesticide exposure for prolonged periods of time results in following consequences:

- Pesticide exposure can cause a range of neurological health effects such as loss of coordination and memory, reduced visual ability and reduced motor signalling (Lah 2011).
- Long-term pesticide exposure damages the immune system (Culliney et al. 1992) and can cause hypersensitivity, asthma and allergies.
- Pesticide residues have been found in the bloodstream of cancer patients compared to normal individuals. Pesticides have been associated with leukaemia, brain cancer, lymphoma, cancer of the breast, prostate, ovaries, and testes (Pesticides and Human Health n.d.).
- The presence of pesticides in the body for a longer time also affects reproductive capabilities by altering the levels of male and female reproductive hormones. Consequently, it results in stillbirth, birth defects, spontaneous abortion and infertility (Pesticides and Human Health n.d.).
- Lon-term exposure to pesticide also damages liver, lungs, kidneys and may cause blood diseases.

Ingestion of organochlorines causes hypersensitivity to light, sound, and touch, dizziness, tremors, seizures, vomiting, nausea, confusion and nervousness (Lah 2011). Exposure to organophosphates and carbamates causes, symptoms similar to those of increased neurotransmitter-acetylcholine. These pesticides interfere with the normal nerve signal transduction and exposure to them causes headaches, dizziness, confusion, nausea and vomiting, muscle and chest pain. Difficulty breathing, convulsions, coma and death may occur in severe cases (Pesticides and Human Health n.d.)

Pyrethroids can cause an allergic skin response, aggressiveness, hyper-excitation, reproductive or developmental effects in addition to causing tremors and seizures (Lah 2011). It is observed that there is a relationship between pesticides and Parkinson's disease and Alzheimer's disease (Casida and Durkin 2013).

8 Conclusion and Future Prospects

Pesticides have proved to be a boon for the farmers as well as people all around the world by increasing agricultural yield and by providing innumerable benefits to society indirectly. But the issue of hazards posed by pesticides to human health and the environment has raised concerns about the safety of pesticides. Although we cannot completely eliminate the hazards associated with pesticide use, but we can circumvent them in one way or the other. Exposure to pesticides and hence the harmful consequences and undesirable effects of this exposure can be minimised by several means such as alternative cropping methods or by using well-maintained spraying equipments. Production of better, safe and environment friendly pesticide formulations could reduce the harmful effects associated with the pesticide usage. If the pesticides are used in appropriate quantities and used only when required or necessary, then pesticide risks can be minimised. Similarly, if a less toxic formulation or low dose of a toxic formulation is used, the havoc can be curbed. As Paracelsus also once said "*The right dose differentiates a poison from a remedy*".

There are organochlorines, which are used as pesticides. These pesticides are least biodegradable and their use is banned in many countries. Besides this fact, organochlorines are highly used in many places. This results in serious health hazards. Water pollution is on the rise due to these pesticides, even at low concentration, these pesticides have serious threat to the environment (Agrawal et al. 2010). The majority of farmers are unaware of the potential toxicities of pesticides. They have no information about types of pesticides, their level of poisoning, hazards and safety measures to be taken before use of those pesticides. Due to this reason, toxic and environmentally persistent chemicals are used to kill pests which can also lead to intentional, incidental or occupational exposure. These compounds have long term effects on human health. Awareness should be arranged for these farmers to reduce the uses of toxic pesticides (Sharma et al. 2012).

In future chemical pesticides can be used in combination with natural treatments and remedies which result in more sustainable elimination of pests and insects. This combination not only promises environmental sustainability, but also has diverse applications in controlling of urban pests and invasive species (Gentz et al. 2010). Pesticides have also posed a serious threat on biological integrity of marine and aquatic ecosystems. It is the need of time to integrate the studies of different disciplines including toxicology, environmental chemistry, population biology, community ecology, conservation biology and landscape ecology to understand direct and indirect effects of pesticides on the environment (Macneale et al. 2010).

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Microbial Composting of Rice Straw for Improved Stability and Bioefficacy

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Abstract Rice is an important cereal crop in the world. Annually, a large amount of straw is produced as by-product from rice cultivation. Proper disposal of rice straw is a concern across the world due to its bulk volume. Composting is an alternative way for recycling of rice straw into a valuable end product for agricultural use. However, composting of rice straw is time consuming as it is composed of lignocellulosic material. Therefore, the aim of this chapter is to summarize the pioneering and recent composting studies and provide information about the uses of potential lignocellulolytic microorganisms in composting as an alternative method for sustainable management of rice straw. In addition, the role of rice straw composts in maintaining of soil health, plant growth promotion and disease suppression as bioenhancer and bioprotectant is discussed. This knowledge could help build a platform for researchers in this area to understand the recent developments in rice straw composting by means of addressing the environmental pollution concerns as well.

Keywords Rice straw • Bioconversion • Lignocellulolytic • Growth enhancer • Bioprotectant

1 Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world, with approximately 87 % currently grown in Asia. Rice is the crop that has shaped the diets, cultures, and economics of billions of Asians. For them, rice is more than food, rice is life. Approximately 120,000 varieties are grown across the world in a wide range of climate, water, and soil conditions (Raboin et al. 2014).

The disposal of rice straw is a problem, as it takes up a large area due to its low bulk density, and harbors pests and diseases. Rice straw cannot be used as animal

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feed due to its low digestibility, high lignin and silica contents which lead to low animal production (Van Soest 2006). Recycling of straw in the field is not feasible because of its slow decomposition rate. In addition, rice straw adds large organic carbon, which leads to net immobilization of nitrogen in soil and the succeeding crops undergo nitrogen deficiency, resulting in lower yield. In Malaysia, a large portion of rice straw is disposed of by open-field burning which causes serious environmental problems. The burning of rice straw emits smoke and dust particles that are harmful to human health, causing asthma and other respiratory problems. It also emits greenhouse gases, namely CO_2, CH_4, and N_2O. Global warming has adverse effects on world climate such as the increase in global temperature, rising water table, melting icebergs, unpredictable weather patterns, and increasing pest infestation and diseases (Gadde et al. 2009; Chang et al. 2013). Attention has to be given to environmentally friendly, nonhazardous, and sustainable methods for proper management of rice straw in a short period of time.

Composting is a promising alternative for the recycling of rice straw (Sanchez-Monedero et al. 2002; Yu et al. 2007; Mishra and Nain 2013; Sharma et al. 2014; Hottle et al. 2015). Composting has long been recognized as one of the environmentally friendly and cost effective alternatives for organic waste recycling (Sanchez-Monedero et al. 2002). Compost is a valuable asset to farmers due to their local availability as a source of multiple plant nutrients (Khaliq et al. 2006). It improves soil characteristics by lowering bulk density, increasing cation exchange capacity, water-holding capacity, soil aeration, buffering capacity, and infiltration rates.

Recent researches have shown that composts suppress plant diseases caused by soil-borne pathogens (Yu et al. 2015; Wei et al. 2015). Composts suppress phytopathogens through various complex biological and physiochemical characteristics (Wei et al. 2015; Ullah et al. 2015). The physiochemical properties reduce disease severity by affecting the growth of pathogen or host plant, while the biological characteristics include the antibiotic production, lytic and other extracellular enzyme production, induction of host-mediated resistance in plants, competition, parasitism, and predation, and other interactions between beneficial microorganisms and pathogens that decrease the disease incidence. Compost sterilization reduces or eliminates disease suppressiveness and colonization by the diverse range of microorganisms resulted in enhanced suppressiveness of diseases (Reuveni et al. 2002; Noble and Roberts 2004; Yogev et al. 2006; Faheem et al. 2015).

One of the imperative aspects of compost application is the degree of maturity and stability. Immature compost may produce phytotoxic effects or enhance anaerobic conditions. Maturity refers to the degradation of phytotoxic compounds produced during the early phases of composting and the proportion of stable humus in compost (Wu et al. 2000; Makan 2015). An optimum level of maturity is attained when compost is stable, but active enough to sustain microbial activity when applying as a biocontrol agent for the control of phytopathogen. Compost maturity and stability are also influenced by the structure and composition of organic materials, and the potential of microbes which decomposed the macromolecules in the substrates.

Phytotoxic compounds are accumulated during composting of lignocellulosic rice straw as it decomposes slowly (Jurado et al. 2015). However, humification process is fed by the intermediate metabolites generated from the bioprocess (Perez et al. 2002). Hence, the successes of composting as well as the usefulness of compost as an organic amendment are highly dependent on the ability of microorganisms. Though, the natural microbial population in rice straw can perform the composting, the inoculation with lignocellulolytic microorganisms could be a strategy that perhaps enhances the bioprocess (Elorrieta et al. 2002; Jurado et al. 2015). In addition, composts need to be colonized by a specific antagonist during the composting process to prepare specific disease suppressive composts (Blaya et al. 2013). As, for example, inoculation of compost with fungal antagonists *Trichoderma viride* gave fruitful results in suppressing *Sclerotium* root rot in chilli (Kausar et al. 2014).

The above information implies that composting of rice straw through inoculation with lignocellulolytic antagonists at optimum conditions might be a promising technique for producing disease suppressive compost in a short period of time. The composting process of rice straw inoculated with lignocellulolytic bioenhancer and its uses for crops have not been widely investigated. Therefore, in the present chapter we integrate different methods of microbial composting of lignocellulosic rice straw and their efficacy in enhancing plant growth and disease suppression as well as in maintaining soil fertility.

2 Production and Properties of Rice Straw

2.1 Rice Straw Biomass

Global rice production was 741.3 million tons in 2014 (USDA 2015). Approximately ~1.5 t straw remains in the field as residue for every ton of harvested grain. Thus, nearly 740–1110 million tons of straw are accumulated annually as a by-product.

Table 1 Rice production of ten leading rice producing countries in the world in 2013 (Statista 2014)

Number	Country	Rice production (Million metric tons)
1	China	203.61
2	India	159.2
3	Indonesia	71.28
4	Bangladesh	51.5
5	Vietnam	44.04
6	Thailand	36.06
7	Myanmar	28.77
8	Philippines	18.44
9	Brazil	11.78
10	Japan	10.76

Table 2 Chemical composition of rice straw (Garay et al. 2014; Kausar et al. 2010; Liu et al. 2013)

Parameters	Rice straw
Cellulose (%)	42–49
Hemicelluloses (%)	23–34
Lignin (%)	11–16
Ash (%)	15–20
Silica (%)	9–14

The world's leading rice producing country is China followed by India. A list of 10-top leading rice producing countries is presented in Table 1.

2.2 Properties of Rice Straw

Rice straw is a complex and highly heterogeneous lignocellulosic material consisting of nodes, internodes, leaves and chaff. It contains three major components, namely cellulose, hemicelluloses, and lignin (Table 2). Cellulose and hemicelluloses are nonlinear and lignin is a three-dimensional polymer (Perez et al. 2002). Cellulose is surrounded by a matrix of hemicelluloses and lignin.

2.2.1 Cellulose

In nature, cellulose is the most abundant linear biopolymer. It comprises approximately 35–45 % dry weight of rice straw (Lynd et al. 2002). It acts as structural and energy-storage components and provides rigidity to the cell wall. In cellulose, glucose unit linked by β-1, 4-glycosidic bonds. Its degree of polymerization can be up to 15,000 units. Each repeating glucose unit is rotated 180° relative to its neighbors. It is classified according to different intermolecular hydrogen bonding patterns as α (insoluble in 17.5 % NaOH) and β (soluble in 17.5 % NaOH) cellulose (Kuhad et al. 1997).

2.2.2 Hemicelluloses

Hemicelluloses are the second largest natural biopolymer after cellulose. It comprises of over 30 % of dry matter in rice straw. It is a branched biopolymer of low molecular weight sugar where the degree of polymerization ranges from 80 to 200 units. Hemicelluloses consist of different sugar units such as xylose, arabinose, glucose, galactose, mannose, rhamnose, fructose, and various methylated neutral sugars. It is amorphous in nature and degraded more easily than cellulose (Perez et al. 2002). Naturally, it remains chemically associated or cross-linked to other biopolymers such as cellulose, lignin, proteins, and pectin. Besides, hemicelluloses form a matrix in primary cell wall together with pectin and protein as well as with lignin in secondary cell wall of rice plants (Hammel 1997).

2.2.3 Lignin

Lignin is the most abundant aromatic biopolymer in the biosphere. It ranges from 5 to 30 % of plant dry weight in lignocellulosic materials (Lynd et al. 2002). It is a high-molecular mass, water-insoluble, three-dimensional compound consisting of phenylpropane-based monomeric units. Its complicated structure, high molecular weight, and non-hydrolyzable bonds make lignin highly resistant to biodegradation. Oxidative enzymes catalyze the biodegradation of lignin. Lignin provides mechanical support, strengthens the cell in vascular tissues, and protects cellulose and hemicelluloses from biodegradation by reducing the surface area available to enzymatic attack. It also plays a role as an antioxidant, as a water-proofing agent ,and as a UV stabilizer (Duval and Lawoko 2014).

2.2.4 Other Cell Wall Components

Besides cellulose, hemicelluloses, and lignin, rice straw also contains silica, terpenes, resins, phenols, low molecular weight carbohydrates, gums, alkaloids, and other chemicals. Carbonates, oxalates, fat, starch, pectin, protein, and various other cytoplasmic constituents are found in the cell wall of straw. These extraneous materials provide a shield against the biodegradation of straw (Kuhad et al. 1997; Lee et al. 2015).

3 Biodegradation of Lignocellulosic Materials in Rice Straw

3.1 Biodegradation of Cellulose

A large number of microorganisms produce cellulolytic enzymes on lignocellulosic materials. Both cellulolytic and non-cellulolytic microorganisms establish synergistic relationship to break down the cellulose during the biodegradation of lignocellulosic materials. The biodegradation of cellulose requires the production of either free or cell-associated extracellular cellulases. The biochemical analyses of cellulose systems from aerobic and anaerobic microorganisms performed during the past two decades have revealed that multiple enzymatic activities are required to hydrolyze cellulose into soluble sugar monomers (Zhang and Lynd 2004; van Zyl et al. 2007; Hasunuma et al. 2013). Three major cellulase enzymes take part during the biodegradation of cellulose. These include endo-1,4-β-D-glucanase, cellobiohydrolase (exo-1,4-β-D-glucanase) and 1,4-β-D-glucosidase. Endoglucanase randomly cleaves the glycosidic bonds of internal amorphous regions in cellulose to produce oligosaccharides of various degrees of polymerization and generate new chain ends. Cellobiohydrolase acts on the non-reducing end of the cleaved cellulose chain and removes cellobiose units from cellulose chains. Finally β-glucosidase acts on cellobiose and converts it into glucose units. The correct combination of the activities and production level of each cellulase enzyme is critical for efficient cellulose bioconversion (Chandel et al. 2012).

3.2 Biodegradation of Hemicelluloses

Hemicelluloses are a heterogenous group of branched and linear polysaccharides that are bound via hydrogen bonds to the cellulose microfibrils in the plant cell wall. They are covalently attached to lignin, forming a highly complex structure together with cellulose. Hemicelluloses require the synergistic action of hemicellulases enzymes for its complete degradation. Hemicellulases are modular proteins, in addition to their catalytic domains, include other functional modules. The most important modules are carbohydrate-binding modules, which facilitate the targeting of the enzymes to the insoluble polysaccharides, and dockerin modules that mediate the binding of the catalytic domains via cohesin-dockerin interactions, either to the microbial cell surface or to large enzymatic complexes (Bourne and Henrissat 2001; Shallom and Shoham 2003). The catalytic modules of hemicellulases are either glycoside hydrolases that hydrolyze glycosidic bonds and carbohydrate esterases, which hydrolyze ester linkages of acetate or ferulic acid side groups. Xylanases are the best studied hemicellulase enzymes. Endoxylanases and xylosidases found in *Trichoderma* spp. and *Aspergillus* spp. can completely breakdown xylan polymers. Endoxylanases cleave the backbone of xylan into smaller oligosaccharide xylobiose, which is further broken down to xylose by xylosidases (Malherbe and Cloete 2002).

3.3 Biodegradation of Lignin

Lignin-degrading mechanisms are extracellular and unspecific as lignin is a large and highly branched biopolymer. Oxidative enzymes cleave stable ether and carbon–carbon bonds in lignin (Yang et al. 2013). The most important lignin-modifying enzymes are lignin peroxidases, manganese peroxidases, functional hybrids of both enzymes (versatile peroxidases VP) and laccases (phenol oxidases). All extracellular peroxidases and laccases catalyze oxidation reactions resulting in the formation of radicals that initiate several spontaneous reactions. These enzymes use low molecular mass mediators during lignin biodegradation which cleave various bond cleavages including aromatic ring fission (Kirk and Farrell 1987; Zeng et al. 2013) in lignocellulosic materials.

4 Composting of Rice Straw

Composting is the bioconversion of organic materials under moist, self heating, and aerobic conditions. It is characterized by a series of different microbial populations. There are a few main factors affect the composting process: temperature, C/N ratio, aeration, moisture content, porosity, and pH (Table 3). Temperature, pH, and nutrients change constantly during composting (Ryckeboer et al. 2003). It reduces the bulk volume of organic materials, destroys weed seeds and pathogenic microorganisms in the end product (Bernal et al. 2009). Typically composting results in a 25–35 % weight reduction of the starting materials. This weight loss is due to the liberation of CO_2 and H_2O by microbial activity (Fig. 1).

Composting is different from natural rotting. Natural rotting occurs in an unmanaged waste pile, sanitary landfill and/or open dump. However, composting is a controlled biochemical process. Different microbial populations mainly bacteria, actinobacteria, and fungi convert organic materials into humus-like substances during bioprocess. Microorganisms need food and energy during bioprocess. They use carbon as an energy source and nitrogen to build up cell structure, proteins, enzymes, and hormones. They take their necessary foods and nutrients from com-

Table 3 Factors affecting the composting of rice straw (Shafawati and Siddiquee 2013; Malińska and Zabochnicka-Świątek 2013)

Parameters	Reasonable range	Preferred range
Temperature (°C)	42–68	55–60
Carbon to nitrogen ratio (C:N)	20.1–30.1	25.1–30.1
Aeration (% of oxygen)	>5.0	>5.0
Moisture content (%)	45–70	50–60
Porosity (%)	30–60	30–36
pH	5.5–8.0	6.5–7.5
Particle size (diameter—cm)	0.5–5.0	0.5–2.5

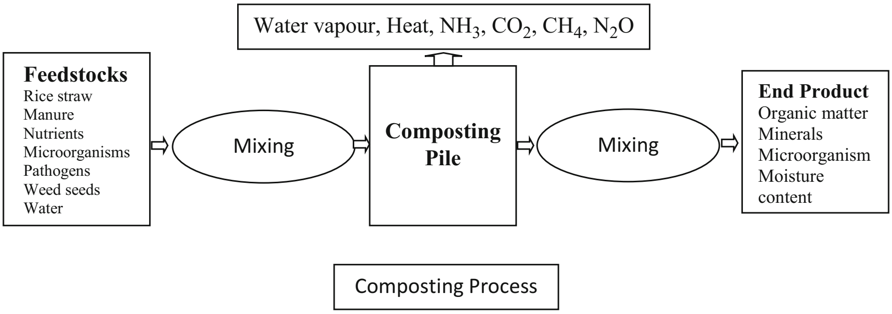

Fig. 1 Conventional composting process (British Columbia Agricultural Composting Handbook 1998)

plex organic substances. Nutrients released during the composting process remain in the compost as humus and the dead bodies of microorganisms (Zainudin et al. 2013; Qian et al. 2014; Vázquez et al. 2015).

Lignin shields cellulose, hemicelluloses, and other cell wall components in rice straw. Only a few microorganisms can cleave the lignin barrier. Lignocellulolytic fungi have an advantage in solid state bioconversion as they are filamentous and produce prolific spores. Mixed microbial cultures have higher influence on substrate colonization through resistance to contamination and increased enzyme production. Strain compatibility is another important determinants in mixed microbial consortium that influences the density, distribution, organization, and ecological balance of communities (Martínez-Sanz et al. 2014; Mishra and Malik 2014; Vázquez et al. 2015). Thus, a compatible microbial consortium perhaps plays an important role in the rapid bioconversion of rice straw.

Composting of rice straw with sewage sludge was evaluated in static piles with passive aeration for 90 days. Compost piles prepared with shredded rice straw reached the maximum temperatures remained above 55 °C revealed that rice straw and sewage sludge were compatible and shredding of straw was necessary to prepare a good blend for composting of these wastes and to guarantee quality compost in sanitation terms (Roca-Pérez et al. 2009).

Rice straw with different organic amendments and lignocellulolytic actinomycete strains of the genera *Micromonospora*, *Streptomyces*, and *Nocardioides* were composted for 3 months under aerobic condition. Results showed that thermally treated municipal sludge and actinomycetes accelerated the composting where bulk volume was reduced by 38.6–64 %, after 3 months, compared to 13.6 % in uninoculated control (Abdulla 2007). In another study, Mishra and Nain (2013) documented composting of rice straw amended with poultry manure or urea co-inoculation of *Cellulomonas cellulans* and *Phanerochaete chrysosporium* in perforated cemented pits for 3 months. Microbial activities reached the highest after second month of composting. After 3 months, the carbon content decreased, but nitrogen content increased. In addition, pH and EC of the end product was found to be within the desirable limits for agricultural use at the end of 3 months of composting.

A fungal consortium comprising of *A. niger* and *T. viride* was found to decompose rice straw amended with chicken manure significantly over control treatment where the C/N ratio was reduced to 19.5 from an initial value of 29.3 in 3 weeks of composting (Kausar et al. 2010, 2013). In another study, Raut et al. (2009) found that municipal solid waste amended with *P. chrysosporium* and *T. reesei* was composted within 9–12 days as indicated by the reduction of C/N ratio and enzyme activities.

A study was conducted to monitor the chemical changes during composting of rice straw and cattle dung, biogas slurry and a consortium of *A. awamorii*, *Paecilomyces fusisporus*, and *T. viride*. At day 90, maximum 17.4 %, dropping in organic C was observed in the treatment containing fungal consortium where C:N ratio of compostable material reduced from 73.7 to 16.6 %. At day 30, cellulase activity was increased from 88 to 252 mg reducing sugar kg^{-1} dry matter h^{-1}, xylanase activity was from 9 to 111 mg reducing sugar kg^{-1} dry matter h^{-1} in microbial amended treatment. Total humic substances were 121 mg g^{-1} and 127 mg g^{-1} compost in finished product amended with fungal consortium and cattle dung, respectively. Carbon dioxide evolution in finished product in cattle dung and fungal consortium amended treatment was 188 mg 100 g^{-1} and 174 mg 100 g^{-1} compost, respectively. About 81–87 % seeds of wheat and 78–86 % seeds of mustard were germinated in compost extract amended with fungal consortium and cattle dung showing their potentiality to be used in the composting of rice straw at large scale (Goyal and Sindhu 2011).

5 Role of Rice Straw Compost in Soil Health, Plant Growth, and Disease Suppression

Composts have been shown to improve soil organic matter, content, resistance against soil erosion, water holding capacity and the subsequent mineralization of soil, plant nutrients (Puppala et al. 2007; Hejduk et al. 2012). It increases soil fertility and contains plant growth promoting substances, e.g., vitamins, hormones, enzymes that enhance plant growth and development (Gutierrez-Miceli et al. 2007; Pramanik et al. 2007; Zaller 2007; Ievinsh 2011; Papathanasiou et al. 2012; Zhang et al. 2012). Composts promote plant root elongation and density, which improves soil aggregation (Daynes et al. 2013). The incorporation of composts in soil improved the retention of nutrients, including magnesium, copper, and iron as well as of nitrogen, phosphorus, potassium, and sequestered carbon (C) (Lehmann et al. 2003; Barrow 2012; Cheng et al. 2012; Borchard et al. 2012; Clough and Condron 2010; Clough et al. 2013; Farrell et al. 2014).

Composts have been used in controlling soil-borne pathogens for a long time now. Composts suppress soil borne diseases by complex interactions between biotic and abiotic factors (Borrero et al. 2004; Litterick et al. 2004; Rotenberg et al. 2007). Composts increase labile carbon pools and soil microbial activities in soils. The disease suppressive potential of composts depends on the level of maturity and the presence of antagonists (Scheuerell et al. 2005). Mature composts sustain biocontrol agents by providing all essential nutrients. On the other hand, immature composts do not support biocontrol agents. They contain pathogenic populations and negatively affect plant growth (Litterick et al. 2004; Trillas et al. 2006).

Composts induce plant disease resistance by increasing the biocontrol agents in the rhizosphere. Plant resistance is induced when biocontrol agents cross the certain threshold size in the rhizosphere. Once resistance is induced the populations may decline without affecting the plant resistance. Composts containing biocontrol agents including *Penicillium*, *Trichoderma*, *Aspergillus*, *Gliocladium*, and *Paenibacillus* antagonize the causal organism of damping-off, stem and root collar rot. The interactions in between the saprophytic microbes and the pathogens and/or the systemic and local resistance of composts are involved in this effect (Kavroulakis et al. 2005; Suárez-Estrella et al. 2007). Composts increase the resistance in chilli, tomato, cucumber, wheat, and barley against *Fusarium* wilt, *Pythium* root rot, anthracnose, and powdery mildew (Lashari et al. 2013; Cao et al. 2014; Verma et al. 2015; Yu et al. 2015).

Rice straw compost rich is in nitrogen, potassium and silicon (Belal and El-Mahrouk 2010). It enhances plant growth, development, and disease suppression in chilli cultivation (Siddiqui et al. 2008; Dukare et al. 2011; Kausar et al. 2014). Rice straw composts were used for chilli cultivation under glasshouse condition. Chilli seeds cv. Kulai were sowed in *Sclerotium rolfsii* challenged soil where microbial infused straw compost increased seed germination, seedling establishment, plant growth and suppressed development of foot rot disease compared to using commercial compost and fungicide Benomyl (Table 4). Use of 15 Mg ha^{-1} microbial infused rice straw compost yielded optimum seed germination (98.1 %), seedling establishment (96.8 %), and disease suppression (84.6 %) (Fig. 2).

Microbial fortified rice straw compost was applied with *Pyricularia oryzae* challenged inoculation at 14, 56, and 80 days after sowing for plant growth promotion, resistance, induction, and yield increment on rice variety M4 under greenhouse conditions. Microbe amended compost significantly increased plant biomass and productivity. Productive tiller number ($r=0.96$), leaf area index ($r=0.96$), area under disease progress curve ($r=-0.62$), and infected panicle ($r=-0.59$) were highly correlated with rice yield with *P. oryzae* inoculation at 80 days after sowing. Low productivity was found with *P. oryzae* infection at the later growth stage due to increase in panicle blast that caused deterioration of grain quality and resulting in severe yield loss (30.99 %) as compared to early infection at 14 days after sowing (Ng et al. 2012).

Siddiqui et al. (2008) compared the efficacy of *Trichoderma* fortified rice straw and empty fruit bunch of oil palm compost extracts on occurrence and morphophysiological growth of Choanephora wet rot of okra. They found shoot and tap root length, leaves per plant, and leaf area were significantly higher in

Table 4 Effect of rice straw compost on seed germination, seedling establishment, and dry matter accumulation on chilli in *Sclerotia rolfsii* infested and non-infested soil

	Seed germination (%)		Seedling establishment (%)		Dry weight	
Treatment	Non-infested	Infested	Non-infested	Infested	Non-infested	Infested
T1	88.1 d	23.1 d	85.0 c	16.2 d	0.8 d	0.4 d
T2	91.8 cd	26.8 d	89.3 bc	19.3 d	1.0 d	0.6 d
T3	94.3 ac	87.5 b	92.5 ab	84.3 b	3.0 b	1.7 b
T4	98.1 a	94.3 a	96.8 a	92.5 a	4.5 a	2.8 a
T5	93.1 bc	81.2 c	90.0 bc	75.0 c	2.5 bc	1.3 c
T6	95.6 ac	88.7 b	91.8 ac	83.7 b	3.4 b	2.2 b
T7	96.8 ab	93.7 a	94.3 ab	91.8 a	3.2 b	2.0 b

T1 = Untreated soil (control); T2 = Soil + basal fertilizer; T3 = Soil + basal fertilizer + 7.5 t/ha *microbial infused* rice straw compost; T4 = Soil + basal fertilizer + 15 t/ha *microbial infused* rice straw compost; T5 = Soil + basal fertilizer + 7.5 t/ha Best Flora compost (commercial); T6 = Soil + basal fertilizer + 15 t/ha Best Flora compost (commercial); T7 = Soil + basal fertilizer + Benomyl @ 0.55 kg/ha

Means within columns followed by the same letter are not significantly different, 5 % level of probability, least significant difference (LSD) test

Fig. 2 Effect of microbial infused rice straw compost on plant growth and disease incidence on chilli in *Sclerotia rolfsii* infested soil. (**a**) Chilli plants treated with 15 t/ha microbial infused rice straw compost; (**b**) Plants in control treatment; (**c**) Single plant from the treatment treated with 15 t/ha microbial infused rice straw compost; and (**d**) Single control treatment

rice straw compost extract treated plants than that of empty fruit bunch compost extract. Similarly, net photosynthetic rate and chlorophyll content were also higher in plant receiving Trichoderma-enriched straw compost extract with a 76.2 % reduction in Choanephora wet rot incidence compared with rest of the treatments.

Man and Ha (2006) found that rice straw compost in combination with 50 % NPK fertilizer increased yield of rice from 26.98 to 37.04 % in the dry season and from 33.45 to 48.08 % in wet season. They also found that after compost application pH value was from 4.60 to 6.74 in dry soil and from 6.38 to 6.83 in wet soil where pH was not toxic to plant growth.

Rice straw composts amended with rock phosphate and *A. niger*, *T. viride* and/or farmyard manure were applied as organic phosphate fertilizers on cowpea plants in pot experiments. All types of rice straw fertilizers were better than superphosphate fertilizer in providing the cowpea plants with phosphorus. *A. niger* and *T. vir*ide inoculated rice straw composts provided the maximum amount of soluble phosphorus (1000 ppm). Cowpea plants receiving compost inoculated with farmyard manure, *A. niger* and *T. viride* resulted in maximum amount of phosphorus uptake (295 ppm). The highest numbers of phosphate dissolving fungi were found in rhizosphere soil treated with *A. niger* and *T. viride* composts, while the highest phosphate dissolving bacterial numbers were found in soil receiving farmyard manure and rice straw compost (Zayed and Abdel-Motaal 2005).

Composting of rice straw with poultry manure and oilseed rape cake and its effects on growth and yield of faba bean and soil properties was studied in pot experiments at Gifu University, Japan in 2001–2002. Compost was rich in organic matter and mineral nutrients with higher level of stability. The use of compost (20–200 g pot-1) increased total N, total C and CEC, decreased particle density and increased soil respiration rate. Application of compost at a rate of 20 g/pot significantly increased growth, yield, yield components, and total crude protein of faba bean (Abdelhamid et al. 2004).

6 Mechanisms of Disease Suppression

Composts serve as a potential alternative to chemical fungicides in controlling plant diseases. The biocontrol agents, metabolites, plant nutrients, and humic acids present in compost suppress diseases. The biocontrol agents compete for infection sites with the pathogens. They leave little spaces for pathogens to proliferate or to secrete secondary metabolites on the plant surface. They also directly parasitize plant pathogens (Bernard et al. 2012; Daguerre et al. 2014), produce different antibiotics which suppress plant pathogens and enhance natural plant defense responses (Souleymane et al. 2010).

In general, biocontrol mechanisms of composts are grouped into two classes. These include general and specific suppression. The biocontrol agents in composts induce the general suppression of phytopathogens such as *Pythium* and *Phytophthora* (Shen et al. 2013; Mehta et al. 2014). Propagules of these pathogens do not germinate in compost amended substrates due to the metabolic activity of biocontrol agents (Dukare et al. 2011; Cray et al. 2015). On the other hand, *Rhizoctonia* spp. which produce sclerotia are not controlled by the general suppression phenomenon. To control damping-off caused by *Rhizoctonia* spp. the presence of specific antagonists such as *Trichoderma* spp. is required. This type of biocontrol is termed as specific suppression (Hoitink and Boehm 1999; Trillas et al. 2006; Olson and Michael Benson 2007).

The antagonistic potential of microorganisms is based on four basic principles: competition for space and nutrients, direct parasitism, antibiosis, and the induction of systemic resistance in host plants. Compost nutrients serve an indirect role with the production of antibiotics, siderophores in phyllosphere or rhizosphere giving fungistatic or fungistasis effect on pathogens (Termorshuizen and Jeger 2008; Bonanomi et al. 2013). Biocontrol agents including bacteria (*Bacillus*, *Pseudomonads*), actinobacteria (*Streptomyces*, *Micromonospora*), and fungi (*Trichoderma*, *Gliocladium*) induce these mechanisms during plant disease suppression.

Fluorescent *Pseudomonads* are the most frequently used rhizobacteria which suppress the growth of pathogenic rhizosphere microflora (Singh et al. 2011; Ahemad and Kibret 2014). Production of antifungal metabolites such as antibiotics and siderophores-mediated iron competition are primary mechanisms of these bacteria to suppress diseases. Siderophores serve to chelate the ferric ion (Fe^{3+}) from the environment into microbial cells and reduce the iron availability to pathogens.

Nonpathogenic *F. oxysporum* suppress Fusarium wilt of tomato (McGovern 2015). Competition for nutrients is the major mechanism of this strain. They compete with pathogens for colonization to the root surface and tissues and induce systemic resistance in host plants (McGovern 2015). *Trichoderma* is an effective antagonist against Fusarium wilt diseases. Some *Trichoderma* isolates compete and colonize potential infection courts and others induce systemic resistance in plants (Marzano et al. 2013). *T. hamatum* isolated from compost was reported to suppress diseases caused by *F. oxysporum* (Shafawati and Siddiquee 2013).

T. viride, *T. virens*, *T. harzianum*, and *T. hamatum* have been used as antagonists against soil and seed-borne diseases, diseases in the phyllosphere and storage rots (Coventry et al. 2005; Siddiqui et al. 2008). The mycoparasitic activities of *Trichoderma* spp. include competition, antibiosis, and production of cell wall degrading enzymes or a combination of these activities. *Trichoderma* spp. produces non-volatile antibiotics that inhibit the hyphae of phytopathogen. When *Trichoderma* recognizes the host, it attaches itself to the host and either grows along the host hyphae or coils around them and secretes lytic enzymes such as chitinase and hydrolase. Subsequently, disorganization of host cell wall occurs, resulting in osmotic imbalance followed by intracellular disruption. It has been shown that chitinolytic enzymes isolated from *T. harzianum* inhibit spore germination and germ tube elongation in several plant pathogens (Viterbo et al. 2001).

7 Conclusions and Future Perspectives

Microbial composting reduces the bulk volume of rice straw, destroys pathogens, converts nitrogen from unstable ammonia to stable inorganic forms, avoids air pollution, and satisfies the fertilizer needs for agricultural use. Composting is highly dependent on C:N ratio, pH, temperature, moisture content, particle size, and the potential of microorganisms present in the substrates. Under natural conditions, composting of rice straw usually takes as long as 6 months, but inoculation with lignocellulolytic microbial consortium at optimized conditions could reduce the bioprocess only to 3–4 weeks as well as enhance the maturity of end product. Fortification with biocontrol agents further enhances rice straw compost as biofertilizer and bioprotectant. However, future composting experiments on industrial scale and trials of compost amendment soil on different crops and field conditions are suggested to ensure the consistency of the obtained results which will expand our current knowledge on the sustainable management of bulky rice straw more precisely.

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Soil Bacteria for Polycyclic Aromatic Hydrocarbon (PAH) Remediation: Application Potentialities and Limitations

H. Deka and J. Lahkar

Contents

Abstract The PAH pollution in the crude oil-contaminated sites is a perennial problem in several parts of the world. About 130 PAH species have been identified, and out of them 16 are reported to be as priority pollutants by the United States Environmental Protection Agency (USEPA) which include toxic, carcinogenic, and mutagenic compounds. PAHs are also ubiquitous in the environment due to atmospheric deposition resulting from the incomplete combustion of organic matter such as diesel, coal, and wood. These compounds are persistent pollutant in the soil system, and their persistency increases with the increase in molecular weight. Several remediation techniques including both chemical and biological method have been tested for decontamination of the PAH-polluted soil ecosystem. Each method has

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advantages as well as limitations. Bacteria are the dominant microorganism in the PAH-contaminated soil and play a crucial role in degradation of a wide range of PAH species. Both aerobic and anaerobic biodegradation are important and their mechanism has been studied. However, aerobic bacteria and their degradation metabolism on PAHs have been well studied as against their anaerobic counterpart. Besides, PAH bioremediation is governed by several factors such as type of organism, bioavailability of the compounds, soil type, microbial enzyme, etc., that are associated with the successful remediation of the PAH pollutant from the environment. In this chapter, an attempt has been made to discuss the application potentialities as well as limitations of soil bacterial candidates in terms of degradation of PAH compounds.

Keywords Bacteria • Polycyclic aromatic hydrocarbons (PAHs) • Biodegradation • Limitations

1 Introduction

Pollution from organic as well as inorganic sources is a burning problem in several parts of the world. Polycyclic aromatic hydrocarbons (PAHs) are a group of organic pollutants that are abundantly present in the crude oil-contaminated sites. PAHs are multiple aromatic ring compounds and are ubiquitous in all main components of the environment, i.e., air, water, and soil (Wang et al. 2013). They may enter to the environment by several ways such as incomplete combustion of solid and liquid fuels and natural combustion that includes forest fires and volcanic eruptions. However, it is the anthropogenic activities such as industrial production, transportation, refuse burning, gasification, and plastic waste incineration which mainly contribute a lot for release of PAHs to the environment. Nevertheless, it is the crude oil leakage during drilling operation and transportation which contributes a significant portion of PAHs to the soil and water ecosystem besides the other industrial activities such as steel-making factories, gas plant, and wood treatment processes that are responsible for release of several PAHs and its derivatives to the environment (Lemaire et al. 2013). It has been heavily reported that abandonment of crude oil drilling sites, accidental spillage from crude oil production unit, refining, and distribution processes results in contamination of the PAHs to the environment (Sarma Roy et al. 2014).

Polycyclic aromatic hydrocarbons (PAHs) are fused-ring hydrocarbon compounds. They are reported to be highly recalcitrant under normal conditions due to their structural complexity and strong molecular bonds. PAHs consist of fused benzene rings in linear, angular, or clustered arrangements and can be grouped under two categories, i.e., low molecular weight (LMW) and high molecular weight (HMW) compounds (Figs. 1 and 2). Although, by definition, PAHs contain only carbon and hydrogen atoms, however sometimes, nitrogen, sulfur, and oxygen atoms may also substitute in the benzene rings to form heterocyclic aromatic

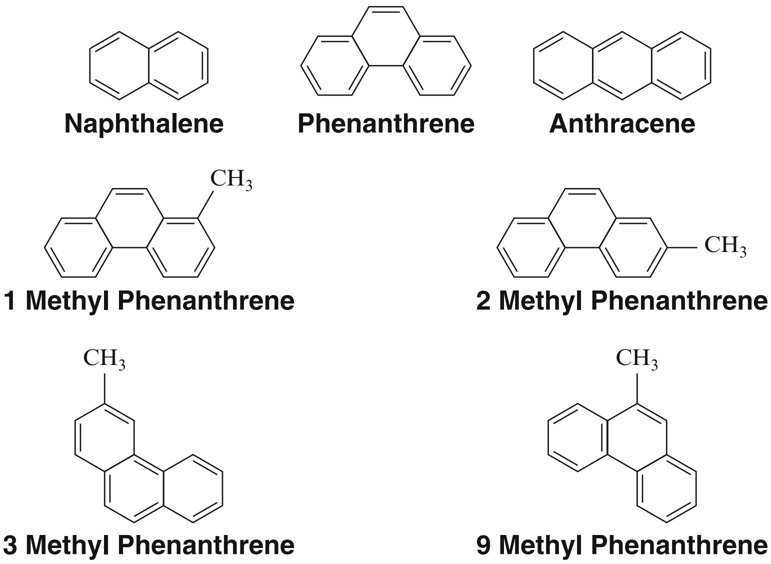
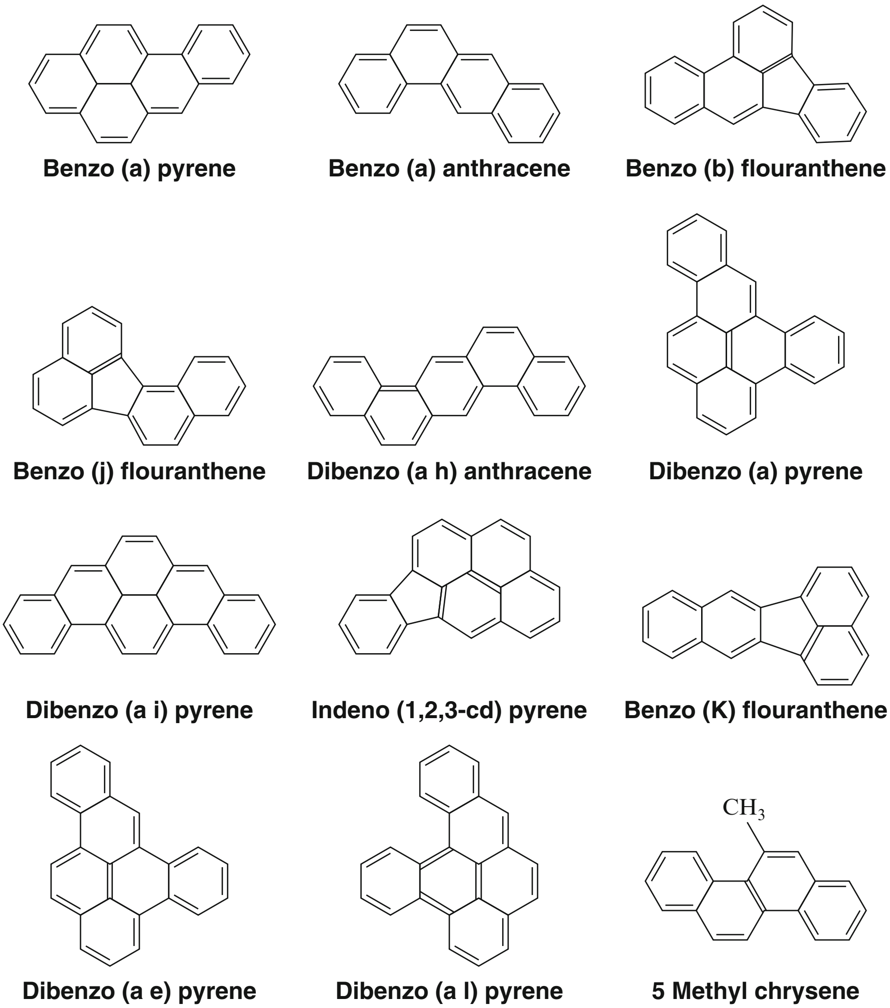


Fig. 1 Examples of some low molecular weight (LMW) PAH compounds along with its structure and molecular mass (Adapted from Anyika et al. 2015)

compounds, which are commonly grouped with the PAHs. Furthermore, the PAHs substituted with alkyl groups are normally found together with the PAHs in the environment. As a whole the group of PAHs and its related compounds are known as polycyclic aromatic compounds (PACs).

Till date, approximately more than 130 PAH compounds have been reported, and among them 16 are classified as priority pollutants by the United States Environmental Protection Agency (USEPA) due to their mutagenic and carcinogenic properties (Loick et al. 2009). Several workers enumerated about the adverse effects such as immunotoxicity, genotoxicity, and carcinogenicity of PAH species on living organisms including human beings (Sahinkaya and Dilek 2005; Qiao et al. 2006; Haritash and Kaushik 2009).

Soil and sediment pollution by PAHs is of great concern due to the potential ecological and human health risks posed by these compounds, more particularly by the high molecular weight compounds (HMW-PAH) which are reported to be not only carcinogenic and mutagenic but also teratogenic (Wu et al. 2008). Sediments represent biologically important environmental habitats, but the ability to accurately determine the effects of sediment-associated contaminants is hampered by uncertainties in the quantification of receptor exposure (Spasojevic et al. 2015). Soils represent a major sink for organic xenobiotic contaminant like PAHs in the environment, and their retention in the soil system is controlled by soil properties, such as organic matter and the physicochemical properties of the contaminant. Following their entry into the soil environment, PAHs may diffuse and be occluded in soil micropores (Semple et al. 2003).



Benzo (a) pyrene
Benzo (a) anthracene
Benzo (b) flouranthene
Benzo (j) flouranthene
Dibenzo (a h) anthracene
Dibenzo (a) pyrene
Dibenzo (a i) pyrene
Indeno (1,2,3-cd) pyrene
Benzo (K) flouranthene
Dibenzo (a e) pyrene
Dibenzo (a l) pyrene
5 Methyl chrysene

Fig. 2 Examples of some high molecular weight (HMW) PAH compounds along with the structures and molecular mass (Adapted from Anyika et al. 2015)

2 Polycyclic Aromatic Hydrocarbon (PAH) Toxicity and Health Hazards

The rapid absorption of PAHs by humans results in a high potential for biomagnifications in the food chain. Again, toxicity of the PAH compound depends on the number of benzene rings it possesses, and in general, the greater the number of benzene rings, the greater the toxicity. Till date, maximum works on PAH toxicity have been carried out by employing fish as a model organism.

The carcinogenic, toxic, and mutagenic properties of some PAHs have been reported by various workers (Hong et al. 2015a; Samanta et al. 2002). More than 400 PAH compounds and their derivatives are reported as carcinogenic (Ren et al. 2015). The accumulation of polycyclic aromatic compounds (PACs) negatively affects the health and productivity of fish, and accordingly, it also affects the nutritional status and health of human populations who consume these fish (Benjamin et al. 2014). For example, high molecular weight PAH compound BaP is reported as a mutagenic and carcinogenic precursor and impairs reproductive functions of fish (Booc et al. 2014). Several countries as well as the WHO have therefore set maximum concentration limits of PAHs in fish above which the health of humans who consume them is threatened (Balk et al. 2011; Scarlett et al. 2011). The risks arising from contaminated fish are imperatively important for some countries of the world where fishing is a major source of employment and foreign exchange. Additionally, fish constitute about 60 % animal intake which signifies the serious effect of PAH contamination in fish. Health risks resulting from dietary intake of PAHs are frequently assessed using the benzo(a)pyrene (Yi et al. 2011). Polycyclic aromatic hydrocarbons (PAHs) also reported to disrupt the endocrine system and affect the reproductive function in humans and wildlife (Horng et al. 2010). Besides the carcinogenicity, some workers also reported about the cardiotoxicity of PAH compound on fish. For example, three-ring PAH such as phenanthrene can cause direct toxicity to the developing heart of fish embryos (Incardona et al. 2004; Brette et al. 2014).

PAHs are highly lipid soluble and hence easily absorbed from the gastrointestinal tract of mammals (Cerniglia 1984). They are rapidly distributed in a wide variety of tissues with a marked tendency for localization in body fat. On uptake into the body tissue, some PAHs form epoxides, and carcinogenicity of these epoxides has already been reported (Samanta et al. 2002). Again, some PAHs also contain a “bay-region” as well as “K-region,” both of which allow metabolic formation of bay- and K-region epoxides, which are also highly reactive. Therefore, many PAHs are considered to be environmental pollutants that can have a detrimental effect on the flora and fauna of contaminated habitats, resulting in the uptake and accumulation of toxic chemicals in food chains. In some instances uptake of these pollutants causes serious health problems and/or genetic defects in humans (Samanta et al. 2002).

Interaction of PAHs with atmospheric nitrogen resulted nitro-PAHs. The effect of nitro-PAH toxicity has been reported by several workers (Onduka et al. 2012; Manzetti 2012; Shimada et al. 2013). Some nitro-PAHs directly act as mutagens and carcinogens on living organisms (Onduka et al. 2015). In mammals, these chemicals have strong genotoxic potencies, which are similar to or higher than those of benzo(a)pyrene (Busby et al. 1989; Wislocki et al. 1986). DNA damage has been detected in brown trout (Mitchelmore and Chipman 1998a), mussels (Mitchelmore and Chipman 1998a), and marine organisms (Onduka et al. 2015) when exposed to 1-nitropyrene. There are an impaired immune response and metabolic functions as well as genotoxic damage in the earthworm species *Eisenia andrei* when exposed to a minimum concentration of PAHs in laboratory condition (Sforzini et al. 2015).

Reports are also available on immunotoxicity of PAHs which are extensively studied in benthic invertebrates. Phagocytosis is the predominant mechanism of immunity in bivalves which tend to decrease when they are exposed to PAHs (Frouin et al. 2007). Experiments exposing bivalves to PAHs via contaminated phytoplankton, oiled sediments, and fine residues from combustion of fuels have caused reduction in activity and efficiency of phagocytosis (Frouin et al. 2007). A few studies have reported about the increase in pathogen susceptibility of fish species on exposure to PAHs. For example, exposed fish were found to have an increased susceptibility to the marine bacterium pathogen, *Vibrio anguillarum* (Arkoosh and Casillas 1998).

The toxic effect of PAHs on plants is well documented in literature (Desalme et al. 2011; Ahammed et al. 2012a, b; Wei et al. 2015). Even, the low molecular weight PAH compound like phenanthrene inhibited seed germination, affected the growth and chlorophyll level of wheat seedlings. Furthermore, phenanthrene also enhanced the levels of lipid peroxidation (LPO) and induced H_2O_2 accumulation in leaf tissues in a dose-dependent manner, accompanied by the changes in the antioxidant status. The activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), displayed a decreasing trend with the increasing of phenanthrene concentration. Thus, phenanthrene could exert oxidative damages in the early development stage of wheat, and the harmfulness occurred mainly in samples with higher concentrations (Wei et al. 2014). The degree of toxicity varies with the kind of PAHs and the species of plants (Ahammed et al. 2012a, b; Maliszewska-Kordybach and Smreczak 2010). Liu et al. (2009) suggested that excessive production of redox oxidative stress (ROS) in plants was a biochemical response to PAHs. In general, oxidation of PAHs results in the generation of ROS, which in turn causes oxidative stress and subsequent damage to plant cells (Wei et al. 2014).

3 PAH Remediation Technology: A Brief Overview

Various remediation techniques have been proposed and tested for removal of recalcitrant and toxic pollutants like polycyclic aromatic hydrocarbons (PAHs). These include mainly chemical treatment or chemotechnology, phytoremediation or phytotechnology, and bioremediation technology. An overview of all this technique has been presented briefly.

3.1 Chemotechnology or Chemical Remediation Method

Chemical remediation of PAHs involves the use of chemicals to extract the pollutants from contaminated soil. The two major methods of chemical remediation are solvent extraction and chemical oxidation. Solvent extraction is particularly applied where soil contaminants do not dissolve in water and therefore show a tendency to stick with the soil and sediment. PAHs are the recalcitrant pollutants that are known to show this property. Solvents initiate desorption of the pollutants (PAHs) and remove them from the polluted soil. On the other hand, chemical oxidation involves the use of oxidants to the contaminated sites. The oxidants start chemical break down of complex compounds into harmless simpler molecules such as water and carbon dioxide. Chemical remediation methods are effective and of low cost as they can be conducted directly at the site of contamination and hence minimize the need of soil or water transport off-site for treatment. Use of chemical additives such as surfactants, organic and inorganic acids, sodium hydroxide, water-soluble solvents, displacement, complexing agents, acids, and oxidizing/reducing agents has also been addressed for remediation of PAHs particularly in the case of high molecular weight (HMW) PAH compounds. The chemical additives help in the solubilization of complex ring PAH compound. Recently, use of chemical surfactant in PAH-polluted soil has been reported by López-Vizcaíno et al. (2012) where they used combined surfactant-aided soil washing (SASW) process and coagulation treatment, using iron and aluminum salts. Another traditional chemical remediation method is the Fenton treatment which can be integrated with other processes and is categorized as physical-Fenton, biological-Fenton, electro-Fenton, and photo-Fenton. As a chemical oxidant, Fenton's reagent ($H_2O_2 + Fe^{2+}$) was very efficient in the destruction of PAHs. Nama et al. (2001) tested Fenton chemistry on a mixture of PAHs (i.e., naphthalene (NAP), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), pyrene (PYR), chrysene (CHR), and benzo(a)pyrene (BaP)) in the model soil and reported up to 96.7 % degradation of PAH mixture as against the initial concentration of the compounds.

Besides, persulfate activation method is one of the important methods for polycyclic aromatic hydrocarbon (PAH) remediation, and it includes thermal activation, citrate-chelated iron, alkaline, and a hydrogen peroxide (H_2O_2)–persulfate binary mixture for treatment of the PAH pollutant. Thermal activation at 60 °C has been found to result in the highest removal of PAHs (99.1 %), and persulfate consumption during thermal activation varied (0.45–1.38 g/kg soil). Persulfate consumption (0.91–1.22 g/kg soil) and PAH removal (73.3–82.9 %) varied using citrate-chelated iron. Greater removal of high molecular weight PAHs has been reported with persulfate activation method, and differences in oxidative ability of the activated persulfate were also related to different radicals generated during activation (Zhao et al. 2013). Further, use of chemical oxidant treatments such as $KMnO_4$, H_2O_2, and Fenton also showed efficient degradation of PAHs in the soil samples, and the preheating (at 100 °C under N_2 for 1 week) improved the efficiency in increasing the available fraction of PAHs (Biachea et al. 2015). Although chemical remediation techniques are found to be effective for PAH decontamination, it cannot be successfully adopted for field condition due to their environmental incompatibility and high cost of chemicals. The applicability of chemical remediation of PAH can be further enhanced by combining it with the biological treatments. Strategies have been developed where some chemical may play the role of bioaugmentors of microbial community. For example, bioaugmentation by methyl-β-cyclodextrin (MCD) on

Paracoccus sp. strain HPD-2 in aged PAH-contaminated soil has been tested, and a significant enhancement of up to 34.8 % degradation of total PAHs was recorded (Sun et al. 2012).

3.2 Phytotechnology or Phytoremediation

Phytoremediation is the use of plants to remove, destroy, or sequester hazardous substances from the environment. Phytoremediation technology has been applied extensively in the habitats that are contaminated with PAHs. Several workers reported about the suitability of the plants for removal of PAH pollutant from contaminated soil (Pilon-Smits 2005). Phytoremediation may have several forms such as phytoextraction, the absorption and concentration of contaminants from the soil into the roots and shoots of the plant; rhizofiltration, the use of plant roots to remove metals from effluents; phytostabilization, the use of plants to reduce the spread of metals in the environment; phytovolatilization, the uptake and release of volatile contaminants into the atmosphere; phytostimulation, the stimulation of microbial biodegradation in the rhizosphere, the area around the roots of plants; and phytotransformation, the absorption and degradation of the contaminants by the plant.

Plants with its extended rhizosphere possess extensive and fibrous root systems and can act as effective stimulators for degradation of pollutants like PAHs. It has been reported that plant species such as *Cymbopogon jwarancusa* and *Helianthus annuus* have ability to remove PAHs (D'Souza et al. 2014). Alfalfa plants (*Medicago sativa* L.) in monoculture or in intercropping with other plants have reported to be promising in situ bioremediation strategy for PAH-contaminated soils (Hamdia et al. 2012; Sun et al. 2011). The main mechanism behind the efficient phytoremediation is the rhizodegradation of the pollutants. Many species of plants produce special enzymes and chemical exudates which can help in phytotransformation followed by degradation of PAHs (Liua et al. 2014; Martina et al. 2014).

Although phytoremediation is a cost-effective and efficient method for remediation of PAHs from soil, it takes more time of even up to 15–20 years to completely remove the contaminants from the soil. Moreover, the high levels of PAH have toxic effect on plants and can impose stress condition, and thus phytoremediation alone cannot become the ultimate solution for removal of PAHs from the soil.

Recent works on the plant rhizosphere microbes and endophytes have provided a new dimension toward an integrated approach where these microbes could effectively assist the plants for a better and faster degradation of the PAH compound and more particularly complex ring compounds (Kang 2014; Hong et al. 2015b). Microbes inhabiting in the contaminated soil surrounding the plants further enhance the availability of essential elements for growth and development of the plant. Another group of research is focused on the identification and isolation of PAH-degrading bacteria from contaminated soil followed by genetically introducing the genes responsible for degradation of the compounds into the plant system to improve their ability of PAH metabolism (Peng et al. 2015). Hence, a strategy involving both plant and microbes will provide a viable and suitable technique for speedy remediation of PAH-contaminated soil.

3.3 Bioremediation

Biological remediation involves the microorganisms to neutralize or degrade contaminations from soil. According to the EPA, bioremediation is a "treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non toxic substances." Bioremediation can be classified as in situ which means treatment of the pollutant is carried out directly at the contaminated site and ex situ which means treatment of pollutants carried out away from the contaminated sites.

PAH-degrading microbes are already an integral part of the soil system where PAH pollution is evident and can exist as natural degrader of the pollutants in PAH-polluted environment for a long time. The advanced bioremediation techniques mainly focus on the biostimulation, i.e., providing some stimulators in the form of carbon and nitrogen source, moistening and aeration of the contaminated site, etc., to encourage the process of degradation more efficiently by the resident microbes in the site. Sayara et al. (2011) reported about the degradation of PAHs (initial concentration 1 g of total PAHs kg^{-1} dry soil) by applying white-rot fungi *Trametes versicolor* along with compost of municipal solid waste as biostimulators. They reported that *T. versicolor* did not significantly enhance the degradation of PAHs and only 29.5 % of degradation is possible with this soil indigenous microorganism without supplying any co-substrate in the media. However, biostimulation using the compost as supplementary material in the media improves the PAH degradation rate, and up to 89 % of the total PAHs were degraded by the end of the experimental period.

In some cases bioremediation involves the screening of microbes from the polluted site and then adding matching species of microbes but with enhanced efficiency of contamination removal. The process may include introduction of a single type of microbe, a consortium of microbes, and the genetically modified microbes on the contaminated site (Mao et al. 2012). Bioremediation studies mostly involve the use of bacteria for decontaminating the sites while fungi on the other hand also have the potential to eliminate the complex compounds from the soil. Bioremediation involving the use of fungal mycelia for remediation is known as mycoremediation. Winquist et al. (2014) used fungal culture of *Phanerochaete velutina* for a laboratory-scale treatment (starting concentration 3500 mg kg^{-1}, sum of 16 PAH compounds) and showed that the high molecular weight PAHs were degraded significantly which account about 96 % of four-ring PAH and 39 % of five-/six-ring PAH degradation, respectively, within 3 months. Nevertheless, bioremediation of PAHs in the natural environment/field condition is a complex process (Bewley and Webb 2001). A successful PAH biodegradation in soil requires acclimation periods of the organism for several weeks prior to the start of degradation, and it is always affected by supply of nutrients, amendment of co-substrates, pH, salinity, abundance of humic acid, particle size of sediment, and organic content of the soil. Alongwith that, applications of biochar, biosurfactants, and cosolvent significantly enhance the PAH bioremediation potentiality in the contaminated environment (Rondon et al. 2007; Anyika et al. 2015; Szulc et al. 2014).

4 Soil Bacteria for PAH Remediation

Microorganisms play an essential role in the degradation of PAHs in the soil (Bacosa and Inoue 2015). There are large numbers of bacterial species isolated from different environments which showed ability to degrade a wide range of PAHs (Ruberto et al. 2006). A few recent references have been presented in Table 1.

Table 1 A few recent references on use of bacteria for PAH degradation during the last 5 years

PAH compounds	Bacterial strain	Experimental condition	Reference
Phenanthrene	*Brevibacillus* sp.	Liquid culture	Reddy et al. (2010)
Pyrene	*Alphaproteobacteria* (*Rhizobium*)	Not specified	Sun et al. (2010)
	Betaproteobacteria (*Hydrogenophaga*)		
Anthracene, phenanthrene, naphthalene, fluorene, pyrene, benzo(k) fluoranthene, benzo(a) pyrene	*Ochrobactrum* sp. VA1	Liquid culture	Arulazhagan and Vasudevan (2011)
Phenanthrene, pyrene, benzo(a)pyrene	*Rhodococcus* sp. P14	Liquid culture	Song et al. (2011)
Fluorene, phenanthrene, fluoranthene, pyrene	*Pseudomonas aeruginosa* DQ8	Liquid culture	Zhang et al. (2011)
Naphthalene, phenanthrene	*Streptomyces* sp. ERI-CPDA-1	Liquid culture	Balachandran et al. (2012)
*15 PAH mixture	*P. putida*	Contaminated soil	Chen et al. (2012)
Phenanthrene, pyrene, benzo(a)anthracene	*Staphylococcus warneri*, *Bacillus pumilus*	Liquid culture	Moscoso et al. (2012)
Anthracene, fluorene, phenanthrene, pyrene	*Serratia marcescens* L-11	Liquid culture	Pandey et al. (2012)
Naphthalene, phenanthrene, pyrene	*Halobacterium piscisalsi*, *Halobacterium salinarum*, *Halorubrum ezzemoulense*, *Haloarcula hispanica*, *Haloferax* sp., *Halorubrum* sp., *Haloarcula* sp.	Liquid culture	Erdogmuş et al. (2013)

(continued)

Table 1 (continued)

PAH compounds	Bacterial strain	Experimental condition	Reference
Phenanthrene, benzo(a)pyrene	*Rhizobium tropici* CIAT899	Liquid culture	Yessica et al. (2013)
Pyrene	*Enterobacter hormaechei*, *P. pseudoalcaligenes*	Liquid culture	Hesham et al. (2014)
Benzo(a)pyrene	*Cupriavidus* spp., *Luteimonas* spp.	Contaminated soil	Jones et al. (2014)
Benzo(k)fluoranthene	*Sphingobium* sp. KK22	Not Specific	Maeda et al. (2014)
Anthracene and pyrene	*Klebsiella pneumonia* PL1	Liquid culture	Ping et al. (2014)
Phenanthrene	*P. xanthomarina*	Liquid culture	Sopena et al. (2014)
Fluorene, fluoranthene, indeno[1,2,3-cd] pyrene	*Kocuria* sp. P10	Liquid culture and contaminated soil	Sun et al. (2014)
Fluorene, phenanthrene, pyrene	*Sphingomonas*, *Pseudomonas*, *Sphingobium*, *Dokdonella*, *Luteimonas*	Consortia in soil and liquid culture	Bacosa and Inoue (2015)
Naphthalene	*P. putida* G7	Liquid culture	Chang et al. (2015)
Naphthalene	*Streptomyces* spp.	Liquid culture	Ferradji et al. 2014
Naphthalene, phenanthrene, pyrene	*P. monteilii* P26, *Pseudomonas* sp. N3, *Rhodococcus* sp. P18, *Gordonia* sp. H19, *Rhodococcus* sp. F27	Liquid culture	Isaac et al. (2015)
Phenanthrene, naphthalene	*Corynebacterium* sp. SA07, *Ralstonia* sp. SA09	Liquid and solid culture	Mujahid et al. (2015)
Anthracene	*Burkholderia* sp. SA011 and SA042; *Stenotrophomonas* sp. SA012; *Pseudomonas* sp. SA013, SA044, and SA049; *Staphylococcus* sp. SA046, SA058, and SA061; *Micrococcus* sp. SA048		
Anthracene, pyrene	*P. indoxyladons*, *B. benzoevorans*	Liquid culture	Patel et al. (2015)
Naphthalene, anthracene, pyrene, fluoranthene	*Amycolatopsis* sp. Poz14, *Gordonia* sp. Poz20, *Rhodococcus* sp. Poz54	Liquid culture	Ortega-González et al. (2015)

It has been reported that the crude oil-contaminated sites harbor the bacterial candidate that showed the capacity to degrade both low molecular weight PAHs and high molecular weight PAHs. However, most of the bacterial species are reported to degrade low molecular weight PAHs rather than high molecular weight PAHs with more than four benzene rings. In general, high molecular weight PAHs are degraded slowly by indigenous or augmented bacterial community as the persistence of PAHs increases with their molecular size (Jain et al. 2005). It was Gibson et al. (1975) who reported for the first time about the ability of the bacteria to degrade PAHs particularly high molecular weight PAHs. They showed about the involvement of *Beijerinckia* sp. in oxidation of benzo(a)pyrene and benzo(a)anthracene to dihydrodiols. However, in the later period during the late 1980s, several other workers reported about the biodegradation of high molecular weight (HMW) PAHs (Mahaffey et al. 1988; Heitkamp and Cerniglia 1988; Mueller et al. 1989). Nonetheless, it was Mueller et al. (1989) who reported for the first time about the utilization of PAHs with four or more aromatic rings as a sole source of carbon and energy by bacterial isolates. They concluded that a seven-member bacterial consortium that has been isolated from creosote-contaminated soil can degrade not only fluoranthene but also other HMW-PAHs co-metabolically. It has been also reported that a consortium of *Mycobacterium*, *Pseudomonas*, and *Alcaligenes* sp. could carry out mineralization of fluoranthene, an LMW-PAH compound (Jain et al. 2005). The degrading ability of several bacterial strains such as *Pseudomonas putida*, *Neptunomonas naphthovorans*, *Burkholderia*, *Flavobacterium* sp., and *Arthrobacter sulfureus*, isolated from different soils of India, has been reviewed (Jain et al. 2005).

Bacterial strain has already been isolated that showed the ability to degrade the benzo(a)pyrene, the most carcinogenic and toxic PAH when grown on alternative carbon source in liquid culture experiment (Haritash and Kaushik 2009). Some species of *Mycobacterium*, *Pseudomonas*, *Sphingomonas*, *Rhodococcus*, *Agrobacterium*, *Bacillus*, *Burkholderia*, and *Flavobacterium* have the ability to degrade a wide range of PAHs including the most toxic and carcinogenic compound benzo(a)pyrene (Li et al. 2007; Aitken et al. 1998). *Pseudomonas aeruginosa* isolated from polluted soil of petroleum refinery can remove high doses of phenanthrene within 30 days of incubation period (Haritash and Kaushik 2009; Romero et al. 1998). Rehmann et al. (1998) isolated a *Mycobacterium* sp. strain KR2 from a PAH-contaminated soil of a gas work plant, which was able to utilize pyrene as sole source of carbon and energy. Yuan et al. (2002) reported that the rod-shaped bacteria like *Pseudomonas fluorescens* and *Haemophilus* spp. have been found to degrade acenaphthene, fluorene, phenanthrene, anthracene, and pyrene in a range of 70–100 % within 40 days of the treatment. In another study, Dean-Ross et al. (2002) also reported that *Mycobacterium flavescens* and *Rhodococcus* spp. isolated from river sediment can effectively mineralized pyrene and fluoranthene in laboratory environment. Different strains of bacteria such as *Pseudomonas*, *Mycobacterium*, *Gordona*, *Sphingomonas*, *Rhodococcus*, and *Xanthomonas* which degrade polycyclic aromatic hydrocarbons (PAHs) have already been characterized for genes encoding degradation enzymes for PAH (Haritash and Kaushik 2009). The crude oil-contaminated soil is a good habitat for potent hydrocarbon degraders of the genus *Lysinibacillus*, *Brevibacillus*, *Bacillus*, *Paenibacillus*, *Stenotrophomonas*, *Alcaligenes*, *Delftia*, *Achromobacter*, and *Pseudomonas* strain. Pure culture of the bacterial strain as well as consortia of several strains might have contributed to improve the quality of oil-contaminated soil (Sarma Roy et al. 2014).

4.1 Degradation Types and Metabolism

Mainly two types of biodegradation pathways along with two categories of bacteria are found to be involved in the biodegradation of polycyclic aromatic hydrocarbons (PAHs). These are namely aerobic and anaerobic bacteria/degradation metabolism. Among the biodegradation pathways, aerobic biotransformation of PAHs has been extensively studied and reviewed by several workers, and hence details of degradation mechanism are not presented in this chapter. The anaerobic degradation mechanism has been less intensely studied and is available only for low molecular weight PAH breakdown. The principal basis of the aerobic mechanisms is the oxidation of the aromatic ring, followed by the systematic breakdown of the compound to PAH metabolites and/or carbon dioxide, whereas anaerobic metabolism of PAHs is thought to occur via the hydrogenation of the aromatic ring (Bamforth and Singleton 2005).

4.1.1 Aerobic Biodegradation

Aerobic degradation means the biotransformation of the PAHs into several intermediates in the presence of oxygen. Several bacterial candidates have been found to be associated with aerobic biodegradation, and their metabolic pathways have already been proposed for a wide range of PAHs including both low molecular weight and high molecular weight compounds.

The basic principle of aerobic catabolism as highlighted above is similar for all aerobic PAH-degrading bacteria. However, the intermediate and end products of the catabolism are different for different bacterial strains. The first step in the aerobic catabolism of a PAH molecule by bacteria is the oxidation of the PAH to a dihydrodiol by a multicomponent enzyme system incorporating both atoms of molecular oxygen into the PAH nucleus (Gibson et al. 1975). However, some workers reported about the involvement of dioxygenase or aromatic ring hydroxylation dioxygenase (ARHD) for incorporation of oxygen at two carbon atoms in the benzene ring of the PAHs (Samanta et al. 2002; Baboshin and Golovleva 2012). The dehydrated intermediates after oxidation undergo either through an *ortho*- or a *meta*-cleavage pathway and further converted to the intermediates of the tricarboxylic acid cycle (Kanaly and Harayama 2000). A generalized degradation pathway has been presented in Fig. 3, and details of pathway of *ortho*- and *meta*-cleavage pattern are shown in Fig. 4.

When there is cleavage in the aromatic ring of the PAH compound in an intradiol manner, i.e., between the carbon atoms that carry hydroxyl groups, the cleavage type is known as *ortho*-cleavage; on the other hand, when cleavage occurs in an extradiol pattern, i.e., cleavage takes place in another C–C bond, then it is called *meta*-cleavage. Dicarboxylic aromatic acid is formed from *ortho*-cleavage of the aromatic ring, whereas *meta*-cleavage results in the formation of monocarboxylic aromatic acids and in maximum cases the aromatic substituents of 2-keto-3-butenoic

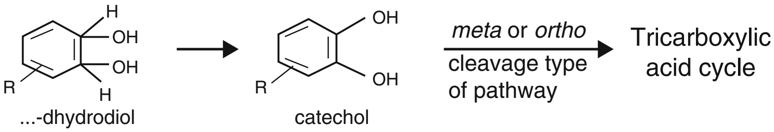


Fig. 3 A generalized degradation pathway of PAH by aerobic bacteria (Adapted from Loick et al. 2009)

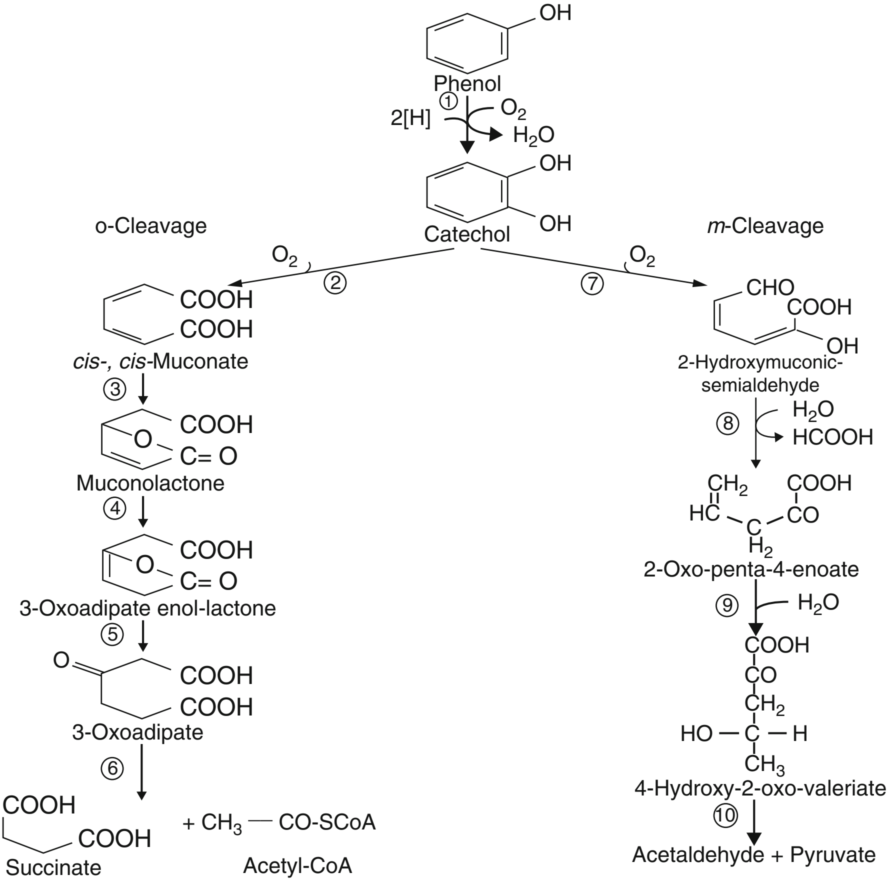


Fig. 4 Aerobic bacterial degradation pathways showing *ortho*- and *meta*-cleavage type. Different numbers showing enzyme involved: (*1*) phenol monooxygenase, (*2*) catechol 1,2-dioxygenase, (*3*) muconate lactonizing enzyme, (*4*) muconolactone isomerase, (*5*) oxoadipate enol-lactonase, (*6*) oxoadipate succinyl-CoA transferase, (*7*) catechol 2,3-dioxygenase, (*8*) hydroxymuconic semialdehyde hydrolase, (*9*) 2-oxopent-4-enoic acid hydrolase, and (*10*) 4-hydroxy-2-oxovalerate aldolase (Adapted from Fritsche and Hofrichter 2000)

acid. This product further converted into pyruvate and a PAH derivative with a number of rings less by 1. If the bacteria contain the respective enzyme activities, all rings comprising the PAH molecule are sequentially cleaved with formation of the compounds of central metabolism. Furthermore, it has been reported that degradation pathways of various PAHs coincide at certain stages and vary only in the initial reactions (Fritsche and Hofrichter 2000).

4.1.2 Anaerobic Biodegradation

The aerobic environment such as soils and sediments where PAHs are common contaminant may develop anaerobic zone due to depletion in molecular oxygen during aerobic respiration. The depletion in the molecular oxygen is due to stimulating affect of indigenous microbial community. The oxygen is not replaced at the same rate as its depletion, and as a result there is formation of anaerobic zones in the PAH-contaminated soil. Therefore, anoxic remediation of PAHs is more important in the oil-polluted sites. However, anaerobic or anoxic biodegradation of PAHs has been less intensely studied as against aerobic degradation. As compared to the aerobic, the anaerobic biodegradation is slow and its biochemical mechanism is yet to be established (Haritash and Kaushik 2009). But it is considered as a practicable, inexpensive remediation technique for soil and sediment contaminated by PAHs. Even anaerobic PAH degradation especially by indigenous microorganisms is more desired for PAH-polluted sediment remediation (Sun et al. 2012). It has been heavily reported that low molecular weight (LMW) PAHs like naphthalene and phenanthrene could be biodegraded under anaerobic situation such as denitrifying conditions (Yuan and Chang 2007). Some researchers found that high molecular weight (HMW) PAHs tended to be more easily biodegraded under sulfate-reducing conditions which is an anaerobic situation (Rothermich et al. 2002). In a study, Lu et al. (2012) reported that up to 77 % biodegradation of two-/four-ring PAHs is possible under anoxic condition. A number of studies have demonstrated that some of PAHs including phenanthrene, naphthalene, acenaphthene, and fluoranthene can be biodegraded in the absence of oxygen in several microcosm studies with nitrate, ferric iron, or sulfate as electron acceptors and even under methanogenic conditions (Bergmann et al. 2011).

Microbial metabolisms for anaerobic degradation of PAHs have been studied primarily under sulfate- and nitrate-reducing conditions. However, some authors also studied the metabolic pathways of PAH degradation under methanogenic condition. It has been documented that PAHs are dissipated under nitrate- and sulfate-reducing conditions (Haritash and Kaushik 2009). Both fermentative and respiratory metabolism of anaerobes are important for effective degradation of PAHs. Anaerobic degradation of fluorene, phenanthrene, and pyrene in soil under denitrifying conditions is possible through fermentative and respiratory metabolism of the bacteria in the presence of low molecular weight co-metabolites and suitable electron acceptors such as nitrate (Ambrosoli et al. 2005). Generally, anaerobic bacteria initiate the biodegradation of PAHs by introducing both atoms

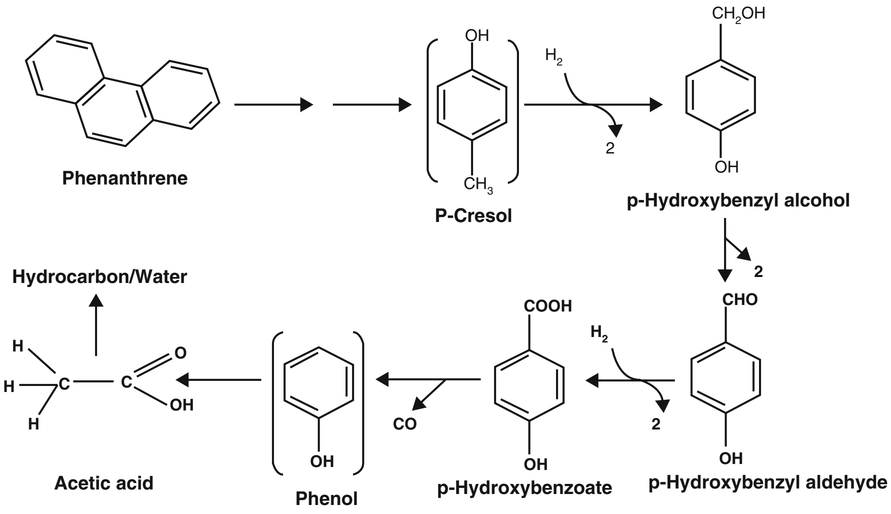


Fig. 5 Proposed biotransformation pathway for phenanthrene by anaerobic bacteria under sulfate-reducing conditions (Adapted from Tsai et al. 2009)

of molecular oxygen to the aromatic nucleus of the PAHs and the reaction being catalyzed by a multicomponent dioxygenase which consists of a reductase, a ferredoxin, and an iron–sulfur protein (Harayama et al. 1992). Further, the anaerobic degradation/transformation pathways for PAH compound such as naphthalene and phenanthrene have already been proposed (Tsai et al. 2009) and presented in the following figures (Figs. 5 and 6).

5 Limitations in PAH Remediation

The most important limiting factor in the bioremediation/microbial remediation of PAH compound from the environment (mainly from soil system) is the bioavailability of these compounds to the microbial populations of the contaminated sites. This is because of low solubility of the PAH compound and absorption as well as adsorption into the soil particles. Soil bioremediation with bacteria involves the transfer of PAH from soil to bacterial cells, and low bioavailability of the PAH compounds results in a low mass transfer rate from soil to bacterial cells. Therefore, optimization of PAH biodegradation with bacteria involves in increasing contaminant bioavailability. However, contaminant desorption can also result in environmental risk because of the possibility of contaminant spreading (Garcia-Delgado et al. 2015). Again, low bioavailability and accessibility of pollutants are associated with biphasic (“hockey stick”) kinetics of biodegradation. It shows an initial period of fast degradation, followed by a much slower phase in the later period. It is commonly observed in soils and sediments during bioremediation. Therefore, to overcome this biphasic constraint, there are constant demand for developing innovative

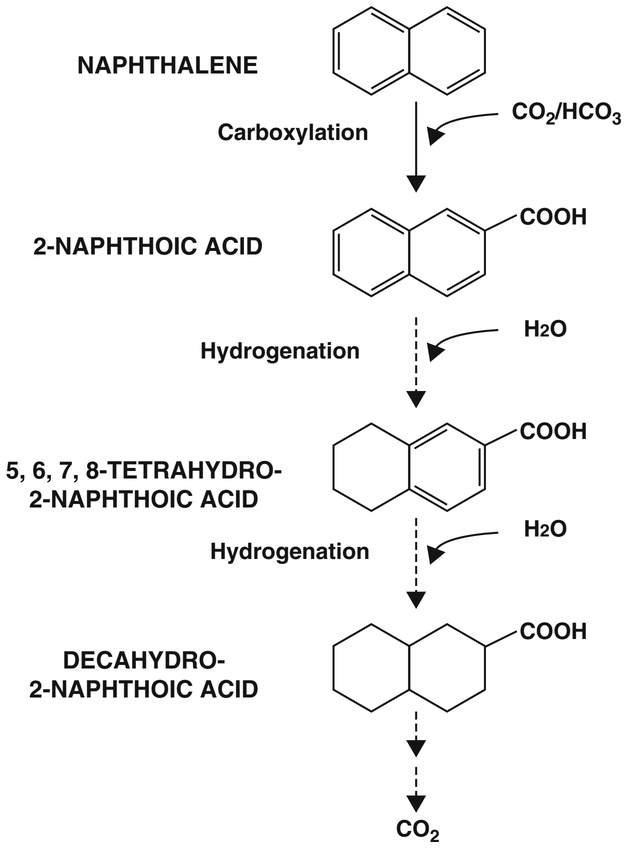


Fig. 6 Proposed metabolic pathway for naphthalene under sulfate-reducing conditions (Adapted from Zhang et al. 2000; Meckenstock et al. 2000)

treatment methods for decontamination of PAH-polluted sites (Semple et al. 2003; Megharaj et al. 2011).

The stability of PAH molecules and its hydrophobicity, soil properties, and soil–PAH contact time (i.e., aging) are some factors that determine the bioavailability of PAHs to the microbes (Mihelcic et al. 1993; Volkering et al. 1995; Alexander and Alexander 2000; Antizar-Ladislao et al. 2006). Besides, other potential limitations to PAH bioremediation include the presence of other toxic compounds, inadequate supply of nutrients, and insufficient biochemical potential of the organism for effective biodegradation (Dua et al. 2002). It has been reported that indigenous soil microorganisms have limits of tolerance for particular environmental conditions as well as optimal conditions for peak performance in terms of degradation of the pollutants. It has also been established that low biodegradability of high molecular weight PAHs (HMW-PAHs) with four and more rings is due to their low aqueous solubility and high sorption to soil particles thus preventing their elimination from the contaminated soil (Sun et al. 2014).

In case of any bioremediation process, the toxicity of the degraded products is not always known. For example, information on the isolation and identification of metabolites from the bacterial degradation of benzo(a)anthracene (BaA) is very limited. It has been reported in the recent past that the partially degraded product of the contaminants is sometimes more toxic than its parent compounds and accumulation of these intermediate compounds also poses a serious threat to the environment. Even the degradation by-products/intermediates may be mobilized in groundwater or bioaccumulated in animals (Bavarva 2015). For use in bioremediation, the PAH degrader should ideally mineralized and grow on PAHs as sole carbon and energy source. Effective mineralization is important to reduce the generation of toxic water-soluble by-products that not only pose threat to the environment but also hamper growth of the strain (Boonchan et al. 2000).

Again in some cases, the sites where the concentration of the pollutants is very high are where the growths of the microbes are inhibited, thereby hampering the bioremediation processes. Moreover, in case of ex situ bioremediation process, the volatilization of the pollutants (i.e., PAHs) is difficult to control perfectly (Sayara 2010). Furthermore, bacterial bioremediation process is frequently affected by competition of the PAH degraders with other bacteria and fluctuating environmental conditions in the contaminated sites (Chen et al. 2005; Teng et al. 2015).

The major limitations of the bioremediation techniques that have been made in the recent past are the poor capabilities of microbial communities in the field, besides, lesser bioavailability of contaminants on spatial and temporal scales (Megharaj et al. 2011). Moreover, the absence of benchmark values for efficacy testing of bioremediation for widespread field application also posed as a potential problem for the environmental scientists worldwide.

It has been heavily reported that the degrading ability of the indigenous microbial candidate including bacteria is mainly limited to low molecular weight (LMW) PAH compounds. For example, BaA is degraded to a limited extent by indigenous microflora in PAH-contaminated soil and sediment. The HMW-PAHs such as benzo(a) anthracene (BaA), benzo(a)pyrene (BaP), chrysene, and dibenz(a,h)anthracene resist to extensive microbial degradation in soils and sediments (Moody et al. 2005). Very little is known about the bacterial oxidation of benzo(a)pyrene (BaP). Till date, no bacteria have been isolated which shows the ability to utilize BaP as a growth substrate except a few successes in liquid culture experiments where bacteria can degrade BaP if an alternative carbon source is provided (Juhasz and Naidu 2000). However, addition of chemicals, for example, NH_4Cl and KH_2PO_4, improves the degrading ability of the bacterial strain. Nevertheless, in maximum cases, degradation was not found up to the mark when mixture of PAHs was added as feedstock for the bacteria. But it is utmost important to achieve effective degradation for mixture of PAH compounds as they commonly found as mixture in the environment (Sun et al. 2014).

The poor predictability of end points associated with the bioremediation of polycyclic aromatic hydrocarbons (PAHs) is a large limitation when evaluating its viability for treating contaminated soils and sediments (Ortega-Calvo et al. 2013). Large-scale application of bioremediation and its success are still constrained by the factors related to the unpredictable end point PAH concentrations in the contaminated sites, the lack of adequate monitoring tools that guarantee the occurrence of active biodegradation processes, and the not entirely accurate risk assessment policies (Vila et al. 2015).

The residual concentrations of the PAHs after bioremediation are crucial because they may limit the use of the area after treatment, or land use might not even be possible if the residual concentrations do not meet the legal requirements (Ortega-Calvo et al. 2013). Therefore, proper understanding of the mechanisms involved in remediations much required for the development of novel remediation technologies. While traditional approaches remove mainly the PAH fraction that undergoes rapid desorption (Reichenberg et al. 2010), hence the new technology must be effective in removing the fraction that desorbs more slowly to reduce residual contaminant concentrations after treatment (Ortega-Calvo et al. 2013).

The association of PAHs with co-pollutants such as hydrocarbons and heavy metals is another factor that can prolong their residence time in the environment and inhibit the biodegradation process. As stated above, aliphatic hydrocarbons including the LMW-PAHs are easily biodegradable by the indigenous microbial community as compared to the more complex chemical and HMW-PAHs. This results in the depletion of available oxygen in the surrounding environment and the onset of anaerobicity in the contaminated sites. Though recent work has shown that there is a real potential for the biodegradation of PAHs in the absence of molecular oxygen (see "Anaerobic Metabolism of PAHs"), details regarding the efficiency and scale of PAH degradation in anaerobic environments are still limited. In addition, it is possible that the presence of heavy metals in soil could inhibit microbial growth and hence limit the metabolism of contaminants under anaerobic conditions (Dua et al. 2002).

Genetically engineered microorganisms (GEMs) have also been applied for PAH remediation with a limited success. Few GEMs have been used for field application because of strict regulations for the release of GEMs into the environment (Ezezika and Singer 2010). *Pseudomonas fluorescens* HK44 is the only GEM approved for field testing in the USA for bioremediation of PAHs, and it contains a naphthalene catabolic plasmid (pUTK21), mutagenized by transposon insertion of lux genes (Ripp et al. 2000). The drawbacks of genetically engineered microorganisms (GEMs) are mainly associated with the field release which also include the decreased levels of fitness and the extra energy demands imposed by the presence of foreign genetic material in the cells (Saylor and Ripp 2000; Singh et al. 2011). Besides, there remains a great risk of entering of mobile genetic elements to the environment which may be acquired by undesirable organisms on release of GEMs to the field condition (Megharaj et al. 2011). The transition of genetically engineered microorganisms from the laboratory to the field environments is also hampered due to the lack of information on the population dynamics of introduced genetically engineered microorganisms in the field and poor physiological control of catabolic gene expression in the engineered organisms under nutrient and other stresses (Cases and de Lorenzo 2005). The bioengineering and environmental release of those engineered microorganisms has to overcome several obstacles which include inconsistencies in risk assessment procedures and public health concerns before their effective application in the field.

6 Conclusion and Future Direction

Soil bacteria are the integral part of the polycyclic aromatic hydrocarbon (PAH)-contaminated soil system and play an important role in the degradation of these compounds. Several works have been published regarding the degrading ability of indigenous bacteria on both low molecular weight (LMW) and high molecular weight (HMW) PAH compound. However, success of bacterial degradation of PAHs is still limited in laboratory environment, and in maximum cases desired results have not been found in field environment. Bioremediation of PAHs is a complex process, and certainly the lone application of indigenous bacteria has some limitation in terms of removal of this recalcitrant components from the soil system. Even a lone application of a single community (i.e., bacteria) in the polluted site is not enough for a permanent solution of PAH pollution problem. Application of plant–microbe consortia and fungal–bacterial coculture can be encourage in this context, and more research is still needed in this line. Besides, metagenomic approach to study the bacterial community has also been carried out in recent past, and more research is yet to come in this track. Nevertheless, a wide range of innovations in recent years, such as the use of biosurfactants, the chemotactic mobilization of bacterial inoculants, the selective biostimulation at pollutant interfaces, rhizoremediation, and electrobioremediation, increase the bioavailability of PAHs and improved the bioremediation potentialities of the bacteria.

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Entomopathogenic Nematodes for the Management of Subterranean Termites

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Abstract Termites cause economic losses by directly injuring and destroying both living and dead vegetation. They can damage right from sowing the crops till harvest. Billions of dollars are spent annually throughout the world to control and prevent termite infestation. Many bacteria, fungi, and nematodes occurring naturally in soils are known to suppress termite activity. Entomopathogenic nematodes (EPNs) and their associated bacterial symbionts are highly specific in their host range and compatible with many pesticides. EPNs, also called beneficial nematodes, are commercially used to control insect pests. These nematodes offer an environmentally safe alternative to chemical insecticides, and a wide range of EPNs are effective against various termite species. Only a limited number of field studies have been conducted using EPNs as control agents for termites. New isolates of EPNs may prove potential against termite pests in the field. This chapter outlines the potentials of entomopathogenic nematodes in termite management.

Keywords Termite • Biological control • Entomopathogenic nematode

1 Introduction

For many decades organochlorines formed the backbone of termite management worldwide. Synthetic insecticidal compounds became popular due to the long residual action and the wide toxicity spectrum. However, these pesticides were banned or withdrawn from the market for human health and environmental reasons from an increasing number of countries in the late 1980s and the 1990s. Indiscriminate, inadequate, and improper use of these synthetic organic pesticides had caused tremendous damage to the environment. Consequently, negative impacts on soil, groundwater quality, human health, wildlife, and ecological balance within agroecosystems are increasingly becoming a concern. To overcome the hazards associated with chemical pesticides, the use of biopesticides is increasingly being adopted. As a consequence of these developments, the focus in termite management has shifted increasingly to alternative methods in dealing with termite problems.

Biological control constitutes a more environmentally acceptable alternative to traditional chemical control measures. When successfully implemented, it can yield permanent, cost-effective management of pest populations with minimal environmental disturbance. It refers to the application or manipulation of predators, parasitoids, or pathogens in order to suppress and manage insect pest population. The literature contains numerous reports of microorganisms that may have potential to cause the death of termites. Lenz (2005) highlighted the potential of nematodes and fungal pathogens in termite management. A partial review by Myles (2002) lists two viruses, five bacteria, 17 fungi, and five nematodes. The full list of such organisms is no doubt larger. Entomopathogenic nematodes have been found parasitizing species in the orders Hemiptera, Diptera, Hymenoptera, Lepidoptera, Orthoptera, Coleoptera, Thysanoptera, Siphonaptera, as well as Isoptera (Nickle and Welch 1984). These nematodes offer an environmentally safe alternative to chemical insecticides in the management of termites. This chapter outlines the potentials of entomopathogenic nematodes in termite management.

2 Termites

Termites are a group of social insects which are widely distributed throughout the tropical and subtropical regions, with the highest diversity found in tropical forests (Eggleton 2000) while few occur in different temperate areas of the world. They are of paramount importance as pests and belong to the insect order Isoptera, an ancient insect group that dates back more than 100 million years. The Latin name Isoptera means "equal wing" and refers to the fact that the front set of wings on a reproductive termite is similar in size and shape to the hind set. Since the first truly scientific work on termites in 1779, which was carried out in India by J. G. Konig, much work has been done all over the world. The presence of termites is often not readily noticed because of their hidden activities. They act as herbivores as well as decomposers, feeding on a wide range of living, dead, or decaying plant material (Bignell and Eggleton 2000; Traniello and Leuthold 2000). Their feeding habits make them ecosystem engineers, which over long periods of time can modify the physical properties of soil, at various spatial scales (Dangerfield et al. 1998). These termites comprise some 2900 species (Krishna and Grimaldi 2003), of which 300 species are of economic importance as pests in agriculture, forestry, and urban situations worldwide.

Termites present in a locality are pests and do not depend on their species and a number of colonies, more precisely their food habits, whether they are competitors for food resources or not. Based on their habitat, termites can be grouped into three general categories: subterranean, damp-wood, and dry-wood termites (Paul and Rueben 2005). Subterranean termites live in the soil and wood that is in contact with soil. Dry-wood and damp-wood termites live inside wood of varying levels of decay and moisture content. Subterranean termites derived their name because of their association with the soil. These are serious pests of wooden structures causing tremendous amounts of damage (Su and Scheffrahn 1998) and are reported responsible alone for at least 80 % of losses caused by termites (Su and Scheffrahn 1990).

Dry-wood or powder-post termites are primitive termites whose damage often goes unnoticed by homeowners. Dry and powdery pellets, occasionally kicked out by dry-wood termites from infested wood, are very characteristic of their presence. Unlike the subterranean termites, dry-wood termites form colonies within sound dead wood rather than in the soil below. Their ecology and behavior are distinctly different from the subterranean termites, a fact which alters their monitoring and controls procedures from those methods used for standard subterranean termites. They have been reported to cause less than 20 % of damage (Su and Scheffrahn 1990). The pest status of damp-wood termites, however, is minor compared to the other termite groups. The wide distribution, large colonies, cryptobiotic lifestyle, and aggressiveness of subterranean termites make it difficult to manage and control them (Su and Scheffrahn 1988; Culliney and Grace 2000). The annual economic cost of termite damage and prevention worldwide is estimated in billions. Yu et al. (2008) reported damage to wooden structures attributed to termites in the United States can exceed $3 billion annually. Rust and Su (2012) reported global economic impact of termite to be at least $40 billion. Estimates, however, vary considerably by region. High frequency of damage caused by termites and the high losses sustained have resulted in multimillion-dollar pest control industry.

2.1 Biology and Habitat

Termites undergo hemimetabolous (incomplete) metamorphosis. Important biological roles are divided among physically distinct termites, called as castes. Termite colonies contain three principal castes: workers (pseudergates), soldiers, and reproductives (king, queen, alates or swarmers). The worker caste is responsible for the damage that makes termites an economically important pest. They are wingless and sterile and comprise the majority of the population within a colony. Their mouthparts are adapted for chewing through wood or other cellulose materials. Workers do all the physical labor like building nests, foraging, cleaning, feeding all other dependent castes, grooming the queen, etc. Soldiers, however, have only one mission, to defend the colony against invaders with their specialized strong mandibles. They cannot forage for food or feed themselves and depend on the workers to care for them. Soldiers, when disturbed, readily attack approaching object and may secrete a white gluey defensive secretion. Termite queen lays about 3000 eggs a day through its enlarged abdomen (Thompson 2000). The eggs are yellowish-white and hatch after 50–60 days of incubation. The queen lives around 25 years.

Winged reproductives are also called swarmers or alates. Alates are flying form of termites, which fly out in great numbers from mature colonies at certain times of the year. This process is known as swarming. Environmental factors trigger the emergence of swarmers, and they leave the colony in large numbers during spring or early summer in search of new habitats. Following swarming, alates shed their wings, and males and females pair off and seek out a suitable place to establish a new colony. Swarming alates are usually the first sign of infestation noticed. Swarming time, season, and conditions differ among species and locations. Only a small percentage of swarmers survive to develop a new colony. The colony reaches its maximum size in approximately 4–5 years.

The size of a termite colony depends on location, food availability, and environmental conditions. Some colonies remain small; others contain up to several thousand individuals. A mature colony of *Coptotermes formosanus* Shiraki may have 1–4 million termites (Su et al. 1984). While other termites like *Reticulitermes flavipes* (Kollar) may contain ca. 200,000 termites in a colony (Howard et al. 1982) with a foraging range up to 100 m in any direction (King and Spink 1969) (Fig. 1). These data indicated the presence of a large subterranean termite colony beneath an infested structure. A termite mound is the most familiar form of termite nest. Typically, each species builds a characteristic mound, although there may be geographical variation in the size and shape of the mound within species. Apart from grass-eating species, which forage in the open, all termites build tunnels between their nest and source of food through covered runways. These shelter tubes connecting soil with structures are common signs of subterranean termite infestation. Termites construct a variety of tube types as summarized by Ebeling (1978). These covered tunnels provide humidity conditions, thus preventing desiccation, and darkness necessary for their movement and protection against predators. Symbiotic flagellate protozoa are found in the hindgut of lower termites, namely, Kalotermitidae, Rhinotermitidae, Hodotermitidae, Mastotermitidae, Serritermitidae, and Termopsidae. Higher termites, the Termitidae, host no or very few protozoa.

2.2 Distribution and Identification

Many ecological factors influence termite distribution but vegetation and soil type remain the most important. Termites are widely distributed by people who unknowingly transport infested furniture. As a result, many pest termites have very wide distributions. The Formosan subterranean termite *C. formosanus* is the most widely distributed and most economically important. This species of termite is probably endemic to southern China and was apparently transported to Japan prior to the

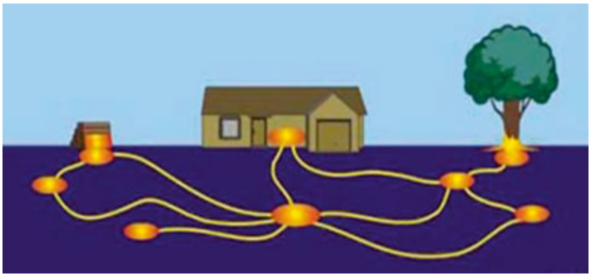

Fig. 1 A single colony of the Formosan subterranean termite *Coptotermes formosanus* Shiraki may contain several million individuals that forage up to 300 ft in soil (*Source*: University of Florida, Publication No. EENY-121)

1600s and to Hawaii in the late 1800s (Su and Tamashiro 1987). Another species of termite, *C. havilandi*, was supposed to have been introduced from Southeast Asia to parts of South and North America and to a number of Caribbean islands, where it became as a serious pest of woodwork in buildings (Scheffrahn et al. 1994; Su et al. 1997a, b). However, in the countries from where it was supposed to have originated, it was never considered a serious pest. Environmental changes can drastically change the species composition, and some rare species in the natural landscape can be more easily detectable in these areas (Dambros et al. 2012). Formosan termites attack a variety of wood products and cause significant damage within a short time period. *C. formosanus*, *R. flavipes*, and *R. hesperus* Banks are the three most important subterranean termite in the United States (Su and Scheffrahn 1990). *C. formosanus* coexists with *Reticulitermes* spp. in some areas of Florida (Scheffrahn et al. 1988a). Termite fauna of Saudi Arabia is predominantly subterranean; however, surveys revealed the presence of dry-wood termites in the Western region, most probably introduced with wood and timber imports from abroad (Faragalla 2002). The majority of the pest species in India are soil inhabiting, either as mound builders or as subterranean nest builders. The major mound-building species are *Odontotermes obesus*, *O. redemanni*, and *O. wallonensis*. The major subterranean species are *Coptotermes ceylonicus*, *C. heimi*, *Heterotermes indicola*, *Odontotermes homi*, *Microtermes obesi*, *Microcerotermes beesoni*, and *Trinervitermes biformis* (Rajagopal 2002) (Table 1).

Soldier termites and alates are the only types of termites that can be accurately identified (Scheffrahn and Su 1994). Soldiers are generally large than the workers, with brownish heads and, in most species, large, toothed mandibles. Unfortunately,

Table 1 Important termite genera in subcontinents

Country	Important termite genera and species
Southern Africa	Anacanthotermes (family Hodotermitidae), Psammotermes and Reticulitermes (family Rhinotermitidae), Amitermes, and Microcerotermes (family Termitidae), and several species of Kalotermitidae
Eastern Africa	Macrotermitidae: Macrotermes (family Termitidae), Hodotermes (family Hodotermitidae), and Schedorhinotermes (family Rhinotermitidae)
Western Africa	Ancistrotermes, Macrotermes, Odontotermes, Microtermes, and Cubitermes (Termitidae)
N. America	Damp-wood termites: (genus *Zootermopsis*,family Termopsidae); dry-wood termites *Incisitermes*, *Neotermes*; subterranean termites *Reticulitermes*, *Heterotermes*, *Amitermes*; and introduced species of *Coptotermes*
S. America	*Cryptotermes* and *Neotermes* (family Kalotermitidae), *Coptotermes* and *Heterotermes* (family Rhinotermitidae), and *Nasutitermes*
China	*Coptotermes, Reticulitermes* (family Rhinotermitidae), *Macrotermes* and *Odontotermes* members of the *Cryptotermes* (Kalotermitidae) and Hodotermitidae
Australia	Subterranean, dry-wood, harvester, and mound builders *Mastotermes*, *Porotemes*, *Stolotemes* (primitive genera)
Europe	*Reticulitermes*

Source: www.chem.unep.ch/pops/termites dry-wood termites do not maintain large number of soldiers in their colonies, and majority of the nest is composed of worker but there is no identification key available for worker individuals. Identification must be done with care, as the alate castes of some dry-wood species found in Alabama were similar in size and color with those of the Formosan subterranean termite. Kirton and Brown (2003) mentioned that *C. gestroi* and *C. havilandi* are, in fact, the same species. *C. gestroi* had been described from the soldier form of the termite by Erich Wasmann in 1896, while *C. havilandi* had been described later by Nils Holmgren in 1911 from the alates of the same species. The difficulty of matching alates to soldiers had led to this situation. If there is taxonomic confusion or the wrong name is applied to the species, then the pest management decisions we make could be based on misleading information. The importance of accurate termite taxonomy in the broader perspective of termite management was highlighted by Kirton (2005). However, Haverty et al. (2005) studied identification of termite species by the hydrocarbons in their feces. They mentioned that hydrocarbons extracted from fecal pellets were qualitatively and quantitatively similar to cuticular extracts and can be used to determine the termite species responsible without the termites present.

2.3 Economic Importance

The economic importance of termites is twofold, extremely beneficial and extremely injurious to man. These small creatures are a part of the natural ecosystem and contribute significantly to most of the world's ecosystems. Their greatest contribution is the role they play in recycling wood and plant material. The breakdown and release of organic matter as termites eat and digest plant material plays an important role in maintaining soil health. Aeration of the soil due to termite burrowing activities helps in maintenance of soil microflora (Wood 1988). Soil fertility improves when termite mounds, rich in minerals, are crushed down and incorporated into the soil. Review by Freymann et al. (2008) on importance of termites for the recycling of herbivore dung highlights the economic importance of termites in tropical ecosystems. Moreover, termites provide a source of protein-rich food for many organisms including ants, guinea fowl, and other mammals including humans. In African countries, use of termites as a protein source for poultry production has been investigated.

Termite species, however, gain pest status when they damage building materials or agronomic and forestry commodities. As the principal food of some of the termite castes is cellulose, they cause economic losses by directly injuring and destroying both living and dead vegetation, buildings, bridges, dams, etc. Termites are among the few forest insects that are able to live in and on both decayed and living plant tissue. They injure living plants originally by attacking from outside but may continue the destruction of living tissues from outside or from within (Fig. 2). Termites also damage man-made fabrics (textile materials), plastics (polytene, polyvinyl chloride), and some metal foils (Howse 1970). Termites ate

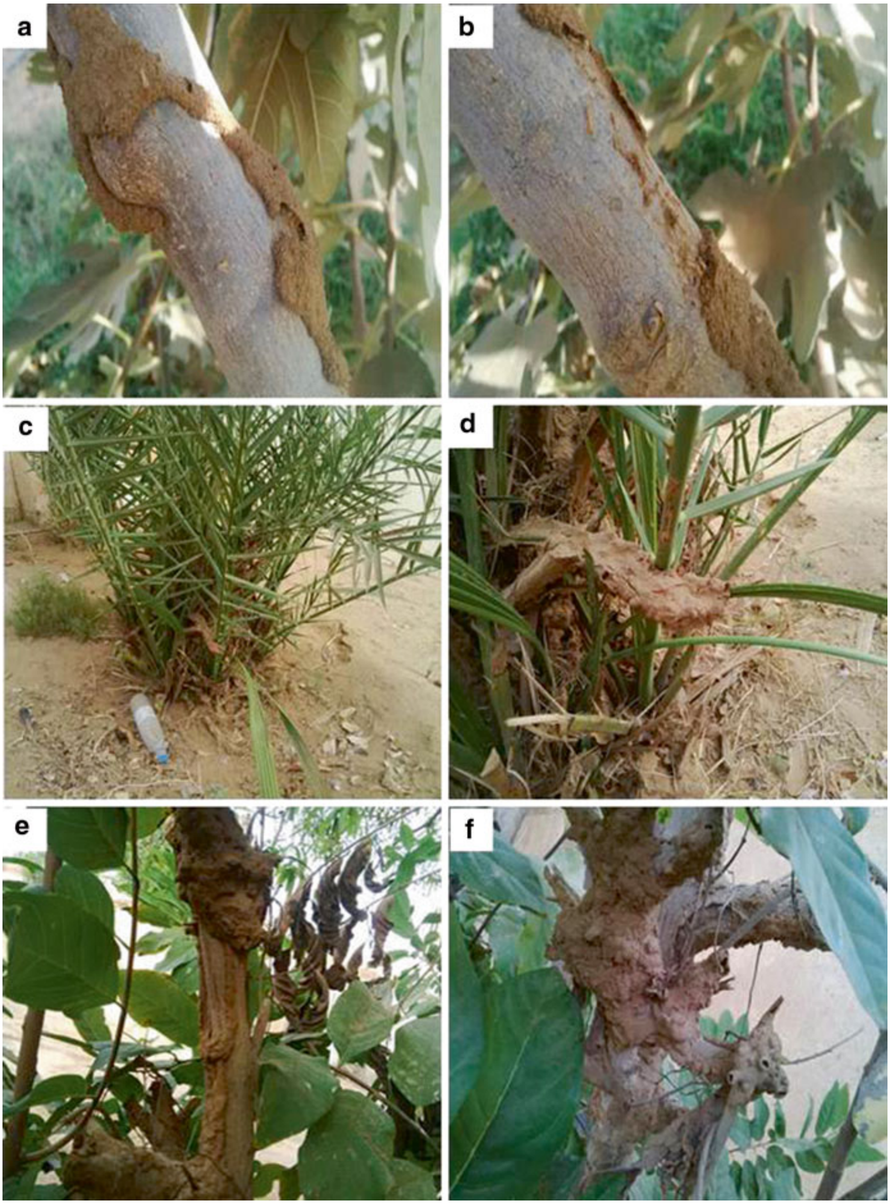

Fig. 2 Termite infestation in various plants

through notes worth Rs. 10 million (about $222,000) stacked in a steel chest inside the State Bank of India, in a northern Indian town (Sacks 2011). The bank was said to be housed in an old building infested with termites. A similar incident happened in Bihar state of India when a trader lost his life savings after termites infested his bank's safe deposit boxes (Tewary 2008). Damage to human habitations by termites varies in its impact. It is estimated that 20 % of Australian homes are infested by termites (Scholz et al. 2010), whereas in China up to 90 % of Chinese homes south of the Yangtze river are affected by termite damage. In what may be the most extreme example of damage by termites, an entire township in India was gradually destroyed by the termite *Heterotermes indicola* and eventually resembled a bombed-out ghost town (Roonwal 1955). Economic losses associated with termite damage in the United States and Japan are around 1000 and 800 million US$ a year, respectively (www.chem.unep.ch/pops/termites), and Japan may be the third largest user of pesticides for structural pest control in the world. Economic losses due to termite in India have been estimated around 35.12 million US$ (Joshi et al. 2005); however, in Malaysia 8–10 million US$ are spent toward termite treatment every year (Lee 2002). Heavy infestation of termite on agricultural crops (field and vegetables) including maize, sorghum, sweet peppers, tomatoes, okra, and millets have also been reported from the western region of Saudi Arabia (Faragalla et al. 1998). Annual economic losses associated with termite activities have also been mentioned by Ghaly and Edwards (2011) (Table 2). Characterization of termite pest problem starts with identification of termite species, knowledge of basic biology and ecology of the species, and evaluation of the magnitude of the economic damage.

2.4 Losses in Agriculture

Termites can damage right from sowing the crops till harvest. It is difficult to locate termites and usually it is too late when the typical termite damage symptoms are noticed in field, leading to severe losses. Credible information on the economic losses caused by termites is difficult to obtain. Ground-based monitoring devices, however, have been developed and used experimentally for identification of subterranean termite colony ramifications (Potter 1997). Termites damage was greater in rain-fed than irrigated crops (Sharma et al. 2009). By increasing the number of irrigations, termite damage was reduced (Sharma et al. 2004). Hence, in dry areas where proper facilities for irrigation are not usually

Table 2 Annual economic losses associated with termite activity

Country	Number of termite species	Costs associated with termite activity (US $ 10^6/year)
Malaysia	NA	8–10
India	NA	35.12
Australia	NA	100
China	482	300–375
Japan	21	800
USA	50	1000

available, termite infestation is inimical. In the dryer parts of India, infestation by termite has also been reported by Roonwal (1979) in maize, pearl millet, pulses, sugarcane, cotton, paddy, groundnut, potato, citrus, vegetables, spices, and fruit crops. Annual crops are attacked toward harvest time while perennial crops are attacked most destructively during dry seasons. Severe losses in different regions of India have been recorded on highly susceptible crops such as wheat and sugarcane in North India; maize, groundnuts, and sunflower in South India; cotton in Western India; and tea in Northeast India (Rajagopal 2002). Subterranean termites attack sugarcane crop from its germination through shoot emergence, and finally it affects the quality of canes. At germination stage, the losses up to 90–100 % have been recorded (Salihah et al. 1988). As many as 13 species of termites are reported to cause damage to sugarcane in India. Alam and Miah (1997) reported five species of termites destructive to sugarcane in Bangladesh. Sharma et al. (2004) conducted a survey to determine termite damage on wheat crop covering five mega wheat-growing zones in India. They reported that termites were a real problem in Rajasthan and some parts of Madhya Pradesh. The range of infestation indicated that ~50 % of the fields had low termite damage, ~30 % had medium damage, and ~20 % had severe termite damage. The damage was low in clay and black soils, high in sandy loam soils, and severe in red soils. About 16 species of termite were found to damage the wheat crop in India, of these two species, viz., *Odontotermes obesus* (Rambur) and *Microtermes obesi* Holmgren, were found dominant (Chhillar et al. 2006). Soft wooded tea plants are known to be easily attacked by termites. Live wood-eating termite *Microcerotermes* and dead wood-eating termite *Odontotermes* are tea pest in India (TBI 2014). Ohiagu (1979) reported that termites damage three major cereal crops, maize, millet, and sorghum, in various parts of Northern Nigeria (Table 3).

Table 3 Crop losses and termite genera of different continents

Country	Crop	Losses (%)	Termite species
Africa	Groundnut	Between 10 and 30	*Microtermes* and *Odontotermes*
	Maize	30–60	*Microtermes*, *Ancistrotermes*, *Macrotermes*, *Odontotermes*, *Allodontermes*, and *Pseudacanthotermes*
	Sugarcane	5–10 (Central Africa) 18 (Sudan)	*Amitermes*, *Pseudacanthotermes*, *Macrotermes*, *Odontotermes*, *Microtermes*, and *Ancistrotermes*
Southeast Asia	Sugarcane	12 (Pakistan)	*Microtermes*, *Coptotermes*, *Odontotermes*, *Macrotermes*, *Trinervitermes* and *Heterotermes*
Australia	Cashew, mango, and avocado trees	30	*Mastotermes*, *Heterotermes*, *Coptotermes*, *Amitermes*, and *Microcerotermes*

Source: www.chem.unep.ch/pops/termites

3 Entomopathogenic Nematodes

Due to environmental concerns and human safety, efforts have been focused on biological control using entomopathogenic nematodes (EPNs). EPNs are soft-bodied, non-segmented roundworms. They are mobile, highly virulent insect parasites with high reproductive potential. Naturally they occur in soil environments and are capable of parasitizing many economically important insect pests. They locate their host in response to carbon dioxide, vibration, and other chemical cues and in some cases provide a level of insect control equivalent to that of chemical insecticides (Poinar 1986; Georgis 1990). Despite their broad host range and high virulence, these nematodes have shown no mammalian pathogenicity (Gaugler and Boush 1979) and are safe to vertebrates, plants, earthworms, honeybees, and other nontarget organisms (Kaya and Gaugler 1993). In cryptic habitats, EPNs have proven superior to chemicals in controlling the target insect (Gaugler 1981).

The first entomopathogenic nematode was described by Steiner as *Aplectana kraussei* (now *Steinernema kraussei*) in 1923. In 1929, Glaser and Fox (1930) found a nematode, *S. glaseri* (Steiner), infecting grubs of the Japanese beetle *Popillia japonica*, at New Jersey. Jaroslav Weiser described *S. carpocapsae* in 1955. The genus *Heterorhabditis* was described in 1976. The genus *Steinernema* and *Neosteinernema*, family Steinernematidae, and genus *Heterorhabditis*, family Heterorhabditidae (Rhabditida: Nematoda), contain the most important species of entomopathogenic nematodes. Now *Steinernema* contains more than 50 species and *Neosteinernema* only one species, *N. longicurvicauda. Heterorhabditis*, however, contains more than a dozen species. Steinernematids and heterorhabditids received great attention as a group of biological control agents in the 1990s. The history of entomopathogenic nematology is briefly reviewed by Poinar and Grewal (2012).

Steinernematids and heterorhabditids are obligate insect parasites (Poinar 1979) and are associated with symbiotic bacteria of the genera *Xenorhabdus* spp. and *Photorhabdus* spp., respectively (Akhurst and Boemare 1990). These *Xenorhabdus* and *Photorhabdus* are motile, gram-negative, facultative, non-spore-forming anaerobic rods in the family Enterobacteriaceae. Together, nematodes and their symbiotic partners form an insecticidal complex that is effective against a wide range of insect hosts (Gaugler and Kaya 1990; Kaya and Gaugler 1993). Symbiotic bacterium of *H. bacteriophora* was earlier characterized as *Xenorhabdus luminescens* in 1979 which was later transferred to the genus *Photorhabdus* (Boemare et al. 1993). Most *Photorhabdus* spp. are luminescent and catalase positive, whereas *Xenorhabdus* spp. have no luminescence and are catalase negative. Studies suggest each *Steinernema* species has an apparent specific natural association with only one *Xenorhabdus* species, though a single *Xenorhabdus* bacterial species may be associated with more than one nematode species (Fischer-Le Saux et al. 1999; Boemare 2002). Poinar (1966) and Poinar and Leutenegger (1968) demonstrated the location of bacteria in the infective-stage juveniles, using light microscopy and later electron microscopy. In *Steinernema* bacterial symbionts are harbored in a specialized structure known as “bacterial receptacle.” In *Heterorhabditis* bacterial symbionts are distributed along a broad stretch of the anterior portion of the nematode intestine. Entomopathogenic nematode species and isolates show substantial variation in behavior, host range, infectivity, reproduction, and environmental tolerances. EPNs find their hosts either actively or passively (Smart 1995). Directional response to electrical fields varies among EPN species which may be used by nematodes for host finding or other aspects of navigation in the soil (Shapiro-Ilan et al. 2012a).

Entomopathogenic nematodes, also called beneficial nematodes, are commercially used to control insect pests. *S. carpocapsae* was the first nematode product marketed while *S. scapterisci* became available commercially only in 1993 and *S. riobravis* in 1994 (Smart 1995). These EPNs offer excellent potential for control of insects in soil habitats but are not well suited for foliar application, as they are sensitive to desiccation and ultraviolet radiation. In laboratory tests, *S. carpocapsae* alone infected more than 250 species of insects from over 75 families in 11 orders (Poinar 1975). The broad host range and high virulence of entomopathogenic nematodes make them suitable for use as augmentative-release biocontrol agents (Hui and Webster 2000). They can be mass produced easily in liquid culture at economically reasonable costs (Ehlers et al. 1998) and are sufficiently small to pass through standard spraying equipment. These nematodes are compatible with many pesticides, can be mass produced and formulated, and have been exempt from registration in many countries. Insects controlled with entomopathogenic nematodes have been reviewed by various researchers (Klein 1990; Georgis and Manweiler 1994; Smart 1995; Hazir et al. 2003; Lacey and Georgis 2012). Several books on the potential use and field techniques of ENPs in various cropping systems has been published (Gaugler and Kaya 1990; Bedding et al. 1993; Lacey and Kaya 2000; Gaugler 2002). Similarly, Lacey and Georgis (2012) highlight EPN development for control of insect pests, above and below ground, including those from foliar, soil surface, cryptic, and subterranean habitats.

Subterranean termites live and forage in habitats that are moist, cool, and without direct sunlight such as soil or wood materials. These environmental conditions are ideal for the survival and movement of steinernematid and heterorhabditid nematodes and, therefore, provide the basis for the interest in their role in control of termites. Research shows that the entomopathogenic nematodes have a potential to use as environmentally safe alternative control tactic for termites (Wang et al. 2002; Yu et al. 2006). The rapid development in the study and commercial application of EPNs in pest control during the last 30 years instigated further interest in finding useful nematodes and better application methods to control subterranean termites. EPNs market continues to grow rapidly with US$14.5 million in 2007 and >US$20 million in 2013 (CPL 2006, 2013a, b).

3.1 Biology

Nematode infective juveniles (IJs) live in soil where they search for susceptible insect hosts. Nonfeeding juveniles invade the body of the susceptible insect by direct penetration either through natural openings (mouth, anus, and spiracles) or, in some cases, through the cuticle. *Heterorhabditis* use an anterior tooth to penetrate directly into the hemocoel. The parasitic cycle of nematodes is initiated by the third-stage infective juveniles (IJs), the only free-living stage. These infective juveniles act as vectors to transport the bacteria (*Xenorhabdus* and *Photorhabdus* spp.) into an insect host within which they can proliferate, as bacteria alone are incapable of penetrating the alimentary tract and cannot independently gain entrance to the host's hemocoel. IJs penetrate the alimentary tract of the host insect and release symbiotic bacteria, which are held in the intestine. At this point in their life cycles, bacteria and nematodes exist separately although in close proximity to one another. The released bacteria cause a lethal septicemia, killing the host within 24–48 h. The bacteria produce extracellular enzymes that break down proteins and lipids in the insect carcass, thereby providing nutrients for bacterial and nematode growth. The bacteria also suppress secondary infection of the host by producing antibiotic substances. Inside the hemocoel, IJs of *Heterorhabditis* release the symbionts by regurgitation, while bacteria are defecated by *Steinernema* spp. (Ciche et al. 2006). Nematode and its symbiotic bacteria act together in overcoming the insect's immune response (Dowds and Peters 2002). *Heterorhabditis* spp., if deprived from their symbionts, are nonpathogenic to insects (Han and Ehlers 1999), whereas symbiont-free *Steinernema* spp. will kill their insect hosts, although it takes them about ten times longer to do so (Ehlers et al. 1997). A review by Stock and Blair (2008) on entomopathogenic nematodes and their bacterial symbionts provides detailed information. Even though the bacterium is primarily responsible for the mortality of most insect hosts, the nematode also produces a toxin that is lethal to the insect (Burman 1982).

Symbiont bacteria create conditions necessary for nematode survival and reproduction within the insect cadaver. The nematode initiates its development, feeding on rapidly multiplying bacterial cells and host tissues that have been metabolized by the bacterium. Nematodes develop through four juvenile stages to the adult and then reproduce. They complete 1–3 generations within the host cadaver, depending on host size. When nematode numbers become high and nutrients become limiting in the insect cadaver, nematode progeny reassociate with bacteria and differentiate into the colonized, nonfeeding IJ form that emerges into the soil to forage for a new host (Kaya and Gaugler 1993). Reproduction differs in heterorhabditid and steinernematid. IJs of heterorhabditid nematodes become hermaphroditic adults, but individuals of the next generation produce both males and females, whereas in steinernematid (with the exception of *Steinernema hermaphroditum* (Griffin et al. 2001)), all generations are produced by males and females (gonochorism) (Grewal et al. 2005).

Infective juveniles do not feed but can live for weeks on stored reserves as active juveniles and for months by entering a near-anhydrobiotic state. The length of time that juveniles survive in the soil in the absence of a host depends upon temperature, humidity, natural enemies, and soil type. Generally, survival is measured in weeks to months and is better in a sandy soil or sandy loam soil at low moisture than in clay soils. The heterorhabditids do not survive as well as do steinernematids (Molyneux 1985). Their survival is better in sterilized soil than in non-sterilized soil. EPN population in non-sterilized soil is reduced by bacteria, fungi, mites, predatory nematodes, tardigrades, and other soil organisms (Smart 1995). Mites appear to be especially voracious nematode feeders.

3.2 Rearing

EPNs in the genera *Steinernema* and *Heterorhabditis* are commercially available for the control of soil-inhabiting insects. Several companies in Asia, Europe, and North America mass-produce EPNs either on a small scale or in large scale using bioreactors (Shapiro-Ilan and Gaugler 2002). EPNs can be reared by different methods either in vivo or in vitro (Bedding 1984; Georgis 1990; Shapiro-Ilan and Gaugler 2002; El-Sadaway 2011). For solid medium culture, a substrate such as beef kidney or liver or chicken offal may be used. The substrate usually is made into a paste that is coated onto a porous substrate such as sponge. The medium is sterilized and inoculated with the bacterium and then nematodes are added 24 h later. Infective juveniles are harvested after about 15 days. This method is labor intensive and is particularly well suited for laboratory use and small-scale field experiments. Production in liquid medium can be done in small containers or in fermentation tanks. Greater numbers of juveniles can be produced per unit area in fermentation tanks, which makes this method especially suited for large-scale commercial production at reasonable quality and cost (Shapiro-Ilan et al. 2012b). Ru (2001) and Sharma et al. (2011) highlight mass production, commercialization, and utilization of EPNs as microbial biopesticides for plant protection.

3.3 Distribution and Dispersal

Steinernematids and heterorhabditids are ubiquitous in distribution and have been recovered from soils throughout the world (Hominick et al. 1996). *S. carpocapsae* and *S. feltiae* are widely distributed in temperate regions, *H. bacteriophora* is common in regions with continental and Mediterranean climates, and *H. indica* is found throughout much of the tropics and subtropics. Other species such as *S. rarum*, *S. kushidai*, *S. ritteri*, and *H. argentinensis* appear to have a much more restricted distribution (Hazir et al. 2003). Juveniles of nematodes can be dispersed at great distances, vertically and horizontally, both actively and passively (Kaya 1990; Parkman et al. 1993). Passively, they may be dispersed by rain, wind, soil, phoresis, infected hosts, or human activity which may, in part, account for their widespread global distribution. Zadji et al. (2014b) observed nematode dispersal occurred by infected termites or phoresis. In addition to jumping for some nematode species, the infective juveniles can disperse in soil up to 90 cm in both the horizontal and vertical directions within 30 days (Kaya 1990). Soil porosity can also affect nematode dispersal with less dispersal occurring as soil pores become smaller. Although some entomopathogenic nematodes have been isolated from insects naturally infected in the field, they are most commonly recovered from soil by baiting with susceptible insects (Bedding and Akhurst 1974). The wax moth larva *Galleria mellonella* (L.) is most commonly used as bait. Molecular techniques have also been applied to measure genetic diversity of the nematodes and provide an initial screen to identify useful strains.

3.4 Genetic Manipulation

A limitation of nematodes for insect control is their susceptibility to environmental stress like extreme temperature, solar radiation, and desiccation, which prevents them from being used to maximum advantage as bio-insecticides under field conditions. The ability of different species to tolerate adverse conditions varies enormously. The potential of genetic engineering to enhance nematode environmental tolerance is being explored. To enhance tolerance of high temperatures, *hsp70A* (heat shock protein genes) from the free-living nematode *Caenorhabditis elegans* was introduced to *H. bacteriophora* juvenile (Hashmi et al. 1995; Wilson et al. 1999). Resulting transgenic strain, however, was not successful at field level. Isolation of natural populations that can survive harsh environments, such as deserts, indicated that some populations have enhanced abilities to survive desiccation. Fodor et al. (2010) isolated a *tps-1* gene from the yeast *Saccharomyces cerevisiae* and transformed it to *H. bacteriophora* which showed increased osmotolerance. In another study, indigenous gut bacteria of the subterranean termite *C. formosanus* were genetically modified with entomopathogenic bacterium *Photorhabdus luminescens* subsp. laumondii TT01, a symbiont of the entomopathogenic nematode *H. bacteriophora*. When termites were fed on filter paper inoculated with these recombinant bacteria, the chromosomal expression of the introduced genes showed that there were insecticidal activities against termite workers and soldiers. Termite mortality was reported 3.3 % at day 5, and it increased from 8.7 % at day 9 to 93.3 % at day 29 (Zhao et al. 2008).

4 Control Measures of Termite

Successful termite management requires many special skills and varies depending on the species causing infestation. An understanding of termite biology and its identification can help to detect problem and methods of control. Subterranean and, less frequently, damp-wood termites can have nests at or near ground level, so control methods for these can be similar. However, dry-wood termites nest above ground; therefore the approach for eliminating them is different. The predominant control strategies for subterranean termite consist of chemical treatment and baiting systems using a matrix containing a slow-acting insecticide or insect growth regulator.

Dry-wood termites, however, must be treated by removing infested wood, using spot treatments or by fumigation. An integrated program to manage termites must be practiced.

4.1 Cultural Practices

Cultural practices regulate termite numbers rather than eliminate them so that the benefits provided by termites are not lost. Removal of the queen or destruction of the nest has frequently been used by farmers as a traditional method for control of mound-building termites. Mounds are physically destroyed, flooded, or burnt with straw to suffocate and kill the colony. Deep plowing exposes termites to desiccation and to predators; therefore repeated plowing and digging of the soil may reduce termite damage. The removal of residues and other debris from the field may reduce potential termite food supplies and hence lead to a reduction in termite numbers and subsequent attack. Wood ash around the base of the trunk of coffee bushes and date palms has been recorded as preventing termite infestations. Intercropping maize and beans is the most effective cultural practice used by small-scale farmers in sub-Saharan Africa. Although termites may damage healthy plants, water-stressed and diseased plants are generally more susceptible to termite attack. Inorganic fertilizers enhance plant vigor and hence the ability to withstand pest damage. Application of nitrogen, phosphorus, and potassium in wheat, barley, and yam has been observed to reduce termite incidence. Sowing of indigenous varieties is recommended. In general, crops susceptible to termites are exotic while resistant crops are indigenous.

4.2 Barrier Control

Barriers can be physical, chemical, or hybrid. Physical barriers are good for prevention of termite damage in buildings and storage structures. As chemical barriers, soil applications of liquid termiticides to form a termite-impermeable barrier are in vogue these days especially for protecting buildings and other structures. The standard measure of acceptable performance is that the chemical barrier must keep termites from penetrating 90 % of the barriers for at least 5 years (Gold et al. 1996).

4.3 Chemical Control

For the last four decades, pest control industry has depended heavily on soil termiticides, and still chemical control is playing a vital role in controlling subterranean termites (Su and Scheffrahn 1998). With cryptic insects such as termites, effective delivery of insecticides to kill the population is particularly challenging. Reproductive and nymphs of subterranean termites are concentrated in nests near or below ground level, out of reach of other control methods. Therefore, common methods for controlling these termites are the application of termiticides or baiting campaigns. Active ingredients in currently available termiticides can be broadly classified as repellent or nonrepellent.

Repellent means that the termites are able to detect the insecticide, which basically serves as a barrier, and termites are repelled by it without receiving a dose that will kill them. Therefore, when using these materials, it is important to make sure there are no gaps remaining in the barrier. Repellent termiticides provide immediate protection for the structure. Currently available repellent termiticides include pyrethroids such as permethrin (Dragnet® FT, Prelude®), cypermethrin (Prevail® FT, Demon® TC), deltamethrin (Suspend® SC), and bifenthrin (Talstar®). Apart from repellent, some termiticides prevent termite invasion by nonrepellent or lethal contact. They include imidacloprid (Premise®), fipronil (Termidor®), chlorfenapyr (Phantom®), etc. Chlorpyrifos, iodofenphos, isofenphos, carbosulfan, and carbofuran have been used as alternatives; however their low persistence often necessitates repeated applications. Environmental factors, viz., soil type, weather, and application techniques, influence the mobility of insecticides in the soil. Use of pesticides containing conventional active ingredients in both agriculture and urban areas had led to increased exposures and unacceptable risks (NRC 1993; Wright et al. 1994). They were also a threat to water quality in many areas (Johnson 2005). Due to increasing concerns about these side effects, there has been great interest in finding other methods of controlling termites and reducing the use of chemicals. Moreover, treatment of soil under the structure may kill only a small portion of the colony. It is very likely that the majority of the colony population will survive to re-infest the structure, either by flying alates or foragers entering through an untreated or an inadequately treated portion of soil.

4.4 Baits

The last decade has seen the rapid development of baiting technology. Termite baits are a promising alternative to soil termiticide treatment. Where liquid termiticides are not normally suitable to apply, termite baits can be used. Bait matrix may contain a slow-acting insecticide or chitin synthesis inhibitor. Foraging termites feed on termite baits and carry the digested cellulose and termiticide back to the colony where they regurgitate their stomach contents to feed other termites, a process known as trophallaxis, leading to the demise of the entire colony population. Effectiveness of these treatment methods varies depending on the skills of the applicator, type and dosage of chemical used, and target species (Rust and Su 2012).

4.5 Trap-Treat-Release

Trap-treat-release (TTR) was developed by Dr. T. G. Myles at the University of Toronto. TTR is a technique for suppressing or killing social insect colonies, particularly subterranean termite colonies. In this technique the toxicant is applied externally to termite bodies as a groomable coating. Coated termites carry effectively larger loads of toxicant than do bait-fed termites. Under laboratory conditions, it is possible to achieve extraordinarily high kill ratios among members of the colony (one treated termite can kill over 1000 untreated termites). Under field conditions, an estimated 50–100 termites are killed for each termite treated.

4.6 Host Plant Resistance

Natural resistance of wood to termite attack is due in part to chemicals deposited during heartwood formation (Kumar 1971; Scheffrahn et al. 1988b). Chemicals in termite-resistant wood may be contact toxic to termites or act as antifeedants, repellents, or protozoacides (Carter et al. 1983). A specific chemical that causes resistance to insects may occur only in one plant species and not in others. Cornelius and Osbrink (2015) evaluated the wood consumption of the subterranean termite *C. formosanus* on ten different species of wood, used as commercial lumber. They reported that teak was the most resistant wood tested, and toxic chemical components of teak hold the most promise as wood preservatives. Indigenous crops are more resistant to termites than exotic crops. In Africa, sorghum and millet are more resistant to termites than maize and cowpea. The indigenous crop bambara nuts are not attacked while groundnuts suffer serious damage.

4.7 Biopesticides

Some plant biomass contains insecticidal activity which can be exploited for termite control. A rich source of new pesticides is plant essential oils. Singh and Kumar (2008) evaluated the anti-termite activity of *Jatropha curcas* Linn. oil and its toxic fraction at 1, 5, 10, and 20 % dilutions against *Microcerotermes beesoni* Snyder. They reported maximum wood protection against termites of both the treatments at 20 % concentration. Orange oil is currently available as an insecticide and has been registered in California under the brand name XT-2000™ for control of dry-wood termites (Mashek and Quarles 2008). Hu et al. (2011) reported anti-termitic properties of *Lantana camara* cultivars against subterranean termite *Reticulitermes flavipes*. Elango et al. (2012) evaluated the anti-termitic activity of crude leaf hexane, ethyl acetate, acetone, and methanol extracts of medicinal plants against *C. formosanus*. All the crude extracts were reported to show anti-termitic activity in a dose-dependent manner and exhibited a significant activity after 24 and 48 h of exposure.

4.8 Biological Control Agents

Natural enemies of insect pests, also known as biological control agents, include predators, parasitoids, and pathogens. Biological control is generally perceived as both providing more permanent insect control and as having less potential damage to the environment or nontarget organisms. Therefore, biocontrol should be considered as a long-range research goal rather than an immediate solution. Brazil has a history of success with biological control projects involving the use of parasitoids, insect pathogenic fungi, and viruses (Campanhola et al. 1995). The shortcomings associated with conventional chemical control methods have prompted policymakers and scientists to evaluate the potential for natural enemies to suppress termite populations. The use of biological control agents to hunt or to infect termites within their hidden galleries is appealing. Reviews by Grace (1997)on biological control strategies for the suppression of termite, by Culliney and Grace (2000) on prospects for the biological control of subterranean termites, and by Verma et al. (2009) on biological alternatives for termite control provide complete knowledge for nonchemical control of termite.

Specialized natural enemies of termites are rather limited in numbers, possibly because of cryptic and protected habitats in which termites live. As termites provide a good source of protein, their predators include spiders, beetles, flies, wasps, and especially ants. Other predators include frogs, reptiles, birds, and mammals such as pangolins, bats, and monkeys. Bushes and trees around farms are a home for many of these useful creatures. Termite predators are both opportunist and specialist, but ants are the greatest predators of termites and may have a considerable local impact on termite populations in some areas of the world. Although ants limit termite numbers under natural conditions, their suitability for use as biological control agents for target termite management has yet to be ascertained. Larvae of *Lomamyia latipennis* (Neuroptera: Berothidae) are carnivorous, live within termite nests, and prey upon damp-wood termites, *Zootermopsis angusticollis* (Hagen) (Tauber and Tauber 1968; Johnson and Hagen 1981). A few parasitoids of termites are known, but their potential for regulating termite populations seems negligible. A larval parasitoid *Verticia fasciventris* Malloch (Diptera: Calliphoridae) was reported to develop in the head of soldier termite in some species of *Macrotermes* (Sze et al. 2008). Parasitized soldiers possess a short and square-shaped head capsule, and these soldiers were statistically less aggressive than healthy soldiers (Neoh and Lee 2011). The use of naturally occurring pathogens of termites offers unique advantages over chemically based termiticides. Characteristics of the colony, such as a protected, underground location, are likely to limit the impact of predators and parasitoids on subterranean termites. The study of pathogens for termite control started as early as 1965 (Yendol and Paschke 1965). Since then there has been renewed interest in using pathogenic organisms, such as bacteria, viruses, nematodes, and most fungi for controlling termites in recent years.

4.8.1 Entomopathogenic Fungi

Fungal diseases are known to cause insect mortality naturally (Vimaladevi and Prasad 2001). Entomopathogenic or disease-causing fungi have received considerable attentions as they are exceptionally virulent and function as lethal parasites of insect pests. These fungi are among the first microorganisms to be used for the biological control of insect pests. They are cosmopolitan organisms, invade their host directly through the cuticle, and have been isolated from soils and infected insects from around the world. More than 700 species of fungi from around 90 genera are pathogenic to insects (Wraight et al. 2007; Hemasree 2013); however, only a few have been thoroughly investigated for their use against insect pests in agriculture. Fungal pathogens for the management of spodopteran pests were reviewed by Khan and Ahmad (2015).

These fungi also offer the best prospect as termite control agents. Conidia of entomopathogenic fungi could be spread through the colony by contact and grooming between contaminated and uncontaminated termites. Investigations with termites have largely focused on two fungal species, *Beauveria bassiana* and *Metarhizium anisopliae*, and recently also *Paecilomyces fumosoroseus*. Green muscardine fungus *M. anisopliae* is especially recommended for practical control of termites as a bio-insecticide and is virulent to all species of termites tested. Conidia from *M. anisopliae* can survive >18 months in termite nests (Milner and Staples 1996). Myles (2002) isolated a virulent strain (pathotype) of *M. anisopliae* from the eastern subterranean termite *Reticulitermes flavipes* in Toronto. Dong et al. (2007) evaluated the efficacy of a new virulent of *M. anisopliae* var. dcjhyium (obtained from *Odontotermes formosanus* in China) against the subterranean termite *O. formosanus* in the laboratory. The new isolate was reported to be highly infectious and virulent against termites and could cause approximately 100 % mortality of termites 3 days post-inoculation at a concentration of 3×10^8 conidia/ml. In the market, there is a bio-control product BioBlast™ containing *M. anisopliae* as active ingredient to be used against subterranean termites. Strains of *M. anisopliae* and *B. bassiana*, when employed in baiting schemes, can be transferred among nest mates and may offer the potential for subterranean termite control (Jones et al. 1996; Wright et al. 2002, 2004). Isolates of *B. bassiana* from soil were as effective as those isolated directly from insect hosts for *Heterotermes tenuis* Snyder (Almeida et al. 1997). *B. bassiana* ATCC 90519 was reported sufficiently pathogenic against subterranean termites (Wright and Lax 2013). Germ tubes of *B. bassiana* can penetrate the integument of *Reticulitermes flavipes* as early as 16 h after application. On the other end antifungal volatile compounds have been identified in *C. formosanus* nests that may contribute to the inability of entomopathogenic fungi to propagate within the colony (Wiltz et al. 1998). Review by Hussain et al. (2012) on the current status of entomopathogenic fungi as mycoinsecticides is worthy to mention.

4.8.2 Bacteria and Viruses

Among the bacteria, *Bacillus thuringiensis* stands out, representing approximately 95 % of microorganisms used in biological control of insect pests in different cultures (Lambert et al. 1992). Castilhos-Fortes et al. (2002) evaluated the effects of *B.*

thuringiensis subspecies against termite *Nasutitermes ehrhardti* under laboratory conditions. They reported that *Bt. kurstaki* registered <72 % mortality at the seventh day after the bacterial application. Singha et al. (2010) evaluated *B. thuringiensis* and *B. thuringiensis* subsp. *israelensis* for their pathogenicity against two species of tea termites, viz., *Microtermes obesi* and *Microcerotermes beesoni*. They reported that *B. thuringiensis* strains caused >80 % mortality in both the termite species. *B. thuringiensis* subsp. *israelensis*, however, was noticed to be more virulent compared to *B. thuringiensis*. Osbrink et al. (2001) isolated *Serratia* from dead termites and reported that three of the *Serratia* isolates were recorded to induce >85 % mortality within 19 days in Petri dish tests. In another study *Pseudomonas fluorescens* CHA0 was reported to kill subterranean termite *Odontotermes obesus* by inhibiting cytochrome c oxidase of the termite respiratory chain (Devi and Kothamasi 2009). Insect viruses can provide safe, effective, and sustainable control of a variety of insect pests. They are highly specific in their host range, usually limited to a single type of insect. Al-Fazairy and Hassan (1988, 1993) reported that nuclear polyhedrosis virus isolated from *Spodoptera littoralis* infects termite castes of *Kalotermes flavicollis*. They mentioned that all tested castes of termites were quite equal in their response to the virus infection and produced over 90 % mortality at 6.4×10^7 polyhedra/ml concentration.

5 Entomopathogenic Nematodes

Majority of the termite control practices are ineffective and ecologically unsustainable and, above all, do not address the root cause of termite infestation and thus merely provide temporary relief to the problem (Mugerwa 2015). Most termite management practices are focused on total elimination of termite population rather than sustaining their population. EPNs are being produced commercially and used as biological control agents in many parts of the world. Because these nematodes are adapted to the soil environment, the principal hosts are soil insects and insects in cryptic habitats. These nematodes have been utilized in classical, conservative, and augmentative biological control programs. *S. scapterisci* originally isolated from Uruguay was successfully introduced into Florida for the classical biological control of mole cricket pests (Parkman and Smart 1996). In the field, however, entomopathogenic nematodes attack a significantly narrower host range than in the laboratory. Indigenous nematodes are exempted from registration in many European countries, Australia, and the United States, while in other countries, they are subject to similar registration procedures as for a chemical pesticide. Use of these nematodes offers a number of advantages and will be useful in countries where alternative control approaches are needed to replace the more toxic insecticides that are currently in use. With the general public becoming increasingly concerned about pesticide usage, the use of nematodes for termite control is a potentially promising market. Current use of *Steinernema* and *Heterorhabditis* nematodes as biological control organisms has been summarized by Shapiro-Ilan and Gaugler (2010) (Table 4).

Table 4 Current use of *Steinernema* and *Heterorhabditis* nematodes as biological control organisms (Shapiro-Ilan and Gaugler 2010)

Crop(s) targeted	Pest common name	Pest scientific name	Efficacious nematodes
Artichokes	Artichoke plume moth	*Platyptilia carduidactyla*	Sc
Vegetables	Armyworm	Lepidoptera: Noctuidae	Sc, Sf, Sr
Ornamentals	Banana moth	*Opogona sachari*	Hb, Sc
Bananas	Banana root borer	*Cosmopolites sordidus*	Sc, Sf, Sg
Turf	Billbug	*Sphenophorus* spp. (Coleoptera: Curculionidae)	Hb, Sc
Turf, vegetables	Black cutworm	*Agrotis ipsilon*	Sc
Berries, ornamentals	Black vine weevil	*Otiorhynchus sulcatus*	Hb, Hd, Hm, Hmeg, Sc, Sg
Fruit trees, ornamentals	Borer	*Synanthedon* spp. and other sesiids	Hb, Sc, Sf
Home yard, turf	Cat flea	*Ctenocephalides felis*	Sc
Citrus, ornamentals	Citrus root weevil	*Pachnaeus* spp. (Coleoptera: Curculionidae)	Sr, Hb
Pome fruit	Codling moth	*Cydia pomonella*	Sc, Sf
Vegetables	Corn earworm	*Helicoverpa zea*	Sc, Sf, Sr
Vegetables	Corn rootworm	*Diabrotica* spp.	Hb, Sc
Cranberries	Cranberry girdler	*Chrysoteuchia topiaria*	Sc
Turf	Crane fly	Diptera: Tipulidae	Sc
Citrus, ornamentals	Diaprepes root weevil	*Diaprepes abbreviatus*	Hb, Sr
Mushrooms	Fungus gnat	Diptera: Sciaridae	Sf, Hb
Grapes	Grape root borer	*Vitacea polistiformis*	Hz, Hb
Iris	Iris borer	*Macronoctua onusta*	Hb, Sc
Forest plantings	Large pine weevil	*Hylobius albietis*	Hd, Sc
Vegetables, ornamentals	Leaf miner	*Liriomyza* spp. (Diptera: Agromyzidae)	Sc, Sf
Turf	Mole cricket	*Scapteriscus* spp.	Sc, Sr, Sscap
Nut and fruit trees	Navel orangeworm	*Amyelois transitella*	Sc
Fruit trees	Plum curculio	*Conotrachelus nenuphar*	Sr
Turf, ornamentals	Scarab grub[3]	*Coleoptera*: *Scarabaeidae*	Hb, Sc, Sg, Ss, Hz
Ornamentals	Shore fly	*Scatella* spp.	Sc, Sf
Berries	Strawberry root weevil	*Otiorhynchus ovatus*	Hm
Beehives	Small hive beetle	*Aethina tumida*	Hi, Sr
Sweet potato	Sweet-potato weevil	*Cylas formicarius*	Hb, Sc, Sf

Nematode species are abbreviated as follows: Hb = *Heterorhabditis bacteriophora*, Hd = *H. downesi*, Hi = *H. indica*, Hm = *H. marelata*, Hmeg = *H. megidis*, Hz = *H. zealandica*, Sc = *Steinernema carpocapsae* Sf = *S. feltiae*, Sg = *S. glaseri*, Sk = *S. kushidai*, Sr = *S. riobrave*, Sscap = *S. scapterisci*, Ss = *S. scarabaei*

5.1 Under Laboratory Conditions

Hundreds of different species from most orders of insects were susceptible to various entomopathogenic nematodes under laboratory conditions. Entomopathogenic nematodes *Steinernema carpocapsae*, *Steinernema riobrave*, *Heterorhabditis bacteriophora*, and *Heterorhabditis indica* were evaluated by Wang et al. (2002) for their activity against *Reticulitermes flavipes* and *Coptotermes formosanus* in the laboratory. All were noticed effective against *C. formosanus* at ≥400 nematodes per termite. *S. riobrave*, however, had no detectable effect against *R. flavipes* even at a rate of 2000 nematodes per termite. They also demonstrated that *H. indica* was more efficacious (*H. indica* > *H. bacteriophora* > *S. carpocapsae* > *S. riobrave*) at $\alpha = 0.10$ level, and nematodes were able to reproduce from *R. flavipes* and *C. formosanus*. In contrast, Manzoor (2012) reported that *S. carpocapsae* and *H. bacteriophora* when applied individually against the eastern subterranean termite *R. flavipes* showed no detrimental effects on workers and nymphs of the termites. These efficacy differences of the same nematode species between two studies may be due to the differences in the strains of nematode species used in each study.

In another study, Yu et al. (2006) evaluated parasitism of subterranean termites by *S. riobrave*, *S. carpocapsae*, *S. feltiae*, and *H. bacteriophora* in laboratory sand assays. They reported that all tested EPNs were capable of infecting and killing termite species *Heterotermes aureus*, *Reticulitermes flavipes*, and *Gnathamitermes perplexus* (Banks). *S. riobrave*, however, was found to be particularly effective in sand assays and caused significant mortality (≥80 %) of worker *H. aureus* and *G. perplexus* at 22 °C. Nematode concentration and incubation time had significant effects on the mortality. Yu et al. (2008) reported that *S. carpocapsae*, *S. riobrave*, and *H. bacteriophora* successfully reproduced in termite *H. aureus* and infective juveniles (IJs) exited the termite cadavers successfully. However, no progeny were produced by *S. feltiae*. However, Yu et al. (2010) compared virulence of three novel strains of *S. riobrave* (3-8b, 7-12, and TP) against subterranean termites *H. aureus*, *R. flavipes*, and *C. formosanus* workers. *H. aureus* was very susceptible to all the *S. riobrave* strains, and termites in all nematode treatments were dead after 4 days. The TP strain of *S. riobrave*, however, caused 75 % and 91 % mortality in *R. flavipes* and *C. formosanus*, respectively, which was more than 300 % and 70 % higher than the mortality caused by other strains. Screening indicated superior virulence in the 355 (=TX) strain of *S. riobrave* to *H. aureus* compared with the virulence of *S. carpocapsae*, *S. feltiae*, and *H. bacteriophora* (Yu et al. 2006). Thus, *S. riobrave* 355 may have substantial potential to be developed as a biocontrol agent for *H. aureus*.

Higher mortalities in subterranean termite *Macrotermes* caused by *S. pakistanense* were reported by Shahina and Tabassum (2010) in a filter paper and sand assay. Mankowski et al. (2005) examined the attachment and infectivity of two entomopathogenic nematode species, *S. carpocapsae* and *H. indica*, on soldiers and workers in two subterranean termite species, *C. formosanus* and *C. vastator*. In attachment tests with *S. carpocapsae*, they noticed that more nematodes attached to soldiers of *C. formosanus* and *C. vastator*. When soldiers alone or workers alone are exposed to the nematodes, there is a differential susceptibility of soldiers and workers to nematode infection with soldiers being more susceptible than workers. The reason for this differential response to nematode infection is that soldiers do not exhibit grooming behavior. El-Bassiouny and El-Rahman (2011) reported the possibility of using entomopathogenic nematodes *H. baujardi* and *H. indica* against the Egyptian subterranean termite *Psammotermes hypostoma* (Desn.) and *Anacanthotermes ochraceus* (Burm.). The bioagent *H. baujardi* was noticed more effective for control of the two tested termites, but *P. hypostoma* was highly susceptible than *A. ochraceus*. Overall, the mortality increased as the nematode concentrations increased and vice versa.

Differential susceptibility of two termite species, *Macrotermes bellicosus* and *Trinervitermes occidentalis*, against EPNs isolates *H. indica* Ayogbe1, *H. sonorensis* Azohoue2, *H. sonorensis* Ze3, and *Steinernema* sp. Bembereke, from Benin (West Africa), was studied by Zadji et al. (2014a). The results at forty-eight hours post-exposure of workers of *M. bellicosus* to 50 IJ of *H. indica* Ayogbe1, *H. sonorensis* Azohoue2, *H. sonorensis* Ze3, and *Steinernema* sp. Bembereke for each termite were 96.3 %, 87.9 %, 94.5 %, and 75.0 % mortality, respectively. In the case of workers of *T. occidentalis*, under the same conditions, these EPN isolates caused 91.7, 98.5, 75.0, and 95.0 % mortality. Based on concentration-mortality data, they reported that isolates *H. indica* Ayogbe1 and *H. sonorensis* Ze3 were more virulent against *M. bellicosus* with LC_{50} values of 11 IJ, whereas *Steinernema* sp. Bembereke was the most virulent against *T. occidentalis* with LC_{50} values of 12 IJ. All tested EPN isolates can be recycled in both *M. bellicosus* and *T. occidentalis* and the soldiers of both termites studied were noticed more susceptible than workers. Twenty-nine Beninese isolates of *H. sonorensis* and one local isolate of *H. indica* were screened by Zadji et al. (2014b) for their pathogenicity against the termite pest *Macrotermes bellicosus*. Most of the isolates (73 %) killed more than 80 % of the insects. Bioassays, however, showed significant differences among isolates of all tested traits. The greatest survival of infective juveniles to heat (8 h), desiccation (8 h), and hypoxia (72 h) was observed with the *H. sonorensis* isolates Kassehlo (72.8 %), Setto1 (72.5 %), and Kissamey (81.5 %, respectively). Sri Lankan isolates of ENPs were compared with two commercial formulations of entomopathogenic nematodes, namely, Biosafe (*S. carpocapsae*) and Nemasys (*S. feltiae*), against the live-wood termite *Postelectrotermes militaris*. Sri Lankan isolate *Heterorhabditis* spp. (HSL 6) and *S. carpocapsae* were reported better controlling agents against *P. militaris* than other isolates tested (Amarasinghe and Hominick 1993a). Wilson-Rich et al. (2007) reported that damp-wood termite *Zootermopsis angusticollis* exhibits a dose-dependent susceptibility to the soil nematode *S. carpocapsae* (Weiser) (Mexican strain).

5.2 Under Field Conditions

Only a limited number of field studies have been conducted using EPNs as control agents for termites. Certain species of nematodes although effective in laboratory control is often quite variable under field conditions. Soil moisture and soil type appear to limit the nematode's ability to move in the soil and locate termites. In the 1930s Glaser and colleagues applied *S. glaseri* to 73 different field plots in New Jersey to control the Japanese beetle, *Popillia japonica* (Glaser 1932; Glaser et al. 1940). They recovered parasitized grubs from 72 plots 2 weeks after application. Parasitism of the grub population by the nematode in the various plots ranged from 0.3 to 81.0 %. A study testing the efficacy of *S. carpocapsae* (Weiser) against foraging workers of *Reticulitermes tibialis* in pastureland was published by Epsky and Capinera (1988). Study showed potential for control of *R. tibialis* in laboratory and field trials. They also established LD_{50} values for specific nematode/termite combinations. Amarasinghe and Hominick (1993b) reported that higher doses of *S. carpocapsae* and *S. feltiae* showed promising control of live-wood termite *Postelectrotermes militaris* in tea plantations. Nguyen and Smart (1994) isolated EPN *Neosteinernema longicurvicauda* Nguyen and Smart from naturally infected subterranean termites, *Reticulitermes flavipes*.

Heterorhabditis sp. have given encouraging results in Australia. Studies indicate that colonies of *Neotermes* attacking in the South Pacific Islands can be eliminated using *Heterorhabditis* sp. from palms and other trees (Dolinski and Lacey 2007). Populations of the damp-wood termite *Glyptotermes dilatatus* that form colonies of only several thousand members have also been successfully managed with *Heterorhabditis* sp. with a dose of 4000 ml and 8000 ml nematode suspension in doses of 40 ml and 30 ml per tea bush, respectively (Danthanarayana and Vitarana 1987).

In contrast, workers reported that EPNs showed effectiveness against subterranean termites in the laboratory but did not cause colony elimination in the field with *C. formosanus* (Fujii 1975; Tamashiro 1976), *R. flavipes* (Mauldin and Beal 1989), or *R. tibialis* (Epsky and Capinera 1988) which have shown to be minimally controlled by nematodes. Nematodes sold commercially in the United States failed to eliminate colonies of *R. flavipes*, in controlled field experiments (Mauldin and Beal 1989). The reasons suggested were termite social behavior like walling-off dead termites and avoiding foraging in nematode infested areas (Fujii 1975; NPCA 1985). Epsky and Capinera (1988) also reported an ability of the subterranean termite *R. tibialis* to detect and avoid *S. feltiae*. Yanagawa et al. (2012) hypothesized that termites can perceive pathogens and this ability plays an important role in effective hygiene behavior. For the successful application of biopesticides to termites in nature, it would be beneficial to identify substances that could disrupt the termite's ability to perceive pathogens. Different isolates or species of entomogenous nematode species that are tolerant to higher temperatures are required for control of subterranean termite in the fields. Insect susceptibility to entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae varies among insect species and is influenced by nematode species, strain, and an assembly of abiotic and biotic factors. Entomopathogenic nematodes also differ in their abilities to survive different environmental conditions.

5.3 Synergism of EPNs

Several previous studies have demonstrated additive or synergistic relationships between the combined use of low-impact insecticides and biological control agents (Kaakeh et al. 1997; Quintela and McCoy 1998; Koppenhofer and Kaya 1998; Nishimatsu and Jackson 1998). Infective juveniles are tolerant of short exposures (2–6 h) to most agrochemicals including insecticides, herbicides, fungicides, and acaricides (Rovesti and Deseo 1990; Ishibashi 1993) and, therefore, can often be tank mixed. However, some pesticides can reduce nematode infectivity and survival (Grewal et al. 1998). Heterorhabditids tend to be more sensitive to physical challenges, including pesticides, than steinernematids (Grewal et al. 2001). Murugan and Vasugi (2011) evaluated bioactivities of *S. glaseri* in combination with neem seed kernel extract (NSKE) against *Reticulitermes flavipes*. On the fourth day 40 % and 70 % mortality were reported at lower (1.0 %) and higher (4.0 %) concentrations of NSKE, respectively. Neem at various concentrations did not affect the survival of nematodes but had considerable impact on the survival of termites which may be due to the presence of bioactive compounds azadirachtins, salanin, etc. Entomopathogenic nematodes and the chloronicotinyl insecticide imidacloprid interact synergistically on termite mortality. The degree of interaction, however, varies with nematode species. Manzoor (2012) reported synergism between imidacloprid and nematodes species *S. carpocapsae* and *H. bacteriophora* that caused more than 50 % mortality of workers and nymphs of *R. flavipes* within all three colonies tested.

6 Survey of New Species/Strains

Screening of entomopathogenic nematode species or strains for the control of specific insect pest, which are adapted to local environmental conditions, could achieve a higher level of efficacy. Therefore, it is important to survey and preserve indigenous entomopathogenic nematode population. Kary et al. (2009) conducted survey in Iran throughout the three provinces in the northwest of the country and concluded that out of the 833 soil samples, 27 (3.2 %) were positive for entomopathogenic nematodes with 17 (2.0 %) containing *Heterorhabditis* and 10 (1.2 %) *Steinernema* isolates. *H. bacteriophora* and *S. feltiae* were the common species. A survey of entomopathogenic nematodes was conducted for the first time in Nepal by Khatri-Chhetri et al. (2010). Of the 276 soil samples studied, 29 (10.50 %) were positive of EPNs containing seven samples of heterorhabditids (24.14 %) and 22 samples

(75.86 %) of steinernematid. To determine the occurrence of EPNs, Zepeda-Jazo et al. (2014) conducted a survey on the Pacific coast of the State of Colima, Mexico. Of the 19 soil samples collected, 14 (73.7 %) were positive for EPNs, containing 12 steinernematid isolates (85.7 %) and two heterorhabditid isolates (14.3 %). Further they reported that most of the isolates were recovered from cultivated habitats, suggesting that there is a higher prevalence of EPNs in cultivated soils. Jawish et al. (2015) conducted a survey of EPNs of the families Steinernematidae and Heterorhabditidae from January 2011 to December 2013 in Damascus countryside of Syria. Of 189 soil samples studied, 17 (9 %) were positive of entomopathogenic nematode with 11 (65 %) samples containing *Heterorhabditis* and six (35 %) *Steinernema* isolates. Therefore survey and evaluation of new species or strains of entomopathogenic nematodes against termite pests may achieve a higher level of management.

7 Conclusion and Future Prospects

EPNs and their associated bacterial symbionts have been proven safe to vertebrates and other nontarget organisms. They are highly specific in their host range and compatible with many pesticides, can be easily produced in vivo and in vitro, can be applied with standard spray equipment, and have been exempt from registration in many countries. A wide range of entomopathogenic nematodes are effective against various termite species. However, limited number of field studies have been conducted against termites using EPNs. New isolates of EPNs from various parts of the world are being studied, which may prove effective against termite pests in the field. Therefore use of EPNs would bring several benefits to sustainable agriculture and develop a better understanding of biodiversity.

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Foliar Application of Polyamines to Manage Water Stress for Improved Grain Filling Formation and Yield in Rice Plants

Berahim Zulkarami, Omar Mohd Husni, Mohd Saud Halimi, Mohd Monjurul Alam Mondal, Ismail Mohd Razi, and Hossain Kausar

Contents

Abstract Water stress is one of the key restriction factors in rice cultivation worldwide. It reduces the production of rice, the incomes of farmers, and stockpiles of rice and leads to increases in rice imports. Under conditions of limited water supply, filled grains are the most affected part of rice plants, especially at

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the booting stage. However, plants can adapt to such situations, using different approaches in combination with better management practices. The foliar application of polyamines to rice plants could represent an alternative method of managing water stress. The application of polyamines could affect the synthesis of starch and sucrose and enhance enzyme activity in rice grains. This chapter discusses the role of polyamines in enhancing the grain filling of rice by improving starch and sucrose synthesis in both greenhouses and field trials under water-stressed conditions as a possible approach to developing sustainable rice cultivation practices.

Keywords Drought • Rice • Polyamines • Foliar spray • Filled grains • Grain yield

1 Introduction

Rice (*Oryza sativa* L.) is the cheapest source of carbohydrate and protein in Asia and a good source of minerals and fiber. It is susceptible to drought, which causes huge yield losses in many Asian countries (Noorzuraini et al. 2012; Alimullah Miyan 2015). Tuong and Bouman (2003) expect that by 2025, 15–20 million ha of irrigated terrain will experience a water shortage (Fig. 1). In addition, many environmental factors, such as water stress, can reduce the development and yield of rice (Xu et al. 2010; Lobell et al. 2011). Water stress adversely affect rice plants, leading to reduced growth and yield, especially during grain filling. The filling of grain is a crucial stage in rice growth, where the activities of starch-biosynthesizing enzymes

Fig. 1 Effect of water stress on rice plant

actively occur and contribute to rice yields (Mohapatra et al. 2011; Sekhar et al. 2015). In the presence of water stress, the filled grain and yield of rice are reduced. As a result, the incomes of farmers and local stockpiles of rice diminish, a situation that is exacerbated in areas where rice cultivation takes place only once a year. In such a scenario, where rice plants must be managed under conditions of water scarcity, it is desirable to develop methods for maintaining plants for survival while at the same time improving the grain filling and yield of rice plants. The foliar application of polyamines, which is economical, easy to do, and accessible to farmers, could represent a solution to rice plant adaptation in a water-stressed environment (Farooq et al. 2009; Alcazar et al. 2010; Ahmad et al. 2010). This chapter discusses the role of polyamines in enhancing the grain filling of rice by improving starch and sucrose synthesis in both greenhouses and field trials under water-stressed conditions as a possible approach to developing sustainable rice cultivation practices.

2 Effect of Water Stress on Plant Behavior and Responses

Water is the most important natural resource for living beings. Plants are exposed to water stress either when the water that reaches their roots becomes restricted or the transpiration rate becomes excessive. Water stress is mainly caused by drought or high soil salinity (Vinocur and Altman 2005; Shrivastava and Kumar 2015).

Drought is a common occurrence in various parts of the world and a regular phenomenon in agricultural fields in arid and semiarid climates (Fig. 2) (Ahmed

Fig. 2 Impact of drought stress in rice field

et al. 2015). Drought stress affects a combination of critical physiological and biochemical processes in plants, leading to a decrease in growth and yield. Several plant species have modified certain mechanisms to deal with the stress, including drought escape, dehydration evasion, or dehydration tolerance. These mechanisms are the results of a massive amount of morphoanatomical, physiological, biochemical, and molecular changes (Ashraf et al. 2011). Drought stress represents a massive challenge to agricultural production around the world. It results in the loss of stability between the production and drenching of reactive oxygen species (ROS) (de Carvalho 2008), thereby diminishing the actual production potential of crop species. ROS are extremely reactive and can badly harm plants by protein degradation, DNA division, lipid peroxidation, and, finally, cell death (Vinocur and Altman 2005; Ashraf et al. 2011). Filled grains and yield are the most affected aspects of rice plants under water stress, especially at booting stages. Usually, when this happens, empty grains are more numerous than filled grains, which may result in a lower yield of rice.

3 Understanding Grain Filling Formation and Yield in Rice Plants

Grain yield is a quantitative trait which is greatly influenced by different environmental and genetic factors. Rice yield is determined by several agronomic characters such as days to heading, days to maturity, filled grain period, the number of reproductive tillers, number of grain filling per panicle, length of panicle, thousand grains weight and grain weight (Halil and Necmi 2005; Badshah et al. 2014). Different yield components of rice are presented in Fig. 3. The number of tillers and

Fig. 3 Yield components of rice [Tiller/(unit area) x spikelets/panicles x % filled grains x grain weight=YIELD]

number of grains per panicle can be determined at the vegetative and reproductive phases, respectively. The thousand grains weight is an important trait that is normally determined during the ripening phase. A higher number of tillers can be expected in a longer vegetative phase, but the available space or optimum growth will determine the number of reproductive tillers. The tillering stage is important for determining the number of panicles (Wang et al. 2008; Badshah et al. 2014).

Grain filling is an ultimate stage of development in rice grains (fertilized ovaries develop into caryopses) and depends on current assimilates from photosynthesis and assimilates reallocated from storage pools in vegetative tissue (Thameur et al. 2012). Culms and sheaths provide 10–40 % of the ultimate yield (Takai et al. 2005; Asli and Houshmandfar 2011). Remobilization and relocation of reserve assimilates in vegetative tissues to the grain in monocarpic plants such as rice and wheat involve the initiation of whole plant senescence (Nooden et al. 1997; Asli and Houshmandfar 2011). Normally, water stress in a filled grain period encourages early senescence and reduces the time of filled grains but enhances the remobilization of assimilates from straw to grains (Asseng and Van Herwaarden 2003; Plaut et al. 2004; Thameur et al. 2012). Slowly filled grains can frequently be related to the delay of whole-plant senescence (Mi et al. 2002; Gong et al. 2005; Mohapatra et al. 2011).

In grains, sucrose synthase, ADP glucose pyrophosphorylase (AGP), starch synthase (StS), and starch branching enzyme (SBE) are the main enzymes involved in the conversion of sucrose into starch. These enzymes are associated with the starch accumulation rate in grains (Yang et al. 2003; Pandey et al. 2012). In the source (stems), better activities of α-amylase and sucrose phosphate synthase result in the fast hydrolysis of starch and enhanced carbon remobilization in the source (stem), whereas in the sink (grains), an improved grain-filling rate is attributed to increased sink activity resulting from the management of the key enzymes that are involved in the conversion of sucrose into starch (Yang and Zhang 2010; Pandey et al. 2012). We have summarized all the aforementioned grain-filling formations in Fig. 4.

3.1 Polyamines and Their Roles in Adaptive Drought Tolerance Mechanisms

Polyamines reduce water stress in plants through osmotic adjustment (Ahmad et al. 2012). They include spermidine (*Spd*), spermine (*Spm*), and putrescine (*Put*), which are small, ubiquitous nitrogenous compounds that resemble plant growth regulators and also serve as secondary messengers signaling pathways during stressful conditions (Liu et al. 2007; Kusano et al. 2008; Ahmad et al. 2012; Tiburcio et al. 2014). However, polyamines are involved in various physiological activities in plants such as growth, senescence, and stress responses (Tiburcio et al. 2014). Richards and Coleman (1952) for the first time reported on the association of polyamines with abiotic stress tolerance. Subsequently, several other studies were conducted on the role of polyamines in abiotic stress tolerance (Nayyar et al. 2005; Alcazar et al.

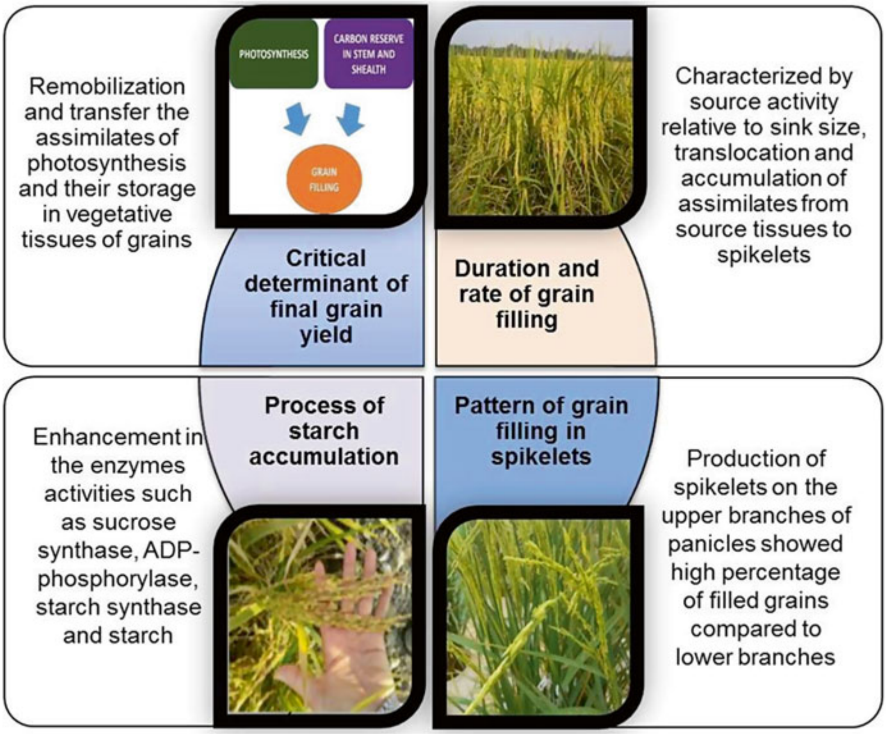

Fig. 4 Schematic procedure of grain filling in rice

2006; Hamdani et al. 2011). Although polyamines can function as free radical scavengers, they also serve to protect membranes against oxidative damage (Besford et al. 1993). Stress-tolerant plants have been found to gather more polyamines than sensitive ones (Lee 1997). Nayyar et al. (2005) reported that exogenous application of *Put* and *Spd* significantly enhanced soybean drought tolerance. Earlier researchers reported that the endogenous products of polyamines are related to enhanced water stress tolerance in rice (Yang et al. 2007; Farooq et al. 2009; Hamdani et al. 2011). In addition, additional functions of polyamines are emerging, such as signaling by inducing nitric oxide (Tun et al. 2006).

To understand this complex mechanism, a diagrammatic representation of drought stress and the effect of polyamines on plants is shown in Fig. 5. Polyamines are protonated at a normal cellular pH, and their biological purpose was initially identified as being related to their ability to bind with different anionic macromolecules (such as DNA, RNA, chromatin, and proteins), thereby restricting them as substances with a structural role. However, it was subsequently confirmed that in addition to stabilizing macromolecules, polyamines serve as regulatory molecules in various basic cellular processes like cell division, differentiation and proliferation, cell death, DNA and protein synthesis, and gene expression (Childs et al. 2003; Seiler and Raul 2005; Kusano et al. 2008; Alcazar et al. 2010). In plants, polyamines

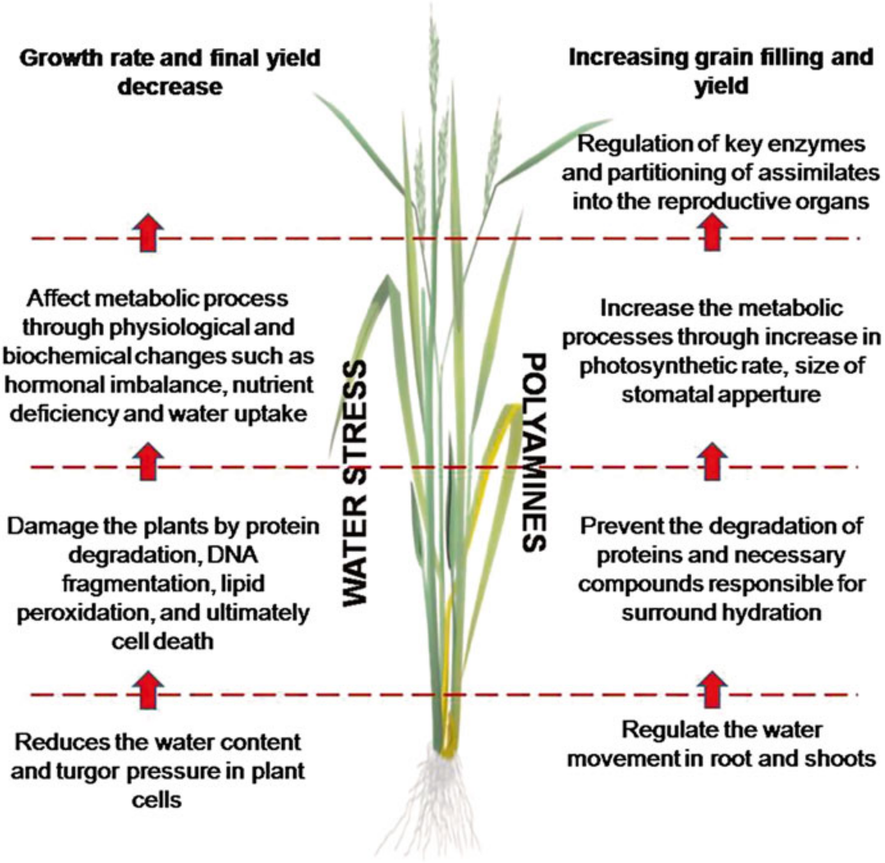

Fig. 5 Overview of effect of drought stress and polyamines on rice plants

have been involved in numerous physiological processes such as organogenesis, embryogenesis, floral initiation and development, leaf senescence, fruit development and ripening, and abiotic and biotic plant stress responses (Alcazar et al. 2006; Kusano et al. 2008; Ahmad et al. 2012). Several studies also showed that polyamines play a significant role in the transduction of signals involved in cross talk with hormonal pathways needed for the regulation of abiotic stress (Alcazar et al. 2010; Ahmad et al. 2012). Abscisic acid (ABA) is an endogenous antitranspirant that decreases water loss via stomatal pores on leaf surfaces. Liu et al. (2000) and An et al. (2008) reported that *Put*, *Spd*, and *Spm* also control responses of stomata by decreasing their opening and inducing closing. It is therefore assumed that polyamines participate in ABA-mediated stress responses occupied in stomatal closing. In this observation, observations point to relations between polyamines with ROS production and nitric oxide (NO) signaling in ABA-mediated stress responses (Yamasaki and Cohen 2006; Pottosin et al. 2014). The production of ROS is strongly connected to polyamine catabolic processes because amino oxidase produces hydrogen peroxide (H_2O_2), which is a ROS related to plant protection and abiotic stress responses (Cona et al. 2006; Pottosin et al. 2014). All together, the available data indicate that polyamine ROS (H_2O_2) and NO act synergistically in encouraging ABA responses in protector cells (Alcazar et al. 2010).

Our group has conducted several experiments to evaluate the efficacy of polyamine formulations on water-stressed rice plants in a greenhouse and in the field. Foliar application of polyamines produced significantly higher grain filling and yield, even under conditions of water stress (increased by 51 % in field trials and 41 % in a greenhouse under cyclic water stress at 10 day intervals) (Table 1). More interestingly, the sucrose and starch contents were improved by 28 and 13 % in unpolished grain and 36 and 22 % in polished grain at 105 days after sowing with the application of polyamines. An additional application of polyamines enhanced starch and sucrose accumulation in grains proportionally to grain yield. These results confirmed that even under conditions of water stress, rice plants still maintain cell turgor pressure to produce sucrose and starch in grains, especially at the reproductive stage. The better yields of rice with the foliar application of polyamines are presumably due to their ability to maintain the turgor pressure of cells in water-stressed rice plants (Ashraf et al. 2011). They may protect plants from damage or water loss and directly affect grain filling and yield. This approach helps plants to thrive under conditions of water stress (Figs. 5 and 6) by maintaining their normal metabolic processes and growth (Blum 2005; Chimenti et al. 2006).

Table 1 Effect of foliar application of polyamines on yield attributes in rice plants

Treatments	No. of grains per panicle	No. of panicles per hills/m^2	Panicle length (cm)	Grain filling (%)	1000 grain weight (g)	Yields (t ha^{-1})	Increase in yield (%)
Greenhouse conditions (UPM, Malaysia)							
Control	118b	13b	37.7b	91.0b	24.2b	9.1b[a]	–
Normal	132a	16a	40.4a	94.4a	25.6a	10.9a[a]	17
Control	97b	11a	NA	91b	23.1b	6.6b[a]	–
Saturated	118a	10a	NA	94.5a	24.4a	7.5a[a]	12
Control	107b	9b	15.2b	93.2b	20.6b	5.0b[a]	–
CWS at 10 days	130a	17a	29.3a	95.0a	22.7a	8.5a[a]	41
Field conditions (KADA, Melor, Kelantan)							
Control	114b	77b	23.0b	47.2b	19.1b	2.2b	–
Water stress	130a	93a	25.0a	64.8a	22.0a	4.5a	51

CWS at 10 days = cyclic water stress at 10 day intervals; UPM = Universiti Putra Malaysia; KADA: Kemubu Agricultural Development Authority; NA = not analyzed; Means within column followed by the same alphabets are not significantly different ($p < 0.05$) by Duncan's multiple range test (DMRT); Foliar applications of polyamines at 35 and 55 days after sowing (until wet at glasshouse and 180Lha^{-1} at the field conditions) in normal, saturated, CWS at 10 days and field conditions.

[a] Estimated yield based on grain weight per pot

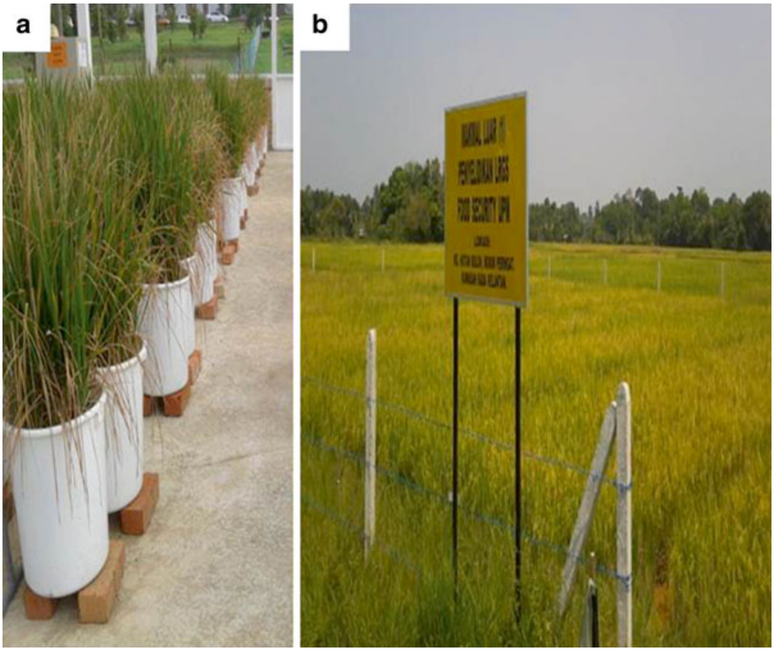

Fig. 6 Effect of foliar application of polyamines on water-stressed plants (**a**) in a greenhouse and (**b**) under field conditions

The grains in polyamine treatments show more filled grain (better sucrose and starch accumulation) even under water stress (Table 1, Figs. 7 and 8). Polyamines might be involved in improving assimilate partitioning in favor of spikelets on secondary branches and raise the number of high-density grains in panicles in order to achieve a high yield potential (Kamoshita et al. 2008; Mohapatra et al. 2011) and enhance the remobilization of assimilates from straw to grains (Asseng and Van Herwaarden 2003; Plaut et al. 2004). They also affect several important enzyme activities, such as sucrose synthase, adenine diphosphoglucose, pyrophosphorylase, starch synthase, and starch branching enzyme, believed to take part in major functions of processes that determine starch quality and grain filling (Yang et al. 2003; Mohapatra et al. 2011). It has been found that increasing sucrose and starch accumulation produces better hydrolysis of starch and improves carbon remobilization; on the sink side (grains), an enhanced grain-filling rate mostly contributes to enhanced sink activity by regulating key enzymes involved in sucrose-to-starch alteration those similar findings with Yang and Zhang (2010).

From a commercial point of view, the additional 5.2 % cost of polyamines (foliar applications with 180 L ha^{-1}) is associated with a better harvest index. The income of farmers could be increased to between RM 293 and RM 1138 per month per hectare. The extra income of farmers brought about by the use of foliar applications of polyamines was enhanced, especially in water-stressed conditions in glasshouse and field experiments (Table 2).

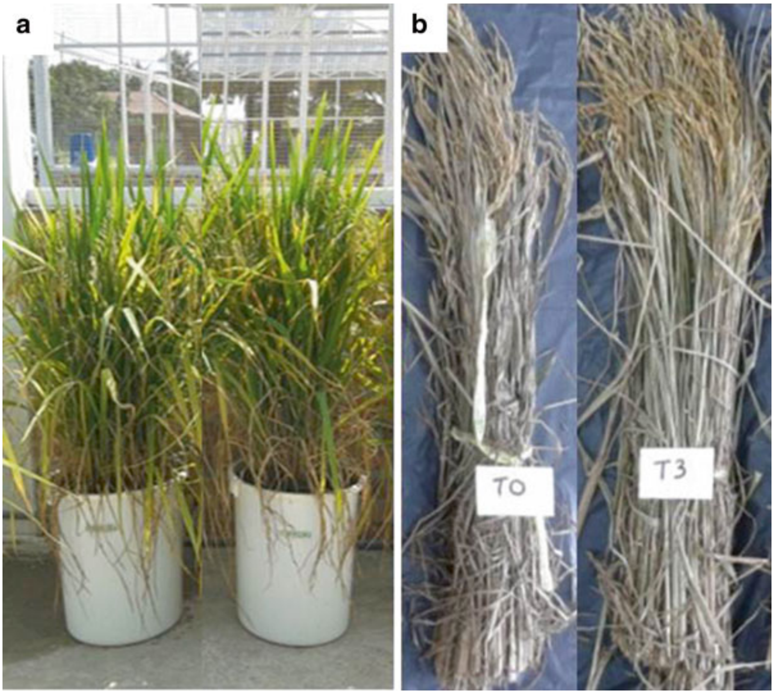

Fig. 7 Effect of polyamines on growth performance of rice (**a**) under greenhouse conditions and (**b**) dried rice plants from field experiments

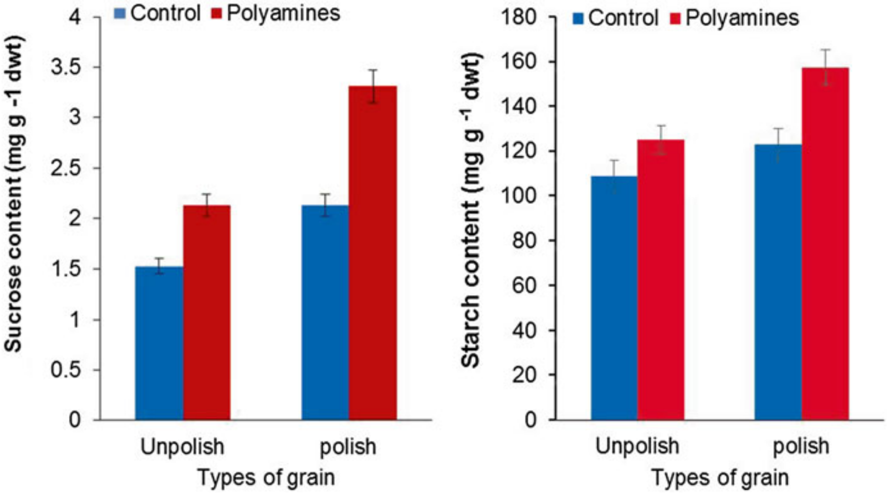

Fig. 8 Effect of sucrose and starch content on grains at 105 days after sowing (DAS) in between control and polyamines under water-stressed conditions

Table 2 Effect of foliar spray of polyamines on rice harvest index, yield gap, increased farmer income, and monthly income

Treatments	Harvest index (%)	Yield gap (t ha^{-1})	Increase in income (RM ha^{-1} season^{-1})	Increase in monthly income (RM ha^{-1})	Percentage increase in cost (ha^{-1})
Glasshouse conditions (UPM, Malaysia)					
Control	37a	–	–	–	–
Normal conditions	36a	1.8	2340	585	5.2
Control	NA	–	–	–	–
Saturated conditions	NA	0.9	1170	293	5.2
Control	32b	–	–	–	–
CWS at 10 days	42a	3.5	4550	1138	5.2
Field conditions (KADA, Melor, Kelantan)					
Control	61a	–	–	–	–
Water stress	51b	2.3	2990	748	5.2

CWS at 10 days = cyclic water stress at 10 day intervals; UPM = Universiti Putra Malaysia; KADA: Kemubu Agricultural Development Authority; NA = not analyzed; Means within column followed by the same alphabets are not significantly different ($p < 0.05$) by Duncan's multiple range test (DMRT).

4 Conclusions and Future Prospects

It is important that we understand the foundation of the rice plant's finely tuned sensitivity to water stress, because if we can overcome it, the advantages for crop growth and food production may be substantial. In the later stages of the plant's development, carbohydrate supply to the developing grains can be crucial if yield is to be maximized. Grain-filling processes are highly sensitive to soil drying, and these processes are another important target for biotechnologists who are interested in enhancing crop yield under dry land environments.

This chapter emphasized the ways in which an enhanced understanding of the physiology of plant growth and development under conditions of stress may be exploited using relatively simple technology to sustain crop yield as water resources are becoming increasingly limited, especially under a changing climate. The way forward is to establish a research program that will make it possible to achieve short-, intermediate-, and long-term outcomes for food security on the basis of ongoing research that addresses climate change challenges using polyamines.

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