Rizwan Ali Ansari · Irshad Mahmood
Editors

Plant Health Under Biotic Stress

Volume 1: Organic Strategies

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Editors
Rizwan Ali Ansari
Section of Plant Pathology
and Nematology, Department of Botany
Aligarh Muslim University
Aligarh, Uttar Pradesh, India

Irshad Mahmood
Section of Plant Pathology
and Nematology, Department of Botany
Aligarh Muslim University
Aligarh, Uttar Pradesh, India

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Preface

In order to understand the plant fitness entirely, it is high time for researchers to relinquish the obsolete theories and must unravel unexplored aspects pertaining to plant health. The book “Plant Health Under Biotic Stress” is being published in two volumes to provide the articulated, justified, and updated information which are either directly or indirectly related to soil and plant health. Plant Health Under Biotic Stress – Volume 1 (Organic Strategies) flashes light on the key literature related to application of organic matters including phytoextracts, chopped leaves, composted organic manures, and liquid manures in eco-friendly agriculture. The mechanisms underlying the effectiveness of these organic amendments in promoting plant health has also been presented and discussed in understandable ways. Although most of the chapters of volume 1 are limited to organic and their applications in the management of biotic/pathogens stress, however, some chapters related to actinobacteria have also been included just to widen the horizon of our understandings. We hope this book will be useful to advisers, extension officers, educators, and advanced researchers who are concerned about the protection of environment and plant health.

A sincere acknowledgment is extended to Prof. Tariq Mansoor, Hon’ble Vice Chancellor, Aligarh Muslim University, Aligarh, India, for being a constant source of inspiration for the researchers.

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This book would have remained just a dream if Dr. Rose Rizvi has not come and taken up each hurdle translating it into an enjoyable moment. She assisted us from onset of this journey and therefore indeed deserves to be acknowledged with great appreciation. In addition, Dr. Sartaj A. Tiyagi, Dr. Safiuddin, Dr. Aisha Sumbul, Mr. Hari Raghu Kumar, and Ms. Aiman Zafar were constantly surrounded with us whenever we felt like giving up – sincere thanks to all of them.

Editors would have not completed this task without endless support, prayers, and encouragements of their elders during light and dark situations.

We can never forget our "little doctor," Mr. Ayan Mahmood, who used to practically look up and smile at us with two lovely and twinkling eyeballs, each time muttering words of comfort and encouragement.

We hope that our efforts to forward the readers toward the better state of plant science shall be fruitful.

Aligarh, India

Rizwan Ali Ansari
Irshad Mahmood

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About the Editors

Dr. Rizwan Ali Ansari is a young and active member of the Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India. He obtained his PhD from the same university and has been involved in the research and development strategies of plant pathology/nematology. He has been engaged in the formulation of management modules involving various microorganisms, antagonists and organic additives active against a wide range of soil-borne plant pathogens infesting several agricultural crops. He has also attended several national and international conferences so far and received prestigious awards by various scientific societies like the Society of Plant Protection Sciences (SPSS) and Nematological Society of India (NSI) for his outstanding contribution in the field of plant pathology/nematology. He has published several book chapters, research and review articles pertaining to the utility of organic additives, mycorrhizal fungi as well as plant growth-promoting bacteria in the sustainable management of plant pathogens in various journals/books of great repute. Judicious application of organic additives and biological agents in the management of plant diseases, amelioration of soil and crop health and survey on disease prevalence caused by soil-borne pathogens on various economically important crops are the current research domain of Dr. Ansari.

Dr. Irshad Mahmood is working as a Professor of plant pathology and nematology in the Department of Botany, Aligarh Muslim University, Aligarh. He obtained his PhD from Aligarh Muslim University in the field of plant pathology and nematology. Promotion of organic farming across the world by utilizing organic additives and potent microorganisms for the sustainable management of phytoparasitic nematodes and plant pathogenic fungi resulting to augment soil and plant health is the domain of his research. He has been engaged with teaching programme of undergraduate and postgraduate-level students for the last 30 years and has many overseas visits including the United States, France and the United Kingdom. He has attended a significant number of national and international conferences pertaining to wide area of agricultural sciences and published around 150 original research papers, review articles and book chapters in various refereed national and international publication media, most of them in very high impact factors. He has successfully completed many training courses in various ICAR-sponsored research institutes in India and also in North Carolina State University, Raleigh, USA. He is also an active member of national and international scientific organizations; an expert for selection committee; a reviewer of journals, doctoral theses and funding agencies; and a recipient of Scientist of the Year award in the field of plant pathology and nematology. He has guided ten PhDs, several MPhil and a large number of MSc dissertations. He has also been engaged in the establishment of a joint government project with Aligarh Muslim University for improvement of infrastructural facilities in botanical garden to facilitate ex situ conservation and propagation of rare, endangered and threatened plants and the plants endemic to the region.

Chapter 1
Organic Soil Amendments: Potential Tool for Soil and Plant Health Management

Rizwan Ali Ansari, Aisha Sumbul, Rose Rizvi, and Irshad Mahmood

Abstract Utilization of organic matter as a chief substrate for agricultural crops and beneficial microorganisms is gaining interest of plant pathologists, agronomists, manufacturing and processing industries, regulators, growers, tycoons and consumers. These organic inputs provide energy and nutrients to soil leading to a considerable change in the environment which becomes appropriate for survival of crops and proliferation of microorganisms. More likely, this exercise is further reinforced by the consumers' demand as they are more conscious towards their health. Moreover, use of organic matter rather than disposal is preferred because it imparts in the market value and recycles back to the land leading towards the enhanced sustainable agricultural system. Various types of organic materials are now available and growers have been familiar with these wastes. However, efficacious nature of each organic matter is different maybe partly due to their chemical constituents, types, origin and duration of decomposition. Henceforth, the results of these natural products are inconsistent from site to site as well as from field to field. Similarly, there is no single mechanism which can advocate the queries prudently pertaining to disease management caused by various soilborne plant pathogens. Some common instances have, however, been exemplified like secretion of pathogen toxic compounds, alteration in soil physico-chemical properties, enhanced microbial activities and induction of host resistance against wide spectrum of soilborne pathogens. Moreover, soil is indistinct part of the ecosystem which may regulate the plants response. Application of low rate of organics is suggested as this will be affordable to the growers. In our opinion, this may be possible through appropriate site selection, formulation, storage and handling as well as consortia of organic matter with other compatible modules. Major problem in the adoption of this technology is insufficient supply of ready-made organics which needs a prudent optimization in order to attain sustainable agriculture.

Keywords Soil · Organic inputs · Microorganisms · Physico-chemical properties · Disease suppression · Growth enhancer

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R. A. Ansari (✉) · A. Sumbul · R. Rizvi · I. Mahmood
Section of Plant Pathology and Nematology, Department of Botany, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

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1.1 Introduction

Organic soil amendments such as animal manures composts, green manures, cover crops, crop residues, straws, etc. are used to augment soil and plant health that leads to sustainable agriculture (Ansari et al. 2017a; Akram et al. 2016; Rizvi et al. 2015; Hadar et al. 1992; Muchovej and Pacovsky 1997; Trankner 1992). There were no synthetic pesticides, insecticides, inorganic fertilizers for application to the field during the beginning of the agriculture. The agriculture practices were totally dependent on cultural practices such as organic inputs, crop rotation, soil solarisation, deep ploughing, etc. Besides, agriculture as an occupation provided very important basic necessities of human being – food, shelter and clothes. However, in the nineteenth century, pesticides, inorganic fertilizer and pest-resistant varieties had replaced the classical practices almost in toto which results in a considerable breakage of the link between organic fertilizers and soil fertility (Hoitink and Boehm 1999; van Diepeningen et al. 2006; Willer et al. 2010). Consequently, organic matters like animal manure, green manure, industrial wastes (after treatments), households waste, etc. transformed into solid wastes. Long-term storage of such wastes started to cause soil, air and water pollution. Various plant diseases caused by soilborne pathogens become more aggressive to the crop plants. Henceforth, public concern and adverse effects of inorganic fertilizers on human health have received attention in organic fertilizers (Lazarovits 2001). In addition, new emerging technologies are being added in order to fulfil the ever-growing demand for food due to significant increase in population. Organic inputs grant the energy and become the ample source of nutrients to soil which creates suitable environment for the proliferation of microorganisms (Drinkwater et al. 1995). A wide range of biofertilizers has been used to control the different soilborne diseases including plant parasitic nematodes (Ansari et al. 2017b; Khan et al. 2014; Akhtar and Malik 2000; Rodríguez-Kábana 1986). Moreover, composts derived from various sources are also being used in the management of various plant pathogens (Hadar and Mandelbaum. 1992; Hoitink et al. 1993). Organic matter is used as soil amendments in order to maintain good health of soil which create conducive environment to the plant. Also, incorporation of organic inputs into soil with or without any beneficial microorganisms offers pollution-free environments (Jindo et al. 2016).

Soil is an intimate part of ecosystem but its conservation in the present scenario has been a big challenge. Moreover, it has the capability to interact with a wide spectrum of organisms in order to maintain better quality and conducive environment for microflora and rhizospheric organisms. Generally, soil quality is quickly deteriorated due to improper intensification of agricultural systems. However, proper management strategies, if applied, improve soil quality in terms of physical, chemical and biological characteristics. Interestingly, organic matter application in the soil plays a very important role in the maintenance of soil ecosystem. Organic matter becomes the substrate for the decomposers which in turn provides nutrients to the soil and plant (Abiven et al. 2009). More likely, proper incorporation of organic matter also increases the soil suppressiveness against wide range of phytopathogenic propagules (Bonanomi et al. 2010) and minimizes toxicity level of heavy metals (Park et al. 2011). Moreover, a number of organic matters, viz. compost, keep much importance in the ameliorations of soil structure (Scotti et al. 2013), biological activity (Ross et al. 2003; Ansari and Mahmood 2017; Franco-Andreu et al. 2016) and reductions in soilborne pathogens (Pane et al. 2016). Besides, depletion of soil organic carbon is directly correlated between the organic inputs and amount of organic matter present in the soil. These are mainly regulated by some environmental factors such as temperature and available water content. Generally, it has been noticed that plant debris amendments to soil contain high amount of organic. This type of organics when amended into the soil decomposes very rapidly and almost disappears within a few months (Bonanomi et al. 2013). Moreover, such organic input provides marginal contributions for the sustenance of soil organic C sink. In addition, transport of such organic C with high biochemical quality may be stimulated through microbial activities (Steiner et al. 2007; Fontaine et al. 2007).

Moreover, it is known worldwide that phytopathogens are responsible for many diseases of crop plants that exert physical as well as mental stress on farmers (Anonymous 2017). Around 50% of plant diseases of main crops in the United States were caused by soilborne phytopathogens (Lewis and Papavizas 1991). Awareness towards the maintenance of harmonious environment pertaining to agriculture has given an impetus to search out alternative to conventional agriculture. Now farmers and researchers have began to use organic matter as fertilizers in order to meet out the goal of sustainable agriculture. There is a wide range of organic matter which is being used in the sustainable agriculture, where compost is considered to be one of the best organic fertilizers. Moreover, composting has been the chief tactics in order to minimize the nutrient loss and rapid decomposition. Subsequently, microbial activities are enhanced providing a balanced nutrients to the soil and ultimately to the crop plants. This way, organic matters are transformed into valuable assets that remain embedded in the soil. Therefore, present collection of literature has been designed in order to explore the recent development in organic soil amendments.

1.2 Possible Sources of Organics

Integration of organic matter to ameliorate soil physical, chemical and biological properties dates back since beginning of the agriculture. It has been extracted from literature that Greeks and Roman had applied animal manures to soil for better yield of crop plants (Goss et al. 2013). A wide range of organic matters such as seashells, vegetable waste, farmyard manure and other waste products are used to enhance plant growth and productivity. There are various types of organic materials and difficult to mention in a short passage. Applications of such organic input vary and controlled by various significant factors. Some important organic materials have been used more commonly such as animal manure, compost, different types of shells, saw dusts, straws, green manures, crop residue, phytoextracts, etc. They are used first hand as plant growth enhancer while on the other hand, considerable amount of disease suppression (Tiyagi et al. 2015). However, the same time an appropriate treatment (such as municipal solid waste) prior to application is given to rescue the environment from pollution. Nowadays, compost is the most common organic matter used as plant growth enhancer (Jouquet et al. 2011). Soil application of compost derived from various sources not only strengthens the plants but also induces resistance in host against wide range of phytopathogens. Besides, animal manures, peat moss, wood chips, straw and municipal wastes are also used to strengthen the plants against various soilborne pathogens leading to enhanced crop productivity (Misra et al. 2016; Smith et al. 2016).

1.3 Types of Organic Amendments Applied to Soils

Various forms of organic matter as soil amendments have been noticed to promote crop productivity and maintain the soil health. They have been categorized in six major categories (Goss et al. 2013).

1.3.1 Animal Manure

Most of the manure produced is applied in land to enhance the soil fertility, plant growth and yield attributes. In the 1950s removal of manure along with water was started to reduce labour cost and improve hygiene. Later on, these liquid manures were applied in the field for the enhancement of crop yield. Generally, beef and dairy systems generate the highest amount of manures followed by pork industry, while poultry farm contributes very small amount as compared to cattle or pig. Later on, these manures are applied in land in different manners such as 84% in croplands and 16% grassland (Beusen et al. 2008). Nature of manures, however, is inconsistent and varies from time to time and depends upon the storage duration prior to its application to the land. Moreover, liquid or slurry manures may have number of layers, and property of each layers vary considerably with each other in terms of space and time (Patni and Jui 1987). More broadly, organic manures aerobic decomposition results in the generation of CO_2 and wide spectrum of organic compounds, while anaerobic decompositions begin in the stored manures. In the absentia of free oxygen, the organic inputs are converted to C compound having low molecular weight chiefly volatile organic substances and eventually CH_4 is released (Lazarovits 2001). In addition to these organic C and volatile substances, breaking down of the proteins may lead to the generation of H_2S. Likewise, generation of volatile fatty acids due to organic matter breakdown leads to reduced pH of the manure which is readily available to microbes as C sources (Lazarovits 2001). Moreover, the rate of breaking down process in aerobic conditions is faster than the anaerobic one. Likewise, disintegration of organic matter under aerobic conditions is much faster than the anaerobic conditions. Besides, liquid swine manure incorporations in the dried soil was more effective than the moist soil, apparently, because active chemical constituents were diluted in the moist (Lazarovits 2001) (Fig. 1.1).

1.3.2 Municipal Biosolids and Septage

Municipal wastes after proper treatment are applied in agricultural land in order to promote crop productivity. But, prior to application of municipal waste (solid or liquid), are subjected to pass through regulatory norms (Kumar 2016). Generally organic inputs are separated through sedimentation (primary treatment) following to digestion of easily metabolized fractions by microorganisms (secondary treatments) and, lastly, removal of N and P (tertiary treatments) (Goss et al. 2013). In addition, stabilization of the materials by heating and drying process is carried out. The stabilization is performed to eliminate the propagules of wide range of phytopathogens. However, not all European countries are applying the municipal wastes into the agricultural land. But, recently a figure has come out such as during 1996–1998 France used 60%, Spain and the United Kingdom 46%, Germany 40% and Italy 16% (Epstein 2003). It has also been observed that around 50–70% of sewage solids are applied into agricultural lands. Many rural areas of the world do not have proper sewage systems, however, they have established a holding tank which is essential to be pumped out periodically. There are many jurisdictions in the application of municipal biosolids; nevertheless, many people have started to use these untreated materials directly to the fields. Such application of municipal waste may create an environmental perturbation. Henceforth prior to application to the agricultural land, certain confirmatory test as per prescription made by the Pollution Control Board is needed to carry out to avoid ambiguity amongst the researchers or growers of the crop plants.

Fig. 1.1 Types of organics used in organic agriculture

1.3.3 Green Manures and Crop Residues

Green manures are grown to augment the nutrient status of the soil leading to improved plant health. Generally, legumes are grown in the agricultural land because they fix atmospheric N and leave some amount of it for the succeeding crop (Reddy 2008). A wide spectrum of green manures has been identified throughout the world. Green manuring have exerted a beneficial impact on soil health through various improved ways like chemical (Ebelhar et al. 1984) and biological properties (Fageria et al. 2005). Incorporation of green manure has enhanced plant growth in terms of higher biomass production. Application of green manures into soil provides good habitat for beneficial microorganisms. In addition, some properties of soil such as water holding capacity, infiltration of water and percolation of water were considerably increased (Raimbault and Vyn 1991).

Green manure crops grown in summer remain on the field for a short period of time, whereas warm seasons cover crops may be utilized to replenish the niche in crop rotation, to keep fragile soil from weathering, to prepare land for a perineal crop or to supply extra animal feeds. Some examples of summer green crops may be seen which are being grown in our surroundings such as *Vigna unguiculata* (Singh et al. 2010), *Glycine max* (Creamer and Baldwin 2000), *Melilotus indicus* (Sarrantonio and Gallandt 2003), *Sesbania* spp. (Sugumaran et al. 2016), *Crotalaria* spp. (Wortmann et al. 2009) and *Mucuna pruriens* (Whitbread et al. 2004). These crops add N along with organic matter to soil. Most of the beneficial impacts expected from the green manuring come from the aerial parts of the plant (Goss et al. 2013).

1.3.4 Food Residues and Wastes

The foods which are discarded or lost uneaten is called as food wastes or food discharges. Fresh produce from the supermarket or other sources of materials from urban centres that have not been sold timely or unusable – food discharges. Later on they are eventually applied to agricultural land after composting (Muchovej and Obreza 2001; Obreza and O'Connor 2003). There are numerous reasons of food wastes and found at the stage of food production, processing, retaining and also on consumption (Galanakis 2015). Total food wastes world widely have been estimated to be around 1.3 billion tonnes (F.A.O. 2011). In developing countries 400–500 calories per day per person are wasted, while in developed countries this figure has significantly enhanced and been found to be 1500 calories per day per person (Kim 2014). Likewise, around 30–50% (1.2–2 billion tonnes or 1.8×10^9 long tonnes or 1.32×10^9–2.20×10^9 short tonnes) of all produced food remain unconsumed (Fox and Fimeche 2013). Country wise, Singapore wasted 788,600 tonnes of food wastes in 2014 (http://www.straitstimes.com/print-edition), the United Kingdom 6,700,000 tonnes (Jowit 2007, https://www.theguardian.com/

environment/2007/oct/28/food.foodanddrink), the United States 30% of food valuing 162 billion US dollar (Elizabeth 2014) and Denmark 700,000 tonnes per year of food wastes (Juul 2016). To tackle with food wastes problems, there are some ways through which these food wastes can be recycled such as use of fertilizers after decomposition (https://en.wikipedia.org/wiki/Food_waste). Moreover, food wastes can be biodegraded after composting and recycled the nutrients into soil (https://www.usda.gov/oce/foodwaste/resources/recycle.htm).

1.3.5 Wastes from Manufacturing Processes

Organic matter may also include the residual organic matter obtained from various manufacturing industries as discharge (Dotaniya et al. 2016). Biosolids are produced in large amounts annually as residues in paper making industries, but only a small amount of these wastes products are used in agricultural system (Thacker 2007). Some examples of manufacturing waste are very common which are used to support the crop production such as sugar extracts from sugar beet (*Beta vulgaris* L.) and distillery waste (Douglas et al. 2003; Hachicha et al. 2012; Kumar et al. 2009). In addition, use of wastes of sugarcane processing industries has been found to be beneficial in agricultural system as this has improved the soil physico-chemical properties leading to enhanced plant biomass (Dotaniya et al. 2016). Several other industries are discharging its wastes in significant amount and started to use in crop production (Arvanitoyannis et al. 2006). Exclusively, waste collected from wine industries can be potentially used as soil conditioners as well as fertilizers (Ferrer et al. 2001). Moreover, different types of wastes have been determined such as grape pomace characterized by abundant phenolics due to poor extraction during the wine preparations. Henceforth, their utilization in cultivable land supports crop production leading to improved sustainable agriculture (Kammerer et al. 2004). Likewise, different types of wastes from sugarcane industries are characterized by soft, spongy, amorphous and brown to black in colour containing higher amount of nutrients of wide spectrum (Ghulam et al. 2012; Dotaniya et al. 2016). Moreover, press mud is generated during sugar purification through various processes like sulphitation and carbonation (Dotaniya et al. 2016). Press mud is a good source of organic matter and provides sufficient nutrient to plant and also improves soil health (Bokhtiar et al. 2001; Razzaq 2001). Similarly, bagasse is another discharge generated by the sugarcane industries which can be used to support agricultural system. Constituent wise, bagasse contains cellulose (47–52%), hemicelluloses (25–28%), lignin (20–21%) and other compounds (0.8–3%) (Rocha et al. 2011). Henceforth, it can be concluded that bagasse may be used to support crop production. As far as molasses are concerned, it is generated when raw juice is used to produce sugar. They are viscous liquid in nature and may separate through massecuite. Molasses are having various types of nutrients which contain enhanced microbial activities being utilized for alcohol production (Dotaniya et al. 2016; Sardar et al. 2013). More broadly, raw spent wash are acidic in nature and produced after fermentation and distillation and leaving unpleasant smell especially just after its generation. Later on, these raw spent wash are treated for its further use in various sectors of agriculture. Biomethanation is considered to be most reliable process which can purify such organically rich wastes. Biomethanated spent wash are rich in various nutrients and enhance the microbial activity when applied in the field as liquid manure (Dotaniya et al. 2016).

1.3.6 Compost

Composts derived from wide range of sources have been top ranked amongst the organic inputs being used in the various agricultural sectors (Goldstein et al. 2000; Martínez-Blanco et al. 2013; Cesaro et al. 2015; Alsanius et al. 2016; Oliveira et al. 2017). It has generally been observed a significant loss of C during the decomposition maybe because of significant displacement of fungal microbes to bacterial-rich microflora (Hu et al. 2017). Generally, organic wastes having highest amount of C:N ratio are allowed to mix with wastes which are rich in N; however, final products of the compost have comparatively lower C:N ratio. Normally, fast activities of microbes in the mixture of composts trigger a significant rise in temperature. Mixing of such materials maintain the temperature which are appropriate for composting for long time. It is assumed that all materials are needed to pass through increased temperature in order to eliminate harmful microbes and propagules of weeds (Al-Turki 2010; Sanmanee 2011). Such significant rise in temperature may sometime hamper the activity of beneficial microbes if water is not properly added (Allison et al. 2010). Moreover, there is a significant loss of N during composting which is a matter of considerable deliberations (Handa et al. 2014; Chan et al. 2011; Chan et al. 2016). In this regard, Kirchmann and Lundvall (1993) recommended not using aerobic process for decomposition of organic matter containing high amount of NH_4^+–N because there is a significant loss of N. Similarly, Ramaswamy et al. (2010) also reported a figure of 60% loss of N and 2% C from loose piled poultry manure. Furthermore, significant loss of N as N_2O from the households organics during composting along with a considerable loss of CH_4 has been observed (Beck-Friis et al. 2000). Another interesting fact has come out from the research that if composting is done in open, a significant loss through leaching may be recorded. Likewise, if windrowing of manure is done without covering, a considerable loss in N and K content may be obtained (Lampkin 1990). In addition, considerable amounts of reduction in antibiotic concentration have been observed in the soil due to composting process (Dolliver et al. 2008).

1.4 Role of Organic Amendments in Soil Health Improvement

There is huge burden on soil in terms of biotic as well as abiotic stress. Also, heavy load of pesticides, insecticides, weedicides, inorganic fertilizers, etc. has accelerated the rate of extinction of a wide range of flora and fauna. Henceforth, to obviate the soil from these stresses, it is essential to frame a module which is conducive to the soil ecosystem. Organic soil amendments are considered to be the chief option for the soil management (Zhang et al. 2015a, b; Shahbaz et al. 2017). Generally, all kind of organic matter helps to impoverish the soil health (Tejada et al. 2001; Jindo et al. 2016). A wide range of organic matters are integrated into soil and different methods for their processing are being used. Compost are mostly used to enhance the soil C stock providing essential nutrients like N and P and also help in the augmentation of microbial activities. It is presumed that quality and quantity of organic inputs directly affects the soil physical, chemical, biological features (Albiach et al. 2000; Saison et al. 2006; Bonilla et al. 2012a, b). Impact of organic soil amendments in microbiota of soil has been correlated to the suppressiveness of the soil for many plant diseases (Weller et al. 2002; Mazzola 2004; Steinberg et al. 2007; Van Bruggen and Finckh 2016).

1.4.1 Physical Properties

Incorporation of organic inputs into soil not only increases organic matter content but also improves soil physical property (Thangarajan et al. 2013; Khaliq and Abbasi 2015; Williams et al. 2017). For instance, some physical properties such as soil aggregate stability, water holding capacity and soil porosity are considerably enhanced (Celik et al. 2004; Leroy et al. 2008). Consortium of compost and wood scraps under intensive farming system enhanced pore size by formation of organo-mineral aggregates which have beneficial impacts on soil structure and soil aeration (Scotti et al. 2013). Moreover, soil integration with cow manure, sheep manure, reeds, wheat straw and rice husk enhanced soil aggregation stability and reduced bulk density (Karami et al. 2012). In another study, farmyard manure and straw application exerted decreased soil bulk density and increased soil organic C and porosity (Zhao et al. 2009). Henceforth, it is concluded that soil organic C is inversely proportional to soil bulk density after application of soil organic matter (Bauer and Black 1994). Organics generated from various types of by-products, like biochar, affect directly the particle size distribution and aggregate stability. Application of biochar improves the soil structure by increasing the soil aggregation significantly (Liu et al. 2014). However, it has also come to notice that organic soil amendments having higher contents of bioavailable C encroached from cellulose help in the proliferation of fungal colonies harbouring in the soil. It also helps in the soil aggregation and promotion of soil microbial activities which ultimately maintain good health of soil (Lucas et al. 2014).

Similarly, as far as C sequestration is concerned, organic soil amendments improve C sequestration process considerably (Müller-Lindenlauf 2009). Organic amendments promote agroforestry systems and augment C sequestration leading to enhanced plant growth and biomass production (Geier 2007; Twarog 2008; Johnson et al. 2007; Berthrong et al. 2013; Bowles et al. 2015; Bhowmik et al. 2016, 2017). In addition, organic agriculture also minimizes the biomass burning contributing a huge amount of CO_2 which impart in global warming (Müller-Lindenlauf 2009). In grassland ecosystem C sequestration was enhanced when organic inputs are amended in a considerable amount (Liebig et al. 2005; Acharya et al. 2012). Moreover, crop rotations and less deep ploughing ameliorate soil organic matter and accelerate C sequestration (Niggli et al. 2009).

1.4.2 Chemical Properties

Without appropriate organic input in the agricultural land, restoration of soil health will remain just a dream of the researchers. It is because use of chemical fertilizers not only changes the physico-chemical properties of the soil but also produces deleterious effects on soil enzymes and microbial diversity and increases soil salinity (Bonanomi et al. 2011a; Wang et al. 2017). Research under different agroclimatic conditions has revealed that organic matter is a potential tool for the replenishment of soil organic C stock (Hargreaves et al. 2008; Zhang et al. 2015a, b). Interestingly, only few studies have revealed the importance of organic amendments under plastic tunnel system so far. For instance, there were no significant differences in organic C recovery stock after 3 consecutive years of application of composts which may be due to rapid mineralization (Morra et al. 2010; Iovieno et al. 2009). Plants require a limited amount of minerals to satisfy their demands. Generally, microbial population rely on substrate derived from organic matter relatively in a fixed manner; however, the microbial activity is hampered when C/N ratio is above threshold, and the threshold values are ~25–30. The rate of organic matter decomposition is significantly decreased when the C/N ratio reaches above the thresholds which allow long-term C storage. Besides, incorporation of organic inputs containing high C/N ratio into soil and mobilization of nutrients are temporarily suspended leading to enervated plant growth and yield attributes (Hodge et al. 2000). No doubt, suspension of N mobilization is unacceptable under intensive agriculture where plant nutrition is regulated to meet the crops demand. To satisfy the demand of a healthy soil, it is needed to identify organic amendments which can balance the trade-off between organic C recovery and mineralization of nutrients. Eventually, after reaching into soil, the organic C retainability not only depends upon biochemical quality but also certain features of soil minerals such as sand, silt, clay, carbonate and organic C contents (Piccolo 1996; Clough and Skjemstad 2000; Scotti et al. 2015). Moreover, soil having low organic content and high clay fraction absorbs exogenously applied organic inputs faster and easier and makes them not easily available to microbial attack (Bonanomi et al. 2014a, b). In addition, sandy soil having high C content is adverse to microbial population because most of the mineral particles are unable to make proper and appropriate interaction. This improper reaction gives much more compounds which are enough to devastate beneficial microbial colonies.

Likewise, rampant use of chemical pesticides accelerates mineral N release; however, in contrast, organic incorporation triggers lower mineral N release for a long time (Claassen and Carey 2006; Weber et al. 2007). It is apparent that mineralization of N in slow mode under organically derived compost ameliorates soil biology (Weber et al. 2007). Generally, there is a significant increase in humic/fulvic acids in soil amended with composts which may be partly due to presence of humic acids in composts that dominate over fulvic acids. In such soil, humic acids are always significantly greater than fulvic acids (Weber et al. 2007). Besides, use of composts as soil amendments promotes the nitrification process leading to reduction in contamination of groundwater (Montemurro et al. 2007). Broadly, application of organic inputs increases some important variables pertaining to soil health such as organic C stock and soil cation exchange capacity. Maximum values of cation exchange capacity permit to retain essential nutrients cation and make possible for them to be available for crop productions (Bulluck lii et al. 2002). Similarly, anions are found to increase subsequent to organic inputs application (Zaccardelli et al. 2013b; Scotti et al. 2015). But, a significant challenge has come out in the use of organic matter especially compost derived from municipal solid waste. Municipal solid waste-derived compost increases the electrical conductivity into soil and subsequently salinity, and solidity increases which impacts negatively on crop yield (Mass and Hoffman 1977; Bonanomi et al. 2014b) and also on soil biological activity (Rietz and Haynes 2003). Such MSW-derived compost increases the soil salinity especially in the soil cultivated under plastic film due to limitation in soil leaching (Bonanomi et al. 2011a, b).

1.4.3 Biological Properties

Organic matter decomposition is the result of considerable work performed by microbes (Thangarajan et al. 2013; De Baets et al. 2016). They play a very crucial role in making soil fertile and help in the organic C mineralization (Burauel and BaBmann 2005; Whitman et al. 2016; Zheng et al. 2017). Amended organic matter into soil favours in proliferation of microbial population; hence, there is a strong correlation between organic C, soil biological activity and enzymatic activities (Chakraborty et al. 2011; Tejada et al. 2001). However, biological properties of soil are considered to be a good indicator of soil health due to their rapid responses to environmental perturbations (Nannipieri et al. 1990; Paz-Ferreiro et al. 2009). Soil with no input of organic matter exhibited a significant reduction in the soil microbial biomass, enzymatic activity and beneficial fungal colonies under intensive agricultural system (Bonanomi et al. 2011a). Use of compost as soil amendments surprisingly enhanced soil fertility such as soil enzymes and microbial activities (Thangarajan et al. 2013). A quick response in enzymatic activities such as dehydrogenase, phosphomonoesterase and β-glucosidase has been obtained after organic amendments. This specific quality of organic inputs has accelerated the repeated use of organic amendments which has subsequently enhanced the microbial population and leading to improved soil fertility (Scotti et al. 2015; Zaccardelli et al. 2013a). Use of seed meals derived from *Brassica carinata* and *Helianthus annuus* as an organic amendment enhanced the soil enzymatic activity like phosphomonoesterase, dehydrogenase, fluorescein diacetate hydrolase, arylsulphatase and β-glucosidase, thereby improving soil biology (Zaccardelli et al. 2013b). Incorporation of composts obviates the stress caused by high saline content and improves the biological fertility of soil (Lakhdar et al. 2009). Likewise, application of compost derived from municipal solid waste and palms waste at different doses such as 0, 50, 100 and 150 T/ha registered a significant improvement in the microbial activities. But, hindrances were observed at the dose level of 150 T/ha; it may be due to the presence of the heavy trace elements in municipal solid wastes (Ouni et al. 2013; Garcia-Gill et al. 2000; Crecchio et al. 2004).

Acceleration in microbial activities and biomass has been the chief aim of some cultural practices like integration of organic matter in the soil ecosystem (Janvier et al. 2007). Various types of organic amendments into agricultural land have been helpful in the enhancement of the microbial biomass than non-amended soil or inorganic fertilizers (Bonilla et al. 2012a, b; Tiquia et al. 2002; Peacock et al. 2001). Many earlier studies have revealed that compost, composted almond shells and composted yard wastes have enhanced the heterotrophic bacterial population (Saison et al. 2006; Perez-Piqueres et al. 2006; Boniall et al. 2012a, b). Soil amendments with manures, yard wastes and compost influence the microbial diversity (Yang et al. 2003; Bonilla et al. 2012a, b). It is pertinent that microbial diversity is a very complex component. Henceforth, measurement of microbial diversity quantitatively and qualitatively is needed to ventilate the unexplored reasons. Measurement through diversity index may give haphazard information; therefore, qualitative community structure analysis is more reliable than other sampling procedures. Moreover, many reports have revealed the impact of organic soil amendments which involved a significant influence on some enzymes such as urease, β-galactosidase, protease, phosphatase or dehydrogenase. In other words enzymatic activities of soil are directly correlated with level of soil organic matter incorporated, which is why soil amendments are considered as an appropriate soil indicator (Garcia et al. 1994; Ros et al. 2003; Tejada et al. 2006; Pascaud et al. 2017). Besides, single enzymes activity cannot reveal complete structure of information pertaining to nutrients status. However, organically rich soils are more complex and depend on soil physico-chemical nature (Albiach et al. 2000; Goyal et al. 1999). Such soil is characterized by abundant heterogeneous populations of microbes. Also, they are difficult to identify up to the last hierarchy level. Therefore, some advanced approaches pertaining to identification of various species are needed. In this context, terminal restriction fragment length polymorphisms (T-RFLP) have now been proven to be a milestone in the characterization of bacterial and fungal communities isolated from various localities (Pérez-Piqueres et al. 2006). Similarly, many researchers have shown that organic amendments may influence the bacterial and fungal communities; however, further verifications are needed by using advanced techniques like direct extraction of lipids (PLFA) and nucleic acids (T-RFLP, ARISA, ARDRA, DGGE) (Bonilla et al. 2012a, b; Tiquia et al. 2002; Peacock et al. 2001; Edel-Hermann et al. 2004; Dimitrov et al. 2017). These novel approaches have been adopted by various researchers globally so far (Van Elsas and Costa 2007).

1.5 Significance of Organic Amendments in Plant Health Amelioration

1.5.1 Plant Biomass Promotion

Researchers have focussed their study on environment protection prompting the research on nutrient management strategies and lowering down the use of chemical pesticides (Ghimire et al. 2017). Moreover, utilization of resistant varieties and pesticides is unable to eliminate the soilborne fungal propagules from the agricultural system. Therefore, proper management modules having high efficacious nature and low costs are needed for contemporary agriculture (Martin 2003). Effect of wide spectrum of organic amendments on different crop yield in various studies has been investigated (Sumbul et al. 2015; Horrocks et al. 2016). For instance, single application of olive pomace at 10 or 20 Mg per hectare enhanced wheat yield by 50%, by increasing of kernel weight and their number (Brunetti et al. 2005). Long-term application of soil organic amendments has increased the growth and yield attributes (Johnston et al. 2009; Xie et al. 2016). Organic integration into soil not only improves physico-chemical feature but also plays beneficial role on crop productivity. Broadly, organic soil amendments are the best option available in many developing countries for compensation of soil nutrients (Lal 2005; Kaur and Verma 2016). Moreover, application of certain easily available organic inputs such as buckwheat (*F. esculentum* L.), millet (*Echinochloa crus-galli* L.), colza (*Brassica campestris* cv. *oleifera* L.), clover (*Trifolium pratense* L.) and mustard (*Brassica hirta* Moench) has successfully improved the yield (N'Davyegamiya and Tran 2001). Besides, different industrial wastes have been applied in order to predict their response on crop yield. Wastes generated from sugarcane industries have been applied in the land and increased crop biomass recorded (Dotaniya et al. 2016). Consortium of N fertilizers and sugar press mud (derived from sugarcane industrial wastes) increased plant growth attributes such as dry matter, cane, sugar yield, etc. (Bangar et al. 2000). Likewise, in another experiment, 25 t ha^{-1} sugar press mud significantly improved the sugarcane yield (Venkatakrishnan and Ravichandran 2013). In addition, application of press mud enhanced the sugarcane quality and biomass-related attributes providing sufficient nutrients by ameliorating soil health (Sarwar et al. 2010).

Bagasse (another generated by-product) is being judiciously used in agricultural crop production system to reduce the application of inorganic fertilizers (Dotaniya et al. 2016). Properly chopped bagasse, applied 1 month before sowing is very sound for the proper decomposition that leads to production of organic acids and mobilization of insoluble phosphorus from soil to soil solution in labile form (Rocha et al. 2011; Dotaniya et al. 2016; Hofsetz and Silva 2012). Moreover, incorporation of 3000 kg ha^{-1} enhanced the crop growth attributes significantly and that may be due to enough P supplementation (Ferrer et al. 2001). Some wastes, derived from tomato, cork residue, olive husk and tannery sludge, improved the crop growth and yield variables (Vallini et al. 1983).

1.5.2 Plant Disease Management

Application of organic amendments like composts derived from various sources, manures, etc. is well studied in context of suppression of pest pathogens and plant diseases (Bailey and Lazarovits 2003; Bonilla et al. 2012a, b; Noble 2011; Noble and Coventry 2005; Van Elsas and Postma 2007; Faye 2017). Organic incorporation has frequently been found to reduce wide range of soilborne diseases infesting different agricultural plants (Aviles et al. 2011; Bonilla et al. 2012a, b; Hadar and Papadopoulou 2012; Noble 2011; Yogev et al. 2006). Generally, compost amendments are found to be associated with soilborne diseases reduction; however, there are certain dependent factors (Bonanomi et al. 2010; Noble and Coventry 2005). Wide ranges of compost were evaluated against various plant pathogens and plant diseases and resulting in significant diseases management (Termorshuizen et al. 2006; Mishra et al. 2017). Generally, it has been seen in various studies that compost has the ability to reduce the disease with a figure of 55% of disease. Some important factors such as compost material, age and quality keep prime importance determining whether compost will be suppressive or not (Bonanomi et al. 2010; Hoitink and Boehm 1999; Noble and Coventry 2005; Termorshuizen et al. 2006).

In a trial, composted dairy manure as a soil amendment along with other composts significantly enhanced microbial populations (Bernard et al. 2014; Zhang et al. 2015a, b). Some reports have suggested that crop yields are increased, but there is no considerable reduction in pathogen population (Bernard et al. 2014). Henceforth, any compost before applying in a large scale should be tested under a small level of field to avoid environmental perturbation (Ansari et al. 2017a, b). Some organic manure in non-composted form has shown inhibitory effects against many phytopathogens; however, results showed inconsistency (Bononomi et al. 2007). For instance, more than 50% of the trials have shown inhibitory effects by un-composted manure and industrial by-products against soilborne diseases, while less than 12% attributed to increase the disease incidence (Bononomi et al. 2011b). The reason behind such inconsistency may be the nature of organic inputs such as quantity, quality, origin, etc. affecting soil physico-chemical properties leading to changed microbial diversity. There are some abiotic factors that have been found associated with disease management practice. Many eminent researchers have stressed their studies to dig out the actual mechanisms pertaining to disease suppression (Bonanomi et al. 2010; Noble 2011). But ample studies have revealed that disease suppression is related to overall enhancement in microbial population and activity developing deleterious environment to the plant pathogens (Bonilla et al. 2012a, b; Bonanomi et al. 2010). This is further advocated that diseases suppression is of biological origin, because suppression nature of organic matter is lost when it is sterilized (Bonilla et al. 2012a, b). For example, incorporation of a wide array of organic manure and organic wastes were highly suppressive to *Verticillium* sp., but this result was inconsistent for site to site (Lazarovits 2001; Lazarovits and Subbarao 2010). Similarly, organic soil amendments reduced soilborne pathogens by forming ammonia or nitrous acid which is lethal to pathogens (Lazarovits 2001). Likewise, liquid swine manure minimized the disease incidence by forming volatile fatty acids in acidic soil (Lazarovits 2001; Lazarovits and Subbarao 2010). In addition, composted teas, water-based compost, contain diverse types of constituents found to be having disease-suppressive nature (Schuerell and Mahaffee 2002; Lazarovits 2010; St. Martin and Brathwaite 2012).

Ample studies have revealed that organic amendments can combat plant diseases caused by various plant pathogens such as bacteria, fungi and phytonematodes (Hoitink and Boehm 1999; Bailey and Lazarovits 2003; Ansari et al. 2017a, b). Composted materials are found showing pernicious effects on root rots as compared to non-composted materials (Hoitink and Boehm 1999). Yogev et al. (2006) found that compost derived from plant waste residue reduces disease caused by different formae speciales of *Fusarium oxysporum*. In another such incident, *Phytophthora cinnamomi* causing avocado root rots was suppressed by application of vegetable produced compost (Downer et al. 2001). Generally, composted materials have constantly been shown to be suppressive on various soilborne diseases including damping off and root rots (*Pythium ultimum*, *Rhizoctonia solani*, *Rosellinia necatrix*, *Phytophthora* spp.) and wilts (*Fusarium oxysporum* and *Verticillium dahlia*) infecting wide range of crop plants (Lazarovits 2001; Yogev et al. 2006; Yogev et al. 2010; Malandraki et al. 2008; Erhart et al. 1999; Pane et al. 2011; Tamm et al. 2010; Bender et al. 1992). Some other pathogens have been significantly controlled by organic matter application that are *Gaeumannomyces graminis* f. sp. *tritici* (Tilston et al. 2002), *Fusarium* spp. (Borrero et al. 2004), *Pythium* spp. (Erhart et al. 1999), *Rhizoctonia solani* (Pérez-Piqueres et al. 2006), *Phytophthora* spp. (Szczech and Smolińska 2001), *Verticillium dahliae* (Paplomatas et al. 2005) and *Sclerotinia minor* (Pane et al. 2011). Nevertheless, the suppressing quality varies greatly depending on organic matter type, plant hosts and pathogens spp. involved, etc. Few reports related to negative impacts of organic amendments have also been documented such as enhanced phytotoxicity and disease severity (Smolinska 2000; Tilson et al. 2002; Scheurell et al. 2005; Delgado et al. 2010). Termorshuizen et al. (2007) showed that organic amendments caused disease suppression in 54%, no considerable suppression in 42.7% and enhancement of disease in 3.3%. Similarly, Bonanomi et al. (2010) found suppressiveness of organic amendments in 45% of the cases, no significant suppressiveness in 35% cases but enhancement of the disease in 20% of the cases studied. Due to such inconsistent results, practical application of composts for disease suppression is still a matter of debate. Moreover, facts to be analysed regarding organic soil amendments derived from wide spectrum of animal and plant residue, composting methods, feedstock origin (municipal wastes, plant pruning, crop residues, animal manures, etc.), rate of application (Serra-Whittling et al. 1996) and level of maturity (Tuitert et al. 1998).

1.6 Mechanisms Implicated in Action of Organic Amendments on Soil and Plant

Amendment of soil with organic matter boosts up soil physical, chemical, biological properties leading to improved plant health (Bonanomi et al. 2010; Lazarovits 2010). No single mechanism can be implicated in such a concerted effort of ameliorating soil health with nutrients and enhancement of plant growth attributes. The quantity and quality of organic matter amended in the soil impose its impact on soil physico-chemical as well as on biological activities (Mazolla 2004; Abbasi et al. 2002; Bulluck and Ristaino 2002; Stark et al. 2008; Saison et al. 2006; Bonilla et al. 2012a, b; Gomez et al. 2006; Ceja-Navarro et al. 2010). Physico-chemical and biological properties of soil collectively make a soil suppressive which may result inhibitory to soilborne plant pathogens and stimulatory to multiplication of beneficial microorganism (Huang et al. 2015). Baker and Cook (1974) described suppressiveness of soil where in suppressive soil disease, severity or incidence remains low in spite of the presence of a virulent pathogen, a susceptible host plant and climatic conditions favourable for disease development (Hiddink et al. 2005; Janvier et al. 2007). Various mechanisms of disease suppression by organic soil amendments have been proposed that include biological control (Abbasi et al. 2007; Fu et al. 2017), stimulation of systemic resistance in plants (Hoitink and Boehm 1999; Alkooranee et al. 2017) and production of compounds lethal to plant pathogens such as ammonia, nitrous acid and volatile fatty acids (VFAs) (Mazzola 2002, 2004; El-Abbassi et al. 2017).

A number of physico-chemical parameters like soil pH, N, C and organic C content, various cations and oligoelements have been found to be associated with plant disease suppression (Bonilla et al. 2012a, b). Emphasis has been given to find out the role of physico-chemical properties in the soil and plant health improvement. Moreover, conservation of C reservoir in agricultural soil system is much helpful in nutrient delivery (Tian et al. 1992), amelioration of soil structure (Abiven et al. 2009), enhancing microbial activities (Mäder et al. 2002) and sustaining the soil suppressiveness for soilborne pathogens (Bonanomi et al. 2010; Scotti et al. 2013). Besides, enhanced soil organic matter content, soil pH alteration, type of clay and improved soil texture on microbial populations maintain soil suppressiveness (Alabouvette 1999; Fang et al, 2005; Mazzola 2002). Soil amended with high nitrogen containing organic inputs such as chicken manure, meat and bone meal, chitin and chitosan, neem and soy meals resulted in N compounds such as ammonia and nitrous acid (Lazarovits et al. 2005). Plethora of the mechanisms is involved through which organic amendments suppress the plant diseases leading to enhanced soil and plant health. To understand the web of mechanisms, a high level of instrumentation and expertise is needed. However, current assay has helped us to elucidate the possible mechanisms involved in the plant biomass enhancement as well as the plant disease suppression (Fig. 1.2). In addition, various complementary mechanisms have been proposed so far to illustrate the suppression capacity of an organic matter, for instance, (i) enhanced rhizospheric microbial activities (Hoitink and Boehm 1999; Li et al. 2017), (ii) food competition amongst the microbes (Lockwood 1990), (iii) secretion of pathotoxic compounds from decomposed organic matter (Tenuta and Lazarovits 2002) and (iv) systemic resistance induction in host plants (Zhang et al. 1996; Pharand et al. 2002; Keswani et al. 2017). Lazarovits (2001) reported that decomposition of organic matter leads to the production of nitrogenous compounds.

Generally, soil microorganisms rapidly start degrading high nitrogenous organic input leading to production of N in its different forms and more than sufficient for microorganism proliferations. The excess amount of N is localized to soil solution as ammonia (NH_3) (Lazarovits 2001). Eventually, this is swiftly converted to ammonia (NH_4^+), and pH of soil significantly enhanced. Likewise, as soon as pH rises, some amount of NH_4^+ converted back to NH_3. Interestingly, ammonia is very toxic even at very low levels, while no such pernicious effect of ammonium has been observed (Warren 1962). However, NH_3 is generated when the soil pH is significantly increased and reaches above 8.5, but this occurs in some special soil (Lazarovits 2001). Similarly parallel mechanism to former is proposed look to be more considerable. Ammonium conversion leads to formation of nitrite (NO_2^-) and subsequently nitrate (NO_3^-) by the bacterial nitrification. Such mechanisms lead to drastic reduction in the pH attaining 5.5. As soon as pH drops to 5.5, NO_2^- converts to HNO_2. NO_2^- is non-toxic, while HNO_2 is highly toxic to a wide range of soil-borne pathogens (Lazarovits 2001). In addition, soil pH is the driving factor that reduces the quantity of toxic (NH_3 or HNO_2) and non-toxic (NH_4^+ or NO_2^-) compounds (Lazarovits 2001). In addition, liquid swine manure is rich in various nutrients essential for the growth of crop plans. Various fractions are found in the liquid swine manure, but specifically, acetic acid is the chief organic compounds playing crucial role in disease suppression (Lazarovits 2001). The existence of effective biocidal products depends highly on composition of the organic amendment added to the soil, pH of the soil and soil buffering capacity (Tenuta and Lazarovits 2002). It is a well-established fact that adding fresh organic amendments to soil transiently enhances ammonium concentration and pH, followed by increased nitrification and a fall in ammonium and pH (Zelenev et al. 2005).

Moreover, volatile fatty acids (VFAs) that can be injurious to some pathogens in low pH soils are produced by the decomposition of certain organic amendments such as liquid swine manure (Conn et al. 2005). Besides, VFAs can also be achieved by adding fresh organic matter like broccoli (*Brassica oleracea* L. Convar. Botrytis (L.)

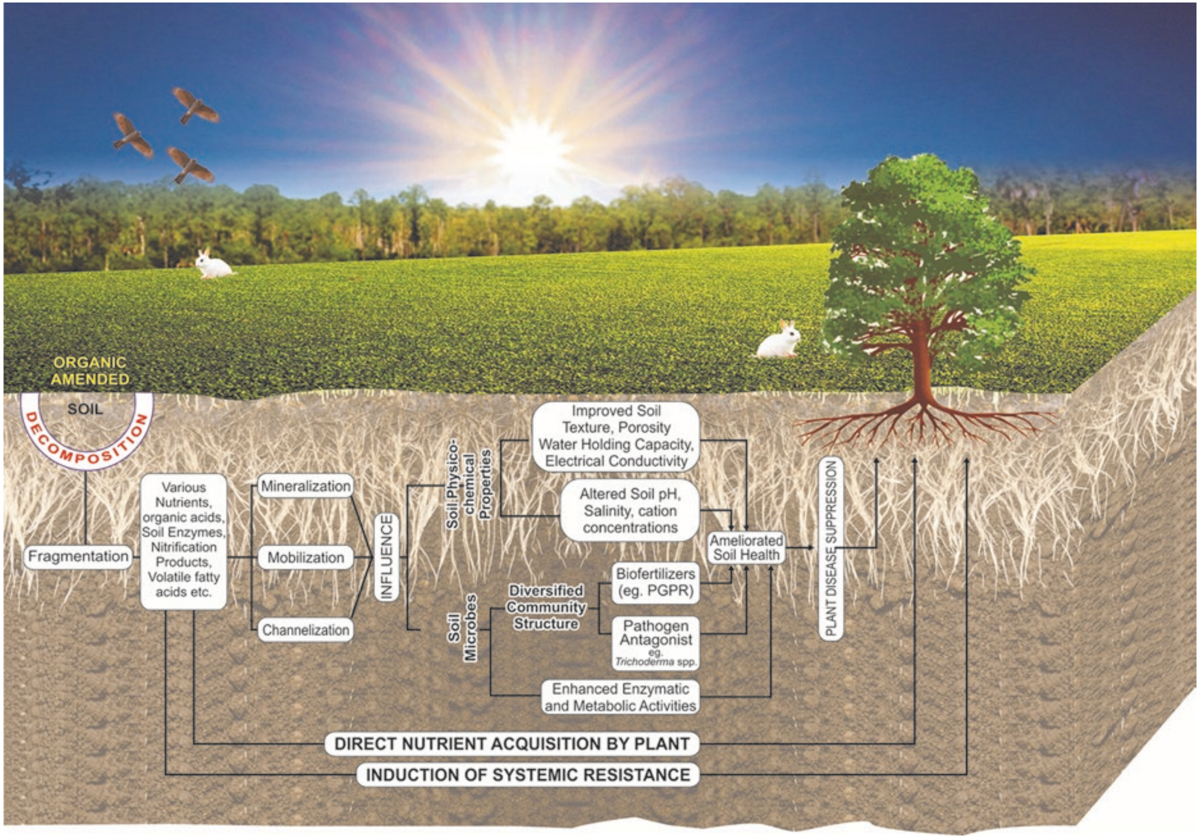

Fig. 1.2 A possible mechanism of soil and plant health ameliorations via organic matter decomposition (a hypothetical view)

Alef var. Cymosa Duch.) or perennial ryegrass (*Lolium perenne* L.) (Blok et al. 2000). Besides, concentration of calcium in the soil has also been implicated showing negative effect on wide spectrum of soilborne pathogens. Heyman et al. (2007) reported that *Aphanomyce*s root rot of pea was affected to varying extent by various calcium-containing compounds, and this was directly correlated with water-soluble Ca in soil. Similarly, Bonanomi et al. (2010) revealed that pH of amendment was not correlated to disease suppression except in the case of *Fusarium* species. Borrero et al. (2004) showed that high soil pH was effective in reducing *Fusarium* wilt of tomato. Also, there is inconsistence report related to physiological parameters pertaining to plant disease control (Janvier et al. 2007). Thus physiological issues have been found less informative in terms of plant disease suppression as compared to enzymatic and microbiological variables (Bonanomi et al. 2010; Castano et al. 2011).

Moreover, amendments of soil with organic matter generate soil suppressiveness against wide range of soilborne pathogens (Klein 2011; Sumbul et al. 2015). The impact of organic amendments on soil suppressiveness has commonly been associated with a general suppression mechanism. Incorporation of organic matter is responsible for enhancement of total microbial biomass and activity in soil, leading to impediment of the pathogen through competition for resources or by other direct forms of antagonism (Mazolla 2002). Alabouvette (1999) propounded that suppressiveness of soil for *F. oxysporum* f. sp. *lini* depends on partially competition for carbon amongst the pathogen and the microorganisms present in soil. Moreover, dissolved organic carbon is the readily available carbon sources which are consistently used, discharged upon cell death. Thereafter, eventually this organic C is reused by rapidly multiplying microorganisms creating competitive environment for plant pathogens thriving in the rhizosphere, and eventually root disease development gets hampered (Zelenev et al. 2005; Raaijmakers et al. 2009). Besides, microbes are united tightly generating an environment unfavourable to plant pathogens and later on disease development (Hoitink and Boehm 1999; Weller et al. 2002; Penton et al. 2014). Moreover, in order to ascertain the amendment suitable for specific disease suppression, it is necessary to determine a particular amendment instigating the microbial population leading to suppressed disease (Steinberg et al. 2007; Bonilla 2012a, b; Penton et al. 2014). Appropriate alteration in microbial community structure is shown to be widely associated with suppression of plant diseases. Thus, enhanced diversity of microbes provoked by organic soil amendment is responsible for successful suppression of pathogen (Cohen et al. 2005; Pérez-Piqueres et al. 2006). Moreover, *Gaeumannomyces graminis* var. *tritici* causing wheat take-all disease was found to be reduced by various strains of *Pseudomonas* spp. both in greenhouse and field experiments (Weller and Cook 1983). Besides, in Western Australia, *Trichoderma* spp. that form a major proportion of total microbial community have been implicated in the control of the wheat take-all disease (Simon and Sivasithamparam 1989). However, the recognition of a particular microbe surely involved in disease suppression does not indicate the sole responsibility of that microbe in the process, but a number of other factors both biotic and abiotic may also play partial role in disease suppression. In addition, different types of organic amendments instigate a different array of microbial community, found to be responsible for their specific efficacy against different pathogens. Such impact of organic amendment on soil suppressiveness may also vary with time, level of decomposition and environment change leading to newly evolved microbial communities (Alabouvette et al. 2004; Bonanomi et al. 2010). Besides, the concept of disease suppression may also be associated with particular activities or functions performed by microorganisms rather than only the presence or abundance of a specific population in soil.

Another aspect related to the suppressiveness of organic amendment may be attributed to the alterations efficiency of microbes in the metabolic and enzymatic activities. Multivariate analysis of this facet has been successfully carried out, but only a few cases proved to be the actual reason behind the soil suppressiveness (Gomez et al. 2006), allowing discrimination between suppressive and conductive soils (Pérez-Piqueres et al. 2006; Pane et al. 2011). For instance, take-all decline of wheat was recorded where fluorescent *Pseudomonas* spp. were found to be related to the production of phenazine (Thomashow et al. 1990) and particularly to 2,4-diacetylphloroglucinol (Raaijmakers and Weller 1998). Thus, the presence and abundance of fluorescent *Pseudomonas* in a soil are considered to be suppressive to take-all disease of wheat (Raaijmakers et al. 1997). Besides, *Trichoderma* spp. reduce take-all disease of wheat by antibiotic production, particularly pyrone compounds, however, non pyrone producing strains of *Trichoderma* are also shown to suppress disease, suggesting other mechanisms being run simultaneously. Moreover, certain other pathways of disease suppression include competition for resources and niches, incitement of plant defences, parasitism and predation, and also hydrolytic activities like chitinases and glucanases have also been reported (Mukhopadhyay 2016). Chitinase and glucanase activities have invariably been associated with soil suppressiveness and biocontrol of soilborne pathogens. Chitin, the main component of fungal cell walls, plays a pivotal role in such kind of disease suppression. Chitinolytic microorganisms instigated by specific organic amendment effectively control fungal pathogens in the soil (Bouizgarne 2013). For instance, chitin compost consisting of crab shell at 30% was used against *Phytophthora capsici* and found reduced pathogen population (Chae et al. 2006). Also, the number of chitinase-producing bacteria in the rhizosphere and the enzymatic activities like chitinase and β-1,3-glucanase were greater in plants amended with the chitin compost than unamended. Overall, no single mechanism is involved in disease suppression, plant and soil health improvement in organic rich soil. It is generally hypothesized that biological activities instigated by organic input are mainly responsible for plant and soil health amelioration. Physico-chemical variables can have an impact on growth and activities of soil microbes leading to improved soil and plant health.

1.7 Conclusions and Future Prospects

The inference can be drawn from above literatures that applications of soil organic amendments have been beneficial to the growers not only in the developed countries but also in underdeveloped one. Moreover, incorporation of wide range of organic matters into soil has culminated the cost burden especially on small landholders. Organic inputs into soil have augmented the soil biology and health. Some important variables pertaining to soil health like microbial activity, microbial diversity, pH, soil respiration, electrical conductivity, etc. have significantly influenced the soil environment. Moreover, it is assumed that as decomposition starts secretion of some organic compounds such as humic/fulvic acids, VFAs and N in its various forms begin simultaneously. These nutrients and volatile substances become ultimately a source providing sufficient nitrogen to microbes and crops. Some organic compounds are secreted by organic matter upon decomposition which are lethal to wide spectrum of soilborne pathogens. On the other hand, microbial activity especially rhizobacterial activity is considerably increased leading to enhanced systemic resistance against various phytopathogens. Moreover, responses of host plants are inconsistent due to diverse nature of organic inputs. Each organic matter differs in terms of their chemical constituents, contents, period of retention, origin, etc. Henceforth, prior to recommendation of any organic matter, a long-term application of targeted organic matter should be screened under various agroclimatic conditions. Besides, physico-chemical properties of the soil like electrical conductivity, porosity, pH, C, C:N ratio, etc. may influence the plant growth. Overall, organic soil amendments may have a lot of beneficial role in microbial activities. A genuine question arises, why farmers are refraining to adopt organic amendments? Prudently, the answer may be unawareness of inorganic fertilizers pertaining to its negative impacts on human health. Also long-term processing of organic matters before its application into the field which charges a heavy labour cost and less appropriate in perspective of cost-benefit ratio. To address these issues, a considerable number of research at various station houses are needed under various agroclimatic conditions. Scientific interaction with landholders, farmers and growers pertaining to use of organic matter into land may indeed support the use of organic inputs into agricultural system. A focus should also be on the identification of different agroclimatic circumstances where it can be best applied. Finally it may be added that organic farming is preferable than that of using chemicals in agriculture.

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Chapter 2
Grafting, Agrochemicals, and Oxidative Enzymes as Factor for Plant Biotic Resistance

**Gean Charles Monteiro, Rumy Goto, Igor Otavio Minatel,
Edvar de Sousa da Silva, Ewerton Gasparetto da Silva, Fabio Vianello,
and Giuseppina Pace Pereira Lima**

Abstract Grafting has been practiced to overcome yield problems associated to soil-borne diseases or declines in production. Usually this agricultural method involves the choice of better stock and scion species, procedures to improve the graft union and subsequent healing, and acclimation of the grafted plant to soil. Similarly, to agrochemicals used in vegetable production, this method may induce changes in oxidative enzymes production by plants and consequently provide a higher resistance to biotic stresses. Microorganisms are one of the major concerns in agricultural practices, mainly by its increasing resistance to chemical products regularly applied in cultures. Nevertheless, the association of grafting methods to agrochemicals usage is a wide field of study that should be better interpreted by considering not only the resulting production, but their interactions and consequences in the plant metabolism. The purpose of this chapter is to review the

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G. C. Monteiro · G. P. P. Lima (✉)
Department of Chemistry and Biochemistry, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil
e-mail: gpplima@ibb.unesp.br

R. Goto
Department of Horticulture, School of Agriculture, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil

I. O. Minatel
Faculdade Sudoeste Paulista, Avaré, São Paulo, Brazil

E. de Sousa da Silva
Instituto Federal do Acre, Rio Branco, AC, Brazil

E. G. da Silva
Instituto Federal do Piauí, Campus Uruçuí, Uruçuí, PI, Brazil

F. Vianello
Department of Comparative Biomedicine and Food Science, University of Padova, Padova, Italy

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resistance induced in plants by grafting methods or agrochemicals. In addition, the role of oxidative enzymes produced for resistance mechanisms is discussed.

Keywords antioxidant activity · phenolic compounds · polyamines · salicylic acid · reactive oxygen species

2.1 Grafting

Grafting is a process known by the Chinese since the beginning of the fifth century, and it is used in Korea since the seventeenth century aiming to control, in a preventive manner, the disease caused by *Fusarium oxysporum*, using resistant rootstocks (Yamakawa 1982). In Japan, the grafting technique began to be used in the mid-1920s as a way of prevention against soil pathogens in the watermelon culture (*Citrullus lanatus*), using the squash rootstock (*Cucurbita moschata* Duchesne ex Poir) (Davis et al. 2008). This technique was intensified since 1950 in eggplant (*Solanum melongena*). In 1955 it began to be described in melon (*Cucumis melo*) and in 1965 in cucumber (*Cucumis sativus*), always aiming to control the soil pathogens (Oda 1995). In Europe, the grafting is used in vegetables since the 1940s by Dutch horticulturists. This technique has been very valued for many researches and employed to induce resistance to soil pathogens and promote modification of the sexual expression, compatibility, nutrition, development, production, and fruits quality (Cañizares et al. 2002).

The grafting can be used to reduce the use of agrochemicals and to improve the production. When compared to other methods for biotic control, the grafting technique may be more interesting because it promotes tolerance to adverse temperatures, soil salinity, physiological disorders, and nutrients (Colla et al. 2010) and minerals stress (Savvas et al. 2009; Rouphael et al. 2008) and induces the production of fruits with better quality (Colla et al. 2006; Venema et al. 2008).

Grafting is the union of two portions of live vegetal tissue aiming the development of only one plant. The morphological and physiological union, with a later adequate growth and development (Cañizares et al. 2002), represents its success. For the success of the grafting is fundamental the choice of the species of the rootstocks and scion, the union of graft and rootstock by manipulation process, the perfect healing, and, finally, the acclimatization (Lee et al. 2010). In this process, the recently cut tissue with meristematic activity gets in close contact with the similar freshly cut tissue of the rootstock. External cells with meristematic activities begin the production of parenchymal cells that, soon after, combine and intertwine, forming the callus tissue. Another factor that affects the grafting success is the rootstock quality, which should present a high tolerance against the harmful agent. However, this degree of tolerance varies between the plants, depending on the rootstock genotype.

In the compatible combinations, there is the reabsorption of the necrotic tissue before the formation of the secondary plasmodesma between the cells, near the formed vascular bundles. These new cells form a new vascular tissue: xylem in the interior and phloem in the exterior, establishing the vascular connection between graft and rootstock (Peil 2003). Frequently, in the beginning of the union, anastomoses are formed, which are connections between the vascular bundles, and the new tissue is completely reconstituted at the end of the second week. The union process in vegetables can be visible 1 day after the grafting and ends between 1 and 3 weeks, with the complete connection between xylem and phloem. The callus formation and the grafting union formation end when the wound heals and reestablishes the water and nutrients circulation from the root to aerial part and the circulation of photo-assimilated compounds from the aerial parts to the root (Cañizares et al. 2002).

In order to avoid problems caused by pathogens that threat the cultivation success in greenhouse, mainly in the ones that involve successive productions, the grafting can be a promissory technique. The soil pathogens can cause injuries in the roots, which reflect in the leaf area, stems production, flowering, and fruit quality, being also able to promote premature death of the plants (Rivero et al. 2003). Soil diseases are a challenge for the producers that cultivate plants in protected environments. The obtaining of resistant varieties has been an important alternative in controlling physiological races, strains, and groups of different soil pathogens. In this way, the use of grafting with a resistant rootstock, along with good commercial characteristics, constitutes an alternative of control in lower time.

In the beginning, many farmers had no knowledge of nutritional handling of plants grafted in protected cultivation and promoted, in many cases, soil salinization, due to the excessive use of fertilizers. With the intensive use of these environments and the lack of diseases prevention, mainly caused by soil pathogens, the cultivation in these environments became unviable (Sirtoli et al. 2011). Many soil pathogens made the vegetable cultivation in protected environment unviable due to structures of resistance that allow them to remain in the soil for a longer time. The grafting aims to avoid the contact of the plant with a possible contaminated soil by grafting a susceptible cultivar on a resistant rootstock. The resistant rootstock is kept healthy, assuming the function of absorbing water and nutrients from the soil, and, at the same time, it is responsible for isolating the grafted plant from the pathogen (Peil 2003). The resistance caused by the grafting is also associated with the improvement of the photosynthesis and increased activity of antioxidant enzymes (He et al. 2009). In a study with grafted tomato in a rootstock resistant to salinity, it was observed that grafted plants had a higher photosynthetic efficiency regarding the stomatal conductance (g_s) and the water use efficiency (WUE), besides maintaining a higher photochemical activity of the photosystem II (PSII) and increasing the activities of the enzymes peroxidase and catalase (He et al. 2009).

Various grafting methods in vegetables can be employed, and the method choice should consider, besides the species, their own advantages and disadvantages, in order to choose the most efficient method (Cañizares et al. 2002). In addition, to avoid problems with the biotic stress, some considerations should be done regarding the grafting. It can be performed on a rootstock of the same cultivar, species, or genus (intraspecific) or between different species or genus (interspecific). According to Louws et al. (2010), a rootstock of different grafts (cultivar, species, genus) includes grafts that contribute to increase the productivity, fruit quality, or graft compatibility, showing less negative effects. The use of resistant rootstocks is important, because it assumes the function of absorbing nutrients and water from the soil, besides separating the graft from the soil, which can contain microorganisms that might induce stress.

Generally, the wrong choice of rootstocks results in damages that can be observed during the adaptation to the environment, decreased quality of fruits and plants, and low resistance of the rootstock. Positive results have been obtained by employing rootstocks different from the graft. Sirtoli et al. (2011) used seven tomato rootstocks ("R601," "R602," "R603," "Guardião," "Protetor," "Spirit," and "Magnet") grafted in the hybrid Platinum and in their respective ungrafted. The evaluations showed that there was no incompatibility between graft and rootstock and the combination of the rootstock "R603" with "Platinum" was resistant to *Ralstonia solanacearum* and increased the plants productivity. Similar results were obtained in the control of *R. solanacearum*, *Fusarium oxysporum*, *Pyrenochaeta lycopersici*, *Meloidogyne incognita*, and *Verticillium dahliae* in tomato culture (Oda 1995) and for control of viruses such as TYLCV (tomato yellow leaf curl virus, transmitted by *Bemisia tabaci*). Thus, history shows that this technique can be efficient against a variety of virus, bacteria, fungi, and nematodes, related diseases (Giannakou and Karpouzas 2003; Bletsos 2005).

Ralstoniasolanacearum is one of the phytopathogens that cause problems in vegetables, mainly in Solanaceae. It is a gram-negative bacteria globally distributed and responsible for one of the most common diseases in plants, the bacterial wilt. It is a soil-borne pathogen with tissue-specific tropism that infects roots and starts to multiply in the xylem vessels. This bacterium is well adapted to a wide range of host plants, however, easily survive in the soil in the absence of hosts. Its genome has been sequenced completely (Salanoubat et al. 2002), whereas the functional analysis of its pathogenic mechanisms remains a challenge and generates increased difficulty for the farmers to efficiently control diseases. The control of *R. solanacearum* is extremely hard, mainly due to its wide range of hosts, high genetic variability, and the survival capacity in the soil for long years, and in great depths, making the chemical control unviable and expensive. The soil disinfestation can reduce the bacteria levels, but the soil can be quickly re-infested, probably by water from the underground or from the surface (Louws et al. 2010).

The bacterial wilt is one of the tomato diseases that can be controlled with the grafting. Some studies show that tomato hybrids grafted in rootstock resistant to *R. solanacearum* have presented symptoms of the bacterial wilt, which can affect the plants commercial production. To evaluate the rootstock resistance to certain pathogens, some parameters could be observed as the pathogen presence in the graft, the gaseous trades in the plants leaves, the total phenols content, and the antioxidant enzymes activity (Silva et al. 2016).

2.1.1 Grafting and Production

Grafted plants can show a higher production in comparison to the ungrafted ones, as described by Pogonyi et al. (2005) in hybrid tomato cv. Lemance F1 using "Beaufort" as a rootstock. The research described that there were higher fruit productions, a result that reflected in a higher productivity. However, organic acids and carbohydrates content were lower in grafted plants. A thesis developed in our laboratory (Laboratory of Plant Biochemistry, Institute of Biosciences, São Paulo State University, Botucatu, São Paulo, Brazil) (Silva et al. 2016) analyzed the qualitative characteristics of tomato production in function of different grafting methods. There was a statistical difference in the ascorbic acid content in tomatoes between the cleft grafting and the other treatments (splice graft, supported graft, and ungrafted plants). In opposition, no difference was observed when we analyzed lycopene, β-carotene, titratable acidity (TA), soluble solids (SS), soluble solids/titratable acidity ratio (SS/TA – *Ratio*), hydrogenionic potential (pH), and fruit firmness (Table 2.1). The fruit from the plants grafted by the cleft grafting method showed a higher ascorbic acid content (17.8 mg ascorbic acid 100 g^{-1} pulp) than the other treatments. The fruit from ungrafted plants (control) showed the lowest ascorbic acid content (15.8 mg ascorbic acid 100 g^{-1} pulp), but was not different from the treatments splice graft and supported graft (Table 2.1).

A research developed with tomatoes (*L. esculentum* Mill.) cultivars "Yeni Talya," "Swanson," and "Beril" (graft) and "Beaufort" and "Arnold" (rootstocks), using cleft grafting, showed that grafted tomatoes on suitable rootstocks showed a positive effect in the production and fruit index (diameter/length). The number of fruits/truss and fruit weight were also significantly influenced by grafting (Turhan et al. 2011).

Thus, grafted plants can produce more fruits with superior qualitative characteristics, as found in (Alan et al. 2007) study, using watermelons that were grafted onto commercial hybrids of *Cucurbita maxima* x *C. moschata* and *Lagenaria siceraria* in low tunnel and open field. The results show that grafted plants have higher growth and production, mainly when cultivated in low tunnel, which had no influence of

Table 2.1 Ascorbic acid (AA, mg 100 g^{-1} pulp), lycopene and β-carotene (mg 100 g^{-1} pulp), titratable acidity (TA, g malic acid 100 g^{-1}), soluble solids (SS, °Brix), soluble solids/titratable acidity ratio (SS/AT), hydrogenionic potential (pH), firmness (N), in function of the grafting method in tomatoes

Treatments	AA	Lycopene	β-carotene	TA	SS	SS/AT	pH	N
Splice graft	16.0^{b}	0.25	0.05	0.05	4.55	87.65	4.59	272.00
Supported graft	16.0^{b}	0.26	0.04	0.05	4.75	90.51	4.57	163.83
Cleft graft	17.8^{a}	0.22	0.06	0.04	4.2	86.61	4.56	281.58
Ungrafted	15.8^{b}	0.23	0.07	0.05	4.6	87.61	4.62	287.25
F	7.47*	0.38^{ns}	3.16^{ns}	0.33^{ns}	3.82^{ns}	0.14^{ns}	2.19^{ns}	4.17^{ns}

*or ns F test significant or nonsignificant at the probability level of 5%. Means followed by the same letter do not differ between themselves by Turkey test at 5% probability low temperatures. Other studies with watermelon also show the positive effect of grafting. When watermelon (*Citrullus lanatus* [Thunb.] Mansf., cv. Crimson Sweet) was grafted onto four rootstocks (Long gourd, Early Max, Max-2, and F-14 gourd), even though there was no increase on the number of fruits per plants, there was an increase in the fruit size, weight, and thickness of the peel (Alexopoulos et al. 2007). Other researches show an increment in the fruit size of watermelon grafted on interspecific squash hybrid (*C. maxima* Duchesne × *C. moschata* Duchesne) (Yetisir and Sari 2003).

2.1.2 Agrochemicals

In order to counter the diseases problem in seedling that affect the production, the use of chemical substances is a frequent option. The use of some chemical substances, as some fungicides, has induced the genetic resistance in some plants, showing a fungistatic action that affects the pathogenic fungi, becoming incompatible to the plant, which is generally susceptible and compatible. According to Oostendorp et al. (2001), this effect causes an imbalance between the plants compatible and susceptible to the pathogens for the disease development occurs, producing incompatible reactions. However, it is not always easy to determine whether the compound effect happens in the plant or in the pathogen.

Fungicides have the capacity to inhibit fungal growth by disrupting/blocking critical cellular processes. However, the four main mechanisms by which fungi can become resistant to fungicide are the alteration of the target site and consequently reducing the fungicide sensitivity, detoxification of the fungicide, overexpression of the target, and exclusion or expulsion from the target site (FRAC 2014). The class of fungicides has increased in the last years. However, the list of fungicides, by which microorganisms have acquired resistance, has increased too. Some groups of agrochemicals frequently used are the sterol biosynthesis inhibitors (SBIs), quinone outside inhibitors (QoIs), and succinate dehydrogenase inhibitors (SDHIs) (Sierotzki and Scalliet 2013).

SDHIs are a class of fungicides structurally very diverse that display an essential common feature, the presence of amide bond (Sierotzki and Scalliet 2013). SDHIs are composed of benodanil, benzovindiflupyr, bixafen, boscalid, carboxin, fenfuram, fluopyram, flutolanil, fluxapyroxad, furametpyr, isopyrazam, mepronil, oxycarboxin, penflufen, penthiopyrad, sedaxane, and thifluzamide (FRAC 2017). Carboxin, originally named carboxamide, are a class of compounds belonging to SDHI that act inhibiting complex II of the mitochondrial respiratory chain of fungal respiration. These fungicides were first used for basidiomycetes control in plant seeds. Nevertheless, the first carboxamide with truly broad-spectrum foliar activity was boscalid (Glättli et al. 2010). Despite SDHI fungicides broad-spectrum activity against various fungal species, the prolonged use of these fungicides can result in the selection of resistant fungal genotypes and decline in the fungicide performance (Avenot and Michailides 2010).

QoIs fungicides constitute a class of fungicides that has been developed from natural fungicidal derivatives such as strobilurin A, oudemansin A, and myxothiazol A and are classified as strobilurin and strobilurin-related fungicides and are derivatives of the toxins produced in nature by the basidiomycete fungi *Strobilurus tenacellus* and *Oudemansiella mucida* and the bacterium *Myxococcus fulvus* (Karadimos et al. 2005). The mechanism of action is based on the linkage to Qo site of cytochrome *b*. This process leads to inhibition of mitochondrial respiration by blocking the electron transport between cytochrome *b* and cytochrome *c1* (Ammermann et al. 2000). Compounds belonging to this class of fungicides include azoxystrobin, famoxadone, fenamidone, fluoxastrobin, kresoxim-methyl, metominostrobin, picoxystrobin, pyraclostrobin, and trifloxystrobin.

Some agrochemicals can exert physiological effects in the plants. Between these agrochemicals, the active ingredients from the group of the strobilurins and anilides and of the biostimulants are generally the most studied. Such products are employed mainly in great cultures due to its higher economical return to the manufacturing companies. In studies performed on these agrochemicals effects, it was possible to observe the positive alterations in the metabolism and growth (Table 2.2). These metabolic and/or physiological changes can result in higher productivity and profit to the farmer.

Pyraclostrobin is a relatively new strobilurin fungicide, and few information about its efficacy against plagues are available. In addition to direct inhibition of fungi (Vicentini et al. 2007), pyraclostrobin has an efficient effect against spore germination, even when used at very low concentrations (Karadimos et al. 2005). This fungicide has shown efficiency in the control of diseases as anthracnose, rust, mildew, powdery mildew, and blight in many cultures of great economic importance; among them are corn, cotton, potato, carrot, onion, melon, watermelon, soybean, tomato, and grape. In addition, it contributes positively in the increase of liquid photosynthesis, increases the nitrate reductase activity, and causes a green effect due to the higher chlorophyll content and reduction of stress associated to the reduction of the ethylene synthesis. Thus, it allows a larger duration of the leaf area, increases the proteins content and biomass, reduces the cellular respiration, and increases the cultures yield (Kozlowski et al. 2009).

Some of the physiological effects caused by the strobilurins were related. Köhle et al. (1994) verified that, even without any alteration caused by pathogenic fungi, the plants treated with this substance show a higher vigor and production, when compared to the plants without any treatment. These effects are attributed to higher auxin levels, higher indoleacetic acid (IAA), and cytokinins synthesis, stimulating the cell division and elongation, initial development of the roots, and retardation of the leaf senescence and fruit ripening. This effect reduces the fungi respiratory process and blocks the energy supply, inducing it to death. Another aspect of the strobilurins utilization is the modification in the CO_2 concentration in plants. Studies already performed indicate that there is a temporary increase in the alternative respiration route, potentially reducing the CO_2 emission due to the mitochondrial respiration inhibition.

Table 2.2 Physiological effects with the application of fungicides in different cultures

Physiological effect	Fungicide	Culture	Author/year
↑ yield	Pyraclostrobin + metiram	Tomato	Guimarães et al. (2014)
	Pyraclostrobin	Soybean	Fagan et al. (2010) and Joshi et al. (2014)
		Bean	Kozlowski et al. (2009)
	Azoxystrobin	Wheat	Zhang et al. (2010)
			Beck et al. (2002)
			Bertelsen et al. (2001)
	Boscalid	Cucumber	Sirtoli et al. (2011)
↑ ACC-synthase activity	Pyraclostrobin	Wheat	Grossmann and Retzlaff (1997)
		Banana	Lima et al. (2012)
↑ chlorophyll content	Azoxystrobin	Wheat	Beck et al. (2002)
↑ mean grain weight	Pyraclostrobin	Wheat	Gooding et al. (2000)
		Soybean	Joshi et al. (2014)
↑ plant growth	Pyraclostrobin	Wheat	Gooding et al. (2000)
		Soybean	Joshi et al. (2014)
	Azoxystrobin and pyraclostrobin	Banana	Lima et al. (2012)
↑ dry matter	Azoxystrobin and pyraclostrobin	Banana	Lima et al. (2012)
	Boscalid	Cucumber	Sirtoli et al. (2011)
↑ leaf area	Boscalid	Cucumber	Sirtoli et al. (2011)
	Pyraclostrobin	Bean	Kozlowski et al. (2009)
↑ number of pods	Pyraclostrobin	Bean	Kozlowski et al. (2009)
↑ levels of polyamines in plants	Pyraclostrobin + metiram	Tomato	Guimarães et al. (2014)
↑ fruit weight	Pyraclostrobin + metiram	Tomato	Guimarães et al. (2014)
	Boscalid	Melon	Macedo et al. (2017)
↑ photosynthetic rate	Pyraclostrobin	Soybean	Fagan et al. (2010)
	Azoxystrobin	Wheat	Dimmock and Gooding (2002)
			Beck et al. (2002)
↑ nitrate reductase activity	Pyraclostrobin	Wheat	Gooding et al. (2000)
		Banana	Lima et al. (2012)
		Soybean	Fagan et al. (2010)
	Boscalid	Cucumber	Sirtoli et al. (2011)
↓ level of superoxide (O_2^-) and ↑ antioxidative system	Azoxystrobin	Barley	Wu and von Tiedemann (2002)
		Wheat	Wu and von Tiedemann (2001)
			(Beck et al. 2002)
			Zhang et al. (2010)

(continued)

Table 2.2 (continued)

Physiological effect	Fungicide	Culture	Author/year
Senescence slows	Azoxystrobin	Wheat	Wu and von Tiedemann (2001)
			Beck et al. (2002)
			Zhang et al. (2010)
	Pyraclostrobin	Wheat	Grossmann and Retzlaff (1997)
		Bean	Kozlowski et al. (2009)
	Boscalid + pyraclostrobin	Tomato	Domínguez et al. (2012)
			Ramos et al. (2015)
↑ carbohydrate in the plant	Boscalid + pyraclostrobin	Tomato	Domínguez et al. 2012)
			Ramos et al. (2015)
↑ fruit quality	Pyraclostrobin	Melon	Macedo et al. (2017)
	Boscalid + pyraclostrobin	Tomato	Ramos et al. (2013)
↓ post-harvest mass loss	Boscalid + pyraclostrobin	Tomato	Domínguez et al. (2012)

↑ increase, ↓ decrease

The boscalid from the anilides chemical group is a systemic fungicide, which presents positive physiological effects in the plants, similarly to the strobilurins, besides providing a preventive antifungal protection. The substance was developed for mildew control, *Alternaria* spp., *Botrytis* spp., *Sclerotinia* spp., and *Monilia* spp., in a great variety of fruits and oleracea trees (Mueller and Bradley 2008).

2.1.3 Agrochemicals and Systemic Acquired Resistance (SAR)

These defense mechanisms have been extensively studied in many species, and important results have been obtained for the control of plagues and diseases. Some chemical substances can activate the systemic acquired resistance (SAR) or induced systemic resistance (ISR) against a series of pathogens, which could be fungi, bacteria, or virus. Both in SAR, as in ISR, the acquired resistance or tolerance depends on the plants defense in response to an infection or treatment. SAR influences on the increased resistance against many leaf pathogens, and this effect can be transmitted to the roots (Gessler and Kuc 1982) and to the progeny (Luna et al. 2012). The SAR and/or ISR are related to a systemic defense, induced by the genes activation, which also activate other metabolic systems, offering protection against many types of stress (LaMondia 2009; Ansari and Mahmood 2017; Pretali et al. 2016).

The plants have a wide range of active defense mechanisms by which they can respond to a variety of abiotic or biotic stresses that are found in the vegetables lifetime. However, this perception and/or response to a stress can make the difference between defending or succumb. The SAR are mechanisms by which plants recognize common characteristics of organisms interacting with its structures and induce responses directed against the specific invader (Jones and Dangl 2006). The first events that occur before the SAR establishment include the change in the plasmatic membrane potential, degradation of the membrane lipid, and increase of the membrane permeability, associated with the lipoxygenase activity. These changes contribute to the plants defensive response, including the direct inhibition of the pathogen and accumulation of phytoalexins (Lin et al. 2009).

The literature has focused on the effects of pathogenic bacteria, fungi, insects, and metallic compounds on the SAR induction or on the application of chemical products (isolated molecules, pesticides, fungicides, herbicides) in the ISR and/or SAR. Some researches show that, in rice, products as the herbicide diclofop-methyl can induce the mediated pathway by salicylic acid from SAR, decreasing the stress against diseases caused by pathogenic bacteria (Chen et al. 2017).

Among the agrochemicals that can be applied and that have been studied for increasing the resistance is included the benzo (1,2,3) thiadiazole-7-carbothioic acid-S-methyl ester (acibenzolar-S-methyl, ASM, or BTH), which can activate the plant resistance against pathogenic agents, interfering in physiological and biochemical processes. ASM has been described for inducing the resistance against fungi, bacteria, and virus in many plants species, as studied by Soylu et al. (2003) with tomato seedling regarding the induced resistance against *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), which causes bacterial canker. The results show that the use of 200 mL per seedling reduced the disease severity (up to 75%) and the damages decrease was related to the low bacterial growth (up to 68.2%), during the time course of infection.

In order to induce the resistance against whitefly *Bemisia tabaci* (Gennadius) biotype B in cucumber, Correa et al. (2005) applied ASM in combination or not with calcium silicate (62% SiO_2 and 18.5% CaO) as a silicon source, which has been used to decrease the incidence of insect pests and diseases. These products could be an alternative to the isolated use of ASM. In the study, the results show that the application performed separately or as a mixture on leaves was efficient to induce the defense system and reduce the whitefly population, as well as the preference of *B. tabaci* biotype B for oviposition.

Against tobacco blue mold caused by *Peronospora tabacina* Adam (*Peronospora hyoscyami* f. sp. *tabacina* Skalicky 1964), LaMondia (2009) applied dimethomorph plus mancozeb and azoxystrobin fungicides as well as acibenzolar-S-methyl on disease severity over 2 years in both shade-grown and broadleaf tobaccos. The result showed that the application of 17.5 g a.i. ha^{-1} of ASM reduced damages (up to 99%) caused by blue mold and dimethomorph plus mancozeb treatments decreased the lesions compared with the paired fungicide-only treatments by 28 to 94%. In opposition, Perez et al. (2003) reported that the application of lower acibenzolar-S-methyl doses was not efficient to control the blue mold in tobacco leaves.

Thus, the used dose of ASM can determine the response level against some pathogens. The use of 100 μg ASM mL^{-1} applied 5 days after the transplant of three beans cultivars with different levels of resistance to bacterial wilt, cultivated in greenhouse, was not efficient enough to induce resistance on the susceptible cultivar (IAC Carioca), neither it increased the resistance levels in IAC Carioca Akytã or IAC Carioca Pyatã (Soares et al. 2004).

Some biological control agents (BCA) also demonstrated to induce the SAR and ISR. An example of a BCA that induces SAR is *Bacillus mycoides*, which provided control of diseases caused by fungal, bacterial, and viral pathogens in different plants species (Jacobsen et al. 2004; Neher and Jacobsen 2008). The combination of agents that induce resistance (SAR and ISR), such as handling of fungicides, insecticides, and herbicides or biological control agents or with hosts resistance to diseases, can provide effective diseases control, especially in situations when the disease control is difficult (Moya-Elizondo and Jacobsen 2016).

Besides the mentioned agrochemical, the SAR can be induced in response to the genes activation by substances as salicylic acid (SA) and its derivative SA-glucoside (SAG) or pathogenesis-related proteins (Dempsey et al. 2011). Molecules as jasmonic acid (JA), ethylene, and salicylic acid have been indicated as messengers or signal transducers (Jung et al. 2009; Shah and Zeier 2013), inducing SAR. Salicylic acid (2-hydroxybenzoic acid) is a phenolic compound, and the SA-mediated defense signaling pathway is activated by the biotrophs infection, in a live vegetal tissue. SA accumulation after a pathogenic infection induces expression of multiple pathogenesis-related (*PR*) genes, which are denominated markers of the onset of SAR (Durrant and Dong 2004).

Salicylic acid in plants under pathogenic attack is synthesized from trans-cinnamic acid, producing o-coumaric (decarboxylation) or benzoic acid (hydroxylation) as intermediates. The key enzyme for the occurrence of this pathway is the phenylalanine ammonia lyase (PAL) (phenylpropanoid pathway), which is regulated by many types of stress, producing defense compounds (Anand et al. 2007). Methyl salicylate (MeSA) is synthesized by the SA carboxyl methyltransferase (SAMT) (Loake and Grant 2007) and is a volatile ester, which can be produced after the pathogens infection and transmitted by air, inducing the expression of defense genes in neighbor plants (Koo et al. 2007). MeSA and SA are related to the defense of plants against pathogens and phloem-feeding insects, acting as an endogenous SAR signal (Vlot et al. 2008).

Differences in disease resistance are frequently observed. In two cultivars of kimchi cabbage (*Brassica rapa* var. glabra Regel) seedlings, one moderately resistant (CR-Hagwang) and the other susceptible (Buram-3-ho) regarding anthracnose (*Colletotrichum higginsianum*), black spot (*Alternaria brassicicola*), and black rot (*Xanthomonas campestris* pv. *campestris*, Xcc), researchers verified that the pathogenesis-related (PR) was higher in the resistant cultivar treated with SA than in the susceptible cultivar (Lee and Hong 2015). Lee et al. (2014) investigated the exogenous application of SA, methyl jasmonate (MJ), and 1-aminocyclopropane-1-carboxylic acid (ACC) in the same plants previously described. SA induced an increase of susceptibility in the cultivar susceptible to *A.*

brassicicola e Xcc but increased the disease resistance to Xcc in the resistant cultivar. The MJ use did not affect the disease resistance to *C. higginsianum* and Xcc. The ACC application, a precursor of the ethylene synthesis, did not promote modifications in the resistance to *C. higginsianum* and Xcc.

When there is the attack of wounding and necrotrophic pathogens, another pathway can be activated, using the jasmonic acid (JA) and ethylene (Verhage et al. 2010; Dempsey and Klessig 2017). JA and derived compounds are denominated as jasmonates, synthesized from polyunsaturated fatty acids, and are called oxylipins. Methyl jasmonate (MJ) is a volatile compound, derived from JA that also acts as an indicator in response to pathogens attacks. Some results show the inhibitory action against some pests after the exogenous JA application. Black et al. (2003) tested JA spray against leaf miners and other pests on celery and verified that there was a decrease of adult leaf miners occurrence in plants sprayed with JA. The exogenous application in other cultures also has shown a positive effect. In tomatoes, JA or MJ induced the mechanism of increasing enzymes such as polyphenol oxidase (PPO), peroxidase (POD), proteinase inhibitors, and lipoxygenase, induced by herbivore feeding (Thaler 1999; Cipollini and Redman 1999).

2.1.4 Oxidative Enzymes

The vegetables can endure various types of stress, such as oscillations in the temperature, humidity, solar radiation, plagues and pathogens attacks, and handling, among others. However, the plants have a response mechanism, with alterations related to the defense and protection of its molecules. These mechanisms were developed against damages and diseases during the vegetables evolution, which are activated immediately after the attack recognition (de Wit 2007).

In natural environmental conditions, the reactive oxygen species (ROS) protect the plants but are produced in great quantity in stress situations, becoming toxic and causing damages through oxidation reactions that affects nucleic acids, proteins, and lipids (Strid et al. 1994). High ROS quantities cause the oxidative stress characterized by damages, as DNA fragmentation, protein modifications, chlorophyll disintegrations, ionic leakage, lipid peroxidation, and, consequently, cell death (Møller et al. 2007). The oxidative stress induces the synthesis of most of the reduced forms of H_2O_2, O_2•-, and the hydroxyl radical (OH•) (Halliwell and Gutteridge 1999).

In order to survive to stress conditions, which are frequently imposed by the environment, the plants developed systems of removing the reactive oxygen species (ROS) and have two antioxidant defense systems: one nonenzymatic and the other enzymatic. The nonenzymatic mechanism refers to some molecules with the capability of scavenging free radicals, as carotenoids phenolic compounds, vitamin C, and alkaloids, among others. The enzymatic mechanism is composed of a series of proteins, with the function of scavenging free radicals, as the superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR), and glutathione S-transferase

(GSTs). Zhang et al. (2014) mention POD, ß-1,3-glucanase, chitinase, phenylalanine ammonia lyase (PAL), and polyphenol oxidase (PPO) as enzymes related to the stress provoked by pathogens.

The ROS formation is important to species survival. Soon after the pathogens attack, the cells can die due to the ROS, and this process is fundamental to limit the pathogen growth. Thus, ROS can lead to a hypersensitive response (HR), and healthy tissues develop SAR against a series of pathogens (Elsharkawy et al. 2013). When the plant presents SAR, there might be necrotic damages after the pathogen attack or after the chemical compounds application, as SA (Loake and Grant 2007) and ASM (Soylu et al. 2003), among others.

SOD (EC 1.15.1.1) is the first enzyme from the plant defense line against the ROS in the cells, among organisms that consume oxygen. Three distinct types of SOD isoenzymes have been detected in plants, which are classified according to their metallic cofactor Fe, Mn, and Cu/Zn (Gratão et al. 2005). The Cu/Zn-SOD are located in the cytosol and chloroplasts, Fe-SOD are located in the chloroplasts, and Mn-SOD are located in the matrix (Scandalios 2005). The enzymes catalyze the dismutation of the superoxide radical (O_2^-) in H_2O_2 and O_2, thus representing one of the main defense mechanisms against the oxidative cell stress (Scandalios 2005).

The CAT (EC 1.11.1.6) converts H_2O_2 in H_2O and O_2, and in the plants they are not found in the peroxisomes and glyoxysomes. They are separated in three classes: class I, responsible for removing the H_2O_2 produced during the photorespiration and are found in the photosynthetic tissue; class II, located in the vascular tissue and participates in the lignification process; and, finally, the class III, found in seeds and plants and degrade the H_2O_2 produced during the acids degradation in the glyoxysome (Resende et al. 2003). The catalases act as a H_2O_2 scavenger. According to Mittler et al. (2004), besides CAT, the POD and the SOD also act in the ROS removal. In the plants infected tissues, a reduction of the catalase activity would lead to the raise of H_2O_2, which could provoke cell death (promoted by the programmed cell death (PCD)), inducing the hypersensibility response (HR) (Coll et al. 2011).

Catalase has been demonstrated by its action on H_2O_2 detoxification. When this system is no longer sufficient, both hydrogen peroxide and organic peroxides will be metabolized by peroxidases (Willekens 1997; Mhamdi et al. 2010). Both POD and CAT have affinities for H_2O_2; however the function of these enzymes is different. Catalases are described by removing excess hydrogen peroxide, and peroxidases would be related with the ROS modulation (Noctor et al. 1998; Ali and Alqurainy 2006). POD (EC 1.11.1.7) is involved in many cell reactions, as the phenolic compounds oxidation, polysaccharides bonding, indol-3-acetic acid oxidation, lignification, wounds healing, and defense against pathogens (Hiraga 2001). They catalyze the oxidation of a great variety of substrates through the reaction with H_2O_2. According to its structural and catalytic properties, they can be divided into three classes: class I, intracellular enzymes in plants, bacteria, and yeasts as the cytochrome *c* peroxidase and ascorbate peroxidase of chloroplast and cytosol; class II, extracellular enzymes from fungi; and the class III, constituted by peroxidases that are secreted in the apoplast (Hiraga 2001). The studies reinforce the hypothesis that the class III has an important role in the plants defense and can act as an enzymatic defense in response to biotic stresses (Saathoff et al. 2013). The POD has an important role in the cell wall biosynthesis, increasing the mechanical barriers against pathogenic attacks, decreasing the speed of the pathogen penetration. The lignification process by the enzyme is observed in many plants species in response to infections by pathogenic agents, and the enzymatic activity has shown to be induced by bacteria, fungi, and viruses (Hiraga 2001; Babu et al. 2008).

The polyphenol oxidase (PPO) (EC 1.14.18.1) belongs to the group of the oxidoreductases, contains the copper as a prosthetic group, and is related to the phenolic compounds oxidation. These compounds are substances resulting from secondary products, which contain a phenol group that is a hydroxyl functional group and an aromatic ring. They are produced by the plants and show a variety of functions in the vegetable. The PPOs are codified by nuclear genes and stored in the plastids to stay separated from its substrates (phenolic compounds), which are located mainly in the vacuoles and have activity only when they are liberated in the cellular rupture (Hunt et al. 1993). Because of that, it is believed that the main role of this enzyme in plants is the defense against microorganisms, insects, and herbivorous mammals (Appel 1993; Steffens et al. 1994). The higher is the PPO activity, the higher are the toxic products concentrations obtained from the oxidation, which provide resistance against the infections. Another function of this enzyme is the lignification, related to the plants protection, due to the toxic action of reactive quinones, produced from the phenolic compounds catalysis (Mayer and Staples 2002). The quinones are considered bactericidal and fungicidal substances and are also highly toxic to pathogenic microorganisms (Cowan 1999; Kim et al. 2004).

The PAL enzyme (EC 4.3.1.5) is a regulator of the phenolic compounds synthesis, which are precursors of lignin and salicylic acid that act in the structural defense and in the process of signalizing a systemic response (Bonas and Lahaye 2002). This is the most intensively studied enzyme of the plants secondary metabolism due to its importance in the metabolic reactions of the phenolic compounds. The PAL is responsible for the deamination of the L-phenylalanine amino acid, transforming it in trans-cinnamic acid and ammonia. The accumulation of phenolic substances in plants submitted to the stress seems to be a consequence of the increasing PAL activity, which generally results in an increase of the other enzymes activities, as, for example, the PPO and POD (Gaspar et al. 1985). This enzyme is regulated by factors as nutrition, light, and pathogens infection. Among the substances formed by this enzyme is the benzoic acid, which originates the salicylic acid, an important compound in the plants defense against pathogens.

Many studies have been performed relating these enzymes to the plants defense against pathogens. In response to the inoculation by *R. solanacearum*, stems from *Pogostemon cablin* (patchouli) presented an increase in the POD, SOD, PPO, and PAL activities in comparison to control plants, which could be a protective mechanism against damages caused by the bacteria (Xie et al. 2017). In the study of Soares et al. (2004), the use of acibenzolar-S-methyl sprayed in three common bean (*Phaseolus vulgaris*) cultivars induced peroxidase activity in leaves, and this induction would promote biochemical defense reactions. The increment of the plant resistance against the pathogen could be verified through changes of peroxidase activity in plant sprayed with ASM.

According to Kohatsu et al. (2013), both peroxidases and polyphenoloxidase activities can be considered as successful grafting processings in grafted “Tsuyoi” cucumber plants grafted onto “Shelper” squash and “Green-stripped” cushaw squash. The authors verified that the POD activity was directly responsible for the process of vessel lignifications, and lower PPO activity would provide a faster and more efficient grafting process, thus changing the transport of nutrients, photo-assimilates, plant hormones, and, consequently, plant vigor.

Some synthetic compounds as SA, ASM, or BTH can activate genes that act in the plants resistance. The exogenous application of JA or MJ induces an increase in the pathogenesis-related proteins (PR) activity, as PPO and POD, forming substances that decrease the pathogens action by forming harmful compounds to these organisms. Against tomato canker promoted by *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), the ASM use can reduce the disease severity up to 75% and promoted the highest peroxidase and glutathione peroxidase activities in comparison to plants without treatment. In this study, the low rate of symptoms and low bacterial growth in plants treated with ASM can happen due to the high activity of oxidative enzymes as a part of the plant antioxidant system (Soylu et al. 2003).

Besides these enzymes (PPO, POD, SOD, CAT, and PAL) mentioned as participants of the plant defense system against pathogens, some amine oxidases have been related to the defense system. The oxidation of some polyamines (putrescine, spermidine, and spermine) produces peroxides, via PAO (polyamine oxidase) and/or DAO (diamine oxidase), which contributes to the host’s cell death. These reactions are related to pathosystems involving biotrophic or hemibiotrophic pathogens (Yoda 2006; Moschou et al. 2009). However, the peroxide production by these oxidase amines is the only responsible for the defense system activation (Jiménez-Bremont et al. 2014). The formation of a compound related to the plants defense, the γ-aminobutyric acid (GABA), is a result from the polyamines oxidation pathway (PUT and SPD) that forms Δ^1-pirroline, a GABA precursor (Bouché and Fromm 2004).

2.1.5 Conclusions

The effects of grafting methods agrochemicals on plant metabolism and physiology should be better studied. Interactions induced by both factors (graft and agrochemicals) have gained attention in the last years; however, a relatively slow progress has been reached. Plant responses are variable and dependent of the vegetable studied, the graft method employed, the agrochemical, and the enzymatic pathway activated in which situation. There are many problems associated to grafting, such as cost of rootstocks, dispended time, and expertise in the technique. Nevertheless, this method has been successfully used at several decades, and associated to agrochemicals is a very important practice to prevent or resist to biotic factors that decreases vegetables production.

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Chapter 3
Management of Plant Biotic Stress with Botanicals and Antagonistic Fungi in the Tropics

David Babatunde Olufolaji and Ayodele Martins Ajayi

Abstract The adverse effect of the living components of the environment on plants is called biotic stress. Food insecurity, environmental degradation and global warming are some of the problems associated with loss of vegetation cover and crops death resulting from the activities of insect pests and pathogenic organisms (biotic stressors). A number of control measures exist for the management of plant biotic stress, but chemical control measures appear to be the most common in several parts of the globe. Mammalian toxicity, carcinogenicity, mutagenicity and disruption of the natural ecosystem are some of the common problems associated with chemical control of biotic stressors. It has therefore become imperative to adopt other management strategies that are environment friendly and less toxic to man and nontarget organisms. Botanicals and antagonistic fungi are two of these alternatives. They are cost effective, safe, environment friendly and sustainable. The tropic, because of its peculiar geography, ecology and economy, will benefit from this approach immensely.

Keywords Biotic stress · Food insecurity · Environmental degradation · Botanicals · Antagonistic fungi

3.1 Introduction

Plants are exposed to numerous environmental factors that can induce stress. These factors are classified as abiotic or biotic. Abiotic stress results from nonliving factors, while biotic stress results from living factors (Nilsen and Orcutt 1996. The impact of abiotic stress can be devastating, resulting in poor plant growth, low productivity, crop failure and poor yield (Agrios 2005). The use of synthetic chemicals in the management of biotic stress is an age long practiced, but this practice is beset with myriads of problems (Richter 2002). The need for alternative control measures has become imperative, especially in the tropics where a very high level of poverty

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D. B. Olufolaji (✉) · A. M. Ajayi
Pest Management Unit, Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Ondo State, Nigeria

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and illiteracy amongst peasant farmers have further aggravated the problems associated with chemical use. Research has shown that bioactive compounds from plants as well as biocontrol agents, which occur in abundance in the tropics, hold great promise in the management of plant pests and disease.

3.2 Meaning of Biotic Stress

In physical science, mechanics to be precise, the word stress is used to describe a condition in which an object A (the stressor) exerts force on another object B to the point where the impact from the stressor is more than the resistance of object B, causing it (object B) to experience strain and deformation (Korner 2012).

In life science, this principle has been adopted in the description of the impact of environmental factors (living and non-living) on plants and animals. Stress induced by the non-living components of the environment is referred to as abiotic stress, while that induced by the living components is referred to as biotic stress. The common biotic and abiotic stressors are listed in (Table 3.1).

3.2.1 Definition of Plant Stress

Plants are living things capable of responding to stress in ways much different from non-living bodies. The situation is made more complex when the stressor is also a living organism. The dynamics created by the interactions between the two living entities sometimes tilt in favour of the host plant, eliciting responses that limit damage from stressors to a minimal level or to effect repair when damage has occurred. These factors have made the definition of plant stress much more challenging than the definition of the same process in mechanics. Expectedly, different definitions have been proposed by different authors. Lichtenthaler (1996) defined it as any unfavourable condition or substance that affect or block a plants metabolism, growth or development. The reaction of a biological system to extreme environmental factors that may cause significant changes in the system depending on their intensity

Table 3.1 Some of the sources of environmental stress in plants

Physical	Chemical	Biotic
Drought	Air pollution	Competition
Temperature	Heavy metals	Herbivores
Radiation	Pesticides	Parasitic plants
Flooding	Toxins	Pathogens
Wind	Soil pH	Diseases
Magnetic field	Salinity	Insects pests

Source: Nilsen and Orcutt (1996). Modified and duration was the definition proposed by Godbold (1998). Gaspar et al. (2002) said it is a condition caused by factors that tend to alter an equilibrium and changes in plant physiology that occur when species are exposed to extraordinary unfavourable condition that need to represent a threat to life but will induce an alarm response.

The most important biotic stressor of plants is pathogenic fungi. A great number of diseases of crop plants are incited by fungi. Bacteria, phycoplasmas, viruses, viroids, protozoans, nematodes, algae and parasitic plants are also known to incite diseases in crops. Insect pests, which may be defoliators or vectors, and parasitic plants are equally very important as biotic stressors of plants (Agrios 2005; Deacon 2006; Kaiser et al. 2015; Brooks et al. 2015).

3.3 Biotic Stressor and Host Plant Relationship

Biotic stress may result in histological damage in the host plant. Cell wall disintegration is common with infection from pathogenic organisms, while the permeability of the plasma membrane can be altered. Protein synthesis and enzymatic processes all become altered when a plant is undergoing biotic stress. The net result is that physiological activities and morphological features in such plants are adversely affected (Agrios 2005; Fraire-Velázquez et al. 2011).

In fungal-induced biotic stress, the impact of the stressor sometimes starts from the cell wall of the epidermis at the point of contact. This is particularly true for fungal pathogens that breakdown the barrier presented by the host plant chemically, through the produced cellulolytic enzymes, or those that do so mechanically, through germ tube or infection peg. Cellulolytic enzymes degrade the cellulose structure of the cell wall, thus creating an entry point, while the germ tube or infection peg exerts force on the cell wall and breaks it down mechanically. During the post entry stage of the pathogen, establishment and invasion of the host's tissue occur. The protein synthesizing apparatus of the host is diverted for the use of the invading pathogen. This action, coupled with the release of toxins into the host's tissue, disrupt photosynthesis, respiration and several other physiological processes in the host plant. Passive invaders like bacteria, viruses, phycoplasmas and algae produce similar trends of events but find their way into plant tissues through natural openings or those created by biotic and abiotic agents (Deacon 2006; Dyakov et al. 2007; Muller and Paschke 2012).

In bacterial stressors, colonies of new bacteria form rapidly shortly after successful entry into the tissue of the host plant. Studies showed that these colonies feed on plant nutrients that leaks into the intercellular spaces. Some of the colonies find their way into the vascular bundle where they disrupt nutrient absorption and translocation of photosynthate. The host's cell wall eventually breaks down, due to the continual increase in the population of bacterial pathogen, growth regulators and the production of toxic metabolites. Growth activities in the host plant stall as it struggles to cope with the pathogen (Muller and Paschke 2012; Mehrotra and Ashok 2006).

Viral stressors find their way into the cells of host plants through natural openings or through the activities of insect vectors. These viral pathogens remove their protein coat as soon as they find their way into the cytoplasm of the host's cells. They then replicate new viral genomes, through the transcription of their mRNA, and form new proteins, making use of the translating apparatus of the host. The nucleic acid genome of stressor virus is then replicated, leading to the formation of new viral particles, which are lysed out of infected cells. Again, these events disrupt the normal functioning of the host plant and can bring about deformation of plant organs and poor growth of the entire plant (Hull 2009; Pallas and Garcia 2011). Nematodes cause mechanical damage to plant roots, disrupting the normal absorption of water and mineral salts, while also transmitting diseases in some cases. Parasitic plants send their haustoria into the conducting tissues of the host plant, competing for nutrient and causing the host plant to struggle for sustenance. *Cassytha filiformis* and *Cuscuta approximata* are two good examples (Kaiser et al. 2015). Insect creates mechanical damage on leaves through their feeding, thus reducing the surface area available for photosynthesis. They are also important as vectors of numerous diseases, especially those caused by viral pathogens (Hull 2009).

The response of plant to biotic stress is usually determined by the type of stressor and other associated environmental factors. The response may be short-lived physiological changes such as the formation of cork cells beyond the point of infection by a pathogenic stressor, formation of tylose, formation of abscission layer or the formation of sheath around the hyphae of fungal pathogen after it had gained entrance (Amrhein et al. 2012). It may also come in form of phytoalexins production, production of salicylic and jasmonic acids and ethylene (Rejeb et al. 2014). Fraire-Velázquez et al. (2011) gave a detailed account of the histological, physiological and molecular responses of plants to abiotic stress. They also elaborated on the impact of abiotic stress on these responses. The response of plants can sometimes ward off an invading pathogenic stressor, helping the host plant to cope with the effect of the pathogen with minimal disruption to its physiological processes (Lam et al. 2001; Greenberg and Yao 2004). At other times, however, the response does not stop disease progression. Symptoms and signs of infection manifest and the host plant may die.

3.3.1 Biotic Stress and Crop Production

Biotic stress in plants can manifest in a number of ways. It can be in form of germination failure, poor seedling establishment and loss of vigour in young seedlings. The extra cost incurred through supplying of un-germinated seeds or dead seedlings has the net effect on increasing the cost of crop production. In standing plants, poor weedy growth, small-sized leaves, leaf chlorosis, stem deformation, premature leaf fall and poor quality yield are some of the most common symptoms of the impact of biotic stress which may results death of the plants (Krishania et al. 2013). The implication is threat to food security, not just in the tropic but in the whole world. The death of plants can also bring about vegetation loss and an increase in the CO_2 concentration of the atmosphere. Rapid loss in vegetation cover can lead to soil degradation, because of its exposure to the agents of denudation, while an increase in the CO_2 concentration in the atmosphere is one of the many factors implicated in the steady increase in global temperature in the last few years. These negative impacts are the reason why millions of dollars are spent annually on the management of plant pests and diseases.

3.3.2 Biotic Stress and Food Security in the Tropics

The desire of man to attain a state of food security is as old as mankind. History has it that in ancient China and Egypt, food was stored in a specialized way during periods of surplus only to be released for consumption when food supply is scarce or during periods of famine. Food security has been defined differently by various authors. At the world food conference that was held in Japan in 1974, food security was defined as the availability at all times of adequate world food supplies of basic foodstuffs to sustain a steady expansion of food consumption and to offset fluctuations in production and prices (Wikipedia 2017a). More recently, the Food and Agricultural Organization arm of the United Nations defined food security as a situation that exist when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meet their dietary needs and food preference for an active and healthy life (FAO 2015).

Globally, about 800 million people are poorly feed (Richard 2005), and the bulk of this population is in the tropic. Several factors have been implicated in global food insecurity, but research has shown that pests and diseases alone account for 20%–40% of annual yield loss in crops (Oerke 2006; Alabi et al. 2016; Cera et al. 2017). This value may even be higher if the significant postharvest losses incurred as a result of the activities of storage pests and diseases are taken into consideration. Plant diseases are not limited to the tropics, as history showed that Europe has had its fair share of major disease outbreak in the past, but the non-adoption of modern farming methods in most parts of the tropics, especially Africa, and the inadequate or non-availability of storage facilities coupled with a very poor state of infrastructures have meant that farmers watch helplessly as their crops are ravaged by pests and diseases. They also have to bear with significant losses in the store (Etebu et al. 2013; Bello et al. 2016). To avoid incurring losses in the store, farmer sell off their perishable produce at ridiculously low prices at harvest. The overall effect of this practice is food scarcity during the off season. In Nigeria, a number of serious pest/disease outbreaks have been reported with serious implication on food security (Alabi et al. 2016). The most recent was the outbreak of tomato leaf miner, *Tuta absoluta*, in several states in the northern part of the country where large-scale cultivation of tomato is done (Borisade et al. 2017). Significant yield losses were recorded, bringing about an increase in the price of tomato above the reach of most households.

3.4 Management of Plant Biotic Stress

Several measures can be adopted in the management of plant biotic stress. The stressor, the host plant, the nature of the interaction between the stressor and the host, the part of plant suffering from the stress and the cost of control are some of the factors to be considered in determining a management option of choice. Cultural control, development of resistant varieties, biological control, chemical control and, most recently, transgenic control are some of these options (Jewell et al. 2010). Since the discovery of Bordeaux mixture, there has been a revolution in the use of chemical disease control agents. All over the world, chemical control of plant disease has gained prominence. One of the biggest breakthrough came when DuPont Company patented the dithiocarbamates group of chemical for the control of fungal and other microbial pathogens of crops. Today, other chemical groups like the organochlorides, organophosphates, heterocyclic nitrogen compounds, aromatic compounds and several others have been developed. Some of the chemical control agents are therapeutic in their action, while others act prophylactically. These chemicals are applied as fumigants, seed protectants, wound dressers, fruit protectant and foliar spray amongst others. The mode of action range from interference with hydrogenation reaction in the cells of pathogens to inactivation of different enzymes, especially those of the Kerb's cycle. Some of these chemicals are also known to disrupt lipid metabolism.

Lately, concern about the numerous problems associated with the use of synthetic chemicals has led to agitation for their discontinued use by some school of thought, while others advocate sparing use in combination with some other disease management strategies. These problems include development of resistance by pathogen, mammalian toxicity, carcinogenicity and mutagenicity (Richter 2002). Others include destruction of beneficial and non-target organisms and disruption of the natural ecosystem (Chaturvedi et al. 2013). In most of the so-called developing and underdeveloped countries, the bulk of which are found in the tropics, syndicates that specializes in the importation or manufacturing of fake and substandard agrochemicals have aggravated the problem. The high level of poverty amongst peasant farmers in Africa, and with non-existent agricultural impute and production subsidy, has made agrochemicals simply unaffordable. Illiteracy of the farmers is another common problem. Even when the right chemicals are provided, misuse is common. It has become imperative, therefore, to find alternatives to synthetic chemicals in the management of plant biotic stress.

3.4.1 Botanicals as Viable Alternative to Synthetic Chemicals in the Management of Plant Biotic Stress in the Tropic

The tropic borders the equator North and South. It is demarcated by the tropic of cancer in the Northern Hemisphere (23° 26′ N) and by the tropic of Capricorn in the south (23° 26′ S) (Wikipedia 2017b) (Fig. 3.1). Countries in this region includes Mexico in North America, 7 countries in Central America, 13 in South America, 26 in the Caribbean, 44 in Africa and 11 in South-East Asia (Hobo Traveler 2017; Tropical foodies 2017). Most countries in this region possess tropical climate with abundant sunshine and very high mean annual rainfall. Lush green vegetation with a wide variety of flora and fauna is common.

Indigenous peoples from this region rely heavily on the healing power of most of the trees, shrubs and herbs that grow luxuriantly and abundantly around them for medicine and personal health. In Africa, common ailments like malaria, abdominal disorder, skin infection, urinary tract infection and several others are treatable with decoction from the appropriate plant or combination of plants. In Nigeria, legend has it that the first man to use plant for medicinal purpose amongst the Yoruba tribe was an ancestral deity called Orunmila (Abayomi 1993). Modern science has elucidated the mysteries and secrecy that were once associated with the use of medicinal plants, making it possible to identify and isolate the active ingredient responsible for their potency.

It is in the light of this that attention is shifting to botanicals as disease control agents in plants. The wide array of different species of plants found in the tropics makes this region a haven of limitless potentials and possibilities when it comes to botanical management of plant diseases. Report abound on the use of different parts of plants, such as leaves, bark, root, seeds and whole fruits sometimes, for the

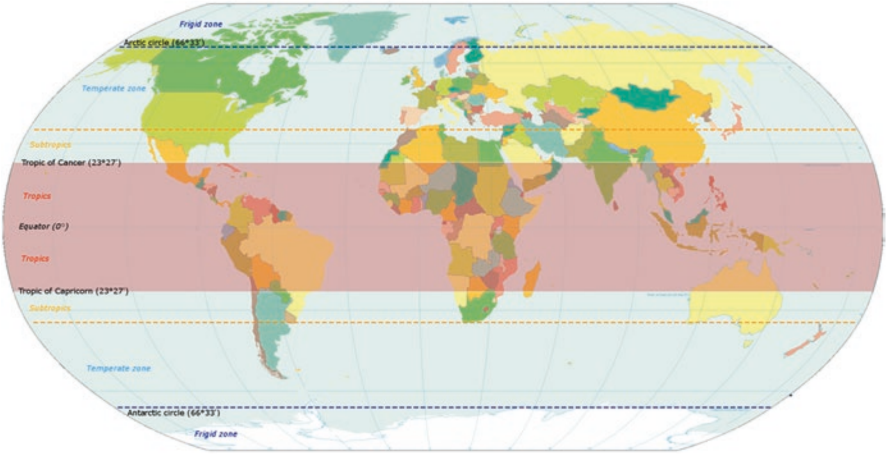

Fig. 3.1 Tropical region of the world. The purple band on the globe/world map represents the tropical region. (Source: Wikipedia 2017a, b)

Table 3.2 Common tropical botanicals and the parts used

English Name	Scientific name	Family name	Used parts
Eucalyptus	*Eucalyptus globulus* lab.	Myrtaceae	Leaves
Lemon grass	*Cymbopogon citratus* Stapf	Gramineae	Leaves
Neem	*Azadirachta indica* Juss.	Meliaceae	Leaves and fruits
Basil	*Ocimum bacilicum* L.	Lamiaceae	Leaves
Peppermint	*Mentha piperita* L.	Labiatae	Leaves and flower tops
Capsicum	*Capsicum frutescens* L.	Solanaceae	Fruits
Garlic	*Allium sativum* L.	Liliaceae	Cloves
Castor beans	*Ricinus communis* L.	Euphorbiaceae	Fruits

Source: El-W'akeil (2013). Modified

Table 3.3 Methods of extracting botanicals from plants

Water	Ethanol	Methanol	Chloroform	Dichloromethanol	Ether	Acetone
Tannins	Alkaloids	Terpenoids	Terpenoids	Terpenoids	Alkaloids	Flavonoids
Saponins	Tannins	Saponins	Flavonoids		Terpenoids	
Terpenoids	Terpenoids	Tannins				
	Flavonol	Flavones			Coumarins	

Source: Gurjar et al. (2012)

management of phytopathogenic organism (Table 3.2). Different methods of extracting the active ingredients, such as the use of organic solvents and water, or with specialized apparatus that can extract the natural and essential oils from such plants (Table 3.3).

Varying percentages of success have been recorded against fungal and bacterial pathogens of crops using botanical extracts. Successful management of some insect pest of plants has also been reported. A significant increase in percentage seed germination of cowpea was recorded by Ajayi and Olufolaji (2009) when extracts of *Gmelina arborea* and *Zingiber officinale* were used as seed treatment for brown blotch infected cowpea. They also achieved significant reduction in disease incidence and severity of brown blotch in cowpea plant treated with the botanicals under screen house condition. Rahman et al. (2012) evaluated 11 plant species for antibacterial activities against *Erwinia carotovora*, the potato soft rot pathogen, and reported that extracts from 2 of the plants, namely, *Corchorus capsularis* and *Swertia chirata* inhibited the pathogen significantly. Amoabeng et al. (2013) reported on the success recorded with the use of extracts from selected African plants against common pests of cabbage. Chandel and Sharma (2014) reported 60.53% inhibition of mycelial growth of *Rhizoctonia solani* in vitro, with the use of commercial formulation of neem (*Azadirachta indica*) extract, called neem gold. A significant inhibition in the mycelial growth of the rice blast pathogen was recorded in vitro by Olufolaji et al. (2015) with the use of crude extracts from selected tropical plants. Significant mycelial growth inhibition of *Ceratocystis paradoxa* (patho-

Table 3.4 Mode of action of selected active ingredient in some tropical plants

Class	Subclass	Mode of action
Phenolics	Simple phenols	Membrane disruption, substrate deprivation
Phenolic acids	Phenolic acids	Binds to adhesins, complex with cell wall, inactivate enzymes
Terpenoids, essential oils		Membrane disruption
Alkaloids		Intercalate into cell wall
Tannins		Binds to proteins, enzyme inhibition and substrate deprivation
Flavonoids		Binds to adhesins, complex with cell wall, inactivate enzymes
Coumarins		Interaction with eukaryotic DNA
Lectins and polypeptides		Form disulfide bridges

Source: Gurjar et al. (2012)

gen of sugarcane sett rot) was recorded with the use of crude extracts from four tropical plants by Ajayi et al. (2016a).

The list of findings on the antimicrobial and anti-insecticidal properties of tropical plants is almost inexhaustible. New plants with promising antimicrobial properties are discovered almost on a daily basis.

Scientific analysis has revealed the active ingredients in most tropical plants with antimicrobial properties to be derived from metabolic activities. These secondary metabolites, as they are commonly called, were developed to confer protection against pathogenic organisms and insect pest in some cases. Several compound and groups of compounds have been identified. They include saponins, phenols and phenolic acids. Others are flavonoids, steroids and tannins (Cowan 1999; Das et al. 2010; Gurjar et al. 2012). These compounds have been isolated from several tropical plants. *Allium sativum*. L., *A. indica* Juss., *Caspsicum frutescens* L., *Eucalyptus globulus* Lab., *Cymbopogon citratus* Stapf, *Ricinus communis* L. and *Ocimum bacilicum* L. are some of the most commonly used botanicals.

The mode of action of these botanicals is not as distinct and clear-cut as synthetic chemicals (Ansari and Mahmood 2017). Some school researchers are of the view that metabolites from botanicals exhibit multiple mode of action, and this may explain why development of resistance by pathogens is not a common feature. Gurjar et al. (2012) listed some of these modes of action to include substrate deprivation, membrane disruption and inactivation of enzymes amongst others (Table 3.4).

Botanicals that are employed in the management of insect pests have several mode of action. These include repellence, disruption of nervous transmission (leading to paralysis and death) or deprivation of oxygen supply (leading to asphyxiation). Some others acts as antifeedants, while others disrupt oviposition (Regnault-Roger and Philogène 2008; El-Wakeil 2013).

The benefits associated with the use of botanical includes the fact that they are eco-friendly, are easily biodegradable, thus leaving no harmful residue in the environment, and are relatively easy to prepare and use. Other benefits include non-toxicity to plants and animals and sustainability. The relatively cheap cost of obtaining most of the botanicals is another very important advantage, especially when one considers the very high level of poverty amongst peasant farmers in most tropical countries.

3.4.2 Antagonistic Fungi for the Management of Plant Biotic Stress in the Tropics

Antagonistic fungi, sometimes referred to as endophytic fungi, were initially thought to be epiphytes, until DeBary (1887) establish a distinction between them and epiphytes. Endophytic fungi inhabit the tissue of host plants causing no apparent harm. Attention has shifted lately towards understanding the relationship between endophytic fungi and their hosts, and it is now very clear that these groups of fungi can confer resistance on their hosts against infection from certain microbial pathogens (Singh 2016). The tropic is home to a wide variety of plants, and the population of associated endophytes is equally very huge. Investigation of some terrestrial and aquatic trees, herbs, shrubs and even grasses has yielded endophyte belonging to several genera of fungi. In India alone, about 306 plants belonging to 97 genera have been investigated for the presence of endophyte with encouraging result (Singh 2016).

The genera of fungi that have been found to contain antagonistic species include *Fusarium, Penicillium, Gliocladium, Cladosporium, Pythium, Rhizoctonia* and *Trichoderma.*

Reports of successful use of antagonistic fungi in the management of plant diseases span several decades. As far back as 1951, *Penicillium claviforme,* previously isolated from lettuce, was inoculated into the same plant to prevent the attack of *Botrytis cinerea* (Wood 1951). Bhatt and Vaughan (1962) reported on the successful suppression of the grey mould disease of strawberry with the use of the antagonistic fungi *Cladosporium herbarum* under field condition. Today, *Trichoderma* spp. is the most investigated genera of antagonistic fungi for the biological control of plant diseases. This is probably due to the ease with which they can be isolated, coupled with the fact that most species in the genus are non-pathogenic to plants, unlike the other genera of antagonistic fungi that may be pathogenic. Significant reduction in the mycelial growth of *Botrytis fabae* (chocolate spot disease of bean pathogen) was recorded with the use of antagonistic fungi *Trichoderma* spp., and it was reported to have performed exceptionally (Sahile et al. 2011). Elad and Freeman (2002) reported that scientific publications on the use of antagonistic fungi especially, *Trichoderma* spp., for the management of plant diseases increased by 1170% in the 1980s and 1990s as compared to what was obtained in the 1970s. This trend is certainly not showing any sign of abating. Soytong et al. (2005) recorded significant reduction in the growth of *Colletotrichum gloeosporioides* (anthracnose of grape pathogen) with the use *T. harzianum*, *T. hamatum* and two other antagonistic fungi. Olufolaji et al. (2014) recorded significant inhibition in the mycelial growth of *Colletotrichum falcatum* (sugarcane red rot pathogen) in vitro with the use of *T. asperellum*. In a similar vein, Ajayi et al. (2016b) evaluated three *Trichoderma* spp. against the pathogen of pineapple disease of sugarcane (*Ceratocystis paradoxa*) and report significant inhibition of the pathogen by each of the *Trichoderma* sp. evaluated. It is interesting to point out that certain antagonistic fungi (*Arthrobotrys oligospora* and *A. anchonia*) have shown great promise in the control of plant parasitic nematodes (Casas-Flores and Herrera-Estrella 2007). Certain fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) are excellent entomopathogens and have been used successfully to control susceptible insect pests of crops (Gul et al. 2014).

The mechanism of *Trichoderma* spp. and, by extension, other antagonistic fungi has been explained to be through, (i) antibiosis, in which antibiotic produced by the fungus acts directly on pathogenic organisms; (ii) mycoparasitism, the antagonistic fungi attack and feed on the pathogen; (iii) competition, the pathogen is deprived of food and space within the host; (iv) cell wall degradation, hydrolytic enzymes produced by the antagonistic fungi break down the cell wall of the pathogen, bringing about its death; and (v) induction of systemic resistance on the host plant, the presence of the antagonistic fungi within the host plant elicits responses in the host. This response confers immunity on the host against infection by specific pathogens (Lo 1998; Elad and Freeman 2002; Soytong et al. 2005; Casas-Flores and Herrera-Estrella 2007). Fungi that are antagonistic to nematodes are known to exhibit various modes of actions. Trapping is one of such mode. In this case, susceptible nematodes are trapped by rings of mycelia formed around it. These rings constrict the nematode, leading to its death. *Arthrobotrys oligospora* is a good example of such antagonistic fungi (Kerry 2000). Other modes of action includes endoparasitism, egg and cyst parasitism and production of toxic metabolites (Casas-Flores and Herrera-Estrella 2007). In entomopathogenic fungi, mechanical puncturing of the exoskeleton of the host and the production of toxins to supress the response of the host, elicited by the presence of the fungi, have been described as the two most important activities involved in the infection process. Reports suggest that infecting fungi produce numerous hyphae within a short period of gaining entrance into the host. The insect soon lose control of its metabolism, while its organs break down. Death of the host results, as the fungus sporulates and breaks down the tissue of the infected insect to effect dispersal (Boguś and Scheller 2002; Charnly and Collins 2007). Entomopathogenic fungi are commonly found in the order Entomophthorales and Hypocreales, with the latter being more common in the tropics (Aung et al. 2008).

3.5 Conclusion and Future Prospects

The potential of botanicals and antagonistic fungi in the management of plant biotic stress is simply overwhelming. It is a massive resource waiting to be tapped. Their relative abundance of both disease control agents in the tropics, the ease with which they can be extracted or isolated coupled with the low cost of large-scale production all points to a long-term sustainability. It is hoped that countries of the tropics will take advantage of this economical and eco-friendly method of plant disease management.

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Chapter 4
Phytosanitation: A Novel Approach Toward Disease Management

Regiane Cristina Oliveira de Freitas Bueno, Rizwan Ali Ansari,
Giuseppina Pace Pereira Lima, and Renate Krause Sakate

Abstract For millennia, man has been producing food, using agriculture, but with increasing cultivated areas, due to the increasing need for food, problems related to production, especially the increase of insect pests, diseases of plants and interferences with weed plants also multiplied. The evolution of plants, through a better genetic approach, transformed the terrestrial environment, making them a very valuable resource for the herbivore community. In ecosystems, plants and insects are just some of the living organisms that continually interact in complex ways and may be the most complex relationships observed in nature. The generated effects of this interaction may be beneficial or harmful to both. To avoid insect attack, plants have developed different mechanisms, such as physical and chemical barriers, defense proteins, volatile substances, secondary metabolism, and trichomes. On the other hand, the insects developed different patterns of associations with host plants, together with different feeding strategies necessary for the exploration of the hosts. Herbivorous insects present complementary adaptations as a response to each defense adaptation in host plants. It is clear that insects are successful in terms of number of species and size of population and as the chemical composition of plants is variable, this represents a challenge for insect feeding. However, insects possess a powerful set of enzymes that constitute the defense against toxic chemicals produced by plants.

R. C. O. de Freitas Bueno (✉) · R. K. Sakate
School of Agriculture, Department of Crop Protection, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil
e-mail: regiane@fca.unesp.br; renatekrause@fca.unesp.br

R. A. Ansari
Section of Plant Pathology and Nematology, Department of Botany, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

G. P. P. Lima
Department of Chemistry and Biochemistry, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil
e-mail: gpplima@ibb.unesp.br

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4.1 Introduction

Plants constantly undergo adverse situations, managing to modulate defense responses to overcome these conditions and return to normal development. Knowing how plants protect themselves is essential to increase plant production and quality.

The factors that can affect overall plant development are considered to be stress conditions, characterized as different conditions that affect growth, development of plants. The factors involved can be biotic, by organisms, or abiotic, due physical or chemical environment.

With trophobiosis being intimately related to physiological stress mechanisms, capable of leading to a state in which free amino acids and reducing sugars are available to feed insects, it is important to observe the factors that promote stress, as well as the agricultural practices capable of minimizing it.

Stress in plants is defined as any condition that affects or blocks metabolism, growth, and plant development (Lichtenthaler 1996). It is the state whereby an increase in cellular metabolic demand leads to the initial destabilization of the functions followed by the normalization and increase of resistance. Thus, the tolerance limit can be changed, and as a consequence, an extra adaptive capacity will be demanded. The result can be permanent damage or even death of the plant (Larcher 2000). Plant stress involves a range of biological and environmental factors. These factors include cold, heat, cohabitation with weeds, insects, and diseases caused by viruses, fungi, bacteria, etc. (Siedow 1995).

A series of natural environmental conditions can cause plant stress, which can be divided into biotic and abiotic factors (Larcher 2000). Among these stress factors, capable of promoting metabolic imbalances that act on proteosynthesis and, therefore, plant resistance, the following are prominent (Chaboussou 1999):

- Biotic factors: spacing, pests and diseases, genetic constitution of the plant (the species and variety, phenological age).
- Abiotic factors: climate (solar energy, temperature, humidity, precipitation, cosmic influences).
- Cultivation factors: soil (chemical composition, structure, aeration), fertilization (organic and mineral), grafting (influence of the rootstock on the physiology of the graft and reciprocally), treatment with pesticides.

Plants generally respond to environmental stress in a similar manner, firstly reducing their growth rate and the rate of resource intake. This is true both for plants adapted via evolution to environments with scarce resources and for any plant that physiologically adjusts to any limiting factor (Pires et al. 1998).

Generally, stress is related to diverse responses in plants and may encompass changes in gene expression and cellular metabolism to changes in growth and productivity rates. Plant responses to stress depend on the duration, severity, amount of exposure, and combination of stress factors, as well as the type of organ or tissue, age of development, and genotype. Some responses allow plants to cope with stress, while others are not so apparent (Kacperska 2004).

Cases in nature where a stress factor acts in isolation are rare. Frequently, multiple stresses are involved, in a combination of factors (Larcher 2000). Some plant species are more tolerant to stress than others are, while others are much less so. Air temperature is one of the most stressful factors, being able to manifest in minutes (both highs and lows); soil humidity can take days, while mineral deficiencies in the soil can take months to manifest themselves (Taiz and Zeiger 2004). To the extent that a plant tolerates more stress, it can adapt. However, it is not adapted, since adaptation involves the level of resistance determined by genetic makeup. Therefore, adaptation to stress results in anatomical, morphological, cellular, biochemical, and molecular aspects (Lopes et al. 2011).

4.2 Plant Stress and Colonization by Pests

In general, plants subjected to abiotic stress factors are more susceptible to herbivorous insects (Larsson 1989), the increase in the concentration of free nitrogen in the leaves, which produces higher amounts of amino acids and results in greater growth, development, survival, fecundity, and, therefore, greater abundance of insects (White 1984), which leads to greater herbivory.

The hypothesis of plant stress presupposes that plants under stress are more vulnerable to insect attacks, because they will be richer in nutrients and less protected by chemical defenses (White 1984). Biotic stress leads to alterations in the pattern of protein expression of plants, with inhibition of the induction of biosynthesis of protein constituents. There is induction of proteinase inhibitors, as a possible defense mechanism against insects (Green and Ryan 1972). Methyl jasmonate action also occurs in the alteration of rubisco enzyme levels and of other proteins (Cavalcante et al. 1999). There are also alterations in rubisco levels with the senescence of *Brassica napus* Linnaeus (Brassicaceae) (Ghosh et al. 2001).

Generally, one can propose two theories to explain the relation between plant stress and pest attacks (Angelo and Dalmolin 2007). The first is that hydric stress is a more significant factor in insect population explosions, given that population increases of diverse insect species occur after periods of drought (Mattson and Haack 1987; Angelo and Dalmolin 2007).

One of the initial studies that presented this hypothesis was produced by White (1970), involving *Cardiaspina densitexta* (Taylor 1962) (Hemiptera: Psyllidae) regarding *Eucalyptus fasciculosa* (F. Muell.) (Myrtaceae) in Australia. The stress hypothesis was expanded by Rhoades (1979), who argued that plants produce toxins under stress, reducing the production of allelochemicals of high metabolic cost and redirecting resources to produce cheaper allelochemicals. Therefore, the production of toxins is increased and the production of compounds that reduce digestibility reduced. The literature contains a large number of citations presenting the hypothesis of stress in plants in relation to herbivores. Examples can be found in Austarå and Midtgaard (1987), with *Neodiprion sertifer* (Geoffrey) (Hymenoptera: Diprionidae) on *Pinus sylvestris* L. (Pinaceae) after acid rain; Cates et al. (1983), with *Choristoneura occidentalis* (Freeman) (Lepidoptera: Tortricidae) on *Pseudotsuga menziesii* (Mirbel) Franco (Pinaceae) after exposure to drought; and Coleman and Jones (1988), with *Plagiodera versicolora* (Laicharting) (Coleoptera: Chrysomelidae) on *Populus deltoides* Bartr. ex Marsh (Salicaceae) after exposure to ozone, among others.

The second theory refers to the balance in the allocation of resources that takes place between the processes related to growth and differentiation under determined environmental conditions. The growth of roots, branches and leaves, or any process requires cell division. However, cell differentiation is the maturation and specialization of existing tissues (Angelo and Dalmolin 2007). Therefore, the allocation of carbon to these different functions cannot occur simultaneously, and equilibrium in the processes of growth and differentiation interacts in the herbivory competition and thus can define the strategies of defense of plants (Herms and Mattson 1992).

Competition in resource-rich environments leads to strategies directed toward growth, while stress from poorer environments leads to differentiation strategies (Stamp 2003), such as products related to differentiation obtained via secondary metabolism, as well as the production of trichomes and the enrichment of leaf cuticles (Herms and Mattson 1992). This involves a cost for enzyme production and the transport and storage structures involved in defense (Angelo and Dalmolin 2007).

4.3 Biotic Factors and Pest Attacks

4.3.1 Density

Plant density during cultivation can contribute to the establishment of a microclimate favorable to pest attacks, though this is not the rule. In coffee plants, where increased planting density allows the obtainment of greater production per unit area, there is a significant increase of phytosanitary problems, mainly from attacks by the coffee borer [*Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae)] (Braccini et al. 2008).

However, in denser spacing, the incidence of mining bug is reduced, and problems with the coffee borer are aggravated. This results from the microclimate formed, which provides, in the closer spacing, greater humidity in the environment (Braccini et al. 2008).

4.3.2 Phenological Age

The hypothesis of phenological age of the host plant predicts that herbivores prefer and/or develop better in younger plants, due to having better nutritional quality, than in older plants.

Plant age interferes in the abundance of sucking insects in citruses, such as *Aleurothrixus floccosus* (Maskell) (Hemiptera: Aleyrodidae) and *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae). The abundance of *A. floccosus* and *T. citricida* is greater in 1-year old plants than in older plants (3, 5, 10, and 20 years).

4.3.3 Variety

The identification of the varieties to be cultivated is one of the most important steps for a farmer, given that not applying the necessary practices for the variety chosen can lead to greater plant stress. In this case, there are various aspects of management to be noted, and up until the present moment, there is no variety resistant to all types of stress that a plant can confront, either biotic or abiotic. Additionally, one should consider that accesses or lineages with different morphological and physiological characteristics can possibly contribute to variations in stress resistance factors.

The use of resistant varieties, selected by traditional genetic improvement programs, contributed considerably to integrated pest management programs. Due to the low cost and better environmental preservation, the use of resistant materials is a highly desirable strategy for insect control.

Some varieties have a certain degree of resistance to insects, and the biosynthesis and regulation of chemical compounds associated with these defenses by plants have been studied for some time now. Currently, it is known that these defenses are found in various plant tissues and among these compounds, antibiotics, alkaloids, terpenes, and proteins can be found, among others. Among the proteins, enzymes such as chitinases, lectins, and digestive enzyme inhibitors are found (Ryan 1990).

4.3.4 Pest Attacks

Herbivory is an interaction between plants and different organisms with important ecological and evolutionary repercussions. This interaction is determined by variation in local biotic and abiotic conditions that affect the quality and quantity of the resources offered by the host plant. Therefore, the intensity of herbivory depends on innumerous characteristics of the plants, including leaf thickness, the carbon-nutrient relation present in the tissues, the concentration of secondary compounds, the water content contained in the plants, etc. (De Moraes et al. 2001).

To avoid injuries caused by herbivores, plants developed defensive strategies based on the presence of chemical compounds, mechanical barriers, or biological associations. Among the defense mechanisms utilized by plants against pathogens and herbivores, the production of secondary metabolites stands out, such as tannin, flavonoids, terpenes, alkaloids, etc. (Moraes 2009).

4.4 Stresses and Species Reactive to Oxygen (EROs)

When submitted to stress, plants show various physiological and biochemical responses. Sources promoting stress are diverse and can be biotic (pathogens, pests) and abiotic (extreme temperatures, hydric availability – excess or lack, wind, radiation, UV, salinity, heavy metal, etc.), requiring adaptations for survival and production (Scansalios 2005).

Stress becomes a challenge for agricultural production compromising food production. Crops are constantly submitted to factors that promote imbalances, which in turn promote the production of antioxidant defenses as a response, inducing the formation of oxygen reactive species (EROs) (Scandalios 1997).

EROs are cellular stress indicators or secondary messengers involved in the translation pathways for signals in response to stress (Mittler 2002; Foyer and Noctor 2005). The main source of EROs is photorespiration, in which neutral and nonreactive molecular oxygen (O_2) during the metabolic process of transference and transport of electrons can produce free radicals, resulting in radical superoxide (O2 • -), radical hydroxyl (OH-), and radical hydrogen peroxide (H_2O_2) (Foyer and Noctor 2009).

When radicals are at high concentrations, they are toxic to the plant. To reduce the damage caused by the EROs, aerobic agents develop nonenzymatic and enzymatic methods to combat them. The nonenzymatic means involve the production of β-caroteno and vitamins C and E. However, the enzymatic defense induces the production of enzymes capable of neutralizing the radicals and/or intermediary oxygen counting on the superoxide dismutase (SOD), peroxidase (PPO), catalase (CAT), polyphenol oxidase (POD), and phenylalanine (PAL), among others (Scandalios 2005).

The EROs can interact with lipids from the membranes, fragment the peptide chain, modify amino acids, cause deletion and mutation in the DNA, degrade the nucleotide bases, and lead to cellular death. Each organelle has a potential target for the damage or accumulation of EROs, and enzymes attempt to maintain cellular homeostasis, which can lead to the activation or disconnection of some genes (Munné-Bosch et al. 2013).

4.5 Stresses and the Production of Species Reactive to Oxygen (EROs) in Soy Plants

Even with growing territorial expansion and agricultural production, soy cultivation, as with many others, its potential for output and quality is influenced by internal and external factors during cultivation, such as chemical and physical characteristics of the soil, climatic and edaphic components, genetic characteristics, and phytosanitary management. The adaptation to the tropical climate and low nodulation are examples of factors referred to as negatives of productivity (Hartman et al. 1991). However, another important factor in this sense is the fairly diversified entomofauna of this culture, which contains an elevated number of insect species, with those that cause serious damage to the culture being considered the main pests. Others, considered secondary pests, occur at lower levels and only under special conditions lead to economic liabilities. A third group corresponds to the beneficial insects that feed on insect pests and therefore act as natural control agents (Carneiro et al. 2010).

To increase soy productivity therefore, that is, to produce more without increasing the plantation area, the improvement of the technology used in the management of the culture is necessary, mainly when it influences factors such as disease incidence, pest populations, and agricultural characteristics that can result in the alteration of production potential. The greater expression of the potential of soy cultivars, however, depends on the conditions of the environment where the plants develop. Therefore, changes in plant population reduce or increase yield because of plant density and spacing (Tourinho et al. 2002).

However, population increases or alterations in the sowing system can favor undesirable factors, such as inter- and intraspecific competition of the plants for environmental resources, especially water and nutrients, morphophysiological changes, damage to optimal soil conditions due to excessive movement of agricultural machinery in the cultivation area, as well as shading between plants caused by an increase in the leaf area index (IAF) (Argenta et al. 2001), which is high in the soy culture that is greater than what the plants really need to carry out photosynthesis and generate energy for development (Truble et al. 1993; Haile et al. 1998; Gazzoni and Moscardi 1998). However, in heavy infestations of defoliating caterpillars, the increase of IAF is an advantage, since leaf loss caused by the caterpillars permits the entry of light through the canopy and therefore guarantees the development of vegetation at the base and middle of the plant, promoting better grain production.

The increase in population density of plants or alteration in the sowing system can further affect plant-arthropod relations, since with a greater number of plants by area, there will be modifications to the environment in which they are located such as changes in intensity of solar radiation that reaches the leaves of the lower third and middle, influencing the microclimate (humidity and temperature) that is a severe limiting factor for the development of a pest population (Rodrigues et al. 2010).

4.6 Influence of the Different Sowing Systems on Soy Culture

Soy cultures have gone through many changes with the use of new technology. The introduction of cultivars with a tendency for undetermined growth with predominant characteristics such as greater precocity, new plant architecture, greater potential for production, and smaller leaflets with a more vertical inclination has raised various questions in terms of the management of soy cultivation leading to research seeking a scientific basis to show which planting system is better for these new cultivars (Procópio et al. 2013).

The sowing system with less intraspecific competition allows better utilization of available resources for the growth and production of soybean grains (Rambo et al. 2003). Reducing the distance between the lines is a worldwide trend and reduces the time for the crop to reach 95% of the solar radiation (Shaw and Weber 1967).

Cross-soybean cultivation has been a common choice among producers. The cross-sowing consists of sowing along parallel lines, followed by a distribution of semenstes in lines forming an angle of 90° in relation to the previous ones; thus a grid of lines is over the cultivation area (Lima et al. 2012).

The double-row system or skip row also seeks to optimize the use of resources and consequently reduce production costs. This type of arrangement is based on two between rows, one internal and the other external (Chiavegato et al. 2010). In the soy culture, the seeds are sown in double rows with a spacing between the internal rows of 0.20 m, while with the external between rows, the spacing utilized is 0.40 or 0.60 m. Changing the spacing configuration for sowing by removing of one or more rows is a technique of the planting system that can favor a higher penetration of light and agrochemicals into the canopy. This improves the rate of photosynthesis and the health and longevity of the leaves nearer to the soil, which, finally, can maximize the productivity of grains. The lack of seeders, however, is a significant barrier to the development of this arrangement.

In the denser cultivation system, the spacing between the rows is reduced and can lead to modifications in the quantity of dry material accumulated by the plants and reduction of the area of the between rows (Scott and Aldrich 1975) and the leaf area and index, which can result in an increase in grain output (Pires et al. 1998).

Some advantages in terms of the conventional system can be considered in the densified system, such as optimization of the factors of soil, machines, tools, and inputs, less degradation of the area used, better weed control, more efficient water use, greater capture of photosynthetically active radiation, and earlier harvesting. However, there are also risks with a greater possibility of incidence of pests and diseases, a lower number of fruits per plant and lower weight per 1000 grains (Chiavegato et al. 2010).

The aim of agricultural practice from the physiological point of view is to maximize the photosynthetic efficiency of the cultures and seek gains in productivity and quality of the final product, highlighting the importance of seeking information regarding CO_2 assimilation (Brandão Filho et al. 2003). The respiratory and photosynthetic variation of soy occurs due to development, resulting from the alteration of the drainage force, in the architecture and leaf structure (Porras et al. 1997; Pereira 2002). The photosynthetic and respiratory rate of the plants from this culture progressively increases from the vegetative to the reproductive stage and reaches maximum values during the period of grain filling. Starting from the moment in which the demand for photosynthates increases, there is an increase in photosynthesis, which can be observed during the stage of grain growth, considered primary drains for the plant (Pereira 2002).

The canopy architecture is considered a determining factor in the photosynthetic capacity of the soy culture (Wells 1991). This is characterized by a dense upper layer of leaves that hampers the penetration of light into the lower strata, such that at the beginning of the reproductive period, around 50% of the liquid radiation reaches the soil surface; however, in the R5 stage (beginning of the grain filling) and R6 (maximum grain volume), 20% reaches the middle part of the plant community and only 10% the lower part. Therefore, even with the increase in leaf area index, there will only be an increase in radiation interception up to a certain point (Pengelly et al. 1999), since, it implies an increase in self-shading, leading to growth and an increase in the coefficient of luminous extinction (Pengelly et al. 1999).

Stomata function is another limiting factor on photosynthetic rate, given that it controls CO_2 absorption (Costa and Marenco 2007). The stomatal pores permit water vapor loss into the atmosphere during transpiration and the entry of CO_2 that takes place from photosynthetic fixation of carbon (Vavasseur and Raghavendra 2005). Generally, when there is competition for water or hydric deficits, plants reduce the degree of stomatal opening, thereby reducing stomatal conductance, to reduce water loss and maintain hydric balance. Therefore, the greater the hydric deficit, the lower the degree of openness of the stomata and, consequently, the greater the resistance to the entry of atmospheric CO_2 (Kerbauy 2004). In terms of transpiration, stomatal conductance diminishes in relation to the water fraction available for the plant and for the incidence of photosynthetically active radiation (Bergonci and Pereira 2002).

Some morphophysiological characteristics (branches per plant, length of branches, and number of fertile nodes) have a relation to the productive potential of the soy plant, representing a greater photosynthetic surface and potential productivity due to the number of sites for the emergence of reproductive buds. However, the number and length of branches can also represent an additional demand redirecting photoassimilates that, in another way, might be used in fixation and in the production of reproductive structures (Navarro Júnior and Costa 2002).

4.7 Biochemical Response of Soy Plants to Sowing Arrangements and to Insect Attack

Sowing arrangements can also influence the plant-arthropod arrangement as a result of modifications in the microclimate caused by greater exposure to solar rays that will reach the leaves of the lower third and middle, which is a limiting factor on the development of a pest-insect population (Rodrigues et al. 2010).

As consequence of the alterations in the soy plant morphology and of insect population fluctuations, the increase in productivity of species reactive to oxygen (EROs) can occur. This is an important metabolic alteration for plants under biotic and abiotic stress conditions (Van Bbreusegem et al. 2001; Apel and Hirt 2004; Foyer and Noctor 2005). The elimination of the EROs and the protection against oxidative damage in plants take place via enzymatic and nonenzymatic antioxidant defense systems. The first group was represented by the enzymes superoxide dismutase (SOD), catalases (CAT), peroxidases (POD), glutathione peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR), and glutathione S-transferase (GSTs). The second was represented by nonenzymatic compounds such as vitamin C and glutathione (GSH), β-carotene, phenolic compounds, tocopherols, and polyamines (Hernández et al. 2001; Blokhina et al. 2003; Scandalios 2005).

Therefore, it is possible to observe that in cultures sown in crossed and reduced planting systems, there is a lower occurrence of defoliating Lepidoptera (Carvalho 2014), and given this, fewer insecticide applications were required for the control of caterpillars, consequently increasing the liquid output of the production (Higley and Peterson 1996). The CO_2 assimilation rate (A, μmol m^{-2} s^{-1}), stomatal conductance (gs, mol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), and internal CO_2 concentration in the leaf (Ci, μmol mol^{-1}) were greater in the conventional and double-row planting systems than in the crossed and reduced planting. The CO_2 assimilation rate is greater in these sowing arrangements, since the rate of photosynthesis of the leaves increases with the development of the plant (Rosa et al. 2007; Pereira 2002). This relationship is observed because during transpiration, the stomatal pores permit the loss of water vapor into the atmosphere and the entry of CO_2, through the photosynthetic fixing of carbon (Vavasseur and Raghavendra 2005), resulting in greater internal concentrations.

The reflex of this alteration is related to greater intraspecific competition that can occur due to competition for essential sources such as water, light, and nutrients (Raventós and Silva 1995). Under these conditions, plants reduce the degree of stomatal opening, in this way reducing stomatal conductance, diminishing water loss, and maintaining hydric balance. The greater the competition and consequently the hydric deficit, the lower the degree of stomatal opening and, therefore, the greater the resistance to the entry of atmospheric CO_2 (Kerbauy 2004).

In the crossed and reduced planting system, there is a formation of areas with high intraspecific regions, particularly in the intersection of the sowing rows. This means that greater competition between plants in these systems leads to a reduction of stomatal conductance and implies a lower rate of CO_2 assimilation, a lower rate of transpiration, and lower internal concentration of $CO_{2.}$ Consequently, there is a lower incidence of defoliating caterpillars, since the insects prefer physiologically healthier plants. The quantity and quality of food have a direct effect on host preference, as well as affecting the growth rate, the development time, body weight, and survival as well as fecundity, longevity, movement, and the competition capacity of adults (Panizzi and Parra 2009).

However, even with more competition between the plants sown in crossed and reduced planting systems, there is no difference in the leaf area ratio ($dm^2.g^{-1}$), which occurs due to the greater efficiency of the photosynthetic in soy plants, independent of leaf area (Campos et al. 2008). In other words, even with the lower rate of CO_2 assimilation in sowing systems that promotes competition, there is no reflection of this stress in the emission of leaf area and consequently in soy plant production. The defoliation of around 30%, which is the control level for the soy culture (Hoffmann-Campo et al. 2012), shows the need to control these insects, which increases production costs. Defoliation in soy plants causes a loss of 10.7 bags.ha^{-1} for sequential defoliation, in the vegetative (33%) and reproductive (17%) stages (Reichert and Costa 2003).

In terms of enzymatic activity, the peroxidase and polyphenol oxidase enzymes are more active in sowing arrangements with greater competition between plants. This generates more stress for soy plants, in this way provoking an increase of reactive species (EROs) and consequently the increase of the activity of these enzymes. However, with the increase of stress caused by the increase of pest populations in plant arrangements, there is an inversion in enzymatic activity due to the stress caused by the feeding on the soy plants.

There are numerous studies that demonstrate the relationship between enzymes and the reaction process to insects (Lattanzio et al. 2006; Frazen et al. 2007; Gustche et al. 2009; Pierson et al. 2011; Marchi-Werle et al. 2014; Timbó et al. 2014; Cruz et al. 2016). Therefore, changes to the oxidative enzyme levels generally occur in response to population fluctuations and feeding by insects, since the greater the insect population density in the arrangements of double sowing rows, the greater the peroxidase and polyphenol oxidase enzyme activity.

Therefore, it is important to underline the importance of sampling pest-insects in the context of Integrated Pest Management (IPM) in soy. The identification of the correct moment for pulverization of the insecticide results in a reduction of the phytosanitary control costs and lower environmental interference in sowing arrangements in which the insect populations do not go beyond control levels.

4.8 Biochemical Response of Corn Plants Under Hydric Stress and Insect Attack

Productivity in corn cultivation is highly prejudicial to elevated occurrence of pests. Among these, the species *Spodoptera frugiperda* (J.E. Smith 1797) (Lepidoptera: Noctuidae) stands out, which is considered a key pest for the culture in Brazil. This caterpillar mainly causes damage in the leaves of the plants. Additionally, high-density infestations can also occur on the ground, attacking the base of the plants, as the dark sword-grass *Agrotisipsilon* (Hufnagel 1766) (Lepidoptera: Noctuidae) habitually does, or feeding on the reproductive structures, as the species *Helicoverpa zea* (Boddie 1850) (Lepidoptera: Noctuidae) does.

Corn genetically modified for insect control (*Bt* corn) is one of the main control tools for *S. frugiperda*. However, in areas with the adoption of technology, which accounts for 80% of the crops in Brazil (Isaaa 2016), the 2013/2014 crop required the realization of on average two applications of insecticide, due to elevated *S. frugiperda* infestations, even in *Bt* corn plantations, in diverse regions of Brazil (Farias et al. 2014).

Initially, the need for additional control was attributed to the loss of resistance of the *Bt* technology; however, it was also found that abiotic factors cause interference in plant physiology and these disturbances are related to the expression of the insecticidal proteins. Physiological alterations are mainly related to the activation of stress enzymes that act in photosynthetic inhibition, reduction of respiration, cell wall breakdown, reduction of leaf expansion, reduction of metabolic and cellular activities, and cell death (Sorg 2004).

The responses of cultures to hydric stress can be complex and vary according to their duration (Liu et al. 2010). When there are hydric deficits, the first metabolic responses to reduce water loss into the environment are the closure of the stomata and a reduction of transpiration. With this, the process of assimilation of carbon and other nutrients is affected, meaning that the development of the culture is slower even leading to smaller size plants. This reduces the distance between nodes and the leaf area and with a smaller leaf expansion; there is a reduction in the liquid photosynthesis rate and in quantities of photosystems present in the leaf. This also induces the formation of species reactive to oxygen.

The hydric stress caused by the greater competition between plants affects the photosynthetic activity, through stomatal closure and the consequent reduction of CO_2 absorption. However, only more severe deficits affect the photosynthetic process of carbon reduction; moderate deficits do not affect the photosynthetic reactions in the chloroplasts (Farias et al. 2007).

The morphological characteristic to avoid water loss is the closure and rolling of the leaves during the hottest hours of the day (Taiz and Zeiger 2004; Ge et al. 2012; Terzi et al. 2010; Farooq et al. 2009). When plants are submitted to hydric deficits, the aerial part has lower development, investing the photoassimilates in root growth and expansion for greater water absorption (De Souza et al. 2016).

The imposition of hydric deficits on corn plants during the vegetative stage promotes biochemical alterations initiating the production of secondary compounds and EROs, such as the accumulation of peroxide in chloroplasts and in the mesophile (Zhao et al. 2016). In addition to the EROs, the secondary compounds formed, such as phenols, have the primary function in metabolism of protecting the plants, such as protecting the plants when attacked by insects, releasing these compounds to deter herbivores. This can also be correlated with stress enzymes such as peroxidase and polyphenol oxidase presenting a synergistic effect with the defense response.

The hydric factor affects overall plant growth and development. The frequency and intensity of hydric stress constitute factors of primary importance for the limitation of global agricultural production (Ortolani and Camargo 1987). Lack or excess leads to disastrous effects on plant development given that various physiological aspects such as openness and closure of stomata, photosynthesis, and leaf growth and expansion can undergo alterations when the plant is submitted to hydric stress. This can consequently generate alterations to secondary metabolism (Moraes 2009).

There are optimal limits for humidity for plant development. The water intake by the root system means that one of the fundamental problems of agriculture is encountered in the water balance of the soil-root system. Excess water in the soil can alter chemical and biological processes, limiting the quantity of oxygen and accelerating the formation of compounds toxic to the roots. On the other hand, the intense percolation of the water provokes the removal of nutrients and the inhibition of the normal growth of the plant. Hydric surpluses, though significant, cause less problems than drought. Hydric deficits, characterized by different forms and intensities, are the main cause of loss of productivity; however, it presents a correlation in the concentration of secondary metabolites, which are important in the insect-plant relationship, acting as allelochemicals toxic to the insects (Moraes 2009).

In the coffee plant, there are variations in secondary metabolite levels, reducing the viability of the *Leucoptera coffeella* eggs (Guérin-Méneville) (Lepidoptera: Lyonetiidae), causing physiological disturbances in the larvae and pupa, and increasing insect mortality (Awmack and Leather 2002). Hydric stress in plants has been considered one of the main factors for attack by insect herbivores. *Eucalyptus* sp. plants, for example, when submitted to a period of hydric stress become more susceptible to attack by the psyllid *Cardiaspina densitexta* (Taylor) (Hemiptera: Psyllidae).

During hydric deficits, in addition to an increase in the concentration of nutritional compounds, turgor pressure, and a reduction in water content in the plants, there is an elevation in the quantity of allelochemicals. Under these conditions, the suckers can benefit from the greater concentration of nitrogen induced by stress, given that they can effectively extract it.

Another example is the interaction of corn plants that express *Bt* proteins, stressed from being submitted to hydric deficits and infested by caterpillars. This is related to the release of phenols, by the synergistic effect to abiotic and biotic damage, which correlates with the fragility of the technology when exposed to hydric deficits, being potentialized when caterpillar attacks occur.

With the oxidative explosion caused by various sources of stress, the abiotic and/or biotic factors promote the increase of the activity of the superoxide dismutase enzyme (SOD), which is instantly activated, since it is considered the first enzyme on the defense line. SOD is responsible for the dismutation of the radical $O_2^{\bullet -}$, generating H_2O_2 and O_2 (Breusegem et al. 2001). The increase of SOD in the corn hybrids stressed from hydric deficits and caterpillar infestations activates the plant defense system (Świątek et al. 2014) and also elevates the rate of production of SOD. SOD action produces the substrate for the activation of other enzymes, such as peroxidase, that breaks down the peroxide of the hydrogen. The activity of this enzyme is characterized by plant-insect interaction, being one of the principal means of plant defense (Van Loon et al. 2006; Kehr 2006).

With all the production of stress enzymes to reduce and control the adverse effects of the EROs in plant metabolism, the production of proteins in the plant is affected. Due to this, there is a failure in the control of the caterpillars, even with *Bt* plants. The plants are restricted in the synthesis of protein and begin to increase enzyme activity related to the combat of EROs, resultant on the transition of the metabolism of the protein synthesis to hydrolysis (Bilgin et al. 2010). As well as insect attacks, in transgenic cultivars under abiotic stress such as hydric deficits, there is a reduction of soluble proteins contained in the leaves affecting the production of *Bt* proteins (Dong and Li 2007). The expression of proteins can also vary according to plant age and is more active in green tissues. Therefore, they decrease during the reproductive phase (Rao 2005).

Change to the physiological processes (photosynthesis, respiration, stomatal conductance, and transpiration) of the plants in response to feeding by arthropods is an important step to understand plant-arthropod interaction (Peterson and Higley 1993). There is a direct relation between the increase of stress with stomatal closure, because with the stomata closed, there is a reduction in the absorbance of carbon (CO_2) by the plant (Larcher 2006), therefore reducing the rate of CO_2 assimilation and the internal CO_2 concentration as well as a reduction in transpiration.

Plants in interaction with biotic factors can also emit molecular signs in this manner activating nuclear genes involved in plant defense responses. This results in an increase of activity of enzymes directly or indirectly associated with plant stress. This takes place through plant-insect interactions wherein the plant is not a passive entity but an active organism, shown through evolutionary processes to be developing protection mechanisms that interfere in its exploitation by insects (Vendramim and Guzzo 2009).

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Chapter 5
Integrated Management of Rice Blast Caused by *Magnaporthe oryzae*

Manish Kumar and Shabbir Ashraf

Abstract Rice (*Oryza sativa* L.) is the world's most important crop and is considered to be a primary source of food for over half of the world's population. In 2017, rice cultivation globally occupied an area of 166 m ha, with a production of 758.8 m t of paddy. More than 90% of the world's rice crop is consumed in Asian countries, which account for about 60% of the earth's population. Rice blast caused by the fungus *Magnaporthe oryzae* is one of the most severe diseases of rice. This pathogen is highly variable in nature. It attacks all developmental stages of rice, causing losses of around 10–30% annually in different rice-producing areas. The pathogen can infect several organs of the rice plant, such as the leaves, collars, necks, and panicles. Chemical agents have been used to combat several soil borne pathogens including *Magnaporthe oryzae*, but our environment is severely degraded by the use of chemicals that pollute the atmosphere and leave harmful effects. The excessive use of pesticides is responsible for the degradation of soil conditions, but this degradation can be limited by the use of targeted bioagents that are antagonistic to pathogens. The reduction of chemical pesticide use in agriculture is achieved by the integration of biocontrol agents, botanicals, and minimum doses of chemicals. Various management strategies, such as the controlled use of nitrogen fertilizers, the application of silica, and the flooding of fields have been used for a long time to control rice blast disease. Scientists are keen to develop durable resistant rice varieties through the pyramiding of quantitative trait loci and major genes. New strategies, such as the characterization of the *R* and *Avr* genes of rice, and biotechnological approaches that lead to the development of resistant cultivars should act against rice blast disease. However, the exploitation of durable host resistance remains a challenge for plant pathologists.

Keywords Rice · *Magnaporthe oryzae* · Management · Pathogens · Productivity

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M. Kumar (✉) · S. Ashraf
Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

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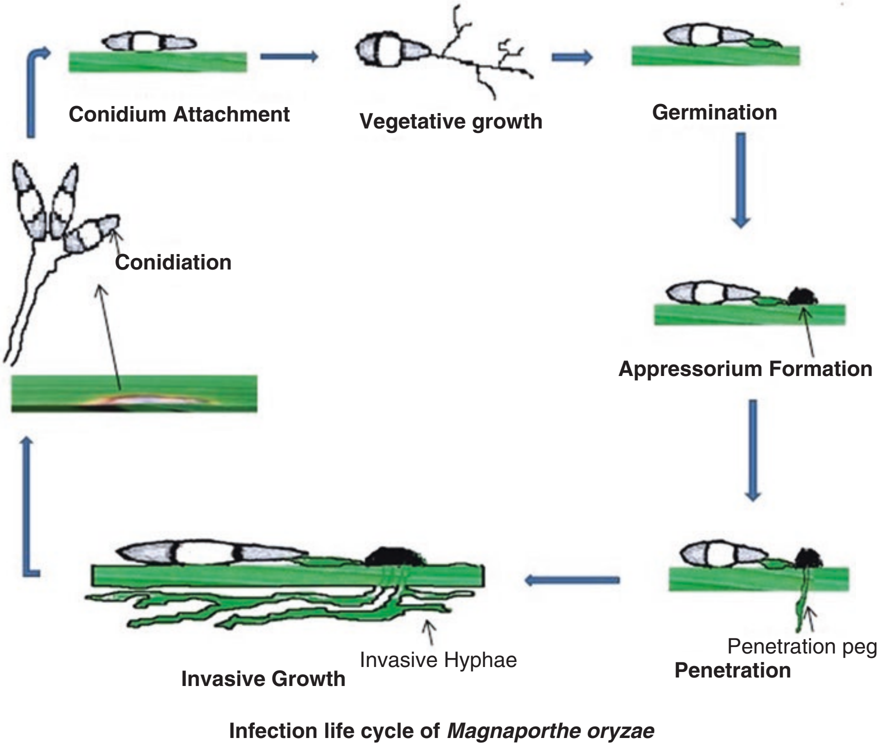
5.1 Introduction

Rice (*Oryza sativa* L.) is the world's most important crop and a primary source of food for more than half of the world's population. More than 90% of the world's rice crop is consumed in Asian countries, which account for about 60% of the earth's population (Kole 2006; Zeigler et al. 1994). In 2017 the Food and Agriculture Organization (FAO) reported that, globally, rice occupies an area of 166 m ha, with a production of 758.8 m t paddy crop. Many pests and diseases attack the rice crops. Rice blast caused by *Magnaporthe oryzae* has more significant economic importance than caused by other pathogenic fungi.

The *M. oryzae* pathogen can infect several parts of the rice plant, such as the leaves, collars, necks, and panicles (Ou 1987; Pinnschmidt et al. 1995). Rice blast caused by *M. oryzae* is an important disease in upland and rain-fed tropical and subtropical areas (Ou 1987; Zeigler et al. 1994). Rice blast is also known as rice fever; neck blast is particularly damaging, as infections damaging the panicle lead to significant yield reduction, of around 20–30% annually (Ou 1987). Rice blast was first reported in China by 1637, then in Japan in 1704, and in the United States and India in 1876 and 1913, respectively (Ou 1971). Blast is one of the major diseases of upland rice (Teng et al. 1990), including that grown in Indonesia (Suwarno et al. 2001). Traditional varieties are generally resistant to blast. However, these varieties generally have a large plant height, a long growth cycle, and a low attainable yield (Suwarno et al. 2001). Chemical fungicides are able to control plant diseases effectively, but the excessive use of chemicals leads to serious concerns for the environment and causes human health problems. Chemical residues in soil make it infertile, and disturb the proper growth and development of plants. Various integrated management strategies have been employed to manage blast diseases of rice, and these strategies involve good cultural as well as agronomic practices, the use of resistant varieties, the application of bioagents and botanicals, and less use of chemicals. Such approaches reduce the chemical load on the environment and enhance the activity of soil microbes, thus both directly and indirectly influencing the productivity, composition, and diversity of plant communities (Barea et al. 2002; Fitzsimons and Miller 2010; Ansari and Mahmood 2017; Lau and Lennon 2011; van der Heijden et al. 2006, 2008).

5.2 Biology of *Magnaporthe oryzae*

M. oryzae damages the aerial parts of the rice plant, and the most commonly affected parts are the leaves and panicles (Sesma and Osbourn 2004). Infection with this pathogen reduces the photosynthetic area of the plant, and panicle infection reduces the yield (Roumen 1992). The blast spores release a special adhesive which attaches them to the leaves, starting the infection (Hamer et al. 1988). The spore attaches to the leaf surface and the forms a specialized cell, the appressorium, that allows the fungus to penetrate the host tissues (Tucker and Talbot 2001; Talbot 2003; Xu et al. 2007). The appressoria of *M. oryzae* develop great cellular turgor due to the accumulation of glycerol as a compatible solute within these cells (de Jong et al. 1997). Owing to the presence of melanin in the appressorium, these cells are impermeable to glycerol efflux, but fully permeable to water. Rapid and continuous influx of water into the cell leads to the development of hydrostatic turgor pressure (Wilson and Talbot 2009; de Jong et al. 1997). The germ tube of the germinating fungal spore recognizes the hydrophobic hard surface of the rice leaf, differentiates in less than 3 h, and invades the underlying leaf tissue (Dean 1997; Hamer et al. 1988); owing to the extremely high turgor pressure (up to 8 MPa), this specialized infection cell ruptures the leaf cuticle. The penetration peg formed at the base of the appressorium (Dean et al. 2005), along with the action of cutinases, allows the appressorium to breach the host cuticle and cell wall, leading to infection of the host.



Life cycle of *M. oryzae* (modified/adopted from http://www.ibwf.de/index.php/fields-of-competence/plant-protection-research-development/molecular-basis-of-plant-microbe-interaction)

5.3 Rice Blast Management

Blast is present in most rice-growing areas; however, the *M. oryzae* pathogen is quite variable and the virulence factors present in one population may not be present in another geographically isolated one. The chemicals used to control blast disease of rice have a bad effect on soil health, and the chemical residues remain in the soil for a long time, affecting the soil microbiome. Such anthropogenic pollution affects human health both directly and indirectly. To reduce the chemical load on the environment, the integration of biocontrol agents (BCAs), which are able to combat pathogens, and botanicals, as well as the proper combination of appropriate nutrients and water, in addition to minimal doses of the required chemicals, should be used for the management of blast diseases of rice.

5.3.1 Water and Planting Time

Under upland conditions rice susceptibility to blast is increased with increasing drought stress (Kahn and Libby 1958). Hence, in upland rice, flooding of the fields would be effective to reduce the severity of blast. Planting time also affects the development of blast on rice crops. In tropical upland rice, early planting, rather than late sown crops, is recommended for preventing blast infection. In upland areas of Brazil, farmers are advised to sow early to escape the inoculum produced on neighboring farms (Prabhu and Morais 1986).

5.3.2 Nutrient Management

Nitrogen and silicon have been found to have a significant effect on the occurrence and development of rice blast disease. Hori (1898) reported that a high soil level of nitrogen led to a high incidence of rice blast. Prabhu and Morais (1986) conducted an experiment in upland rice fields in Brazil and suggested that a limit of 15 kg N/ha reduced blast disease of rice.

Plants with low silica content in the epidermal cells of leaves show low resistance to blast, while the nitrogen level is high (Miyake and Ikeda 1932). The correlation between silica content and disease incidence was studied in different cultivars of rice and it was observed that plants with a high silica content or a large number of silicated epidermal cells had slight damage from blast disease (Onodera 1917). The application of silica slag in the field increased the resistance of rice to blast (Kawashima 1927). Datnoff et al. (1997) reported that the application of silica (calcium silicate slag) reduced the occurrence of rice blast, in terms of the reduced use of a fungicide (benomyl). Now silicon fertilization has become a routine practice in Florida for better rice yields.

5.3.3 Botanical and Biological Management

5.3.3.1 Botanicals

Botanicals are used as alternatives to chemical pesticides, as an ecofriendly method to manage blast disease of rice. The effectiveness of neem (*Azadirachta indica*) oil and neem oil plus neem leaf extract against blast diseases on the Pusa Basmati 1121 rice variety were evaluated and it was found that these agents inhibited the pathogens by around 25% (Kumar et al. 2017). Other botanicals were also evaluated for their antifungal activity against *M. oryzae* and some of them were found to be very effective. The leaf extract of *Atalantia monophylla* was found to inhibit disease by up to 82.22%, while the leaf extract of *Plumbago rosea* inhibited disease by 70.57%. Biochemical studies showed that *A. monophylla* had a higher content of phenols (4.8 mg/g) and flavonoids (24.5 mg/g) than other botanical agents tested (Parimelazhagan 2001). The obnoxious weed eupatorium (*Chromolaena odorata* L.), which has spread extensively in the hill region of Karnataka, India, is known to have antifungal activity; extracts of this weed with different solvents were evaluated for the management of blast disease in rice (Manjappa 2013). Evaluation of the antifungal activity of the leaves of *Ocimum gratissimum*, *Chromolaena odorata*, and *Cymbopogon citratus* the seeds of *Eugenia aromatica* and *Piper guineense*; and the nuts of *Garcinia kola* at different concentrations revealed 70–90% inhibition of mycelial growth (Olufolaji et al. 2015).

5.3.3.2 Biocontrol Agents

Bio-management is an ecofriendly and economic method of managing *M. oryzae* that causes rice blast. Such management is an alternative to the use of chemical agents, and BCAs such as *Trichoderma viride*, *T. harzianum,* and *Pseudomonas fluorescens* have been found to be most effective against *M. oryzae* (Kumar et al. 2017). BCAs can antagonize soil-borne pathogens either directly or indirectly by eliciting a plant-mediated resistance response (Jamalizadeh et al. 2011; Pozo and Azcón-Aguilar 2007). *Trichoderma* spp. synthesize iron-chelating siderophores to cope with the problem of micronutrient scarcity arising from other pathogenic fungi (Benítez et al. 2004). Seeds of four rice varieties; namely, Swarna, IR–64, Samba Mahsuri, and Sahbhagi Dhan, grown under upland rice conditions at Almora and Hazaribagh in India, were treated with isolates of different *Trichoderma* spp. against leaf blast. Compared with control results, the seeds of Samba Mahsuri treated with *Trichoderma* spp. isolate Th–3 showed a maximum increase in plant height (57%), followed by Samba Mahsuri treated with the Tv–12 isolate (44%). Treatment with these isolates also increased root length (by 51–93%), total number of leaves (by 6–60%), number of tillers (by 3–41%), number of panicles (by 4–39%), flag leaf length (by 2–30%), and panicle length (by 5–32%) as compared with results in untreated controls (Aravindan et al. 2016). The biological agent *Chaetomium* *cochliodes* was also found to be effective in the control of *M. oryzae*. When rice seeds were coated with spore suspensions of *C. cochlioides*, early blast infection was controlled and the seedlings were healthy and taller than the controls. In India, studies of BCAs for the control of rice blast were conducted at the Center for Advanced Studies in Botany, University of Madras, and it was found that, among the 400 bacterial isolates collected from the rice fields of the International Rice Research Institute (IRRI), three strains of *Pseudomonas fluorescens*, five of *Bacillus* spp., and one of *Enterobacter* spp., were inhibitory under in vitro conditions (Gnanamanickam et al. 1989; Gnanamanickam and Mew 1992). Microbes have also been engineered to control rice blast. An epiphytic bacterium, *Erwinia ananas*, transformed by the chitinolytic enzyme gene (Chi A) from an antagonistic bacterium, *Serratia marcescens* strain B2, a tomato epiphytic bacterium, was found to be inhibitory against *M. oryzae* (Someya et al. 2004). Other studies on the biocontrol of rice blast showed that *Bacillus subtilis* strain B-332 (Mu et al. 2007) and strains 1Pe2, 2R37, and 1Re14 (Yang et al. 2008), as well as *Streptomyces sindenius* isolate 263, had good antagonistic activity against *M. oryzae*.

5.3.3.3 Chemical Management

The effectiveness of fungicides depends on several factors, e.g., the level of disease present, the compound used, the timing and method of its application, the efficiency of disease forecasting systems, and the rate of emergence of fungicide-resistant strains (Skamnioti and Gurr 2009). Fungicides are frequently used for the control of blast disease of rice. In Japan, a mixture of copper fungicides and phenyl mercuric acetate (PMA) was found to be more effective to control blast disease of rice than copper fungicides alone, while a mixture of slaked lime and PMA was found to be more effective than the mixture of copper fungicides and PMA (Ogawa 1953). However, PMA was eventually banned because of its toxic effects on mammals and because it caused serious environmental problems (Ou 1985). It was suggested that rotating the use of fungicides, or mixing them, rather than continuously relying on a single compound, greatly reduced the risk of developing highly resistant populations (Uesugi 1978). Copper fungicides were found to be effective for rice blast control in India as well, but it was seen that high-yielding varieties were copper-shy; hence, the emphasis shifted to another group of fungicides, viz., dithiocarbamate and edifenphos, but they had shorter residual activity. In 1975 the first-generation systemic fungicides benomyl, carbendazim, and others were evaluated and found to be effective against rice blast. These fungicides have different modes of action, and include anti-mitotic compounds, melanin inhibitors, and ergosterol biosynthesis inhibitors to control blast disease (Siddiq 1996). The fungicides tricyclazole and pyroquilon, as seed dressers, have been found to be effective for providing protection to rice seeds for up to 8 weeks after sowing. Trials of Bavistin (50% carbendazim; 1 g/L spray) at tillering plus Hinosan (edifenphos; 1 g/L) at heading and after flowering resulted in the best yield. In the most recent field evaluation of commercial fungicidal formulations, Rabicide (tetrachlorophthalide), Nativo (tebuconazole + trifloxystobin), and Score (difenoconazole) were found to be most effective (Usman et al. 2009). Fungicides such as azoxystrobin, carpropamid, dithiocarbamate, edifenphos, fenoxanil, tiadinil, tricyclazole, pyroquilon, probenazole, iprobenfos, isoprothiolane, metominostrobin, and propiconazole have been found to control blast disease of rice (Skamnioti and Gurr 2009; Pooja and Katoch 2014).

5.3.3.4 Antibiotics

The first antibiotic that was found to inhibit the growth of rice blast fungus on rice leaves was cephalothecin, produced by a species of *Cephalothecium* (Yoshii 1949), followed by antiblastin (Suzuki 1954), antimycin-A (Harada 1955), blastmycin (Watanabe et al. 1957), and blasticidin-A (Fukunaga et al. 1968), all of which were tested, but due to their chemical instability and toxicity to fish, none of them was put to practical use. In 1955 a new systemic antibiotic, blasticidin S, produced by *Streptomyces griseo chromogenes*, was developed by Fukunaga et al. (1968). It was found to control blast effectively but it was an inferior protectant and was highly toxic to plants and mammals (Ou 1985). A new antibiotic, kasugamycin, produced by the bacterium *Streptomyces kasugaensis*, was discovered and isolated. It showed excellent control of rice blast and had very low toxicity in mammals and rice plants (Okamoto 1972). In the 1970s, after antibiotics had been used extensively and exclusively for blast control, *M. oryzae* began to show resistance to antibiotic compounds (Uesugi 1978). Katagiri and Uesugi (1978) reported mutants of *M. oryzae* that were resistant to different chemicals; resistance was highest for kasugamycin, followed by IBP (Iprobenfos), edifenphos, and isoprothiolane, and was lowest for benomyl.

5.4 Forecasting

Plank (1963) quoted that “Chemical industries and plant breeders forge fine tactical weapons but only epidemiology sets the strategy”. Knowledge of the epidemiology of a disease can help to better implement disease management strategies. For the most economical and most effective use of fungicides, forecasting is essential; the forecasting is based on information on the fungus, host plant, and environment (Ou 1971). Using 13-year data, Padmanabhan (1963) concluded that whenever a minimum temperature of 24 C or below was associated with RH (Relative Humodity) of 90% or above, the conditions were favorable for blast infection. Refaei (1977) found that the number of blast lesions was more closely correlated with the dew point than with the number of airborne spores. Today a number of computer simulation-based forecast models are available, such as:

1. LEAFBLAST (Choi et al. 1988).
2. EPIBLAST (Kim and Kim 1993).
3. EPIBLA (Manibhushanrao and Krishnan 1991).

5.5 Host Resistance

Miyake and Ikeda (1932) reported that the cultivar Bozu, which was resistant to rice blast, contained a greater amount of silicon than a susceptible cultivar. Ito and Sakamoto (1939) found that resistance to mechanical puncture of the leaf epidermis was positively related to resistance to blast. Resistance was reduced by the application of nitrogen fertilizer but increased as the plant became older. Hori et al. (1960) reported that the distribution of starch in the leaf sheath was related to blast resistance. A greater accumulation of starch in the leaves of rice indicates greater resistance to blast disease. Kawamura and Ono (1948) were able to isolate *M. oryzae* from lesions, and they reported that pyricularin and α-picolinic acid produced by *M. oryzae* were toxic to rice plants and caused stunting of seedlings and leaf spotting. Tamari and Kaji (1955) reported that, when combined with chlorogenic acid or ferulic acid, which are both present in the rice plant, pyricularin and α-picolinic acid become nontoxic to the plants. Resistance to *M. oryzae* in rice is usually dominant and is controlled by one or a few pairs of genes (Thurston 1998). Link and Ou (1969) proposed a system of standardization of race numbers of *M. oryzae*.

5.6 Biotechnological Approaches

Biotechnological approaches used in the studies of genome organization and molecular analysis of the rice blast fungus *M. oryzae* have become more frequent (Valent and Chumley 1991). The mechanism of host pathogen interaction at the molecular level involves the mitogen activated protein (MAP) kinase and cyclic adenosine monophosphate (cAMP) signaling pathways (Xu and Hamer 1996). Further research has explored the identification, isolation, cloning, and characterization of the *R* and *Avr* genes of rice. Biotechnological tools have also been exploited for gene pyramiding through marker-assisted selection and for the identification and mapping of quantitative trait loci for partial resistance to blast. Today, a total of 73 *R* genes conferring blast resistance in rice have been identified. Many of them have been mapped, but only 5, viz. *Pi-b*, *Pi-ta*, *Pi-25*, *Pi-5*, and *Pi-9*, have been isolated and characterized using molecular techniques (Tacconi et al. 2010). Several techniques that have found applications in plant pathogen diagnosis have been developed; these include the use of monoclonal antibodies, enzyme-linked immunosorbent assays, and DNA-based technologies.

Table 5.1 Blast resistance genes and their rice cultivars at different genetic locations

S. No.	Gene	Rice cultivar	Location	References
01	Pib2	Lemont	Philippines	Tabien et al. (1996)
02	*Pi44*	Moroberekan	United States	Chen et al. (1999)
03	*Pi42(t)*	DHR9	India	Kumar et al. (2010)
04	*Pikg*	GA20	Japan	Pan et al. (1996)
05	*PiGD1*	Sanhuangzhan 2	China	Liu et al. (2004)
06	*Pikh (Pi54)*	Tetep	India	Sharma et al. (2005)
07	*Pi28(t)*	IR64	France	Sallaud et al. (2003)
08	*Pigm(t)*	Gumei 4	China	Deng et al. (2006)
09	*Pi-tq5*	Tequing	United States	Tabien et al. (2000)
10	*Pi30(t)*	IR64	France	Sallaud et al. (2003)
11	*Pi36*	Q61	China	Liu et al. (2005)

5.6.1 Blast Resistance Genes

Two main rice genera are cultivated in the world: *Oryza sativa* and *Oryza glaberrima*. In Asian countries *Oryza sativa* (an ancient crop species) is cultivated, while *Oryza glaberrima* is confined to African countries. Rice has encountered many biotic and abiotic stressors and these stressors have influenced its growth and development, so that cultivated rice lines show more uniformity than the wild genotype. This uniformity makes rice lines narrow, which favors the better survival of plant pathogens. Meanwhile, a large genetic pool remains unexplored; e.g., the blast-resistant genes *Pi9* from *Oryza minuta* (Sitch et al. 1989; Amante-Bordeos et al. 1992), *Pi-40(t)* from *Oryza australiensis* (Jena et al. 1991), and *Pirf2–1(t)* from *Oryza rufipogon* (Dwinita et al. 2008). The resistant genes are responsible for the resistance to blast disease of rice. The introgression of broad-spectrum blast resistance gene(s) from *Oryza rufipogon* into an *indica* rice cultivar has also been reported (Ram et al. 2007). In plants, resistance to a particular pathogen is governed by incompatible interactions, which follow the gene-for-gene hypothesis (Flor 1955). Gene-for-gene resistance is resulted from the interactions between products of the pathogen avirulence (*Avr*) genes and their matching plant resistance (*R*) genes. *Avr* genes have been cloned from a variety of pathogens including fungi, bacteria, viruses, and oomycetes. (Jones and Dangl 2006). The first cloned *Avr* gene of *M. oryzae* was the *PWL* gene family, consisting of four genes, viz., *PWL1, PWL3, PWL4* (Kang et al. 1995), and *PWL2* (Sweigard et al. 1995) (Table 5.1).

5.7 Future of Rice Blast Management

Molecular and biotechnological tools have changed research on rice blast management. The availability of genome sequences for both the host rice (Dean et al. 2005) and the pathogen has opened many doors for further research. A combination of disease-resistant cultivars, more efficient use of nitrogen fertilizers, and minimal doses of fungicides will lead to better management of blast disease in rice. Nanotechnology in agriculture could prove to be beneficial in future research and management. Studies of the *R* and *Avr* genes and their gene products will add to our knowledge of host-pathogen interactions. For the management of resistance to blast treatments, strategies such as gene rotation, gene pyramiding, spatial and temporal gene deployment, and the use of varietal mixtures will be the best means to reduce blast epidemics. The development and use of transgenic rice could be the best form of rice blast management in the future. The development of genetically engineered bioagents will supplement the environmentally friendly management of rice blast. However, there is still a need for the further development of noble fungicides and fungistatic agents with longer residual effects; this development could be better assisted by biotechnology in future.

5.8 Conclusions

The chemicals used to control blast disease of rice have a bad impact on soil health. The residues of these chemicals remain in the soil for a long time and this affects the soil microbiome. Moreover, these residues inhibit the activity of BCAs that are able to act against pathogens. Anthropogenic pollution affects human health both directly and indirectly. For the management of blast diseases of rice, to reduce the chemical load on the environment, the integration of bioagents and botanicals, along with the proper combination of required nutrients and water, as well as the use of the minimal dose of the required chemical, should be used. The application of the required doses of nitrogen and the proper amounts of silica slag in the field have been shown to increase rice resistance to blast disease. Disease forecasting also plays a crucial role in the management of disease. Scientists should focus on the development of new forecasting systems for the management of blast disease. Biotechnological approaches will also help to manage pathogens, and the exploration of blast-resistant genes should make it easy to develop resistant rice varieties. The best option to manage blast diseases in rice is to develop resistant cultivars. However, owing to the highly variable nature of pathogens, continuous research is needed to develop durable resistant cultivars that will be fruitful for farming communities. Yet the exploitation of durable host resistance still remains a challenge for plant pathologists, although scientists are keen to develop durable resistant varieties through the pyramiding of quantitative trait loci and major genes.

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Chapter 6
Vegetables Quality and Biotic Stress

Carlo Nicoletto, Carmelo Maucieri, Giampaolo Zanin, Fabio Vianello, and Paolo Sambo

Abstract Biotic stresses are one of the most important factor that have a substantial effect on crop growth and development and, finally, responsible for enormous losses of crop yield. Worldwide crop yield is reduced of about 25% due to diseases and insect infestation. Within different crops, worldwide vegetable production and consumption are constantly growing as a result of countless findings that attest their beneficial health properties. The quality target is an aspect that is increasingly considered within productions destined for modern consumption. This objective can be pursued through the improvement of one or more quality attributes. This issue seems to be very complex if we consider the relevant differences that characterize the vegetable production industry starting from crops, genetics, commodities, pedoclimatic conditions, agronomic and technical points of view. Biotic stresses can play a double role in conditioning vegetable quality with positive or negative consequences. Considering the complex modes of stress signaling by the plant, secondary metabolism is greatly affected by the generation of reactive oxygen species (ROS), hormonal components, and enzymatic activity. It is easy to assume that the effect of biotic stress is negative for product quality, but in some cases there is a potential utility of these metabolites for the consumer health. Paradoxically, the presence of stress during the cultivation of some species can be a crop extra value under the health profile. This chapter will seek to provide some guidance on the relationship between vegetables and biotic stresses, highlighting the consequences of biotic stress, the possible impact on the quality of vegetable crops, and some possible solutions.

Keywords Agronomic practices · Antioxidant activity · Metabolites · Nutritional properties · Pest and diseases

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C. Nicoletto (✉) · C. Maucieri · G. Zanin · P. Sambo
Department of Agronomy, Food, Natural resources, Animals and Environment, Agripolis – University of Padova, Legnaro, PD, Italy
e-mail: carlo.nicoletto@unipd.it

F. Vianello
Department of Comparative Biomedicine and Food Science, University of Padova, Padova, Italy

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6.1 Introduction

Vegetable production and consumption are constantly growing worldwide as a result of countless findings that prove their beneficial health properties. In the last 25 years, the vegetable production doubled worldwide, and the global trade value of vegetables is now higher than that of cereals. Beyond their economic value, fruits and vegetables are really important in improving diet quality since they are the best resource to solve micronutrient deficiencies. The World Health Organization (WHO) reveals that low fruit and vegetable intake contributes to 16 million disability-adjusted life years and more than 1.7 million deaths worldwide are due to poor rate of consumption of fruits and vegetables (Faostat 2018). According to WHO and FAO, the minimum recommended daily portion of fruit and vegetables for the prevention of chronic diseases such as heart diseases, cancer, diabetes, and obesity is 400 g, excluding starchy root crops.

Vegetables, as well as other extensive crops, are exposed to a wide range of potential pests and are vulnerable to several biotic and abiotic stresses. Among biotic stresses, fungal, bacterial, and viral plant pathogens are the main causes of poor quality of vegetables in terms of quality and quantity. The presence of biotic stresses represents a notable limitation for the plant, but it is difficult to clearly define how the plants respond to them. Among the various existing limitations, the comprehensive knowledge of plants, pests, and their interactions is difficult to be obtained. Moreover, the injury quantification is complicated for some negative factors such as weeds, plant pathogens, and sucking insects (Peterson and Higley 2001).

Vegetable production is extremely diversified in every production context. The remarkable variety of potentially cultivable species and the edible product types make this class of plants extremely complicated in terms of biotic and abiotic stress studies. This chapter will seek to provide some guidance on the correlation between vegetables and biotic stresses, reflecting the consequences of biotic stress, the possible impact on the quality of vegetable crops, and some possible solutions. Before to consider these aspects, a synthetic description of the main qualitative features characterizing vegetable crops is reported.

6.2 Vegetables and Quality

Quality, even for horticultural products, is a wide and complex issue, involving several figures. The question should be discussed with reference to all factors contributing to its extrinsic nature, taking into account the standpoint of the most important figures in the production chain, where it is possible to identify the farmer, the trader/distributor, and the consumer. Sometimes, these figures express, as intuitive, conflicting goals and different points of view. However, the consumer is the economic entity most involved in the quality judgment. The fulfillment of its needs represents the essential aspect of a production that has become "consumer oriented" by "market oriented". In addition to basic qualitative traits such as exterior appearance, integrity, etc., the consumer's perception of quality involves also factors deriving from organoleptic stimuli (e.g., flavor, touch) or health expectations (real or virtual) associated with healthiness (e.g., organic production) or the content of nutraceutical substances.

The target quality is an aspect that is increasingly considered within productions destined for conscious consumption. This objective can be pursued through the improvement of one or more quality attributes. This issue seems to be very complex if we consider the relevant differences that characterize the vegetable production industry starting from crops, genetics, commodities, pedoclimatic conditions, and agronomic and technical points of view. On the other hand, the quality feature perception may vary and take a different importance depending on the product's intended use.

6.2.1 Qualitative Traits

Vegetable products represent a large group of widely used agricultural products for food, especially for fresh use. Their quality is expressed by different attributes that can be ascribed to the organoleptic, nutritional, hygienic-sanitary, commercial, and technological characteristics.

Organoleptic traits – They cover all the perceptible properties of our senses. The most important ones for vegetable products are the taste and the texture. The first one, in the case of fruits (e.g., tomato, melon), is essentially determined by the sugar and organic acid content. The taste also sums up the component of the typical aromas of each product perceived directly, in the case of volatile compounds, and also through the smell. It is supported by complex system of feelings and therefore difficult to parameterize. The typical taste of different products can be determined by a single or a small number of compounds (e.g., in cucumber and onion). Some compounds may give unpleasant tastes and aromas, thus adversely affecting quality. Significant examples are the bitter flavor of cucumbers for the presence of cucurbitacin and the spiciness of pepper by the presence of capsicin.

The second trait, texture, is a complex feature that can be traced back to a set of mechanical attributes that can be divided into primary (hardness, cohesion, viscosity, elasticity, adhesiveness) and secondary (fracturability, chewiness, gumminess). These are mechanical attributes resulting from the relationships between some cellular compounds (e.g., enzymes, starch, and phytin), turgor pressure, cell wall properties, and the strength of the contiguous cell bonds. Knowledge about the consistency of vegetable and vegetable products in general is of considerable practical interest also in order to establish the optimum harvesting time, to guarantee adequate storability, and to evaluate its handling attitude and resistance to mechanical stresses.

Nutritional characteristics – They refer to the chemical composition and the nutrient content of the products. They are not directly perceived by the consumer and consequently scarcely evaluable at the time of purchase. Traditionally, the composition has gained importance for the evaluation of products intended for industrial processing. In the past, the actual nutritional characteristics (e.g., carotene in carrot) or presumed ones (e.g., available iron in spinach or vitamin C in sweet pepper) have only influenced consumption for fresh consumption. More recently, they have been taken into account because of the increasing awareness of the importance of fruit and vegetable products for regulation of metabolic activity and for protective action due to the presence of mineral salts, vitamins, essential amino acids, raw fiber, and antioxidant compounds.

Hygienic traits – This is a prerequisite; fruit and vegetable products, alike any other foodstuff, can only be used as food if they do not endanger consumer health and safety. For this reason, hygienic and sanitary characteristics are of preeminent importance, and the other quality traits are subordinate to these. In recent times, fruit and vegetables, which have always been considered as genuine foods, are increasingly accounted for possible alterations in their hygienic-sanitary characteristics. This is mainly due to the intensification of the production process that characterizes modern cultivation and marketing systems. To avoid any risk for the consumer health, fruit and vegetables must not carry any harmful physical, chemical, or biological contamination. The most frequent conditions of sanitary-hygiene risk may arise from the presence of pathogenic microorganisms, residues of atmospheric pollutants, natural substances produced by plants, chemicals used in cultivation, and their metabolites. The presence of agrochemical residues and nitrates is regulated by specific guidelines that establish the maximum admitted amount for each product.

Marketable traits – Include all those parameters taken into account for the product classification according to the regulation established by European control agency. Except for rare exceptions (e.g., soluble solids in melons), these are external parameters on the basis of which the product is classified into more or less homogeneous categories, which are considered on trade. Commercial features are based on attributes that can easily be detected from an external examination (e.g., shape, size, color). They do not always affect the organoleptic characteristics but, still, are important for consumer choice. These characteristics define "quality standards" such as presentation and packaging of horticultural products so that they can be marketed within the European community, exported to third countries, or imported from third countries.

Technological traits – Express the suitability of the product to a specific process of industrial transformation. These characteristics are related to the compatibility with the characteristics and operating modes of the processing industries and the parameters that affect the quality profile of the finished product. They became very important as the transition from handmade to industrial processing resulted in a drastic reduction in the elasticity of the process. In traditional processing, the technique was usually adapted to product characteristics, whereas the industrial transformation is characterized by opposite trend.

6.2.2 The Evolution of Quality Concept

The vegetable quality is a multifactorial concept that takes into consideration tangible and intangible aspects and intrinsic and extrinsic characteristics of the product and which can change over time and space. Among the intrinsic features of the product are those related to the chemical-nutritional composition, to the health (related to the presence of mineral salts, vitamins, antioxidants, and other compounds), to the hygienic-sanitary aspects that convey the safety of use, and, finally, to the physical and organoleptic properties, among which there are some strong influencing properties for consumer choice (e.g., color, shape, water content). Very often, all these features are perceived and indicated by the consumer as "freshness," a generic term, not always well defined.

There are also some aspects of the vegetables quality not directly linked to parameters that are somehow measurable on the product itself, but are connected to the quality of the process and the environment in which the product is obtained. Beyond the evaluation of the vegetable as a nutrient food, hedonistic aspects (tasteful) and environmental and cultural/historical traits are also taken into consideration. Nowadays, many consumers want to know the so-called intangible values or positive externalities of foods that contribute to create the concept of global quality. Among these, there is an increasing worldwide interest to the evaluation of production system impact on the environment, biodiversity, workers' welfare, and, last but not least, how these systems are also related to the sociocultural component of the territory they come from. Therefore, enhance the quality of a food also means retrieving this kind of information and knowing how to communicate them.

Vegetables are known to be perishable, and their quality is inevitably destined to deteriorate rapidly over time. Shelf life is of crucial importance in determining their value. In fact, during the distribution and marketing phases, the product quality decreases more or less rapidly depending on the storage and transport conditions. Despite that, in the fresh vegetable industry, two main phases – from production up to harvest and from packaging up to distribution of the finished fresh product – are normally identified, whereas the phases that need to be checked in a global quality system look much more numerous. Each of these phases affects some fundamental aspects of the quality of vegetables that fall into both the intrinsic and extrinsic characteristics of the product.

Shelf life and the intensity of quality decay in vegetables depend in part on the pre-harvest condition and gathering system, partly by the postharvest management that goes from harvest to marketing.

Finally, the quality of horticultural products is a constantly evolving concept that is closely dependent on the choices of the consumer and the product itself and is conditioned by the dynamism of the production systems (fast production and process innovations). A big deal that should be studied and defined nowadays is the consumer's predisposition to spend for a high value product taking into account most of the abovementioned aspects. It is also important to highlight the fact that part of these values need to be communicated and disseminated in order to increase the knowledge of the consumer. In order to meet the increasing consumers' needs, it is possible to hypothesize innovative communication ways concerning the entire horticultural chain. In this field, the margins for improving the quality of production processes are still significant. However, it is necessary to determine whether the innovations aimed at product qualification are justified by the greater market opportunities.

The various qualitative aspects so far reported are expressed in relation to the conditions in which the plant is grown. Removing plants from optimal growth conditions, which means to stress plants, inevitably results in qualitative variations that may improve or compromise the quality of the product.

6.3 Signaling Crosstalk Between Biotic and Abiotic Stress Responses in Vegetables

Physiology gives us a tool to combine our knowledge of biotic stress and plant reaction. Before explaining some of the possible response systems of plant under stress, it is appropriate to define in a concise manner what is meant by "stress." Higley et al. (1993) further clarified the terms injury, damage, and stress, to link them better with physiological processes of plants. Injury is a stimulus which causes an anomalous alteration in a biological process. Damage is something measurable such as a reduction in plant growth or development consequential to injury. Stress, instead, can be considered a deviation from optimal conditions (Peterson and Higley 2001).

Plants are continuously exposed to different biotic and abiotic stresses in their environment. In order to survive, plants, as sessile organism, have evolved complicated mechanisms to recognize external signals, producing an optimal response to difficult environmental conditions (Fujita et al. 2006). Reactions that occur in stressed plants are extremely difficult to be identified and described. With current standing, various important molecules including transcription factors and kinases are considered as potential candidate which play pivotal role in the crosstalk between stress signaling pathways. Plant hormones such as salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and abscisic acid (ABA) primarily regulate plants' responses against biotic and abiotic stresses via synergic and antagonistic actions (Bostock 2005; Lorenzo and Solano 2005; Mauch-Mani and Mauch 2005). Moreover, the free radical synthesis has been proposed as a key process that is shared between biotic and abiotic stress responses (Apel and Hirt 2004; Torres and Dangl 2005). The existence of such crosstalk between signaling networks is confirmed by large-scale transcriptome analyses with DNA microarray technology (Schenk et al. 2000; Cheong et al. 2002; Atkinson and Urwin 2012). The study reveal that reactive oxygen species (ROS) are a common signal that activate stress responses (Fujita et al. 2006; Orsini et al. 2016).

Another mechanism that plants use to defend themselves and adapt to stress conditions involves the sugar metabolism (Van den Ende and El-Esawe 2014). For example, the vacuolar accumulation of fructans and anthocyanins is a common signal observed in both biotic and abiotic stress responses. This mechanism, known as “sweet immunity,” improves abiotic and biotic stress tolerance. Moreover, the production of fructans and anthocyanins is stimulated by sucrose-specific signaling pathways that are strictly dependent on Ca^{2+} (Van den Ende and El-Esawe 2014). In Fig. 6.1 is reported a simplified scheme about some examples of crosstalk networks. From the different established relationships it clearly emerges the intricate complex of chemical response signals and the various actions that each signal can provide to the plant in order to react properly to abiotic and biotic stress.

The evolution and understanding of stress-triggered mechanisms seek to increase knowledge in this area to clarify what is happening within the host due to abiotic and biotic attacks. The host, as reported by Peterson and Higley (2001), may be represented by a black box within which not yet been clarified changes produce not only yield loss but also positive or negative variations of the qualitative characteristics of the product (Fig. 6.2).

The physiology of plants response to biotic stress requires different monitoring systems that range from cellular level to plant populations. In recent years, technological progress has greatly improved the potential to investigate these topics. Some examples can be found in portable infrared CO_2 analyzers able to check plant gas exchange; molecular biology is another tool to understand plant disease physiology and, since instrumentation continues to improve, we can reasonably expect new findings on stress issues.

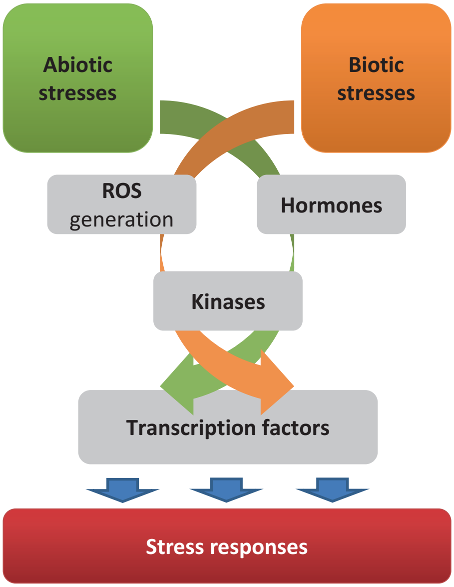

Fig. 6.1 Schematic representation about the complex pathways network induced by stress experiences in plants. (Source: Fujita et al. (2006), adapted by authors)

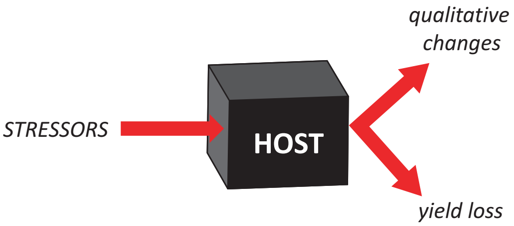

Fig. 6.2 The black box represents up-to-date understandings of the relationship between biotic stressors, plant yield and quality. (Source: Peterson and Higley (2001), adapted by authors)

6.4 Stress Conditions and Vegetables Production

A stress condition, as previously reported, is capable to generate extremely negative phenomena in terms of production, limiting the ability to produce food for the world population. The understanding of stress mechanisms is quite difficult, also because plants are normally cultivated in open fields or in a environment where often there can be more stresses involved at the same time. For this reason, in recent years the attention of researchers is also focusing on the effect of different stress combinations (e.g., biotic + abiotic, biotic + biotic, abiotic + abiotic) on plant physiology. Sometimes it is misleading if biotic and abiotic stresses have rather antagonistic, synergistic, or additive effects, inducing more or less susceptibility to a specific stress. Usually, the exposition to different types of stresses can determine antagonistic responses in the plants (Yasuda et al. 2008; Ton et al. 2009). For example, under drought stress condition, common beans showed more symptoms when infected by *Macrophomina phaseolina*, and applied ABA on tomato (*Solanum lycopersicum*) leaves increases the susceptibility to *Botrytis cinerea* (Kayum et al. 2016).

In field conditions, plants faces multiple stresses and obviously their individual prediction is quite tough task (Atkinson and Urwin 2012). Different levels of sensitivity can be demonstrated by plants depending on environmental condition and the developing stage of the plant (Mittler and Blumwald 2010). In this context, a large number of interactions may take place between the defense mechanism and the stress intensity.

Vegetables, similar to other cultivated species, are subjected to numerous abiotic and biotic stresses (Fig. 6.3). Unfavorable conditions for growth and productivity are more frequent in recent years due to climate changes and the intensification of cultivation process that might affect natural resource availability (Montoya and Raffaelli 2010).

This context is being studied with increasing interest by many authors. Most studies conducted so far have examined widely diffuse crops such as cereals (Balmer et al. 2013; Huang et al. 2016), tobacco (Li et al. 2014; Dimlioğlu et al. 2015), cotton (Abdelraheem et al. 2017; He et al. 2017), etc. About vegetables, the available information are more limited, but it is still possible to find enough data to describe the stress-related problem. From the latest data available, the number of scientific papers related to “stress and vegetables” is over 6500 since 1970 to date. Considering

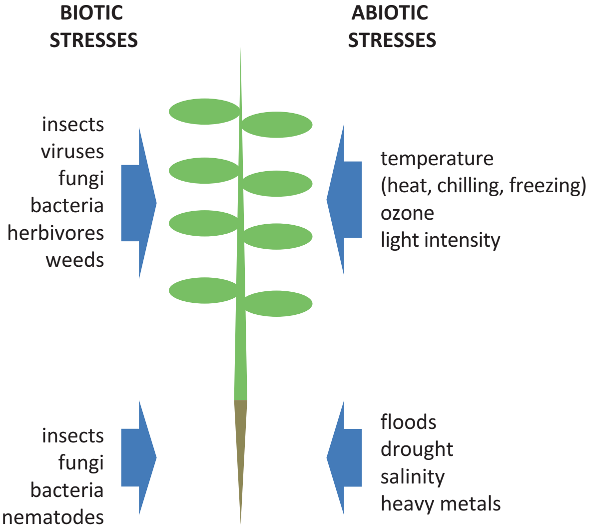

Fig. 6.3 Schematic representation of the most important abiotic and biotic stresses affecting plants

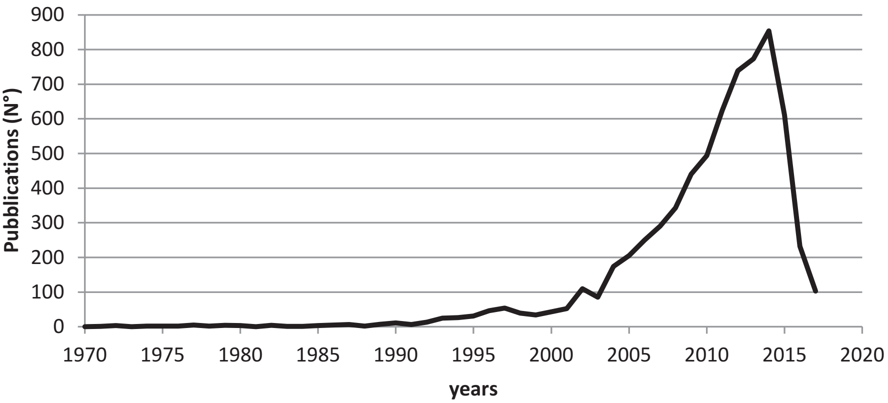

Fig. 6.4 Number of publications concerning "stress and vegetables". (Source: Scopus 2017)

their distribution (Fig. 6.4), it is clear that the interest generated by this issue arises around the 1990s and exponentially increased until 2014 (Scopus 2017).

In general, the largest number of studies conducted on vegetables refers to abiotic stress with about 3000 research papers. With regard to biotic stresses, the number of papers is considerably reduced to around 1200. Among the Most studied horticultural crops, we find tomato, brassicas, and legumes. Most of these studies refer to biotechnological research and physiological detection of plant response mechanisms.

By way of example, some results are reported about the main groups of vegetables studied focusing on the effects of biotic stress on production. Among the most important vegetables under the economical point of view, tomato is one of the most widely cultivated crop. This crop can be affected by a high number of biotic stress, and among the biotic challenges, whiteflies (*Bemisia tabaci*) have been defined as a big problem. *B. tabaci* Group is highly prolific, polyphagous, and invasive crop pest that can be found all over the world causing significant yield decline (Abate et al. 2000). For tomato, *B. tabaci* has been found to be the promising pest which causes direct and indirect damage by (1) direct feeding on the tomato plants (Muigai et al. 2002) and (2) transmission of viral diseases which causes adverse effects on crop yield (Mansoor et al. 2003). Some studies have evaluated the possibility of limiting the presence of this biotic stress in tomato production by chemical, physical, and biological control (Mutisya et al. 2016) using the combination with aromatic species. The organic volatile compounds that characterize aromatic species are well known for their insecticidal, antifeedant, repellent, attractant, and oviposition deterrent effects on insect pests (Song et al. 2010; Deletre et al. 2016). Under the mechanical point of view, in a study conducted by Mutisya et al. (2016) the application of cover net reduces the yield waste to 5.9 t ha^{-1} compared to 9.8 t ha^{-1} in the control and lowered *B. tabaci* tomatoes infestation by 68.7%. The application of companion aromatic species has also been successfully used. For example, basil (*Ocimum basilicum*) had repellent action against flea beetles on pechay (*Brassica pekinensis*) (Roxas 2009). Together with the direct effects on insects, companion planting also enhances the population of beneficial insects (Schader et al. 2005), reducing at the same time chemicals applicability.

Brassica crops have experienced many different types of biotic stresses such as fungal, bacterial, and viral diseases and insect pests (Kayum et al. 2016). In these circumstances, the plant can respond not only by fast acclimations but also by long-term adaptations such as variations in leaf shape (size and thickness), stomata density and distribution, chloroplast structure, and increasing the levels of photo-protecting enzymes and stress metabolites (Obidiegwu et al. 2015).

For legumes, there are numerous studies aimed at identifying possible systems to reduce the incidence of biotic stresses. In general, most of the research seek for resistance mechanisms in the host. Considering the global production of legumes, the major problems related to biotic stress occur in the cultivation of cool season food legumes. These are seriously affected by diseases that cause yield reductions estimated at over 50% on a worldwide basis (Muehlbauer and Kaiser 1994). Recently, research activities have been focused on genetic strategy (Keneni and Ahmed 2016), plant virus disease control (Makkouk et al. 2014), and molecular breeding (Duc et al. 2015; Rodda et al. 2017) to manage biotic stresses.

6.5 Biotic and Abiotic Stress Interaction

Generally, studies on stress try to identify plant responses and behaviors related to one or a few stresses. Such approach, even if needed from a research point of view in order to have comprehensible data, does not necessarily reflect the actual world in which plants grow. Normally, in fact, plants are subject to more stresses at the same time, and this involves vastly different responses from what happens with a single stress individually considered. The current understanding reveal that plants respond to multiple stresses differently from what they behave for individual stress (Atkinson and Urwin 2012; Nguyen et al. 2016). It has been observed that presence of wide range of abiotic stress may reduce or accelerate the vulnerability to biotic pests and vice versa. Such interaction between abiotic and biotic stress are managed by different hormone signalling pathways.

A multi-stress evaluation approach is therefore more interesting and realistic under the plant profile responses. As far as vegetables species are concerned, this approach has not yet been sufficiently taken into account since, so far, research was mainly focused on model species such as *Arabidopsis* (Anderson et al. 2004), rice (Xiong and Yang 2003), and wheat (Atkinson and Urwin 2012; Suzuki et al. 2014). However, there are some research papers that have studied the effect of multi-stress on vegetable species such as tomato (AbuQamar et al. 2009). Authors tested the effect of *B. cinerea*, *Pseudomonas syringae*, NaCl, and ABA evaluating the potential integrated responses to biotic and abiotic stresses by modulating ABA signaling and ion fluxes. Moreover, it has been observed that exposed tomato plants with drought (abiotic) and nematode (biotic) sresses begun to accumulate carotenoids and phenolic compounds, and sugars in the fruits (Atkinson et al. 2011). Further experience was done on hot pepper by Lee et al. (2004) that evaluated the effect of multi-stress such as ethylene, wounding, NaCl, and *Xanthomonas axonopodis*. Results showed target-binding domains in genes involved in pathogen defense and salt tolerance. Simultaneous stress such as heat, drought, viral infection, either individually or different combinations can cause a significant reduction in plant biomass (Atkinson and Urwin 2012; Prasch and Sonnewald 2013). Some authors have also identified positive interactions between stresses of different origins such as wounding (biotic) and salinity (abiotic) in tomato (Orsini et al. 2010; Dombrowski et al. 2011).

6.6 Biotic Stress and Quality in Vegetable Crops

From what has been reported so far, it clearly emerges the complex network of relationships that lays within the plant when it is affected by abiotic and/or biotic stress. The consequences that occur do not only affect production aspects, as pointed out by different authors, but the entire plant quality is conditioned.

Much of the biotic attacks on plants and, consequently, also on vegetables involve variations both in morphological and metabolic traits. In the first case, biotic stress can lead to growth slowdowns, resulting in reduced product size, color and shape variations, low yield, and, finally, lower shelf life.

Generally, a reduction in crop growth is associated with suboptimal growing conditions such as diseases and abiotic stresses. In this area some scientific works aimed at evaluating the effect of organic cultivation on the production of horticultural crops have shown that in general organic crops may produce 5 to 50% less than conventional crops (Del Amor 2007; Benbrook 2009; Quirós et al. 2014). This result can be explained by some crops stresses that could be responsible for the observed yield reduction. Within the stresses potentially experienced by crops, biotic problems are more frequent as chemicals are not allowed. Considering the complex modes of signaling a stress by the plant, secondary metabolism is greatly affected by the generation of ROS, hormonal components, and enzymatic activity. The production of chemical compounds as a defense reaction allows to short- and long-term adaptation to a suboptimal environment (Atkinson and Urwin 2012).

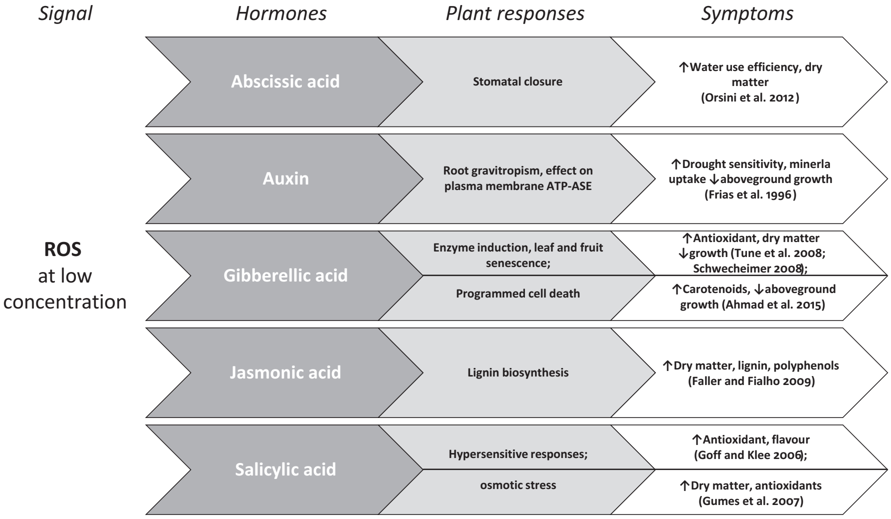

Fig. 6.5 Connected reactions among plant signal and physiological responses affecting product quality. (Source: Orsini et al. (2016), Sharma et al. (2012), adapted by authors)

The chemical components produced by the plant can be numerous, and among which there are ascorbate (Suzuki et al. 2013), carotenoids (Baranski et al. 2014), glucosinolate (Barbieri et al. 2008; Nicoletto et al. 2016), proline (Sperdouli and Moustakas 2012), polyamines (Hussain et al. 2011), and tocopherol (Benbrook 2009). One of the interesting aspects is the nutraceutical potential of these compounds. Paradoxically, the presence of stress during the cultivation of some species can be a crop extra value under the human health profile. Orsini et al. (2016) proposed a summary scheme with the main qualitative impacts on the plant as a stress result (Fig. 6.5). The role that all the components activated by the plant as a result of stress can be easily seen from the figure. The ROS generation appears to be effective for product quality: the main consequences are adjustable in terms of total antioxidant capacity, dry matter content, variation in pigment concentration, and flavor.

Phenolics are secondary plant metabolites which are very important for survival of the plants and they are naturally present in all plant. They are particularly conspicuous in fruit and vegetables determining color, appearance, flavor, and taste. Some changes takes place during the post harvest life of fruits and vegetables and eventually in these metabolites. These metabolic activities are some time responsible for certain essential changes which affects the quality of commodities. Moreover, the plants phenolics also imparts a lot in the brwoning reactions and resistance mechanisms against storage fungi (Lattanzio 2003). Tomato crops contain wide range of organic molecules which are much more benificial to human health, however, quantity and quality of these compunds varies significantly (Dumas et al. 2003; Dorais et al. 2008). Like other species, tomato crops also releases some organic molecules which have the resistance activity and protect the plants from various pest attack (EnglishLoeb et al. 1997; Treutter 2006). In particular, chlorogenic acid is supposed to impart in the resistance activty against root-knot nematode attack (*Meloidogyne* spp.) (Pegard et al. 2005). Chlorogenic acid, non-flavonoid phenolic compounds, powerful antioxidant, have many benificial impact in elimination of stress (Sato et al. 2011). Root-knot nematodes (*Meloidogyne* spp.) are the key attacking agents of most of the solanaceous crops (Fuller et al. 2008). These pathogens have been well studied and produced excellent findings (Atkinson et al. 2011). Inoculation of nematode and water stress exhibited different pattern in the lycopene and β-carotene content. Nematode stress was found to be associated with enhanced flavonoid levels, reduced yields, however, on the other hand, chlorogenic acid content was significantly higher in nematodes, water stress, and the combinely exposed plants. Sugar level was significantly higher only in tomatoes exposed to both stresses (Atkinson et al. 2011).

However, high levels of stress are not always favorable. Very often the presence of biotic stress implies the production of unpleasant bitter compounds. Talcott and

Howard (1999) noted isocoumarin production in carrot when attacked by mold species as it is a secondary metabolite that inhibits the growth of many microorganisms. Furthermore, isocoumarin is produced in the presence of ethylene, a well-known vegetable hormone synthesized by the plant under stress. The production of volatile ethylene compounds due to biotic stress was recorded in tomato (Maes and Debergh 2003). A combined effects of abiotic (constant light) and biotic stress (*Spodoptera littoralis* caterpillar) was carried out on volatiles compounds in the headspace. It was observed that caterpillars infestations caused an immediate higher secretions of the constitutive compounds (mono- and sesquiterpenes) and the induced compounds (linalool and indole). Induced compounds were started to emit approximately one day after the attack, and linalool was even emitted 2 days after removal of the caterpillar (Maes and Debergh 2003). Another example of increasing bitter compounds occurs in radicchio (*Cichorium intybus*) when undergoes stress (Malarz et al. 2007; Graziani et al. 2015). The experimental treatment with methyl jasmonate or salicylic acid on the sesquiterpene lactone content and biomass accumulation were investigated in a hairy root culture of radicchio. Results showed that methyl jasmonate enhanced the biosynthesis of the sesquiterpene lactones in the culture.

As previously reported, the effect of biotic stresses on the quality of horticultural products is positive or negative depending on the species considered and the chemical compounds. It can be observed that the increase of the qualitative value of a product through stress induction can be more easily pursued in the case of abiotic stress, such as salinity or water deficit, as they can be agronomically managed. On the contrary, it is difficult and risky to use biotic stress because it is more difficult to control and manage. In any case, the application of stress during the crop cycle will probably lead to yield reductions. Consequently, the final consumer will have to pay more for a qualitatively improved product and counteract for the yield loss to maintain farmers' adequate income.

6.7 Biotic Stress and Potential Solution for Vegetable Crops

The presence of biotic stresses during the crop cycle is generally a big problem that can compromise production. Consequently, it is interesting to give some synthetic information on the main systems used to contain this problem. In recent years, different solutions have been developed: genetic techniques and biotechnological systems, beneficial organism application, and agronomic techniques. Below are some synthetic information for each control system.

6.7.1 Genetic and Biotechnological Systems

One of the best options to minimize the losses due to disease occurrence is the adoption of resistant or tolerant varieties. In order to achieve a resistant variety is fundamental to find sources of resistance. Such sources may be present in germplasm collections, indigenous cultivars, or landraces of the selected crop (Singh et al. 2009). Wide range of biotic stress resistance in vegetable crops can be seen in many economically crops like tomato (Kissoudis et al. 2015), pepper (Majid et al. 2017), melon (Oumouloud and Álvarez 2016), legumes (Khoury et al. 2015; Mir and Kulwal 2014; Li et al. 2015), and brassicas (Dita et al. 2006). Genetic program or biotechnological approaches targets the genes which imparts disease resistance or tolerance against many pathogens, and the commercial significance of this action depends on the crop, damage caused by the disease, alternative measures for control, availability of resistance sources, and the ease of selection. Moreover, various plant pathogens can be effectively controlled by the developing disease resistance such as most virus and nematode diseases also some fungal diseases such as powdery scab (*Spongospora subterranea*) in potato or black leg (*Plasmodiophora brassicae*) in rapeseed (Miedaner 2016). Therefore, implication of genetic and biotechnlogical approaches in the development of suitable management system may fruitful.

6.7.2 Application of Beneficial Organisms

Another topic that has been, and continues to be, widely studied is the use of beneficial organisms in order to limit the negative effects of biotic stress on crops. Several studies have been carried out on the use of mycorrhizal, plant growth-promoting rhizobacteria (PGPR) and beneficial insects. Some examples can be found in nematode control. The nematicide potential of the bioagents *Bacillus megaterium*, *Trichoderma album*, *Trichoderma harzianum*, and *Ascophyllum nodosum* against the root-knot nematode, *Meloidogyne incognita*, was successfully employed in tomato (Radwan et al. 2012). Application of PGPR in the management of plant pathogens is considered to be novel tool in terms of acceleration of ecosystem sustainability (Ansari and Mahmood 2017; Gupta et al. 2015). Research indicate the PGPR improve the quality of vegetables which may yield a good remuneration (Zaidi et al. 2015). In *Brassica oleracea* the PGPR application ameliorated plant growth, nutrients, and useful hormones in the plants growing under various biotic stress (Turan et al. 2014). The third area, namely, the beneficial insect application, is an extremely large subject that presents a lot of work conducted both in greenhouse and in open field (Yang et al. 2014; Van Lenteren et al. 2017; Devi and Nath 2017). The biological control approaches are very sound and viable which can be used in the sustaibale agriculture for the promotion of plant growth and yield growing under various agroclimatic conditions.

6.7.3 Agronomic Practices

The agronomic practices available to control biotic stresses are numerous, and in recent years, they have considerably evolved. Obviously, the main system currently used to limit damage from biotic attacks (insects, mites, and nematodes) is still represented by the chemical control that allows a prompt action and with a significant impact. As it is known, this practice presents negative aspects ranging from the increasing resistance of pathogens to environmental impact and to the risks for both operators and consumers. At the same time, there are other, complementary, and/or substitute solutions that have been developed over the last 20 years.

There has been consistent losses in the yield when crops are cultivated in adverse environments. Grafted plants, cultivated under adverse environments and soilborne diseases are found to exhibit greater growth and yield, improved photosynthesis, better water and nutritional status, and a reduced accumulation of Na and/or Cl, heavy metals, and excessive amount of trace elements in shoots as compared to ungrafted or self-grafted plants (Colla et al. 2013; Colla et al. 2017).

The use of biostimulants is another tool that can be used to limit the effect of biotic stress (Sharma et al. 2014). For example, application of macroalgae in the improvement of crops by reducing the biotic stress have been very important. It is anticipated that polysaccharides, metabolic copounds which are capable to induce the antisenescence may help in the removal of the plant stress.

In some cases, the crop biofortification may also be helpful in reducing biotic stresses. Particularly the increased presence of some elements like zinc (Poschenrieder et al. 2006), silicon (Farooq and Dietz 2015), and selenium (Oancea et al. 2015; Wu et al. 2015) is a possible alternative strategy to reducing the damage caused by biotic stress.

In the climate change we are facing, the aggressiveness of the various pathogens and crops vulnerability are expected to be further accentuated as a result of increasing abiotic stress.

6.8 Conclusions

As briefly described in this chapter, the combined presence of multiple stresses can dramatically increase the effect on crops. From a qualitative point of view, biotic stresses are able to present conflicting responses which, in some cases, can compromise the quality of horticultural products by reducing their commercial value, in others provide some sort of added value. Very often, in fact, the presence of biotic stresses leads to the increase of nutraceutical compounds which promote consumer health by improving his diet. In any case, the productive world is not helpless about this problem. In recent years, scientific expertise has enabled the development of integrated control systems to avoid damage caused by biotic stress. Application of microbial agents in the aleviation of biotic stress have been proven to be very promising and ecologically sound. Acquired techniques also help in reducing environmental impact by exploiting natural or induced resistances, utilizing beneficial organisms and environmentally friendly agronomic techniques.

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Chapter 7
Management of Soil-Borne Diseases of Plants Through Some Cultural Practices and Actinobacteria

K. P. Roopa and Anusha S. Gadag

Abstract With a growing population, the demand for crop production is an increasing trend. The main hindrance in this regard is the biotic stresses like diseases and pests attacking crop causing huge losses every year. Mainly the soil-borne pathogens are devastatingly affecting the crop production. It is challenging to predict, detect and diagnose a variety of soil-borne pathogens that are causing plant diseases. The soil-borne plant pathogens can be categorized under divisions of bacterium, virus, fungus or plant parasitic nematode. These pathogens are highly effective due to extensive surviving periods even in absence of host plant and also congenial environmental condition. The survivability of soil-borne pathogens in the soil varies for each of them. Most soil-borne pathogens are difficult to control by conventional procedures like the use of resistant cultivars. Extensive use of fungicides is expensive and affects nontarget microflora. The promising strategies used are crop rotation and cover cropping and using organic amendments (manures and composts) and biological control are alternative methods which can replace the use of chemical pesticides in the control of soil-borne pathogens. In recent years, biological control has become very popular and included in plant disease management, and it is considered as a practical and safe approach in many crops. Among these antagonistic microorganisms, actinobacteria are well-known and classified among the most active rhizobacteria. In recent decade actinobacteria have gained importance due to its extensive presence and active colonization ability in rhizosphere of plants, its ability to produce a wide variety of agro-active compounds effective against many pathogens. Actinobacteria, especially *Streptomyces* spp., have biocontrol action against a range of phytopathogens. The actinobacterial mechanisms imparting soil-borne disease control majorly involve antibiosis, hyperparasitism, production of cell wall-degrading enzymes, stimulation of nodulation, etc. Actinobacteria-treated plants showed ameliorated plant health. The role of actinobacteria, as the probable stimulator of ISR (induced systemic resistance), is a major aspect in disease control. It induces signalling pathway involved in plant disease resistance and produces many defence-related enzymes protecting plants against

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K. P. Roopa (✉) · A. S. Gadag
Department of Biotechnology, University of Agricultural Sciences, Dharwad,
Karnataka, India

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pathogen attack and also preventing its further spread. Hence actinobacteria are an effective biocontrol agent and can be used for the control of soil-borne pathogens.

Keywords Management · Soil borne pathogens · Crop rotation · Solarization · Sanitation · Biocontrol · Actinobacteria

7.1 Introduction

The world population is increasing every year and the demand for the food is also high. Hence global crop production needs to double by 2050; however, current estimates are far below what is really required (Ray et al. 2013). It has been estimated approximately 36% reduction in the production of crops worldwide is due to plant diseases, insects and weeds. The yield losses due to diseases alone have been shown to be 4% (Agrios 2005). Therefore, the management of plant diseases has to be emphasized to increase crop production. Among plant diseases, soil-borne diseases are considered to be more limiting than seed-borne or airborne diseases which affect the crop production of 10–20% of yield losses annually (USDA 2003).

The diseases caused by various plant pathogens which persist or survive in the soil matrix and reside on the soil surface are called soil-borne diseases. Therefore, the soil is considered a reservoir of inoculum of these pathogens distributed in soils and on tissues of the infected plant hidden in the soil. Until the soil-borne diseases show symptoms on above-ground (foliar) parts such as damping-off, stunting, wilting, chlorosis, withering of leaves and death (Veena et al. 2014).

Soil borne diseases are the major limitation to crop production, since they are difficult to control, even using standard protocols. These soil-borne pathogens mainly survive on host plant debris, organic matter or as free-living organisms for over long periods. Most of the vegetable crops are highly vulnerable to several soil-borne pathogens. Numerous soil factors such as pH, soil type, texture, temperature, nutrient levels, moisture and ecology affect the activity of these pathogens and contributing to low yields of crop plants. Vegetable crops are most vulnerable to a range of pathogenic organisms that decrease production by destroying whole plants or valued products and make them unfit for use. Plant diseases are responsible for 26% of yield loss in global agriculture, and few instances it may lead to complete crop failure (Khan et al. 2009). Disease development is normal in an ecosystem which becomes a concern when the diseases assume an epidemic form causing huge crop losses (Morsy et al. 2009). Many soil-borne diseases are of soil-inhabitant pathogens which have broad host ranges that include weeds and produce dormant structures. Some important plant pathogens like fungi, fungi-like organisms, plasmodiophorid, bacteria, viruses and phytonematodes (Asma and Shafique. 2016; Baysal-Gurel et al. 2012).

7.2 Soil-Borne Fungal Diseases

Important soil-borne fungal pathogens include species of *Sclerotium*, *Fusarium*, *Verticillium*, *Rhizoctonia* and *Macrophomina phaseolina* causing huge loss of billions of dollars every year. Soil-borne fungi survive in soil by producing resistant survival structures such as chlamydospores, oospores and sclerotia (Baysal-Gurel et al. 2012). *Phytomyxea* (plasmodiophorids) pathogens are species of *Plasmodiophora* and *Spongospora*. Important oomycete pathogens include *Phytophthora* and *Pythium*. *Fusarium solani* and *R. solani* develop in both cultured and non-cultured soils and cause damping-off and root rot diseases in a wide range of vegetable and crop plants (Szczechura et al. 2013). *Rhizoctonia solani* causes the incidence of root rots and 10–80% losses in different vegetables. *Rhizoctonia solani* has detrimental effects on agricultural and horticultural crops causing pre- and post-emergence damping-off, root rot and stem canker. Its host plants are alfalfa, peanut, soybean, lima bean, cucumber, papaya, brinjal and corn (Keijer et al. 1997).

Macrophomina phaseolina incites charcoal rot and found in almost all habitat which keeps prime importance in the reduction of crop productivity especially in arid regions (Ijaz et al. 2013). More than 500 species are attacked by these pathogens and cause various diseases in the plants such as stem canker, stalk rot or charcoal rot (Khan 2007). Charcoal rot causes significant damages and considered as a major constraint in the sunflower production (Khan 2007; Ijaz et al. 2013). Likewise, *Pythium* spp. causes damping-off and root rot and being considered among the most devastating fungus infesting various crops. *Pythium* species are among the most destructive plant pathogens and able to parasitize seeds, seedlings and at later stages whole plants (Agrios 2005). Similarly, *Phytophthora* attacks on roots, crown and fruits of many horticultural crops. The disease is more aggressive stage under wet state, and dissemination happens to be due to soil contaminations. *Phytophthora capsici* affects a wide range of crops and causes great losses under various agroclimatic conditions (Benson et al. 2006). *Phytophthora* pod rot (PPR) ranked first followed by stem cankers in terms of yield losses and estimated to be approximately 10–20%; however, it may go up to 10–30%, with tremendous losses during the rainy season (Erwin and Ribeiro 1996). Some other soil-borne pathogens such as *Plectosporium tabacinum* (El-Tarabily 2003), *Gaeumannomyces graminis* var. *tritici* and *R. solani* (Coombs et al. 2004) and *Fusarium oxysporum* (Cao et al. 2005) have also been documented well by the earlier worker.

7.3 Other Soil-Borne Diseases

The bacterial plant pathogens such as *Ralstonia*, *Pectobacterium*, *Agrobacterium* and *Streptomyces, Pseudomonas* spp. (Baysal-Gurel et al. 2012). There are some which are sometimes considered as soil-borne viruses that affect vegetables, although they perpetuate only in the living cells of the host plant or vectors that transmit these virus. Phytonematodes lower the plant growth and productivity and ultimately cause great losses to the farmers. Root-knot nematodes (*Meloidogyne* spp.) among the phytonematodes cause great losses to the cultivated crops and reduce the plant productivity around the world (Youssef and Lashein 2013). Moreover, they are sedentary endoparasites and cause losses of up to 80% in heavily infested fields (Sikora and Fernandez 2005; Adam *et al.,*2014; Saifullah et al. 2007).

7.4 Disease Management Strategies

The effect of management practices on soil-borne pathogens is a heated topic in the present era in terms of crop management systems that are non-conducive to the growth of soil-borne pathogens. The following approaches are few management practices which are widely employed for the management of soil-borne plant pathogens.

7.4.1 Assessment of Disease Incidence and Severity

Diagnosing of the diseases caused by soil-borne pathogens may be difficult since there is an absence of characteristic symptoms. But chlorosis (yellowing), stunting and wilting are typical symptoms. It shows the root symptoms such as lesions, rotting of root tips and swellings (galls, knots, etc.) and reduced the size of lateral roots. Assessment of plant health is recommended to be done by checking disease incidence, disease severity and crop loss estimates, which are major factors for control strategies (Baysal-Gurel et al. 2012).

7.4.2 Disease-Free Seed Material and Storage Practices

It is very important to select and use of disease-free seeds for sowing to reduce infection of soil-borne pathogens. To reduce the incidence of soil-borne pathogens which includes *Rhizoctonia* (stem canker/black scurf) and *Fusarium* (dry rot) and secondary bacterial soft rot in cut potato seed, a good practice of management and seed storage prior to sowing is necessary.

7.4.3 Variety Selection

Resistant variety usage and the varieties which can tolerant specific pathogen can prevent the occurrence and spread of soil-borne disease.

7.4.4 Organic Matter Amendments

For reducing soil-borne pathogens, application of the organic matter to the soil such as cover crop, green manure crops, dried plant material, compost, peat and other organic waste can be effectively used (Ansari and Mahmood 2017). Many researchers have reported that organic additive amendments can be very effective in the management of some key soil-borne pathogens such as *Rhizoctonia solani* (Diab et al. 2003), *Fusarium* spp. (Klein et al. 2011), *Pythium* spp. (Veeken et al. 2005; McKellar and Nelson 2003) and *Sclerotinia* spp. (Lumsden et al. 1983). Organic matter enhances soil structure and improves its ability to hold water and nutrients; it also gives shelters to microorganisms which improves the soil quality on one hand and reduces the soil-borne pathogens on the other hand. Organic matter amendments have great potential, but sometimes it may exhibit inconsistent results and cause phytotoxicity to the plants. Therefore, judicious application of organic additives is considered as good management practices for the management of soil-borne pathogens infesting various agricultural crops.

7.4.5 Nursery Maintenance

A good water drainage facility can prevent soil-borne diseases and seed decay. For vegetables, transplanting or sowing seeds directly is practised. Proper management of seedlings such as using sterilized growing media is required to control damping-off and root rots. For transplanted crops raised beds are being practised. Heavy irrigation and extreme temperature during transplanting of the crops also minimize the propagules of fungal pathogens. Shallow planting reduces the risk of damping-off and root rot disease problems. Good soil preparations help in proper drainage and reduce soil-borne diseases for seed-sown crops.

7.4.6 Sanitation

In soil-borne diseases pathogens survive on the crop residue of diseased plants, it's important to remove or destroy old infected plant debris as much as possible. Timely ploughing the soil helps in removing the plant residues left from previous crops.

There are many methods for soil sanitization, heat pasteurization, UV radiation, chlorine treatment, slow sand filtration and biofiltration (Katan 2000).

7.4.7 Soil Solarization and Disinfection

Soil solarization is one of the best method to keep soil free from pathogens, it is done before planting. Plastic sheets are used to capture solar radiation in the soil by which upper layer of the soil gets heated up, thereby increasing soil temperature, and it helps to eradicate soil-borne fungal and bacterial pathogens which include *Verticillium* spp., *Fusarium* spp., *Streptomyces scabies*, *Sclerotinia* spp., *Agrobacterium*, nematodes and weeds. Anaerobic soil disinfestation is an alternate method where disinfection is created by anaerobic soil conditions by amending easily decomposable organics such as rice straw, wheat bran, molasses and rice bran into the soil. Anaerobic soil disinfestation can be done by using wheat bran or molasses which is found to be effective against a wide spectrum of soil-borne plant pathogens such as *Fusarium oxysporum* f. sp. *lycopersici*, *Verticillium dahliae* and *Ralstonia solanacearum*, the phytoparasitic nematodes *Meloidogyne incognita* and *Pratylenchus* spp. (Momma 2008).

7.4.8 Crop Rotation and Intercropping

Crop rotation is the most common and effective method used for controlling soil-borne diseases. A good rotation with the resistant crop for the specific pathogen will solve most of the soil-borne infections at field level. Selection of cover crops on the basis of disease suppression ability needs careful attention since the effects can be specific to cultivar. In intercropping systems, disease-suppressive mechanisms such as host dilution, allelopathy and ameliorated microbial populations play important roles in the reduction of soil-borne pathogen populations (Baumann et al. 2002; Bukovinszky 2004).

7.4.9 Biocontrol

Biological control is the utilization of living organisms in the suppression of activities and populations of one or more plant pathogens (Pal and Gardener 2006). In the current situation, the use of plant growth-promoting rhizobacteria (PGPR) might be a suitable and alternative strategy to overcome the issues pertaining to persistent use of hazardous inorganic fertilizers, chemical pesticides, etc. There are several mechanisms by which PGPR operate to deal with pathogens through plant-microbe interactions and PGPR association and affect plant health, including N-fixation, hormonal interaction and bioynthesis antimicrobial compound (Srinivasan et al. 2013).

7.4.10 Actinobacteria

A wide number of studies have previously reported the potentiality of actinobacteria to control diseases caused by a diverse range of phytopathogens such as *Alternaria solani* and *Helminthosporium oryzae* (Chattopadhyay and Nandi 1982), *Alternaria brassicicola* (Tahvonen and Avikainen 1987), *Macrophomina phaseolina* (Hussain et al. 1990), *Phytophthora fragariae* var. rubi (Valois et al. 1996), *Sclerotinia sclerotiorum* (Baniasadi et al. 2009) and *Sclerotium rolfsii* (Pattanapipitpaisal and Kamlandharn 2012). Actinobacteria constitute a morphologically diverse group, belong to the order *Actinomycetales*, and are different from other Gram-positive bacteria by the presence of filamentous growth and GC-rich DNA (Lacey 1997). Actinobacteria metabolically and morphologically are comparatively more complex than sessile bacteria (Doumbou et al. 2001). Metabolically, they are prolific producers of different secondary metabolites, such as antimicrobial agents, siderophores and plant growth-promoting molecules (phytohormones). Morphologically, they produce mycelia and some what spore-like structures which results in a various pattern of colonization as compared to sessile bacteria. They are exploited in sustainable agriculture because they produce bioactive compounds such as lytic enzymes and antibiotics (El-Tarabily et al. 1997; Bérdy 2005; Clardy et al. 2006). The antibiotics produced by *Streptomyces* spp. have been found to check the growth and colonization of a wide range of phytopathogenic fungi and/or bacteria (Berg et al. 2001). Further, these compounds have also been considered as one of the important tools controlling the soil-borne diseases of wide scale (Buchenauer 1998). Utilization of such bioactive compounds leaves no destructive inputs to the environment (Cardoso et al. 2010).

Among the various actinobacteria, *Streptomyces* spp. are commonly occurring and being exploited stridently in nowdays. The antagonistic actinobacteria, *Streptomyces griseoviridis*, are commercially available in the trade name Mycostop® (Tahvonen and Avikainen 1987). Mycostop is nowadays used to control *Alternaria* and *Fusarium* diseases in crucifers and *Fusarium* wilt in carnation crops. It also controls the root diseases caused by *Pythium*, *Phytophthora* and *Rhizoctonia* (Costa et al. 2013).

7.5 Mechanisms of Action of Actinobacteria in Disease Control

There are various mechanisms by which protection against plant pathogens can be achieved. The actinobacteria mechanisms imparting soil-borne disease control majorly involve antibiosis, hyperparasitism, cell wall-degrading enzyme production, stimulation of nodulation, etc.

7.5.1 *Antibiosis*

Antibiotics produced by actinobacteria are an effective strategy to control the plant pathogens. Antibiotics can also suppress or kill other microorganisms even at low concentrations. To be effective against any plant pathogen, antibiotics must be produced in a significant amounts near the plant pathogen which may immediately start the biocontrol activity. Actinobacteria produce a wide range of antibiotics such as polyketides, b-lactams and peptides besides producing of other secondary metabolites which have antifungal, antitumour and immunosuppressive activities (Behal 2000). Another compound, Kasugamycin having bactericidal and fungicidal activity, was discovered in *Streptomyces kasugaensis* is very important and currently used at a commercial level (Umezawa et al. 1965). This antibiotic inhibits protein biosynthesis in microorganisms, and Hokko Chemical Industries have used systemically active kasugamycin in the management of *Pyricularia oryzae* (Sharma 2014).

A new class of fungicide, polyoxins B and D, was isolated from *Streptomyces cacaoi* var. *Asoensis* in 1965 (Isono et al. 1965). Application of polyoxin inhibits the synthesis of the fungal cell wall, and therefore this is widely accepted (Endo and Misato 1969), thereby inhibiting the growth of fungal pathogens infesting agricultural crops such as vegetables, fruits, ornamental and industrial crops. Polyoxin D has also been commercialized and marketed, and available for the management of rice sheath blight caused by *Rhizoctonia solani* Kùhn (Sharma 2014).

7.5.2 *Hyperparasitism*

Several reports are available on actinobacteria hyperparasitism on pathogenic fungi (Tapio and Pohto-Lahdenpera 1991; Yuan and Crawford 1995). Apart from *Streptomyces* spp. even non-*Streptomyces* spp. also exhibit hyperparasitism. *Nocardiopsis dassonvillei* has antibiotic, mycolytic and parasitic activities against the vegetative mycelium of *Fusarium oxysporum* f. sp. albedinis (Sabaou et al. 1983). Upadhyay and Rai (1987) strains of *Micromonospora globosa* parasitized the hyphae of *Fusarium udum* in vitro (Khan et al. 1993). Oodles species of the genus *Actinoplanes* effectively parasitized the oospores of *Pythium* spp. including

P. aphanidermatum, *P. ultimum*, *P.arrhenomanes*, *P. irregular* and *P. myriotylum*, both in vitro and in sterile and non-sterile soils (Khan et al. 1993). Ningthoujam et al. (2009) reported that several actinomycete isolates from various localities in Manipur have the ability to control fungal pathogens like *Curvularia oryzae*, *Pyricularia oryzae*, *Bipolaris oryzae* and *Fusarium oxysporum*, and among 30 LSCH-10C was found more promising. Soil-borne *Actinoplanes* spp. parasitize oospores of *P. megasperma* f. sp. *glycinea* (Sutherland et al. 1984) and *Pythium* spp. (Khan et al. 1993; El-Tarabily et al. 1997; El-Tarabily 2006) in in vitro studies.

7.5.3 Lytic Enzymes

The extracellular hydrolytic enzymes play important role in reducing the population of soil-borne plant pathogens. For example, chitinase and -1, 3-glucanase act against the formation of chitin and -1, 3-glucan, resulting in the degradation of cell wall which ultimately kills the fungal pathogens (Lam and Gaffney 1993; Chernin and Chet 2002). It is clear now that antagonist produces high levels of chitinase and β-13-glucanase causing extensive hypha plasmolysis and cell wall lysis and significantly reduces disease incidence (El-Tarabily et al. 2003). *S. plymuthica*, *Serratia marcescens*, *Paenibacillus* sp. and *Streptomyces* sp. also produce chitinase which was found to be inhibitory against some plant pathogens such as *Botrytis cinerea*, *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *cucumerinum* (Ordentlich et al. 1988; Frankowski et al. 2001). Quecine et al. (2008) observed chitinase production through endophytic actinobacteria and their utilization in the management of *Colletotrichum sublineolum*. The correlation studies between chitinase production and pathogen inhibition have also been conducted, and it was seen that β-1,3-glucanase produced by *Paenibacillus*, *B. cepacia* destructed the cell wall of *F. oxysporum*, *R. solani*, *S. rolfsii* and *Pythium ultimum* (Fridender et al. 1993). Application of ChiA from *S. marcescens* reduced disease incidence of southern blight of bean caused by *Sclerotium rolfsii* (Shapira et al. 1989). *M. carbonacea* suppressed the *Sclerotinia minor*, the pathogen of basal drop disease of lettuce in the United Arab Emirates which are more prominent (El-Tarabily et al. 2003). El-Tarabily (2006) reported that the strains of *Microbispora rosea*, *Micromonospora chalcea* and *A. philippinensis* are the producers of β-1,3-, β-1,4- and β-1,6-glucanases and cause lysis of *P. aphanidermatum* hyphae and also reduce damping-off disease of vegetable like cucumber.

7.5.4 Stimulates Nodulation

Actinobacteria also help in the stimulation of nodulation. Recently, Solans et al. (2009) isolated saprophytic actinobacteria *Streptomyces* MM40, *Actinoplanes* ME3 and *Micromonospora* MM18 from the root nodule surface of the plant *Discaria trinervis*. Application of low level of nitrogen (0.07 mM), the inoculation of the actinobacteria did not register any significant effect on plant growth and yield. On another hand, when actinobacteria were simultaneously inoculated with *S. meliloti*, the nodulation and plant growth promotions were found significantly enhanced. Likewise, under a high level of nitrogen (7 mM), inoculation of *S. meliloti* alone or co-inoculation with actinobacteria improved the nodule formation.

7.5.5 Induced Systemic Resistance (ISR)

Induced resistance is a state of enhanced defensive ability offered by a plant when appropriately accelerated (Van Loon et al. 1998). Induced systemic resistance (ISR) was illustrated as the mode of disease suppression by some non-pathogenic rhizospheric bacteria (Van Peer et al. 1991 and Wei et al. 1991). The implication of ISR in disease reduction has been well documented in a wide range of biological control agents. In ISR, plants are primed with rhizobacteria, and therefore, strong and rapid counterreaction takes place against attacking pathogens by inducing defence mechanisms. ISR is effective in the controlling of diseases caused by various pathogens. ISR implicates jasmonate and ethylene signalling pathways within the plant, and these hormones stimulate the host defence mechanisms against the pathogens (Verhagen et al. 2004). ISR involves in the protection of physical and mechanical barriers of the cell wall as well as changing physiological and biochemical reaction of host leading to the synthesis of defence chemicals against challenge inoculation of pathogens. The defence reactions are shown through the accumulation of defence and PR-enzymes such as chitinase, β-1,3-glucanase, PAL, PO, phenolics and phytoalexins (Kloepper and Beauchamp 1992). The production of the iron chelator, siderophores, competition for food and space and induction of systemic resistance as the chief mechanisms of biocontrol of soil-borne fungal plant pathogens by *Streptomyces* spp. have also been observed (Tokala et al. 2002).

7.5.6 Plant Growth Promotion

Biocontrol agents also produce some plant growth hormones. These hormones help in the suppression of the toxic effects of plant pathogens and promote the plant growth of plants and also improve the crop yield. The key mechanisms involved behind crop protection include the plant growth hormone production, accumulation of important nutrients and iron chelators (siderophores) and antibiotics production. This way plants manage themselves from the attack of deleterious pathogens. Actinobacteria may also increase the plant growth by the production of key hormones such as auxin/IAA, gibberellins and mineralizing phosphates (Poonguzhali et al. 2008; Chitraselvi et al. 2015). Hamdali et al. (2008) assessed the plant growth-promoting abilities of eight indigenous rock isolates from Moroccan phosphate mines and reported that these isolates inhibited the growth of phytopathogen *Pseudomonas aureus*, *Staphylococcus aureus*, *Fusarium oxysporum* and *Pythium ultimum*. Five of these isolates could produce indoleacetic acid. Further, the most potent strain *Streptomyces griseus* (BH7) effectively stimulated the plant growth.

Franco-Correa et al. (2010) isolated 30 actinomycete isolates from the rhizosphere of *Trifolium repens* L. and tested them for their ability to solubilize phosphate sources, nitrogen-fixation and siderophore production. All isolates exhibited phosphate-solubilizing ability; however, only ten isolates were able to grow on nitrogen-free media. Almost all isolates showed siderophore production and inoculation of clover plants with the selected actinobacteria significantly enhanced plant growth and nitrogen acquisition. Recent studies showed that actinobacteria-treated plants registered better plant growth such as chlorophyll content, higher phenylalanine ammonia lyase (PAL) activity and high total phenolic content. Application of these actinomycetes also influenced the qualitative and quantitative studies of phenolic compounds from tomato leaves (Patil et al. 2011). *Actinopoymorpha* spp. improved plant health and reduced the severity of sheath blight infection in rice. The role of actinobacteria, therefore, may be a probable stimulator of ISR (induced systemic resistance) signalling pathway involved in plant disease resistance (Shinde et al. 2014).

7.5.7 Volatile Substances

Volatile substances produced by biocontrol agents play a tremendous role in suppression of pathogens in plants. Apart from antibiotics, volatile compounds like hydrogen cyanide, alcohols, acids, ketones, sulphides and aldehydes are involved in pathogen inhibition (Bouizgarne 2013). Many rhizobacteria including actinobacteria are known to produce hydrogen cyanide and this has been found to play direct as well as an indirect role in the biological control of plant diseases and also responsible for ameliorated plant health. The HCN production by actinobacteria is also responsible factor in the suppression of various soil-borne plant pathogens (Wang et al. 2013; Boukaew et al. 2013).

7.5.8 Conclusion and Future Perspective

In the current scenario of the rapid global increase in population, there is a need for increasing the production and productivity to feed the world. Disease and pest are the major constraints for crop production. Among the plant pathogens, soil-borne pathogens have a deleterious effect on crop growth and limit the total potentiality of the crop. There is a need for more environmental friendly and economically feasible strategy to control these soil-borne pathogenic microorganisms. Biocontrol method is considered as the best alternative option to manage these soil-borne pathogens. Presently, actinobacteria are an important group of biocontrol agents which is gaining more attention mainly due to the production of some important organic molecules, i.e., secondary metabolites, volatile substances, lytic enzymes, hyperparasitism, inducing systemic resistance and plant growth promotion in plants. All these mechanisms play a crucial role in the control of the pathogen growth in soil, thereby preventing the spread and survival of pathogens. Many pieces of evidence prove that actinobacteria can be effectively used in the management of plant pathogens. Currently, the most exploited actinobacteria are *Streptomyces* spp. In future there is a need for the exploitation of more of non-*Streptomyces* spp. of the actinobacteria having great efficiency in the management of plant diseases caused by soil-borne microorganisms which are pathogenic in nature.

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Chapter 8
Organic Amendments to Alleviate Plant Biotic Stress

Khalid Azim

Abstract In nature, plants are exposed to many anomalies that cause severe metabolic disturbances and very often an installation of the disease. These anomalies are either climatic (low or high temperatures) or anthropogenic (heavy metals and pesticides) and are called abiotic stresses. In contrast, biotic stress involves a second living being known as a pathogen (fungus, bacterium, mycoplasma, virus, and viroid) or others (mites, insects, mollusks, nematodes, and herbivores). Plants attacked by a pathogen develop complex defense strategies (hypersensitive reaction and acquired systemic resistance) and often effective to cope with the anomaly. The suppressive effects on biotic stress agents, often observed during the use of organic amendments, open up horizons of new perceptions. Some of the living organisms that live in the organic amendments are also known to inhibit soilborne disease and phytopathogenic nematodes. However, it is only gradually that we begin to understand under what conditions they can be developed and following what approach they can be efficient. Different control approaches propose control of biotic stress by the suppressive effect of organic amendments from plant and animal sources such as compost. Nevertheless, not all the organic amendments have the same ability to effectively inhibit biotic stress agents. The variability observed between the effects of different organic amendments is undoubtedly the biggest obstacle to their use on a large scale. In general, the suppressive ability of organic amendments to plant diseases has been linked to their chemical composition, the bioavailability of their nutrients, and their microbial consortium due to both supply of microorganisms and nutritional stimulation of those in the soil.

Keywords Organic agricutlure · Compost · Manure · Biochar · Soilborne pathogens · Suppressive effect

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K. Azim (✉)
Research Unit of Integrated Crop Production, Regional Center of Agronomy Research, INRA-CRRA, Inzegane, Agadir, Morocco

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8.1 Introduction

The economic development and the rise of consumption worldwide have led to an important rise of the organic waste disposal due to the intense activity of the agricultural production systems (Hofmann 2008). The good management of these organic wastes would be a key answer to fertility rebuilding of soils already degraded by intensive horticultural practices and climate change impact (Azim et al. 2017a). The United Nations Framework Convention on Climate Change stated that the capacity to assess accurately the amount of carbon (C) sequestration through biomass and soil in the agricultural systems is gaining (Regni et al. 2017). The amendment of organic wastes derived from different sources is among the agricultural managements. It represents an effective way to build carbon stock in the agroecosystem soil (Russo et al. 2015). The organic amendments (OA) have extremely suppressive impacts on diseases triggered by telluric pathogens such as *Pythium*, *Phytophthora*, *Fusarium*, *and Rhizoctonia*, both in greenhouse and in open fields (Schönfeld et al. 2003). This suppression is achieved either by reducing growth and saprophytic survival of the pathogen or by reducing the expression of the disease or by both mechanisms (Cotxarrera et al. 2002). However, the majority of soils are not suppressive, and disease control is mainly based on chemical control (Tian et al. 2002), although the interest in using biological control means increased considerably during these two last decades with increasing concern for sustainable and organic agriculture (Małolepsza 2006). Specific rhizosphera and bacterial and fungal antagonists can induce systemic resistance in plants (De Meyer et al. 1998; Yedidia et al. 2003; Harman et al. 2004) and control of soilborne diseases (Raupach and Kloepper 1998; Pieterse et al. 2003). The most outstanding results were obtained with *Trichoderma* species (Chen et al. 2016, Youssef et al. 2016, Doni et al. 2017). OA are all the materials that are used in addition to its fertilizer effect and nutrient deficiency and are used also for telluric disease management. They can be defined also as any agricultural input, such as lime, compost, gypsum, synthetic soil conditioners, animal manures, sawdust, or crop residue that is amended to the soil to improve plant growth other than low nutrient content. OA may enclose important nutritional elements, but it usually denotes the supplementary materials other than those considered mainly as fertilizers. OA refer to external organic matter to the agricultural plot, sometimes spotted as wastes, with a heterogeneous and a multiple composition and from a large panel of animal and vegetal sources (Ruano-Rosa and Mercado-Blanco 2015). The mode of action of OA is very wide due to the complexity of relationship among biological, chemical, and physical variables. Hence, disease control is affected by OA through the improvement of the host nutritional status, hence improving its resistance, or by reducing the telluric pathogen potential in the soil. Furthermore, soil amended with organic amendments rich in nitrogen is less affected by *Fusarium* wilt and other root wilting pathogens, due to the increase in the soil pH and the concentration in ammonia content (Borrego-Benjumea et al. 2014). It is also critical to take care of sources of the OA which they are derived from because they could spread pathogen into the soil and then to the crop (Lopez-Escudero and Blanco-Lopez 1999). The traceability of OA origins, if not appropriately handled, can cause severe environmental pollution and sanitary issues at the air, soil, and groundwater level. The appropriate management of the organic wastes would be an effective way of C sequestration through organic amendment restitution to depleted soils (Azim et al. 2017a). In this chapter, the objective is out of a broad assessment of all resources considered as OA; it's rather a review of different recent breakthrough solutions against biotic stress agents that will be discussed along with some preference to compost as broad medium of potential beneficial microorganisms.

8.2 Effect of Organic Amendments on Soil Biota

Amendment of soil with OA is assessed in general as a way to increase soil fertility and to alleviate the effects of climate change. Nevertheless, OA effect on soil biota has been less studied than the effect on soil physicochemical parameters (Lehmann et al. 2011). Nowadays, the soil is considered as a huge pool of microbial diversity compared with other ecosystems. The use of meta-genomics approaches and specific media for culture is enlightening a much soil microbial diversity than that discovered by classical methods (Daniel 2005). Irrigation, cropping, tillage, fertilizer, and pesticide application are some management practices considered among the most affecting factors that affect the biodiversity of microbial communities at rhizosphera level (Prashar et al. 2013). Consequently, any abiotic/biotic input amended into the soil will lead in short-term and/or long-term fluctuations of the structure of the microbial community. The soil microbiota, either pathogenic or beneficial, is crucial for plant health; potential fluctuations of its organization and functioning as a result of external agricultural inputs (organic or chemical) should be extremely considered to avoid unpredicted collateral effects for the cultivated crop (Lehmann et al. 2011).

OA have the ability to change soil characteristics such as nutrient availability (e.g., P, K, Fe, etc.), pH, nitrate content, organic matter, and soil structure (Ansari and Mahmood 2017). As these parameters are definitively modelling the structure of the microbial community of the soil, OA amendment into the soil will ultimately affect soil-resident microbiota and their activity. Montiel-Rozas et al. (2016) found an improvement in soil quality due to the increase in soil pH and a reduction of trace element availability thanks to the OA. Furthermore, the genetic diversity of the arbuscular mycorrhizal community increased, reaching the highest diversity degree at the maximum rate of biosolid compost. The forms of OA are wide but have not the same impact on soil biota. Indeed, the soil amendment of biochar as soil alternative fertilizer had little effect on soil microbial community and could be a sustainable phosphorus and potassium fertilizer while mitigating climate change by carbon sequestration (Winding et al. 2017). On the other hand, the same form of OA could have a different effect on soil biota if applied to different soil types. In fact, the application of municipal solid waste (MSW) has resulted in an increase in the clay loam soil microbial respiration than in the loamy sand soil. On the other hand, the opposite was found for alfalfa residue amendments (AR) (Yazdanpanah et al. 2016). The application frequency of OA also plays a vital role in its impact on soil biota.

Taking into consideration the application duration of OA on a specific soil, results showed that 1-year amendment of biochar did not alleviate nor additionally lighten the negative effects of previous agricultural management (Pressler et al. 2017). At a medium term (3 years), microbial abundance has doubled after amendment of corn biochar, at its highest addition rate (30 $T.ha^{-1}.year^{-1}$). Meanwhile, mesofauna activity that facilitates litter decomposition was not improved considerably but was positively affected by biochar amendment when these patterns were modelled. Additionally, in short-term lab-scale experiments, biochar mixing with litter increased NO_3 and NO_2 mineralization and decreased the concentration of Cl and SO_4 after the addition of litter. However and at the field scale, those nutrient effects were not revealed to be significant, where only some important increases in soil organic carbon, Cl, pH, and PO_4 were observed. Thus, there has been no negative effect after testing alkaline biochar for 3 years, on soil microbial activities and assessed functions. Although this finding should to be confirmed for other soils and biochar types (Domene et al. 2014), according to Diacono and Montemurro (2010), the long-term application of OA had led to the following major points:

- Many effects should be referred to long-term trials because of potential toxic element accumulation.
- Repeated application of OA to cropland led to an enhancement in soil microbial activities.
- Long-term amendment of OA increased up to 90% of organic carbon as compared to non-amended soil and doubled organic carbon content compared to intensive treatment.
- Continuous addition of OA especially composts improved aggregate stability and decreased soil bulk density without risk of heavy metal accumulation that improve soil physical fertility.
- The best agronomic performance of compost is generally achieved with the highest application doses and frequency. More beneficial effects such as nitrogen slow release were also reported coping with its leaching.
- An increase by up to 250% has been reported through long-term applications of municipal solid wastes at high rates.

Soil carbon sequestration is enhanced by organic amendments which has a tremendous role in climate change mitigation. In summary, there is a large consensus that OA can enhance structures of microbial communities and that these fluctuations can last for a long time. However, the actual significant influence of each OA component is still to be unraveled (Ruano-Rosa and Mercado-Blanco 2015).

8.3 Organic Amendment Suppressiveness Against Soilborne Fungi

Soil-living community contains macro-, meso-, and microorganisms in a large extent. Most of them are plant pathogens like phytoplasmas, viruses, nematodes, parasitic phanerogams, protozoa, fungi, and bacteria. Oomycetes and fungi are probably the most important groups of telluric pathogens because of their diversity, number, and production losses caused by their infestations (Garcia-Jimenez et al. 2010). Various control strategies have been suggested to control soilborne pathogens, such as bio-fumigants, crop rotation, grafting on resistant rootstocks, use of resistant cultivars, decontamination of plant materials (Hsu 1991), biological soil disinfestation (Messiha et al. 2007), and microbial antagonists (Lemessa and Zeller 2007; Messiha et al. 2007). The amendment of manure and compost is considered as a fundamental component of organic production along with cover crops to provide plant nutrients, improve soil quality and structure, and increase natural suppressiveness of the soil against telluric pathogens (Russo and Webber 2007; Janvier et al. 2007).

8.4 Composts and Vermicomposts

In general, addition of composts and vermicomposts significantly enhanced microbial activity, microbial biomass, and the microbial diversity of the growing media. In fact, Huang et al. (2012) have found that cucumber *Fusarium* wilt (*F. oxysporum* f. sp. *cucumerinum*) was effectively suppressed in sludge compost-amended media (peat: compost in a 4:1 ratio [v/v]), whereas pig manure compost didn’t produce the same disease suppression effect. The compost of pig manure contains ammonia significantly higher than that of sludge compost. That might explain its low aptitude of disease suppression. In addition, Ventorino et al. (2016) revealed that the addition of chestnut green waste compost to the peat-based growth substrates exhibited a considerable reduction of 70% or 51% of disease in tomato plants compared to pure peat substrate, respectively, in the presence of *Sclerotinia minor* or *Rhizoctonia solani*. Moreover, reduction of soil suppressiveness quantum against *Fusarium oxysporum* f. sp. *melonis* has been studied after 1 year of solarization. In solarized soil, *Fusarium* disease severity inoculated artificially to melon plants was higher as compared to non-solarized soil. The addition of compost (15% wheat straw and made of 85% cattle manure on a dry weight basis) significantly reduced the disease severity in solarized as well as non-solarized soils. Nevertheless, total control was not reached after solarization, where compost antagonistic effect was clearly apparent on melon plants at this time (Kanaan et al. 2017).

Vermicompost (e.g., by-product of the ingestion of cow manure by *Eisenia fetida* earthworms) amendments (rate of 3 tons.ha^{-1}) exhibited an improvement in the relative abundance of Ascomycota, Sordariomycetes, Chytridiomycota, Eurotiomycetes, and Saccharomycetes and a decrease in Glomeromycota, Zygomycota, Dothideomycetes, and Agaricomycetes. Vermicompost amendment significantly enhanced the relative abundance of antagonistic fungi and reduced those of pathogenic fungi mainly *F. oxysporum*, *F. solani*, *R. vagum*, and *Cladosporium* sp., as compared to the organic fertilizer treatment (Zhao et al. 2017). Furthermore, research has led to the isolation of three bacteria isolates (VBI-4, VBI-19, and VBI-23) from herbal vermicomposts, which were screened for their suppressive ability to control telluric fungal pathogens of chickpea. The control was effective through the total colonization of chickpea rhizosphera and thus preventing fungal pathogens of chickpea (Sreevidya and Gopalakrishnan 2017). Vermicompost has been proven an efficient antagonist agent against *Fusarium oxysporum* and *Phytophthora infestans* on *Thymus vulgaris*. Vermicompost suppressive effect was improved gradually with increasing doses of vermicompost (0, 25, 50, and 75%), with the highest effectiveness obtained with 75% vermicompost rate. β-1,3-Glucanase, polyphenol oxidase and peroxidase, phenylalanine ammonia lyase, and total phenolic content are some of the defense-related enzymes that were correlated to the development of plant disease protection activities. These results advocate the potential of vermicompost in increasing plant yield along with inducing self-defense in *Thymus vulgaris* (Amooaghaie and Golmohammadi 2017).

Matured composts are already valuable even without microbial inoculation, but research results showed that using microbial enrichment in compost seems to enhance its efficiency. Composted manure (as compared to uncomposted one) has a long-term effect in building soil fertility and has been proved more effective in building soil microbial biodiversity and increasing soil enzymatic activity. Similarly, amendments of large quantities of OA were found to create anaerobic conditions that resulted in the reduction of inoculums of plant pathogenic fungi like *Fusarium oxysporum* f. sp. *Asparagi*, *R. solani*, and *V. dahliae* (Shafique et al. 2016). Composts issued from different raw organic wastes present different qualities and stability degrees, which depend closely upon the composition and proportion of raw material that would be composted. The wide range of biological and chemical fluctuations that happen during composting, and the different procedures suggested in literature, has increased the difficulty to come out with simple and practical assessment method of maturity (Azim et al. 2017b). Compost suppressiveness should then be evaluated in case-to-case basis to avoid biased extrapolation and disease dissemination.

8.4.1 Biochar

Biochar is known for its quality as a carbon sequestration approach, soil fertility improvement, and ameliorating crop performance, which can affect foliar and soil-borne phytopathogens. In fact, biochar application suppressed tomato root rot and *Fusarium* crown and at the same time improved tomato cultivation at growth and physiological level. Furthermore, *Fusarium* root colonization was reduced (reduction in CFU by up to 85% at $P < 0.005$) along with its survival in soil and increased the abundance of numerous antagonists and plant growth-promoting microorganisms (PGP). Gene sequencing with 16S rRNA has identified potential antagonistic strains and showed the highest similarity (i.e., 98–99%) to *Pseudomonas fluorescens*, *P. koreensis*, *P. putida*, *P. moraviensis,* and *P. monteilii*, all of which have been formerly identified as PGP in addition to their biocontrol effects. High microbial taxonomic and functional diversity may jointly explain the significant decrease of disease and enhancement in plant health performance observed after application of biochar (Jaiswal et al. 2017). In a contrasting study, results showed that biochars had considerably contrasted effects on root rot caused by *Fusarium virguliforme*, as 75% of tested biochars were not significantly decreasing root rot severity. Furthermore, the study concluded that there is no proof that biochar applications are able to neither induce soybean plants' systemic resistance nor increase its grain yield (Rogovska et al. 2017). Another experiment studied the impacts of rice husk (RH) and biochar from rice husk (RHB) on root rot (caused by *Cylindrocarpon destructans* and *Fusarium solani*) of ginseng (*Panax* ginseng) and on soil microorganisms. RHB application did not reduce the incidence of root rot disease as compared to RH both amended with 5.2 $\mathrm{Mg.ha^{-1}}$ (Eo et al. 2017). However, biochar reduced *Fusarium* root rot by increasing arbuscular mycorrhizal colonization and reducing allelopathic residue (Elmer and Pignatello 2011). In order to illuminate mechanisms promoting "biochar effect," a study systematically monitored the development and the resistance of tomato plant to the foliar fungal pathogen (*Botrytis cinerea)*. The study tested non-amended soils as a control and biochar-amended soil using local biochar and washed biochar. The outcomes revealed that the "biochar effect" is at least partially elicited by the improvement of diversity and changes in metabolic parameters in the rhizosphera microbiome. This change is mostly triggered by the recalcitrant carbon core of the biochar and strongly bound compounds of organic matter. It comes to the emerging consensus that soil amendments, which enhance soil microbial diversity, have significant benefits to soil ecosystem functioning (Kolton et al. 2017). Consequently, biochar amendment has an indirect effect on soilborne fungus by increasing the biota diversity and providing carbon-feeding materials to antagonists that foster enzymatic activities.

8.4.2 Oil Cake Amendments

Alongside a large variety of OA that have been experienced for managing phytopathogens, oilseed cakes can also decrease the population of soilborne pathogens (Sharma et al., 1995). Shafique et al. (2015) have tested cotton cake enrichment (with *Pseudomonas aeruginosa* and *Paecilomyces lilacinus*) for suppressing the root rot fungi and improving the antioxidant status and stimulating the synthesis of polyphenols in okra. Enriched cotton cake significantly eliminated *Macrophomina phaseolina*, *Fusarium solani*, and *Fusarium oxysporum* with full suppression of *Rhizoctonia solani*. Soil amended with cotton cake combined with biocontrol agents revealed highly positive effect on polyphenol and antioxidant activity of okra. In another experiment, effects of soil amendments with oilseed cakes were considered using mustard (*Brassica campestris* L.), almond (*Prunus amygdalus* L.), cotton (*Gossypium hirsutum* L.), and black seed (*Nigella sativa* L.) cakes at a dose of 0.1 and 1% w/w (soil) enriched with antagonists (*Trichoderma harzianum* and *Rhizobium meliloti*). The results indicated that mutual effect of bio-priming with *T. harzianum* spore suspension to seeds at nursery level, and soil amendment with mustard cake (rate 1% w/w) was the most effective for the growing of both legumes and non-legume crops (peanut, okra, chickpea, and sunflower). It was found also suppressive against root rot fungi such as *Macrophomina phaseolina* and *Fusarium* spp. (Rafi et al. 2016). Pandey et al. (2005) led a pot experimentation under greenhouse to study the effect of the application of *Trichoderma viride* and neem oil cake to the soil, in the management of root-knot nematode and *Fusarium* wilt infesting chickpea. Results showed that the wilt of chickpea was better controlled, and there was an increased in the yield and yield contributing parameters. A further experiment has revealed that a mixture of mustard oil cake, wheat bran, and khesari bran was a suitable solid substrate for mass production of *T. harzianum* for controlling the seedling mortality of lentil caused by *S. rolfsii* Sacc. and *F. oxysporum* Schlecht (Faruk and Rahman 2016). The results of triennial experiment showed that *T. harzianum* biofungicides presented in five diverse formulations of substrates among them mustard oil cake were similarly effective to control soilborne seedling disease (*Rhizoctonia solani*) of brinjal in tray soil and seedbed condition (Faruk and Rahman 2017). In cotton crop (*Gossypium hirsutum* L.), neem cake (*Azadirachta indica*) and endophytic bacteria *Pseudomonas aeruginosa* (PGPR) were used to induce systemic resistance, under saline limiting conditions and fungal infection. The biocontrol agent combination with OA induced fungal infection tolerance against *Macrophomina phaseolina* and salinity stress (EC = 17.3 dS.m^{-1}). It has been revealed in field experiment that there was a production of salicylic acid 6.9–8.6 mg.mL^{-1} as compared to the control (2.8–4.6 mg.mL^{-1}) in control plants, while polyphenols were found with 3.1–3.7 mg.mL^{-1} in neem cake + plants treated with bacteria as compared to 2.7–2.9 mg.mL^{-1} found in control plants. Inoculated cotton plants with *M. phaseolina* showed maximum disease severity in control treatment (75%), while only 37.5% of plants were found infested in the treatment with neem cake + *P. aeruginosa* in field experiment (Rahman et al. 2016).

Because of previous findings, it clearly appears that the amendment of soil with oil cake is more used as a medium (through artificial inoculation) of biological antagonists. It aims to enhance their density to achieve higher soil stability level for successful control of soilborne pathogens. Indeed, the native biological antagonists usually persist in low population density at the agricultural soil level. Therefore, low-cost substrates such as oil cake that are locally accessible could definitely be used as substrates to stimulate biological antagonists in the agricultural soil.

8.4.3 Green Manure

Although manure is mainly used for soil fertility purpose, it may also enclose microbial pathogens that constitute a threat to plant and human health (Bradford et al. 2013). Generally, the fungal biodiversity of manure is poorer than in the soil, and both abundance and uniformity are lowest in cow manure. This low level could be due to the difference in the carbon structure of the different components of the organic material (Sun et al. 2016). On the other hand, green manure was largely used as an eco-friendly practice in organic agriculture. The application of green manure or making cover crop as a part of crop rotation will deliver reasonable management of soilborne fungi, along with improving population of soil antagonists, which will activate suppressiveness of agricultural soils (Mawar and Lodha 2015). Fall-planted green manure, *Trifolium incarnatum* or *Vicia villosa*, incorporated in spring as cover crops can suppress *Fusarium* wilt (*Fusarium oxysporum* f. sp. *niveum*) of watermelon (Himmelstein et al. 2016). A further was performed to evaluate the effects of diverse soil managements, like soil amendment forms and application frequencies, on fungistasis. Results showed that frequent applications of OA decreased fungistasis relief and reduced the time required for fungistasis rebuilding regardless of the fungal species and OA forms. This study confirmed that regular amendment of green manure influenced soil fungistasis probably because of higher abundance of microorganisms (Bonanomi et al. 2017). Seed meals from Brassicaceae (SM) were evaluated on wheat to suppress soil charcoal rot (*Macrophomina phaseolina*) of strawberry and as well to study the contribution of seed meal cakes on the soil physicochemical parameters and soil biology in the disease control. Results showed that SM amendments suppressed *M. phaseolina* identified in soil systems. This finding suggests that there is need of functional soil biology to result in optimal SM-induced pathogen suppression. Indeed, observed disease control in natural soil conditions was eliminated after artificial pasteurization of soil amended with SM prior to be infested with *M. phaseolina*. This suggests a functional role of soil microbial biology in phytopathogen suppressiveness (Mazzola et al. 2017). Additional study evaluated five treatments, *B. carinata* seed meal, *Brassica juncea* seed meal, *Sinapis alba* seed meal, and *B. juncea* green manure in comparison with fumigation using methyl bromide/chloropicrin in addition to a non-treated control. The objective was to evaluate the five treatments on potential phytopathogens and antagonistic microorganisms, soil health, and conifer seedling (*Pseudotsuga menziesii*) in nursery fields. Results indicated that treatment with *S. alba* improved soil densities of *Fusarium* spp., while its density was significantly reduced after *B. juncea* green manure application [1.8 log CFU (colony-forming units)] than after chemical fumigation (2.4 log CFU) or in the non-treated control (2.6 log CFU). The soil population of *Trichoderma* spp. was significantly higher in fumigated plots (3.7 log CFU) followed by green manure of *B. juncea* (3.4 log CFU) and obviously lowest in non-treated control (3.2 log CFU). Soil microbial activity of dehydrogenase was higher when treated with *B. juncea* and lower in fumigated soil. These results suggested that green manure of *B. juncea* might have a suppressive effect on phytopathogens that preserve and improve soil and crop health (Paudel et al. 2016). It can be concluded that green manure has a potential effect on controlling of soil-borne fungi through its fumigant effect by releasing toxic molecules and increasing dehydrogenase activity within the soil. However, it could have adverse impact on soil antagonists such as *Trichoderma* spp. In contrast, organic farmers should be aware of the green manure nature in order to avoid allelopathy effect on the crop.

8.5 Organic Amendment Suppressiveness Against Phytopathogenic Bacteria

8.5.1 Composts and Vermicomposts

Bacterial wilt disease causes increasingly high-yield losses in recent years. Therefore, some approaches have been developed to suppress bacterial wilt, such as tolerant and resistant varieties, GMO-resistant rootstocks, and grafting technique. Nevertheless, significant achievement has been realized due to developed survival ability in complex biospheres, wide host species, and broad geographical dispersal and genetic biodiversity of bacterial wilts (King et al. 2008). In addition, composting and vermicomposting are considered as waste recycling approaches for agricultural organic wastes. On the other hand, the quality of compost or vermicompost is not convenient with regard to suppressive effect because of the presence of remaining bacterial pathogenic substances (Soobhany et al. 2017). Besides roots and soils, beneficial microorganisms for agriculture can also be isolated from compost that contains substrates from various sources of nitrogen and carbon that favor the growth of various groups of microorganisms. The disease control ability of compost is mainly facilitated by the activities of beneficial microorganisms (Stella and Sashikala 2016).

Biofertilizer (compost of pig manure 24.24% and rice straw 75.75% w/w) was used to control tobacco bacterial wilt (*Ralstonia solanacearum*) along with enrichment with antagonists, *Streptomyces rochei* (L-9) and *Brevibacillus brevis* (L-25). The control effectiveness of *R. solanacearum* was 95.4% and 30.0% in two different field sites after application of biofertilizer containing L-25 and L-9 to the soil in field condition. On the other hand, densities of *R. solanacearum* in the rhizosphera were much lower than the control rhizosphera in the soil applied with antagonists only. As a result, the antagonists were more efficient when they were used with biofertilizer as compared with the application of antagonistic strains alone. That led to suggest that the combination between the antagonistic strains L-25 and L-9 with biofertilizer can successfully control bacterial wilt through the soil microbial structure modification (Liu et al. 2013). Recently, Liu et al. (2016) have tested soil compost products (SCP) on bacterial wilt of tomato *Ralstonia solanacearum*. Compared to the control, application of SCP at rate of 15 tons.ha^{-1} decreased the incidence of the disease by 32–81% and improved fruit yields by 59–95% for three consecutive trials. It has been found that there is a significant correlation between *R. solanacearum* populations and soil organic carbon, total nitrogen, and N-NO^{3-} contents. Additionally, the bacterial wilt severity was found to be negatively correlated with the β-d-glucosidase and phosphomonoesterase activity, FDA hydrolysis, microbial biomass carbon, soil respiration, and diversity of bacterial community. On the other hand, a positive correlation was found with diversity of fungal community. The results showed that the improvement of the suppressiveness ability of soils against *R. solanacearum* by application of SCP was probably owed to the modified structure of microbial community and increased antagonistic microorganism's competitiveness with phytopathogens. Further in vivo experiment has tested mushroom compost which was inoculated with *Bacillus amyloliquefaciens* (IUMC7). Results showed that the compost enriched with IUMC7 considerably decreased severity of the disease in tomato plants as compared to the control soils. This result was confirmed with the *R. solanacearum* population decreasing after inoculation of soil with IUMC7 even without compost. A TLC-bioautography assay indicated that possibly a lipopeptide similar to "iturin" which was produced by IUMC7 as antimicrobial substances might be responsible for *R. solanacearum* control. These results lead to conclude that these bioactive substances are the origin of the control of the disease and that compost made of mushroom and enriched with IUMC7 has antimicrobial activity potentiality and could be evolved in a biocontrol product (Sotoyama et al. 2017).

Vermicompost enriched by a promising biocontrol agent *Bacillus subtilis* "IIHR BS-2" was assessed against a disease complex of nematode-bacterium (*Meloidogyne incognita-Pectobacterium carotovorum* subsp. *carotovorumin*) on carrot crop under field conditions. Soil amendment of vermicompost (2 tons.ha^{-1}) enriched with *B. subtilis* (5 liter.ha^{-1}) along with seed treatment resulted in the maximum carrot yield improvement by 28.8% nematode population and disease incidence reduction by 69.3% and 70.2%, respectively (Rao et al. 2017). Application of the inoculated substrate combined with seed and root treatment, soil amendment at transplanting, and soil amendment at 30 days after transplanting revealed drastic decrease in the incidence of wilt and results in tomato brinjal and chilli maximum yield. Maximum disease control was achieved using bioformulation based on vermicompost enriched with *P. fluorescens* (81.85%) followed by mustard oil cake (MOC) enriched with *T. viride* (79.07%). Maximum yield was obtained in tomato (36.0 t/ha), when the crop was amended with vermicompost enriched with *P. fluorescens* followed by MOC enriched with *T. viride* (33.35 t.ha^{-1}). On the other hand, maximum similarity was obtained for yield of brinjal (27.60 t.ha^{-1}) and chilli (26.30 t.ha^{-1}) when the crops were amended with bioformulation of vermicompost-based *P. fluorescens* (Bora et al. 2016). Vermicompost (VC) application has been tested to control bacterial blight disease (BBD) (*Xanthomonas axonopodis* pv. *punicae*) on leaves against control. Results showed that VC was not effective compared to the untreated plants to control BBD even while resulting in maximum plant height of 140.0 cm, plant spread with 127.9 cm, and chlorophyll content of 61.4 SPAD value (Marathe et al. 2017).

The suppressive effect of compost and vermicompost is predominantly depends on biological properties of soil. Many antagonistic microorganisms secrete metabolites and enzymes in plant rhizosphere, which are eventually absorbed and translocated to plant system. As a result, these bioactive metabolites convey systemic resistance to plant, thus decreasing crop losses caused by aerial pathogens (Joshi et al. 2009). Several scientific findings have suggested that compost (Margareth and Mangkoedihardjo 2010) or vermicompost (Gopinathan and Prakash 2014) can be an excellent substrate carrier for microbial antagonist's bio-inoculants. It has to be validated and confirmed through more experimental trials through microorganisms' multiplication and their mixing with the compost/vermicompost just before soil or field application.

8.5.2 Biochar

Biochar is broadly used as an amendment for agricultural soils, and it has been confirmed to exhibit positive impacts on soil health, plant growth, and nutrient retaining. Lately, a growing interest is observed on the opportunity of biochar to act as a controlling mean for phytopathogen suppression (Zhang et al. 2017). In a greenhouse experiment, incidence of bacterial wilt caused by *Ralstonia solanacearum* was assessed by two different biochar sizes of 53–120 μm and 380–830 μm on tomato crop. Results showed that biochar with fine size applied with 3% w:w has significantly reduced incidence of bacterial wilt by 19.9%. Moreover, fine biochar was efficient (91%) in pathogen adsorption, while both biochar with different sizes resulted in similar adsorption capacity for root exudates. The chemotaxis ability of pathogen was increased by root exudates and fine biochar, while the latest reduced pathogen swarming motility and rhizosphera colonization (Gu et al. 2016). In another trial, two (BC1 and BC2) different biochars, prepared, respectively, from peanut shell and wheat straw, were added (rate of 2% w/w) to soil infested with *Ralstonia solanacearum* in order to explore the interaction between bacterial wilt of tomato and biochar and microbial properties of the tested agricultural soil. The results revealed that both treatments considerably reduced disease index of bacterial wilt, respectively, by 28.6% and 65.7%. Soil populations of *R. solanacearum* were significantly reduced when biochar were amended. Infection by *R. solanacearum* in control treatment has considerably decreased natural bacteria and actinomycete densities and improved soil fungi/bacteria ratio. Additionally, higher microorganism's metabolic abilities enhanced by biochar amendment were detected at 96 and 144 h in Biolog EcoPlates. Subsequently, there could be a link among plant resistances to the disease and the fluctuations occurred in density and activity soil microbial populations (Lu et al. 2016). Under field conditions, rice straw biochar was evaluated to control *R. solanacearum* the causing agent of tobacco bacterial wilt.

The results revealed that biochar application with a rate of 3 ton.ha^{-1} led to reduction of 76.64% in the disease incidence and 73.87% drop in the disease severity. Particularly, the soil abundance of *R. solanacearum* was reduced by 94.51% and then reducing the causing agent of tobacco bacterial wilt. Statistics suggest that enhanced soil fertility (physical and chemical properties) and improved diversity and bacterial richness increase the biochar ability to manage the bacterial wilt tobacco (Zhang et al. 2017).

In most cases, biochar formulated in rates higher than 25% (v:v) has positive/neutral effects on plant growth as compared to peat media. Nevertheless, studies done on biochar effect on phytopathogens showed that lower rates (≤1%) of biochar suppressed often some diseases, but with higher rates (≥3%), biochar was generally ineffective and induced incidence of plant diseases. In order to use biochar as horticultural peat substitute, biochar raw materials and rates should be optimized so that application of biochar can be suggested at large scale levels (Frenkel et al. 2017). As a conclusion, there are five different potential approaches suggested explaining biochar suppression of plant diseases:

1. Systemic resistance induction in host plants through bioactive translocated metabolites.
2. Enhanced biodiversity of beneficial microbes such as mycorrhizal fungi.
3. Soil fertility modification in terms of bioavailability of nutrient and abiotic factors like liming effect.
4. Biochar direct fungi-toxic effect.
5. Indirect effects through allelopathic and phytotoxic compounds sorption which can directly harm plant rhizosphera and therefore stimulate pathogen occurrences.

Moreover, the possibility of absorbing agrochemicals like insecticides, herbicides, and fungicides has been reported as potential side effects of biochar, thus reducing their efficacy (Ippolito 2016).

8.5.3 Oil Cake Amendment

Amendments of soil with plant based products such as neem, castor, mahua, linseed, and mustard in the oil cakes forming along with dry leaves, seed kernel, seeds, seed coat, and seed powder are commonly used against soilborne pest and diseases (Rizvi and Mahmood 2017). Microbe-hydrolyzed rapeseed cake was used successfully to control tobacco bacterial wilt (*Ralstonia solanacearum*) along with enrichment with antagonists, *Brevibacillus brevis* and *Streptomyces rochei* (Liu et al. 2013). Bioformulation of mustard oil cake (MOC) mixed with virulent cells of biocontrol agents such as *Pseudomonas fluorescens*, *Trichoderma viride*, and *Bacillus subtilis* exhibited promising results in the control incidence of bacterial wilt (*Ralstonia solanacearum*) in tomato, chilli, and brinjal cultivations (Bora et al.

2016). In situ green manuring (GM) using sunn hemp (*Crotalaria juncea* L.) and ex situ GM using Gliricidia (*Gliricidia sepium*), karanj (*Pongamia pinnata*), and neem (*Azadirachta indica*) was tested to control bacterial blight disease (BBD) caused by *Xanthomonas axonopodis* pv. *punicae* on pomegranate. Results indicated that neem GM was an effective control means, recording the lowest incidence of BBD on leaves and fruits with, respectively, 12.8% and 9.5%. Results on BBD index showed that its incidence was higher in sunn hemp (in situ) GM followed by inorganic fertilizer. The disease incidence was lowest in plots treated by neem GM followed by Gliricidia GM. It can be concluded that neem GM could be suggested for effective production of organic pomegranate and controlling of BBD (Marathe et al. 2017).

The improved efficacy of OA in the form of oil cakes mixed with inorganic fertilizers might be due to the fact that application of OA provides some nutrients such as zinc, copper, iron, manganese, etc to the plants. These elements are required in plant metabolism in the early growing phase of the plants. OM like oil cakes act as a nutrient pool, and upon mineralization, important quantities of organic compounds are slowly released in the soil to be absorbed by crop roots in its ionic forms leading to improved plant health (Rizvi and Mahmood 2017).

8.5.4 Manures

Animal manures are regularly amended to agricultural lands to improve crop yield, but the susceptibility to spread microbial phytopathogens during field amendment has not been yet fully considered. A survey was realized to a collection of 49 cattle, horse, swine, sheep, or chicken manure samples in 14 Polish rural circles for monitoring the most important plant pathogenic bacteria – *Ralstonia solanacearum* (Rsol), *Xanthomonas campestris* pv. *campestris* (Xcc), *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc), *Pectobacterium atrosepticum* (Pba), *Erwinia amylovora*, *Clavibacter michiganensis* subsp. s*epedonicus*, and *Dickeya* sp. All tested animal fertilizers were free of these pathogens. Subsequently, the growth dynamics of Pba, Pcc, Rsol, and Xcc in cattle, horse, swine, sheep, and chicken manures sterilized either by autoclaving or filtration was evaluated. Investigated phytopathogens did not reveal any growth in the poultry manure. However, the manure filtrates originating from other animals were suitable for microbial growth, resulting in optical density change ranging 0.03 to 0.22, depending on bacterial species and the manure source. Pcc and Pba were multiplied most efficiently in the cattle manure filtrate. These bacteria grew faster than Rsol and Xcc in all tested manure samples, both filtrates and the autoclaved semisolid ones. Though the growth dynamics of investigated strains in different animal fertilizers was unequal, all tested bacterial plant pathogens were proven to use cattle, horse, swine, and sheep manures as the sources of nutrients (Sledz et al. 2017).

8.6 Conclusion and Further Prospects

Organic farming has considerably been developed recently. It is clear that pesticides and chemical fertilizers can lead to temporary good yield and result good income to farmers at the cost of health risks and environmental pollution. However, OA can achieve similar yields as conventional and descent farmer income under good management practices while managing biotic stress efficiently. For their effective management, it is essential to choose the suitable combination of available methods. Finally, it is concluded that OA have great potential to be explored for the management of biotic stress on cash crops, stimulate the development of soil microorganism's population, and decrease phytopathogens as well as improving soil fertility, such as physical parameters, water retention, permeability, water infiltration, aeration, and plant growth. However, before application a thorough understanding of the mechanisms are needed which may unravel various important information pertaining to optimization of organics. These OA are locally accessible and eco-friendly and help in maintaining good soil health but also sequester atmospheric carbon into soil and thus combat climate change impact. It can be concluded that there is a need to integrate approach to phytopathogen management and that multidisciplinary research is required to solve persistent plant disease problems, particularly for organic farming.

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Chapter 9

Eco-friendly Approaches to the Management of Plant-Parasitic Nematodes

**Everaldo Antônio Lopes, Rosangela Dallemole-Giaretta,
Wânia dos Santos Neves, Douglas Ferreira Parreira,
and Paulo Afonso Ferreira**

Abstract Eco-friendly approaches have been increasingly used for the management of plant-parasitic nematodes because of growing worldwide concern regarding health risks and environmental contamination caused by nematicides. Avoiding the introduction and spread of nematodes to non-infested areas is the most efficient method of control. Cleaning machinery and equipment, use of healthy planting materials, and quarantine procedures are good examples of preventive practices. In infested fields, nematode populations can be reduced by combining cultural, physical, and biological methods and genetic resistance of plants. The use of resistant crops is one of the most efficient and eco-friendly methods for reducing losses caused by plant-parasitic nematodes. Based on the information on which nematode species/races are prevalent in the field, the grower should choose a resistant crop, when available. Soil plowing and irrigation – named humid fallow – have been used for the management of root-knot nematodes in common bean (*Phaseolus vulgaris*), lettuce (*Lactuca sativa*), and okra (*Abelmoschus esculentus*) in Brazil. Soil steaming, treatment of planting materials with hot water, and soil solarization are recommended for the control of several plant-parasitic nematode species, based on the lethal action of high temperatures. Biofumigation with residues from some species

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E. A. Lopes (✉)
Instituto de Ciências Agrárias, Universidade Federal de Viçosa, Rio Paranaíba, MG, Brazil
e-mail: everaldolopes@ufv.br

R. Dallemole-Giaretta
Universidade Tecnológica Federal do Paraná, Pato Branco, PR, Brazil

W. dos Santos Neves
Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), EPAMIG Sudeste,
Viçosa, MG, Brazil

D. F. Parreira
Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG, Brazil

P. A. Ferreira
Universidade Federal de Mato Grosso, Barra do Garças, MT, Brazil

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of Brassicaceae and manures releases volatile toxic gases during the degradation process of the organic matter, including isothiocyanates. Non-host or antagonistic plants are also important tools for the integrated management of nematodes. In this context, marigolds (*Tagetes erecta* and *T. patula*), crotalaria (*Crotalaria spectabilis*), sunn hemp (*Crotalaria juncea*), and velvet bean (*Mucuna pruriens*) are widely used as antagonistic plants. Soil amendment with crop residues of neem (*Azadirachta indica*), castor bean (*Ricinus communis*), velvet bean (*Mucuna pruriens*), crotalaria (*Crotalaria spectabilis*), and *Brassica* spp.; oil seed cakes of neem, castor bean, mustard, and sesame; cattle manure; poultry litter; liquid swine manure; and crab shells release nematotoxic substances during decomposition, provide nutrients to the plants, and increase the population of biocontrol agents. More than 200 species of nematode antagonists have been identified, including fungi, bacteria, nematodes, tardigrades, and collemboles. Fungi and bacteria are the most studied and commercially exploited organisms for nematode control. Several commercial bionematicides have been developed from the nematode-trapping fungi *Arthrobotrys*, *Dactylaria*, *Dactylella*, and *Monacrosporium*, the egg-parasitic fungi *Purpureocillium lilacinum* and *Pochonia chlamydosporia*, the antibiotic bacterium *Bacillus* species, and the obligate parasite bacterium *Pasteuria* spp. The anaerobic soil disinfestation is an ecological alternative to soil fumigation for the control of several soilborne pathogens, including nematodes. This technique consists of incorporating organic material that is easily decomposable (C/N ratio from 8 to 20:1) into the soil, irrigating to saturation, and covering the soil with oxygen-impermeable plastic. Accumulation of toxic products from anaerobic decomposition, antagonism by anaerobic organisms, lack of oxygen, and the combination of all of them are the main drivers that explain the efficacy of anaerobic soil disinfestation. Consumers have been demanding higher food security and environmental quality, and this situation will not be different in the future. In this context, scientists' efforts in discovering new nonchemical strategies for nematode control and improvements in the current methods must be continuous.

Keywords Cyst nematode · Lesion nematode · Nematode control · Root-knot nematode · Sustainable agriculture · Sustainable management

9.1 Introduction

Over 4100 species of nematodes parasitize cash and subsistence crops in all continents (Decraemer and Hunt 2006). Losses caused by nematodes in agriculture are estimated to be between US$78 and 125 billion per year (Sasser and Freckman 1987; Nicol et al. 2011). They can cause direct damage to their host and facilitate subsequent infestation by secondary pathogens; besides, some nematodes are vectors of plant viruses (Nicol et al. 2011; Lopes and Ferraz 2016). Most plant-parasitic nematode species spend all their life-span in soil, feeding on host roots (Lopes and Ferraz 2016). Like other soilborne pathogens, nematodes are difficult to control. In general, nematodes are not eradicated from an infested field, and more than one control method is needed to reduce their population to levels that do not cause economic losses (Ferraz et al. 2010). Because of growing worldwide concern regarding health risks and environmental contamination caused by chemical pesticides, eco-friendly approaches have been increasingly used for the management of plant-parasitic nematodes instead of nematicides. Preventive practices; physical, biological, and cultural methods; and genetic resistance of plants are nonchemical strategies that can be used for nematode management, as will be shown in this chapter. All these strategies will be discussed separately here, although they should be applied as part of an integrated management system.

9.2 Preventive Practices

Avoiding the introduction and spread of nematodes to non-infested areas is the most efficient method of control. Cleaning machinery and equipment, use of healthy planting materials, and quarantine procedures are good examples of preventive practices.

Agricultural implements, machinery, vehicles, and tools can carry nematode-infested soil. In Brazil, infested soil adhered to machinery, equipments, and vehicles was the major driver for the dispersal of *Heterodera glycines* throughout soybean-growing areas (Silva 1999). The first reports of this nematode in Brazil date from 1991 to 1992 in six municipalities in the central region of the country. Five years later, the nematode was found in 98 municipalities, covering an area of two million hectares, including states in the South and Southeast (Silva 1999). To avoid nematode dispersal, farmers must use machinery and implements first in non-infested areas before they can be used in infested fields. Besides, soil must be washed off machinery, vehicles, tools, and implements right after the work in the field (Ferraz et al. 2010).

Long-distance dispersal of nematodes also occurs efficiently via planting materials, such as seeds, seedlings, cloves, tubers, cuttings, and rootstocks. *Anguina tritici*, *Aphelenchoides besseyi*, and *Ditylenchus dipsaci* are instances of nematodes that can survive longer than 10 years within seeds or cloves. Cysts of *H. glycines* also can be found mixed with soybean seeds. *Meloidogyne exigua*, *M. incognita*, *M. paranaensis*, and *M. coffeicola* have become widespread in coffee-growing areas in Brazil via infected seedlings. Thus, farmers must use only nematode-free planting materials.

Quarantine procedures are important to limit nematode spread to new areas. The list of major plant-parasitic nematodes of quarantine importance worldwide is led by the potato cyst nematode, *Globodera rostochiensis* and *G. pallida* (Lehman 2004). The exclusion of plants if accompanied by prohibited articles (soil, hay, straw, forest litter, etc.), the prohibition of all known host plants of nematodes that may represent risks for local agriculture, and the requirement of phytosanitary certificates are key actions to avoid the introduction of quarantine nematodes (Lehman 2004). For instance, South Africa excludes 270 hosts to indirectly exclude *Aphelenchoides* *besseyi*, *Ditylenchus dipsaci*, and *Radopholus similis* (Lehman 2004). In Minas Gerais state, which accounts for more than half of the coffee production in Brazil, the production, commercialization, and transit of coffee seedlings within the state are regulated to avoid dispersal of root-knot nematodes (Ferraz et al. 2010).

9.3 Clean Fallow

Plant-parasitic nematodes are biotrophs, and the longer host plants (crops, volunteer plants, or weeds) are absent from the soil, the lower is the survival of these nematodes in the soil. Weeds can be alternative hosts of nematodes (Rich et al. 2009; Godefroid et al. 2017), and they must be mechanically removed or killed by herbicides. This technique is most effective in the hot and dry summer months between crops (Sikora et al. 2005). However, soil erosion and the costs of keeping the soil free of weeds and crops limit the use of clean fallow.

9.4 Soil Plowing and Humid Fallow

High temperatures and low soil moisture cause desiccation of eggs and vermiform stages of nematodes. Most plant-parasitic nematodes are found up to 30 cm beneath the soil surface. For this reason, soil plowing at a depth of 30 cm during dry and warm seasons reduces nematode populations by exposing them to the deleterious effects of desiccation. Dutra and Campos (1998), for instance, reported the reduction of second-stage juveniles of *M. javanica* by more than 50% after soil plowing. The benefit of this operation is more pronounced when the field is left without any crop or weeds. However, the occurrence of erosion and soil disruption are among the main disadvantages of this approach.

Soil plowing and irrigation – called humid fallow – have been used in Brazil for the management of *M. incognita* in common bean (Dutra and Campos 2003a) and of *M. javanica* in okra (Dutra and Campos 2003b) and lettuce (Dutra et al. 2003). The second-stage juvenile (J_2) of root-knot nematode develops, hatches, and moves in the soil until it reaches a root of a host. Under favorable conditions of temperature and soil moisture, these events happen in about 14 days (Campos et al. 2005). Under adverse conditions, juveniles do not hatch, which ensures nematode survival. However, irrigating the soil to field capacity will stimulate J_2 to hatch if soil temperature is in the range of 21–30 °C. If the field is maintained without host plants for 2 weeks or longer, juveniles will consume much of their body reserves and will die of starvation (Van Gundy et al. 1967).

Irrigation and soil plowing must be done on hot and dry days (Campos et al. 2005). Plowing does not need to be deep, and irrigation must be enough to raise soil moisture to field capacity. In a common bean field infested with 60 J_2 of *M. incognita* per 100 cm^3 of soil, grain yield was four times higher in plots where humid fallow was used in comparison to non-plowed and non-irrigated plots (control) (Campos et al. 2005). Plowed and irrigated plots were maintained free of weeds for 14 days, when common bean was sown. The costs of this tactic were only 4% of those spent by applying the nematicide aldicarb (Campos et al. 2005).

9.5 Heat-Based Methods to Control Plant-Parasitic Nematodes

Most plant-parasitic nematodes die when exposed to soil temperatures exceeding 45–50 °C for 1 h or less (Tsang et al. 2003; Wang and McSorley 2008). Sublethal temperatures (38–45 °C) may also cause nematode death, but a longer exposure time is required (Wang and McSorley 2008). The lethal action of high temperatures is the core principle behind the efficiency of the use of steam, treatment of planting materials with hot water, and soil solarization in the control of plant-parasitic nematodes.

9.5.1 Steam

Soil steaming is used in several countries as an alternative for soil treatment in glasshouses, seed beds, and small areas (Ferraz et al. 2010; Marbán-Mendoza and Manzanilla-López 2012). Temperatures over 70 °C can be reached with this technique and can inactivate propagules of various pathogens, weeds, and insects, as well as part of the beneficial soil microbiota. One of the disadvantages of the method is the formation of phytotoxic substances in the heated soil, such as soluble salts, ammonia, and manganese (Ferraz et al. 2010). Ideally, a waiting period of 20–40 days is required before planting to eliminate phytotoxic compounds (Tihohod 1993). The costs of this method can also be a limitation on its use, including equipment, pipes, water, and fuel or electricity (Marbán-Mendoza and Manzanilla-López 2012).

9.5.2 Treatment of Planting Materials with Hot Water

The immersion of plant material (seeds, bulbs, cloves, seedlings, tubers, rootstocks) in hot water for a certain period may inactivate nematodes. The success of the treatment depends on the adjustment of the binomial water temperature-treatment time. High temperatures may kill nematodes but also damage plants. Thus, sublethal temperatures can be used for a longer period, without any damage to the plants. Immersion of plant materials into cold water prior to hot water treatment can reactivate quiescent juveniles and enhance the effect of the heat on nematodes. For instance, pre-soaking

Table 9.1 Examples of hot water treatments for the control of nematodes in planting materials

Crop	Planting material	Nematode	Temperature/time
Solanum tuberosum	Tuber	*Meloidogyne* spp.	46–47.5 °C/120 min
		Pratylenchus coffeae	52 °C/15–20 min or 53 °C/10–15 min
Vitis vinifera	Rootstock	*Meloidogyne* spp.	54.4 °C/3 min; 50 °C/10 min or 47.8 °C/30 min
		Xiphinema index	52 °C/5–10 min
Triticum aestivum	Seed	*Ditylenchus* sp.	54 °C/15 min
Musa spp.	Rhizome	*M. incognita; Helicotylenchus multicinctus; Pratylenchulus brachyurus; Radopholus similis*	55 °C/20 min
Citrus spp.	Rootstock	*Tylenchulus semipenetrans*	49 °C/10 min 45 °C/25 min
Dioscorea spp.	Tuber	*Meloidogyne* spp.	51 °C/30 min
		Scutellonema bradys	50–55 °C/40 min
Allium sativum	Clove	*D. dipsaci*	45 °C/20 min
Allium cepa	Bulb	*D. dipsaci*	44–45 °C/180 min

Adapted from Ferraz et al. (2010)

rice seeds in cold water for 18–24 h before immersing them in water at 51–53 °C for 15 min controls *Aphelenchoides besseyi* (Bridge and Starr 2007).

Results using this approach can vary, depending on the plant species and cultivar, nematode inoculum density, and the conditions of the treatment. Examples of recommended treatments for nematode management in planting materials are described in Table 9.1.

9.5.3 Soil Solarization

This technique consists of mulching a wet soil with transparent plastic film (50–200 μm thick) during periods of higher solar incidence. Lethal and sublethal temperatures can be reached in the first weeks of the treatment, inactivating nematodes (Table 9.2) and other soilborne pathogens, as well as insects and weeds (Katan and Gamliel 2011). Soil warming also can weaken plant pathogens and increase the population of biological control agents (Katan and Gamliel 2009).

The soil usually remains covered for 4–8 weeks (Katan and Gamliel 2011). The soil must be prepared by harrowing, plowing, and removing sharp objects. Then, the soil is irrigated to field capacity and covered with plastic. The water in the soil activates pathogen propagules and enhances heat conduction. The borders of the plastic should be buried to avoid heat loss.

Table 9.2 Control of plant-parasitic nematodes by soil solarization

Nematode	Crop	Time (days)
Meloidogyne javanica, *M. incognita*	Cucumber	35–60
M. incognita	Olive	21
Meloidogyne spp.	Tomato	21–60
M. javanica	Okra	139
M. javanica, R. reniformis, *Paratrichodorus minor*, *Mesocriconema* spp.	Tomato	32–42
Globodera rostochiensis	Potato	62–63
Meloidogyne spp.	Eggplant	30–60
Pratylenchus thornei	Chickpea	28–56
M. incognita, *M. javanica*	Pepper	45
R. reniformis	Lettuce, cowpea	28–56
P. thornei	Potato	31

Adapted from Ferraz et al. (2010)

In this method, the solar radiation is trapped under the plastic film and raises the temperature of superficial layers of the damp topsoil (up to 20 cm deep) (Katan and Gamliel 2011). During the warmest periods of the year, temperatures in solarizated soil usually range from 35 to 60 °C (DeVay 1991). However, the temperature and the efficiency of the control decrease with depth in soil profile (Katan and Gamliel 2011), which means that soil has to be kept covered for longer periods of time. The efficiency of solarization depends on the occurrence of high temperatures and high luminous intensity. In temperate regions or during cooler times of the year, this technique may not be efficient. The costs of plastic tarp can also limit its use in larger areas.

The thickness of the plastic tarp has no direct influence on the solarization efficiency (Katan and Gamliel 2009). The most used plastic films range from 50 to 150 μm. Thin films (25–30 μm) tend to tear easily. The thicker ones are more expensive; however, they can be reused (150–200 μm). Double layers of plastic can increase control efficiency, increasing soil temperature by more than 10 °C (Katan and Gamliel 2009), although the costs of treatment are also increased.

9.6 Biofumigation

The incorporation of certain organic amendments into the soil, especially residues from some species of Brassicaceae and manures, releases volatile toxic gases during the degradation process of the organic matter. The suppression of pests and pathogens by the release of biocide compounds into the soil is called "biofumigation," because of the microbial decomposition of organic amendments (Kirkegaard et al.

1998). The soil must have sufficient moisture for intense microbial activity and decomposition of organic amendments.

For better results from biofumigation, it is essential to prevent the escape of volatile toxic compounds from the soil. Therefore, the soil can be covered with transparent plastic immediately after crushing and incorporating the organic materials. Alternatively, superficial layers of soil may be compacted with rollers. Transparent plastic cover increases soil temperature and accelerates the degradation of the residues (Kirkegaard et al. 1998; Gamliel et al. 2000). Therefore, the association of biofumigation with solarization may have a synergistic effect on the control of nematodes, and the time that the soil remains covered may be reduced. Thicker plastics (100–150 μm) are recommended for use in biofumigation to avoid the occurrence of holes and the loss of volatile toxic substances. Increasing the population of biological control agents of nematodes is an additional benefit of biofumigation. For example, biofumigation with chicken manure controlled *M. incognita* in lettuce, and the rhizosphere of lettuce plants was rapidly colonized by species of *Bacillus* and *Pseudomonas* after removing soil cover (Gamliel and Stapleton 1993).

The residue of Brassicaceae (*Brassica* spp.) has been the most studied organic material for biofumigation, due to a range of toxic substances released during its decomposition. Brassica plants are rich in glucosinolates, which are hydrolyzed by myrosinase into degradation products, such as isothiocyanates and nitriles (Brown and Morra 1997). Glucosinolates are nontoxic compounds, but isothiocyanates are toxic to nematodes and other soilborne pathogens, such as *Fusarium oxysporum* f. sp. *lycopersici*, *Macrophomina phaseolina*, *Sclerotium rolfsii*, *Pythium ultimum*, and *Ralstonia solanacearum* (Stapleton et al. 1998; Njoroge et al. 2009; Bensen et al. 2009). Papaya seeds are also rich in glucosinolates, and amending soil with this material controls root-knot nematode (Neves et al. 2012). Other organic amendments can also be used in biofumigation for the management of nematodes, such as residues of neem (*Azadirachta indica*), castor bean (*Ricinus communis*), velvet bean (*Mucuna pruriens*), crotalaria (*Crotalaria* spp.), marigold (*Tagetes* spp.) (Gamliel et al. 2000), chicken litter (Leon et al. 2000), and cattle manure (Leon et al. 2001).

9.7 Crop Rotation and Antagonistic Plants

Non-host or antagonistic plants have been used to control nematodes for decades. Nematodes are unable to penetrate the roots of non-host plants. Antagonistic plants can limit nematode penetration by releasing repellent substances into the rhizosphere. Some plants allow nematodes to penetrate the roots, but they do not develop to adult stages. Examples of crops recommended for the control of soybean cyst nematode (*Heterodera glycines*), root-knot nematode (*Meloidogyne incognita* and *M. javanica*), reniform nematode (*Rotylenchulus reniformis*), and lesion nematode (*Pratylenchus brachyurus*) are presented in Table 9.3.

Table 9.3 Antagonistic and non-host plants of *Heterodera glycines* (Hg), *Meloidogyne javanica* (Mj), *Meloidogyne incognita* (Mi), *Rotylenchus reniformis* (Rr), and *Pratylenchus brachyurus* (Pb)

	Nematode				
Crop	Hg	Mj	Mi	Rr	Pb
Black oat	–	+	+	–	±
Pearl millet	–	±	+	–	±
Soybean	+	+	+	+	+
Brachiaria	–	–	–	–	+
Forage sorghum	–	±	±	–	+
Sunflower	–	+	+	–	±
Corn	–	±	+	–	+
Sorghum	–	±	+	–	+
Cotton	–	–	+	+	+
Sugarcane	–	+	+	–	+
Peanut	–	±	–	–	+
Common bean	+	+	+	+	+
Cowpea	+	+	±	±	+
Cassava	–	+	+	+	+
Rice	–	+	+	–	+
Crotalaria spectabilis	–	–	–	–	–
Crotalaria breviflora	–	–	–	–	–
Crotalaria juncea	–	±	±	–	+
Mucuna pruriens	–	±	±	–	+

Adapted from Inomoto and Asmus (2009). (+) Crop increases nematode population. (±) Variation in the response to nematode population. (−) Crop reduces nematode population

Crotalaria (*Crotalaria spectabilis*), sunn hemp (*Crotalaria juncea*), velvet bean (*Mucuna pruriens*), and marigolds (*Tagetes erecta* and *T. patula*) are widely recommended to reduce nematode populations in the soil. *Crotalaria* species and *M. pruriens* have the advantage of producing large amounts of N-rich biomass, acting as green manure, and increasing the soil population of biocontrol agents (Inomoto and Asmus 2014).

Care must to be taken in choosing cover crops or non-host plants to manage nematodes. Certain crops can suppress a prevalent nematode in the field, but they can allow the reproduction of other nematodes. For instance, cotton following soybean in a rotation system will reduce the *M. arenaria* population, but will favor the reproduction of *Pratylenchus brachyurus*, *Rotylenchus reniformis*, and *M. incognita* races 3 and 4 (Ferraz et al. 2010). Thus, local nematode species and their population levels in the field must be known before recommending crops for the management system.

The nematode population can be reduced by half after one cycle of a non-host plant. The population of *R. reniformis* declined from 1102 to 581 nematodes per 200 cm^3 of soil when rye was cultivated following cotton (Asmus and Ishimi 2009). Reproduction factors (RF) of the reniform nematode were about 0.4 and 0.18 in the first and second years of rotation with corn, respectively (Asmus and Richetti 2010). In the case of highly susceptible crops, the nematode population must be at low densities in the field to prevent significant losses. Then, longer periods of rotation may be needed. In the Alto Paranaiba region, a major vegetable production area in Brazil, forage grasses (*Brachiaria decumbens* and *B. ruziziensis*) are cultivated for 2 or 3 years in *Meloidogyne*-infested fields before growing carrots, potato, and red beet.

Host status for nematodes may vary across species within the same genus of plants or among cultivars from the same species. Borges et al. (2010) reported that black oat (*Avena strigosa*) was highly resistant to *P. brachyurus* (RF < 1.0), while Algerian oat (*A. byzantina*) and white oat (*A. sativa*) were susceptible to the nematode (RF from 1.93 to 2.63). However, none of these three types of oats were resistant to *M. incognita* (Borges et al. 2009). Thus, they are not recommended as cover crops in fields with mixed populations of *P. brachyurus* and *M. incognita*. In another study, silage sorghum cultivar BRS 601 was resistant to *M. javanica*, while the cultivars IPA 7301011, BRS 700, and BRS 701 were good hosts (Inomoto et al. 2008).

9.8 Organic Amendments

The nematicidal effect of various materials has been widely reported. Soil amendment with crop residues, animal manure, composts, cakes from oil pressing, chitinous wastes, and other organic materials can release nematotoxic substances during decomposition, increase the population of biocontrol agents, and provide nutrients to the plants. Examples of nematicidal organic amendments are crop residues of neem (*Azadirachta indica*), castor bean (*Ricinus communis*), velvet bean (*Mucuna pruriens*), crotalaria (*Crotalaria spectabilis*), and *Brassica* spp.; oil seed cakes of neem, castor bean, mustard, and sesame; cattle manure; poultry litter; liquid swine manure; and crab shells (Ferraz et al. 2010; Stirling 2014). The organic material added into the soil can act as a soil conditioner, improving biological, chemical, and physical properties of soil. As a result, plants tend to be more tolerant to nematodes (Hoitink and Fahy 1986; McSorley and Gallaher 1995; Ritzinger and McSorley 1998; Bridge 2000). The combination of soil amendment with crucifer residues or animal manures and solarization enhances nematode suppression (Gamliel et al. 2000; Ferraz et al. 2010).

In general, organic amendments with C/N ratio from 14 to 20/1 have nematicidal properties and do not limit plant development (Rodríguez-Kábana et al. 1987). Materials with C/N ratio below 12 can be phytotoxic, and above 23 they are nontoxic to nematodes (Rodríguez-Kábana et al. 1987).

The use of organic amendments can be limited by the amount required for nematode control, usually from 4 to 10 ton ha^{-1} (Rodríguez-Kábana et al. 1987). As pointed out by Marbán-Mendoza and Manzanilla-López (2012), high transport costs, the lack of large-scale manufacturing, and inconsistency in production parameters are other limitations of using organic amendments to manage plant-parasitic nematodes. Application of organics either individually or in consortium of different living organisms may act as soil conditioner leading to ameliorated plant health (Ansari and Mahmood 2017).

9.9 Biological Control

Natural enemies can suppress plant-parasitic nematodes in the soil. More than 200 species of nematode antagonists have been identified, including fungi, bacteria, nematodes, tardigrades, and collemboles (Stirling 2014). Fungi and bacteria are the most studied and commercially exploited organisms for nematode control (Table 9.4).

Experimental and commercial bioproducts based on the nematode-trapping fungi *Arthrobotrys*, *Dactylaria*, *Dactylella*, and *Monacrosporium* and the egg-parasitic fungi *Purpureocillium lilacinum* and *Pochonia chlamydosporia* have been produced for the control of nematodes in several countries (Stirling 2014). These fungi can survive saprophytically in soil, and they can be mass-produced using cheap materials (Stirling 2014).

In Brazil, a commercial bionematicide based on chlamydospores of *Pochonia chlamydosporia* has been used for the management of nematodes in banana (Freitas et al. 2009), carrot (Bontempo et al. 2014, 2017), and lettuce (Dallemole et al. 2013). An experimental formulation based on a mixture of *Arthrobotrys robusta*, *Arthrobotrys oligospora*, *Arthrobotrys musiformis*, *Dactylella leptospora*, and *Monacrosporium eudermatum* controlled *Pratylenchus jaehni* in orange orchard (Martinelli et al. 2012).

Bacillus and *Pasteuria* have been widely studied for biological control of nematodes (Chen and Dickson 2012; Zhou et al. 2016; Rao et al. 2017). Several commercial bionematicides have been developed from *Bacillus* species (Table 9.4). *Bacillus* species are easily mass-produced in vitro; they form resistant endospores and have a broad range of activity against nematodes, such as producing toxins, inducing host resistance, and altering root exudates (Chen and Dickson 2012). In recent research, liquid formulations based on *Bacillus* species controlled *M. incognita* in tomato (Zhou et al. 2016) and carrot (Rao et al. 2017) in field conditions.

Pasteuria parasitizes juveniles and adults of plant-parasitic nematodes, including *Meloidogyne* spp. (parasitized by *Pasteuria penetrans*), *Pratylenchus* spp. (parasitized by *P. thornei*), *Heterodera* spp. and *Globodera* spp. (parasitized by *Pasteuria nishizawae*), and *Belonolaimus longicaudatus* (parasitized by *Candidatus Pasteuria* usage). *Candidatus* P. hartismerei and *Candidatus* P. goettingianae are species with provisional names described as parasites of the plant-parasitic nematodes *Meloidogyne ardenensis* (Bishop et al. 2007) and *Heterodera goettingiana* (Sturhan et al. 1994), respectively. *Pasteuria penetrans* is by far the most studied species of this bacterium (Chen and Dickson 2012). It has been used as a biological control agent of different species of *Meloidogyne* (Freitas et al. 2009; Chen and Dickson 2012). In a 102.4-hectare plantation of jaborandi (*Pilocarpus microphyllus*) in Brazil, a single application on the soil surface (treated area of 170 m^2) of tomato root powder suspension with endospores of *P. penetrans* (10^3 endospores/g of soil at 20 cm depth) controlled *M. incognita* (Freitas et al. 2009). Two years after the application, the soil was suppressive to the nematode (Freitas et al. 2009). For research purposes, large-scale production of *Pasteuria* endospores has been achieved by

Table 9.4 Bionematicides on the worldwide market

Biocontrol agent	Mechanism of action	Product	Company	Country
Arthrobotrys oligospora	Nematode-trapping fungus	Nematofagin	Mycopro	Russia
Arthrobotrys oligospora, *Arthrobotrys botryospora*	Nematode-trapping fungus	Nemout 0.65 WP	Agri - Mart Inc.	USA, Costa Rica
Arthrobotrys sp., *Glomus* sp., *Pochonia* sp.	Multi-spectrum activity	Pochar	Microspore Green Biotechnology	Italy
Bacillus spp.	Antibiotic bacterium	Nemato-Cure	Biotech International Ltd.	India
Bacillus amyloliquefaciens	Antibiotic bacterium	Nemacontrol	Simbiose	Brazil
Bacillus chitinosporus	Antibiotic bacterium	Biostart	Microbial Solutions	South Africa
Bacillus chitinosporus, *B. laterosporus*, *B. licheniformis*	Antibiotic bacterium	Biostart Rhizoboost	Rincon-Vitova	USA
Bacillus firmus	Antibiotic bacterium	BioNemaGon	Agri Life	India
Bacillus firmus	Antibiotic bacterium	BioNem WP, BioSafe	Agrogreen	Israel
Bacillus firmus	Antibiotic bacterium	Andril, Nortica, Oleaje, Poncho, Vortivo	Bayer	USA, Brazil
Bacillus licheniformis, *B. subtilis*	Antibiotic bacterium	Presence, Quartzo	FMC Química do Brasil Ltda	Brazil
Burkholderia cepacia	Antibiotic bacterium	Deny	Stine Microbial Products	USA
Mycorrizhal fungi	Endophytes	Prosper-Nema	Circle One, Inc.	USA
Myrothecium verrucaria	Antibiotics produced by the fungus	DiTera	Valent	USA
Pasteuria nishizawae	Obligate parasite of J_2 to adult	Clariva	Syngenta	USA
Pochonia chlamydosporia	Egg-parasitic fungus	Rizotec	Rizoflora Biotecnologia S.A.	Brazil
Pochonia chlamydosporia	Egg-parasitic fungus	Xianchongbike	Tianjin Blue Ocean Chemical Co. Ltd.	China
Pochonia chlamydosporia	Egg-parasitic fungus	KlamiC	CENSA	Cuba

(continued)

Table 9.4 (continued)

Biocontrol agent	Mechanism of action	Product	Company	Country
Pochonia chlamydosporia	Egg-parasitic fungus	PcMR-1	Clamitec-Myco-Solutions Ltd.	Portugal
Purpureocillium lilacinum	Egg-parasitic fungus	Biomyces	Bio Tropical S.A.	Colombia
Purpureocillium lilacinum	Egg-parasitic fungus	Bionemat, Nemator	Biotech International Ltd.	India
Purpureocillium lilacinum	Egg-parasitic fungus	Bio-Nematon	T. Stanes & Company Ltd.	India
Purpureocillium lilacinum	Egg-parasitic fungus	Bioniconema	Nico Orgo Manures	India
Purpureocillium lilacinum	Egg-parasitic fungus	BiostatWP	Bayer	Chile
Purpureocillium lilacinum	Egg-parasitic fungus	Mytech	Lachlan Kenya	Kenya
Purpureocillium lilacinum	Egg-parasitic fungus	Nemakontrol	Solagro	Peru
Purpureocillium lilacinum	Egg-parasitic fungus	Nemata	Live Systems Technology	Colombia
Purpureocillium lilacinum	Egg-parasitic fungus	Nematofree	International Panaacea Ltd.	India
Purpureocillium lilacinum	Egg-parasitic fungus	BioAct	BioAct Corp.	The Philippines
Purpureocillium lilacinum	Egg-parasitic fungus	BioAct	Biotech Resources for Agriculture and Industry, Inc.	The Philippines
Purpureocillium lilacinum	Egg-parasitic fungus	BioAct WG, Nemacheck	Australian Technology Innovation Corp.	Australia
Purpureocillium lilacinum	Egg-parasitic fungus	BioAct	Intrachem Bio Itala	USA
Purpureocillium lilacinum	Egg-parasitic fungus	Xianchongquaike	Beijing Zhengnong Agri-Tech Co. Ltd.	China
Purpureocillium lilacinum	Egg-parasitic fungus	BioAct WG, MeloCon, Paecil, Nemout WP	Prophyta	Germany
Purpureocillium lilacinum	Egg-parasitic fungus	Yorker	Agriland Biotech Ltd.	India
Purpureocillium lilacinum	Egg-parasitic fungus	FB Nemakill	Parama Agri Clinic	India
Purpureocillium lilacinum	Egg-parasitic fungus	Paecil	Shakti Biotech	India
Purpureocillium lilacinum	Egg-parasitic fungus	Bio-nematicide	ANC Enzyme Solutions Pte Ltd.	Singapore

(continued)

Table 9.4 (continued)

Biocontrol agent	Mechanism of action	Product	Company	Country
Purpureocillium lilacinum	Egg-parasitic fungus	PIPlus	Biological Control Products	South Africa
Purpureocillium lilacinum	Egg-parasitic fungus	PL Gold	Becker Unerwood Co.	South Africa
Purpureocillium lilacinum	Egg-parasitic fungus	MeloCon	Certis	USA
Pseudomonas fluorescens	Antibiotic bacterium	Sudozone	Agriland Biotech Ltd.	India
Streptomyces avermitillis	Toxic metabolites produced by bacterium	Abamectin	Many products	Worldwide
Trichoderma harzianum	Toxins produced by the fungus	Ecosom-TH	Agri Life	India

Adapted from Chen and Dickson (2012) and Dallemole-Giaretta et al. (2014)

growing a host plant (tomato, for instance) infected by *Meloidogyne* parasitized by *Pasteuria*. The high degree of specificity to nematode hosts and the limitation of artificial production of endospores are difficulties involved in using *Pasteuria* as a biocontrol agent (Stirling 2014). Recently, the company Pasteuria Bioscience (Florida, USA) developed a method for mass production of this bacterium. In 2012, Syngenta acquired this company. One year later, they launched a product to manage the soybean cyst nematode, based on *P. nishizawae* (Table 9.4).

9.10 Anaerobic Soil Disinfestation (ASD) or Biological Soil Disinfestation (BSD)

This ecological alternative to soil fumigation was developed in Japan (Shinmura 2000; Shinmura 2004) and The Netherlands (Blok et al. 2000) and has been used since then for the control of several soilborne pathogens, such as *Fusarium*, *Verticillium*, *Rhizoctonia*, *Sclerotium*, *Sclerotinia*, *Pythium*, *Phytophthora*, *Macrophomina*, *Ralstonia*, and nematodes (Rosskopf et al. 2014; Shennan et al. 2014; Shrestha et al. 2016). This technique consists of incorporating organic material that is easily decomposable (C/N ratio from 8 to 20:1) into the soil, irrigating to saturation, and covering the soil with oxygen-impermeable plastic (Rosskopf et al. 2014; Shennan et al. 2014). Carbon source will stimulate rapid growth and respiration of soil microbiota, reducing available oxygen. As soil pore spaces are filled with water, and the plastic cover limits inflow from the atmosphere, anaerobic conditions are created in the soil, stimulating the activity of facultative anaerobic microorganisms (Rosskopf et al. 2014; Shennan et al. 2014; Shrestha et al. 2016).

Accumulation of toxic products from anaerobic decomposition (acetic, butyric, and propionic acids, CO_2, NH_3, H_2S, CH_4, and N_2O), antagonism by anaerobic organisms, lack of oxygen, and the combination of all of them are the main drivers that explain the efficacy of ASD (Runia et al. 2014; Shennan et al. 2014).

Rice or wheat bran, soybean flour, ethanol, molasses, manure, and fresh crop residues have been assessed as carbon sources at rates ranging from 0.3 to 9 kg/m^2 (Shrestha et al. 2016). The incubation period has varied from 3 to 10 weeks (Shrestha et al. 2016). A meta-analysis published recently revealed that ASD suppresses bacterial, oomycete, and fungal pathogens by 59 to 64%, while the effect of the technique on plant-parasitic nematodes ranged from 15 to 56% (Shrestha et al. 2016). The number of studies aiming to assess the effect on nematodes was approximately seven times fewer than for other pathogens, and the authors recognized that this low number of studies influenced the evaluation of nematode suppression, with large confidence intervals due to error (Shrestha et al. 2016). They also encouraged more studies on the effect of ASD for the control of nematodes. Regarding the overall effects on pathogens, an incubation period of 3 weeks was the most effective, and amendments in liquid form (such as ethanol or liquid molasses) were more effective than solid forms.

9.11 Resistant Crops

The use of resistant crops is one of the most efficient and eco-friendly methods for reducing losses caused by plant-parasitic nematodes. Based on the information on which nematode species/races are prevalent in the field, the grower should choose a resistant crop, when available. Ideally, resistant genotypes should control nematodes, be adapted to a wide range of environmental conditions, and have high yield potential.

Resistant crops are developed through conventional breeding approaches or through molecular techniques (Fuller et al. 2008). Introgression of resistance genes from wild relatives into crop cultivars has been widely used to generate nematode-resistant crops. Many resistant crops based on this conventional approach are recommended for use in several countries. In Brazil, conventional resistant genotypes of soybean, coffee, corn, tomato, cucumber, melon, and lettuce are available for the management of nematodes (Ferraz et al. 2010; Matsuo et al. 2012). Recently, genetic engineering has emerged as a powerful approach that may provide novel and durable nematode-resistant crops. Expression of natural resistance genes in heterologous species, cloning of proteinase inhibitor coding genes, anti-nematodal proteins, and use of RNA interference to suppress nematode effectors are transgenic strategies used for nematode resistance in plants (Fuller et al. 2008; Ali et al. 2017). More details on transgenic approaches for nematode control are found in Ali et al. (2017).

Globally, most of the resistant crops available for commercial use target the control of sedentary endoparasites, such as *Meloidogyne*, *Heterodera*, and *Globodera*, or sedentary semiendoparasites, including *Rotylenchulus* and *Tylenchulus* (Roberts

2002). Few resistant genotypes have been released for the management of migratory endoparasites and ectoparasites (Peng and Moens 2003), despite the importance of species such as *Pratylenchus brachyurus*, *Radopholus similis*, *Xiphinema index*, *Ditylenchus dipsaci*, and *Aphelenchoides besseyi* (Jones et al. 2013).

Repeated use of resistant genotypes may select for virulent biotypes or cause a shift in the balance of nematode populations. Soybean cultivars resistant to *H. glycines* races 3 and 1 are widely used in Brazil. Eleven races of this nematode are found in the country (Dias et al. 2009), which increases the chance of the emergence of virulent populations when cultivars resistant to the same races are constantly used (Dias et al. 2009). In the USA, repeated cultivation of soybean cultivars resistant to *M. incognita* created a selective pressure for *M. arenaria* (Fassuolitis 1987). The use, over decades, of potato cultivars that are resistant to *G. rostochiensis* in the UK has exerted selective pressure for *G. pallida* (Thomas and Cottage 2006). Crop rotation with non-host plants should be integrated with the use of resistant crops to avoid the appearance of virulent biotypes and the population growth of other species.

9.12 Concluding Remarks

The demand for eco-friendly methods for nematode control has been increasing. Consumers have been demanding higher food security and environmental quality, and this situation will not be different in the future. In this context, scientists' efforts in discovering new nonchemical strategies for nematode control and improvements in the current methods must be continuous. Advances in biotechnology may contribute to the development of resistant crops, accurate and rapid methods for the diagnosis of quarantine-listed nematodes, and efficient protocols for the screening of biocontrol agents. Multinational companies have been increasingly interested in the production of bioproducts, and this fact may expand the availability of commercial bionematicides. Biofuel production is a potential source of organic amendments for use in agriculture. Even with several different prospects for the control of nematodes, the use of preventive practices and the combination of strategies will have a relevant place in the management of plant-parasitic nematodes.

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Chapter 10
Microorganisms, Organic Matter Recycling and Plant Health

R. N. Lakshmipathi, B. Subramanyam, and B. D. Narotham Prasad

Abstract Organic manures play a very crucial role in maintaining the good nutrient and soil health. With the need for quality products, there has been an implication to address qualitative traits and in turn influence soil microbial biomass and thus depict the soil health. Importance of organics is increasingly felt these days in sustainable crop production systems as nutrient management in soil and maintenance of soil health are important parameters. Organic matter availability is the major constraint; hence all possible sources of organic matter, namely, the agricultural residues, urban residues, and agro-industrial residues, could be explored for utilization as humus source. Organic additives serve as a long-lasting source of plant nutrients, influencing water-air regimes, minimize degradation, and aid in sustaining soil health. Soil microbial biomass is the living component of soil which is ultimately a source of various nutrients. The soil microbial biomass is an essential component of organic matter turnover. The varieties of microorganisms found in the environment, supporting healthy growth of plants, are in a state of dynamic equilibrium due to balance in the sum total of associative and antagonistic physiological activities of the soil microflora. In this dynamic state, the beneficial microflora decompose organic residues; help the root system in nitrogen fixation, mineralization, and immobilization of nutrients; and also contribute to soil health and texture by secretion of vitamins, growth factors, and extracellular products as soil binding factors along with suppressing soil-borne pathogens. Application of antagonistic microbes through soil amendments in the form of organic manures is one of the effective methods of biocontrol. Therefore among the various attributes, organic matter content also determines the soil quality including its fertility and productivity, since it serves as a long-lasting source of nutrients, minimizes degradation, and aids in sustaining soil health.

Keywords Organic recycling · Microorganism · Sustainable agriculture · Bioconversion · Nutrient

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R. N. Lakshmipathi (✉) · B. Subramanyam · B. D. Narotham Prasad
Department of Agricultural Microbiology, College of Sericulture Chintamani,
University of Agricultural Science, Bangalore, Karnataka, India

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10.1 Introduction

Organic matter is referred to as “life of soil,” which is an indispensable component of good soil health (Bardgett 2005). With emphasis on integrated nutrient management, use of organics in a nutrient package is important as it has positive improvement on soil physical, chemical, and biochemical properties (Ansari and Mahmood 2017). Organic matter plays crucial role in the maintenance of natural ecosystem and sustainable agriculture. Different organic components have the ability to act as temporary nutrient reservoir. The proper management of this reservoir has made it possible to increase the efficiency of use of both soil and fertilizer nutrients which has recently been separated into biomass and non-biomass active components (Lützow et al. 2006). The active soil organic component comprises of soil microbial biomass and labile organic inputs, which has recently been added. Microbial biomass is an agent of decomposition and mineralization of organic nutrients; active soil organic matter supplies majority of the nutrients (Paul 1984). Therefore to conserve and to sustain soil fertility, incorporation of organic residues has recently been a topic of discussion among the scientist (Ansari and Mahmood 2017). Rapid industrialization and agricultural production have led to generation of huge quantities of urban wastes and agro-based industrial residues such as pressmud, bagasse, poultry litter, coir pith, etc., which pose disposal and environmental problems. On the other hand, application of organic wastes directly to soil leads to many problems such as immobilization of nutrients and phytotoxicity. Hence organic matter should be sufficiently stabilized. Therefore, the most appropriate approach to manage these wastes is through bioconversion by way of composting (Lim et al. 2016). Composting is a controlled bio-oxidative process in which organic material is partially decomposed due to microbial activity in a suitable environment. During the process, degradable organic substrates undergo physico-chemical changes resulting a humified products which ultimately augment soil and plant health (de Bertoldi et al. 1983).

Soil contains very high number of microorganisms and is a habitat for vast complex and interactive community of soil organisms whose activities reveals the physico-chemical and biological properties of soil (Rivkina et al. 2000). Because of their small size, large surface area, ability to utilize a wide range of carbon and energy sources, rapid metabolic rate, short generation time, and quick adaptability, the microorganisms are more versatile. The varieties of microorganisms found in the environment, supporting healthy growth of plants, are in a state of dynamic equilibrium due to balance in the sum total of associative and antagonistic physiological activities of the soil microflora. Present article will update our understating pertaining to microbial world: organic manures especially the compost and their role in the plant disease management. Application of organic and microorganism can improve the soil and plant health as these both agents may act as soil conditioner.

10.2 Microbes in Recycling of Organic Matter

Composting is the biotransformation process of complex organic matter into simpler or more readily available form by the mixed microbial population under optimum conditions of aeration and moisture (Mathur et al. 1993). Crawford (1983) defined composting as an incomplete, artificially accelerated decomposition of heterogenous organic matter by a wide range of microbial population in an environment conducive to these microorganism. It was noticed that both mesophilic and thermophilic microorganisms would be evenly distributed throughout the compost pit (Davis et al. 1992). As the process of composting commences, the indigenous microorganisms start utilizing organic materials containing C, N, and other nutrients. As the activity continues, the temperature increases, and heat is generated through microbial oxidation and respiration. At this point, the high temperature inhibits the mesophilic microorganisms, and the thermophiles become very active. Eventually, available C and other nutrients are depleted, microbial activity subsides, decomposition slows down, and cooling occurs, and thereafter, the stabilization phase is reached wherein mineralization and humification occur which is known as maturity stage (Jimanez and Garcia 1992). Composts differ dramatically in their content of minerals, biochemicals, humic substances, and billions of different microorganisms in them. The community-level association also influence the microbiomes. Lockhead (1957) reported that the percentage of populations able to produce thiamin, biotin, or vitamin B12 was about 50% higher in soil samples from the rhizosphere of barley plants than from control soil samples. He therefore proposed that this might potentially be the significance in the interrelationships between normal soil microbiomes, soil-borne pathogens, and the plant. There is a lack of knowledge regarding the community-level control on the microbial agents of elemental cycling (Janzen et al. 1995).

10.3 Composition of Organic Residues

10.3.1 Urban Solid Waste

The composition of the waste varies mainly with the type of waste. The sludge collected in Calcutta was alkaline in reaction as it had higher salt content in winter (Maiti et al. 1992). Further the base saturation was above 80%, rich in organic carbon, ammoniacal nitrogen, nitrate nitrogen, available phosphorus, and potassium. Further, it was also found that the micronutrient content was high in sludges. Various waste collection processes were evaluated by Prabhakarachary et al. (1998) in and around Hyderabad, and the physicochemical characteristics of solid wastes and the calorific value were compared. It was observed that the domestic solid wastes offered less calorific value and greater ash content than those from market yards. Addition of urban waste compost was attempted by Karnataka Compost Development Corporation. Two grades of urban composts, viz., Bioagrorich and Bioagro, were released. Bioagrorich had 1.2, 1.8, and 1.0% N, P2O5, and K2O (Shivakumar 1999). Reddy et al. (2000) have analyzed the compost prepared from segregated and unsegregated wastes collected from Bangalore, and it was found that the compost was alkaline in reaction (pH 8.2) with nitrogen (1120 mg/100 g), phosphorus (120 mg/100 g), and potassium (850 mg/100 g). It was estimated that the total waste generated by 217 million people living in urban area was around 23.86 million tonnes per year (1991), and it might exceed even 39 million tonnes by 2001 A.D. The total N, P_2O_5, and K_2O content of this would be around 2.5 lakh tonnes of K_2O (0.67%) with a C/N ratio of 21–31. This is almost equivalent to 5.4 lakh tonnes of urea, 16.2 lakh tonnes of super phosphate, and 4.3 lakh tonnes of muriate of potash (Channabasavanna and Abdul Rahaman 2002).

10.3.2 Coir Pith

The shredding of coconut fiber results in a fine powder, as what is called as coir pith. Its unique property is that it has a large water-holding capacity. Composition of coir dust was found to vary with the variety of coconut, fertility status of coconut gardens, method of extraction, disposal, and other environmental factors (Rahman et al. 2002). According to Anand (1998), coir dust had pH of 6.12, EC 1.26 dSm^{-1}, and it contained 48.22% OC, 0.42% N, 0.094% P, 0.82% K, 1.14% Ca, 0.51% Mg, 0.22% S, 51.33% lignin, and 34.11% cellulose. It was reported by Datta (1998) that the coir dust had 6.68 pH, 1.63 dSm^{-1} EC, and it contained 55.65% OC, 0.46% N, 0.19% P, 0.63% K, 24 ppm Zn, 3.3 ppm Cu, 11.01 ppm Fe, 30.66 ppm Mn, 66.15% lignin, 17.08% cellulose, 309 mg 100 g^{-1} total phenols, and 3.65% total lipids. According to Kadalli (1999), coir dust had a pH 6.25, EC 1.54 dSm^{-1}, and it contained 48.72% OC, 0.47% N, 0.02% P, 0.62% K, 0.46% Ca, 0.31% Mg, 0.07% S, 53 ppm Zn, 15 ppm Cu, 1264 ppm Fe, and 58 ppm Mn. He also reported that it contained 35.50% cellulose, 48.90% lignin, 7.00% hemicellulose, and total phenols of 112.9 mg 100 g^{-1}.

10.3.3 Poultry Waste

Poultry droppings and cage litter are useful organic residues rich in nutrients. However, they need to be conditioned before they are used as compost. The nutrient composition of poultry waste depends on the quality of feed, type of bedding material, and the nature of stocks (Adeniyan et al. 2011). According to Devegowda

(1997), poultry excreta and other wastes are a good source of organic manure. He reported that poultry manure was rich in major nutrients as well as secondary and micronutrients with average values of 4% N, 3% P, 2.5% K, 2.4% Ca, 0.67% Mg, 0.5% S, 451 ppm Fe, 150 ppm Cu, 406 ppm Mn, and 463 ppm Zn. Shepherd and Withers (1999) reported that a major portion of the total phosphorus of poultry manure was in inorganic form (74%) with a C/P ratio of 32:1 and N/P ratio of 3:4 with dry matter content of 62% deep litter manure contained 87.9% total dry matter, 5% nitrogen, 2.5% phosphorus, and 2.04% potassium, while cage layer manure contained 89.85% total dry matter, 4.5% nitrogen, 2.98% phosphorus, 2.33% potassium, 72% organic matter, and 28% ash (Narahari 1999). Toor et al. (2001) noticed that the NPK content of cage system poultry manure and deep litter system poultry manure were 1.82, 2.43, and 1.61 and 1.28, 1.88, and 1.72%, respectively.

10.3.4 Pressmud

Pressmud is a by-product of sugarcane industry and is a major source of nutrients (Ansari 2018). Pressmud is generally soft spongy, amorphous, and dark brown to brownish white material containing sugar, fiber, coagulated colloids including cane wax and albuminoids, inorganic salts, and soil particles (Dotaniya and Datta 2014). The composition and properties of pressmud cake are, however, variable depending upon the quality of cane crushed and process followed for clarification of cane juice in a sugar factory (Sarangi et al. 2008). Narwal et al. (1993) found that sulfitization of pressmud cake had pH 6.5, EC 2.6 dSm^{-1}, 41.3% OC, and 2.9% P_2O_5. This composition was slightly different from the composition he had reported in an earlier study. Hence, depending upon industrial process and quality of cane, the pressmud composition varied to certain limits. Mala et al. (1998) found that pressmud had 20–25% organic carbon, 74.28% organic matter, and 2.5% N with an iron content of 0.01%.

10.4 Microbial Dynamics during Composting

The population dynamics of bacteria, fungi, and actinomycetes have been investigated in various composting systems including straw (Carlyle and Norman 1941; Chang and Hudson 1967), tree bark (Bagstam 1978), mushroom compost (Waksman and Cordon 1939; Chanter and Spencer 1974; Fermor et al. 1979), and city refuse (Strom 1985). Results of these studies vary according to substrates, process management, and microbial technique. A general trend could be observed with a peak bacterial count per gram substrates ranging from 108 to 1012 at temperatures of 50–550 °C, and at mesophilic temperature, the bacterial counts will be at higher magnitude. Thermophilic actinomycetes population peaks later than bacterial peaks and often achieves counts in 10^7–10^9 ranges. Thermophilic fungal populations peak much after those of bacteria, normally in the declining activity stage as temperatures drop into the lower 500 °C and below range, fungal colony-forming units per gram substrates being in the 105–108 range. At temperatures above 600 °C, fungi are normally absent, and above 700 °C, actinomycetes are generally included (Miller 1993). Fungi are commonly recovered from the stage where temperature is more moderate and remaining substrates are predominately cellulose and lignin (Chang and Hudson 1967). Kane and Mullins (1973) isolated thermophilic and thermotolerant fungi, viz., *Aspergillus fumigatus*, *Chaetomium* sp., *Humicola lanuginosa*, *Mucor pusillus*, *Thermoascus aurantiacus*, and *Torula thermophilus*, from unprocessed municipal waste. A relative quantity of research work has been published against the fate of plant pathogenic fungi during composting. Several researchers reported the eradication of different plant pathogenic fungi in litter, viz., *Plasmodiophora*, *Brassica*, *Phytophthora cinnamomi*, *P. cryptogea*, *Rhizoctonia solani*, *Pythium irregulare*, *Botrytis cinerea*, *Armillaria mellea*, etc., during composting (Yuen and Raabe 1984; Hoitink and Fahy 1986).The elimination of pathogens from organic additives may be due to (a) the high temperature, (b) release of toxic products and (c) enhanced microbial antagonists (Hoitink and Fahy 1986).

10.4.1 Bacteria

Carlyle and Norman (1941) reported that there were a great variation in the population of some important bacteria such as *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Micrococcus*, and *Bacillus*. Niese (1959) reported that the mesophilic temperature encouraged the predominance of yellow, red, and white colonies of non-spore-forming short rods. Further, at thermophilic stage he isolated spore formers like *Bacillus subtilis* and *B. stearothermophilus* and confirmed the optimum growth of *B. subtilis* at 50 °C on meat extract yeast agar. *B. stearothermophilus* showed the temperature range of 40–73 °C for its growth, and the optimum growth was at 65 °C. A variety of aerobic thermophilic heterotrophic and autotrophic bacteria from thermogenic composts was isolated by Beffa et al. (1996) from several composting facilities in Switzerland. During thermal cooling or maturity phase of compost, a high number of metabolically diversified mesophilic bacteria including nitrogen fixers, phosphate solubilizers, sulfur oxidizers, nitrifiers, etc. were noticed.

10.4.2 Actinomycetes

Colonization pattern of actinomycetes are slower than bacteria and fungi (Lacey 1973). Chang and Hudson (1967) reported that the number of mesophilic actinomycetes was fluctuating during composting. The late appearance of actinomycetes was a common feature of municipal waste composts regardless of many manipulative and substrate variations (Finstein and Morris 1972). At fixed temperature, Waksman et al. (1939) noticed the development of actinomycetes later than that of bacteria and fungi.

10.5 Factors Influencing Composting Process

Many biotic and environmental factors influence the process of composting. These factors are very much essential for efficient decomposition to obtain quality product (Poincelot 1974).

10.5.1 C/N Ratio

The C/N ratio of the substrate is an important factor to have an efficient decomposition system. The materials with a wide C/N ratio is degraded slowly when compared to materials with narrow C/N ratio. To accelerate the rate of decomposition, C/N ratio of the organic substrate should be balanced (Gaillard et al. 1999). Microorganisms require carbon and nitrogen for growth and protein synthesis, respectively. On an average they require 30 parts of carbon per every single part of nitrogen for synthesis of cell material. Hence C/N ratio of 30 is demandable for effective composting process. If it is below 26, the excess nitrogen is converted to ammonia and wasted into atmosphere (Poincelot 1974). Thus, increasing the C/N ratio by addition of degradable carbon sources to nitrogen-rich material is an effective solution for nitrogen losses (Kirchmann 1985). Increased microbial activity might result from adaptation of microbial ecosystem to changes in available substrate (Ashbolt and Line 1982). Proper adjustment of C/N ratio in composting of agricultural waste will lead to profitable recycling, with a greater degree of N enrichment, low C/N ratio results in a rapid composting accompanied by an increased loss of excess nitrogen through ammonia volatilization. Loss of ammonia a pilot-scale composting trial showed a secondary peak after second week. Nitrogen losses due to low C/N ratio in sewage sludge composting through volatilization of ammonia were maximum in the initial stages of composting (Witter and Lopez-Real 1988).

10.5.2 pH

Hegarty and Curran (1985) observed that different fungi used in their study produced greatest loss in weight wood in a pH range 5–8. Verdonck (1988) found that organic matter with a wider range of pH (3–11) could be composted. The optimum pH levels were between 6 and 8 for composting and between 4 and 7 for end products. Optimum pH for lignin biodegradation by *Phanerochaete chrysosporium* was 4–4.5, and there was a marked suppression above 5.5 and below 3.5 (Kirk and Farrel 1987), whereas lignocellulose degradation by lignin degraders and production of acid-precipitable polymeric lignin at alkaline pH with *Streptomyces viridosporus* were reported by Pometto and Crawford (1986).

10.5.3 Moisture and Aeration

Moisture and aeration are the two factors known to play a prominent role in the decomposition process. Composting is mainly an aerobic process, and anaerobic condition slows down the decomposition process and produces foul smell. However, in some material, the level of oxygen can apparently drop to 0.5% without creating anaerobic condition (Poincelot 1974). He also reported that less than 40% moisture would result in decomposition. More than 60% moisture causes anaerobic condition and results in foul smell. Inbar et al. (1993) found that moisture content has a major effect on oxygen consumption.

10.6 Biochemical Changes during Composting

The chemical and biochemical alteration during composting process determine the extent of decomposition. During composting, there will be a drastic reduction of complex molecules such as lignin, cellulose, hemicellulose, lipids, etc., and these complex molecules break down into simpler components (Datta 1998 and Kadalli 1999). Cellulose is a major component of organic matter and is degraded by a group of functionally similar hydrolytic enzymes called as cellulases, which is a microbial origin. According to Linkins et al. (1990), the activity of cellulases is directly related to the loss of mass of decomposing organic material and inversely related to lignin content. Lignin, second most abundant natural polymer making up to 15–30% of organic matter (Gold and Alik 1993). The degradation of lignin is an important way in the completion of the completion of carbon cycle (Freudenberg 1968). Lignin degradation is mainly brought about by three major enzymes, namely, lignin peroxidases, manganese peroxidases, and laccases (Chefetz et al. 1998). Laccase enzyme may have a role in polymerization of phenolic monomers during humification. The enzyme analysis brings about the hydrolysis of starch. The two amylases commonly concerned with breakdown of starch include α- and β-amylases. The β-amylase acts on both amylase and amylopectin, clearing second glucose-glucose bond from the terminal end of molecules, but is incapable of catalyzing the hydrolysis of branch points of amylopectins, which can be done by α-amylase. Chang Yung (1967) noticed a dry weight loss of 50% in wheat straw decomposition. Proteins are the most abundant organic molecules in the organic wastes and are degraded by the enzyme protease. It hydrolyzes the proteins to polypeptides and oligopeptides to amino acids. This conversion is responsible for the release of ammonia during the process of composting (Alexander 1977). The enzyme urease catalyzes the hydrolysis of urea to CO_2 and NH_4 (Tabatabai 1982). Urease enzyme is widely distributed in and present in varying living cells. It also catalyzes the hydrolysis of hydroxyurea and semicarbazide. The largest fraction of organic P is in the form of phytate and its derivative in the organic material. This organic P must be hydrolyzed to inorganic P before plants can utilize it. Phosphatases are the enzymes secreted by microorganisms and plant roots and are responsible for hydrolysis of C-O-P ester bonds. Phosphatases in decomposition may originate from microbial cells. Spier and Ross (1978) observed increased phosphatase production by organic matter and in presence of organic P compounds. Phosphatase activity had positive relationship with organic P supply and negatively related to the inorganic P availability.

10.6.1 Humification

During the process of composting, the humic fractions and the rate of humification increased depending on raw materials and other factors. Scheid (1989) found that the bulk composts and the paper or sewage sludge mixtures had significantly less HA than straw or organic refuse wastes. The contents of HA in all the composts were positively correlated with the age of the composts. A 2-year-old organic refuse waste compost showed the best humus quality with 23.8% organic substances as HA and 7.5% as FA. The HA and FA obtained from sewage sludge was studied by Boyd and Sommers (1990). They found that HA and FA has complex metal binding sites involving oxygen containing chelating groups and mixed N/O ligand system. They also observed several distinguishing chemical and structural characteristics of HA and FA extracted from sewage sludge which include (i) higher N content; (ii) low N ratio; (iii) higher H/C ratio, indicating higher fraction of aliphatic components; and (iv) lower carboxyl group acidity.

Deiana et al. (1990) characterized HA extracted from sewage sludge, manure, and earthworm compost by chemical and spectroscopic methods and observed meaningful differences in the composition. These differences allow a differentiation among the products depending on the source from which they were obtained. Humic acids extracted from sewage sludge contained highest aliphatic C and have characteristics close to those of aquatic HA. On the other hand, HA extracted from manure and earthworm composts were similar to HA originating from the soil. The relative quantities of HA increased with increase in composting time of wheat straw, and FA production decreased after 30 days of composting (Singh and Amberger 1990).

Garcia et al. (1991) extracted the HA from solid municipal wastes before and after composting and found that the ratio of HA to total humic substances increased, phenolic content decreased, and carboxy and ketonic groups increased. Humic substance (HA and FA) levels are important chemical criteria in defining compost maturity (Jimanez and Garcia 1992). They reported that during composting of municipal solid waste, the amount of HA expressed as percent of OM increased from 7.8% in the raw materials to 12–19% in the mature compost. Chefetz et al. (1996) reported that HA content increased during composting of municipal solid waste and it reached a stable value of 13.5% of the OM after 112 days, whereas the fulvic fraction level decreased. According to them, the increasing level of HA represents the degree of humification and maturity of compost. Kadalli (1999) reported that during composting of coir dust for 120 days, HA content increased up to 120 days, while FA content initially decreased up to 60 days and further increased. He characterized humic and fulvic acid extracted from composts.

10.7 Compost Enrichment

Microbial communities carry out the decomposition process. Just hastening composting process is not important, but the quality of the final compost produced is also important. Therefore, microbiome present in the soil influence the composting process and quality of final product (Sabine et al. 2000). Most of the composts contain relatively low levels of nutrients (Sikora and Enkiri 1999). Therefore the possible way of improving the quality of compost is by improving the nutrient status. Maximum efficiency of nutrient utilization occurs when nutrients released from organic residues synchronize with the plant demand. Appropriate information about decomposition rate, release of nutrients will provide enough information in the utilization of organic additives effectively. The synchronization of nutrient release from organic additives and uptake by plants has been an important instance (Handayanto et al. 1997). Organic materials cannot be used directly as plant nutrients because it can cause serious damage to the plant growth (Zucconi and Bertoldi 1987). Therefore, composting is one of the methods of stabilization of organic waste materials that involves aerobic respiration and passes through a thermophilic stage which results an important end product i.e. compost (Finstein and Morris 1972).

Compost quality can also be improved by enrichment that can be achieved by using microorganisms (N_2-fixers, PO_4-solubilizers, etc.), chemical fertilizers (urea, superphosphate, etc.), or other mineral additions (pyrite, rock phosphate, etc.). These inputs bring about nutrient enrichment of finished compost (Sreenivasa et al. 1993). Inoculation with *Azotobacter* and phosphate-solubilizing microbes, viz., *Aspergillus awamori* and *Bacillus polymyxa*, improved the nitrogen content and manurial value of compost (Kapoor et al. 1983). In recent years, due to the resource crunch, alternative sources are investigated for phosphorus nutrition of crop plants. Cheaper sources such as rock phosphate gained lots of importance. Highly enriched compost from plant wastes and coir pith was prepared by adding 25% Mussoorie rock phosphate (Mishra et al. 1982). During composting, solubilization of rock phosphate took place which in turn enhanced the microbial activity. Such enriched compost is called phospho-compost (Singh et al. 1992). Singh et al. (1992) conducted a study wherein nitrogen- and phosphorus-enriched compost was prepared by incorporation of rock phosphate, pyrite, and urea N at the ratio of 10:1 and 1% of dry weight of the compostable material, respectively. The final compost contained 2% nitrogen and 1.29% phosphorus after 90 days after decomposition. In a study conducted by Singh (1983), solubilization of insoluble low-grade rock phosphate by composting with pearl millet straw in the presence of nitrogen and molasses was rapid up to 15 days and reached a maximum of 60% in 120 days of composting. There was a substantial increase in total nitrogen content where rock phosphate was used during composting. It was attributed to the acceleration of mineralization process upon addition of rock phosphate (Yadav et al. 1992). Combining microbial inoculants and chemical fertilizers, especially mineral, is a common practice of improving the quality of the compost. The phospho-sulfo-nitro compost was prepared by decomposing rice straw along with Mussoorie rock phosphate and obtained compost with 1.6% total N and 3.12% total P (Manna and Ganguly 2000). Present-day research in compost science concentrates on the ability of composts to support plant growth and suppress plant pathogens. Raghavendra Rao and Radhakrishna (2001) noticed a substantial increase in growth of tomato plants treated with composts enriched with plant growth-promoting microorganisms.

The composts produced by inoculation of earthworms, *Azotobacter*, and PO_4-solubilizing fungi, viz., *Aspergillus*, to decomposing pearl millet straw showed 1.29% of total N and C/N ratio of 11.6 when compared to composts developed without inoculation which showed only 0.75% N and C/N ratio of 17.50 (Singh et al. 2000). The conventional method of composting consider more time for producing the compost of good quality which is because of the diverse microbial communities. There are strains that could be the target of isolation and screening for use in efficient processing of organic wastes (Gaur 1990). Even though composting involves thermophilic phase and requires microbes tolerating such temperatures, some reports have clearly mentioned mesophilic efficient cellulolytic cultures for acceleration of composting process (Gaur 1990). Further, this may also prove beneficial in case of in situ application of plant residue where thermic rise is unlikely to be more than 40–45 °C. The thermophilic microorganisms take part to the maximum in decomposition when compared to mesophilic microorganisms. Application of mesophilic cellulolytic fungi, viz., *Aspergillus niger*, *Aspergillus* sp., *Trichoderma viride*, and *Penicillium* sp., in composting of Jowar stalks and wheat straw reduced the period of decomposition by 1 month. Inoculation of *Aspergillus* sp., *Penicillium* sp., and *Trichuris spiralis* along with super phosphates in the decomposition of sugarcane trash markedly hastened the composting process (Rasal et al. 1988).

10.8 Vermicomposting of Organic Matter

Earthworms play an important role in accelerating the process of decomposition, these worms can consume all kind of organic additives which also depend on their body size and weight (Gaur 1982). Earthworms are released into the decomposing wastes after a preliminary decomposition of 15–20 days. They act as good pulverizer of organic matter and promote microbial activity. The organic matter passes through the gut and is released as castings that are rich in nutrients. This process is known as vermicomposting. The common species of earthworms are *Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavatus* which are effective in composting of organic wastes (Nishio 1985; Satchell and Martin 1984). In addition to accelerating composting process, earthworms are also known to improve quality. The vermicompost can promote plant growth (Subler et al. 1998). Muscolo et al. (1999) reported production of humic substances by earthworms, which are endowed with hormone-like activity. Maturity of composts is an important parameter as it critically affects their successful utilization in agriculture. A combination of several parameters is needed to develop a maturity index of compost (Morel et al. 1985). The immature composts with wide C/N ratio when applied to the soil result in the immobilization of mineral nitrogen causing nitrogen deficiency to the plants, while composts with very low C/N ratio cause ammonium toxicity and inhibit plant growth (Inbar et al. 1993). Immature composts induce high microbial activity in soil supporting further decomposition of the applied composts, some causing oxygen deficiency and indirect toxicity problems in the rhizosphere (Zucconi et al. 1981). Immature composts contain high concentration of organic nutrients that support the growth of certain pathogens such as *Pythium* sp. Stabilization and maturity are important characters to prevent the regrowth of plant pathogens with higher saprophytic ability (Morel et al. 1985). All aspects of compost maturity are a function of the degree to which fresh organic matter is being transformed into a stable product. Although it has been demonstrated that application of compost to soil improves soil quality, the negative effect normally is associated with decrease in yield (Chanyasak et al. 1983). Various other tests such as total N, C/N ratio, cation exchange capacity, and respiration rates are also useful parameters for compost maturity. Utilization of direct spectroscopic procedures on bulk organic wastes of diverse origin subjected to process strategy elevates the maturity into a quantitative aspect. The role of composts on the nutrition of soil microorganisms, which in turn induce plant response and disease suppression, has clearly been shown with direct spectroscopy (Inbar et al. 1993).

10.9 Management of Plant Pathogens

Application of soil amendments is one of the effective methods of controlling the plant pathogens (Ansari and Mahmood 2017). Amendment of green manures, farmyard manure, compost, oil cakes, and other organic residues may improve the soil health. Diseases could be reduced by certain amendments due to suppressiveness. Addition of amendments to soil might increase microbial activities in soil to suppress the disease (King et al. 1934; Zentmeyer and Thompson 1967; Mehrotra and Tiwari 1976; Sivaprakasam 1991). Therefore, organic amendments are perhaps more practical for biocontrol of plant pathogens, especially for pathogens of vegetable crops in orchards (Cook and Baker 1983). Management of soil-borne plant pathogens by organic amendments can be achieved through several mechanisms. Organic matter influences soil physical characters and helps in better solubilization of minerals which in turn aid in rapid expansion of the root system, better uptake of nutrients, and finally better plant vigor. In many diseases, this could help the plant to resist the attack by pathogens or replace the damaged roots quickly by new roots (Singh 1983). Decomposition of organic amendments being a biological process stimulates the microbial activity both quantitatively and qualitatively antagonistic to plant pathogens (Bouhot 1981). Mitchell et al. (1941) reported for the first time the germination lysis of sclerotial bodies of *Phymatotrichum omnivorum*, after the addition of organic amendment like oil cakes. Stindt (1990) differentiated groups of microorganisms on selective media and concluded that extracts contain very rich and varied microbial population, which could play an important role in disease suppression. Application of farmyard manure (FYM) at 10 tonnes per hectare reduced seedling disease of cotton caused by *Rhizoctonia solani* by increasing the soil microflora (Ramakrishnan and Jayarajan 1986). Addition of FYM at 10 tonnes per hectare and inoculation of soil with *Trichoderma viride* reduced postemergence mortality and improved germination (Krishnamohan and Kandaswamy 1986). In pot and field experiments, manure application reduced wilt incidence in tomato. A study conducted by Padmodaya (1994) indicated the beneficial effect of FYM application to crop growth and decreased the disease incidence of Fusarium wilt of tomato. Shivanandappa et al. (1989) reported that groundnut shells and pressmud were effective in minimizing the incidence of damping off disease of tobacco caused by *Pythium aphanidermatum.*

Several workers have suggested the use of organic amendments to control soil-borne pathogens. Pigeon pea wilt incidence was reduced in soil supplemented with groundnut cake, molasses, and sweet clover after inoculation with *Bacillus subtilis*. Mahmood (1964) and Bhalla (1966) noticed suppression of *Fusarium* population in soil, when peanut oil cake was amended at more than 1% (w/w). Singh and Singh (1982) reported that application of neem cake checked the radial growth of *F. udum*, when exposed to 1 or 2 weeks after amendment with chitin, cellulose, and starch at 0.1%; also the population of *Fusarium* species in sugarcane soils was reduced (Gupta 1986). Dasgupta and Gupta (1989) studied the effect of different soil amendments on wilt of pigeon pea and noticed that green manure (*Sesbania aculeata*), oil cakes (mustard and *Azadirachta indica*), and urea minimized the pathogens population and wilted plant, and enhanced the plant growth and yield status. Soil application of *Trichoderma harzianum* + FYM was better than individual application and recorded highest chickpea seed yield. It was hypothesized that soil amendments with FYM may have improved soil properties which might have further helped in better solubilization of minerals and checked the disease (Patel 1991). Voland and Epstein (1994) reported that manure and compost were more effective than urea alone in inducing suppression of the damping off of radish caused by *R. solani*. Singh and Singh (1980) reported inhibition of *Fusarium oxysporum*, *Fusarium udum* by soil bacteria *B. subtilis* among the four antagonists, was most effective and produced an inhibition zone of three mm. *Trichoderma* spp., *Curvularia* climatic, and *Streptomyces* sp. overgrew the species of *Fusarium*. Upadhyay and Rai (1988) reported that antagonist, viz., *Penicillium* sp., *T. harzianum*, and *T. viride*, contributed to suppressiveness of soils against *F. udum*. Microorganisms from the rhizosphere soil of pigeon pea were tested for their antagonistic action against *F. udum* and it was noticed that *Trichoderma* was the most effective in controlling the disease followed by *Aspergillus niger*, *Streptomyces* sp., *Penicillium* sp., and *Bacillus* sp. (Gaur and Sharma 1991).

10.10 Microbial Biomass in Soil

The soil microbial biomass (SMB), comprising 2–3% of the total organic carbon a more important repository of plant nutrients than its small size might indicate. Microbial biomass has been found to be a transforming agent and as a source and sink for different nutrient needed for the growth and development. The SMB itself is a part of the soil organic matter, typically about 2% of the total organic carbon which may be defined as the living microbial component of the soil that includes bacteria, actinomycetes, fungi, algae, and microfauna (Alexander 1977). The soil microbial biomass is also referred to as active soil organic matter pool (Parten et al. 1987) and related to quantity, type, and placement of organic residue addition (Sanchez et al. 1989). The effects of cropping systems and residue placement on SMB has been characterized however, the mechanisms need to be analyzed (Singh et al. 1989). The microbial biomass is an essential component of organic turnover (Jenkinson 1990; Duxbury et al. 1989a). The microbial biomass is influenced to a variable extent by macroclimate (Insam et al. 1989) and soil type (Sparling 1991) but, in particular, is responsive to land use and agricultural practices. The interaction among these results in a system which oscillates between nutrient mineralization and immobilization (Mazzarino et al. 1991a). The SMB is considered to be a good indicator of improved soil health (Ayanaba et al. 1976). Contrastingly, others consider SMB as a poor indicator of soil health (Singh et al. 1989; Mazzarino et al. 1991a, b). The most practicable general measure of the biological status is the microbial biomass (Jenkinson 1988; Sparling 1991). Microbial activity was found to be influenced by vegetation type, substrate availability, and abiotic factors in an ecosystem (Gupta and Singh 1981; Rajavanshi and Gupta 1986). It plays significant role in soil nutrient transformations and largely determine the rate of C, N, and other nutrient (Jenkinson 1988). The microbial biomass in soils comprises of a substantial pool of nutrients (Anderson and Domsch 1989), depend upon the growth stage (Duxbury et al. 1989b). Mineralizable N has been related to microbial C and N in a wide range of soils (Singh et al. 1995). SMB is known to respond much more rapidly to any change in organic matter inputs than the soil organic matter (Powlson et al. 1987). Srivastava and Singh (1988) reported that microbial biomass C ranged from 149 to 667 μg N g^{-1} soil which were significantly correlated with 35% of phosphorus.

Sukamoto and Oba (1991) reported a relation between time of application of organic matters and population density of microbes. Aoyama and Nozawa (1993) incubated the soil with addition of organic amendments like chicken manure, cloverleaves, farmyard manure, bark compost, and rice straw. They reported that amendment of organic residues exhibited enhanced microbial nitrogen. Application of organic additives augmented the microbial biomass, however, there were no significant changes in the organic carbon which leads to enhancement in the plant growth and yield. Plant yield attributes of *Sesbania* were significantly higher when farmyard manure was applied (Goyal et al. 1992). Application of plant residue of wheat and cowpea enhanced the microbial biomass C and N, however, cowpea straw showed greater microbial biomass C and N than when compared to wheat straw (Patra et al. 1992).

Oberson et al. (1993) concluded that the SMB and the activity of acid phosphatase activity were significantly higher in the biologically active cropping system. Soil microbial biomass was increased due to the addition of dairy pond sludge which might be due to the increased water-soluble C and nutrients that enhanced the soil microbial growth (Zaman et al. 1998). Soil health is regulated by different factors such as amount of organic additives, quality, time of application which ultimately influence the microbial activity and several nutrient cycling like C and N (Robertson et al. 1994). Grant et al. (1993) reported that C and N mineralization depends upon the rate of substrate consumption by themicrobial community. Soil microbial biomass appeared to be very important in promoting the plant biomass (Srivastava and Lal 1994). Balakrishna (2001) reported that microbial biomass is higher in the root zone of tree species that stimulated due to mycorrhizal colonization. The tree species that stimulated mycorrhizal colonization also showed higher microbial biomass. An incubation study with different organic amendments in an alfisol showed maximum microbial biomass C and N on the 30th day and a sudden decrease on the 45th day followed by a sudden increase in both microbial biomass C and N by the 60th day (Snehalatha et al. 2003). Asha (2003) reported significantly higher soil microbial biomass in wetlands than in dry lands, and further there was a significant increase in microbial biomass up to 30 days of incubation but decreased after 45 days after incorporation of *Chromolaena odorata* to these soils. Boby (2004) reported that the microbial biomass C and N continued to increase from the 20th day of planting of cowpea to the 60th day but later on declined. Higher microbial biomass C and N values were recorded in treatments with AM fungi *G. mossae* and soil yeasts.

10.11 Soil Enzymes

Soil contains various free, non free extracellular enzymes which play important role in the maintenance of ecological balance and nutrient cycling (Skujins 1978). It is clear that the enzymes which are somehow proteins with catalytic properties owing to their specific activation energy, catalyze all biochemical reactions. Enzymes are catalysts which accelerate the rate of reaction without undergoing permanent changes (Tabatabai 1982). Enzymes produced by the proliferating microorganisms carry out many important processes. These enzymes are substrate specific and act from either outside or inside the living cells. Any threat to microbial activity is reflected in the rate of biochemical transformation occurring in soils. The variations in the microbial population might result in an alteration of the enzyme activity (Ansari and Mahmood 2017). Enzymes have biological significance in the nutrient cycling and also in the microbial proliferation under unfavorable conditions (Kiss et al. 1975). Some of the important soil enzymes are dehydrogenases, ureases, cellulases, proteases, and phosphatases. The work carried out with reference to enzymes as influenced by different organic amendments is limited; however, the studies made so far have been reviewed here. Biological oxidation of organic compounds is generally a dehydrogenation process and different dehydrogenase enzymes are there which play pivotal role in the dehydrogenation. Therefore, the results of the assay of dehydrogenase activity exhibit the appreciable activity of active microbial population (Skujins 1976). The variations in activities are attributed to diversities in vegetation, cropping history, soil amendments, soil fauna, environmental factors, etc. (Tabatabai 1982).

Eiland (1981) noted highest dehydrogenase activity in soil treated with sludge with a high content of toxic metals and lowest in soil treated with low content of toxic metals and least in untreated soil. It is observed that the treatment that included addition of nitrogen from continuous leguminous green manure crops significantly increased the dehydrogenase, phosphatase, and urease activities and the soil microbial biomass as compared to the treatment that included FYM fertilized for 30 years (Bolton et al. 1985). Kukreja et al. (1991) noticed that the total microbial biomass and dehydrogenase activity of the soil was significantly increased in plots that received 90 t/ha of FYM continuously for over 20 years. In an inceptisol of Coimbatore, the dehydrogenase and phosphatase activities were found to be highest in FYM treated plots. With higher rates of NPK fertilization, the activities of soil enzymes were also found to be higher, and the effect was more pronounced when FYM was applied along with fertilizers (Singaram and Kamalakumari 1995). They concluded that addition of inorganic fertilizers for over 20 years would not cause any detrimental effect on the enzyme dynamics of the soil. Manna et al. (1996) studied the influence of FYM on dynamics of microbial biomass and its turnover and activities of enzymes under a soybean wheat system. They observed that the microbial biomass contents of the soil were significantly higher under soybean than under wheat crop. All the three enzymes were positively correlated with microbial biomass N and P, and there were also significant correlations between soil organic carbon and biomass C and N and dehydrogenase activity. High organic carbon may support higher microbial populations that lead to higher concentration of various enzymes such as dehydrogenases, ureases, and phosphatases (Druakumar et al. 1992). These soil enzyme activities may serve as useful indicators for changes in the biology and biochemistry of the soil due to external management and environmental factors (Dick 1994). Singaram and Kamalakumari (1995) reported that the addition of inorganic fertilizer over 20 years would not cause any detrimental effect on the enzyme dynamics of the soil. The effect was more pronounced when FYM was applied in combination with fertilizers.

Phosphatases reveals about the wide spectrum of intracellular activities and soil accumulated as well which helps in the catalytic reaction during the hydrolysis of esters and anhydrites of phosphoric acids (Spier and Ross 1978). Organic matter acts as the main source of organic P in the soil. The phosphatase activity was significantly related to the amount of phosphorus in the lipid and nucleic acid fraction (Rzesniowiecka-Sulimierska et al. 1984). Phosphatase activity was significantly correlated with organic carbon, organic phosphorus, and bacterial population but has a negative relationship with pH (Chhonkar and Tarafdar 1984). The regression of phosphatase activity on phosphorus showed that pH of 7.0 is optimal for phosphatase activity in the soil. Halstead and Sowden (1986) in their study on the effect of various types of organic matter added for over 20 years noticed that in comparison with the untreated plots, the organic matter-treated plots showed increased phosphatase activity in soil, but its effect on soil phosphorus was less consistent. Phosphatases also assist in release of inorganic P from organically bound P returned to soil as litter and other organic debris (Druakumar et al. 1992). Kirchner et al. (1993) noticed higher activities of alkaline phosphatase, aryl sulfatase, and beta-glucosidase enzymes in green manured soilscompared to N-fertilized soils. Iqbal et al. (1998) reported that the activity of phosphatase was negatively correlated with pH and inversely correlated with electrical conductivity. The activity had direct relationship between organic matter, nitrogen, and bacterial population in both soils. Oberson et al. (1993) reported a correlation between the phosphatase and residual organic P. The urease activity of the soil may be correlated with the abundance of microorganisms present in the soil (Anderson 1962; Chin and Kroontje 1963). Ureases are of much importance for urea hydrolysis and for performance of urea over other ammonical fertilizers as urea is commonly used in modern agriculture. Much of its activity is found to be in the free state of the enzyme (Kiss et al. 1975). In soil the activity of urease appears to be correlated in general with the number of microorganisms and increased with increasing organic matter. The maximum activity in most soils is found at pH 6.5 to 7.0.

Petit et al. (1976) reported that stability of urease under conditions which rapidly inactivate jack bean urease is controlled by enzymatically active organic extract. Urease enzyme catalyzes extracellular nutrient-mineralizing reaction breaking down the urea into CO_2 and ammonia, hence important in N-economy of soils (Skujins 1976). Mineralization of urea in some soils found good correlation with the microbial population and organic matter content of soil, urea, and moisture. They also observed that hydrolysis of urea was more in acidic soils with higher organic matter than in neutral and alkaline soil. Balasubramanian et al. (1972) observed appreciable increase in urease activity in soil due to application of maize stalk or pongamia cake at 1% organic carbon level. Pancholy and Rice (1973) noted that the activity of urease, phosphatase, and dehydrogenase was related to the type of vegetation and quality of organic material in the soil. Vinodkumar and Waget (1984) reported from an incubation study that urease activity ranged from 19.0 to 26 μg nitrogen hydrolyzed /g of soil at 37 °C. Increase in nitrogen level, temperature up to 35 °C, and moisture content up to field capacity linearly increased urease activity of soil and decomposed organic matter. Nannipieri et al. (1983) reported a higher activity of phosphatase and urease, which coincided mainly with the increase in bacterial biomass and with rapid immobilization of labeled N. Kandeler et al. (1994) reported increased activity of urease at all soil depths as a result of slurry amendment. Bhattacharya et al. (2001) found that application of municipal soild waste significantly enhanced the microbial biomass C, soil respiration, urease and phosphatase activity of the soil. In another study, the changes in activity of soil enzymes like ureases, dehydrogenases, and cellulases were maximum for organic amended soils than unamended soils, and also there was no consistent trend with study intervals. However, cellulase activity was not significantly affected due to application ofthese organic residues (Sajjad et al. 2002).

10.12 Conclusion and Future Prospects

Organic manures play an important role in maintaining the nutrient status and soil health. With the need for quality products, there has been an implication to address qualitative traits, which in turn influence soil microbial biomass and thus depict the soil health. Agro-based industrial wastes and urban solid wastes are composted to recycle the nutrients in soil ecosystem and also to control a wide range of plant pathogens. Application of organic manures significantly reduces the environmental pollution which is the need of present era. The studies delineate the importance of quality organic manures that are to be enriched with beneficial microbial isolates for the wholesome development of soil and plant health which will ultimately help in meeting the food demand of explosive population. There is need to explore the possibility of bioconversion of organic matter for larger land coverage and to relieve the environment from the attack of various plant pathogens. Since the organic matter (raw materials) is derived from diverse sources, the decomposed product also contains a proportion of specific chemical substance of the raw materials used. Such organic manures applied to the soil will have either stimulatory or antagonistic properties on the soil microorganisms. The study of population dynamics and soil microbial biomass turnover under these different organic manures is important to understand the role of these organic manures in soil microbial interactions.

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Chapter 11
Strategies for the Management of Soil-Borne Pathogens and Crop Production Under Saline Environment

M. I. S. Safeena and M. C. M. Zakeel

Abstract Total agricultural land of the globe becomes insufficient due to the progressive nature of primary and secondary salinity. High salt content in the irrigation water or soil is a serious restriction factor to the cultivation of many crops. Salinity has significant influence in maintaining the balance nature of osmosis, the availability of water and nutrients, and the formation of free radicals in plant. The consequence of these factors causes undesirable effect on photosynthesis, growth, and development of numerous economically important plants. This review evaluates the management practices or strategies based on the combination of approaches through management of soil-borne pathogens, root-zone salinity management, quality irrigation, and cultural practices to accelerate the removal of salts and cultivation of salt-tolerant plants. Root-zone salinity is mainly controlled by different irrigation systems and leaching and by the use of appropriate plants to maintain the water table. There is a diverse strategy that can be applied through quality irrigation and cultural practices. Different modes of irrigation, tillage, and bio-drainage, addition of organic matters and gypsum, and application of sulfur are some of them. However, growing salt-tolerant plants along with the traditional methods of managing the saline environment take a momentum to reduce the effect of high salinity. Genetic engineering approach through the deep understanding of physiological response of plants to salinity would augment the identification of potential gens for developing transgenic plants. Application of microbes, organic matters, and green remediation also has proved the improvement of plant health and productivity under salinity and biotic stress. These management strategies provide an insight to the effective crop production under saline environment.

M. I. S. Safeena (✉)
Department of Biological Sciences, Faculty of Applied Sciences, South Eastern University of Sri Lanka, Sammanthurai, Sri Lanka
e-mail: safeenim@seu.ac.lk

M. C. M. Zakeel
Department of Plant Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka, Anuradhapura, Sri Lanka
e-mail: zakeel@agri.rjt.ac.lk

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11.1 Introduction

Obtaining adequate and virtuous water source is an essential factor for agriculture to produce food for the rapidly growing population in the world. This situation will be deteriorated when there is an arable land exaggerated with salinity. Seckler et al. (1999) has stated that nearly 1.5 billion population of the world will demand for all categories of diet from the water shortage and saline-prone land by the year 2025. The amount of irrigated land is shown in Table 11.1 for various regions of the world. Nearly 60% of irrigated land is located in Asian countries where population is rapidly rising with limited land for domestication. Accordingly, salinity in irrigation water and cultivating soil will unquestionably affect the food production. Umali (1993) and Epstein et al. (1980) have revealed that nearly 8–12% of the irrigated land or one billion hectares of the world's land is affected by salinity. Accumulation of salts in soil occurs through weathering of parent minerals or from the unsuitable management practices applied to the land and water resources (Qadir and Oster 2004). Although potassium, calcium, magnesium, and sodium salts are important ingredients for higher yields, the presence of excess amount of salts more than required amount causes the reduction of water intake, dehydration of plants, and finally death of plants. The accumulation of salts in surplus quantities in agricultural land is recognized as a significant factor for the reduction of crop yields in many parts of the world. In addition, irregular irrigation practices, low rainfall, high evaporation, high salt contents in groundwater, and intrusion of sea water are some reasons for the occurrence of salinity. Among the soluble salts, accumulation of sulfate (SO_4^{-2}) and chlorides (Cl^-) in the soil enhances the electrical conductivity (Ahmad and Qadir 1995). When electrical conductivity at 25 °C is higher than 4 dSm^{-1}, the soil becomes saline in nature with pH of <8.5 and 15% of exchangeable sodium percentage (ESP) (Tyagi 2003). Some soils may have both saline and alkaline

Table 11.1 Irrigated areas (millions of acres) of the world by region

E. Asia + S. Asia	131
W. Asia + N. Africa	22
Former USSR	21
North America	19
Europe	17
L. America + Caribbean	16
Sub-Saharan	3
Oceania	2

Adapted from Samad et al. (1992)

characters. As a result, both pH and electrical conductivity are high in these soils. Irrigated agriculture consumes nearly 70% of total water to produce around 36% of global food (Howell 2001). Growth and carbon metabolism of several crops are adversely affected under high salinity because of water deficit, osmotic effect, oxidative influence, and disproportion in nutritional availability (Kim et al. 2008). Salinity causes abiotic stress in plants in different degrees. Sensitive crops such as pulses and some special crops are mostly affected with the substantial reduction in growth and yield. Hence, salinity plays a major role in economy and sustainability of food production, and it is very much concerned especially relevant to cultivating land and irrigation water (Beltran 1999; Andriolo et al. 2005; Ünlükara et al. 2008). Many strategies are available for managing crop production in salinity-affected lands. The improvement and sustainability of food production under salinity can be intensified through two foremost approaches: adapting the environment to suit the plant and adapting the plant to suit the environment. This could be used either single or in combination (Tyagi and Sharma 2000; Tyagi 2003).

11.2 Effects of Salinity on Soil

Both visual observation and chemical analysis help to identify saline lands. The presence of white salt crust on dry soil surface is a common feature in saline lands. The prevalance of high amount of salt in soil forms hard columns of salt deposition inside when soil is exposed to prolonged dries and such depositions do not permit the penetration of root to deeper surface. Hence, the availability of volume of soil to grow, water, and nutrients become limited. There are some plant species which can withstand under high salt condition. Abundance of these plant species help to identify salt-accumulated lands. Chemical analysis of soil for cations is the easiest way of identifying saline soil (Aslam et al. 1993). When soil becomes saline or alkaline, most of the exchangeable sites are filled with cations, such as calcium, sodium, and magnesium. As a result, important nutrients such as potassium are not retained at required quantities. Consequently, soil fertility is declined and hinders the yield. Accumulation of sodium ions in the exchangeable sites caused soil particle dispersion and less porosity.

Major forms of soluble salts in the inland areas such as sodium carbonate and sodium bicarbonate cause a considerable enhancement in the soil pH. A hard layer of $CaCO_3$ and light-textured subsoil layers are prominent in saline soils having high quantity of calcium. The salinity is quantified in terms of electrical conductivity (EC), mg l^{-1} (p.p.m) or meq l^{-1}. This reflects the amount of different types of salt dissolved in soil water. The higher EC of the soil extract reveals the significant quantity of dissolved salt in soil water, and total dissolved solids (TDS) are expressed in mgl⁻1. Quality of irrigation water, extended rate of evaporation from soil surface, and nature of drainage system may also cause variation in EC of the soil (Focht 1979; Handawela 1982; Dulanjalee and Pitawala 2008).

11.3 Effect of Salinity on Crops

Increasing level of soil salinity influences the osmotic pressure of soil solution which negatively impacts availability of water to plant for growth and development. Generally, the effect of salinity on yield is considered for individual crops, but in actual practice it is cropping system (Tyagi 2003). Germination process is initially affected by soil salinity under field condition. The reduced germination is subsequently having an effect on initial growth of plants which results smaller plants with lower leaf area for photosynthesis. Several studies reveal that the either cumulative or interaction of number of factors relevant to plant, soil, and environment, such as the rate of evaporation, concentration of soluble salt, different categories of soil, precipitation, water table conditions, type of crop, and water management practices, determines salinity buildup in the soil and crop performance. Accumulation of higher amount of sodium and chloride in plant cell causes destruction of plant cells due to Na^+ and Cl^- toxicity. Plant cells damaged by salt accumulation resulted in low chlorophyll synthesis, reduction in yield, and poor growth of apical buds due to less availability of Ca^{2+} (Munns and Tester 2008). High osmotic pressure in the saline soil creates physiological drought; hence physiological activities are disturbed which leads to a significant nutrient deficiency for the plant. Plants exposed to the stress show early signaling responses to the high salinity by ceasing the growth. Ionic salts are transported to the cell through non-specific ion channel in the plasma membrane and triggering sequence of changes in the biochemical and physiological activities of plants. During this, plants slow or cease the cell division and growth and produce stress hormones to manage the stress persuaded by salt ions (Rameshwaran et al. 2015). Magdalena and Christa (2015) have stated salt stress disrupts the cell organelles of an individual plant to different degrees. At later stage, plant show slight recovery of growth as salt ions are pumped into vacuoles and storage organs. Salt-stressed plants show variation in morphological architecture compared to nonstressed plants during the same growth period. This is obvious through a number of studies which suggest that the ability of plants either to cope with stress or be tolerant is really due to the changes in growth morphology. Increasing concentration of salt above the maximum tolerance level of crops sharply decreases the yield, and the reduction of yield of different crops may differ depending on their capability for tolerance. Salt tolerance ability of crops vary with different growth stages. Kim et al. (2016) have found that the continuous irrigation with saline water caused observable modifications in crop yields. The commonly recognized soil salinity levels for different crops is shown in Table 11.2. Some crop like *Helianthus tuberosus* L. (Artichoke) is moderately sensitive at threshold EC of 0.4 (Newton et al. 1991), while *Brassica napus* L. (canola or rapeseed) is tolerant at EC of 11.0 (Francois 1994). This observation is according to the tuber and seed yield of artichoke and canola, respectively. An EC of 4 is a general salinity rating for many traditional annual crops. McKenzie (1988) has proposed another type of rating system to assess the salinity level as follow:

1. Nonsaline (0–2 dS/m)
2. Slightly saline (2–4 dS/m)

Table 11.2 Variation in salt tolerance ability of some crops (grains, special crops, vegetables, fruits, and woody crops)

Crops	Salt tolerance parameters			
Botanical and common name	Tolerance based on	Threshold (ECe) dSm^{-1}	Rating[a]	References
Crambe abyssinica Hochst.	Seed yield	2.0	MS	Francois and Kleiman (1990)
Secale cereale L. rye	Grain yield	11.4	T	Francois et al. (1989)
Sorghum bicolor (L.) Moench sorghum	Grain yield	6.8	MT	Francois et al. (1984)
Psidium guajava L. guava	Shoot and root growth	4.7	MT	Patil et al. (1984)
Citrus sinensis (L.) Osbeck orange	Fruit yield	1.3	S	Bielorai et al. (1988)
Solanum melongena L. eggplant	Fruit yield	1.1	MS	Heuer et al. (1986)
Vigna radiata (L.) R. Wilcz. Bean, mung	Seed yield	1.8	S	Minhas et al. (1990)
Portulaca oleracea L. purslane	Shoot yield	6.3	MT	Kumamoto et al. (1992)
Lycopersicon lycopersicum var. *cerasiforme* (Dunal) Alef. Tomato, cherry	Fruit yield	1.7	MS	Caro et al. (1991)
Cucurbita pepo L. var. *melopepo* (L.) Alef. Squash, zucchini	Fruit yield	4.9	MT	Graifenberg et al. (1996)

[a] *S* sensitive, *MS* moderately sensitive, *MT* moderately tolerant, *T* tolerant

3. Weakly saline (4–8 dS/m)
4. Moderately saline (8–15 dS/m)
5. Strongly saline (>15 dS/m)

11.4 Management of Crop Production Under Saline Environment

The world will need a large quantity of food for the fast-increasing population with limited and marginal cultivated land around the world. One of the main causes for the limitation is the rapid changing of soil due to salinity. These types of lands require effective and efficient management systems to increase the cultivation and yield.

It is very obvious that the management practices of single system or technique is not adequate to solve the problems associated with the saline environment. Integrated approaches such as application of modern biology or molecular biology to produce salt-tolerant plants and traditional soil and landscape manipulation are essential to be practiced to improve and sustain the productivity of the lands with high salt. The combination of approaches can include root-zone salinity management, quality irrigation, and cultural practices to accelerate the removal of salts and cultivation of salt-tolerant plants (Rains and Goyal 2003; Goyal et al. 1999; Kaffka et al. 1999; Zeng et al. 2001; Asraf and Akram 2009; Sirisena et al. 2011).

11.4.1 Root-Zone Salinity Management

Several management strategies are practiced to regulate the crop root-zone salinity during the application of saline/alkaline water for irrigation (Boumans et al. 1988; Chandra 2001). They include important and restriction practices such as controlling the flow of saline water, meticulous leveling mostly with lower water table and high frequency irrigation, mixed use of different quality of water in diverse modes, avoiding the sensitive stage of growth for salinity by scheduling the irrigation period, chemical amelioration, etc. Reclamation of saline and saline-waterlogged soils needs drainage for evaluating excess water and salts from the crop root zone. The surface and subsurface drainage systems are considered much during the reclamation of saline soils. Minhas and Gupta (1992) have suggested that subsurface drainage technology has provided boost to control water logging and associated soil salinity by maintaining water table below a specific depth. Tyagi (2003) promoted the usage of brackish water by introducing manipulation of subsurface drainage and water table during a situation like prevailing of high water table with saline water. Salt tolerance crops having the ability of late maturing and deep rooting would be a worthy choice to benefit and lower the water table by utilizing the saline groundwater according to the nature of soil (Goyal et al. 1999). It has been proven through many studies that among the crops, alfalfa is showing the greatest performance not only to help lower the water table but also to be used as a part of a cycle of cultivation or as a long-lasting water barrier when it is necessary to control the flow of salt water from one soil to another (Brown and Hayward 1956; Bernstein and Francois 1973). Similarly, in addition to the supply of nitrogen for the next crop as green manure, sweet clover also helps lower the water table (Gismer and Gates 1988). However, a proper management must be followed to avoid yield reduction in the consequent season due to the water use by sweet clover. Superficial tillage is appropriate or recommended than plowing, when green manures are applied. Hence the salts are not returned to the surface. Therefore, management practices using plants to help lower the water table should be viewed as a long-term implementation plan in respect to root-zone salinity and a neither quick nor permanent renovation technique.

11.4.2 Quality Irrigation and Cultural Practices

It is apparent that good quality irrigation water is important to cut down the salt accumulation and water having EC below 0.5 dSm^{-1} is considered to be a good quality. However, a direct application of saline water is possible if plants grow well, giving acceptable yield and no adverse effect on soil. Boumans et al. (1988) reported that minimal quality water (EC of 4–6 dSm^{-1}) has been applied to different cultivation lands to get acceptable yield. As an alternative measurement to avoid the severe effect of salinity, fresh water and saline water can be used in both methods: blending and cyclic modes (Tyagi 2003). Several factors determine the favorable blending mode such as adequate availability of fresh water, salinity status of other types of water, categories of the crop cultivated, and marginal reduction of acceptable yield to be considered (Tyagi 2001). Many advantages can be obtained using cyclic mode which is a common method in multi-quality irrigation practices (Rhoades et al. 1992). Under the successive use of cyclic mode, both fresh and saline waters are alternatively applied to the field according to a preplanned calendar. Occasionally, there is an inter-seasonal switching of application of fresh water and saline water in different periods of cultivation (Sharma and Rao 1996). A number of researches have recommended that saline irrigation water consisting high amount of salt above the appropriate values can be used effectively for cultivation of several crops for at least 6–7 years without significant loss in the yield. Though there is a great uncertainty prevailing about the prolonged effects of these practices, it should be concerned that some special improvements or treatments are needed along with poor-quality water irrigation compared to good-quality water irrigation in order to maintain satisfactory yield level. The yield is determined not only by the prevalence of salinity in the groundwater but also, it depends on other water sources available to the crop in the form of rainwater, river etc. (Sharma et al. 2001). Sharma et al. (1993) found that pre-sowing irrigation through blended or sequential modes is influencing the final yield because the seeds germination and seedling stage of many crops are more sensitive to salinity. Hence, pre-sowing irrigation avoids early stress exposure and retains crop stand. Therefore, the effect of salinity and its influence on final yield can be reduced by applying some certain techniques such as crop sequencing or crop rotation, crop substitution, cultivating under favorable season, maintaining precision level of land with adequate drainage system, and conserving rainwater for irrigation. Deep plowing can be avoided on saline soils since it will bring salts up to the soil surface. Zero plowing should be considered for strongly saline soils. For sodic soils, deep tillage may be beneficial to break up the hardpan and improve infiltration, as well as to bring any calcium salts present in the subsoil to the surface. A field investigation should be conducted before attempting deep tillage (Gurung and Azad 2013). Adding of carbon-based materials will avoid the surface drying of soil surface rather than decreasing salinity. It will also improve water-holding capacity and permeation, cation exchange capacity of soil as such more plant nutrients are absorbed, and drainage capacity; as a result, washing of unnecessary salts are possible (Qadir and Oster 2002; Qadir and Schubert 2002; Qadir et al. 2007; Murtaza et al. 2009; Murtaza et al. 2013). Green manure, animal manure, charcoal, and rice straw can be used as organic manure. In addition, sodium absorption ratio of soil can be reduced by leaching of salts using monsoonal rains or inundating land with irrigation water. Amount of water required for this purpose depends on the salt contents in the soil and the leaching depth. Salt concentration and quantity of the input water should be lower than the output water to have successful results by reducing accumulation of salts in the lower part of the land through leaching (Minhas and Bajwa 2001). Regardless of all the progressive points related with drainage of salt-affected areas, the drainage technology could gain momentum with the possibility of bio-drainage (growing high water transpiring trees) in waterlogged saline areas, mainly along the irrigation canals (Jeet Ram et al. 2008; Chaudhari et al. 2012a, b).

Applying gypsum to soil will be more effective when it is rich with sodium ions. Gypsum is the source of calcium which is enabling to exchange with excess sodium present in the soil (Raza et al. 2001; Chaudhry et al. 1984; Haq et al. 2007). The cost efficiency of different amendments was explored by Ghafoor and Muhammed (1981) and concluded that gypsum is much economical than the other treatments. Hussain et al. (2000a) have assessed the success of gypsum application and soil ripping to manage saline-sodic soil irrigated with brackish groundwater. The crop yield data of the study has showed that gypsum application in combination with ripping had considerable additional effect than either of the two treatments alone. Addition of equal amount of gypsum to the soil to the content of sodium in the irrigated water evidenced relatively a minimum effect on the crop yield when compare to the similar study conducted with normal soil irrigated with brackish tube well water (Hussain et al. 2000b). Application of sulfur helps to lower the pH of saline soils. Soil microorganisms oxidize the sulfur into sulfuric acid, and H+ ions and sulfuric acid replace the sodium ions. Kahloon and Gill (2003) have stated that sulfurous acid generator can be used to treat the saline water. However, economically this technology was much more costly due to initial cost of the generator and cost of sulfur, and it's burning as compared to other saline water management options (Zia et al. 2006).

11.4.3 Use of Salt-Tolerant Crops

There is a wide-ranging variation in functions among the same and different species of plants when they are exposed to salt stress (Glenn et al. 1999). Therefore, the use of genetically modified salt-tolerant crops would be one of the strategies to improve the productivity under saline environment. However, it is recommended to use the genetically manipulated salt tolerance crops along with the traditional approach of managing the saline soil and water (Epstein 1985). Naturally many crops show different levels of tolerance to salinity; among them canola, rye, oats, millet, sugar beets, cotton, and barley are considered to be the most tolerant crops. Insight understanding of physiological response of plants to the salinity will enlighten the role of potential genes for stress tolerance (McNeil et al. 1999; Epstein and Rains 1987). Gong et al. (2001) has made available a list of genes from sensitive mutants that are regulated by salt stress. In addition, several genes have been identified that affect transmembrane transport channels in membrane including the gene controlling the function of calcium in transport system (Yermiyahu et al. 1994; Lui and Zhu 1998). Zhang et al. (2001) have produced salt-tolerant transgenic plants *Brassica* and tomato which can accumulate increased sodium in vacuoles and foliage, respectively (Zhang and Blumwald 2001).

11.5 Application of Microbes and Green Remediation in the Alleviation of Soil Salinity, and Management of Soil-Borne Pathogens

11.5.1 Disease Suppression Through Microbial Agents Under Saline Environments

Rangarajan et al. (2003) reported that indigenous *Pseudomonas* strains suppressed sheath blight of rice by 19 to 51%. Four strains of *Pseudomonas* suppressed the disease of bacterial leaf blight and sheath blight of rice of which three were potential candidate under natural as well as saline environments. Best performed candidate under saline environments was MSP538 from the site which reduced the bacterial leaf blight by 82%, and this strain was identified as *P. putida*. They further propounded that the level of disease suppression of five of the strains increased marginally under saline conditions. Local strains are more potent in the inhibition of the stress as compared to introduced ones. The efficiency of such strains may also be augmented considerably (Gopalaswamy, 2001).

Application of some potent strains such as MSP377 (*P. fluorescens*), MSP497 (*P. putida*), MSP504 (*P. fluorescens*), and MSP573 (*P. fluorescens*) was tested against bacterial leaf blight and sheath blight. It was seen that these bacterial agents can suppress both bacterial leaf blight and sheath blight diseases under nonsaline and saline soil environments. These isolates possess great potential for the acceleration in the development of biological control agents to be used in coastal environment (Egamberdieva 2012).

Pseudomonas spp. use direct antagonism, production of several metabolites with antimicrobial activity toward fungi and cell wall-degrading enzymes to show the biological control activity (Haas and Keel 2003). *Pseudomonas chlororaphis* produces antibiotics, inhibitory molecules against proliferation of *Helminthosporium solani* (Assis et al. 1998; Chin-A-Woeng et al. 1998; Martinez et al. 2006; Carlier et al. 2008). Moreover, *P. chlororaphis* TSAU13 showed some antagonistic impacts on a wide range of plant-pathogenic fungi like *F. oxysporum f. sp. radicis-lycopersici*, *F. solani*, *Gaeumannomyces graminis pv. tritici*, *Pythium ultimum*, *Alternaria alternate*, and *Botrytis cinerea* (Egamberdieva and Kucharova 2009). *P. chlororaphis* TSAU13 strain was responsible for the enhancement of plant growth and yield of cucumber and tomato due to their competitiveness and persistence under saline soil environment. Moreover, *Bacillus* UW85 has been found to suppress the soil-borne plant due to the production of the antibiotics zwittermicine and kanosamine (Handelsman and Stabb 1996; Milner et al. 1996a, b).

11.5.2 Soil Salinity Removal Through Microbes and Organic Additives

Abiotic stresses usually work together with biotic stresses. For example, a crop that was grown under saline condition frequently shows more vulnerability to attacks from insects, fungi, or mites, and a crop prone to these attacks appears greater sensitivity to salt stress. Naturally colonized and soil-borne beneficial microbes around the root zone facilitate nutrient absorption and maintain health of the root in the course of biotic and abiotic stress (Hashem et al. 2016; Ab-Allah et al. 2015). It has been revealed that above physiological processes can be further enriched by using the plant growth-promoting rhizobacteria (PGPR) as an enhancing and effective agent in plants such as *Pisum sativum* (Meena et al. 2015) and O*ryza sativa* (Yadav et al. 2014). Egamberdieva et al. (2017) have proved two such *Pseudomonas* strains that were tested on soybean (*Glycine max* L.); plants under saline condition and biotic stress (*Fusarium solani*) have performed significantly very well by stimulating and improving the growth, yield, antioxidant enzyme activities, and proline concentration. Similarly, the plant growth-promoting bacteria (PGPB) have shown promising effects in increasing crop tolerance to salinity by avoiding excessive absorption of sodium (Mayak et al. 2004). Ruiz-Lozano et al. (1996) have demonstrated that application of *Glomus* species of arbuscular mycorrhizal fungi to lettuce grown under saline condition improved photosynthesis, stomatal conductance, and absorption of mineral nutrients, minimized effect of salt stress, enhanced osmotic regulation, and had a direct effect on synthesis of plant hormone. Bioremediation is a one of the healthy approaches to maintain clean environment in the plant root zone and suppress the effect of stress to the plant for ultimate improvement of yield. This strategy is practiced through the biological control, improved soil infiltration, and leaching of excess salts from the root zone (Hamideh Nouri et al. 2017). Accordingly, both sterilized and non-sterilized composts and biochar have effectively remediated a saline-sodic soil leached with reclaimed water (Vijayasatya et al. 2015). The adaptation mechanisms of halophytes are maintained through salt accumulation, excretion, and exclusion processes which have made them to use as potential plants of "salt remover" rather cultivating salt-tolerant crops alone to manage the saline soil (Mirza Hasanuzzaman et al. 2014). There are many evidences of the use of halophytes in phytoremediation of salt-affected soil since they are environment-friendly, safe, and clean processes (Rabhi et al. 2008; Ashraf et al. 2010; Qadir et al. 2007; Ravindran et al. 2007). Hence, phytoremediation has shown an active amelioration of saline condition comparable to the chemical amendments that is involving with high cost cultivation and possibly creating polluted environment. In addition, halophytes are considered to be effective means to obtain salt-tolerance genes and gene regulatory sequences (Xiaoqian Meng et al. 2018; Mishra and Tanna 2017). The enhancement of soil quality through organic amendments is another technique to mitigate salts in soil in addition to the enhanced effect on crop production and plant health (Ansari and Mahmood 2017). Youssef Ouni et al. (2013) and Abdelbasset Lakhdar et al. (2009) have stated that application of composted municipal solid waste (MSW) and palm waste (PW) composts with high organic content and less inorganic pollutants could be an alternative and promising material to protect plants from adverse effect of saline soil. Many experiments have revealed that application of organic matters to the soil in the form of composted and uncomposted has significantly suppressed the diseases caused by a number of soil-borne pathogens such as bacteria, fungi, and nematodes (Nuria Bonilla et al. 2012; Bailey and Lazarovits 2003; Aryantha et al. 2000; Hoitink and Boehm 1999). The variation in biotic and abiotic soil parameters and the interaction among many components of them would determine the pathogenicity and disease development by soil-borne pathogens (Alabouvette et al. 2004). However, depending on the pathogenicity and type of organic amendments, there will be variable responses in suppression of biotic stress (Weller et al. 2002). In addition, the plant also plays a significant role in this phenomenon, and the stimulation of plant protection mechanisms could be a vital factor of compost suppressiveness (Hadar 2011; Yogev et al. 2010). Reigosa et al. (2002) have revealed that the allelopathy plays a major role when signal receptors of plant are much affected by severe biotic and abiotic stresses. The tolerance/resistance to the stress conditions would improve either by increasing or decreasing the threshold level of concentration of allelochemicals (Einhelling 2004). Plants which are under stress environments show higher allelopathic activity not only due to increasing concentration of allelopathic compound but also by maintaining correct composition of them (Bais et al. 2004; Gouinguené and Turlings 2002).

11.6 Conclusion and Future Prospects

The progressive nature of high salinity which affects the cultivating agricultural land can be minimized through a number of management strategies. Rather considering an individual crop, many times we have to view in the way of cropping system when some management strategies are applied to control salinity. The combination of strategies such as root-zone salinity management, quality irrigation and cultural practices, and cultivating salt-tolerant plants will provide the new avenues for the sustainable crop production under different saline environments. In addition, the application of microbes and green remediation techniques through the addition of organic matters, bioremediation, phytoremediation, and allelopathy have shown promising effect in alleviating the effect of salinity and managing biotic stress to improve the crop production.

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Chapter 12
Significance of Botanicals for the Management of Plant Diseases

A. Sajeena, Jacob John, B. Sudha, A. V. Meera, and S. R. Karthika

Abstract Thirty six per cent of the crop production is lost annually due to diseases, pests and weeds under field conditions and 14% during storage. Pathogenic fungi are one among the major causes of crop loss during various stages of crop growth including postharvest. Though chemical pesticides should be the last resort in crop disease management, they are still continuing as the most dominant component of disease control. Non-judicious use of pesticides will result in residual toxicity, environmental pollution, health hazards to human and other life forms, non-specificity, resurgence and high cost. Thus, the obvious threats due to synthetic fungicides have resulted in a rethinking for the search of safer alternatives. The use of botanicals and biocontrol agents for disease management is a vital area of research in present day. Naturally occurring plants possess several antimicrobial metabolites having less human adversities and environmental impact. They have been proved as apt substitutes for synthetic pesticides. Plants have several mechanisms to combat diseases caused by fungal pathogens, mostly by preventing physical contact between plants and pathogens as well as through innate defence mechanisms. However, only 10% of the total plants species worldwide have been investigated for their pesticidal activities. Development of plant-based formulations is an important step to achieve their economical and effective use as pesticidal agents. The future strategy should be to identify and select plants with antimicrobial potential, conserve them to obtain sufficient quantity and standardization of the methods for development of pesticidal formulations and utilize them for eco-friendly and safe disease management without endangering life forms and environment.

Keywords Anitfungal metabolites · Botanicals · Eco-friendly disease management

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A. Sajeena (✉) · J. John · B. Sudha · A. V. Meera
Integrated Farming System Research Station, Kerala Agricultural University, Trivandrum, Kerala, India

S. R. Karthika
Department of Plant Pathology, College of Agriculture, Kerala Agricultural University, Trivandrum, Kerala, India

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12.1 Introduction

On a global basis annually, a crop loss of about 36.5% has been estimated due to the attack of pests, diseases and weeds (Cramer 1967). Unpredictable climatic conditions and the capability of plant pathogens to withstand the adverse climate have also become the major factors for crop loss in the present scenario (Kumar 2013). Crop loss incited by insects as well as plant diseases accounts to nearly 36% under field conditions and 14% during storage. Hence, annually a total loss of 50% has been estimated for agricultural crops (Okwute 2012). Among the different factors resulting in crop loss, plant diseases play a pivotal role resulting in the maximum crop loss of 14.1% (Agrios 2004). In developing countries, preharvest and postharvest diseases are estimated to result in 10 to 15% loss of the already low yields (El Khoury and Makkouk 2010). According to Waddington et al. (2010), plant diseases and biotic stresses resulted in 3–14% and 16–37% yield decline, respectively.

Martinez (2012) reported that yield decline resulted by diseases plays a major role in crop disease management and postharvest storage. Plant diseases result in various direct and indirect losses such as reduction in the appearance and amount of farm produce, high production rate, health hazards, environmental pollution, which all adversely affect crop production, disturb the natural resources and leave no option other than less remunerative disease management options (Kumar and Saxena 2009). It has been estimated that more than one lakh fungal species exist which contaminate agricultural and food products (Kacaniova 2003). Pathogenic fungi adversely affect plants during various stages of crop growth from sowing to harvest including postharvest (Pal et al. 2013) and affect the quality, nutritive value, organoleptic characteristics and shelf life of fruits and vegetables (Agrios 2004). Fungal growth may also result in decreased germination percentage, discolouration of grains, loss in weight, adverse biochemical changes as well as production of toxins (Sinha et al. 1993).

Plant diseases can be managed by various techniques including the use of resistant varieties, cultural practices, physical measures and chemical biological control and biointensive approaches. These form the integral parts of integrated disease management practice. However, lack of adequate information on the genetic variability of fungal population and non-availability of appropriate markers as well as resistant donors are some of the limiting factors hindering breeding for resistance to diseases. Completely resistant varieties are present only against a few diseases but are lacking against the majority of diseases. Cultural and physical methods are not among the much adopted crop management measures. Disease management using fungicides, the last resort for plant protection, is still continuing as the predominant plant protection tool (El Khoury and Makkouk 2010). Despite the increased use of pesticides, crop losses have been reported to have increased further (Oerke and Dehne 2004; Kumar and Gupta 2012). Pesticides could result in an appreciably quick and evident disease management but with several serious consequences over the years. To add to this adversity, non-judicious use of pesticides has the consequence of resurgence and outbreak of secondary diseases (Oerke and Dehne 2004). The use of pesticides against postharvest decay has been studied to reveal the presence of chemical residues on or within the tissues of fruits and vegetables (Martinez 2012). The recently released new-generation fungicides have the disadvantage that they inhibit only one main site (organelle/cycle), making the plants vulnerable to resurgence of pathogens (Leadbeater 2012). The best example can be of strobilurins against which pathogens have developed quick resistance (Kumar and Gupta 2012; Kumar 2014). Thus, non-judicious use of pesticides can result in residual toxicity, environmental pollution, health hazards to human and other life forms, non-specificity, resurgence and high cost. Intensive use of synthetic fungicides has resulted in adverse effects on crops, beneficial organisms, predators and parasites. The most serious problem is the resurgence of pathogens, resulting in adversities in crop protection (Waard et al. 1993). Thus overuse of pesticides can ultimately result in reduced food production. The main emphasis of any disease management programme should be to prevent diseases from reaching economically damaging levels. The ultimate aim should be for the adoption of sustainable agricultural systems for preservation of natural resources and mother earth including its life forms (Kumar 2014). These obvious threats have resulted in a rethinking, and there has been an urge to search for safe alternatives to synthetic fungicides (Martinez 2012).

12.2 Plant-Based Fungicides

Plant disease management using botanicals and biocontrol agents forms a vital area of plant protection in present day to avoid environmental pollution. Biological control, besides being cost effective, is eco-friendly, does not leave any residual toxicity and can be successfully exploited in the framework of integrated disease management (Knight et al. 1997). Naturally occurring plants possess several antifungal compounds with low human and environmental adversities. Environmental friendly pesticides with least health hazards have become a major public concern (Martinez 2012). Use of plant based products in the management of plant diseases has recently gained popularity througout the world (Wedge and Smith 2006). Thus eco-friendly, effective and biodegradable biopesticides are the need of the hour (Zaker 2016). Plants having natural antimicrobial metabolites form one among the most desirable source for sustainable plant protection technique (Kim et al. 2002; Rai and Carpinella 2006). Bio fungicides include natural products and microorganisms. These include products from living organisms, selected plants and animals. Living organisms include fungi, viruses, bacteria, nematodes and protozoa (Copping and Menn 2000; Yoon et al. 2013). Compounds isolated from organism are the less accepted products, whereas natural plant products are the most favoured ones. Fungicides of plant origin are selective, biodegradable, less toxic and effective compared to synthetic fungicides (Yoon et al. 2013) and can serve as apt substitutes to synthetic fungicides (Knight et al. 1997). Besides, their eco-friendliness (Dwivedi and Singh 1998; Karnwal and Singh 2006) and cost effectiveness make them more convincible among the users. However, only 10% of the total plant species worldwide has been investigated for their pesticidal activities (Suprapta 2016). Many plant extracts have antifungal activities against plant fungal pathogens as obvious from their potential to prevent the fungal mycelium from advancing forward, spore formation, spore germination and biomass formation (Suprapta et al. 2001; Olufolaji et al. 2015; Sesan et al. 2015; Ikeura and Kobayashi 2015); Rongai et al. 2015); Darmadi et al. 2015).

12.3 Defence Mechanism in Plants

Crop plants have different mechanisms to combat microbial attack. They utilize several metabolites to defend themselves from the attack of various microorganisms (Martinez 2012). They fight against fungal attack through the formation of physical barriers as well as using chemical defence strategies. The most common means of their defence is through some preformed substances, viz. secondary metabolites (Martinez 2012) which are not necessary for their primary metabolic processes but which can ensure their survival in the ecosystem (Verpoorte 2000). Plant disease management can be achieved by the use of extracts of various plant parts as well as oils (Zaker 2016). Plant products possess fungicidal potential, disruption of fungal cell membrane, inactivation of various enzymes as well as metabolic processes (Aye and Matsumoto 2011). Plant extracts have special importance for the development of eco-friendly and safe strategy for plant disease management and several botanicals possess excellent fungicidal properties (Tewari and Dath 1984; Pramanick et al. 1998; Kandhari and Devakumar 2003).

The major compounds in plants responsible for fungicidal or fungistatic activity or which interferes with the pathogen development are phenols, flavanoids, quinones, terpenes, tannins, alkaloids, lectins, polypeptides, saponins, sterols, etc. (Scheuerell and Mahaffee 2002; Halama and Van Haluwin 2004). Martinez (2012) classified the plant secondary metabolites into three major groups, viz. preformed compounds, inducible preformed compounds and induced inhibitory compounds. The preformed compounds further include plant extracts, essential oils, phenolic compounds, hydroxycinnamic acids, flavonoids, plant growth substances as well as regulators, acetaldehydes and other volatile compounds, ethanol, hinokitiol, glucosinolates, latex and steroids. The induced inhibitory compounds include phytoalexins, PR proteins, active oxygen species, plant lectins, etc. (Martinez 2012).

12.4 Commercially Available Plant-Based Fungicides

Yoon et al. (2013) detailed some of the commercial available plant-derived fungicides and antifungal metabolites. They include cinnamaldehyde, L-glutamic acid + gamma-amino butyric acid, jojoba oil, laminarin, milsana, pink plume poppy powder extract, essential oils, fatty acids, phenolic compounds, alkaloids and glycosides (Table 12.1).

Table 12.1 Commercially available plant-based natural fungicides (Zaker 2016)

Product	Source	Controlled disease	References
Milsana	*Reynoutria sachalinensis* (giant knotweed)	Powdery mildew diseases of various important crops such as cucumber, tomato, apple, begonia and downy mildew of grapes, rust of beans	Daayf et al. (1995)
Carvone™	Dill and caraway seed	Pathogens of stored products	Moezelaar et al. (1999)
TomorexGold	*Melaleuca alternifolia*	Powdery mildew, downy mildew, rust, early and late blight in vegetables, grapevines and orchards	Zaker (2016)
E-rase™	*Simmondsia californica* (jojoba) oil	Organic farming	Zaker (2016)
Sporan™	*Rosmarinus officinalis* (rosemary) oil	Organic farming	Zaker (2016)
Promax™	*Thymus vulgaris* (thyme) oil	Organic farming	Zaker (2016)
Trilogy™	*Azadirachta indica* (neem) oil	Organic farming	Zaker (2016)
GC-3™	Mixture of cotton seed oil and garlic extract	Organic farming	Zaker (2016)
Bla-S™	–	Rice blast	Dayan et al. (2009)
Kasugamin™	–	Rice blast	Dayan et al. (2009)
Mildiomycin™	–	Powdery mildew	Dayan et al. (2009)
Delvolan™	–	Fungal diseases of ornamentals	Dayan et al. (2009)
Validacin™	–	*Rhizoctonia* root rot disease	Dayan et al. (2009)

12.5 Benefits of Botanical Fungicides

Yoon et al. (2013) reported that botanical fungicides have enormous potential. They prevent development of resistance, residue formation and also protect our environment. They have several advantages of being eco-friendly and self-degradable. Botanicals are important as far as the safety of the ecosystem is concerned (Adityachaudhury 1991; Ansari and Mahmood 2017; Ogobo and Oyibo 2008). Zaker (2016) proposed that botanicals are specific in their nature without adversely affecting biocontrol agents, having less residual effect and persistence. Botanicals are environmentally safe, commonly available and cost effective. They form a viable substitute to pesticides in integrated disease management programme (Kuberan et al. 2012).

12.6 Drawbacks of Botanical Fungicides

Martinez (2012) reported that botanicals do not possess the capability of inhibiting a wide range of pathogens and their do not have durable activity. Comparatively higher quantity of these products is required for their optimum activity. Okwute (2012) proposed that botanicals need to be processed for better activity, and this makes their production a costly affair. Gurjar et al. (2012) analysed that the various limitations in the use of botanicals include lack of standardized extraction methods, rapid degradation of their active metabolites, laboratory confined efficacy studies, presence of toxic chemical compounds and limited availability of plant-based formulations. Tewari (1992) reported that the major constraints in the use of botanicals include the lack of availability of botanicals in desired quantity, knowledge of its utilization, knowledge on the shelf life of plant extracts and their constituents, knowledge on seasonal and regional variations, etc.

12.7 Plants with Antimicrobial Activity

12.7.1 Common Plants with Antimicrobial Potential

Extracts of various commonly available plants exhibited antimicrobial properties against a wide range of plant pathogens. Steamed aqueous ethanolic extracts from *Achyranthes aspera*, *Annona squamosa*, *Thevetia neriifolia*, *Impatiens balsamina, Cassia fistula, Tagetes erecta, Momordica charantia, Antidesma* sp., *Aegle marmelos*, etc. adversely affect the growth of *Pyricularia oryzae* (rice blast), *Rhizoctonia solani* (sheath blight), *Drechslera oryzae* (brown spot) under laboratory and field conditions (Mitra et al. 1984; Michael et al. 1985; Tewari 1986). *Impatiens balsamina, Dioscorea rotundata and Avena sativa* were fungitoxic to *Pyricularia oryzae*, *Drechslera oryzae*, *Cladosporium* sp. and *Ophiobolus graminis* (Mitra et al. 1984). Schrickel (1986) reported that oats (*Avena sativa*) possesses allelopathic properties and is a better option for crop rotation so as to inhibit the growth and proliferation of soilborne diseases. According to Verma et al. (1998), *Clerodendrum aculeatum* leaf extract induces strong systemic resistance against virus attack in susceptible plants (Verma et al. 1995a, b). A systemic resistance inducing protein (SRIP) was identified as the basic protein with a molecular mass of 34 kDa (Verma et al. 1996). Cold water extracts of *Prosopis juliflora* followed by *Thevetia peruviana* prevented the development and multiplication of *R. solani*, the rice sheath blight fungus. The complete inhibition of sclerotial production is observed in extracts of *Caesalpinia pulcherrima*, *Eucalyptus globulus and Lawsonia inermis and in the hot water extracts of Calotropis gigantea, Ocimum sanctum* and *P. juliflora* at all the concentrations tested. The fungitoxicity of *C. pulcherrima* and *E. globulus* was not lost even after air drying and autoclaving (Kuruchev et al. 1997).

Glycyrrhiza glabra, *Rosmarinus officinalis, Avena sativa, Vaccaria pyramidata, Centaurea behen, Anagallis arvensis* and *Tribulus terrestris* possess antifungal activity against *R. solani*, *Fusarium oxysporum* and *Cochliobolus sativus* (Bahraminejad et al. 2011). *Tridax procumbens* followed by *Chromolaena odoratum* and *Allium sativum* effectively inhibited (40.04, 33.46 and 30.66%, respectively) the mycelial growth of *Cercospora beticola* causing leaf spot of palak in vitro (Poornima et al. 2011). In vivo studies revealed that *Allium sativum* was the most effective botanical exhibiting the lowest PDI (7.26%) followed by *Azadirachta indica* (7.83%), *Chromolaena odoratum* (8.4%) and *Tridax procumbens* (9.95%). Bahraminejad (2012) reported that among 97 plant species tested for their antifungal potential, 17 plant species showed inhibitory activity against *Pythium aphanidermatum* in vitro. Further, glass house experiments showed *Glycyrrhiza glabra*, *Portulaca oleracea*, *Centaurea behen*, *Alhagi camelorum* and *Verbascum* sp. extracts reduced the disease severity caused by *P. aphanidermatum* from 70% to 43%, 43%, 46%, 53% and 56%, respectively. No side effect of the extracts was observed on the sprayed seedlings. Extracts of *Cannabis sativa* (bhang) prepared in distilled water, acetone and methanol possess antifungal activity against *R. solani*, web blight fungus of urdbean. Field studies conducted under artificial inoculation conditions revealed that seed treatment of seeds followed by foliar application (3 times) of the extract resulted in the least disease severity, appreciable yield as well and maximum grain weight (Kumar and Tripathi 2012). Ademe et al. (2013) reported that ethyl acetate extracts of *Lantana camara* resulted in excellent antifungal effect on *Colletotrichum gloeosporioides* causing papaya anthracnose during storage in vitro. In vivo studies revealed that *Echinops* sp. (25%) was highly efficient in preventing the disease incidence and in maintaining the overall quality of papaya fruits.

12.7.2 Medicinal Plants with Antimicrobial Potential

Several medicinal plants besides their medicinal value exhibited antimicrobial potential against plant pathogens. Clove extracts inhibited the growth of *Aspergillus* and *Penicillium* species. Cinnamon inhibited three *Penicillium* species (Davidson 1997). The extract from *Aloe vera* was antifungal against pathogens affecting stored products harvested produce, viz. *Penicillium digitatum, P. expansum, Botrytis cinerea* and *Alternaria alternata* (Barkai-Golan 2001). The extracts of *Aloe vera* are antifungal to four pathogens affecting harvested produce, viz. *Penicillium digitatum*, *P. expansum*, *Botrytis cinerea* and *Alternaria alternata* (Barkai-Golan 2001). Suleiman and Emua (2009) stated that ginger and aloe were studied to completely inhibit the mycelial growth of *Pythium aphanidermatum* under laboratory conditions, but the effect prolonged only for a short period of time under field conditions. Tulsi extract inhibited *Fusarium solani* under laboratory conditions (Pandya et al. 2009). Ethanolic extracts of *Allium sativum, Azadirachta indica, Curcuma longa* and *Zingiber officinale* inhibited *Pythium aphanidermatum* under laboratory conditions (Singh et al. 2010). Ethanolic leaf extracts of *Cymbopogon citratus* possessed antifungal effect against *Rhizoctonia solani* affecting rice. Seed treatment of rice with ethanolic extract of *C. citrus* was observed to induce systemic resistance (two- to fourfold) against the fungus (Pal et al. 2011). Zimmu leaf extract also effectively inhibited *P. aphanidermatum* under laboratory and glass house conditions (Muthukumar et al. 2010). Chakraborty et al. (2012) observed that the maximum per cent of inhibition on the radial growth of *Phoma lingam* affecting *Annona squamosal* was recorded by the leaf extract of *Azadirachta indica* (76.27%) followed by leaf extract *Cymbopogon citratus* (70.97%) and bulb extract of *Allium sativum* (67.83%). Among 13 plant extracts, *Saraca asoca* completely inhibited the mycelial growth of *R. solani*, the rice sheath blight fungus under in vitro conditions (Divya et al. 2014). Garlic, eucalyptus, lemon grass, Gokhru and Van Tulsi significantly inhibited the mycelia and sclerotia of *R. solani* causing web blight of groundnut (Koma et al. 2014). Ravi et al. (2014) reported that the maximum inhibition (78.89%) in the mycelial growth of *R. solani*, affecting *Andrographis paniculata*, was recorded with garlic extract (15%) followed by thuja extract (75.56%) and tulsi extract (54.44%) after 48 hours of incubation. Negi and Kumar (2015) reported that 20% extracts of neem and garlic in vitro inhibited *Xanthomonas axonopodis* pv. *citrii*, the citrus canker pathogen. Five and ten per cent concentrations of *Azadirachta indica* (46.67% and 51.11%, respectively) significantly reduced the mycelial growth of *R. solani* f. sp., the causal agent of banded leaf and sheath blight of maize followed by *Datura stramonium* seed extract (45%) (Rajput et al. 2016).

12.7.3 Weeds with Antimicrobial Potential

Weeds should be judiciously used rather than undertaking their expensive eradication (Webber et al. 1999). Use of botanicals necessitates large-scale availability of their raw materials (Mdee et al. 2009; Aderogba et al. 2014). Weeds can be considered as better raw materials for botanicals (Aderogba et al. 2014). Choosing as raw material for botanical fungicides, the use of various weed species will be an eco-friendly method of disease control as well as a solution for weed management whereby the unwanted species can be economically used (Srivastava and Singh 2011; Rodino et al. 2014). Weedy plants can resist the attack of various plant pathogens (Pal et al. 2013). Dold and Cocks (2000) studied the possibility of development of pesticidal products from weedy plants.

Srivastava and Singh (2011) reported that the dried leaf powder (20 mg/ml) of two weeds, viz. *Lantana camara* and *Parthenium hysterophorus*, inhibited the mycelial growth (59.5% and 45.9%, respectively) of *Alternaria* species. Bahraminejad (2012) studied the potential of several plants against *Pythium aphanidermatum* and reported that most of the plant species (especially weeds) possess inhibitory effect on *Pythium aphanidermatum.* It can be concluded that weeding and returning the plant species to soil to retain soil healthy is a way to reduce the incidence of phytopathogenic fungi and will be effective in a long-term crop production programme. Pal et al. (2013) reported that the chloroform and methanol extracts of the weeds, viz. *Ageratum conyzoides*, and methanol extract of *Parthenium hysterophorus* exhibited fungicidal potential against *Alternaria* spp. Rodino et al. (2014) observed that 10% ethanolic weed (*Xanthium strumarium*) extract inhibited the mycelial growth (86.7%) of *Alternaria alternata*. According to Aderogba et al. (2014)weeds possess biologically active metabolites with the potential of plant pathogens inhibition. They reported that the acetone leaf extract of *Pseudognaphalium luteoalbum* had strongly inhibited *Aspergillus niger, Aspergillus parasiticus, Colletotrichum gloeosporioides, Fusarium oxysporum, Penicillium expansum, Penicillium janthinellum, Phytophthora nicotiana, Pythium ultimum.* The antifungal compounds, viz. hispidulin-7-o-glucopyranoside and stigmastero-3-o-beta-glucopyranoside from *Pseudognaphalium luteoalbum* inhibited the growth of selected plant pathogenic fungi. Rodino et al. (2014) observed that the ethanolic extracts of the common plants, viz. *Artemisia absinthium* (absinth), *Rosmarinus officinalis* (rosemary), *Datura stramonium* (jimson weed) and *Xanthium strumarium* (cocklebur), exhibited good antifungal properties against *Alternaria alternata*. Srivastava and Shukla (2015) reported that the locally available noneconomical weed plant, viz. *Ipomoea cairica* commonly found in waste lands, has a great pharmaceutical potential. It has many phytochemicals which are responsible for various pharmacological medicinal properties. The study revealed that the plant has a leading capacity for the development of new good efficacy drugs against many diseases in future. Distilled water leaf extracts of *Parthenium hysterophorus*, a weedy plant, inhibited appreciably the mycelial growth of *Sclerotium rolfsii* and *Alternaria brassicae*. The presence of antifungal compounds, viz. terpenoids, saponins, flavonoids, tannins and alkaloids, was detected in the extracts (Devkota and Sahu 2017).

12.8 Conclusion and Future Prospects

Several plants and their constituents have displayed strong toxicity against various disease-causing organisms and insect pests and hence proved their potential for pesticidal usage. Development of plant-based formulations is an important step to get economical and effective use of plant extracts as pesticidal agents. Various future challenges in the use of botanical pesticides are being standardized, so that application of plant extract not only in laboratory conditions but also under glass house and field situations could be ensured. The isolation and identification of active substances in plants responsible for antifungal activity is required to predict their mode of action and side effects. However, standardization of methods of extraction of antifungal agents from plant extracts is the utmost requirement to search new biologically active plant products. Thus, the future strategy in the use of botanical pesticides should be in the identification and selection of suitable plant materials with antimicrobial potential, conservation of the plants and ensuring their sustainable supply in desired quantity.

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