Chapter 11
Biocontrol of Plant Parasitic Nematodes by Fungi: Efficacy and Control Strategies

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11.1 Introduction

Nematodes are filiform roundworms belonging to phylum Nematoda commonly found in plants, animals, and soil. They have the ability to utilize the various organic sources for the production of energy (Akhtar and Panwar 2011). Some plant parasitic nematodes usually feed on plant cells by choosing and establishing a single feeding site known as sedentary feeders, while others are migratory feeders which means they move from site to site on the root and rarely feed on plant single cell. In general, the plant parasitic nematodes are documented as the utmost vicious

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pests for several economically important crops worldwide. Bowers et al. (1996) reported that the nematode had the ability to alter the root exudates in qualitative and quantitative fashion, which may influence the activity of beneficial and pathogenic microbes in the rhizosphere. The estimated average annual yield loss of various crops by plant parasitic nematodes is about 12.3 % (Sasser and Freckman 1987), but it varies from 8.8 to 14.6 % from developed to developing countries (Nicol et al. 2011; Palomares-Rius and Kikuchi 2013). Among the sedentary feeders, *Meloidogyne* species are predominant and are considered as the most damaging genera throughout the world. About 95 % of the total nematode populations are represented only by four major species such as *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla*.

Suppression of plant diseases in the presence of a pathogen, suitable host plant, and favorable climatic conditions is known as soil suppressiveness (Mazzola et al. 2004; Weller et al. 2007). It is directly associated with the nature and fertility level of the soil and the types of soil microorganisms. However, the level of disease suppressiveness is directly proportional to the level of soil microbial activity, meaning the larger the active microbial biomass, the greater the soil capacity to use carbon, nutrients, and energy, thus lowering their availability to pathogens (Kumar et al. 2012). Any treatment to increase the microbial activity in the soil enhanced the suppression of pathogens by increasing competition for nutrients, but overall it is a very tough task to control all types of soilborne pathogens by suppressive soils. To control the diseases caused by plant parasitic nematodes, frequent use of chemical nematicides has been increased in the past few decades globally (Gupta and Dikshit 2010; Leng et al. 2011). But these chemical nematicides possess several toxic effects on the human health, soil microbiota, and environment. Thus, several cultural practices have been adopted for the management of nematodes, but gradually the annual losses observed in the quality and quantity of crop yields revealed that there is a decisive need to develop a new eco-friendly way to control the plant parasitic nematodes. In this regard, biological control strategies provide an alternative tool for management of plant parasitic nematodes over the conventional chemical control strategies (Mazzola 2007). The biological control of nematodes could be achieved either by managing the natural habitats to marmalade by increasing the activity of native fungi or by introducing new beneficial rhizospheric fungi or by the combination of both (Timper 2011). Nevertheless, the augmentation of the beneficial microorganisms in the agricultural fields and their potential benefits on the various crops is feasible through the adoption of various management practices such as reduced tillage, crop rotation, and lowering the micronutrient uses.

The rhizosphere is the immediate microenvironment surrounding the plant roots which provides novel environments for microbes due to change in increased levels of nutrients and intense microbial population (Giri et al. 2005; Gupta et al. 2012; Yadav et al. 2015). The rhizoplane and the surrounding rhizosphere soil are colonized and occupied by a wide range of microorganisms. Of the various microorganisms present, opportunistic fungi and arbuscular mycorrhizal (AM) fungi play a key role in the biocontrol of diseases caused by plant parasitic nematodes. Consequently, the plant parasitic nematode and beneficial rhizospheric fungi share common ecological niche and also influenced the plant growth and yield attributes in various means (Akhtar and Siddiqui 2008; Akhtar and Panwar 2011). Because of multifaceted nature, it is very hard to generalize the overall underground interaction processes taking place between the plant parasitic nematodes, opportunistic fungi, and AM fungi. The aim of this chapter is to provide an overview of the biocontrol potential of opportunistic as well as AM fungi on the growth and improvement of various crop plants and population of plant parasitic nematodes in different pathosystems. The chapter also focuses on the cost-effective technologies used for the mass propagation of opportunistic fungi and AM fungi and their ample application in the expansion of practical control system desired for the sustainable agricultural practices.

11.2 Opportunistic Fungi

Fungi have the immense miscellany in their metabolic pathways and offer numerous important classes of commercial compounds having nematicidal activity (Li et al. 2007; Anke 2010) and limit the nematode densities by the production of nematotoxic compounds due to their parasites and antagonistic or predatory actions between fungi and plant parasitic nematodes (Lopez-Llorca and Jansson 2007; Akhtar and Panwar 2011). Lopez-Llorca and Jansson (2007) found that the opportunistic fungi either directly parasitize the nematodes or secrete some nematicidal metabolites which may affect the viability of one or more stages of the nematode life cycle or having deleterious effects on reproductive structures of a nematode. The secondary reproductive stage of the nematode is highly susceptible against the opportunistic fungi. The obese females are highly prone to fungal attack similarly like the parasitism of egg masses. The opportunistic fungi when come in contact with nematode eggs grow more rapidly and parasitize the eggs during initial embryonic developmental stages. This may reduce the parasitic actions of nematode juveniles. Among the various known opportunistic fungi, *P. lilacinus* and *P. chlamydosporia* have been extensively studied by several previous researchers for their nematophagous knack and biocontrol potentiality (Khan et al. 2004; Kiewnick and Sikora 2006; Siddiqui and Akhtar 2009a, b; Akhtar and Panwar 2011; Azam et al. 2013).

11.2.1 Paecilomyces lilacinus

Paecilomyces lilacinus (Thom) Samson is a mutual Hyphomycetes and is ubiquitously distributed especially in warmer climates (Samson 1974). It is encompassed in the group of frequently tested biocontrol agents against the plant parasitic nematodes (Brand et al. 2010; Pau et al. 2012; Azam et al. 2013). It is basically a saprophyte but could also compete for extensive range of substrates (Holland et al. 2003; Pau et al. 2012).

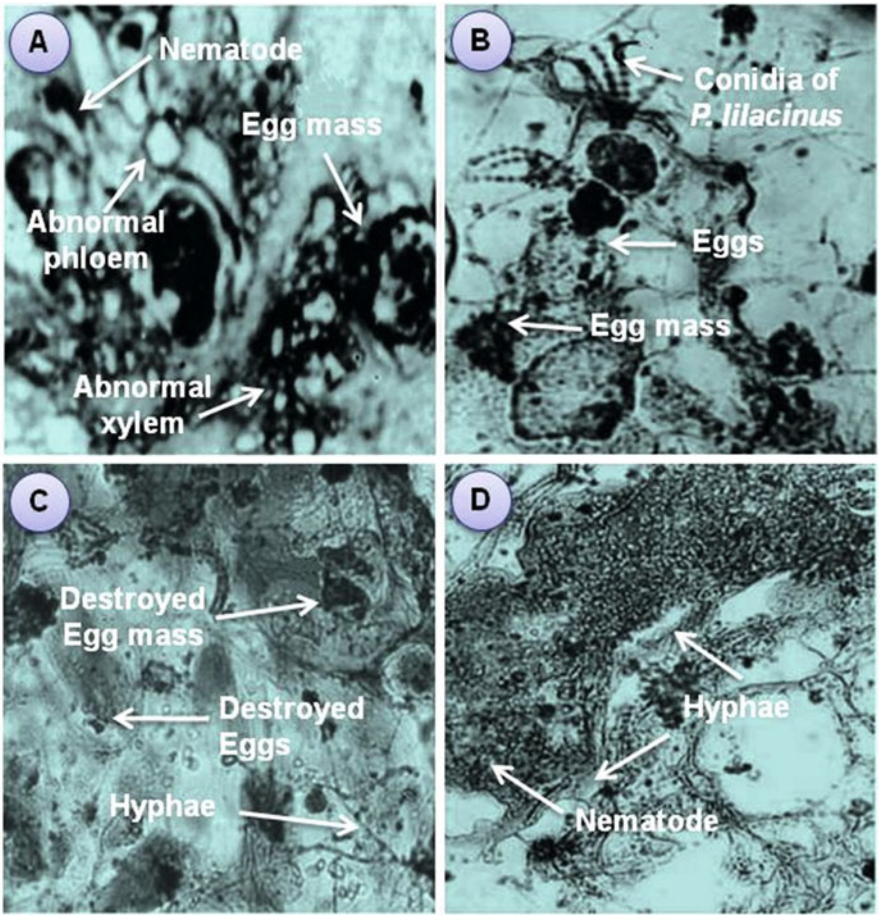

Fig. 11.1 Cross section of tomato root infected with root-knot nematode; (**a**) showing presence of nematode, egg masses, abnormal phloem, and abnormal xylem in the cortical region; (**b**) showing conidia of *P. lilacinus* surrounding the nematode eggs and egg masses; (**c**) disruption of eggs and egg masses by *P. lilacinus* hyphae; (**d**) complete disintegration of nematode eggs by *P. lilacinus* hyphae

Jatala (1986) reported that *P. lilacinus* infects eggs and females of plant parasitic nematodes and destroyed the embryo within 5 days under laboratory conditions. He found that the infection of nematode eggs starts in a gelatinous matrix with the development of fungal hyphae which latter surrounds the entire nematode eggs. The colonization of nematode eggs occurred through the diffusion of egg cuticle by the fungal hyphal network by enzymatic or mechanical actions. His experiments clearly indicated that *P. lilacinus* grow well between 15 and 30 °C. It also had the adaptability to grow in a wide range of soil pH which made it a pretty modest organism in most of the cultivated fields. The suppression of plant parasitic nematode by *P. lilacinus* is ascribed by disintegration of the embryo, inhibition of hatching, and parasitism of adult females (Fig. 11.1). However, after establishment of *P. lilacinus* in soil, it grows faster and spread rapidly within a short span in the introduced area as dominant species. Moreover, the production of secondary metabolites such as chitinases, leucinotoxins, and proteases has also been associated with *P. lilacinus* infection (Park et al. 2004).

11.2.2 Pochonia chlamydosporia

Pochonia chlamydosporia (Goddard) Zare and Gams is a well-known nematophagous fungus and ubiquitously distributed in all parts of the world. It is naturally occurring as a facultative parasite of females, eggs, cyst, and plant parasitic nematodes (Lopez-Llorca et al. 2008; Manzanilla-Lopez et al. 2013). In the rhizosphere, this fungus could settle the host root as endophytes preferably with the plants belonging to families Gramineae and Solanaceae and provide numerous benefits to host plant defense against the soilborne pathogens (Macia-Vicente et al. 2009a, b). *P. chlamydosporia* have been extensively studied for its biocontrol potential against plant parasitic nematodes (Kerry and Hirsch 2011; Manzanilla-Lopez et al. 2013). The efficacy of this potential biological fungus against the plant parasitic nematode is affected by three major factors: (1) the amount of fungus in the rhizosphere, (2) the rate of development of eggs in the egg masses, and (3) the size of galls in which female nematode develops.

The population of *P. chlamydosporia* could be identified on the basis of position and shape of conidia, the plethora of dictyo-chlamydospores, and the development of conidia either in heads or chains (Zare and Gams 2004). *P. chlamydosporia* infects the nematode eggs through the expansion of aspersoria at the tip or lateral position of hyphae, which encompasses tightly to the surface of eggshells (Fig. 11.2), and finally penetrated into eggshells by the formation of an infection peg (Holland et al. 1999). A postinfection bulb leads to the expansion of mycelia within the eggs that caused almost the complete devastation of their contents (Tikhonov et al. 2002; Esteves et al. 2009a). Khan et al. (2004) reported that the eggshells and juvenile cuticles both have been physically disrupted, and the fungal hyphae willingly multiplied inside the eggs and juveniles due to enzymatic activity and biosynthesis of diffusible toxic metabolites. *P. chlamydosporia* are reported to secrete serine, protease, and chitinase responsible for the major structural changes inside the nematode eggs which may result in the disintegration of lipid and vitelline layers. Application of *P. chlamydosporia* as soil inoculants could reduce the natural nematode population up to 90 % under field condition (Bordallo et al. 2002), but the fungus differs in virulence toward nematode competence to colonize the root and production of chlamydospore (Bordallo et al. 2002; Yang et al. 2007; Macia-Vicente et al. 2009a, b). All these specific features make *P. chlamydosporia* a successful biocontrol agent under different pathosystems (van Damme et al. 2005; Rumbos et al. 2006; Esteves et al. 2009b).

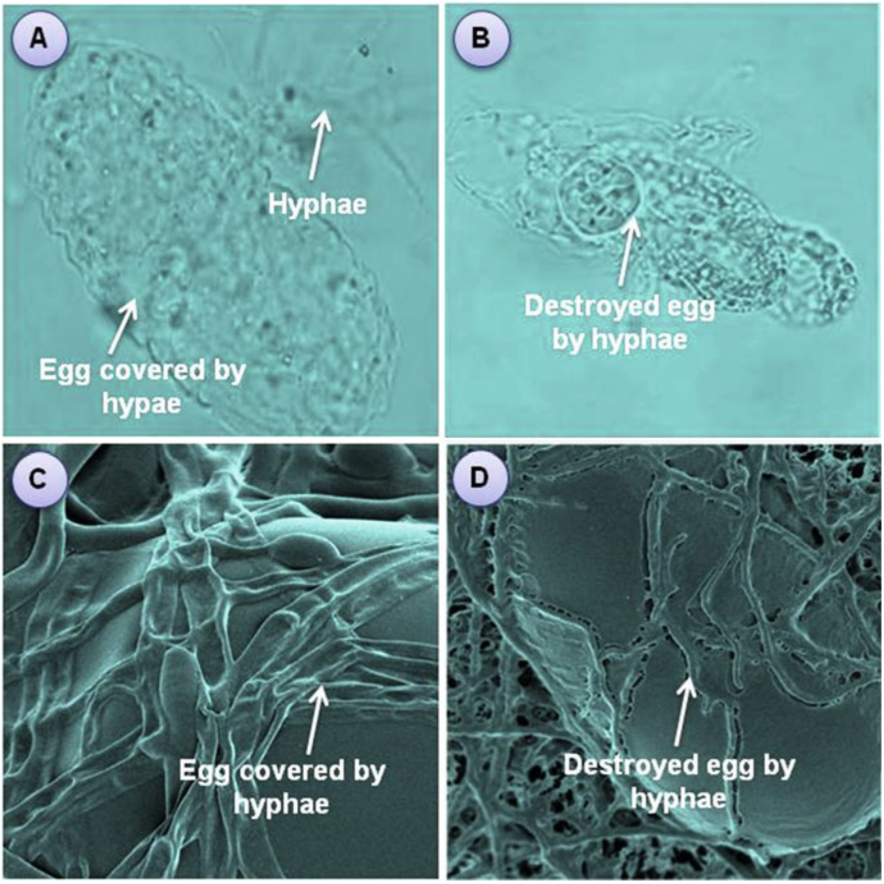

Fig. 11.2 Classical and electron microscopic images of root-knot nematode infected by *P. chlamydosporia*; (**a**) egg of a nematode infected by *P. chlamydosporia* hyphae; (**b**) complete disintegration of nematode egg by *P. chlamydosporia* hyphae; (**c**) electron microscopic view of *P. lilacinus* hyphae covering the nematode egg; (**d**) disruption of nematode egg by *P. chlamydosporia* hyphae

11.3 Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal (AM) fungi are the key components of soil microbial populations with ubiquitous distribution in almost all the agroclimatic conditions of the world and form symbiosis with most of the land plants, in any kind of terrestrial ecosystem (Akhtar and Siddiqui 2008). Currently, AM fungi have been cited in the phylum Glomeromycota (Redecker and Raab 2006), and over 200 morphospecies of Glomeromycota have been described (Schüßler 2008). AM fungi have been categorized on the basis of extra-radical mycelium and branched haustoria-like structure within the cortical cells, termed as arbuscules. These

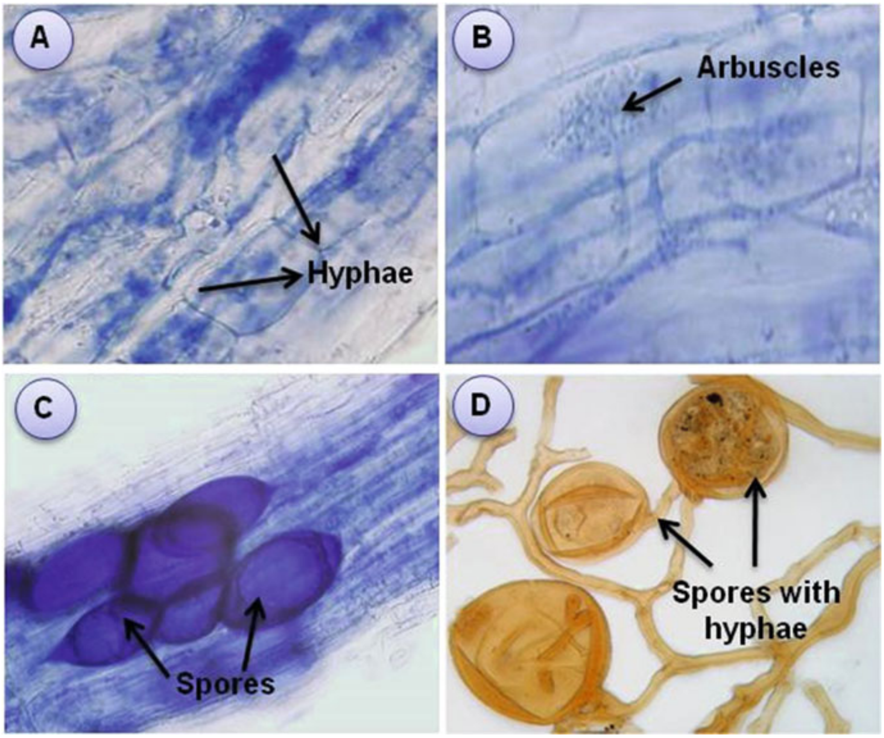

Fig. 11.3 Microscopic view of colonization pattern of AM fungi inside the tomato root; (**a**) showing hyphae of AM fungi; (**b**) showing formation of arbuscles; (**c**) visualization of AM spores inside the cortical tissue; (**d**) AM spores with hyphae stained with Melzer's reagents

arbuscules are the core sites for the nutrient exchange (Fig. 11.3), where the fungi supply water and nutrients like N and P to plants and in turn receive carbon from plants (Bonfante and Genre 2010).

Due to their unique ability and adaptability in different agroclimatic conditions, the AM fungi improved plant health by the acquisition of essential mineral nutrient and water from soil and enhanced production of growth regulations, tolerance toward various abiotic conditions, and mutualistic relationship with additional rhizospheric microorganisms existing in the same ecological niche (Akhtar and Siddiqui 2008; Akhtar 2011).

11.4 Efficacy and Biocontrol Strategies of Beneficial Rhizospheric Fungi

Persistence of plant parasitic nematodes is the most serious problem worldwide because they nourish and multiply their population on live host plants and also actively migrate inside the plants and aerial parts or in the rhizosphere. Among all the available options, chemical control has been extensively used against the plant parasitic nematode, due to its nonselective nature. However, use of chemicals to control plant parasitic nematodes has been restricted in many countries due to their environmental toxicity and ability to leach into the soil. They may cause the hazardous effect on the soil microbial flora and fauna as well as on the environment (Akhtar 1997). In the beginning, most of the fumigants were effectively used to control the plant parasitic nematodes due to their nematicidal properties, but later the detection of their remains in soil, water, and edible crops has caused awareness among the global scientific community concerned about the safety of human health and the environment (Alphey et al. 1988). Methyl bromide was the first fumigant which was widely used against the pathogens causing soilborne diseases, but it has been now banned and completely withdrawn from the market by imposing an international agreement in most of countries worrying about the environment safety (Oka et al. 2000).

Nowadays, several control measures such as the use of green manure, organic or inorganic soil amendments, crop rotation, resistant variety cultivation, unplanted treatment, and biological control have been used to limit the population of plant parasitic nematodes in the soil. But, unfortunately, all these control methods have led to limited success (Barker and Koenning 1998). Integrated pest management provides a working methodology for pest management in sustainable agricultural systems. With the increasing cost of inorganic fertilizers and the environmental and human health hazards associated with the use of pesticides, opportunistic and AM fungi may provide a more suitable and environmentally acceptable alternative for sustainable agriculture. Several comprehensive reviews have been published time to time exploring the possibilities of using AM fungi (Barea et al. 2005; Akhtar and Siddiqui 2008; Smith and Read 2008; Akhtar and Panwar 2011) and opportunistic fungi in the biocontrol of plant diseases (Atkins et al. 2005; Hildalgo-Diaz and Kerry 2008). We have summarized some recently published results of interaction studies between opportunistic fungi, AM fungi, and plant parasitic nematodes in tabular forms (Tables 11.1, 11.2, and 11.3).

Table 11.1 Effect of *Paecilomyces lilacinus* on the plant growth and reproduction of plant parasitic nematodes

Plant	Nematode	Effect on plant growth	Effect on nematode population	References
Faba bean	*M. incognita*	Pre- and posttreatment of plants with *P. lilacinus* increased the shoot dry weight from 11.1 to 13.3 % and 9.1–12.1 %, respectively	Pre- and postinoculation of plant with *P. lilacinus* reduced the number of juveniles from 95.4 to 97.4 % and 91.1–98.9 %, respectively, compared to control	El-Shanshoury et al. (2005)
Tomato	*M. incognita*	Pre- and postinoculation of nematode to *P. lilacinus* significantly reduced the dry weight of plant by 26.15–56.92 %	Pre- and postinoculation of fungus parasitized the nematode eggs by 72.0 % and 68.0 %, respectively	Esfahani and Pour (2006)
Tomato	*M. incognita*	Use of *P. lilacinus* increased the root and shoot weight of plants up to 27.83 % and 46.8 %, respectively	Inoculation of fungus reduced the number of galls per plant, egg masses per root system, and eggs per egg mass up to 44.74 %, 34.23 %, and 16.90%, respectively	Goswami et al. (2006)
Tomato	*M. incognita*	Use of various glucose formulations of *P. lilacinus* increased the shoot weight by 1.83–9.89 % and root weight by 5.0–14.2 % compared to control	Soil treated with fungus reduced root galling, number of egg masses, and final nematode population in the roots by 66 %, 74 %, and 71 %%, respectively, compared to control	Kiewnick and Sikora (2006)
Tomato	*M. incognita*	Use of single or combined application of *P. lilacinus* with bacterial inoculants increased the plant height up to 4.3 %	Treatment with *P. lilacinus* reduced the number of eggs per egg mass up to 18 % compared to untreated control	Anastasiadis et al. (2008)
Tomato	*M. incognita*	Inoculation of *P. lilacinus* increased plant	Use of fungus caused the 44.0 % and 76.0 %	Siddiqui and Akhtar (2008a)

(continued)

Table 11.1 (continued)

Plant	Nematode	Effect on plant growth	Effect on nematode population	References
		length and shoot dry of plants by 42.82 % and 42.25 %, respectively, over nematode-infected plants	parasitism on females and eggs of nematode	
Tomato	*M. incognita*	Enhancement in shoot length (72.66 cm), shoot weight (42.66 g), and root length (36.66 cm) was recorded when *P. lilacinus* was applied in dose 10 g /kg soil compared to control treatment	Inoculation of *P. lilacinus* caused the highest reduction in nematode population, galling, and egg mass per gram root on nematode-infested plants	Kannan and Veeravel (2008)
Tomato	*M. incognita*	Treatment with different spore inoculums of *P. lilacinus* increased the root weight from 37.94 to 65.58 %	The galling is reduced from 89.89 to 97.31 % by the application of different loads of spore inoculum of *P. lilacinus*	Oclarit and Cumagun (2009)
Lettuce	*Meloidogyne* spp.	Application of *P. lilacinus* increased the yield of lettuce by 59.33 % in nematode-infested soil under field conditions	The reduction in galling and nematode population was achieved by 34.89 % and 61.76 % with the application of *P. lilacinus*	Prakob et al. (2009)
Banana	*M. incognita*	Use of *P. lilacinus* significantly increased the plant length (23.09 %) and pseudo stem girth (39.61%) compared to nematode-infected plants	Treatment with *P. lilacinus* reduced the nematode population in soil root by 91.18 % and 81.82 %	Sundararaju and Kiruthika (2009)
Ashwagandha	*M. incognita*	Treatment with *P. lilacinus* increased the shoot dry weight by 84.23% over nematode plants	Use of *P. lilacinus* reduced the root-knot indices approximately up to 50.0 % compared to	Sharma and Pandey (2009)

(continued)

Table 11.1 (continued)

Plant	Nematode	Effect on plant growth	Effect on nematode population	References
			nematode-inoculated plants	
Chickpea	*M. incognita*	Application of *P. lilacinus* caused 26.83 % increase in shoot dry weight of plants as compared to nematode-infested control treatments	Inoculation of *P. lilacinus* caused 42 % and 70 % of re-isolation of females and eggs from a nematode-infested plants	Siddiqui and Akhtar (2009a)
Tomato	*M. javanica*	Simultaneous inoculation of *P. lilacinus* was found better in terms of plant growth than sequential inoculation and causes 41.26 % increase in shoot dry weight of plant compared to control treatments	Concurrent use of *P. lilacinus* reduced the galling, egg masses, egg per egg mass, and final nematode population by 31.44, 33.39, 46.40, and 47.13 %, respectively, compared to control treatments	Ganaie and Khan (2010)
Tomato	*M. incognita*	Results showed that there is no significance difference between the treatments observed in terms of plant growth compared to control under growth chamber experiment	Preplanting soil treatment with *P. lilacinus* reduced the galling, egg masses per root system, and final nematode population by 66 %, 74 %, and 71 %, respectively, compared to the inoculated control under growth chamber experiment	Kiewnick et al. (2011)
Guava	*M. enterolobii*	ND	Application of *P. lilacinus* reduced the egg and egg masses up to 40 % over control treatments	Carneiro et al. (2011)
Okra	*M. incognita*	Application of *P. lilacinus* as soil inoculants with neem cake increased shoot weight up to	Use of various combinations of *P. lilacinus* as seedling treatment and soil inoculants reduced the galling	Kannan and Veeravel (2012)

(continued)

Table 11.1 (continued)

Plant	Nematode	Effect on plant growth	Effect on nematode population	References
		4.17 %, but the results were more pronounced (28.33%) when this combination was applied with seedling dip treatments of *P. lilacinus*	from 26.50 to 64.96 % and juvenile population from 9.96 to 28.18 % over control	
Tomato	*M. incognita*	Application of *P. lilacinus* increased the shoot dry of plants up to 73.19 % over untreated control	Treatment with *P. lilacinus* reduced the galls and egg masses up to 88.23 % and 76.94 %, respectively	Khalil et al. (2012)
Pepper	*M. incognita*	Use of *P. lilacinus* as seed and substrate treatment increased the seedling length from 4.68 to 7.03 %	Seed and substrate treatment with *P.* significantly lowered the root-knot indices from 6.3 to 5.8 %	Rao et al. (2012)
Brinjal	*M. incognita*	Inoculation of *P. lilacinus* reduced the shoot dry weight of nematode-infested plants from 33.70 to 37.76 %	Use of *P. lilacinus* lowered the root-rot indices from 1.20 to 1.28	Usman and Siddiqui (2012)
Tomato	*M. incognita*	ND	Alginate-formulated *P. lilacinus* pellets at 1.6 % (w/w) with soil mixture reduced the root galling by 66.7 %	Aminuzzaman et al. (2013)
Tomato	*M. incognita*	One-week prior inoculation of *P. lilacinus*, nematode increased the shoot dry weight by 57.0 %	One-week prior inoculation of *P. lilacinus*, nematode reduced the root-knot indices and egg-mass indices from 11 to 30 %	Azam et al. (2013)
Tomato	*M. incognita*	Inoculation of *P. lilacinus* increased the root length by 59.49 %	Treatment with *P. lilacinus* reduced the galling up to 58.58 % and egg masses by 65.18 %	Khalil (2013)
Okra	*M. incognita*	Treatment with various concentrations	Application of various concentrations	Mukhtar et al. (2013)

(continued)

Table 11.1 (continued)

Plant	Nematode	Effect on plant growth	Effect on nematode population	References
		of *P. lilacinus* propagules increased the shoot dry weight by 4 to 8 %	of *P. lilacinus* reduced the number of galls from 14 to 37 %, egg masses from 15 to 37 %, nematode reproduction factor from 20 to 52 %	
Tomato	*M. incognita*	Single and twice application of *P. lilacinus* increased the shoot dry weight of plants by 31.40–37.00 %, respectively, over control	Single or twice treatment with *P. lilacinus* reduced the number of galls and egg mass per root system by 52.86–67.71 % and 75.86–87.58 %, respectively	Udo et al. (2013)
Potato	*M. arenaria*	Use of Bio-Nematon (*P. lilacinus* at 10^8 unit/cm^3) increased the plant height, number of leaves, and number of branches by 68.2 %, 106.9 %, and 137.0 %, respectively, compared to control treatments under field condition	Treatment with Bio-Nematon (*P. lilacinus* at 10^8 unit/cm^3) reduced the number of galls and number of egg masses in root system by 77.4 % and 83.3%, respectively, under field condition	Abd-El-Khair and El-Nagdi, (2014)
Chickpea	*M. incognita*	Use of *P. lilacinus* increased the shoot length of plants by 40.62 % compared to control treatments	Application of *P. lilacinus* reduced the number of juvenile in root and galling by 44.42 % and 65.88 %, respectively	Mishra et al. (2014)
Brinjal	*M. incognita*	Treatment with *P. lilacinus* increased the shoot and root length by 45.62 % and 29.41 %, respectively, compared to control treatments	Inoculation of *P. lilacinus* reduced the root-knot indices up to 63.88 % compared to control treatments	Ravindra et al. (2014)

Table 11.2 Effect of *Pochonia chlamydosporia* on the plant growth and reproduction of plant parasitic nematodes

Plant	Nematode	Effect on plant growth	Effect on nematode population	References
Hollyhock Petunia Poppy	*M. incognita*	Root dip treatment with *P. chlamydosporia* increased the flower production by 7–15 % on various tested ornamental plants under field conditions	The frequency of colonization of eggs, egg masses, and females by *P. chlamydosporia* was recorded as 25–29 %, 47–60 %, and 36–41 %, respectively, under field conditions	Khan et al. (2005a)
Chickpea	*Meloidogyne* spp.	Application of *P. chlamydosporia* increased the plant growth by 28 % and yields by 25 % of nematode-infected chickpea plants	Use of *P. chlamydosporia* reduced the galling by 23 % and egg mass production by 18 %	Khan et al. (2005b)
Faba bean	*M. incognita*	Application of *P. chlamydosporia* reduced the population density of nematodes on faba bean	Application of *P. chlamydosporia* reduced the population density of nematodes on faba bean either with post- or preinfection with the range of 97.1 to 98.9 % compared to control	El-Shanshoury et al. (2005)
Cabbage Tomato	*M. incognita*	ND	Use of *P. chlamydosporia* reduced nematodes population by 51–78 % in the tomato compared to cabbage	Tahseen et al. (2005)
Tomato	*M. incognita*	Treatment with *P. chlamydosporia* increased plant length and shoot dry weight by 36.71 % and 36.63 %, respectively, compared to nematode-infested plants	Use of *P. chlamydosporia* caused the parasitism on females and eggs of nematodes by 30.0 % and 67.0 %, respectively	Siddiqui and Akhtar (2008a)
Okra	*M. incognita*	Combined application of *P. chlamydosporia* with neem cake or carbofuran significantly increased the plant growth and yield by 53 % and 64 %, respectively, over non-inoculated control	Use of *P. chlamydosporia* with neem cake or carbofuran reduced the galling, egg production, and nematode population by 89 %, 90 %, and 81 %, respectively	Dhawan and Singh (2009)

(continued)

Table 11.2 (continued)

Plant	Nematode	Effect on plant growth	Effect on nematode population	References
Chickpea	*M. incognita*	Use of *P. chlamydosporia* caused 22.41 % increase in shoot dry weight of plants as compared to nematode-infested control plants	Inoculation of *P. chlamydosporia* caused 28 % and 66 % of re-isolation of females and eggs from nematode-infested plants	Siddiqui and Akhtar (2009a)
Okra	*M. incognita*	Use of *P. chlamydosporia* increased the shoot length, shoot weight, root length, and root weight of plant by 80.9, 74.1, 73.9, and 80 %, respectively, over control treatment under pot conditions	Treatment with *P. chlamydosporia* reduced galls and egg masses per plant and eggs per egg mass by 54.8, 53.7, and 46.5 %, respectively, under pot condition	Dhawan and Singh (2011)
Guava	*M. enterolobii*	ND	Application of *P. chlamydosporia* reduced the disease severity up to 61.5 % as compared to control under glasshouse conditions	Carneiro et al. (2011)
Tomato	*M. javanica*	ND	Among the various tested isolates of *P. chlamydosporia*, isolates 64 and 10 were most efficient in reducing the number of eggs by 72.0 % and 60.0 %, respectively	Dallemole-Giaretta et al. (2012)
Tomato	*M. javanica*	Inoculation of *P. chlamydosporia* Pc123gfp increased the root and shoot growth of plants 20 days after inoculation compared to nematode-inoculated plants	Treatment with *P. chlamydosporia* Pc123gfp reduced the number of galls and egg masses per root system by 53.6 % and 32 %, respectively, compared to control	Escudero and Lopez-Llorca (2012)
Tomato	*M. incognita*	Use of chlamydospore inoculum of *P. chlamydosporia* (strain 4) increased the shoot dry weight up to 12.14 % compared to non-inoculated control treatment	Use of chlamydospore inoculum of *P. chlamydosporia* (strain 4) reduced the number of egg per root system by almost 50 % compared to non-inoculated control treatment	Yang et al. (2012)

(continued)

Table 11.2 (continued)

Plant	Nematode	Effect on plant growth	Effect on nematode population	References
Tomato	*M. incognita*	ND	Use of alginate-formulated *P. chlamydosporia* pellets at 1.6 % (w/w) with soil mixture reduced the nematode density by 90 % on tomato under greenhouse conditions	Aminuzzaman et al. (2013)
Okra	*M. incognita*	Use of various concentrations of fungal propagules enhanced the shoot dry weight from 5 to 10 %	Treatment with various concentrations of fungal propagules suppressed the number of galls from 12 to 32 %, egg masses from 11 to 30 %, and reproduction factor from 20 to 43 %	Mukhtar et al. (2013)
Tomato	*M. javanica*	Treatment with *P. chlamydosporia* increased the shoot by 7.38 % and root mass by 4.64 %	Application of *P. chlamydosporia* reduced the number of galls per plant by 12.68 % and number of eggs per plant by 17.39 %	Podestá et al. (2013))
Brinjal	*M. incognita*	Use of *P. chlamydosporia* increased the shoot length and root length by 29.46 % and 33.88 %, respectively, compared to control treatments	Inoculation of *P. chlamydosporia* reduced the root-knot indices by 58.33 % compared to control treatments	Ravindra et al. (2014)
French bean	*M. javanica*	Pre- and postinoculation of fungus to nematode in soil increased the shoot dry weight of plant by 43.39–48.36 % and 13.79–29.24 %, respectively	Pre- and posttreatment of plants with fungus to nematode reduced the number of galls per root system up to 55–62.5 % and 2.5–7.5 %, respectively	Sharf et al. (2014)
Cucumber	*M. javanica*	Application of *P. chlamydosporia* to the soil increased cucumber root mass by 12.03 % compared to control plants	The application of *P. chlamydosporia* reduced the number of galls per gram of roots by 49.44 % and the number of eggs per gram of roots by 40.58 %	Viggiano et al. (2014)

Table 11.3 Effect of AM fungi on the plant growth and reproduction of plant parasitic nematodes

Plant	Nematode	Effect on plant growth	Effect on nematode population	References
Chickpea	*M. incognita*	Inoculation of *G. intraradices* increased the shoot and root dry weight up to 9.68 and 14.75%, respectively	Application of AM fungus reduced the galling up to 28.57% and nematode population up to 27.32%	Akhtar and Siddiqui, (2006)
Mentha	*M. incognita*	Use of *G. aggregatum* increased the herb yield up to 16.61 % and oil yield up to 37.25 % compared to control treatments	Inoculation of *G. aggregatum* reduced root-knot indices up to 27.3 % over control treatments	Pandey (2005)
Tomato	*M. incognita*	Application of both isolates of *G. fasciculatum* increased the shoot weight up to 8.20–10.93 % and yield up to 9.75–10.40 %	Treatment with both isolates of *G. fasciculatum* reduced galling up to 41.3–44.7 % and 60.1–63.1 %, respectively	Kantharaju et al. (2005)
Banana	*M. javanica*	ND	Results showed that AM fungus-inoculated plants had 20 % less galling compared to non-mycorrhizal plants	Rodríguez Romero and Jaizme-Vega (2005)
Papaya	*M. incognita*	Inoculation of *G. mosseae* and *G. manihotis* significantly increased the plant growth, but the increase in plant growth was marginal when each AM fungus was compared individually	Inoculation of *G. mosseae* and *G. manihotis* reduced the galling by 84–44–99.59 % and number of nematodes per root by 83.33–99.54 %	Jaizme-Vega et al. (2006)
Tomato	*M. incognita*	Use of *G. mosseae* and *G. margarita* both increased the shoot dry weight of plant by 35.34 % and 31.74 %, respectively, but the results were more pronounced when the AM fungi were used with tested organic manures	Treatment with *G. mosseae* and *G. margarita* both reduced the galling by 60.22 % and 51.14 %, respectively, and nematode population by 60.27 % and 50.41%, respectively, but the results were more pronounced when the AM fungi were used with tested organic manure	Siddiqui and Akhtar (2007)
Chickpea	*M. incognita*	Inoculation of *G. intraradices* increased the shoot dry	Use of *G. intraradices* reduced the galling by 25.0 % and nematode	Akhtar and Siddiqui (2007)

(continued)

Table 11.3 (continued)

Plant	Nematode	Effect on plant growth	Effect on nematode population	References
		weight by 8.5 % compared to nematode-infested control	population by 25.83 % compared to control	
Tomato	*M. incognita*	Inoculation of AM fungus increased the plant dry weight by 34.80 % and yield by 54.54 % compared to nematode-infested plants	Inoculation of AM fungus reduced the galling by 66.09 %, number of egg masses by 66.47 %, and nematode population by 55.20 %	Shreenivasa et al. (2007)
Sunflower	*M. incognita*	Pre- and posttreatment of AM fungi to nematode increased the plant length by 6.02 % and 2.41 %, respectively, compared to nematode-inoculated control treatment	Pre- and postinoculation of AM fungi reduced the nematode infestation by 83.33 and 33.33 %, respectively, compared to nematode-inoculated control treatment	Jalaluddin et al. (2008)
Tomato	*M. incognita*	ND	Inoculation of *G. intraradices* reduced the galling by 24 %, while the results were more pronounced (60 %) with the combination of *R. etli*	Reimann et al. (2008)
Tomato	*M. incognita*	Treatment with AM fungus increased the shoot dry weight by 30.69 % compared to nematode-infested control plants	Inoculation of AM fungus reduced the galling by 30.30 % and nematode population by 38.44%	Siddiqui and Akhtar (2008b)
Cucumber	*M. incognita*	Inoculation of *G. mosseae* and *G. versiforme* significantly increased the shoot dry of plants by 39.38 % and 50.17 %, while the *G. intraradices* was found least effective in terms of plant growth	All the tested AM fungi reduced the galling index by 3.0, 2.4, and 2.0, respectively. However, inoculation with *G. versiforme* decreased the number of galls per gram root by 45 %, while the other two fungi also showed the similar propensity, but the trend was not significant	Zhang et al. (2008)
Chickpea	*M. incognita*	Use of AM fungus increased the shoot dry weight by 15.11 %, grain weight by 16.23 %, and yield by	Use of AM fungus reduced the galling by 27.27 % under field conditions	Akhtar and Siddiqui (2009)

(continued)

Table 11.3 (continued)

Plant	Nematode	Effect on plant growth	Effect on nematode population	References
		15.13 % under field conditions		
Tomato	*M. incognita*	Treatment of AM fungi increased the shoot dry weight of plants by 29.9–30.9 % compared to untreated control	Use of AM fungi reduced the galling up to 26.08–29.71 % and nematode population up to 24.59–33.33 % compared to untreated control	Siddiqui and Akhtar (2009b)
Cucumber	*M. incognita*	Application of both levels of P with *G. intraradices* increased the shoot dry of plants by 25.0 % and 28.42 %, respectively	Use of both levels of P with AM fungus reduced the galling approximately up to 50–54 %	Zhang et al. (2009)
Sweet passion fruit	*M. incognita*	Inoculation of AM fungus stimulated the root biomass of plants up to 35.71 % and 10.94 % in the non-disinfected and disinfected soil	AM fungus-treated plants showed 72.0 % reduction in the number of galls per gram of roots and 87.7 % in egg masses per gram of roots in disinfested soil, while in noninfected soil the number of eggs and galls per root system were recorded 44.0 and 26.5 %, respectively	Anjos et al. (2010)
Cowpea	*M. incognita*	ND	Inoculation of AM fungus suppressed the root galling and nematode reproduction up to 12.80–72.73 % and 24.24–55.43 % on various tested varieties of cowpea in both pot experiments	Odeyemi et al. (2010)
Acacia farnesiana *Acacia saligna*	*M. incognita*	Treatment of AM fungi together with oxamyl increased the shoot dry weight of both plants by 66.57–72.90 and 61.73–65.18 %, respectively	Application of AM fungi together with oxamyl decreased no. of egg masses, eggs per egg mass, final nematode population, and buildup of nematode approximately by 80.40 %, 47.90 %, 79.70%, and 89.80 %, respectively, in both tested plant species	Soliman et al. (2011)

(continued)

Table 11.3 (continued)

Plant	Nematode	Effect on plant growth	Effect on nematode population	References
Pea	*M. incognita*	Use of AM fungus significantly increased the growth (24.54 %), in the nematode-inoculated plants	Inoculation of AM fungus reduced the number of galls and nematode population up to 30.13 % and 32.23 %, respectively	Akhtar and Panwar (2013)
Tobacco	*M. incognita*	Combined inoculation of *G. aggregatum* with neem cake caused the maximum increase in the shoot dry (48.81 %) of plants over nematode-infested soil	Combined inoculation of *G. aggregatum* with neem cake reduced the nematode population by 60.87 % and nematode reproduction rate by 58.96 %	Serfoji et al. (2013)
Mays	*M. incognita*	Use of AM fungus increased shoot weight (17.58 % and 11.63 %) and the yield (64.92 % and 20.07 %) of plants under pot and field conditions	Treatment with AM fungus reduced the galling (47.56 % and 44.81 %) and nematode population (98.23 % and 80.81 %) under pot and field conditions	Odeyemi et al. (2013)
Tomato	*M. incognita*	Results showed that all the tested AM fungi increased the shoot dry weight of plant compared to control, but *G. deserticola* caused the height increase (40.17 %) in shoot dry weight compared to other tested fungi	Among all the tested AM fungi, *G. deserticola* reduced the number of galls per root system by 44.28 % and number of eggs per root system by 72.42 %	Udo et al. (2013)
Potato	*M. arenaria*	Treatments with Stanes symbion vam (mixture of *G. fasciculatum* and *Gigaspora* sp.) increased the plant height, number of leaves, and number of branches by 64.5 %, 82.2 %, and 113.4 %, respectively, compared to control treatments under field condition	Inoculation of Stanes symbion vam (mixture of *G. fasciculatum* and *Gigaspora* sp.) reduced the number of juveniles in soil, eggs, and egg masses on root system by 86.1 %, 69.8 %, and 71.9 %, respectively, compared to control treatments under field condition	Abd-El-Khair and El-Nagdi, (2014)

11.5 Mass Propagation Strategies of Opportunistic Fungi and AM Fungi

11.5.1 Mass Production of Opportunistic Fungi

Several media have been extensively used for the mass production of opportunistic fungi. For the mass production of *P. lilacinus* potato dextrose broth (Rangaswami 1972), Richard's medium, 10 % molasses (Rangaswami 1972), and semi-selective medium (Mitchell et al. 1987) can be used. The highest mycelium weight and spore production were achieved by using the semi-selective medium followed by 10 % molasses medium (Prabhu et al. 2008). Corn meal agar and potato dextrose agar media have also been used for the mass production of *P. lilacinus* (Robl et al. 2009). Similarly, the mass production of *Pochonia* spp. was achieved by using shrimp agar medium (Moosavi et al. 2010). Besides this wheat, bran and barley grain were also used for the mass production of *Pochonia* spp. (de Leij and Kerry 1991; Crump and Irving 1992). For the large-scale commercial production, liquid fermentation method is generally used because of difficulties to improve spore production on solid medium (Khan and Anwer 2011).

11.5.2 Mass Production of AM Fungi

AM fungi have the unique ability to improve the uptake of water and mineral nutrients from the soil and also to guard the plants against the pathogen attack (Smith and Read 2008). AM fungi also scavenge the available P through their extraradical hyphae and upsurge the secretion of various amino acids (such as serine and isoleucine) and defense-related proteins (Akhtar and Siddiqui 2008; Akhtar et al. 2011), which augments their importance toward the modern and profitable agronomic practices. Due to their obligate nature, the AM fungi could not be cultured in vitro, which may limit the mass production of AM fungal propagules. In the conventional method of propagation, the AM fungi are propagated through the pot or pan culture usually with single spore culture, swiftly spread on the substrate, and finally colonize the root of host plants (Akhtar and Abdullah 2014). This method is quite useful for the production of clean fungal inoculum with high potentiality in a short span of time. Similarly, aeroponic culture systems allow the production of cleaner spores and enable even nourishment of AM fungi-colonized plants (Jarstfer and Sylvia 1999). Propagation of any AM fungal strains on root-organ culture permitted the propagation of monoxenic strains that could be used either directly as inoculum or as a starter inoculum for the mass production of AM fungi. A very simple and low-cost technique of single spore pot culture has been developed by Panwar et al. (2007). It permits undistributed growth of the mutualistic partners and visualization of germinating AM fungal spores and their mass multiplication. Moreover, the mass production of AM fungal inoculum requires control and optimization of both host growth and fungal development. The microscopic sizes of AM fungi, together with the complex identification processes, also contribute to the drawbacks of inoculum propagation.

Nevertheless in vitro bulk production of AM fungal inoculum is a promising approach, offering clean, viable, contamination-free fungal propagules. The cost of in vitro inoculum may appear expensive compared to the greenhouse-propagated fungal inoculum, but its use as starting inoculums is a warranty of purity (Akhtar and Abdullah 2014). The main purpose of this cultural method is to provide pure, clean, and reliable material as starter inoculum for the fundamental and applied research. There were several reports which indicate that mycorrhizologists were able to produce 25 spores/ml in 4 months' incubation time (Chabot et al. 1992), while the other workers claimed for the production of 3250 spores/ml in 7 months (Douds 2002). Recently another work justifies the production of more than 2400 spore/100 g of soil after 120 days from single spore culture (Panwar et al. 2007).

11.6 Conclusions

The present chapter provides an overview on the interactions between opportunistic fungi, AM fungi, and plant parasitic nematodes. Use of opportunistic and AM fungi will not only reduce the load of nematicides in agricultural practices but also increase the plant vigor through the uptake of essential mineral nutrients and also reduce the nematode buildup in the plant and soil. Moreover, use of these biocontrol agents has an eco-friendly approach toward the environment as well as human health. The protection of nematode diseases by the application of these biocontrol agents is a complex process which may depend upon the molecular interactions between hosts, biocontrol agents, and pathogenic microorganisms. Application of single or mixed inoculum of opportunistic fungi, AM fungi were found to be effective in controlling the nematode diseases under greenhouse, pot, and field conditions in various agroclimatic conditions. An overview of the recent cost-effective technologies used for the mass propagation of these beneficial rhizospheric microorganisms is discussed. The success of mass propagation of indigenous biocontrol agents depends upon its selective nature toward edaphic, environment, and other rhizospheric biota, but it is still a challenge to develop these biocontrol agents in the sustainable agricultural practices to understand real underground mechanisms involved between the host, biocontrol agents, and pathogenic microorganisms.

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