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RESEARCH ARTICLE

Nematode biocontrol agents: diversity and effectiveness against phytonematodes in sustainable crop protection

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ABSTRACT: Plant parasitic nematodes are generally soil borne in nature, and attack underground plant parts. Since, soil population of nematodes do not build-up suddenly, biocontrol approach may serve as a practically feasible, low cost, long lasting and ecofriendly method. The nematode biocontrol fauna is highly diversified, and fungi and bacteria constitute a vast group of nematode antagonists. But, only some fungi and a few bacteria have demonstrated antagonism to a level that has been exploited commercially to control nematodes in crop fields. Commercial formulations of *Dactylaria candida*, *Arthrobotrys robusta*, *Purpureocillium lilacinum*, *Pochonia chlamydosporia, Pasteuria penetrans*, *Trichoderma harzianum, T. virens, Aspergillus niger*, *Pseudomonas fluorescens* and *Bacillus subtilis* have been developed and are being currently used for nematode/fungus management. In view of greater effectiveness of two or more biocontrol agents in cohabitance, a consortium of microorganisms such as *P. lilacinum/T. harzianum + P. fluorescens/B. subtilis* may prove more suppressive to nematodes, and hence is recommended for nematode control and soil health improvement. However, there is legal biosafety restriction on the development of a formulation with two or more microorganisms. Continued research efforts in this direction shall pave a way to overcome the existing and upcoming issues to offer suitable technology(ies) for safer and effective nematode management. Further, effectiveness of biocontrol agents vary with the area and climate, hence, local efficient isolates are required to be searched regularly.

Key words: Biological control, plant parasitic nematodes, bacteria, fungi, quorum sensing

Pests and diseases are important challenges the agriculturists do face today in enhancing the golabal food production. In developing countries including India, where a vast proportion of farmers are illiterate and have small holdings, around half of the total produce is lost quantitatively or qualitatively due to the attack of pests and diseases at pre- and post-harvest stages (Fig. 1). The diseases caused by the pathogenic fungi, bacteria, viruses and nematodes have been found to contribute around 25% of the total losses inflicted by pests and pathogens. Among the diseases, greatest losses are inflicted by fungi (42 %), followed by bacteria (27%), viruses (18%) and nematodes (13%, Fig. 1, Khan and Jairajpuri, 2010a).

Plant parasitic nematodes constitute an important group of pathogens that attack every agricultural crop throughout the world. The yield loss inflicted by nematodes varies with crop, inoculum level and climatic conditions (Table 1). Plant parasitic nematodes are vermiform or thread-like animals. Their body is thin, flexible, generally elongated, cylindrical to fusiform and tapering towards both ends (Fig. 1). Adults of plant nematodes may be 0.3 to 11 mm long. They constitute 80-90% of all the multicellular animals. Fortunately, only a fraction of this number (around 7%) possesses ability to parasitize plants, and rests are free-living surviving on various substrates.

Nematodes cause damage to plants by injuring and feeding on the root hairs, epidermal cells, cortical and stealer cells (Khan, 2008). A large number of nematodes are ectoparasites feeding on root surface e.g., *Rotylenchus, Tylenchorhynchus, Belonolaimus, Hoplolaimus, Trichodorus, Longidorus* etc. However, a considerable number of nematodes fully enter inside the host root and are called endoparasitic nematodes such as root-knot nematodes (*Meloidogyne* spp.), cyst forming nematodes (*Heterodera* spp.) and root-lesion nematode (*Pratylenchus* spp.). Whereas some nematodes such as citrus nematode (*Tylenchulus semipenetrans*) and reniform nematode (*Rotylenchulus*

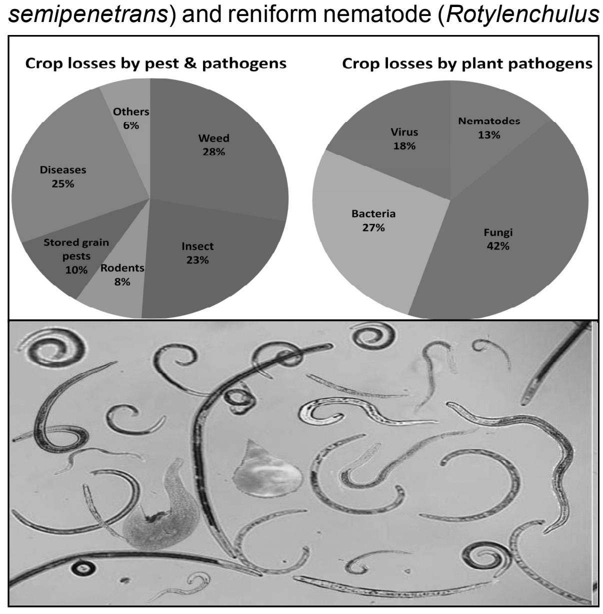

Fig. 1. Pie chart showing crop losses caused by pests and pathogens and nematodes (L). Plant parasitic nematodes of different shape and size (R).

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reniformis) are semi-endoparasites as they partially enter the host tissue. The most common effect of nematode parasitism is debilitation of the plant even without appearance of any symptom. In addition to direct damage, nematodes often aid or aggravate the diseases caused by fungi, bacteria and viruses or may break host resistance. Wilts and root-rots generally become severe in the presence of nematodes. Further, nematodes transmit some bacteria, fungi and viruses that result in some serious diseases in important agricultural crops. Although, nematodes cause substantial damage to crops, but the damage usually remains unrecognized due to non-appearance of specific symptoms. The general symptoms of nematode infestation appear as patches of plants irregularly distributed in a field showing stunted growth, sparse and dull green or yellowish foliage. The severely infected plants show incipient wilting during the day time despite availability of adequate soil moisture, but recover at night. Some nematodes, however, cause recognizable symptoms on roots, shoots or influorescence, such as root-lesions, root-rot, root pruning, stubby root, dirty root, root-galls, cyst attached roots, leaf necrosis, white leaf tip, red ring and seed galls (Fig. 2).

Although, a large number of nematode species attack agricultural crops (Khan and Jairajpuri, 2010a, 2010b, 2012), but the nematode species from the genera, *Meloidogyne, Pratylenchus, Ditylenchus, Heterodera, Radopholus, Globodera, Rotylenchulus, Tylenchulus, Aphelenchoides, Xiphinema, Longidorus, Helicotylenchus, Hemicycliophora, Belonolaimus* spp. etc. are most important with regard to distribution, host range and parasitic potential (Sasser, 1989). Majority of these nematodes attack underground parts, and cause 5-43% or even greater yield loss to the crop (Sasser, 1979). According to an estimate, around 6% loss in field crops, 12% in fruit and nut crops, 11% in vegetables and 10% in ornamental crops occur annually due to nematode infection (Khan and Jairajpuri, 2012).

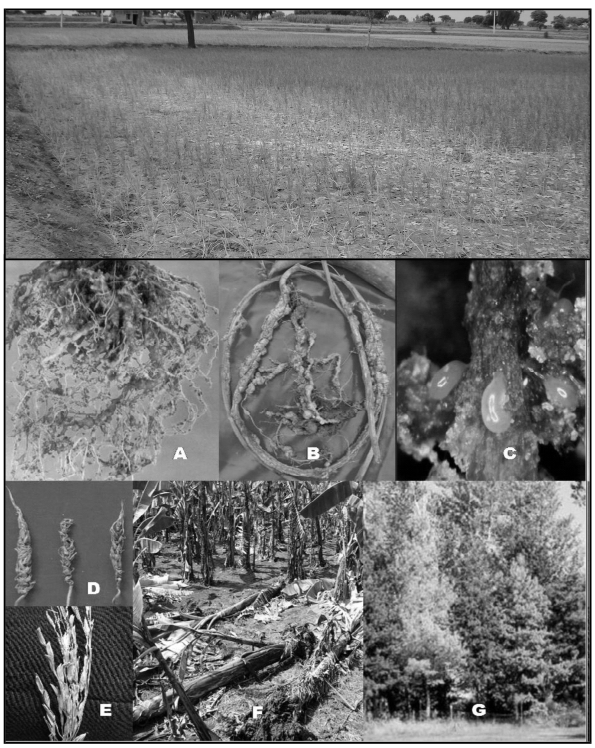

Fig. 2. General symptoms of nematode attack in a rice field, plants in patch show stunted growth with chlotetic foliage (top). Specific symptoms caused by *Meloidogyne* (root-knot on tomato, A and sponge gourd, B), *Rotylenchulus reniformis* (dirty root, C), *Anguina tritici* (seed gall, D), *Aphelenchoides besseyi* (penicle chaffiness, E), *Radopholus similis* (toppling of banana, F), *Bursaphelenchus xylophillus* (pine wilt and needle browning, G). Source: http:// vegetables-mdonlineppath.cornell.edu. (A), C: Charles Overstreet (C), Nandal *et al.,* (D) Prasad *et al*., (E), Sheela and Sudraraju, 2012 (F), S. C. White (G).

A number of options for the management of plant parasitic nematodes are available to farmers. The effectiveness of the management measures, however, varies with the nematode species, crop, time of application and climate, and is coupled with several limitations and illeffects. Among the nematode management options, biocontrol is one of the effective, widely acceptable and ecofriendly methods. In the present scenario of the climate change and environmental contamination, biocontrol becomes a most obvious choice for nematode management in a wide array of crops. Biological control of plant nematodes can be defined as "a reduction of nematode population accomplished through the action of living organism(s), which occur naturally by manipulation of environment or through introduction of antagonists" (Stirling, 1991). The microorganisms, which can antagonize the nematodes are commonly referred to as biocontrol agents (BCA). History of nematode biocontrol dates back to 1852, 1874 and 1877 when Fresenius, Lohde and Kuhn, respectively suggested that parasites and predators can suppress nematode population in soil.

A widely diversified range of soil organisms from almost all the major groups such as fungi, bacteria, viruses, rickettsias (Shepherd *et al*., 1973), protozoans (Small, 1988), nematodes, collembolla, tardigrades, turbellarians, enchytraieds and mites (Stirling, 1991) have demonstrated parasitism or predation against soil inhabiting nematodes under laboratory or field conditions (Khan, 2007). A critical perusal of the relevant information has revealed that biocontrol fungi and bacteria have shown greater potential and prospects for commercial use in nematode management, if explored and exploited judiciously. This chapter attempts to present the diversity among the biocontrol fungi and bacteria, so as to explore the relative biocontrol potential of these organisms. Special emphasis has been given to the research conducted under field condition in order to analyze the perspective of large scale use of biopesticides in nematode management. The diversity among the BCAs belonging to fungi and bacteria is described under.

Biocontrol fungi

Fungi constitute a highly diversified group of biocontrol agents of phytonematodes. The biocontrol fungi are ubiquitous in occurrance and have wide host range.

Table 1. Crop losses caused by nematodes to different agricultural crops

Crop	Yield loss (%)	Crop	Yield loss (%)
Vegetables	5-43	Soybean	11-27
Pulses	10-20	Pea	12-19
Cereals	7-22	Maize	10-16
Fruit Crops	9-28	Wheat	7-21
Sugar Crops	7-19	Rice	10-32
Commercial Crops	10-28	Grapes	8-28
Fiber Crops	8-37	Citrus	7-18
Ornamentals	11-32	Banana	14-26
Okra	9-31	Pineapple	6-16
Tomato	11-43	Sugarbeet	11-19
Eggplant	7-40	Sugarcane	5-15
Potato	5-22	Tea	8-22
Pepper	4-17	Coffee	5-15
Chickpea	7-15	Tobacco	10-28
Mungbean	11-23	Cotton	10-27

The fungi antagonize the nematodes through the mechanism of predation or parasitism, and on this basis they are grouped as under.

Predacious fungi

The predacious fungi, also called as nematode trapping or nematophagous fungi are probably oldest BCA known to mankind (Fresenius, 1852). The fascinating behaviour of the predacious fungi generated interest in early workers to explore their role in nematode management. At present around 150 fungal species from Hyphomycetes are reported to predate upon nematodes. Members of the family Moniliaceae (Hyphomycetes) constitutes a single largest group of nematophagous fungi. These fungi develop various devices to capture a prey nematode. The fungal mycelium produces some chemicals that attract nematodes to the trap device. Some of the common trap devices formed in the mycelium of these fungi are sticky traps such as, sticky branches (*Dactylella labata*), sticky network (*Arthrobotrys oligospora*) and sticky knobs (*Dactylella elliposponal*). Predacious fungi also trap nematodes mechanically with the help of constricting (*Monacrosporium bembicodes*) or non-constricting mycelial rings (*Dactylaria candida*, Fig. 3).

Predacious fungi especially, *Arthrobotrys, Dactylella, Dactylaria, Hirsutella* and *Nematoctonus* species have been found effective against different plant nematodes atleast under laboratory condition (Khan, 2007). Kerry and Jaffee (1997) reported that application of *A. oligospora* controlled the root-knot in green house grown vegetables. Other researches have also shown suppression of root-knot nematode by the action of *Arthrobotrys* (Nordbring-Hertz, 2004) and *Nematoctonus* spp. (Guima and Cooke, 1974). *Nematoctonus* spp. secretes nematoxic chemical that causes rapid immobilization of the nematode followed by penetration of the fungus germtube (Guima *et al*., 1973). Predacious fungi have also proved effective against *Pratylenchus* spp., *Radopholus similis* and *Heterodera schachtii* (Stirling, 1991). Important predacious fungi and their effects on prey nematode spp. have been enlisted in the Table 2.

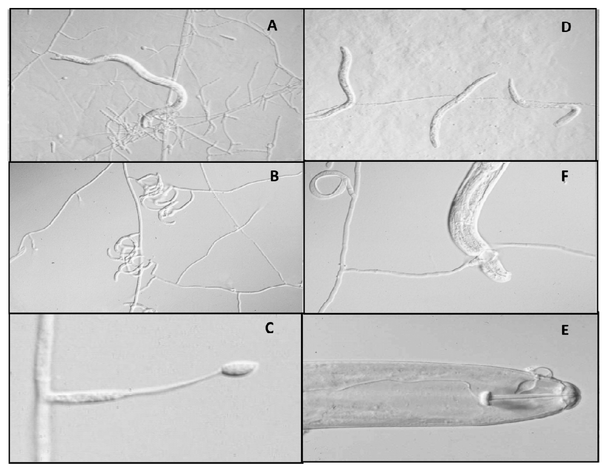

Fig. 3. Predaceous fungi showing different trapping devices as adhesive knobs (A), adhesive hyphae by *Genicularia* sp. (B), adhesive networks (C), and constricting rings by *Arthrobotrys dactyloides* (D); An adhesive conidium of *Hirsutella rhossiliensis* on conidiophore (E), and Conidium of *H. rhossiliensis* on the cuticle of a nematode with infection bulb inside the body cavity (F), Courtesy photo: (A-F) B. A. Jaffee, and P. Timper (E); (http://www.cpes.peachnet.edu).

Parasitic fungi

This is another diversified group of nematode biocotrol agents. The parasitic fungi have shown relatively greater potential to control nematode infestations. The fungus spores/myceliua invade the nematode body through natural orifices or at any point. The fungus disseminates when nematodes come in contact with the infested nematode. The nematode parasitic fungi are also known

Table 2. Important predacious fungi, prey nematodes and their effects.

Predacious fungi	Prey nematode	Effect	Reference
Arthrobotrys oligospora, A. botryospora, Dactylella brochophaga, Drechmeria coniospora	*Radopholus similis*	Controlled the nematode population in banana plantation.	Martinuz *et al.* (2007)
A. oligospora,	Root-knot nematodes	Reduced in galling in green houses vegetables and soil population of the nematode.	Kerry and Jaffee (1997), Nordbring-Hertz (2004)
A. suporba,			
Dactylella pseudoclavata,			
Genicularia sp., *A. oligospora, Candelabrella* sp.	*Pratylenchus penetrans, P. crenatus*	Reduced the nematode population of cover crops	Hendricks (2002)
A. dactyloides, A. oligospora, D. ellipsospora, D. haptotyla	*Heterodera schachtii*	Significantly reduced the nematode population at 10^2 CFUs/g soil.	Jaffee (2003, 2004)
H. rhossiliensis	*Criconemella xenoplax*	Nematode population was suppressed in a peach orchard.	Jaffee *et al.* (1994)

as endozoic or endoparasitic fungi. The endozoic fungi are generally encountered in soil and act as a natural force to maintain equilibrium of nematode population; hence are also referred to as natural antagonists. Around 50 endozoic fungi from Phycomycetes and a few from Deuteromycetes are known to parasitize nematodes (Stirling, 1991). These fungi are often obligate parasites and all of them have a limited saprophytic phase. Usually they do not produce mycelium in soil and complete their life cycles within the nematode body. On the basis of type of nematode and the developmental stage they attack, the parasitic fungi are categorized into parasites of vermiform nematodes, and parasites of eggs and adult females.

Parasites of vermiform nematodes

These fungi parasitize vermiform nematodes in a uniform manner, but initiation of infection varies widely. Some of these fungi produce motile zoospores, which follow the gradient formed by the nematode exudates and upon approaching the nematode, the spores germinate, sending the germ tube into anus, vulva or buccal cavity or directly at any point, eg, *Catenaria anguillulae* and *Lagenidium caudatum* (Jaffee, 1986; Singh *et al*., 1996, Fig. 4). Conidia of *Drechmeria coniospora, Hirsutella rhossiliensis* and *Verticillium balanoides* are provided with some sticky material that helps the spores in adhering to the nematode cuticle. The germinating spores of *Nematoctonus* spp., secrete nematoxic compounds which cause mobilisation of the nematode (Guima *et al*., 1973). Considering the mode of parasitism, these fungi can also infect second stage juveniles of sedentary nematodes.

Parasites of eggs and adult females

This group of parasitic fungi infects eggs and sedentary stages of phytonematodes such as *Meloidogyne, Heterodera, Globodera* and *Rotylenchulus*. These fungi are also called as opportunistic fungi, as they readily invade eggs when available, otherwise survive saprophytically in soil (Khan, 2007). Certain species of *Purpureocillium*, *Pochonia*, *Nematophthora*, *Fusarium*, *Clindrocarpon*, *Cephalosporium*, *Trichoderma*, *Exophila*, *Phoma*, *Dactylella* etc. have been frequently found associated with the eggs, females and cysts of plant nematodes (Khan and Jairajpuri, 2012). The sedentary stage of root-knot nematodes and cyst forming nematodes when become exposed on the root surface are particularly vulnerable to the infection by these fungi. Egg masses or eggs released into soils are easily and preferably parasitized by the opportunistic fungi (Morgan-Jones *et al.,* 1984; Fig. 4). *Purpureocillium lilacinum, Pochonia chlamydosporia, A. niger, Trichoderma* spp. and *Cylindrocarpon* spp. are some of the most important fungi which parasitize eggs and adults of plant nematodes.

Pochonia chlamydosporia

It is a promising opportunistic fungus, and it readily infects the eggs and unhatched larvae of nematodes (Kerry, 2000). The invasion of egg shell may take place with the help of specialized perforation organs or by lateral branches of the mycelium (Stirling, 1991). The eggs and larvae infected with *P. chlamydosporia* generally do not hatch, and if hatched, the larvae fail to invade roots. Only a few isolates of the fungus are, in fact, parasitic to eggs and adults of plant parasitic nematodes. The fungus appears to contribute towards natural balance of nematode population at least in some parts in Europe (Stirling, 1991). Intended application of *P. chlamydosporia* has also been found to satisfactorily control the root-knot disease in vegetables (Godoy *et al.,* 1983) and ornamentals (Khan *et al*., 2005) under glass house or field condition.

Researchers have tested the effectiveness of the fungus against plant nematodes under field condition.

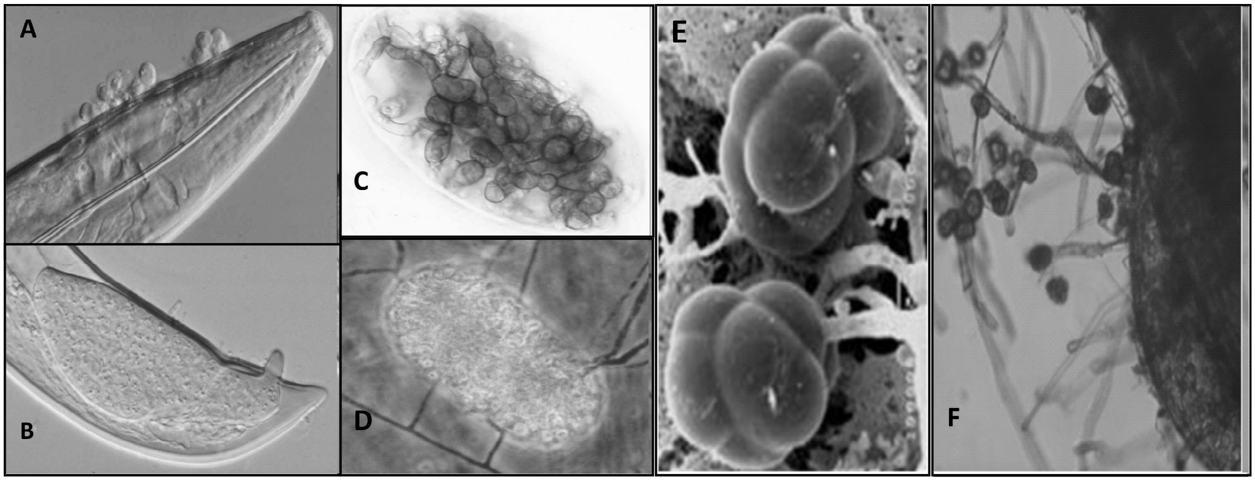

Fig. 4. Zoospores of *Catenaria anguillulae* encysted on *Xiphinema americanum* (A), Sporangium and discharge tube in the nematode tail (B), Root-knot nematode eggs parasitized by *Cylindrocarpon destructans* (C) and *Pochonia chlamydosporia* (D). Spores of *Pochonia chlamydosporia* (E) and a *Globodera* cyst emerged on potato root infected with the fungus (F). Source: A,B&C: A. Jaffee (http://www.cpes.peachnet.edu), P. Timper; D: http://www.cpes.peachnet.edu), and E & F: rothamsted.bbsrc.ac.uk.

Soil application of the oat kernels colonized by *P. chlamydosporia* @ 0.5 and 1.0% (w/w, soil: seed) considerably reduced the population of root-knot and cyst nematodes (Godoy *et al*., 1983; Rodriguez-Kabana *et al*., 1984). Khan *et al.* (2001) reared *P. chlamydosporia* on various substrates viz., leaf litter, saw dust-soil-molasses and bagasse-soil-molasses etc, that yielded 10^{6-7} CFUs /g material. Successful encapsulation of liquid suspension of spores and hyphae of *P. chlamydosporia* was done with sodium alginate containing 10% (w/v) Kaolin or wheat bran (De Leij and Kerry, 1991). Dry powder formulation of *P. chlamydosporium* has also been prepared on a flyash based carrier, and its seed treatment significantly controlled the root-knot in chickpea and pigeonpea (Khan *et al*., 2011).

Purpureocillium

The *Purpureocillium lilacinum* (Luangsa-Ard *et al.* 2011) *Paecilomyces lilacinus* is a hyphomycetous fungus and has demonstrated tremendous biocontrol potential against a number of nematodes, such as *Meloidogyne, Heterodera, Globodera, Rotylenchulus, Tylenchorhynchus* etc. on vegetable and other crops (Stirling, 1991). The fungus is widely distributed in warmer climates. It is a specialized parasite of eggs and sedentary female nematodes (Jatala *et al*., 1979). The fungus has been found parasitizing eggs of *Meloidogyne* spp. and several other plant parasitic nematodes in different geographical locations (Jatala, 1986). Infection by *P. lilacinum* on eggs initiates with hyphal growth on the gelatinous matrix leading to its invasion. The hyphae become prostrate or spiral over the smooth egg surface (Fig. 5).

Khan *et al*. (2012) in a field trial reported that application of *P. lilacinum* with dry neem leaves reduced the gall formation, egg mass production and fecundity of *M. incognita* and increased the yield of okra and tomato. Application of *P. lilacinum* to soil suppressed the root-knot disease on various crops such as tomato (Khan and Tarannum, 1999), okra (Sharma and Trivedi, 1997), cowpea (Midha, 1985), black pepper vine (Sosamma and Koshy, 1997), watermelon and banana (Devrajan and Rajendran, 2002). The fungus treatment has also been reported to suppress the infection of semiendoparasitic nematodes, *Rotylenchulus reniformis* (Fazal *et al*., 2011) and *Tylenchulus semipenetrans* (Maznoor *et al*., 2002) on various field crops.

Aspergillus niger

In recent years, biocontrol potential of *Aspergillus* spp., in particular *A.niger* in nematode management has been explored (Khan and Anwer, 2008). The fungus produces nematoxic metabolites, which cause mortality to the larvae of plant parasitic nematodes within 48 h (Molina and Davide, 1986). The culture filtrates of *A. niger* isolates, AnC2 and AnR3 efficiently suppressed hatching of eggs and caused mortality to *Meloidogyne* juveniles (Khan and Anwer, 2008). The cultures of *A. terreus, A. nidulans* and *A. niger* parasitized 53% of the nematode eggs, inhibited upto 86% egg hatching and induced upto 68% mortality to the juveniles (Singh and Mathur, 2010). Treatments with *A. niger* has been found to suppress the galling, egg mass production and soil population of *M. javanica* in chilli (Shah *et al*., 1994), *M. incognita* in okra (Sharma *et al*., 2005) and tomato (Khan and Anwer, 2008). In a field experiment, soil application of *A. niger* in castor beans abated the population of *R. reniformis* up to 71% (Das, 1998).

Trichoderma species

Strains of *Trichoderma* species are strong opportunistic invaders, fast growing, and prolific producer of spores and powerful antibiotics. Besides being a potent antifungal agent, *Trichoderma* spp. have also demonstrated great potential for antagonism against plant nematodes with a variable degree of parasitism and mode of action (Mohiddin *et al*., 2010). Among the *Trichoderma* spp., *T. harzianum*, *T. viride*, *T. koningii, T. virens, T. longibrachiatum T. atroviride, T asperellum* and

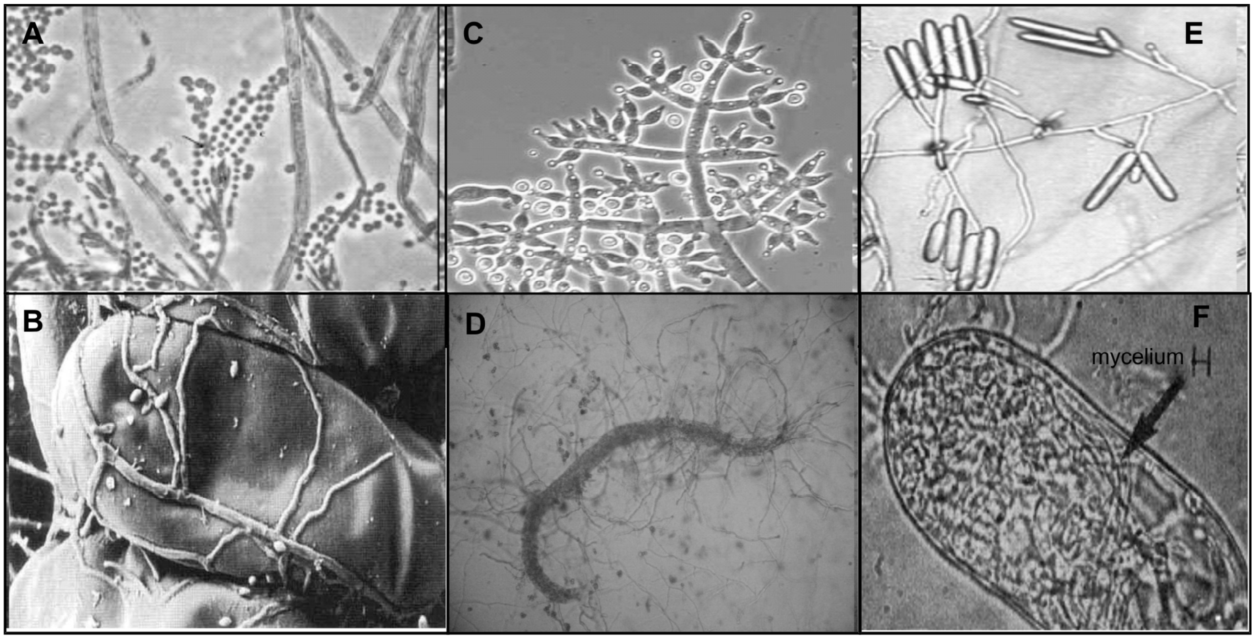

Fig. 5. Divergent phialides and long chains of elliptical conidia of *Purpureocillium lilacinum (=Parcilomyces lilacinus)* (A), the fungus hyphae in and around female of *Meloidogyne incognita* (B), phialides and *Trichoderma harzianum* (C), a nematode larvae infected with the fungus (D), conidia of *Cylindrocarpon destructans* (E) and an infected egg of *Heterodera* (F). Source: Dr. Rajan Laboratories, http://195.98.196.121/en/technology/ nematode-diseases/mode-of-action/ (A) & Bhat *et al*. 2009 (B), http://nt.ars-grin.gov/ taxadescript ions/keys/framephotogallery.cfm?gen= Trichoderma (C), Chanu *et al*., 2015 (D), http://digilander.Lib ero.it/bubicig/funghi/pages/ Cylindrocarpon%20 destructans %2002.html (E) N. Vovlas and S. Frisullo (F).

T. reesei have been found to be the best colonizers of eggs and egg masses of root-knot and other nematodes (Bokhari and Firdos, 2009, Sharon *et al.,* 2009, Khan and Jairajpuri, 2012). *Trichoderma* spp. suppressed the root-knot disease in nursery by 58-69% (Eapen and Venugopal, 1995). Ivanova *et al*. (1996) also observed the infection of *Trichoderma* spp. on *Meloidogyne* spp. in cucumber and tomato. Treatments with the culture filtrate and organic substrate of *T. viride* was reported to suppress the root galling in egg plant (Kumar and Chand, 2015). *T. harzianum* was found infecting the eggs and sedentary female of *T. semipenetrans* in soil and roots (Reddy *et al.,* 1996). Sharon *et al*. (2009) recorded enhanced parasitism of the *T. asperellum*-203 and *T. atroviride* on the egg masses and eggs of *Meloidogyne* spp. Application of culture filtrate of *T. harzianum, T. viride, T. koningii, T. reesei* and *T. hamatum* controlled the reniform nematode (*R. reniformis*) and root-knot nematode (*M. javanica*) on eggplant (Bokhari and Fardos, 2009). Singh and Prasad (2016) reported maximum suppression of reniform nematode population due to continued application of neem oil and *T. harzianum* on chickpea. Application of *T. harzianum* decreased the egg masses production of *M. arenaria* (Windham *et al.,* 1989). Seed and soil application of *T. harzianum* and *T. viride* increased the plant growth and yield of okra and controlled *M. incognita* infection (Prasad and Anes, 2008) and black pepper (Santhosh *et al*., 2009). Treatments with *T. harzianum*, *T. virens*, *T. viride* and *T. ressei* were found to suppress the infection of *M. incognita* (Dew and Hassan, 2002), *M. graminicola* (Pathak *et al*., 2005), *Helicotylenchus exallus* and *Pratylenchus zeae* (Ismail *et al*., 2006) in the field crops.

Cylindrocarpon species

Cylindrocarpon species are commonly soilborne and are especially prevalent within the rhizosphere of many plants. *Cylindrocarpon* spp. rapidly colonize the rhizosphere, and are pathogens of herbaceous and woody plants. Some specie of *Cylindrocarpon* have ability to parasitize eggs and adults of plant nematodes (Tribe, 1977), but thier biocontrol potential has not been exploited fully (Fig. 6, Khan, 2007). Two species *viz*., *C. destructans* and *C. radicicola* have been commonly encountered infecting nematode eggs. The former attacks eggs and cysts of *Heterodera* and *Globodera* spp. (Tribe, 1979; Crump, 1987). These species occur at low frequency, and hence, considered to play only a minor role in the management of nematode populations. Khan *et al.* (2002) have recorded some decline in the root galling on mungbean by the seed or soil application of *C. destructans* under field condition. Freitas *et al*. (1996) reported that *C. destructans* reduced the galls of *Meloidogyne* spp. on citrus and tomato.

Biocontrol bacteria

Bacteria, constituting a major share of microbial community in soil, aacan interact with nematodes directly or indirectly (Khan *et al*., 2009). Among various biocontrol agents of nematodes known to occur in soil, bacteria rank next to fungi with regard to their effectiveness and volume of information available. Three major groups of biocontrol bacteria are stipulated, herewith, on the basis of the mode of antagonism exerted on nematodes. These are nematoxic metabolite producing bacteria, nematode parasitizing bacteria and plant growth promoting bacteria.

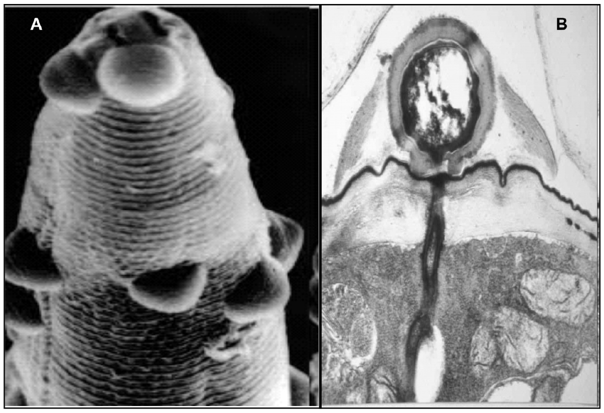

Fig. 6. Spores of Pasteuria penetrans adhered to the head region of a Meloidogyne incognita juvenile (A), and germ tube penetrarted the nematode body (B) Source: rothamsted.bbsrc.ac.uk (A) and R. M. Sayre (B).

Nematoxic metabolite producing bacteria

There is a wide diversity in the bacteria that produce metabolites toxic to nematodes. A number of bacteria are known to produce metabolic by-products, enzymes, toxins, fatty acids, hydrogen sulphide, ammonia, and other compounds, which are generally suppressive to nematodes. Johnston (1957) reported reduction in the population of *Tylenchorhynchus martins* in a saturated rice field due to production of volatile fatty acids, especially butyric acid by *Clostridium butyricum*. Other nematoxic compounds like hydrogen sulphide and ammonia produced by *Desulfovibrio desulfuricans*, *Nitrosomonas* and *Nitrobacter* species have also been found suppressive to *Hirschmanniella oryzae* and *Meloidogyne* spp., respectively (Rodriguez-Kabana *et al*., 1965; Jacq and Fortuner, 1979). Some bacteria, especially those belong to actinomycetes produce antibiotics such as avermectins that has been found quite effective against nematodes (Burg *et al*., 1979). An extensive screening programme involving more than 800 actinomycetes carried out revealed that some 10-15 actinomycetes possessed nematicidal activity (Mishra *et al*., 1987). The valinomycin produced by *Streptomyces annulatus* was found responsible for the suppression of tested nematode species. The toxin produced by *Bacillus thuringiensis* has also been reported to suppress *Meloidogyne* spp. (Zuckerman *et al.,* 1993).

Nematode parasitizing bacteria

There are a few bacteria that are true parasites of nematodes. The bacterial infection affects the egg hatching, larval movement, host penetration, development and reproduction of the infected nematode. Adams and Eichenmuller (1963) were probably the first to notice the infection of *Pseudomonas denitrificans* in *Xiphinema americanum*. True parasitism on plant parasitic nematodes is, however, caused by *Pasteuria* species. Among its three parasitic species viz., *P. penetrans*, *P. thornei* and *P. nishizawae*, the former has demonstrated greatest potential of being an efficient parasite of plant parasitic nematodes (Stirling, 1991). The species has been reported infecting more than 200 nematode species from different parts of the world (Sturhan, 1988).

Pasteuria penetrans is commonly referred as *P. penetrans* group, and consists of mycelium and endospore forming bacteria that are strictly obligate parasites of nematodes (Stirling, 1984). Life cycle of the bacterium starts with the encumbersence of endospores (Sayre and Wergin, 1977) to the cuticle of a vendering nematode, especially second stage juvenile, including *Meloidogyne, Globodera, Heterodera, Pratylenchus* etc. in the soils (Fig. 6). Spore germination occurs generally when spore encumbered juveniles enter the roots and start feeding (Davies *et al*., 1988). A germ tube that develops at least a week after root invasion, penetrates nematode cuticle (Fig. 6) and forms a micro colony in the hypodermis. The colony consists of dichotomously branched, vegetative septate mycelium that proliferates and sporulates internally and eventually body of the adult nematode is filled with the spores. Feeding, moulting and growth of the infected nematode proceed normally, but its reproductive capacity is destroyed and females fail to lay eggs (Sayre and Wergin, 1977). The bacteria infested larvae incite the root galls, but egg masses are not formed, as a result an expected population of the nematode does not emerge. When remnants of roots and infected females decompose; the bacterial spores are liberated in soil. The bacterial endospores can survive acute desiccation and high temperature for prolonged duration. The air-dried powder of the roots infested with *P. penetrans* parasitized root-knot nematodes serve as a source of inoculum of the bacteria for lab or field application. Seed treatment with the dry root powder having *P. penetrans* reduced the galling and egg mass production in okra and chickpea (Vikram and Walia, 2015).

Plant growth promoting bacteria

The free nitrogen fixing bacteria (*Azotobacter*, *Azospirillum*, *Beijerinkia* etc.), and phosphorus solubilizing bacteria (*Bacillus subtilis, B. megaterium*, *Pseudomonas fluorescens, P. eautiginosa, P. striata* etc.) contribute immensely in enhancing the nutritional status of soil by fixing and solubilizing the nutrients. These microorganisms may also contribute in protecting plants from nematode attack or at least minimize the yield losses (Khan *et al.,* 2009). The phosphate solubilizing microorganisms produce nematicidal toxin such as bulbiformin (Brannen, 1995), agrocin (by *B. subtilis*, Kim *et al*., 1997), membrane disrupting lipodepsi peptidase, syringotoxins and syringomycines (by *P. fluorescens*, Woo *et al.,* 2002). These chemicals may suppress the nematode pathogenesis (Khan *et al*., 2009). Root-dip treatments with *P. fluorescens* PF- I significantly enhanced the growth of tomato and reduced the infection by *M. incognita* (Santhi and Sivakumar, 1995). Soil application or root-dip treatment with *P. fluorescens* PRS-9 in field plots also produced similar effects on tomato and *M. incognita*. In a field trial, *P. fluorescens* reduced the population of rice nematode, *Hirschmanniella gracilis* when applied on seeds, seedlings (root dip) and soil application. The bacterium also increased the plant growth parameters and grain yield (Seenivasan and Murugan, 2011). Field application of *P. fluorescens* suppressed the population of burrowing nematode, *Radopholus similis* in banana (Senthilkumar *et al*., 2008). Treatments with native *P. fluorescens* KBR-16 decrease the soil population of *Pratylenchus coffee* and enhanced the yield of coffee beans (Senthilkumar and Deivamani, 2016). Application of *Azotobacter chroococcum* and *Bacillus polymyxa* has been reported to promote the growth of *M. incognita* inoculated tomato plants (Khan and Akram, 2000). Effect of seed treatment (Khan and Kounsar, 2000) and soil application (Khan *et al.,* 2002) of *A. chroococcum, Azospirillum lipoferum, B. subtilis* and *Beijerinkia indica* have been investigated on root-knot of mungbean under field conditions; soil application of *B. subtilis* or *B. indica* significantly decreased the galling leading to 20-26% yield improvement. Out of the three species of blue green algae, *Nostoc calcicola* considerably increased the plant growth and reduced the gall formation and egg mass production of root-knot nematodes (Youssef and Ali, 1998). A mixture of *Anabaena oryzae*, *N. calcicola* and *Spirulina* sp. however, proved much superior in checking the nematode disease and promoting plant growth of cowpea.

Quorum sensing and consortium of BCAs

Micoorganisms in soil, especially bacteria become highly interactive and express social behaviours like swarming, motility, antibiotic resistance, biofilm maturation, virulence etc. in the presence of other bacteria and at or above a specific cell density (Li and Tian, 2012). This phenomenon is commonly refferred to as “Quorum Sensing”. The quorum sensing is also suspected to be involved in the expression of antagonism of biocotrol agents against nematodes and other pathogens. It is probably the quorum sensing which is responsible for increasing the effectiveness (virulence) of the BCAs when in co-occurrence with other microorganisms. It has been frequently reported that presence of phosphate solubilizing microorganisms especially, *B. subtilis* or *P. fluorescens,* the effectiveness of *Trichoderma* spp. against pathogens is enhanced due to increase in the production of α-glycosidase and other pathogen suppressive molecules (Raupach and Kloepper, 1998, Woo *et al.,* 2002, Hanudin *et al*., 2012). In view of greater effectiveness of BCA when in a combination of two or more, the concept of “consortium of microorganisms” has emerged and offer promising results.

Application of *T. virens* and *Burkholderia cepacia* (bacterium) as seed coat followed by root drenches suppressed the root-knot nematode infestation in bell pepper much better than single microorganism treatments (Meyer *et al.,* 2000). Pant and Pandey (2001) reported maximum reduction in the population of *M. incognita* due to combined treatment with *T. harzianum, P. lilacinum* and *A. niger*. Sharma *et al*. (2007) reported effective reduction in the population of root-knot nematode and considerable yield enhancement in okra with seed and soil application of *T. viride* plus *A. niger*. Siddiqui and Shaukat (2004) observed greater effectiveness of combined treatment of *T. harzianum* and *P. fluorescens* in reducing the *M. javanica* population densities in tomato roots. Another combined application of *P. fluorescens* with *P. chlamydosporia* against *M. incognita* on brinjal in farmer’s field was found highly effective over single microorganism treatment (Dhawan *et al*., 2008). Combined application of *T. viride, P. lilacinum, P. chlamydosporia* and *P. fluorescens* provided highest control of root-knot of tomato (Senapati *et al*., 2016).

Combined application of *A. niger, Epicoccum purphurascum, Penicillium vermiculatum* and *Rhizopus utgricans* effectively controlled the root-rot complex caused by *R. solani* and *M. incognita* and greatly increased the seed germination of tomato cv. Pusa Ruby (Rekha and Saxena, 1999). Similarly, combined application of *T. harzianum* and *P. fluorescens* had greater suppressive effect on the disease complex caused by *M. incognita* and *R. solanacearum* on brinjal (Barua and Bora, 2009).

CONCLUSION

Critical analysis of relevant information scattered in the literature has revealed that there is a wide diversity in the occurrence of nematode biocontrol agents. The spatial distribution of the BCAs vary with the climate, nematode species and host plant. Overall, *Purpureocillium lilacinum (=Parcilomyces lilacinus) A. niger, Pochonia chlamydosporia,* and *Trichoderma* spp., *Pseudomonas fluorescens, Pasteuria penetrans,* are the widely occurring BCAs and have been found quite effective against a large number of plant nematodes. Hence, these microorganisms have substantial potential and feasibility for commercial application in sustainable nematode management. Effectiveness of these BCAs varies greatly with the isolate/strain, and some times efficient isolates fail to establish and antagonize nematodes in an exotic environment. This situation necessitates the search for efficient indigenous strains for commercial exploitation in a particular geographical area. The bacterium, *P. penetrans* possesses high degree of parasitic capability but the strict obligate nature makes it difficult for rearing to produce the cultures for commercial use. By and large, strains of *Purpureocillium lilacinum, Pseudomonas fluorescens, A. niger* and *Trichoderma* spp. may prove effective in controlling the nematodes in agricultural crops under subtropical and tropical climates. Further, the application of a consortium of synergistically interacting microorganisms may prove more effective and offer a superior management of nematode infestation in agricultural crops. A combination of *P. lilacinum/ Trichoderma* spp./ *A. niger* + *P. fluorescens/ B. subtilis* shall work better in subtropical, tropical or temperate climates. Although, commercial formulation of consortium of microorganisms are not available, but the individual formulations shall work when applied in combination. Further, the quality and standard of the formulations is needed to be maintained properly, otherwise the application will not yield expected degree of nematode control, consequently the farmers shall lose faith in the biocontrol. This also warrants an immediate need for strict implementation of biopesticide regulations by the central and state plant protection agencies.

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