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Microbial control: A potential tool for management of plant parasitic nematodes in black pepper (*Piper nigrum* L.)

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Abstract
Black pepper (*Piper nigrum* L.) is a highly valued spice crop and probably the oldest known spice to mankind. Globally, black pepper production is adversely affected by several biotic and abiotic factors. Among biotic factors plant parasitic nematodes play an important role which causes significant yield losses and affects their productivity. Various synthetic chemicals have been used for the control of several plant parasitic nematodes, but due to serious non-target effects and environmental hazards, most of the pesticides have been withdrawn from the market. The search for environment friendly alternatives to manage plant parasitic nematode populations has, therefore, become increasingly important and have identified microbial agents as an alternative. Fungal pathogens and bacterial pathogens have been studied and proved as potential agents for microbial control of plant parasitic nematodes in black pepper. This article discusses the important plant parasitic nematodes on black pepper with special reference to root knot nematode and burrowing nematode and the different microbial agents to manage plant parasitic nematodes.

Keywords: Black pepper, parasitic nematode, pathogens, root knot nematode

Introduction
Black pepper (*Piper nigrum* L.) known as 'The King of Spices' is a native of the Malabar Coast in south western India. India is worldwide known as the home of spices and the crop is grown in an estimated area of 1,37,378 ha with an annual estimated production of 61,000 tonnes in 2019-20 [1]. The black pepper production in India is facing sustained losses due to several reasons. Among them, one of the major constraints are nematode infesting diseases, which causes significant yield losses and affects their productivity. The major plant parasitic nematodes infesting these crops include burrowing nematode *Radopholus similis*, root knot nematode *Meloidogyne incognita* [2].

It was reported that 250 species from 43 genera fulfilled one or more of the criteria to be considered to present a phytosanitary risk. The genera and number of species (in parentheses) are considered as posing phytosanitary risk included: Achlysiella (1), Anguina (8), Aphasmatylenchus (1), Aphelenchoides (12), Aphelenchus (1), Belonolaimus (2), Bitylenchus (3), Bursaphelenchus (4), Cactodera (3), Ditylenchus (8), Dolichodorus (1), Globodera (3), Helicotylenchus (7), Hemicriconemoides (3), Hemicycliophora (3), Heterodera (25), Hirschmanniella (5), Hoplolaimus (5), Ibipora (3), Longidorus (10), Macroposthonia (2), Meloidogyne (38), Merlinius (3), Nacobbus (1), Neodolichodorus (2), Paralongidorus (2), Paratrichodorus (11), Paratylenchus (3), Pratylenchus (24), Punctodera (3), Quinisulcius (3), Radopholus (5), Rotylenchulus (3), Rotylenchus (1), Scutellonema (5), Sphaeronema (1), Subanguina (3), Trichodorus (5), Tylenchorhynchus (8), Tylenchulus (2), Vittatidera (1), Xiphinema (15) and Zygotylenchus (1). Almost all of these 250 species were also associated with economically important crops and some also acted as vectors for viruses [3].

Plant parasitic nematodes in black pepper
Plant parasitic nematodes belonging to 29 genera and 48 species are reported from black pepper [4]. So far, seventeen genera are reported in association with the crop in Kerala and Karnataka. Jacob and Kuriyan [5] stated that endoparasitic nematodes *Meloidogyne incognita*, *Rhadopholus similis* and *Holicotylenchus sp*. were commonly associated with black pepper in Kerala of which *M. incognita* was the predominant species.

A new semi-endoparasitic nematode, *Trophotylenchulus piperis* was also found infesting black pepper in Kerala [6]. One new species of root knot nematode, namely *Meloidogyne piperi* sp.n., collected from the roots of *Piper nigrum* growing in Kerala state of India, had been described and illustrated [7]. It was identified *Radopholus similis, Meloidogyne incognita* and *Trophotylenchulus piperis* (new record) infecting the roots of black pepper (*Piper nigrum*) in Uttara Kannada and Dakshina Kannada in Karnataka, India [8]. The presence of 14 plant parasitic nematode species in 14 genera associated with the crop, *Meloidogyne incognita, Radopholus similis, Trophotylenchulus piperis, Rotylenchulus reniformis* and *Helicotylenchus* sp. were the major species found. The concomitant infestation of *M. incognita, R. similis* and *T. piperis* in the roots of black pepper was reported high compared to their solitary infestation [9].

Thirty-five plant parasitic nematodes belonging to 19 genera and 11 families were identified associated with pepper plant in Vietnam [10]. Five taxa of plant-parasitic nematodes were present in all provinces surveyed: *Meloidogyne spp., Rotylenchulus reniformis, Tylenchus sp., Aphelenchus avenae, and Ditylenchus ausafi*. *Meloidogyne spp*. was the most abundant taxon present and all the Meloidogyne populations collected were identified as *M. incognita.* Pot experiments conducted in Sri Lanka indicated that the nematodes *Meloidogyne arenaria, M. incognita, M. javanica, Hoplolaimus seinhorsti* and *Xiphinema ifacolum* adversely affect the growth of black pepper (*Piper nigrum*). All nematode species reduced plant height and root development. Severely stunted plants, infested by *M. incognita and H. seinhorsti*, showed yellowing of the leaves [11]. The common plant parasitic nematodes attacking black pepper are *Acontylus sp, Aphelenchus sp, Criconemoides sp, Helicotylenchus sp, Hoplolaimus sp, Longidorus sp, Meloidogyne incognita, Pratylenchus sp, Radopholus similis, Rotylenchulus reniformis, Scutellonema sp, Trophotylenchulus piperis, Tylenchorhynchus sp* and *Xiphinema sp* [12].

M. incognita and *R. similis* are suspected to be responsible for the slow wilt/yellows disease of black pepper [13]. Slow decline disease of black pepper is due to a feeder root damage caused by *R. similis* and *M. incognita or Phytophthora capsici* either alone or in combination.

a. Root knot nematode

Root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood is one of the most lingering and difficult to manage pest and is one of the major hurdles due to its damage-causing potential [14] particularly in Brazil and India, where the infestation levels over 90% [15]. It was reported *Meloidogyne spp*. as the cause of decline and unthrifty growth of black pepper [16, 17] in India, in Malaysia [18] and in Brazil [19]. As few as 10 juveniles per plant can reduce black pepper growth by 16% under potted condition [20]. The root-knot nematode is an obligate endoparasite that spends its entire life inside the plant roots. After entry inside the plant roots, root-knot nematode induces the formation of "giant cells" in the vascular tissues [21]. The feeding by root knot nematode makes disturbances in water and nutrient uptake by the plant roots, moreover, the giant cells are metabolically active cells that act as nutrient sink for the fulfilment of increasing nutritional demands of nematode females for their reproduction [22]. *M. incognita* is the cause of the yellowing and wilt disease attacking pepper plant. Wilting occurrs two to three months after heavy infection followed by sunny, warm and dry weather [23].

b. Burrowing nematode

Burrowing nematode, *Radopholus similis* (Cobb) Thorne, has been observed infecting more than 300 plant species [24, 25]. Association of burrowing nematode with black pepper was first reported in India [16]. *R. similis* on black pepper in India had been identified as 'banana race' through a host differential study using various species of citrus and through cytological studies [26]. High populations of *R. similis* (>250 nematodes/gram of roots) were found in black pepper gardens throughout the year except during summer months. The buildup of nematode population starts from June/July and reaches maximum during September/ October [27]. The black pepper is a more preferred host for *R. similis.* Pepper fields suffering from burrowing nematode infestation will display yellowing symptoms coupled with root necrosis and canopy dieback [24].

Impact of plant parasitic nematodes

Annual crop losses caused by plant-parasitic nematodes are estimated at 8.8–14.6% of total crop production and 100 - 157 billion USD worldwide [28, 29]. Yield loss data is difficult to obtain because of the complex interactions of plants, nematodes, other soil organisms and soils [30, 31, 32]. In India the production of black pepper is threatened by several biotic and abiotic stresses [33]. Among the biotic stresses, plant-parasitic nematodes are one of the major limiting factors and are responsible for the yield losses of up to 15–35% [34]. Among pests of black pepper, nematodes had been observed to be a major reason for yield decline incredibly early [16, 17]. The slow wilt prevalent in many pepper growing tracts is suspected to be a complex of nematode fungal infection combined with nutritional deficiency [35]. Pathogenicity tests conducted under simulated field conditions showed that *M. incognita* and *R. similis* caused significant reduction in growth and productivity of black pepper vines [36]. In addition to direct feeding and migration damage, nematode feeding facilitates subsequent infestation by secondary pathogens, such as fungi and bacteria [37]. Perhaps more importantly, only ~0.2% of the crop value lost to nematodes is used to fund nematological research to address these losses [38].

Several nematodes have been reported from the rhizosphere of black pepper in all the pepper growing districts of Kerala [39]. The association of the root-knot nematode was reported exceedingly early [40] on black pepper vine and subsequently of the fungus *Fusarium*. sp [41] associated with the 'wilt disease'. It was suspected slow decline disease to be due to a complex of nematode and fungus infection coupled with nutritional deficiency [35]. Studies revealed that both organisms induced a synergistic pathogenic effect on plant resulting in wilting of the plants and that presence of initial nematode infection enhanced the growth suppression [42]. The reason that led to slow decline of black pepper is rather complex, not only due to a species of nematode or fungi, but an interaction of nematode and fungi [43].

Microbial control of nematodes in black pepper

The management of nematodes is more difficult than other pests because nematodes mostly inhibit the soil and usually attack the underground parts of the plants [44]. For the past decades, management of plant parasitic nematodes is being

practiced with chemical nematicides but gradually are being reappraised in respect of health and environmental concern and limited availability in developing nations. On the other hand, microbial control agents are gaining popularity in integrated nematodes management due to their promising results in nematode control and are safer than synthetic nematicides [45, 46]. Biological control offers an alternative to chemical control and is an economical and eco-friendly strategy for nematode management along with safer crop protection to overcome the nematodes stress [47]. In nematode control the potential of microbial pathogens, endophytes, and antagonists for biological control of *Meloidogyne* spp. is great when one considers the microbial-based efficacy within a suppressive soil [48]. Many researchers have reported on the effectiveness of several culturable microorganisms viz. bacteria and fungi as biocontrol agent against plant parasitic nematodes in different crops [49, 50].

a. Fungus

Nematopathogenic fungi are carnivorous fungal species that use their spores or mycelial structures to capture vermiform nematodes or use their hyphal tips to parasitize the eggs and cysts of nematodes or produce toxins to attack nematodes [51]. Over 200 species from 6 different classes of fungi were reported to parasitize on nematode eggs, juveniles, adult and cysts [49]. The common fungi groups with pathogenic activity against plant parasitic nematodes are listed in Table 1 [52].

Table 1: Common fungi groups with pathogenic activity against plant parasitic nematodes [52].

Fungi	Pathogenic effects on nematodes
Aspergillus fumigatus, A. niger	Kill J2 larvae of root knot nematode
Beauveria bassiana	Produce secondary metabolites bassianin, bassiacridin, beauvericin, bassianolide, beauverolides, tenellin and oosporein
Metarhizium anisopliae	Produce cyclic peptides and destruxins toxin that parasitize J2 larvae of target nematodes
Paecilomyces lilacinus	Produce serine protease toxin which parasitize egg and egg mass of target nematodes
Pochonia chlamydosporia	Infects nematode eggs and sedentary females of cyst nematodes by hyphae produced on actively growing mycelium
Trichoderma harzianum, T. viride	Produce nematode cuticle degrading enzymes which are toxic to juveniles of root-knot nematodes and inhibit egg hatch.
Verticillium lecanii	Infect eggs and kills J2 larvae of target nematodes

Paecliomyces lilacinus

Paecilomyces lilacinus (Thorn.) Samson, a soil inhabiting hypomycetes fungus was reported to be highly effective in suppressing root-knot nematodes [53, 54, 55, 56], *Tylenchulus semipenetrans*, *Rotylenchulus reniformis* [55, 57, 58] and cyst nematodes [59]. It is commonly known as natural facultative egg parasite of root knot and cyst nematodes [60] and has drawn attention of many researchers for the past decade owing to its efficacy in parasitizing and controlling populations of phytonematodes. It was reported with high frequency of occurrence in tropics and subtropic [61, 62] and can be found in most of the agricultural soils.

Ten indigenous isolates of *Paecilomyces lilacinus* (PL), isolated from two black pepper farms in Sarawak [63] heavily infested with root-knot nematodes showed varying degree in colonizing female nematodes. Out of the ten isolates two indigenous strains demonstrated highly significant colonization (90%, $P \leqslant 0.01$) on female root knot nematodes and hatching of nematode eggs incubated in spore suspension were significantly reduced. Though *Paecilomyces* could not suppress nematodes completely, it significantly reduced nematode infestation and thereby increased root mass in black pepper [64]. The fungus showed greater efficacy on *Meloidogyne incognita* than *Rhadopholus similis*. The specificity of *P. lilacinus* as an egg parasite of root-knot nematodes was pointed out to be the reason for reduction in Root Knot Index (RKI) in plants inoculated with *M. incognita* and *P. lilacinus*. The efficacy of *P. lilacinus* for the control of *M. incognita* on black pepper was studied in pots under greenhouse condition. *P. lilacinus* was found to be more effective compared to *P. penetrans*, but the combination of both the organisms was found to be highly effective in the management of root-knot nematode in black pepper [65].

Pochonia chalamydosporia

Pochonia chlamydosporia (Goddard) (=*Verticillium chlamydosporium*) was first reported in 1974 as a parasite of nematode eggs by Wilcox and Tribe in the UK. *Verticillium chlamydosporium* was isolated and identified for the first time [66] from cases of a semi-endoparasitic nematode, *Trophotylenchulus piperis*, from an infested black pepper (*Piper nigrum*) garden in Calicut District of Kerala, India. The fungus suppressed hatching of root knot nematode (*Meloidogyne incognita*) eggs by 41.4% within 5 days in an *in vitro* bioassay and appeared promising for the control of root knot nematodes of spice crops. The efficacy of biocontrol agents in suppressing root knot nematodes, considering the ease of mass multiplication, saprophytic nature and the resilience of chlamydospores produced, only *Pochonia chlamydosporia* evidenced better management of root knot nematodes infesting black pepper [67]. Organic soils have been a better substrate for the growth of *P. chlamydosporia* than mineral soils [68]. The tritrophic interaction between root-knot nematodes, *P. chlamydosporia* and the host plant was found to be complex [69].

Arthrobotrys oligospora

Arthrobotrys oligospora Fres., the first recognized nematode-trapping fungus is the most commonly isolated and by far the most abundant nematode-trapping fungus in the environment [70, 71, 72]. Nematophagous fungi belong to three main genus: *Arthrobotrys* (53 species), *Dactylellina* (28 species) and *Drechslerella* (14 species). The activity of the fungus in the soil contributed to decrease in the number of nematodes, reducing the damage caused by the nematode [73]. They comprise more than 200 species of taxonomically diverse fungi that share the ability to attack living nematodes (juveniles, adults and eggs) and use them as nutrients [74]. Three nematophagous fungal strains of *Arthrobotrys oligospora* isolated and selected from 60 coffee and pepper planted soil samples in Dak Lak and Gia Lai provinces had a multi-trap ability for different nematodes species, especially

to *Meloidogyne incognita* and *Pratylenchus coffea* that caused harm on pepper and coffee in Vietnam [75].

Vesicular Arbuscular Mycorrhizae (VAM)

The role of VAM in reducing the harmful effect of root infestation by many parasitic nematodes in crop plants is now well recognized [76]. Four species of vesicular arbuscular mycorrhizae were effective in suppressing nematode infestations in black pepper, which was on par with phorate treatment. Pre inoculation of pepper vines with VAM will be useful in reducing the degree of root infestation by *M. incognita*. The reduction in root-knot index was to the extent of 32.4% with *Glomus fasciculatum* and 36% with *G. etunicalum*. The maximum plant growth in terms of vine length, number of nodes, no. of existing leaves, shoot and root weight in black pepper was recorded in plants that received AMF alone [77]. AMF when introduced prior to nematode inoculation, the population of burrowing and root knot nematodes was suppressed, thereby reducing the root-lesion index and root-knot index.

b. Bacteria

A variety of nematopathogenic bacterial groups have been isolated from soil, host-plant tissues, and nematodes as well as their eggs and cysts [78]. They form a network with complex interactions among bacteria, nematodes, plants and the environment to control populations of plant parasitic nematodes in natural conditions [50, 78]. The common bacterial groups with pathogenic activity against plant parasitic nematodes are listed in Table 2 [52].

Table 2: Available bacterial groups with pathogenic activity against plant parasitic nematodes [52]

Bacteria	Pathogenic effects on nematodes
Bacillus subtilis	Penetrate the nematode cuticles and eventually digest the target Organism
Bacillus thuringiensis	Produce cry proteins that show toxicity to larval stages of parasitic Nematodes
Pasteuria penetrans; *P. thornei;* *P. nishizawae;* *P. usage*	Major economically important plant parasitic nematodes have been observed to be parasitized by *Pasteuria*
Pseudomonas fluorescens	Colonizes the rhizosphere, which facilitates the infection of egg masses protruding from female root knot nematodes on infected roots

Bacillus subtilis

Bacillus subtilis (Ehrenberg) Cohn, have been reported to be effective in boosting the plant vigour and found to be deleterious to the plant pathogens and nematodes [79]. A study conducted in Vietnam to select an active antinematode rhizobacteria [80] showed that strain RB.DL.28 of *Bacillus subtilis* isolated from roots of black pepper demonstrated the greatest inhibition on nematode egg hatching of root knot nematode (82%). Chitinase and protease were strongly associated with the inhibition of egg hatching, while it was newly discovered that natural compounds with thermal stability play a key role in killing J2 nematodes. Prophylactic application of *B. subtilis*, *P. fluorescens*, *T. viride* and AMF, produced a soil condition capable enough to suppress the population buildup of nematodes in soil and roots of *Piper longum* and kept the infection at a lower level. Maximum reduction in root knot index was observed in *P. longum* treated with *B. subtilis* [81].

Bacillus thuringiensis

Bacillus thuringiensis, Berliner (Bt) which is used for insect pest control [82] has studied on its nematicidal effects against economically important phyto parasitic nematodes [83, 84, 85]. *B. thuringiensis* culture was effective in killing freshly hatched 2nd stage juveniles (J2) of *Meloidogyne javanica* [86]. 80% mortality of nematodes after in vitro treatment with Bt [87].

Pasteuria

Pasteuria is a gram-positive, endospore-forming bacterial parasite of a wide range of invertebrates originally observed parasitizing water flea, *Daphnia* spp. [88]. Six species of *Pasteuria* parasitizing plant parasitic nematodes [89] and one species parasitizing bacterivorous nematode have been identified [90]. Among the various biocontrol agents, *Pasteuria* spp. are one of the most promising bacterial bioagents for many nematode species as they have the potential to completely suppress the nematode reproduction by acting as an ovarian parasite [91, 92].

Black pepper being a perennial crop was reported as a particularly good host for the maintenance of *P. penetrans* on *M. incognita* [65]. The efficacy of *P. penetrans* for the control of M. incognita on black pepper under greenhouse condition was found to reduce nematode population, root-gall indices, improved the growth and root mass production significantly [65]. The host range study revealed that the identified in Kerala and Karnataka states of India *Pasteuria* strain was specific to *M. incognita* and completed its life cycle in root knot nematode [93]. Infected females laid no eggs or egg masses; thus, *Pasteuria* prohibited the total fecundity of the nematodes.

Pseudomonas fluorescens

The effectiveness of *Pseudomonas fluorescens* Migula, as a potential biocontrol agent against root knot nematode was due to their ability to envelop or bind the root surface with carbohydrate and lectin thereby interfering with normal host recognition [94]. The efficacy of different biological agents such as *Pseudomonas fluorescens* (Pfbv 22), *Bacillus subtilis* (Bbv 57), *Trichoderma viridi*, Biodynamic compost and AM fungi on root knot nematode management in black pepper was found to have potential to increase significant plant growth in terms of number of leaves and plant biomass [95]. FYM enriched *Pseudomonas fluorescens* @ 50 g/plant was adjudged to be the best of all bio-management treatment in terms of reduction of final nematode population in black pepper [96].

Endophytic bacteria

Endophytic bacteria are one of the antagonist organisms which are usually used in biological control [9]. It colonizes the internal plant tissue, as do endoparasitic nematodes, which makes them ideal candidates for control of such a pathogen [48]. Endophytes would be an effective way of control when compared to chemical control because they move to internal plant tissue and find pathogen by itself [9]. Consortia of endophytic bacteria (Bacillus spp., Pseudomonads,

Arthrobacter spp., Micrococcus spp., Curtobacterium sp. and Serratia) were able to suppress nematodes, *M. incognita* and *Radopholus similis*, significantly [97]. The biocontrol properties of endophytic bacteria isolated from plant roots of black pepper against root knot nematodes were explored for their potential as biocontrol agents and their activities against *Fusarium oxysporum* and *Meloidogyne incognita* [98]. Nine endophytic bacteria isolate from pepper plant were safe and potential to be used as the biocontrol agent against *F. oxysporum* and *M. incognita*. The bacteria could produce such enzymes as protease (4 isolates) and chitinase (7 isolates), play roles in nitrogen fixation (5 isolates) and phosphate solubilisation (5 isolates) [98].

Naturally occurring endophytic bacteria from black pepper vines were found to exhibit strong antagonistic activities against *Phytophthora capsici* and *Radopholus similis*. Stem bacterisation with endophytic *Pseudomonas spp*. was found to suppress *P. capsici* infection (over 90% reduction in lesion length) on cut shoots [99]. Pre-plant root bacterisation with *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Bacillus megaterium* yielded over 60% of plantlets free from *P. capsici* infection on roots. *Curtobacterium luteum* and *Bacillus megaterium* recorded over 70% reduction of nematode population in soil with concomitant production of over 65% of nematode-free plantlets. Besides protecting the plants from the pathogens, the bacteria were also found to enhance the growth of rooted cuttings [99].

Irrespective of the black pepper varieties screened, significantly higher nematode suppression was observed with TC 10 (*Curtobacterium luteum*) followed by BP 17 (*Bacillus megaterium*) [99]. This is first report of suppression of *R. similis* by these two bacteria.

Conclusion

The options for controlling root knot nematodes are becoming increasingly limited due to the potential risk involved in environmental and health hazards. Various synthetic chemicals have been used for the control of several plant parasitic nematodes, but due to serious non-target effects, most of them have been withdrawn from the market. Though several nematicides are effective in checking nematode infestations in black pepper their usage is limited due to high cost and environmental pollution. Nematicides with proper label claim are missing in countries like India where plant parasitic nematodes pose a serious threat to spice production. There is an instant need to adopt an alternative, economical, and eco-friendly strategy for nematode management that can be easily accepted by the farmers. Hence there is urging need for integrated nematode management programmes.

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