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Review

Biological control: An effective approach against nematodes using black pepper plants (*Piper nigrum* L.)

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ABSTRACT

Black pepper (*Piper nigrum* L.) is one of the oldest spices in the world, additionally, it is highly demanded. Several biotic and abiotic variables pose black pepper production worldwide. Plant-parasitic nematodes play a key role among biotic factors, causing considerable economic losses and affecting the production. Different synthetic nematicides were used for controlling plant nematodes, however the majority of pesticides have been pulled from the market due to substantial non-target effects and environmental risks. As a result, the search for alternative eco-friendly agents for controlling plant-parasitic nematodes populations. Microbial agents are a precious option. In this review the bacterial and fungal agents used as an alternative nematicides, they were studied and confirmed as essential anti-microbial agents against plant nematodes which infected *Piper nigrum* L. This work examines the most common plant nematodes infected *Piper nigrum* L., with a focus on root knot and burrowing nematodes, in addition, how to control plant parasitic nematodes using microorganisms.

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Contents

1. Introduction	2048
2. Plant parasitic nematodes in black pepper	2048
2.1. Root knot nematode	2049
2.2. Burrowing nematode	2049
3. Impact of plant parasitic nematodes	2049

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4. Biological control of nematodes in black pepper 2049

 4.1. Fungus 2049

 4.1.1. Paeclomyces lilacinus 2049

 4.1.2. Pochonia chlamydosporia 2050

 4.1.3. Arthrobotrys oligospora 2051

 4.1.4. Vesicular arbuscular mycorrhizae (VAM) 2051

 4.2. Bacteria 2051

 4.2.1. Bacillus subtilis 2051

 4.2.2. Pseudomonas fluorescens 2052

 4.3. Endophytic bacteria 2052

5. Conclusion 2052

Declaration of Competing Interest 2052

Acknowledgements 2052

References 2052

1. Introduction

Black pepper (*Piper nigrum* L.) is an Indian origin. It is grown on an estimated 1,37,378 ha in India, with an evaluated yield production of 61,000 tons in 2019–2020 (Subha and Balamurugan, 2020). Karnataka produces the majority of pepper in India, which has been evaluated to deliver about 30 thousand metric tons of pepper

Table 1
Nematode’s genera and number of species that badly affect black pepper crops.

Genera	Number of species
Achlysiella	1
Anguina	8
Aphamatylenchus	1
Aphelenchoides	12
Aphelenchus	1
Belonolaimus	2
Bitylenchus	3
Bursaphelenchus	4
Cactodera	3
Ditylenchus	8
Dolichodorus	1
Globodera	3
Helicotylenchus	7
Hemicricnemoides	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
Zygotylenchus	1
Achlysiella	1

annually. It was expected that the perennial woody vine of pepper is going to grow in about 40 ha of land (Statista, 2021). Many factors were affected the black pepper crop for several years (Karmawati et al., 2020). One of the most earnest restrictions is parasitic nematodes, which cause massive yield losses and lower productivity as a result of biotic and abiotic stressors such as in (Desoky et al., 2020a). Burrowing nematode, *Radopholus similis* and Root knot nematode, *Meloidogyne incognita* are the most common plant parasitic nematodes infesting black pepper crops (Gómez-Rodríguez et al., 2017; Pervez, 2018). Table 1 presented 250 species from 43 genera that meet one or more of the criteria for being classified as a phytosanitary risk. The majority of these species affected the economically important crops, and the others were vectors for viruses (Singh et al., 2013).

2. Plant parasitic nematodes in black pepper

Black pepper was infected with plant 29 genera and 48 spp, of parasitic nematodes. Seventeen genera have been linked to the crop thus far. *Meloidogyne incognita*, *Rhadopholus similis*, and *Helicotylenchus* spp. usually infest black pepper in Kerala (Subila and Suseela Bhai, 2020) *M. incognita* is the most prevalent species. *Trophotylenchulus piperis* also attack black pepper (Ravindra et al., 2015). *Meloidogyne piperi* sp. is a new species of root-knot nematode discovered in the roots of *Piper nigrum* growing in Kerala, India, has been described and illustrated (Nisha et al., 2019). The varieties, *Radopholus similis*, *Meloidogyne incognita*, and *Trophotylenchulus piperis* attack the roots of *Piper nigrum*. In Vietnam, 35 plant-parasitic nematodes from 19 sp. and 11 families hurt pepper plants (Thuy et al., 2012). *Meloidogyne* spp., *Tylenchus* sp., *Rotylenchulus reniformis*, *Ditylenchus ausafi*, and *Aphelenchus avenae* are five plant-parasitic nematodes found in all provinces studied. *Meloidogyne* spp, were the common taxon found, and all *Meloidogyne* were recognized as *M. incognita*. In Brazil, the check results revealed that *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, *Hoplolaimus seinhorsti*, and *Xiphinema ifacolum* affect the growth of black pepper. Plant height and root development were both lowered by all nematode species, where the leaves of stunted plants attacked with *H. seinhorsti* and *M. incognita* revealed yellow color (De Souza et al, 2021). Additionally, *Cricnemoides* sp., *Xiphinema* sp. *Aphelenchus* sp., *Longidorus* sp., *Trophotylenchulus piperis*, *Radopholus similis*, *Hoplolaimus* sp., *Meloidogyne incognita*, *Pratylenchus* sp., *Scutellonema* sp., *Helicotylenchus* sp. *Rotylenchulus reniformis*, *Tylenchorhynchus* sp., and *Acontylus* sp. (Ramana et al., 1994). The gradual wilt/yellows disease in the black pepper is caused by *M. incognita* and *R. similis* (Brooks, 2021). The slow decline infection in black pepper is induced by *M. incognita*, *Phytophthora capsica*, and *R. similis*, revealing feeder root damage. Generally the bad effects of nematodes were briefed in Fig. 2.

2.1. Root knot nematode

Meloidogyne incognita (Kofoid and White) Chitwood, a root-knot nematode (RKN), is one of the highly persistent pests because of its damage-revealing potential (Ravindra et al., 2014) see Fig. 2, the infection levels exceed to 90% in Brazil and India. *Meloidogyne* spp. has been implicated in the decreased unprofitable development of black pepper in India (Narayana et al., 2018), Malaysia (Leong et al., 2021), and Brazil (De Souza et al., 2021). Under potts conditions, ten juveniles per plant can reduce black pepper growth by 16%. The root-knot nematode is an obligatory endoparasite that lives entirely within the roots of plants. The RKN causes “giant cells” bloating in the vascular tissues after entering the plant roots (Jones and Goto, 2011). The root-knot nematode’s feeding disrupts water and nutrients intake via plant roots, while the gigantic cells are metabolic active cells which consider as feed sinks to meet the nematode females’ rising nutritional demands for reproduction (Mhatre et al., 2015). The yellowing and wilt disease that affects pepper plants is caused by *M. incognita*. Wilting appears 2–3 months after a severe infection in conditions of sunny, warm, and dry weather (Shahnazi et al., 2012).

2.2. Burrowing nematode

Radopholus similis (Cobb) Thorne, a burrowing nematode, found to be infesting over 300 plant species (Sathyan et al., 2020). Via the host variable analysis using multiple *Citrus* sp. and cytological research in India, proved that *R. similis* infected black pepper was known as the ‘banana race’ (Ramana, 1992). *R. similis* population increase in black pepper gardens throughout the year except in the summer, when they are estimated to be more than 250 nematodes/gram of roots. The nematode growth population begins in June/July and reached the peak in September/October. *R. similis* prefers black pepper to white pepper as a host of *R. similis*. Yellowing symptoms, root necrosis, and canopy dieback are all signs of burrowing nematode infestation in pepper crops (Kumar et al., 2018).

3. Impact of plant parasitic nematodes

Plant-parasitic nematodes posed 15% of annual crop yield estimating losses of 100–157 billion USD all over the world (Abd-Elgawad and Askary, 2015; Phani et al., 2021). Due to the complicated relationship between plants, nematodes, soil organisms and soils, yield loss data is difficult to acquire (Coyné and Plowright, 2000). Several biotic and abiotic stressors are threatening black pepper production (Thangaselvabal et al., 2008; Negi et al., 2021). Plant parasitic nematodes are one of the principal limiting factors among the biotic stressors, causing yield losses of up to 15–35% (Abd-Elgawad and Askary, 2015). Nematodes have been identified as a primary cause of early decrease in black pepper output (Thuy et al., 2012). Slow wilt is thought to be caused by a combination of nematode fungal infection and nutritional inadequacy in many pepper growing areas (Naik et al., 2017). *M. incognita* and *R. similis* produced considerable reductions in black pepper growth and productivity in pathogenicity experiments conducted under simulated field conditions (Mohandas and Ramana, 1991). Nematode feeding combined by secondary diseases such as fungus and bacteria, in addition to direct feeding and migratory harm (Mitiku, 2018). More crucially, just 0.2% of the infested crops were used to fund nematological research to remedy these losses (Pervez and Eapen, 2015). The root-knot nematode’s relationship with black pepper vines was first observed in 1906 (Butler, 1906), followed by the fungus *Fusarium* sp. connected with the ‘wilt sickness’ (Krishna Menon, 1949). Considerable decline infection was thought to be caused

by a combination of worm and fungus disease, as well as nutrients deficiencies (Abd-Elgawad and Askary, 2018). Both organisms had a synergistic pathogenic effect on plants, causing the plants wilting, and the presence of an initial nematode infection increased the growth inhibition. The decline in black pepper yield is not only due to nematodes or fungi, but also the synergy of nematodes and fungi causes a massive decrease in pepper yield (Ramana et al., 1992; Usman et al., 2020).

4. Biological control of nematodes in black pepper

Because nematodes primarily block the soil and attack the plants’ roots, nematode treatment is more complex than other pests (Mian, 1998). Chemical nematocides have been used to control plant parasitic nematodes for decades, but they are progressively being re-evaluated due to health and environmental concerns, as well as restricted availability in developing countries. Various studies demonstrated several techniques i.e., conventional breeding for black pepper (Hassanin et al., 2020), eco-friendly natural compounds which may have nematocidal activity among these compounds are bioactive peptides (Saad et al., 2020a; El-Saadony et al., 2021a,b; Saad et al., 2021a), polyphenolic extracts (Saad et al., 2020b; Saad et al., 2021b,c), essential oils (El-Tarabily et al., 2021; Abd El-Hack et al., 2022; Alagawany et al., 2021), and nanomaterials (Saad et al., 2021d; El-Ashry et al., 2022; El-Saadony et al., 2021c), additionally microbial control is a safe and an effective attitude in controlling parasitic nematodes (Dong and Zhang, 2006; Mukhtar and Pervaz, 2003). Biological control is a cost-effective and environmentally acceptable alternative to chemical control for worm management, as well as a safer crop protection technique to combat nematode stress (Mhatre et al., 2019). When considering the microbial-based efficacy inside a suppressive soil, the effectiveness of pathogens, endophytes, and opportunities for biological management of *Meloidogyne* spp and other stressors, is high (Desoky et al., 2020b; Hallmann et al., 2009). Therefore, the use of these natural practices is safer, environmentally friendly, and more effective than chemical formulations and limits the spread of diseases (El-Saadony et al., 2020; Swelum et al., 2020). Many studies have shown that numerous culturable microorganisms, such as bacteria and fungi, can be used as biocontrol agents against plant parasitic nematodes in a variety of crops (Mukhtar et al., 2013; Rahanandeh et al., 2012). Table 2, Figs. 1 and 2 showed recent brief about microbial control of nematodes and their mechanisms of action.

4.1. Fungus

Nematopathogenic fungi are carnivorous fungi, which trap vermiform nematodes with their spores, mycelial structures or hyphal tips to parasitize nematode cysts and eggs or create toxins to assault nematodes (Khan and Haque, 2011). More than 200 fungus species from six different classes have been found to parasitize nematode eggs, juveniles, adults, and cysts (Mukhtar et al., 2013).

4.1.1. *Paeclomyces lilacinus*

Paeclomyces lilacinus (Thorn.) Samson, a soil-dwelling hypomyces is potent against root-knot nematodes recently, it has piqued the interest of different scientists because of its effectiveness in controlling phytonematode propagations. It has been discovered in most agricultural soils with repeated occurrence in the tropics and subtropics (Chen et al., 1996). Ten local strains of *Paeclomyces lilacinus* (PL) obtained from two extensively infested root-knot nematode-infested black pepper plantations (Pau et al., 2012), colonized female nematodes to varied degrees. Two indigenous strains out of ten showed highly substantial colonization (90%)

Table 2
Microbial control of black pepper parasite nematodes.

Microorganisms	Mode of action	References
Fungi	<i>Paecilomyces lilacinus</i>	extensively infested root-knot nematode-infested black pepper plantations where colonised 90% of female nematodes, inhibited egg hatching kept in spore suspension
	<i>Pochonia halamydosporia</i>	reduced the eggs hatching of RKN by 41.4% in five days, indicating that it could be used to manage root knot nematodes in spice crops
	<i>Arthrobotrys oligospora</i>	The fungal activity in the soil lead to a drop in nematodes count, minimizing the nematode's destruction
	Vesicular Arbuscular Mycorrhizae (VAM)	Pre-inoculating pepper vines with VAM will help to reduce the severity of <i>M. incognita</i> root infestation. <i>Glomus fasciculatum</i> had a reduction in root-knot index of 32.4%, whereas <i>Glomus etunicalum</i> had a reduction of 36%. In black pepper plants
Bacteria	<i>Bacillus subtilis</i>	revealed the strongest inhibition on root knot nematode egg hatching by 92% where, Chitinase and protease were found to be highly linked to egg hatching suppression, while natural chemicals with thermal stability were recently identified to be important in killing J2 nematodes
	<i>Bacillus thuringiensis</i>	Freshly born 2 nd stage juveniles (J2) of <i>Meloidogyne javanica</i> were killed by <i>B. thuringiensis</i> culture by 80%
	<i>Pasteuria spp</i>	One of the most promising bacterial biocontrol agents for many worm species because they can totally limit nematode reproduction by functioning as an ovarian parasite. It is found to be specific to <i>M. incognita</i> from completed its life cycle
Bacteria	<i>Pseudomonas fluorescens</i>	is attributed to bind the root surface with carbohydrate and lectin, competing with the host, made it a promising biocontrol agent against root knot nematode
Entophytic bacteria	<i>Serratia spp.</i>	reduced nematode populations in soil by over 70% while simultaneously producing over 65% nematode-free plantlets
	<i>Pseudomonads spp.</i>	
	<i>Arthrobacter spp</i>	
	<i>Curtobacterium spp.</i>	
	<i>Micrococcus spp.</i>	
	<i>Bacillus spp.</i>	

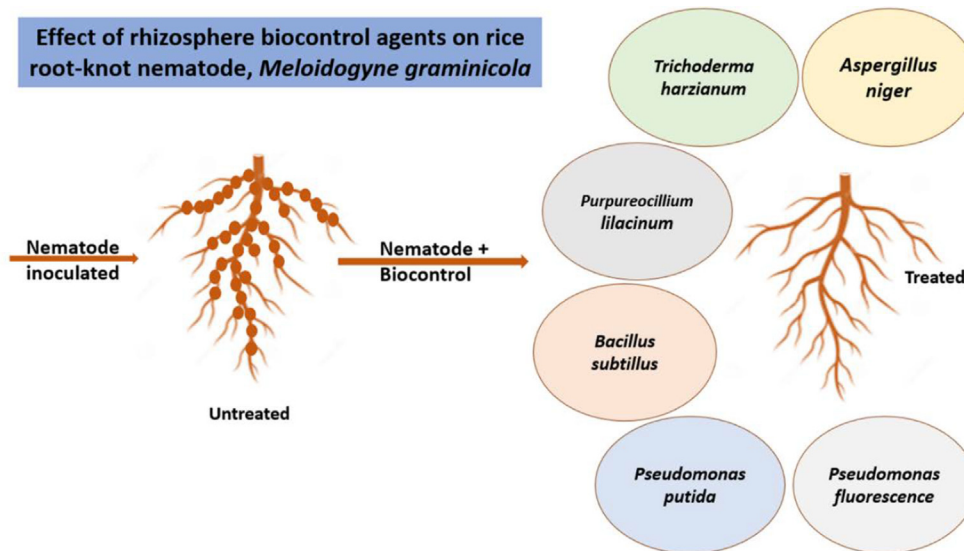


Fig. 1. Effect of rhizosphere biocontrol against RKN.

on RKN females and dramatically inhibited egg hatching kept in spore suspension. *Paecilomyces* did not entirely suppress nematodes, but it can reduce nematode infection and enhance root mass in black pepper (Hano and Khan, 2016). The fungus was more effective on *Meloidogyne incognita* than *Rhadopholus similis*. The low values of Root Knot Index (RKI) in plants treated with *P. lilacinus*, while *M. incognita* was attributed to the specificity of *P. lilacinus* parasites RKN eggs. In pots under greenhouse conditions, the potency of *P. lilacinus* to overcome of *M. incognita* on black pepper was investigated. In the treatment of RKN in black pepper, *P. lilac-*

inus was more effective than *P. penetrans*, although the usage of both species together was beneficial (Sosamma and Koshy, 1997).

4.1.2. *Pochonia chlamydosporia*

Pochonia chlamydosporia (Goddard) (*Verticillium chlamydosporium*) was reported as a parasite of nematode eggs in 1974. For the first time (Sreeja et al., 1996), *Verticillium chlamydosporium* was isolated and identified from infected black pepper by a semi-endoparasitic nematode. In an in vitro experiment, the fungus reduced the eggs hatching of RKN by 41.4% in five days, indi-

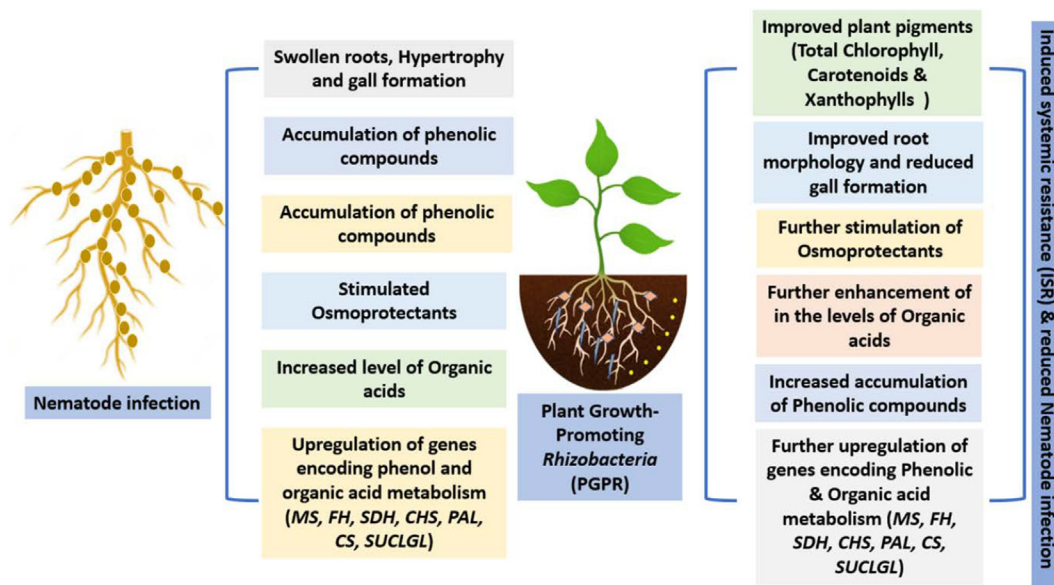


Fig. 2. RKN Infection and the benefit of biocontrol.

cating that it could be used to manage root knot nematodes in spice crops. Owing to the mass population, saprophytic character, and durability of chlamydo spores, only *Pochonia chlamydosporia* demonstrated valuable control of root knot nematodes attacking black pepper (Eapen et al., 2009). Organic soils have proven to be a better substrate for *P. chlamydosporia* growth than mineral soils (Kerry et al., 1993). It was discovered that the tritrophic relationship between root-knot nematodes, *P. chlamydosporia*, and the host plant is complex (Kerry, 2001).

4.1.3. *Arthrobotrys oligospora*

Arthrobotrys oligospora is a species of *Arthrobotrys*. The widely isolated and the most ubiquitous nematode-catching fungus in the environment, the first reported nematode trapping fungus (Farrell et al., 2006; Jaffee, 2004; Wachira et al., 2009). *Arthrobotrys* (53 sp.), *Dactylellina* (28 sp.), and *Drechslerella* are the three primary genus of nematophagous fungi (14 sp.). The fungal activity in the soil lead to a drop in nematodes count, minimizing the nematode's destruction (Jaffee et al., 1996). They are made up of about 200 species of taxonomically varied fungi that all have the potential to feed on living nematodes (juveniles, adults, and eggs) and use them as nutrients (Nordbring-Hertz et al., 2006). Three strains of nematophagous fungus *Arthrobotrys oligospora* were isolated from sixty coffee and pepper planted soil samples have multi-trap effect for different nematode sp., especially *Meloidogyne incognita* and *Pratylenchus coffea*, which harmed pepper and coffee in Vietnam (Hiep et al., 2019).

4.1.4. *Vesicular arbuscular mycorrhizae (VAM)*

VAM's contribution to minimizing the damaging effects of root invasion by several parasitic nematodes in crop plants is now widely acknowledged. Four kinds of vesicular arbuscular mycorrhizae were equally potent as phorate in controlling worm infections in black pepper. Pre-inoculating pepper vines with VAM will help to reduce the severity of *M. incognita* root infestation. *Glomus fasciculatum* had a reduction in root-knot index of 32.4%, whereas *Glomus etunicatum* had a reduction of 36%. In black pepper plants, the highest plant development was recorded in the shape of vine length, nodes numbers, existing leaves number, shoot and root weight in plants, which received only AMF (Koshy et al., 2003). The multiplication of burrowing and root knot nematodes

was decreased when AMF was administered prior to nematode inoculation, lowering root-knot indices and the root-lesion indices.

4.2. Bacteria

Host-plant tissues, soil and nematodes, as well as their eggs and cysts, have all yielded a range of nematopathogenic bacterial groupings (Tian et al., 2007). To manage populations of plant parasite nematodes in natural conditions, they build a net with intricate interactions between the environment, bacteria, nematodes and plants (Tian et al., 2007; Rahanandeh et al., 2012).

4.2.1. *Bacillus subtilis*

Bacillus subtilis (Ehrenberg) Cohn is useful in increasing plant vigour while also being toxic to plant diseases and nematodes. Active nematocidal rhizobacteria, *Bacillus subtilis* strain (RB. DL.28) was isolated from black pepper roots in Vietnam, revealed the strongest inhibition on root knot nematode egg hatching with 82% (Nguyen et al., 2019). Chitinase and protease were found to be highly linked to egg hatching suppression, while natural chemicals with thermal stability were recently identified to be important in killing J2 nematodes. Prophylactic application of *B. subtilis*, *P. fluorescens*, *T. viride*, and AMF resulted in a soil environment capable of suppressing nematode population formation in the soil and roots of *Piper longum*, as well as keeping infection at a lower level. *P. longum* treated with *B. subtilis* showed the greatest reduction in root knot index (Subhagan, 2008). additionally, *Bacillus thuringiensis* Berliner (Bt) had nematocidal effects for insect management (Brar et al., 2006), also, it examined against commercially important phyto parasitic nematodes (El-Sherif et al., 2007; Khan et al., 2010; Mohammed et al., 2008). Freshly born 2nd stage juveniles (J2) of *Meloidogyne javanica* were killed by *B. thuringiensis* culture (Carneiro et al., 1998). After *in vitro* treatment with Bt was reduced the nematodes population with 80% (Mozgovaya et al., 2002). *Pasteuria* is a Gram positive, endospore forming bacterial parasite of a high range of invertebrates that was first discovered parasitizing *Daphnia* spp., a type of water flea. *Pasteuria* parasitizes plant parasitic nematodes in six species (Mohan et al., 2012) and one parasitizes bacterivorous nematodes in one species (Mohan et al., 2012). *Pasteuria* spp. are one of the most promising bacterial bio-control agents for many worm species because they can totally limit nematode reproduction by functioning as an ovarian parasite

(Perrine-Walker and Le, 2021). Black pepper is a perennial crop that has been reported to be an excellent host for *P. penetrans* on *M. incognita* (Sosamma and Koshy, 1997). Under greenhouse conditions, the efficiency of *P. penetrans* in controlling RKN in black pepper was considerably reduced nematode propagation, root-gall indexes, and improve development and root mass productivity (Sosamma and Koshy, 1997). The *Pasteuria* strain found to be specific to *M. incognita* and ruined its life cycle (Mhatre et al., 2020). *Pasteuria* prevented nematode fecundity by preventing infected females from laying eggs or egg masses.

4.2.2. *Pseudomonas fluorescens*

The capacity of *Pseudomonas fluorescens* Migula is attributed to bind the root surface with carbohydrate and lectin, competing with the host, achieving a promising biocontrol agent against root knot nematode (Oostendrop and Sikora, 1990). Different biological agents, as *Bacillus subtilis* (Bbv 57), *Pseudomonas fluorescens* (Pfbv 22), *Trichoderma viridi*, AM fungi and Biodynamic compost were discovered to have the ability to boost considerable plant growth in terms of number of leaves and plant biomass in black pepper (Senthilkumar and Ananthan, 2018). In terms of nematode population decrease in black pepper, FYM enriched *Pseudomonas fluorescens* is considered the best of all bio-control agents (Bina and Sarodee, 2019).

4.3. Endophytic bacteria

One of the antagonist species commonly used in biological control is endophytic bacteria (Ryan et al., 2008). It like endoparasitic nematodes, colonizes inside plant tissue, making them great candidates for pathogen control (Hallmann et al., 2009). When compared to chemical control, endophytes are more effective since they migrate to internal plant tissue and detect pathogen on their own (Ryan et al., 2008). Endophytic bacteria consortiums (*Serratia*, *Pseudomonads*, *Arthrobacter* spp., *Curtobacterium* sp., *Micrococcus* spp., and *Bacillus* spp.) reduced nematodes, *Radopholus similis* and *M. incognita* (Aravind et al., 2009). Endophytic bacteria isolated from black pepper plant roots were studied for their biocontrol characteristics against root knot nematodes, as well as their activity against *Fusarium oxysporum* and *Meloidogyne incognita* (Wiratno et al., 2019). Nine endophytic bacteria isolated from pepper plants were safe and potent against *F. oxysporum* and *M. incognita*. Protease and chitinase are among the enzymes produced by the bacteria, which also play a role in nitrogen fixation and phosphate solubilization (Wiratno et al., 2019). *Phytophthora capsici* and *Radopholus similis* were discovered to have strong antagonistic effects against naturally existing endophytic bacteria from black pepper vines. On cut shoots, stem bacterisation with endophytic *Pseudomonas* spp., suppressed *P. capsici* infection (almost 90% lowering the length of the lesion) (Aravind et al., 2012). The majority of plantlets which inoculated with *Pseudomonas aeruginosa* were free of *P. capsici* infection on roots. *Pseudomonas putida*, and *Bacillus megaterium*. *Curtobacterium luteum* and *Bacillus megaterium* reduced nematode populations in soil by over 70% while simultaneously producing over 65% nematode-free plantlets. The bacteria were discovered to boost the growth of rooted cuttings in addition to protecting the plants against infections (Aravind et al., 2012). *Curtobacterium luteum* TC 10 was found to have much greater nematode suppression than *Bacillus megaterium* BP 17 regardless of the black pepper cultivars evaluated (Aravind et al., 2012).

5. Conclusion

Because of the possible risk of environmental and health issues, the alternatives for managing root knot nematodes are becoming

increasingly limited. Several synthetic compounds have been employed to control plant parasitic nematodes; however, most of them have been pulled from the market due to substantial non-target effects. Several nematode-controlling nematicides are potent in controlling nematode infections in black pepper, but their use is minimized due to large costs and pollution. In places like India, where plant parasitic nematodes represent a severe danger to spice production, nematodes with adequate label claims are lacking. There is an immediate need for farmers to accept an alternative, cost-effective, and environmentally acceptable nematode management technique. As a result, the demand for comprehensive nematode management programs is pressing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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