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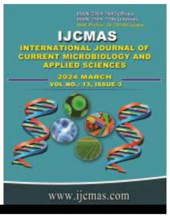

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Management of Root-Knot Nematodes in Peas Using Bacterial and Fungal Bio-Agents: A Review

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ABSTRACT

Pea, *Pisum sativum* L. is one of the most important vegetable crops grown in *rabi* season throughout the world. It is cultivated worldwide over 5.9 million hectares with a production of about 11.7 million tons. In India, it is grown over 0.7 million hectares yielding about 0.6 million tons. Among various obstacles (biotic stress) in cultivating this crop, root-knot nematode (RKN) which is one of the most economically important plant-parasitic nematodes (PPNs) has been reported to cause severe yield losses of up to 20%-56%. RKNs are polyphagous, sedentary endoparasites which is estimated to cause an annual loss of $ 78 billion in agricultural production around the world. In order to control the infection level, frequent and excessive application of chemical nematicides have caused high toxicity level to the soil ecosystems as well as to the environment. An alternative approach of application i.e., biological control agents is an environmentally safe and effective method for sustainable management of RKNs. Among various bioagents, fungal and bacterial agents were reported to reduce RKN density by inhibiting egg hatching, repelling, immobilizing and killing J_2s. Nematophagous fungi are capable of capturing, killing, and digesting nematodes. As a group of important natural enemies of nematode pests, nematophagous bacteria also exhibit diverse modes of action including parasitizing, producing toxins, antibiotics, enzymes, competing for nutrients, inducing systemic resistance of plants and promoting plant health.

Keywords

Root knot nematode (RKN), Plant-parasitic nematodes (PPNs), Biological control agents, Sustainable

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Introduction

Pea, *Pisum sativum* L. belonging to the legume family (Fabaceae) is one of the most cultivated vegetable crops grown in *rabi* season throughout the world. They are highly rich in starch, fiber, protein, vitamin A, vitamin B, vitamin C, vitamin K, phosphorus, magnesium, copper, iron, zinc and lutein (Pownall *et al.,* 2010). In addition, seeds have micronutrients like phytic acids, α-galactosides, polyphenolics, and saponins whose potential health benefits are still being investigated. Peas come in a variety of forms that humans can consume such as, whole or ground dry seeds which are cooked in a variety of dishes, fresh seedlings, immature pods, and seeds which serve as a green vegetable. Whole seed structural and functional characteristics have been evaluated for food improvement, and high quality starch, protein, or oligoside isolates are being extracted from dry pea seeds. Due to their low levels of anti-nutritional factors, dry seeds are primarily used as a source of protein in diets that are monogastric without compromising growth or production characteristics. Pea hay is fed to ruminants as fodder. The origin of pea and its ancestors have been suggested to be Ethiopia, the Mediterranean region, and Western and Central Asia. While Southern Asia and the South and East Mediterranean regions are regarded as secondary centers of diversity, Ethiopia and Western Asia are regarded as the primary centers of diversity. About 10,000 years ago, Neolithic farmers in the fertile crescent domesticated peas. After then, pea quickly spread throughout Europe, the Mediterranean region, and Southwest Asia. Pea landraces and varieties now show an amazing diversity of forms and growing types, adapted to diverse environments and cropping systems. This is likely related to their wide range of cultivation and the diversity of their use as food, feed, or fodder. Globally, it is cultivated over 5.9 million hectares with a production of about 11.7 million tons (Singh, 1983). Some of the top producers in the world includes China, India, USA, France, Egypt, United Kingdom, Pakistan, Algeria, Peru and Turkey. In India, it is grown over 567 thousand hectares yielding about 5846 million tonnes (GoI, 2021). Major pea producing states include Uttar Pradesh, Madhya Pradesh, Bihar and Maharashtra.

In Manipur, it is cultivated over an area of 7.51 thousand hectares with a production of 7643 tonnes in the year 2020-21 (GoI, 2021). Among various pathogens attacking pea plants, the RKN, *Meloidogyne incognita*, is the most important one that attacks the roots (Anwar and Mcknery, 2010). *M. incognita* has been reported to cause severe yield losses of up to 20-56 per cent in pea (De *et al.,* 2000). Root-Knot Nematodes (RKNs) are polyphagous, sedentary endoparasites that pose a serious threat to agricultural production (Machado, 2014; Peiris *et al.,* 2020). The RKNs are in the genus, *Meloidogyne*, which has about 100 described species including four of the most important species, *M. incognita, M. javanica, M. hapla* and *M. arenaria*, which are responsible for substantial losses in agriculture around the world (Coyne *et al.,* 2018; Sikandar *et al.,* 2020). In India, 14 species of the genus, *Meloidogyne* have been identified. *Meloidogyne* species, such as *M. javanica* and *M. incognita*, pose major threats to the production of a wide range of crops (Barros *et al.,* 2018). It was estimated that RKNs cause an annual loss of $ 78 billion in agricultural production around the world (Lima *et al.,* 2017). Plant-parasitic nematodes (PPNs) are microscopic animals that belongs to the phylum Nematoda. More than 4100 PPN species are thought to exist (Decraemer and Hunt, 2006). PPNs are responsible for approximately 12.3% of annual global agricultural production losses which is estimated to be $ 157 billion per year (Singh *et al.,* 2015). The infection in RKN begins at the root level, when second stage juveniles (J_2) hatch in soil from eggs encased in egg masses laid by the females on the infected roots. The juvenile matures into a globose adult female which deposit its eggs in a gelatinous matrix on the surface of a galled root. Due to the close relationship between the host and the RKN, which is controlled by genetics in both species, many crop species have evolved resistance genes (Sidhu and Webster, 1981). RKNs are known to cause significant financial losses, so it is imperative to control them with appropriate measures as soon as possible.

To date, a range of strategies have been employed to manage them, such as the use of chemicals, antagonistic organisms, crop rotation, soil solarization, host plant resistance, etc. Crop rotation is a already a popular method of controlling nematodes, but it requires enough land to grow crops that are not hosts for the nematodes. These alternative crops also need to be profitable for the grower. Although chemical treatments have been found to be highly effective in managing nematodes, resistance developed in nematodes as a result of their unavailability, high cost, and short-term effects. Additionally, use of persistent chemicals may pose a serious threat to the ecological balance and can kill useful microorganisms. Nematicides may be carcinogenic to animals and toxic to reproduction. Therefore, the development of affordable and environmentally friendly bio-agents has become imperative due to restrictions on the use of nematicides.

When it comes to combating and suppressing the pathogenic RKNs in crop ecosystems, biocontrol offers a viable alternative to nematicides and conventional methods that is also environmentally friendly. So the adoption of biological control, which involves the use of predators and parasites to reduce the RKNs at various life stages (eggs, juveniles and adults), as well as the secretion of poisonous diffusible inhibitory metabolites which are known to hamper the reproduction ability of RKNs. Soils which are rich in organic matter are generally colonized by some fungi such as *T. harzianum* that improve biological control activity. In recent year, use of nonchemical means for the control of nematodes is gaining importance because of increased awareness of environmental and human health hazards associated with the use of chemicals. By colonizing the root system, plant growth-promoting rhizobacterias (PGPRs) promote plant growth. However, certain PGPR have also been shown to have nematicidal activity against plant-parasitic nematodes. One such example is the production of secondary metabolites by *Pseudomonas fluorescens* CHA0 that induce mortality of nematode eggs and second-stage infective juveniles J_2s (Siddiqui and Shaukat, 2003). In one of a research conducted by Zhao *et al.,* (2018) from 860 strains of bacteria which are collected from the rhizosphere, 5 showed high efficacy as bio-control agents against *Meloidogyne javanica*, i.e., *Bacillus cereus, B. subtilis, Pseudomonas putida, P. fluorescens,* and *Serratia proteamaculans*. Hence, PGPRs, in addition to increase plant growth, have great potential through direct interaction against nematodes.

Symptoms of RKNs in peas

They are generally classified into two different types.

Above ground and Below ground symptoms

Above ground symptoms

- Stunting which is usually seen in patches due to the uneven distribution of nematodes in the field.
- Yellowing of plants are occurred nematode feeding which adversely affects the uptake and translocation of nutrients upwards.
- Wilting of plants also occurred as damage caused by nematode to the vascular tissues disrupts the flow of water.
- Pods produced by infected plants are mostly less developed and smaller in size.

Below ground symptoms

- The root system is severely reduced and exhibits rotting and degeneration of the infected root parts.
- Development of root nodules is highly affected, as infected roots have fewer functional nodules (pink and intact) and more non-functional nodules (brown and degenerated).
- Formation of galls or knots on the roots (diagnostic symptom).

Life cycle of RKNs

RKNs are obligatorily sedentary endoparasites of plants that exhibit marked sexual dimorphism, with males being vermiform and females pyriform or saccate. There are six stages that make up the life cycle: an egg, four juvenile stages, an adult stage, and so on. In between every juvenile and adult stage is a moulting phase. Temperature has a major impact on how long the RKN life cycle lasts. The life cycle, which starts with the egg and ends with adults following a succession of moults, is successfully completed and comprises stages that are distinct in terms of both morphology and function. The juveniles in their second stage, known as J_2s, undergo additional morphological changes, become sedentary, and feed on specific nurse cells. 200–500 partially or un-embryonated eggs are contained in the gelatinous egg matrix that the female lays. The shells of the eggs are transparent and contain chitin. A first stage juvenile (J_1s) develops over 4-6 days and becomes a J_2 in 2-3 days. In terms of RKN, the parasitic cycle starts at the moment the J_2 enters a root within the elongation zone. The juveniles in their second stage, known as J_2s, undergo additional morphological changes, become sedentary, and feed on specific nurse cells. J_2 then manages to pass through the cortex intercellularly and reach the root tip without harming the root cells. Simple penetration and roaming inside the root are caused by the nematode's stylet's mechanical force as well as its secretions, which include enzymes that break down cell walls that are made by specialized glands. Once it reaches the area where protoxylem is just starting to form, J_2 reverses course and travels back up into the differentiating vascular cylinder, where it establishes a long-term feeding site. Five to seven parenchyma root cells are induced by the J_2 to undergo redifferentiation in order to develop the structure of the nematode feeding site. These feeding cells develop into multinucleate giant cells when secretions from J_2's dorsal oesophageal gland are injected. When J_2 begins to feed, it becomes sedentary and, following the second and third molts that distinguish the feeding phase from the non-feeding phase (J_3 and J_4), immediately changes form from a vermiform to a fusiform shape. When the temperature and humidity are just right (20–35°C), the infectious J_2s are prepared to hatch from the egg. Using their chemosensory amphids, these J_2s detect chemical gradients secreted from the host root and migrate toward the susceptible roots. The number of exterior cuticles from previous moults and a non-functional stylet set apart the next two stages (J_3 and J_4); J_4 is the stage at which female and male nematodes differ in terms of sexual dimorphism.J_4 then goes through its fourth moult in order to differentiate into the adult stage. Under ideal circumstances, nearly all J_4 differentiate into young females, and giant cells allow feeding to resume.

At temperature 25°C, the normal life cycle usually completes within 21 days from the first infection to the laying of egg masses by the females. Males can differentiate and leave the root without feeding in certain situations, such as during a drought or in a host that is resistant, depending on the environmental factors, plant response, and nutrient availability. RKN reproduce primarily by parthenogenesis, though males are occasionally observed and appear to play no part in sexual reproduction.

Bio-control Agents (BCAs) against RKNs

Microorganisms are essential for managing plant diseases as well as for the health of the soil, plant growth and development. These microorganisms can be used as bio-control agents by targeting various soil-borne pathogens with their natural mode of action. Utilizing beneficial organisms and their byproducts to boost positive reactions, decrease negative ones, and support significant increase in the productivity is known as biocontrol. Bio-agents prevent disease while posing little or no threat to the environment. These agents could remain in the soil for a very long time.

Through biological control, diseases can be controlled by various mechanisms, such as:

Competition: Intraspecific and interspecific competition, primarily for resources such as space, nutrients, and water, inhibits the growth, activity, and reproduction of the involved organisms and may have an impact on the fitness of nematodes.

Antibiosis: When plant compounds are discharged into the soil from the roots, antibiosis may occur. Some antibiotics and toxins that bacteria produce and release may be harmful to nematodes during their infectious stage. It is also known that allelochemicals damage plant-parasitic nematodes.

Parasitism: Most nematophagous bacteria feed on nematodes and may use them as a source of nutrients. Through enzymatic action, they can also pierce the cuticle and destroy the nematode host.

Plant growth promotion: By promoting better nutrient solubilization, increased nutrient uptake, and nutrient sequestration, bio-agents help control plant diseases by promoting plant development. More plant-parasitic nematodes in the roots can be tolerated by plants with higher nutritional status.

Induced systemic resistance: Many bacterial products cause plants to produce systemic signalling, which can help plants develop resistance to different pathogenic organisms or shield the entire plant from diseases brought on by different pathogens.

Fungi and bacteria are one of the most important bio-agents used in the context of biological control of root-knot nematodes.

Nematophagous Fungi as BCAs

Numerous fungi can infect different nematode groups and cause diseases. Approximately 200 different taxa are capable of attacking live nematodes. As nematode antagonists by nature, nematophagous fungi present a promising avenue for biocontrol. By compromising the physical and physiological integrity of nematode cuticles, extracellular enzymes play a critical role in the fungal infection of nematodes, facilitating fungal penetration and colonization. Research has demonstrated that extracellular enzymes, including chitinases, collagenases, and serine proteases, function as crucial virulence factors by specifically targeting the chemical components that make up the nematode's cuticle and eggshell. Nematodes can be caught, killed, and digested by nematophagous fungi. They live inside and outside of the host organism, taking advantage of it for their own needs. These fungi attach themselves to nematodes by producing toxins through conidia, hyphae tips that parasitize females and eggs, and specialized traps that ensnare prey.

The most studied nematophagous fungal species used as biocontrol agents are *Trichoderma harzianum, Paecilomyces lilacinus* and *Pochonia chlamydosporia*.

They are traditionally classified into four groups:

(1) The group of fungi that prey on nematodes using specialized traps (Predators)
(2) Egg and Female parasitic fungi
(3) Endoparasitic fungi
(4) Toxin-producing fungi

Predators

In order to catch nematodes, a number of fungal groups make specialised trapping tools. Usually called "nematode-trapping fungi," these fungi produce mycelial traps that kill and capture nematodes as a response to their presence.

Five types of trapping device are produced, they are:

Adhesive networks-The most popular method of trapping is this one. The vegetative mycelium gives rise to lateral branches, which loop around to form an arch. The majority of species produce multiple loops, which combine to create a network of traps that is covered in adhesive material and can be two or three dimensions.

Adhesive knobs-Typically, a spherical adhesive knob on an erect, short stalk serves as the trapping device. Normally, the knobs catch the nematode, but if it escapes while the knobs are still attached, the nematode will still be parasitized by the fungus.

Constricting rings-The most advanced trapping mechanism is this kind. Situated on a short, robust stalk, a three-celled ring with an inner diameter of approximately 20µm forms. The ring's cells instantly expand to catch the victim when a nematode enters it and hold them in place. The prey's body is then filled with assimilating hyphae, which devour its contents.

Non-constricting rings-Constricting rings and these traps are comparable, but when a nematode enters the ring, the cells do not expand. Additionally, when a nematode becomes lodged in the ring, the longer support stalk frequently snaps.

Adhesive branches-These adhesive-coated, erect trapping structures are produced by short laterals that emerge from prostrate hyphae.

Egg and Female Parasitic Fungi

Research on egg and female parasitic fungi has been in progress since the 1990s. These fungi usually employ appressoria (*Purpureocillium* spp. and *Pochonia* spp.), zoospores (*Nematophthora gynophila*), lateral mycelial branches and penetration pegs in order to parasitize eggs, females, and other growth stages of the PPNs.

Endoparasitic Fungi

Spores are produced by nematophagous fungi known as endoparasitic fungi, which infect nematodes. These spores can attach to the nematode's epidermis and cause an infection, or they can be consumed by the nematodes and internalized by them. Spores, like zoospores and conidia, can be ingested or attached to the nematode's cuticle by endoparasites for infection.

Toxin Production

Toxins produced by certain nematophagous fungi affect plant defense and resistance mechanisms against parasitic nematodes while killing nematodes. Fungi that produce toxins come from a variety of orders and families. Without coming into contact with the nematodes directly, the fungus attacks them by secreting inhibitory metabolites that immobilize them. Following immobilization, the hyphae pierce the cuticle of the nematode. Strong enzymatic (proteolytic and chitinolytic) activities, low-molecular-weight metabolites, and particular non-volatile oil components found in the culture filtrates of these fungi kill larvae or prevent them from hatching.

The metabolites secreted by the fungi change the embryonic development of nematode eggs, preventing them from hatching because of their irregular sizes and shapes. Similarly, aside from enzymes, fungi also produce toxic chemicals that immobilize and subsequently eat nematodes. The most common type of fungi that produce toxins are called basidiomycetes.

Nematophagous bacteria as BCAs

Numerous studies conducted recently have shown how rhizobacteria, such as *Pasteuria, Bacillus*, and *Pseudomonas* affect RKNs. The three most common nematophagous bacteria found in soil are *Pseudomonas, Bacillus*, and *Pasteuria*. These bacteria are highly effective first line of defence against pathogens that target plant roots in the rhizosphere. In order to combat RKNs, these bacteria used a variety of strategies, including direct parasitism, antibiosis, competition for food or ecological niches, inducing systemic resistance in the host plant, and reducing root penetration. Gaining an understanding of nematophagous bacterial populations and their molecular mechanisms of action against nematodes will lay the groundwork for developing novel biological control strategies, enhancing the pathogenic activity of potential biocontrol strains, and investigating their roles in an integrated nematode management system. The majority of organisms found in soil are bacteria and have demonstrated significant promise for the biological regulation of nematodes. Over the past 20 years, extensive investigations has been done to determine whether they have the ability to control plant-parasitic nematodes. These investigations have revealed that nematophagous bacteria have wide host ranges, are widely distributed, and have a variety of action modes. Numerous bacterial groups that feed on nematodes have been identified through isolation from soil, tissues of host plants, and nematodes' eggs and cysts. They can impact nematodes through multiple means, such as parasitizing, generating toxins, antibiotics, or enzymes, hindering the nematode's ability to recognize its host, posing a competition for nutrients, causing plants to become resistant to their environment, and enhancing the well-being of plants. These bacteria suppress a wide variety of nematode species, including predatory and free-living nematodes as well as plant- and animal-parasitic nematodes. *Bacillus* offers exceptional benefits in terms of production, transportation, storage, and application when compared to other biological control bacteria. Numerous *Bacillus* isolates, including *Bacillus cereus, Bacillus subtilis, Bacillus firmus,* and *Bacillus amyloliquefaciens*, have been shown to exhibit nematicidal activity against RKNs both *in vitro* and *in vivo*. Since *Bacillus* from the plant rhizosphere readily colonizes the roots and shields plants from pathogens while simultaneously fostering plant growth, its role in microbial application has gained prominence in recent years."Rhizobacteria" is the name given to bacteria that successfully colonize roots. The process by which bacteria live on seeds, grow in the spermosphere in response to sugar- and amino-rich seed exudates, adhere to the surfaces of the roots, and colonize the growing root system is known as "root colonization." As a result, root colonization is an ongoing process rather than a fleeting interaction between bacteria and roots in the soil. Free-living bacteria known as PGPR colonize plant roots and, when applied to seeds or crops, cause infections that are not immediately noticeable. They can also be used to boost plant growth or lessen the harm that soil-borne plant pathogens cause. In addition to increasing crop productivity, PGPR causes a spectrum of resistance against pests and diseases in a variety of crops. PGPR as biopesticides (which control diseases primarily by producing antibiotics and antifungal metabolites), phytostimulators (which promote plant growth by degrading organic phytohormones), and phytofertilizers (which increase the availability of nutrients to the plant). Siderophores are low-molecular-weight compounds that bind iron that are produced and secreted by soil microbes. Fe(III) is bonded by siderophores with extreme affinity. The iron siderophore complex is taken up by the original siderophore-producing bacteria through a receptor found in the outer membrane of the bacterium that is unique to the complex. Iron is released inside the cell and becomes available to support the growth of microorganisms. Through the production of siderophores, which bind the majority of the Fe(III) in the vicinity of the plant root, PGPR can stop the infection of nematodes and other pathogens. As a result, there is less iron in the area, which stops infections from spreading. The pathogens are killed by the PGPR because they outcompete them for the iron that is available. Localized soil iron depletion has no effect on plants because most plants can grow at much lower iron concentrations than microorganisms-roughly a thousand times lower. In a similar vein, gram negative bacteria also produce cyanide, a secondary metabolite. Glycine is converted to hydrogen cyanide (HCN) and carbon dioxide (CO_2) via the action of HCN synthase. *Pseudomonas* strains that produce HCN are able to suppress diseases, but mutant strains that are faulty in their HCN synthesis are unable to shield plants from illness. Furthermore, it was noted that when the plant was infected by the fungus, there was a greater availability of endogenous free indole-3-acetic acid (IAA) and a quicker defence response. Plant resistance to pathogens may be markedly increased if IAA is overexpressed. This could have a positive effect on other defence mechanisms of the host plant, including phenolic infusion, enzyme synthesis (glucanase, for example), gel formation, phytoalexin synthesis, and tylose formation. Biologically active volatile organic compounds (VOCs) are produced by a wide variety of bacteria. For instance, it has been demonstrated that certain bacteria release VOCs. Small chain alcohols, ketones, diacetyl, and esters are all naturally occurring substances that pseudomonas produces and can serve as nematode chemoattractants. Nematicidal VOCs can be produced by seven different bacterial species: *Bacillus simplex, B. subtilis*, *B. weihenstephanensis, Microbacterium oxydans, Stenotrophomonas maltophilia, Streptomyces lateritius*, and *Serratia marcescens*. VOCs produced by *Bacillus megaterium* have the ability to prevent nematode egg hatching and minimize infection by *Meloidogyne incognita.* The VOCs of a newly discovered species, *Lysinibacillus mangiferahumi*, which was isolated from rhizosphere soil, also demonstrated nematicidal activity against *M. incognita.* One of the most promising bacteria *Bacillus firmus*, has received extensive description and characterization in recent years.

Table.1 Some of the fungal BCAs for the management of PPNs

Name of fungi	Mixed with	Effectivefor nematode	Crop	References
T. harzianum	Carbofuran and neem cake	*M. incognita*	Pea	Brahma and Borah, 2016
T. viride	Neem cake	*M. incognita*	Tobacco	Raveendra *et al*., 2011
T. harzianum	Combination of neem cake and *P. fluorescens*	*M. incognita*	Brinjal	Singh *et al*., 2013
T. harzianum	Carbofuran	*M. incognita*	French bean	Gogoi and Mahanta, 2013
Paecilomyces lilacinus	Groundnut cake, neem cake, castor cake, mahua cake, and linseed cake	*M. javanica*	Brinjal	Ashraf and Khan, 2010
P.chlamydosporia	Mustard cake and neem cake	*M. incognita*	Brinjal	Parihar *et al*., 2015

Table.2 Bacterial species as antagonists against RKNs

Species Name	Concentration Used	Reduction in Diseases (Result)	Crop	Nematode Managed	References
Bacillus isolates (BC 27, BC 29, and BC 31)	10^8 spores mL^{-1}	BC 27 and BC 29 caused100% mortality after 24 h. BC 31 was less effective compared to BC 27 and BC 29, as it caused only 84% mortality after 24 h.	Soybean	*M. javanica*	Chinheya *et al*., 2017
Pasteuria penetrans	50% spore suspension	Number of J_2/100 cm^3 was reduced to 9.2 in soil compared to 16.6 in control.	Babchi	*M. incognita*	Mehtab *et al*., 2013
Pseudomonas fluorescens	10^7–10^9 CFU/mL	69.8% reduction of *Meloidogyne incognita*	Cowpea	*M. incognita*	Abd-El-Khair *et al*., 2019
Bacillus pumilis	10^7–10^9 CFU/mL	81.8% reduction of nematode population	Cowpea	*M. incognita*	Abd-El-Khair *et al*., 2019
Serratia plymuthica	1×10^9	Mortality rate of 92.67% was observed in J_2 24 h after treatment (*in-vitro*), and Gall index lowered to 38.67% compared to 49.33% in control, and biocontrol efficacy of 21.62% was observed	Tomato	*M. incognita*	Zhao, 2021

Numerous investigations have shown that *B. firmus* is effective against *M. incognita*, *Heterodera spp., Rodopholus similis, Ditylenchus dipsaci, Tylenchulus semipenetrans*, and *Xiphinema index*. It is capable of killing, paralyzing, and inhibiting PPN egg hatching capacity. The nematicidal effects of the *B. firmus* are most likely the cause of the production of secondary metabolites.

The early inoculation of crop plants with PGPR strains enhances biomass production through various mechanisms such as, by directly affecting root and shoot growth, as well as seedling germination, stand health, plant vigor, plant height, shoot weight, nutrient content of shoot tissues, early bloom, chlorophyll content, and increased nodulation in legumes. RKN infected plants which are treated with PGPR showed an increase in root length, shoot freshness, and dry weight per plant. As PGPR has the ability to colonize the rhizosphere and directly contribute to plant growth, through rhizoremediation, biofertilization, root growth stimulation, and plant stress management.

Types of nematophagous bacteria based on their mode of action

Parasitic Bacteria

- Endospore forming bacterial parasite of plant parasitic nematode.
- The spores get attach to the cuticle of J_2, germ tubes penetrate the cuticle and proliferate through the body of the developing female.
- Gradually, the reproductive system of the female nematodes gets degenerated and the matured endospores are again released in the soil.
- Example: *Pasteuria penetrans*

Opportunistic parasitic bacteria

- In fact, most nematophagous bacteria, except for obligate parasitic bacteria, usually live a saprophytic life, targetting nematodes as one possible nutrient resource.
- They are also able to penetrate the cuticle barrier to infect and kill a nematode host in some conditions.
- They are described as opportunistic parasitic bacteria here, represented by *Brevibacillus laterosporus* strain G4 and *Bacillus* sp. B16

Rhizobacteria

- They have been studied for the biological control of plant-parasitic nematodes (Sikora, 1992). Aerobic endospore-forming bacteria (AEFB) (mainly *Bacillus* spp.) and *Pseudomonas* spp. are among the dominant populations in the rhizosphere which are able to antagonize nematodes (Rovira and Sands, 1977).
- Numerous *Bacillus* strains can suppress pests and pathogens of plants and promote plant growth. Some species are pathogens of nematodes (Gokta and Swarup, 1988; Li *et al.,* 2005).
- The most thoroughly studied is probably *B. subtilis* (Siddiqui and Mahmood, 1999; Lin *et al.,* 2001; Siddiqui, 2002).

Cry protein-forming bacteria

- The nematicidal and insecticidal toxins of Bt are believed to share same modes of action.
- Cry protein exerts its effects by forming lytic pores in the cell membrane of gut epithelial cells (Crickmore, 2005).
- After ingesting the toxin by target nematode larvae, the crystals dissolve within the gut of the nematode, and followed by proteolytic action (Crickmore, 2005).
- Cry toxicity is directed against the intestinal epithelial cells of the midgut and leads to formation of vacuole and pores, pitting, and eventually degrading the intestine (Marroquin *et al.,* 2000).

Limitations and Future Prospects

The frequently restricted host range of nematophagous fungi and bacteria as bio-control agents is a major drawback. A great deal of species have evolved to target particular species of nematodes. This limits their use in scenarios involving the coexistence of several nematode species. Future studies could concentrate on identifying and analyzing strains with wider host ranges or look into methods to improve their capacity to adapt to various nematode species in order to overcome this constraint. To accomplish this multifaceted objective, methods like selective breeding and genetic modification may be investigated. The effectiveness of these bio-agents can be affected by a range of environmental factors because these fungi are sensitive to changes in soil type, temperature, and humidity. Extreme weather, such as excessive heat or dryness, can hinder their capacity to successfully establish and procreate. Researchers ought to look into ways to make these bio-agents more resistant to unfavourable environmental circumstances, such as creating stronger strains or coming up with creative application methods. They typically act more slowly than chemical nematicides, which can be a drawback in situations requiring quick nematode control, particularly in high-value farming environments. Research in the future might concentrate on increasing these fungi's rate of action, perhaps by creating more virulent strains or improving administration techniques. Different applications of bio-agents may have different levels of effectiveness due to variables like nematode densities and soil microbial communities. In order to create strategies that can increase the consistency of nematode control using fungi, these factors should be looked into more thoroughly. This may involve creating tools for tracking and forecasting fungal performance under various circumstances. Because of legal restrictions and the requirement for safety evaluations, BCAs registration and approval for commercial use can be difficult and time-consuming processes. Therefore, cooperation between scientists, government organizations, and business partners is necessary to expedite this procedure and promote the use of nematophagous fungi and bacteria as BCAs. Furthermore, it is important to investigate and creating fresh and enhanced formulations, like encapsulation or granules, which can eventually increase their stability and shelf life and make them more useful for field applications. To guarantee effective distribution, research into cutting-edge delivery techniques like sprays and seed coatings is also beneficial. To guarantee the safety and environmental compatibility of non-target organisms, thorough research that provides insights into the effects of BCAs on these organisms and the environment should be promoted.

Developing safer and more sustainable bio-control strategies can be guided by a deeper understanding of their ecological interactions. To increase virulence and adaptability of BCAs to various environmental conditions and nematode species, biotechnological tools must be investigated. This may entail locating and modifying the genes encoding essential predation mechanisms. Furthermore, to establish more comprehensive and successful nematode control programs where integrated pest management approaches can be developed and tested for particular agricultural systems, use of BCAs should be integrated with other nematode management strategies, such as crop rotation, resistant crop varieties, and chemical nematicides. In order to expedite the approval process for BCAs-based products and increase their accessibility to farmers and industry partners, interdisciplinary work and collaboration with regulatory bodies should be given priority. This will ultimately lead to the scaling up of production and distribution channels for these bio-control agents. Finally, in order to convince farmers to adopt biological control method and show its efficacy in real-world settings, large-scale field trials should be carried out. This calls for the establishment of an appropriate monitoring system to track the long-term impacts on crop productivity and soil health and to generate actionable suggestions for nematode management that are sustainable.

This review emphasized the use of biological methods in the control of *Meloidogyne* species. These tactics are great substitutes for synthetic pesticides and are currently receiving a lot of attention in the field of plant protection research. Through various processes, soil microorganisms, including bacteria and fungi, play a significant role in the management of nematodes. Adopting such methods to control root-knot nematodes in various cropping systems appears to be difficult, though. To understand how the introduction of biocontrol agents affects soil biodiversity, more research is required. Conversely, research should be done on the intricate relationships that exist between biological control agents (or their product) and the targeted pathogen, infected plant, soil structure, environmental factors, and other field-related considerations. In order to ensure effective management, biocontrol must be implemented with greater precision and timing, considering the nematode life cycle under ideal development conditions.

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Author Contribution

Moirangthem Chanu Lankubee: Investigation, formal analysis, writing—original draft. Kshetrimayum Sumita: Validation, methodology, writing—reviewing. Yengkhom Arun Singh:—Formal analysis, writing—review and editing. Abhiraj Atul Patil: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Abd-El-Khair, H.; El-Nagdi, W.; Youssef, M.; Abd-Elgawad, M.M. and Dawood, M.G. (2019). Protective effect of *Bacillus subtilis*, *B. pumilus*, and *Pseudomonas fluorescens* isolates against root-knot nematode *Meloidogyne incognita* on cowpea. *Bull. Natl. Res. Cent.* 43:1–7. https://doi.org/10.1186/s42269-019-0108-8

Anwar, S.A. and Mcknery, M.V. (2010). Incidence and reproduction of *Meloidogyne incognita* on vegetable crop genotypes. *Pak.J. Zool.*42(2):135-141.

Ashraf, M.S. and Khan, T.A. (2010). Integrated approach for the management of *Meloidogyne javanica* on eggplant using oil cakes and biocontrol agents. *Arch. Phytopathol. Plant Protect.* 43(6):609–614. https://doi.org/10.1080/03235400801972434

Barros, A.F.; Campos, V.P.; Souza, L.N.; Costa, S.S.; Terra, W.C. and Lessa, J.H. (2018). Morphological, enzymatic and molecular characterization of root-knot nematodes parasitizing vegetable crops. *Hortic. Bras.* 36:473–479. https://doi.org/10.1590/S0102-053620180408

Brahma, U. and Borah, A. (2016). Management of *Meloidogyne incognita* on pea with bioagents and organic amendment. *Indian J. Nematol*. 46(1):58–61.

Chinheya, C.C.; Yobo, K.S. and Laing, M.D. (2017). Biological control of the root-knot nematode, *Meloidogyne javanica* (Chitwood) using *Bacillus* isolates, on soybean. *Biol. Control*. 109:37–41. https://doi.org/10.1016/j.biocontrol.2017.03.009

Coyne, D.L.; Cortada, L.; Dalzell, J.J.; Claudius-Cole, A.O.; Haukeland, S.; Luambano, N. and Talwana, H. (2018). Plant-parasitic nematodes and food security in Sub-Saharan Africa. *Annu. Rev. Phytopathol.* 56:381-403. https://doi.org/10.1146/annurev-phyto-080417-045833

Crickmore, N. (2005). Using worms to better understand how *Bacillus thuringiensis* kills insects. *Trends Microbiol*. 13: 347–350. https://doi.org/10.1016/j.tim.2005.06.002

De, R.K.; Ali, S.S. and Dwivedi, R.P. (2000). Interaction between *Fusarium oxysporum* f. sp. *lentis* and *Meloidogyne javanica* in lentil. *Indian Phytopathol.*53:353.

Decraemer, W. and Hunt, D.J. (2006). Structure and classification. *In*: Perry, R.N. and Moens, M. (eds.). Plant nematology, CABI Publishing, Wallingford, pp 3–32.

Gogoi, D. and Mahanta, B. (2013). Comparative efficacy of *Glomus fasciculatum*, *Trichoderma harzianum*, carbofuran and carbendazim in management of *Meloidogyne incognita* and *Rhizoctonia solani* disease complex on French bean. *Ann. Plant. Prot. Sci.* 21(1):172–175.

GoI. (2021). Agricultural statistics at a glance 2021. Department of Agriculture, Cooperation and Farmers Welfare, Directorate of Economics and Statistics, Government of India. https://eands.dacnet.nic.in/. Accessed 22 November 2023.

Gokta, N. and Swarup, G. (1988). On the potential of some bacterial biocides against root-knot cyst nematodes. *Indian J. Nematol.* 18: 152–153.

Li, B.; Xie, G.L.; Soad, A. and Coosemans, J. (2005). Suppression of *Meloidogyne javanica* by antagonistic and plant growth promoting rhizobacteria. *J. Zhejiang Univ. Sci.* 6B: 496–501. https://doi.org/10.1631/jzus.2005.B0496

Lima, F.S.; Correa, V.R.; Nogueira, S.R. and Santos, P.R. (2017). Nematodes affecting soybean and sustainable practices for their management. *In*: Soybean–basis of yield, biomass and productivity, pp 95–110. https://doi.org/10.5772/67030

Lin, D.; Qu, L.J.; Gu,H. and Chen, Z. (2001). A3.1-kbgenomic fragment of *Bacillus subtilis* encodes the protein inhibiting growth of *Xanthomonas oryzae* pv. *oryzae*. *J. Appl. Microbiol.* 91: 1044–1050. https://doi.org/10.1046/j.1365-2672.2001.01475.x.

Machado, A.C.Z. (2014). Current nematode threats to Brazilian agriculture. *Curr. Agric. Sci. Technol.* 20(1):26–35.

Marroquin, L.D.; Elyassnia, D.; Griffitts, J.S.; Feitelson, J.S. and Aroian, R.V. (2000). *Bacillus thuringiensis* (Bt) toxin susceptibility and isolation of resistance mutants in the nematode *Caenorhabditis elegans*. *Genetics*155: 1693–1699. https://doi.org/10.1093/genetics/155.4.1693

Mehtab, A.; Javed, N.; Khan, S.A. and Gondal, A.S. (2013). Combined effect of *Pasteuria penetrans* and neem extract on the development of root-knot nematode in medicinal plants. *Pak.J. Nematol.* 31:55–59.

Parihar, K.; Rehman, B.; Ganai, M. A.; Asif, M. and Siddiqui, M. A. (2015). Role of oil cakes and *Pochonia chlamydosporia* for the management of *Meloidogyne javanica* attacking *Solanum melongena* L. *J. Plant Pathol. Microbiol.* 1:1–5. https://doi.org/10.4172/2157-7471.S1-004

Peiris, P.U.S.; Li, Y.; Brown, P. and Xu, C. (2020). Fungal biocontrol against *Meloidogyne* spp. in agricultural crops: A systematic review and meta-analysis. *Biocontrol.* 144:104235. https://doi.org/10.1016/j.biocontrol.2020.104235

Pownall, T.L.; Udenigwe, C.C. and Aluko, R.E. (2010). Amino acids composition and antioxidant properties of pea seed (*Pisum sativum*) enzymatic protein hydrolysate fractions. *J. Agric. Food Chem.*58(8):4712-4718. https://doi.org/10.1021/jf904456r

Raveendra, H.R.; Krishna, M.R. and Mahesh, K.R. (2011). Management of root-knot nematode *Meloidogyne incognita* by using oil cake, bioagent, trap crop, chemicals and their combination. *Int. J. Sci. Nat.* 2:519–523.

Rovira, A.D. and Sands, D.C. (1977). Fluorescent *Pseudomonas*– a residual component in the soil microflora. *J. Appl. Bacteriol.* 34: 253–259. https://doi.org/10.1111/j.1365-2672.1971.tb02284.x

Siddiqui, I. A. (2002). Suppression of *Meloidogyne javanica* by *Pseudomonas aeruginosa* and *Bacillus subtilis* in tomato. *Nematol. Mediterr.* 30: 125–130.

Siddiqui, I. A. and Shaukat, S. S. (2003). Suppression of root-knot disease by *Pseudomonas fluorescens* CHA0 in tomato: importance of bacterial secondary metabolite, 2, 4-diacetylpholoroglucinol. *Soil. Biol. Biochem*. 35:1615–1623. https://doi.org/10.1016/j.soilbio.2003.08.006

Siddiqui, Z. A. and Mahmood, I. (1999). Role of bacteria in the management of plant parasitic nematodes: a review. *Bioresource Technol.* 69: 167–179. https://doi.org/10.1016/S0960-8524(98)00122-9

Sidhu, G.S. and Webster, J.M. (1981). Genetics of Plant Nematode Interaction. *In*: Zuckerman, B.M. and Rohde, R.A. (eds.). *Plant Parasitic Nematodes.* Vol. III, New York: Academic Press, pp 61–87.

Sikandar, A.; Zhang, M.; Wang, Y.; Zhu, X.; Liu, X.; Fan, H. and Duan, Y. (2020). Review Article: *Meloidogyne incognita* (Root-Knot Nematode) A Risk To agriculture. *Appl. Ecol. Environ. Res*. 18:1679–1690. http://dx.doi.org/10.15666/aeer/1801_16791690

Sikora, R. A. (1992). Management of the antagonistic potential in agriculture ecosystems for the biological control of plant parasitic nematodes. *Annu. Rev. Phytopathol.* 30: 245–270. https://doi.org/10.1146/annurev.py.30.090192.001333

Singh, C. (1983). Field Pea (*Pisum* spp.) In: Singh, C. (ed.). Modern Techniques of Raising Field Crops, New Delhi: Oxford and IBH Publ. Co. Pvt. Ltd, pp219–228.

Singh, S.; Singh, B. and Singh, A.P. (2015). Nematodes: a threat to sustainability of agriculture. *Procedia Environ.Sci.* 29:215–216. https://doi.org/10.1016/j.proenv.2015.07.270

Singh, U.B.; Sahu, A.; Sahu, N.; Singh, B.P.; Singh, R.K.; Renu, S.; Jaiswal, R.K.; Sharma, B.K.; Singh, H.B.; Manna, M.C.; Subba Rao, A. and Prasad, R.S. (2013). Can endophytic *Arthrobotrys oligospora* modulate accumulation of defence related biomolecules and induced systemic resistance in tomato (*Lycopersicon esculentum* Mill.) against root-knot disease caused by *Meloidogyne incognita*. *Appl. Soil. Ecol*. 63:45–56.

Zhao, D.; Zhao, H.; Zhao, D.; Zhu, X.; Wang, Y.; Duan, Y.; *et al.,* (2018). Isolation and identification of bacteria from rhizosphere soil and their effect on plant growth promotion and root-knot nematode disease. *Biol. Control*. 119:12–19. https://doi.org/10.1016/j.biocontrol.2018.01.004

Zhao, J.; Wang, S.; Zhu, X.; Wang, Y.; Liu, X.; Duan, Y. and Chen, L. (2021). Isolation, and characterization of nodules endophytic bacteria *Pseudomonas protegens* Sneb 1997 and *Serratia plymuthica* Sneb 2001 for the biological control of root-knot nematode. *Appl. Soil. Ecol*. 164:103924. https://doi.org/10.1016/j.apsoil.2021.103924

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