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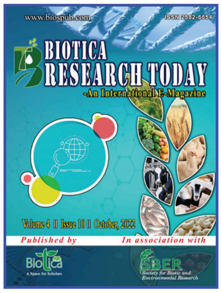

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Bionematicides in India: Opportunities and Challenges

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Abstract

Plant parasitic nematodes are considered as a hidden enemy of crop plants and responsible for a 12.6% annual yield loss. In addition to causing direct damage to crops, they interact with fungi and bacteria to aggravate diseases. Chemical nematicides are used to control plant parasitic nematodes. They have, however, been demonstrated to be harmful to humans, animals, and the environment, causing pollution of natural resources and the environment. Chemicals are unreliable because they cannot provide protection over the long term. Some chemical nematicides have recently been banned, and those that are still in use are quite expensive and out of reach for small farmers. Therefore, scientists are searching for a suitable replacement that will make the control of nematodes sustainable. Bionematicides have several advantages over chemical nematicides, including being environmentally friendly and cost effective. When established in soil, they can provide long-term protection against nematodes.

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Introduction

Plant parasite nematodes are regarded as an unseen foe of crop plants and cause severe crop damage, leading to production and economic losses. It is estimated that the plant parasitic nematode causes an annual yield loss of 8.8% in developed countries and 14.6% in tropical and subtropical regions. Abd-Elgawad and Askary (2015) reported a global average crop loss of 12.6% (equivalent to $ 215.77 billion) attributable to these nematodes for the top 20 life-sustaining crops based on production data and prices from 2010 to 2013. In addition, the subsequent group of food or export crops experienced an average annual yield loss of 14.45% ($ 142.47 billion). In addition to directly damaging crops, they interact with fungus and bacteria to exacerbate plant diseases. The majority of them feed on plant roots, and their symptoms are nonspecific and difficult to diagnose. Because the majority of symptoms are misdiagnosed as water or nutritional deficit, the nematodes' symptoms went undetected. Due to their complex life cycle and presence within the plant, parasitic nematodes are extremely difficult to control.

Chemical nematicides are applied to control plant parasitic nematodes in general. However, it has been demonstrated that they are harmful to humans, animals, and the environment, polluting both natural resources and the ecosystem. The inability of chemicals to give long-term protection renders them unreliable. Some chemical nematicides have been banned recently, and the ones that are still used are very expensive and out of reach for small farmers. Development of resistance-breaking pathotypes, effects of climate change on nematode population, greater adoption of intensive agriculture, and a recent report on the existence of quarantine nematodes all pose a threat to crop plant cultivation.

Therefore, scientists are searching for a suitable substitute that would make the control of plant-parasitic nematodes sustainable.

Significant Advantages of Bionematicides

Bio-nematicides are preferable to chemical nematicides due to the following factors:

- Specific to targets and harmless to beneficial organisms.
- No toxic residues are left in the soil.
- The growth of beneficial soil organisms is unaffected.
- Environmentally friendly.
- Economical.

Disadvantages of Bionematicides

- Establishing nematode antagonists in the soil can require more time.
- Multiple applications are necessary.
- Some bionematicides are slow in action

Important Bionematicides

Purpureocillium lilacinum (=Paecilomyces lilacinus)

This is an egg parasitic fungus which is isolated from soil, decomposing organic debris, insects, and nematodes. *P. lilacinum* has been the subject of the most research on biocontrol of nematodes, and various products are commercially marketed throughout the world. It is widespread in tropical and subtropical soils and can be propagated on a variety of substrates, making it a potential bionematicide.

P. lilacinum produces antibiotics, such as leucinostatin and lilacin, and enzymes, such as protease and chitinase. Protease induces eggshell breakdown and prevents hatching. Chitinase degrades the eggshell allowing the fungus to penetrate through the cuticle. Chitin degradation produces ammonia, which is harmful to root-knot nematode second-stage juveniles (RKN). Its hypha also invades through vulva and anus. Infected eggs expand and deform. As penetration progresses, the vitelline layer of the egg divides into three bands and a significant number of vacuoles; the lipid layer dissolves at this point. Rapidly expanding hyphae kill the developing juvenile inside the egg. Numerous conidiophores are generated, and the hypha migrates to the eggs next to them. In addition, the fungus stimulates the production of substances that promote plant growth (indolacetic acid, cytokinins, 1-aminocyclopropane-1-carboxylate deaminase, and citric acid).

Trichoderma spp. has significant potential as a BCA, not only against root-knot nematodes but also against cyst-forming nematodes via direct parasitism of eggs and larvae. It increases the level of extracellular enzymes such as chitinase and protease, which allow the penetration of the fungus into the eggs by directly affecting very structural components of the eggshell, thereby decreasing the number of eggs and, consequently, the number of infective J2.

Trichoderma harzianum

T. harzianum parasitizes the eggs of *Meloidogyne* and *Globodera* through the secretion of lytic enzymes including chitinase, glucanases, and proteases. Enzymatic activity causes the dissolution of the chitin layer. Its hyphae invade the eggs and juvenile cuticle, grow within them, and produce toxic metabolites. It is commonly recommended for the management of root knot nematode.

Trichoderma viride

It is widely distributed in tropical and subtropical soils and can be multiplied on a wide range of substrates. It produces antibiotics like trichodermin, dermadin, trichoviridin, and sesquiterpene heptalic acid which are involved in the suppression of nematodes. *T. viride* is also recommended for the management of the fungal nematode disease complex.

Pochonia chlamydosporia (=Verticillium chlamydosporium)

It is widely studied nematophagous fungus for the management of root knot and cyst nematodes. It parasitizes the eggs and adult females of plant-parasitic nematodes. The root-knot and cyst nematodes are the primary hosts of this fungus, but it is also known to parasitize citrus, burrowing, and reniform nematodes. The fungus enters the nematode cysts either through natural openings or it may directly penetrate the wall of the cyst. It forms a branched mycelial network when in close contact with the smooth eggshell. The fungus produces an appressorium that adheres to the eggshell by mucigens and from which an infection peg develops and penetrates the eggshell. Penetration also occurs from lateral branches of the mycelium. This results in disintegration of the eggshell's vitelline layer and also the partial dissolution of the chitin and lipid layers, possibly due to the activity of exoenzymes. Egg hatching is inhibited due to toxins secreted by the fungus. In addition, *P. chlamydosporia* is a root endophyte that improves the growth of host plant species and their defence mechanisms against various plant pathogens.

Pseudomonas fluorescens

Products based on this bacterium are recommended and commercially available for the management of nematodes. It produces antibiotics *viz*., phenazines, tropolone, pyrrolnitrin, pyocyanin, and 2,4-diacetylphloroglucinol which have suppressive effect on plant-parasitic nematodes. *P. fluorescens* is recommended for the management of fungal nematode disease complex also.

Bionematicides based on *Trichoderma harzianum, Trichoderma viride, Verticillium chlamydosporium, Purpureocillium* *lilacinum* and *Pseudomonas fluorescens* are recommended for the management of nematodes in India. All the recommended formulations are wettable powder based and being used for seed treatment, nursery treatment and field applications (Table 1). They are generally recommended to be mixed with soil/ FYM so that they can survive in the soil for longer time.

Table 1: List of bionematicides recommended for the management of plant parasitic nematodes in India

Crop	Target nematode	Recommendation
Trichoderma harzianum 1.5% WP (Strain No. IIHR-TV-5 Accessions No. ITCC 6889)		
Tomato, Brinjal, Carrot and Okra	Root-knot nematode, *Meloidogyne incognita*	Seed treatment: *T. harzianum* 1.5% WP @ 20 g kg^{-1} of seeds. Nursery bed treatment: *T. harzianum* 1.5% WP @ 50 g m^2. Soil application: *T. harzianum* 1.5% WP @ 5 kg ha^{-1} enriched FYM @ 5 t ha^{-1} to the soil before transplanting.
Trichoderma viride 1.5% WP (Strain No. IIHR-TV-5 Accessions No. ITCC 6889)		
Tomato	Root-knot nematode, *Meloidogyne incognita*	Seed treatment: *T. harzianum* 1.5% WP @ 20 g kg^{-1} of seeds. Nursery bed treatment: *T. harzianum* 1.5% WP @ 50 g m^2. Soil application: *T. harzianum* 1.5% WP @ 5 kg ha^{-1} enriched FYM @ 5 t ha^{-1} to the soil before transplanting.
Brinjal	Root-knot nematode, *Meloidogyne incognita*	Seed treatment: *T. harzianum* 1.5% WP @ 20 g kg^{-1} of seeds. Nursery bed treatment: *T. harzianum* 1.5% WP @ 50 g m^2. Soil application: *T. harzianum* 1.5% WP @ 5 kg ha^{-1} enriched FYM @ 5 t ha^{-1} to the soil before transplanting.
Carrot, Okra	Root-knot nematode, *Meloidogyne incognita*	Seed treatment: *T. harzianum* 1.5% WP @ 20 g kg^{-1} of seeds. Soil application: *T. harzianum* 1.5% WP @ 5 kg ha^{-1} enriched FYM @ 5 t ha^{-1} to the soil before planting.
Verticillium chlamydosporium 1.0% WP, (2×10^6 CFU g^{-1}min) Strain No. IIHR-VC-3 Accession No. ITCC-6898		
Tomato, Brinjal	Root-knot nematode, *Meloidogyne incognita*	Seed treatment: V. chlamydosporium 1.0% WP @ 20 g kg^{-1} of seeds. Nursery bed treatment: *V. chlamydosporium* 1.0% WP @ 50 g m^2. Soil application: *V. chlamydosporium* 1.0% WP @ 5 kg ha^{-1} enriched FYM @ 5 t ha^{-1} to the soil before transplanting.
Carrot, Okra	Root-knot nematode, *Meloidogyne incognita*	Seed treatment: *V. chlamydosporium* 1.0% WP @ 20 g kg^{-1} of seeds. Soil application: *V. chlamydosporium* 1.0% WP @ 5 kg ha^{-1} enriched FYM @ 5 t ha^{-1} to the soil before transplanting.
Paecilomyces lilacinus 1.15% WP		
Brinjal	Root-knot nematode, *Meloidogyne incognita*	Mix 3.0 kg with 500 kg of Organic manure/ Organic fertilizer.
Pseudomonas fluorescens 1.0% WP (Strain No. IIHR-PF-2, Accession No. ITCC- B0034)		
Tomato, Brinjal, Carrot, Okra	Root-knot nematode, *Meloidogyne* spp.	Seed treatment: *P. fluorescens* 1.0% WP @ 20 g kg^{-1} of seeds. Nursery bed treatment: *P. fluorescens* 1.0% WP @ 50 g m^2. Soil application: *P. fluorescens* 1.0% WP @ 5 kg ha^{-1} enriched FYM @ 5 t ha^{-1} to the soil before transplanting.
Trichoderma harzianum 1.0% WP (Strain No. IIHR-TH-2 Accessions No. ITCC 6888)		
Tomato, Brinjal	Root-knot nematode, *Meloidogyne incognita*	Seed treatment: *T. harzianum* 1.0% WP @ 20 g kg^{-1} of seeds. Nursery bed treatment: *T. harzianum* 1.0% WP @ 50 g m^2. Soil application: *T. harzianum* 1.0% WP @ 5 kg ha^{-1} enriched FYM @ 5 t ha^{-1} to the soil before transplanting.
Carrot	Root-knot nematode, *Meloidogyne incognita*	Seed treatment: *T. harzianum* 1.0% WP @ 20 g kg^{-1} of seeds. Soil application: *T. harzianum* 1.0% WP @ 5 kg ha^{-1} enriched FYM @ 5 t ha^{-1} to the soil before sowing.

Table 1 continue...

Crop	Target nematode	Recommendation
Okra	Root-knot nematode, *Meloidogyne incognita*	Seed treatment: *T. harzianum* 1.0% WP @ 20 g kg^{-1} of seeds. Soil application: *T. harzianum* 1.0% WP @ 5 kg ha^{-1} enriched FYM @ 5 t ha^{-1} to the soil before sowing.
Gerbera	Root-knot nematode, *Meloidogyne incognita*	Apply the Nemastin @ 50 g m^2 at the time of planting.
Carnations	Root-knot nematode, *Meloidogyne incognita*	Apply the Nemastin @ 50 g m^2 at the time of planting.
Tuberose	Root-knot nematode, *Meloidogyne incognita*	Apply 2 Kg Nemastin 1% WP mixed in 2 t of FYM $acre^{-1}$ to the soil before planting.
Banana	Root-knot nematode, *Meloidogyne incognita*	Apply 2 kg Nemastin enriched FYM @ 2 kg $plant^{-1}$ at the time of planting and at an interval of 3 months after planting for a period of one year.
Acid lime	Citrus nematode, *Tylenchulus semipenetrans*	Apply 2 kg Nemastin enriched FYM @ 2 kg $plant^{-1}$ at the time of planting and at an interval of 3 months after planting for a period of one year.
Papaya	Root-knot nematode, *Meloidogyne* spp. and Reniform nematode, *Rotelenchulus reniformis*	Apply 2 kg Nemastin enriched FYM @ 2 kg $plant^{-1}$ at the time of planting and at an interval of 3 months after planting for a period of one year.

(Source: CIBRC, 2022)

Development of Bionematicides

Bionematicide production consists of four core areas of research: (i) selection of biocontrol agent, (ii) formulation type, (ii) manufacturing and packaging process, and (iv) application methods. The entire process of the development of bionematicide is still facing some technological challenges like poor shelf-life, lack of efficacy in wide range of climate and lack of awareness among end users.

Future Directions

Farmers utilise bionematicides seldom despite their immense potential. Although various laboratories are working on nematode biocontrol, very few products have been commercialised. For eco-friendly and sustainable nematode control in India, it is necessary to develop bionematicides that are equivalent or superior to chemical nematicides, exhibit broad-spectrum action, have a longer shelf life, and have a high resistance to environmental conditions (De La Cruz *et al.*, 2019; Poveda *et al.*, 2020). Utilizing cutting-edge technology, like metabolomics, to improve the virulence of bionematicides is urgently required. The availability of whole-genome sequencing will increase our understanding of the biology and genetics of various biocontrol agents being studied for the control of plant-parasitic nematodes. Considering the rich biodiversity of microorganisms in India, novel microbes with a broad spectrum of activity can be explored to manage not only nematodes but also other biotic stresses. In addition, despite the fact that all active ingredients have demonstrated efficacy in laboratory and/or small plot trials, there is a dearth of independent data demonstrating product efficacy in target markets which needs to be addressed (Wilson and Jackson, 2013).

Conclusion

As production, formulation, and packaging of formulated products are crucial processes for the commercialization of bionematicides, research breakthroughs are required to overcome the obstacles associated with these processes in order to make bionematicides as effective as chemical nematicides. In addition, knowledge of nanotechnology is essential for the identification of novel nematicidal molecules, the development of nano formulations of bionematicides, and the improvement of their stability and encapsulation. In order to make bionematicides a viable alternative to chemical nematicides for sustainable and environmentally friendly nematode management, it is essential to increase farmers' awareness of them.

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