Chapter 2

BIOLOGICAL CONTROL OF PLANT PARASITIC NEMATODES OF SPICES

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2.1 Introduction

Plant parasitic nematodes are a limiting factor in spice production and productivity in India. Apart from feeding and damaging the host root system they are also responsible for rendering the plants susceptible to attack by several fungal pathogens. Nematological problems in spices and condiments have been reviewed recently by Koshy and Bridge (1990), Koshy and Geetha (1992) and Ramana and Eapen (1995).

Root knot nematodes (*Meloidogyne* spp.) occupy a unique place as they infest almost all spice crops. The burrowing nematode (*Radopholus similis*) is another important nematode pathogen of spices. *R. similis* and *M. incognita* are the primary incitants of 'slow decline' disease in black pepper. They along with *Phytophthora capsici* cause havocs in black pepper gardens. Yield losses varied from 38.5% to 64.6% in *R. similis* inoculated black pepper plants, alone or in combination with *M. incognita* (Mohandas and Ramana 1991). In cardamom, root knot nematodes pose serious problems in nurseries and also in plantations. Microplot studies under simulated field conditions showed 46.6% yield loss in cardamom at an initial inoculum level of four nematodes/100cm^3 soil (Eapen 1994). Root knot nematodes, *R. similis*, *Pratylenchus coffeae* and *P. zeae* are the major nematode parasites of ginger and turmeric. *M. incognita* reduced yields of coriander, cumin and fennel by 52%, 43% and 42%, respectively (Midha and Trivedi 1991). However, not much is known about the economic significance of different nematodes reported on tree spices.

Chemical pesticides are generally used for nematode management in all crops. The exposure of human populations and natural habitats to increasing levels of pesticides is becoming unacceptable and has prompted the search for new strategies of nematode control, which are sustainable and eco-friendly. Biological control, involving antagonistic microorganisms that compete with, or directly attack the pathogen, is an excellent option in this direction.

2.2 Biological control - different approaches

Many soil organisms like fungi, bacteria, protozoans, viruses, turbellarians, enchytraeids, mites, predatory nematodes, collembolens, tardigrades, etc., are reported as either parasites or predators of plant parasitic nematodes. These biocontrol agents (BCAs) have been considered for four main approaches in control of nematodes.

1) Application of selected isolates of BCAs, 2) soil amendments to enhance the activity of native soil microbes, 3) exploitation of naturally suppressive soils, and 4) use of microbial enzymes and toxic metabolites (Kerry 1990).

2.2.1 Application of selected isolates of BCAs

Though several organisms were found to possess the antagonistic potential against nematode pests, a few fungi and bacteria are generally considered as the best candidates for the control of nematodes. The fungi used for biocontrol of nematodes can be classified into categories like predacious or trapping fungi, endoparasitic or endozoic fungi and egg parasitic or opportunistic fungi based on their mode of action. Bacterial antagonists of nematodes can be categorised into two groups those which directly parasitise nematodes (eg. *Pasteuria penetrans*) and others which release metabolites that are toxic to nematodes (eg. *Bacillus* spp., *Pseudomonas* spp. etc.) (Dhawan and Anju 1995).

2.2.2 *Paecilomyces lilacinus* (Thom.) Samson

Paecilomyces lilacinus is an opportunistic fungus prevalent in many soils. The effect of this fungus was studied in detail on several crops against root knot and cyst nematodes. When black pepper rooted cuttings were inoculated with *P. lilacinus*, 20 days prior to *R. similis* and *M. incognita* inoculations, loss in root mass was reduced from 64.8 to 47.3 per cent in the case of *R. similis* and 31.6 to 21.2 per cent in *M. incognita* inoculated plants. Similarly, root knot index was brought down from 4.4 in plants inoculated with *M.incognita* alone to 2.4 in plants treated with the fungus (Ramana 1994). Similar results were observed by Sosamma and Koshy (1995). However, this fungus was not very effective against *R. similis* (Geetha 1991; Ramana 1994). *P. lilacinus* was found to parasitise root knot nematode eggs and suppressed their infection rate on black pepper seedlings in Brazil (Freire and Bridge 1985).

In cardamom, *P. lilacinus,* multiplied on coffee husk or neem oil cake , reduced root knot nematode multiplication by 48.5 to 57.0 per cent (Eapen 1995). In a field trial, *P. lilacinus* was applied in sick cardamom nurseries at the time of sowing, at the rhizome formation stage and at tillering stage to study its effect on nematodes and rhizome rot. In beds treated with *P. lilacinus,* root knot nematode population in cardamom seedlings was reduced by 74.2% and rhizome rot disease by19.7% (Fig. 2.1, Eapen and Venugopal 1995, Table 2. 1).

2.2.3 *Trichoderma* spp.

Trichoderma spp. are hyphomycetous fungi widely used against several disease causing fungi. The effect of several *Trichoderma* spp. on root knot nematodes of cardamom was studied under *in vitro* and field conditions. Isolates of *T. harzianum, T. hamatum, T. viride, T. aureoviride, T. polysporum, T. longibrachiatum, T. koningii, T. pseudokoningii* and *Gliocladium virens* were tested under *in vitro* conditions to study their interaction with root knot nematode, *M. incognita,* collected from

Fig. 2.1 Field performance of *Paecilomyces lilacinus* treated cardamom seedlings in a primary nursery.
a. *P. lilacinus;* b. without *P. lilacinus.*

Table 2.1. Effect of *Trichoderma* isolates and *Paecilomyces lilacinus* on root knot nematodes and growth of cardamom seedlings in nurseries

Treatment	No. of tillers	Biomass (g/seedling)	Standard seedlings (%)	Root knot nematodes (per g of root)
Trichoderma spp.	3.16 [a]	42.99 [a]	63.16 [a]	163.06 [ab]
P. lilacinus	3.36 [a]	54.14 [c]	60.94 [a]	100.62 [bc]
Trichoderma + *P. lilacinus*	3.33 [a]	51.67 [bc]	66.43 [a]	51.17 [c]
Check	3.22 [a]	43.29 [ab]	51.94 [b]	390.74 [d]

Means followed by the same superscript in a column are not significantly different at 5% level. Data are means of four replications combined over three trials.

black pepper roots. All these fungi colonised the egg masses of root knot nematodes. But, *T. harzianum, T. longibrachiatum, T. koningii, T. viride* and *G. virens* were found to be bettter colonisers. Although direct parasitization was not observed with any of these isolates, distortion of nematode eggs was frequently seen in the case of *T. harzianum, T. viride* and *G. virens.* Culture filtrates of all the isolates had nematicidal properties (IISR 1995).

Isolates of *T. harzianum, T. viride,* an unidentified *Trichoderma* sp. and a *Gliocladium* sp. from cardamom plantations of Coorg were tested against root knot nematodes infesting cardamom seedlings under greenhouse condition. Among these, one *T. harzianum* isolate and an unidentified *Trichoderma* sp. suppressed root knot nematodes and improved the growth of cardamom seedlings (NRCS 1991, 1994, IISR 1995). The suppression was more significant when native soil was used as the potting mixture (Fig. 2.2).

Trichoderma isolates were incorporated in soil beds in 'sick' cardamom nurseries, where there was high incidence of root knot nematodes and rhizome rot/ damping off. On incorporation of the biocontrol agents, the mean incidence of rhizome rot was reduced to 26.44%, compared to 42.47% in untreated plots. The nematode suppression was more than 60% in *Trichoderma* treated nursery beds (Table 2. 1, Fig 2.3, Eapen and Venugopal 1995). Therefore, *Trichoderma* isolates that are broad spectrum biocontrol agents, can be easily multiplied and used in spice nurseries and plantations.

2.2.4 Vesicular arbuscular mycorrhizae (VAM)

Vesicular arbuscular mycorrhizal fungi are obligate dependants on plants for nourishment. Their symbiotic association with roots increases the plant's ability to absorb water, phosphorous and other elements. VAM fungi often increase host tolerance to nematode infection due to the improved 'P' status of the host or by competition or antagonism between the nematode and fungus (Smith 1987). Sivaprasad and others (1990, 1992) observed suppression of root knot nematodes infesting black pepper when the plants are colonised by VAM fungi.

Significant increase in growth of black pepper vines and reduction in root knot nematode, *M. incognita* infestation and their multiplication were observed when the plants were inoculated with VAM fungi, namely, *Glomus mosseae, G. fasciculatum, Acaulospora laevis* and *Gigaspora margarita* (Anandaraj and others 1991). In black pepper, feeder root loss, caused by *P. capsici, R. similis* and *M. incognita* either alone or in combination, leads to slow decline (Anandaraj and others 1996a,1996b). Black pepper cuttings when inoculated with VAM fungus *G. fasciculatum* and subsequently challenged by these three root pathogens either singly or in combination, showed suppression of root rot (Anandaraj and others 1993). In VAM inoculated plants, root rot was considerably reduced even when all the three pathogens were inoculated (Fig. 2.4). In case of *P. capsici* and *M. incognita,* colonization of VAM first deprives the

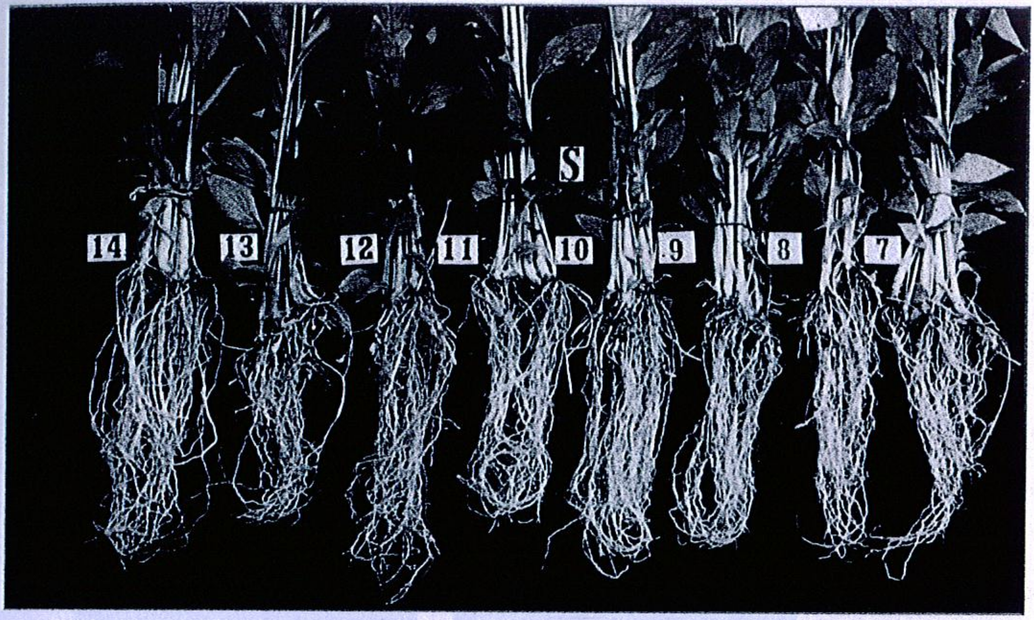

Fig. 2. 2. Effect of *Trichoderma* isolates on root knot nematode infestation in cardamom seedlings. 7 to 12 - Different isolates of *Trichoderma* + root knot nematode; 13. Root knot nematode alone; 14. Check.

Fig. 2. 3. Evaluation of *Trichoderma* isolates for control of rhizome rot and root knot nematodes in a cardamom nursery

a. *Trichoderma* treated; b. Cardamom plants treated with biocontrol agents (from left) *Trichoderma* treated; check; *P. lilacinus* treated.

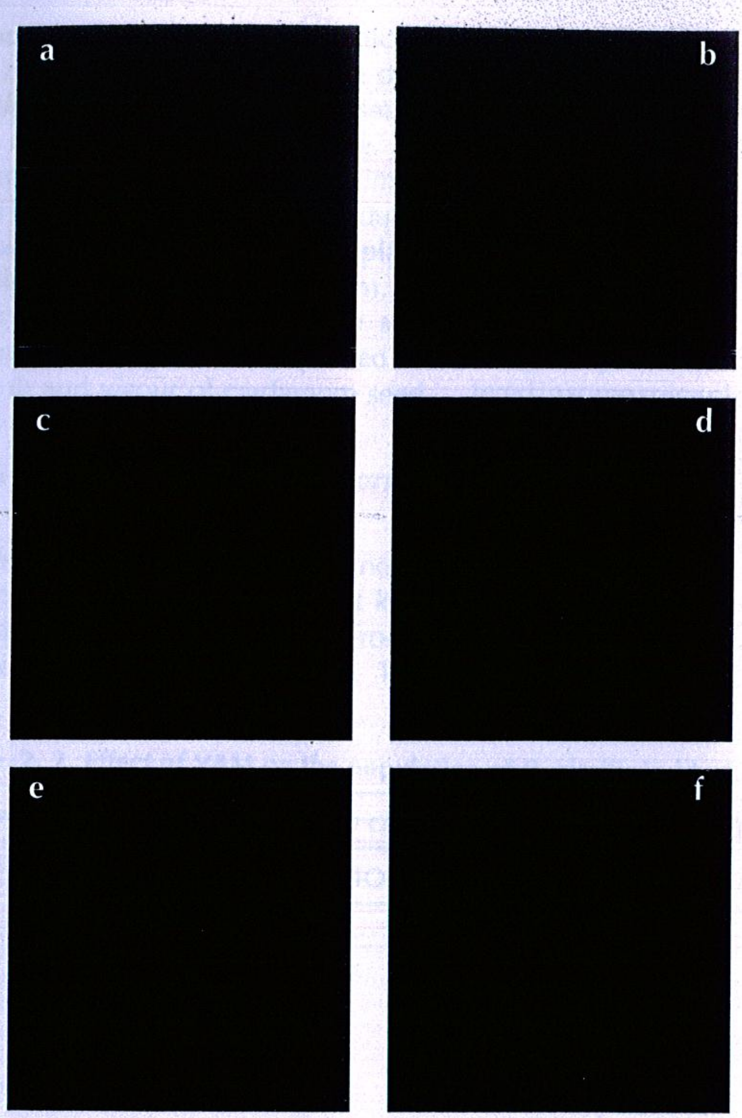

Fig. 2.4. Suppression of root rot of black pepper by VAM

a. *P. capsici* inoculated roots without VAM (left) and with VAM (right)

b. *R. similis* inoculated roots without VAM (left) and with VAM (right)

c. *P. capsici* + *R. similis* without VAM (left) and with VAM (right)

d. *R. similis* + *M. incognita* without VAM (left) and with VAM (right)

e. *P. capsici* + *M. incognita* without VAM (left) and with VAM (right)

f. Combined inoculation of *P. capsici, R. similis* and *M. incognita* with VAM (left) and without VAM (right).

pathogens the point of entry, which is the zone of elongation. In case of *R. similis,* the enhanced rooting offset the root rot caused by this nematode. The population of *M. incognita* and *R. similis,* both in soil and roots, were also reduced considerably in VAM inoculated plants (Table 2.2, 2.3). In cardamom, *G. margarita* and *G. fasciculatum* suppressed *M. incognita* and improved the growth and vigour of cardamom seedlings (Thomas and others 1989).

2.2.5 *Verticillium chlamydosporium* Goddard

Verticillium chlamydosporium, a known biocontrol agent of cyst and root knot nematodes, was isolated recently from a black pepper garden in Kerala. This fungus parasitized root knot nematode eggs and suppressed their hatching by 56.3% within 24 hours of exposure (Sreeja and others 1996, Fig. 2.5). It colonized black pepper roots and is a promising biocontrol agent in spices.

2.2.6 *Pasteuria penetrans*(Thorne) Sayre & Starr

Pasteuria penetrans has been encountered in some spice plantations and black pepper plants inoculated with *P. penetrans* reduced *M. incognita* and *R. similis* and thereby improved the vegetative growth of the plants (Geetha 1991, Sosamma and Koshy 1995). In cardamom also, they gave excellent protection against root knot nematodes (Fig. 2.6)

Table 2. 2. Effect of VAM on the population of *R. similis* in black pepper

Treatment	Soil (100 cc)		Root (1g)	
	VAM	NON VAM	VAM	NON VAM
Con	0.0^{e}	0.0^{e}	0.0^{e}	0.0^{e}
Pc	0.0^{e}	0.0^{e}	0.0^{e}	0.0^{e}
Rs	30.0^{a}	49.0^{a}	175.0^{a}	250.0^{a}
Rs + Pc	15.5^{c}	16.3^{d}	75.0^{c}	50.0^{c}
Rs + Mi	18.8^{b}	29.3^{b}	130.0^{b}	210.0^{b}
Pc + Rs + Mi	14.0^{d}	17.0^{c}	40.0^{d}	30.0^{d}
Pc + Mi	0.0^{e}	0.0^{e}	0.0^{e}	0.0^{e}
Mean	11.1	15.9	60.0	77.1

Con - Control Rs - *Radopholus similis*
Pc - *Phytophthora capsici* Mi - *Meloidogyne incognita*

Means followed by same superscript in a column are not significantly different at 5% level.
Means are average of 4 replications.

Table 2.3. Effect of VAM on the population of *M. incognita* in black pepper

Treatment	Soil (100 cc)		Root (1g)	
	VAM	NON VAM	VAM	NON VAM
Con	0.0[d]	0.0[d]	0.0[c]	0.0[d]
Pc	0.0[d]	0.0[d]	0.0[c]	0.0[d]
Rs	0.0[d]	0.0[d]	0.0[c]	0.0[d]
Rs + Pc	0.0[d]	0.0[d]	0.0[c]	0.0[d]
Rs + Mi	43.8[a]	112.3[a]	100.0[a]	295.0[a]
Pc + Rs + Mi	17.5[c]	24.0[c]	90.0[b]	65.0[b]
Pc + Mi	25.0[b]	27.3[b]	100.0[a]	60.0[c]
Mean	12.3	23.4	41.4	60.0

Con - Control; Rs - *Radopholus similis*

Pc - *Phytophthora capsici*; Mi - *Meloidogyne incognita*

Means followed by same superscript in a column are not significantly different at 5%. Means are average of 4 replications.

2.2.7 Plant health promoting rhizobacteria (PHPR)

Several plant health promoting rhizobacteria (PHPR) exhibit antagonistic potential against plant parasitic nematodes. Sheela and others (1993) inoculated five *Bacillus* spp. for their effect on root knot nematodes and growth of potted black pepper vines. *Bacillus pumilus, B. macerans* and *B. circulans* significantly reduced *M. incognita* population and improved the growth of black pepper plants.

Pseudomonas fluorescens is a rhizobacteria commonly present in rhizosphere. *P. fluorescens* isolates obtained from black pepper gardens were tested against root knot nematode, *M. incognita*. Preliminary observations have indicated that some isolates possess inhibitory effect on nematode multiplication (Fig. 2.7, IISR 1995, Eapen and others 1996).

2.3 Soil amendments to enhance the activity of native soil microbes.

A wide range of soil amendments have been used for control of nematode populations (Rodriguez - Kabana and others 1987). Nematode control observed in such amendments is attributed to the nematicidal products released during their breakdown and also the associated enhancement of indigenous microflora. Soil amendments using various organic cakes have been recommended for nematode control in various spices. Application of well decomposed cattle manure or compost

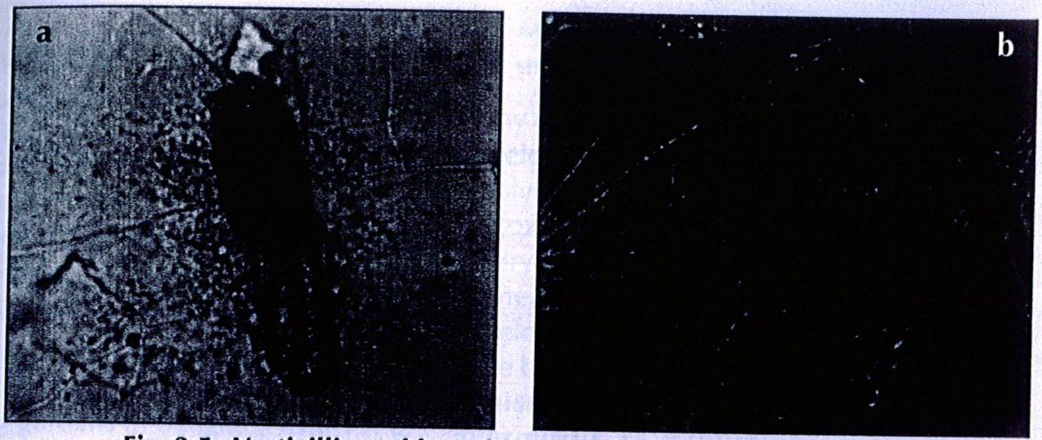

Fig. 2.5. *Verticillium chlamydosporium* on root knot nematodes
a) Parasitisation of eggs b) Chlamydospores

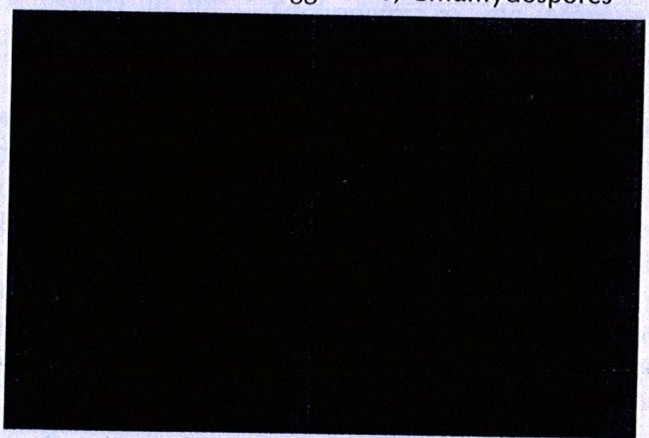

Fig. 2.6. A root knot nematode larva parasitised by *Pasteuria penetrans*

Fig. 2.7. Suppression of root knot nematodes and growth enhancement in black pepper seedlings by *Pseudomonas fluorescens.* From left - Root knot nematode alone; *P. fluorescens* alone; *P. fluorescens* + Root knot nematodes and check.

or saw dust or green leaves or neem oil cake helps in reducing the nematode multiplication in ginger (Colbran 1974, Pegg and others 1974, Mohanty and others 1992). In black pepper and cardamom, neem oil cake application reduced nematode populations and increased the yield (Ali 1987, Ramana and others 1992).

Mulching with green manure plants reduced slow decline incidence in black pepper gardens in Bangka (Pasril 1976, de Waard, 1979) and *M. incognita* populations on black pepper in the Amazonian region (Ichinohe 1980, 1985).

Leaf extracts of *Glyricidia maculata, Ricinus communis* and *Crotalaria juncea* were found lethal to *R. similis* at a dilution of 1:5 within 24 hrs. Addition of chopped leaves of *G. maculata* (10g/kg soil) as green manure also reduced population of *R. similis* and increased growth of black pepper in pot culture experiments (Jasy and Koshy 1992).

2.4 Exploitation of naturally suppressive soils

Soils that suppress nematode multiplication usually contain a range of natural enemies which differ in their biocontrol potential. Antagonistic microflora can develop and persist in a sufficiently stable ecosystem as in the case of perennial crops or those grown in monocultures (Kerry 1987). In spices, exploitation of suppressive soils has not been attempted so far. This is mainly because of the difficulty in demonstrating experimentally non specific natural control and also due to the lack of rapid methods for identifying suppressive soils.

2.5 Microbial enzymes and toxic metabolites

Many nematophagous fungi and bacteria release enzymes or toxins which are involved in parasitism. These enzymes or toxic metabolites that affect the embryonic development and hatching of nematodes can be exploited to develop 'natural' nematicides. Toxins have been found in a range of microorganisms including *Streptomyces avermitilis* (Avermictin), *Bacillus thuringiensis,* several *Pseudomonas* spp., *Trichoderma* sp., etc. Mass production of such 'natural' or biological nematicides can be attempted or efficient strains of nematophagous microorganisms can be identified and used. Genes coding for such materials can be transferred to suitable soil bacteria or plants to disrupt nematode development. Chitinases from egg parasitic fungi or collagenases from nematode trapping fungi could be used in this approach (Kerry 1990).

2.6 Conclusions and future prospects

Research on biological control of nematodes of spices has been intensified only recently. Although a few achievements have been made, much remains to be done in this field. There is a tremendous scope for biological control in the tropical, perennial cropping systems common to many spices. Agents that are endemic in agricultural soils have the highest potential for nematode control in such cropping systems.

Such organisms can be induced to proliferate by methods compatible with conventional farming systems. Though several organisms that are antagonistic to nematodes are known, still there may be many more which need to be exploited. The latest biotechnological advances offer opportunities to develop genetically engineered biocontrol agents that are more efficient than natural ones. Biological control agents are not replacement for nematicides. They have to be used in integration with other methods, such as soil solarization, plant resistance and low rates of nematicides.

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