MANAGEMENT OF *MELOIDOGYNE INCOGNITA* ON MEDICINAL COLEUS BY COMMERCIAL BIOCONTROL FORMULATIONS

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Summary. The effects of commercial formulations of the plant growth promoting rhizobacterium *Pseudomonas fluorescens*, the antagonistic fungus *Trichoderma viride,* the egg parasitic fungi *Paecilomyces lilacinus* and *Verticillium lecanii,* and arbuscular mycorrhizal fungus *Glomus mosseae,* on root-knot nematode, *Meloidogyne incognita,* infecting medicinal coleus, *Coleus forskohlii*, were assessed under glass-house and field conditions and compared with that of the nematicide carbofuran. Dipping of stem cuttings in 0.1% *P. fluorescens* before planting gave the greatest reduction of *M. incognita* population density in soil, suppression of root-gall development and production of heavier root tubers of medicinal coleus. The root colonization of *P. fluorescens* was higher (38-110 $\times 10^4$ colony forming units/g root) in softwood cutting dip treatment than in soil application. The next best treatment was soil application of *P. lilacinus* at 2.5 kg/ha, which parasitized the 51.5-71.5% of the nematode eggs. The effects of these treatments were comparable with that of the nematicide carbofuran. Application of *V. lecanii* as dip treatment of softwood cuttings was the least effective treatment.

Key words: Bioformulations, *Coleus forskohlii*, control, root-knot nematodes.

Medicinal coleus, *Coleus forskohlii* (Wild) Briq is one of the commercial medicinal plants cultivated in India, Nepal, Sri Lanka, Africa, Burma and Thailand. It is a perennial aromatic herb and produces root tubers, which are used as condiments in the preparation of pickles and for extraction of the diterpenoid forskolin by drug industries. Forskolin has the unique property of activating almost all hormone sensitive adenylate cylase enzymes in biological systems. It is useful in the treatment of congestive heart failure, glaucoma, asthma, cancer and in preventing immature greying of hair (Joy *et al*., 1998). It is in great demand in Japan, USA and European countries. Investigations by bio-medical chemists and botanists revealed that *C. forskohlii* is the only source of forskolin. The growing demand for forskolin in international trade has made Indian farmers take up commercial cultivation of medicinal coleus. The crop has now become an important medicinal cash crop in India, where the root-knot nematode (RKN) *Meloidogyne incognita* (Kofoid *et* White) Chitw. is one of the major constraints to its production. Severely infected plants often fail to produce root tubers and yield up to 86% less than healthy plants (Senthamarai *et al*., 2006a). Treatments with chemical nematicides control RKN to a certain extent, but their use is strictly limited in the cultivation of medicinal plants in order to maintain their active principles and medicinal properties. Biological control appears to be a promising alternative strategy in the management of root-knot nematodes. The biocontrol potential of the plant growth promoting rhizobacterium *Pseudomonas fluorescens* Migula (Siddiqui and Shaukat, 2003), the antagonistic fungus *Trichoderma viride* Weindling *et* Fawcett (Dababat *et al*., 2006), the egg parasitic fungi *Paecilomyces lilacinus* (Thom.) Samson (Brand *et al*., 2004) and *Verticillium lecanii* Zimmermann (Meyer, 1998), and arbuscular mycorrhizal fungus *Glomus mosseae* Nic *et* Gerd. (Jaizme-Vega *et al*., 1997) to control RKN on various crops has already been demonstrated. In the present study, the efficacy of commercial formulations of *P. fluorescens*, *T. viride*, *P. lilacinus*, *V. lecanii* and *G. mosseae* was evaluated against *M. incognita* in medicinal coleus under glass-house and field conditions.

MATERIALS AND METHODS

Commercial biocontrol formulations. *Pseudomonas fluorescens* (6×10^8 CFU/g) and *T. viride* (6×10^8 CFU/g) were provided by the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India, *P. lilacinus* (6×10^8 CFU/g) and *V. lecanii* (6×10^8 CFU/g) by the Horticultural Research Station, Tamil Nadu Agricultural University, Udhagamandalam, India, and *G. mosseae* (100 spores/g) by the Horticultural Research Station, Tamil Nadu Agricultural University, Yercaud, India.

Root-knot nematode isolate. *Meloidogyne incognita* was isolated from infected medicinal coleus plants and pure cultures were obtained and maintained on tomato (*Lycopersicon esculentum* Mill.) cv. Co1. Egg masses

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from tomato roots were hand picked with sterilized forceps and placed directly on a double layer of tissue paper in a perforated plastic tray fitted inside a solid plastic tray containing sufficient distilled water to touch the bottom of the perforated tray. Hatched juveniles (J_2) were collected at 24 hr intervals for a period of 10 days whilst maintaining the water level and 1- to 9-day-old J_2 were used as inocula for glass-house experiments.

Plant material. Terminal stem cuttings of 10 cm length of medicinal coleus cv. Selection-K (Karnataka type), provided by M.G.P. Herbal Care Ltd, were used as planting material in both glass-house and field experiments.

Glass-house experiment. This experiment was conducted at the Regional Research Station, Aruppukottai, Tamil Nadu, India during January-June, 2006. Clay pots of 15 cm diameter were filled with steam sterilized soil mixture (red soil, sand and farm yard manure in equal proportion). The commercial formulations of the bio-agents were applied to the soil (1 g/pot) and used as stem cuttings dip in 0.1% water suspension. *Glomus mosseae* was only applied to soil (Table I). The effects of the bio-agent treatments were compared with that of the nematicide carbofuran 3G (1 g/pot). The bio-agents and carbofuran were mixed with the soil just before planting. The stem cuttings were dipped in one litre of 0.1% suspension for one minute and immediately transferred to the pots. Ten days later (when cuttings had started rooting) the plants were inoculated with 5,000 J2 of *M. incognita*/pot (Pi). Untreated control plants were also inoculated with the nematode. Each treatment (Table I) was replicated four times and the pots were arranged in a completely randomized block design. The plants were maintained at 28 ± 5 °C in a glass-house and irrigated every three days. Plants were fertilized with 20-20-20 (N-P-K) fertilizer at 0.1 % concentration at one month intervals. No pests or diseases were noted during the study.

The experiment was terminated 180 days after planting (DAP). The plants were carefully removed from their pots and root tuber weight was recorded. The soil from each pot was thoroughly mixed and J_2 population densities assessed from 100 cm^3 sub-samples by Cobb's decanting and sieving technique followed by modified Baermann's funnel technique (Southey, 1986). Root-gall index was assessed on a 0-5 scale: 0 = no galls; 1 = 5 galls; 2 = 6-20 small galls; 3 = >20 galls distributed over the whole root system; 4 = reduced and deformed root system with some larger galls; 5 = completely deformed root system with few but large galls (Di Vito *et al*., 1979). The number of eggs per g of root was estimated using the sodium hypochlorite method (Hussey and Barker, 1973). J_2 population density per pot (5,000 cm^3) was calculated by multiplying the number of J_2 per cm^3 by the total volume of soil. Egg density in roots was calculated by multiplying the number of eggs in 1 g root by the total weight of the roots. The sum of the eggs calculated per root system and J_2 population density estimated in the soil per pot was considered as the final population (Pf). The reproduction factor (Rf) was calculated by the formula Rf = Pf/Pi.

Root colonization by *P. fluorescens* and *T. viride* was assessed from 1 g root from each replicate at harvest using the serial dilution plate technique (Rodriguez-Kabana, 1967). Nematode egg parasitism by *P. lilacinus* and *V. lecanii* was also recorded. For this, ten egg masses per root system were randomly selected and shaken in 0.5% sodium hypochlorite solution for two minutes. The eggs were then plated on potato dextrose agar medium, incubated at 25 °C for ten to fifteen days and the number of infected eggs was counted and expressed as a percentage. The root infection level by *Glomus mosseae* was assessed from 1 g of randomly selected root material after clearing in KOH and staining in tryphan blue (Phillips and Hayman, 1970) and the percentage of root colonization was determined (Giovannetti and Mosse, 1980).

Field experiment. This experiment included the same treatments used in the glass-house and was also conducted during January-June 2006 in a farmer's holdings with natural infestation of *M. incognita* (average of 89 J_2/100 cm^3 of soil) at Dindukkal, Tamil Nadu, India. Seven sets of ridges and furrows were formed within plots of 20 m^2 (5 m × 4 m). Each plot was planted with 70 stem cuttings spaced 60 cm × 40 cm on one side of the ridges. Each plot was separated by raised bunds leaving 0.5 m space between bunds. There were four replicates per treatment arranged according to a randomized block design. The bio-agents (2.5 kg/ha) and carbofuran (1 kg a.i./ha) were mixed with sterile sand (1:10 v/v) and applied in ridges just two hours before planting. Stem cuttings were dipped in 20 l of 0.1% of each bio-formulation for one minute and immediately planted. Standard agronomic practices were followed for raising the crop.

The soil nematode population density in each plot was determined at planting (Pi) and 180 DAP. Each sample consisted of ten cores, randomly collected at a depth of 15-20 cm in the rhizosphere of the plants, pooled together into a composite sample from which a 100 cm^3 sub-sample was collected by coning and quartering. The samples were processed using Cobb's decanting and sieving technique, followed by the modified Baermann's funnel technique (Southey, 1986). The plants were carefully uprooted 180 DAP and root gall index, number of eggs per g of root, root tuber yield, root colonization by *P. fluorescens, T. viride* and *G. mosseae*, and percentage of egg parasitism of *P. lilacinus* and *V. lecanii* were assessed as mentioned before. For the purpose of comparing the treatments, the sum of J_2 from 100 cm^3 soil and eggs per g of root was considered as final population density (Pf).

Statistical analysis. All the data collected were analyzed using analysis of variance and means separated with Duncan's Multiple Range Test following Panse and Sukhatme (1989).

Table I. Effect of bio-control agents on *Meloidogyne incognita* and yield of medicinal coleus under glass-house conditions.

Treatment	J_2/100 cm^3 soil at 180 DAP	Root gall index	Eggs/g root	Rf	Root tuber yield (g/plant)	Colonization/ parasitism of biocontrol agents.
Soil application of *Pseudomonas fluorescens* (1 g/pot)	640 c	4.0 b	1576 c	14.0 c	157.5 f	8×10^2 CFU*
Stem cutting dip in 0.1% *P. fluorescens*	480 a	3.1 a	836 a	9.2 a	232.5 h	110×10^4 CFU*
Soil application of *Trichoderma viride* (1 g /pot)	705 cd	4.3 bc	1964 cd	15.8 d	120.5 c	22×10^2 CFU*
Stem cutting dip in 0.1 % *T. viride*	738 d	4.3 bc	2126 d	16.7 d	121.5 c	6×10^2 CFU*
Soil application of *Paecilomyces lilacinus* (1 g/pot)	490 a	3.1 a	961 a	9.9 a	228.7 h	71.5 %**
Stem cutting dip in 0.1% *P. lilacinus*	703 cd	4.3 bc	2000 d	16.0 d	146.2 e	21.5 %**
Soil application of *Verticillium lecanii* (1 g/pot)	660 c	4.0 b	1598 c	14.4 c	135.0 d	28.0 %**
Stem cutting dip in 0.1% *V. lecanii*	783 e	4.3 bc	1871 c	15.4 cd	105.0 b	11.0 %**
Soil application of *Glomus mosseae* (1 g/pot)	700 c	4.3 bc	2040 d	15.0 c	109.5 b	17.5 %***
Carbofuran 3 G (1 g/pot)	550 b	3.6 ab	1195 ab	11.5 b	189.0 g	-
Untreated control	870 f	4.6 c	4285 e	24.3 e	71.2 a	-
SEd	15.6	0.24	121.6	0.6	3.6	-
CD (P = 0.05)	36.2	0.51	338.4	1.4	8.3	-

* Root colonization of *P. fluorescens/T. viride* in colony forming units(CFU)/g root.
** Percentage of egg parasitism by *P. lilacinus/V. lecanii.*
*** Percentage of root colonization by *G. mosseae.*
J_2 = Second stage juveniles of *M. incognita.*
DAP = Days after planting.
Rf = Reproductive factor.
Data are means of four replicates. Means followed by the same letter in columns are not significantly different at P<0.05 according to Duncan's multiple range test.

Table II. Effect of bio-control agents on *Meloidogyne incognita* and yield of medicinal coleus under field conditions.

Treatment	J_2/100 cm^3 soil		Root gall index	Eggs/g root	Rf	Root tuber yield (Kg/plot)	Colonization/ parasitism of biocontrol agents.
	At planting	At harvest					
Soil application of *Pseudomonas fluorescens* (2.5 kg/ha)	92	218 c	3.3 b	1024 c	13.5 c	25.02 b	2×10^2CFU*
Stem cutting dip in 0.1% *P. fluorescens*	85	166 a	2.6 a	542 a	8.3 a	26.23 c	38×10^4 CFU *
Soil application of *Trichoderma viride* (2.5 kg/ha)	90	238 d	3.6 bc	1276 dc	16.8 d	24.02 ab	2×10^2 CFU *
Stem cutting dip in 0.1 % *T. viride*	88	251 de	3.6 bc	1381 d	18.5 de	23.80 a	4×10^2 CFU *
Soil application of *Paecilomyces lilacinus* (2.5 kg/ha)	93	171 ab	2.6 a	624 a	8.5 a	25.80 bc	51.5%**
Stem cutting dip in 0.1% *P. lilacinus*	88	239 d	3.6 bc	1300 d	17.4 d	24.41 b	8.5%**
Soil application of *Verticillium lecanii* (2.5 kg/ha)	96	220 c	3.3 b	1038 c	13.1 c	24.82 b	15.0%**
Stem cutting dip in 0.1% *V. lecanii*	84	266 e	3.6 bc	1216 cd	17.6 d	23.30 a	3.0%**
Soil application of *G. mosseae* (2.5 kg/ha)	92	239 d	3.6 bc	1326 d	17.0 d	23.72 a	6.5%***
Carbofuran 3 G (1 kg a.i./ha)	83	186 b	3.0 ab	776 b	11.5 b	25.82 bc	-
Untreated control	91	295 f	4.0 c	2785 e	33.8 f	23.00 a	-
SEd	NS	8.9	0.29	108.1	0.7	0.46	-
CD (P = 0.05)	NS	17.6	0.61	220.4	1.5	1.24	-

* Root colonization of *P. fluorescens/T. viride* in colony forming units(CFU)/g root.

** Percentage of egg parasitism by *P. lilacinus/V. lecanii.*

*** Percentage of root colonization by *G. mosseae.*

J_2 = Second stage juveniles of *M. incognita.*

DAP = Days after planting.

Rf = Reproductive factor.

Data are means of four replicates. Means followed by the same letter in columns are not significantly different at P<0.05 according to Duncan's multiple range test.

RESULTS

Glass-house experiment. Biocontrol agents reduced *M. incognita* population density in the soil and formation of galls on the roots (Table I). *Pseudomonas fluorescens* as stem cutting dip treatment and *P. lilacinus* at 1 g/pot as soil application were the most effective in controlling *M. incognita*. These treatments reduced the nematode population in soil by 44.8 and 43.6%, respectively, being significantly superior to other treatments, including carbofuran. Dipping stem cuttings in *V. lecanii* 0.1% resulted in the smallest reduction of soil nematode population (10%) over the control. The numbers of eggs/g of root were also signficantly less in the *P. fluorescens* as stem cutting dip treatment and *P. lilacinus* as soil application as compared to the carbofuran and control plants. Compared to the nematode-alone control, all biocontrol agent treatments restricted root gall development and reproduction of *M. incognita* on medicinal coleus plants. Gall index ranged from 3.1 to 4.3 for the biocontrol agents treated plants compared to 4.6 for the nematode control. The root gall index was least (3.1) in the *P. fluorescens* stem cutting treatment and *P. lilacinus* at 1 g/pot. The reproductive factor for nematode alone on medicinal coleus was 24.3 versus 9.2 to 9.9 for plants treated with *P. fluorescens* as stem cutting dip treatment and *P. lilacinus* as soil application. Also, these treatments produced significantly heavier root tubers (232.5 g/plant and 228.7g/plant, respectively) than the other treatments. Among the bioagents, *V. lecanii* as a softwood cutting treatment resulted in the lowest yield (105 g/plant).

All the biocontrol agents, applied either to the soil or as stem cutting dipping, were re-isolated at harvest. Root colonization by *P. fluorescens* was greater (110×10^4 CFU/g root) in the stem cutting dip treatment than in the soil application treatment (8×10^2 CFU/g root). Egg parasitism was greater (71.5%) when the *P. lilacinus* treatment was as a soil application than as a stem cutting dip (21.5%). Egg parasitism by *V. lecanii* ranged from 11 to 28%. *Glomus mosseae* colonized 17.5% of roots in medicinal coleus.

Field experiment. All biocides suppressed the nematode population with small differences in effectiveness among treatments (Table II). Stem cuttings dip in 0.1% *P. fluorescens* and soil application of *P. lilacinus* at 2.5 kg/ha were significantly superior to the other treatments. These two treatments produced mean populations as low as 166 and 171 J2/100 cm^3 soil, respectively. Compared to the untreated control, root gall index (2.6) was significantly reduced by stem cutting dip in 0.1% *P. fluorescens* and soil application of *P. lilacinus* at 2.5 kg/ha (Table II). These treatments significantly restricted the number of eggs per g of roots and thereby the reproduction factor as compared to other treatments and *M. incognita* alone. Accordingly, the root tuber yield was significantly greater following stem cutting dip in 0.1% *P. fluorescens* (26.2 kg/plot) and soil application of *P. lilacinus* at 2.5 kg/ha (25.8 kg/plot), with increases over the control of 14.0 and 12.1%, respectively.

Pseudomonas fluorescens as stem cuttings dip resulted in greater root colonization (38×10^4 CFU/g root) than as soil application (2×10^2 CFU/g root). *Paecilomyces lilacinus* as soil application resulted in greater egg parasitism than when used as stem cutting dip. Although it was possible to re-isolate *V. lecanii* from *M. incognita* eggs and *G. mosseae* from medicinal coleus roots, their efficacy against *M. incognita* on medicinal coleus was less than that of *P. fluorescens* and *P. lilacinus*.

DISCUSSION

There has been renewed interest in the use of microbial inoculants to suppress the activity of plant-parasitic nematodes on several crops (Rodriguez-Kabana and Morgan-Jones, 1988; Kerry and Jaffee, 1997; Stirling *et al*., 1998; Dong and Zhang, 2006). However, biocontrol agents are not universally effective in all crops and against all nematodes. In this investigation, *P. fluorescens* and *P. lilacinus* were found to be potential biocontrol agents against *M. incognita* on medicinal coleus under both glass-house and field conditions. Recently, Senthamarai *et al*. (2006b) also observed successful control of *M. incognita* in medicinal coleus with *P. fluorescens* treatment. The presence of *P. fluorescens* in the roots and rhizosphere is known to suppress plant parasitic nematodes by alteration of root exudates, which influence nematode egg hatch, attraction and penetration behavior (Oostendrop and Sikora, 1989). Also, this bacterium produces antibiotics such as 2,4 diacetyl phloraglucinol (DAPG), which reduce *Globodera rostochiensis* J2 mobility (Cronin *et al*., 1997), toxic metabolites that cause mortality of infective *Hirschmaniella gracilis* juveniles (Seenivasan and Lakshmanan, 2001), and induces systemic resistance by synthesis and accumulation of peroxidase, chitinase and glucanase enzymes in plant root systems (Kalaiarasan *et al*., 2006). The re-isolation of the bacterium from the roots after treatment confirmed the endophytic colonization potential of *P. fluorescens* in medicinal coleus. Also, the results of our investigation revealed that the use of *P. fluorescens* as a stem cutting treatment was very effective for the control of *M. incognita*. This may be due to more intensive root colonization by the bacterium after the stem cutting treatment than from a soil application. Seenivasan and Devrajan (2002) also found that the nematode suppressing ability of *P. fluorescens* is related to its root colonizing ability. Soil application of *P. lilacinus* at 2.5 kg/ha was the next best treatment, which parasitized 71.5% of the egg masses under glass-house conditions and 51.5% of the egg masses under field conditions. The efficacy of *P. lilacinus* as an egg parasite of *M. incognita* has been reported (Hewlett *et al*., 1990; Jonathan *et al*., 2000), as has the reduction of *M. incognita* by *T. viride* (Rekha Arya and Saxena, 1998; Senthilkumar and Rajendran,

2004), *V. lecanii* (Meyer, 1994) and *G. mosseae* (Jothi and Sundarababu, 2001).

The effects of the treatments under field conditions were similar to those observed in the glass-house. However, relative suppression of nematode population density, enhancement of root tuber yield and re-isolation of introduced bio-agents were less under field conditions than in glass-house conditions. Reduction of soil nematode population densities was 74.7% in the glass-house and 65.4% in the field. Colonization of the roots by *P. fluorescens* was 110×10^4 CFU/g root in the glass-house and 38×10^4 in the field, while root tuber yield increase was 69.3% under glass and 14.0% under field conditions for stem cutting dip in 0.1% *P. fluorescens*, which was the best treatment. These differences may be attributed to competition from the indigenous microbial community with the introduced bioagents. Enhanced survival and efficacy of introduced *Pseudomonas* sp. for the management of *Mesocriconema xenoplax* (Raski) Luc *et* Raski on peach tree, by pre-plant solarization (Kluepfel *et al*., 2002), supports our finding reduced efficacy under field conditions. Hence, under the conditions of these experiments the application of bioformulations alone is not sufficient to control *M. incognita* in medicinal coleus. Stem cuttings dip in 0.1% of *P. fluorescens* and soil application of *P. lilacinus* at 2.5 kg/ha could be exploited as components of a nematode management system, although further efforts are needed to integrate this strategy with other control means in medicinal coleus.

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LITERATURE CITED

Brand D., Roussos S., Pandey A., Paulo C., Pohl J. and Soccol C.R., 2004. Development of a bionematicide with *Paecilomyces lilacinus* to control *Meloidogyne incognita*. *Applied Biochemistry and Biotechnology*, *118*: 81-88.

Cronin D., Loccoz Y.M., Fehton A., Dunne C., Dowling D.N. and Gara F.O., 1997. Role of 2,4-diacetyl phloroglucinol in the interaction of the biocontrol of pseudomonas strain F113 with the potato cyst nematode *Globodera rostochiensis*. *Applied Environmental Microbiology, 63*: 1357-1361.

Dababat A.A., Sikora R.A. and Hauschild R., 2006. Use of *Trichoderma harzianum* and *Trichoderma viride* for the biological control of *Meloidogyne incognita* on tomato. *Common Agricultural Applied Biological Science, 71*: 953-961.

Di Vito M., Lamberti F. and Carella A., 1979. La resistenza del pomodoro nei confronti dei nematodi galligeni: Prospettive e possibilità. *Rivista di Agronomica, 13*: 313-322.

Dong L. and Zhang K., 2006. Microbial control of plant-parasitic nematodes: a five-party interaction. *Plant and Soil*, *288*: 31-45.

Giovannetti M. and Mosse B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist, 84*: 489-500.

Hewlett T.E., Dickson D.W., Itchell D.J and Kannwischker M.K.E., 1990. Evaluation of *Paecilomyces lilacinus* as a biological control agent of *Meloidogyne javanica* in tobacco. *Journal of Nematology*, *20*: 578-584.

Hussey R.S. and Baker K.R., 1973. A comparison of methods of collecting eggs of *Meloidogyne* spp. including a new technique. *Plant Disease Reporter*, *57*: 1025-1028.

Jaizme-Vega M.C., Tenoury P., Pinochet J. and Jaumot M., 1997. Interactions between the root-knot nematode *Meloidogyne incognita* and *Glomus mosseae* in banana. *Plant and Soil*, *196*: 27-35.

Jonathan E.I., Arulmozhiyan R., Muthusamy S. and Manuel W.W., 2000. Field application of *Paecilomyces lilacinus* for the control of *Meloidogyne incognita* on betelvine, *Piper betel*. *Nematologia Mediterranea, 28*: 131-133.

Joy P.P., Thomas J., Samuel Mathew and Skaria B.P., 1998. *Medicinal plants*. Kerala Agricultural University Publication, Kerala, India, 211 pp.

Jothi G. and Sundarababu R., 2001. Evaluation of optimum dose of *Glomus mosseae* for the control of *Meloidogyne incognita* in brinjal. *Indian Journal of Nematology, 31*: 90-91.

Kalaiarasan P., Lakshmanan P.L., Rajendran G. and Samiyapan R., 2006. Chitin and chitinolytic biocontrol agents for the management of root-knot nematode. *Meloidogyne arenaria* in groundnut (*Arachis hypogaea* L.) cv. Co3. *Indian Journal of Nematology, 36*: 200-205.

Kerry B.R. and Jaffee B.A., 1997. Fungi as biological control agents for plant parasitic nematodes. Pp. 203-218. *In*: The Mycota: a Comprehensive Treatise on Fungi as Experimental Systems for Basic Applied Research (Wicklow D.T. and Soderstrom B.E., eds). Springer-Verlag, Berlin Heidelberg, Germany.

Kluepfel D.A., Nyczepir A.P., Lawrence J.E., Wechter W.P. and Leverentz B., 2002. Biological control of the phytoparasitic nematode *Mesocriconema xenoplax* on peach trees. *Journal of Nematology*, *34*: 120-123

Meyer S.L.F., 1994. Effects of wild type strain and a mutant strain of the fungus *Verticillium lecanii* on *Meloidogyne incognita* populations in greenhouse studies. *Fundamental and Applied Nematology*, *17*: 563-567.

Meyer S.L.F., 1998. Evaluation of *Verticillium lecanii* strains applied in root drenches for suppression of *Meloidogyne incognita* on tomato. *Journal of the Helminthological Society of Washington, 65*: 82-86.

Oostendorp M. and Sikora R.A., 1989, Utilization of antagonistic rhizobacteria as a seed treatment for the biological control of *Heterodera schachtii* in sugar beet. *Revue de Nématologie*, *12*: 77-83.

Panse V.G. and Sukhatme P.V., 1989. *Statistical methods for Agricultural Workers*. ICAR, New Delhi, India, 359 pp.

Phillips J.M. and Hayman D.S., 1970. Improved procedures for clearing roots and observing parasite and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society, 51*: 158-161.

Rekha Arya and Saxena S.K., 1998. *Trichothecium roseum* together with *Rhizoctonia solani* and *Meloidogyne incognita* on germination of seeds of tomato c.v. Pusa Ruby. *Indian Journal of Nematology*, *28*: 217-221.

Rodriguez-Kabana R., 1967. An improved method for assessing soil fungus population density. *Plant and Soil*, *26*: 393-396.

Rodriguez-Kabana R. and Morgan-Jones G., 1988. Potential for nematode control by microfloras endemic in the tropics. *Journal of Nematology*, *20*: 191-203.

Seenivasan N. and Devrajan K., 2002. Biocontrol potential of *Pseudomonas fluorescens* against rice root nematode, *Hirschmanniella gracilis* on rice. *Current Nematology*, *13*: 35-38.

Seenivasan N. and Lakshmanan P.L., 2001. Effect of culture filtrates of *Pseudomonas fluorescens* on rice root nematode, *Hirschmanniella gracilis*. *Pestology*, *25*: 11-12.

Senthamarai M., Poornima K. and Subramanian S., 2006a. Pathogenicity of *Meloidogyne incognita* on *Coleus forskohlii* Briq. *Indian Journal of Nematology, 36*: 123-125.

Senthamarai M., Poornima K. and Subramanian S., 2006b. Bio-management of root knot nematode, *Meloidogyne incognita* on *Coleus forskhlii* Briq. *Indian Journal of Nematology, 36*: 206-208.

Senthilkumar T. and Rajendran G., 2004. Biocontrol agents for the management of disease complex involving root knot nematode, *Meloidogyne incognita* and *Fusarium moniliforme* on grape (*Vitis vinifera*). *Indian Journal of Nematology, 34*: 49-51.

Siddiqui I.A. and Shaukat S.S., 2003. Suppression of root-knot disease by *Pseudomonas fluorescens* CHAO in tomato: importance of bacterial secondary metabolite, 2,4- diacetypholoroglucinol. *Soil Biology and Biochemistry, 35*: 1615-1623.

Southey J.F., 1986. *Laboratory methods for work with plant and soil nematodes*. Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationery Office, London, UK, 202 pp.

Stirling G.R., Licastro K.A., West L.M. and Smith L.J., 1998. Development of commercially acceptable formulations of the nematophagous fungus *Verticillium chlamydosporium*. *Biological Control*, *1*: 217-223.

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