FIELD APPLICATION OF BIO-ORGANICS IN THE MANAGEMENT OF *MELOIDOGYNE INCOGNITA* IN *MENTHA ARVENSIS*[**]

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Summary. A field trial was undertaken to determine the efficacy of *Trichoderma harzianum* isolate U, *Glomus aggregatum*, oil seed cakes of neem (*Azadirachta indica*), and mustard (*Brassica compestris*) in the management of *Meloidogyne incognita* and their impact on yield of menthol mint (*Mentha arvensis*) cv. Kosi. Significant reductions in nematode populations and root-knot indices were noticed in plots receiving oil seed cakes and bioagents, whose effects were equal to that of carbofuran. Application of oil seed cakes and *T. harzianum* significantly enhanced crop yield.

Root-knot nematodes (*Meloidogyne* spp.) are the main pest of medicinal and aromatic plants. Menthol mint (*Mentha arvensis* L.), an important aromatic herb, is widely cultivated for its essential oil, which is a potential source of natural menthol, menthyl acetate, menthone and terpenes. Different constituents of menthol mint oil are extensively used in pharmaceutical, perfumery, food and cosmetic industries all over the world. Introduction of *M. arvensis* as a commercial crop into sandy loam soil in India showed that a threat was posed by the root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw. (Pandey, 1998). As this species is endoparasitic, its spread is facilitated by infected suckers/roots that are used in the vegetative propagation of the crop.

Chemical nematicides have been the primary means of controlling nematodes for over fifty years. However, they are considered major threats to human health, with problems like drinking water contamination, adverse effects on useful organisms, depletion of stratospheric ozone. Therefore, there is urgent need for development of alternative, effective management tools that are environmentally sound but which minimise nematode populations and thus ensure high crop production. To meet these needs, it became imperative to carry out an experiment on the potential of field application of bio-organic products, in comparison with traditional nematicides such as carbofuran. Data on population development and root-knot severity of the nematode, as well as on herb and oil yield in the menthol mint crop are reported herein.

MATERIALS AND METHODS

The experiment was conducted in a root-knot nematode infested field with sandy loam soil at CIMAP experimental farm, Lucknow. *Mentha arvensis* cv. Kosi was used in the study. Healthy suckers (500 kg/ha), 6-cm-long and bearing at least two to three buds, were transplanted into beds (plots) at 10 cm spacing along the row and 30-45 cm between rows. There were 30 beds, each of 6 m^2, allocated according to a randomized block design with five replications.

Treatments were oil seed cakes of neem (*Azadirachta indica* A. Juss) and mustard (*Brassica campestris* L.) at 1,000 kg/ha, the bio-agents *T. harzianum* Rifai isolate U and *Glomus aggregatum* Schenck *et* Smith emend. Koske mixed with suckers at the time of planting, and untreated control. They were compared with carbofuran 2 kg a.i./ha. A twenty-day-old culture of *T. harzianum*, maintained on sand maize meal medium at 27 °C with a population density of $2x10^8$ cfu/g soil, was mixed with suckers in the ratio of 1:4 just before planting. The inoculum of *G. aggregatum* was maintained in a glasshouse with palmarosa (*Cymbopogon martini* (Roxb. J.F. Watson) and 40 g soil/bed, containing 10 chlamydospore/g, were placed along with suckers at the time of transplanting. The chlamydospores were extracted from the soil by wet sieving and decanting techniques (Gerdemann and Nicolson, 1963). CFU densities of *T. harzianum* were determined in soil (Elad *et al*., 1981).

Twenty randomly selected soil samples were collected from each bed and mixed to form a composite representative sample of 1 dm^3, out of which a sub-sample of 200 cm^3 was processed by Cobb's sieving and decanting technique followed by Baermann funnel (Southey, 1986). Soil sampling and determination of phytonematode populations were made before the beds were treated with different bio-organic products (15 Februay) and during the crop cycle at one month intervals (15 March, 15 April, 15 May, and 15 June). The herb yield was harvested from each plot 100 days after transplanting. Then oil content was determined by hydro-distillation of 100 g fresh herb from each plot using Clevenger

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apparatus (Clevenger, 1928).

Immediately after harvest, root-knot index (RKI) was assessed using a 1-5 scale (1 = no galling, 2 = 1-25%, 3 = 26-50%, 4 = 51-75% and 5 = 76-100%) of the galled menthol mint root systems.

Differences among the treatments were tested by the critical difference (CD) test at the 5% probability level (Cochran and Cox, 1957). Correlation coefficients between yield parameters and root-knot index were also calculated.

RESULTS AND DISCUSSION

At the beginning of the experiment, the species of phytonematodes present in the soil were *M. incognita*, *Tylenchorhynchus vulgaris* Upadhyay, Swarup *et* Sethi and *Pratylenchus thornei* Allen *et* Sher. The average population of *M. incognita* juveniles in all beds was greatest at 618/200 cm^3 soil, followed by *T. vulgaris* and *P. thornei* in the ratio of 7:2:1. As the population densities of *T. vulgaris* and *P. thornei* were low and did not increase even in untreated plots during the experimentation, detailed studies were conducted only for *M. incognita*. During the growing season, the soil population density of *M. incognita* juveniles was always larger in untreated control plots than in the treated ones (Table I). The maximum reduction in the population of *M. incognita* was recorded in neem cake, followed by carbofuran, mustard cake and bioagents treated beds. After treating, the population of the root-knot nematode declined to 200-400 juveniles/200 cm^3 soil. At the time of crop maturity, a small increase in juvenile densities was observed in June. Root-knot indices were much lower in the treated plots, indicating that the treatments inhibited the development of *M. incognita* (Table II).

Significantly higher herb yield, % herb oil content and oil yield occurred in treated plots. The increase was, however, more pronounced in neem and mustard cake treated beds (Table II). This gain may be due to additional nitrogen obtained from the organic materials applied to the crop and reduction in nematode reproduction potential. The inhibition of *M. incognita* reproduction in soil might be due to enhanced microbial activity resulting from the addition of oil seed cakes, leading to greater antagonism in the soil environment, or due to release of some nematode inhibitory chemicals from these cakes. Increase in essential oil yield may also be attrib-

Table I. Influence of bio-organics and carbofuran on the soil population density of *Meloidogyne incognita.*

Treatment	Root-knot nematode J_2 population/200 cm^3 soil*			
	March	April	May	June
Untreated control	680	680	680	680
Carbofuran	400	280	300	300
Mustard cake	460	300	360	360
Neem cake	400	220	200	260
Glomus aggregatum	460	400	340	360
Trichoderna harzianum isolate U	400	280	300	340
C.D. at 5% level	28.4	31.0	33.4	27.3

* Pre-treatment population of *M. incognita* 618 juveniles/200 cm^3 soil.

Table II. Effects of bio-organics and a pesticide on herb and oil yield of menthol mint and root-knot indices of *M. incognita*.

Treatment	Herb yield (q/ha)	% oil content	Oil yield (kg/ha)	Root-knot indices (RKI)
Untreated-Control	146.6	1.8	70.4	3.66
Carbofuran	188.0 (+28.4)*	2.3	114.2 (+62.2)*	2.33 (-34.7)**
Mustard cake	199.8 (+36.3)	2.7	143.6 (+104.0)	2.00 (-45.4)
Neem cake	210.0 (+43.2)	2.7	151.0 (+114.5)	2.00 (-45.4)
Glomus aggregatum	175.8 (+19.9)	2.4	112.2 (+59.4)	2.66 (-27.3)
Trichoderma harzianum isolate U	175.0 (+19.4)	2.5	116.4 (+65.3)	2.66 (-27.3)
C.D at 5% level	28.4	0.5	40.4	0.1

* In brackets, per cent increase as compared to untreated control.
** In brackets, per cent decrease in root-knot indices over untreated controls.

Table III. Pre-treatment and post-harvest soil populations of *Glomus aggregatum* and *Trichoderma harzianum* isolate U fungal spores.

Treatment	Pre-treatment population (/g soil)	Post-harvest population (/g soil)*
G. aggregatum	10 chlamydospores	18 chlamydospores
T. harzianum isolateU	2×10^8 spores	2×10^{12} spores

* Estimated 100 days after inoculation

uted to the observed significant reduction in RKI by the application of different organic materials (Sikora, 1992; Tanu *et al.*, 2004).

Treatments with *T. harzianum*, apart from inducing a significant increase in total oil yield of the crop, also reduced the nematode population density in the soil, with an increase in the population densities of *G. aggregatum* and *T. harzianum* (Table III). *Trichoderma harzianum* is well-known to possess nematicidal properties (Windham *et al*., 1993; Rao *et al*., 1996; Spiegel and Chet, 1998; Sharon *et al*., 2001; Suarez *et al*., 2004) and also colonizes eggs and infects second stage juveniles of cyst and root-knot nematodes (Saifullah and Thomas, 1996; Sharon *et al*., 2001). It is possible that VA fungi in association with the root system of the plant secrete some enzymes or other metabolites that lower the multiplication of *M. incognita* in the rhizosphere (Sikora and Sitaramaiah, 1995; Nagesh and Reddy, 2004). The significant correlation between herbage and oil yield and their negative significant association with RKI confirm that a decrease in RKI does enhance crop growth significantly (Table IV).

Table IV. Correlations among herbage, oil yields and root-knot indices of *M. incognita* in menthol mint.

Parameter	Root-Knot Index	Oil yield
Herb yield	- 0.994**	0.974**
Oil yield	- 0.957**	

** Correlation coefficient at $P < 0.01$.

Thus, it is concluded that both oil seed cakes and *T. harzianum* may be used as eco-friendly nematode managing agents to restrict inoculum potential of *M. incognita*, enhancing the yield of menthol mint under field conditions and possibly of the succeeding crops as the fungus population becomes established in the soil.

ACKNOWLEDGEMENTS

The author is grateful to Dr. S.P.S. Khanuja, Director CIMAP, Lucknow, for facilities and encouragement and Drs A. Kalra, and M.L. Gupta, for providing necessary materials and help during the course of the present investigation.

LITERATURE CITED

Cochran W.G. and Cox G.M., 1957. *Experimental Designs.* Vol. II. John Wiley and Sons Inc., New York, U.S.A., 611 pp.

Clevenger J.E., 1928. Apparatus for the determination of volatile oil. *Journal of American Pharma Association, 17*: 346.

Elad Y., Chet I. and Henis Y., 1981. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. *Phytoparasitica, 9*: 59-67.

Gerdeman J.W. and Nicolson T.H., 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society, 46*: 235-244.

Nagesh M. and Reddy P.P., 2004. Biochemical changes in *Glomus fasciculatum* colonized roots of *Lycopersicon esculentum* in presence of *Meloidogyne incognita*. *Indian Journal of Experimental Biology, 42*: 721-727.

Pandey R., 1998. Nematode pests of medicinal and aromatic plants and their management. Pp. 177-216. *In*: Phytonematology - Aspects and Prospects, CBS Publishers & Distributors, Delhi, India (Ed. Trivedi P.C., Professor), Department of Botany, University of Rajasthan, Jaipur, India.

Rao M.S., Reddy P.P. and Nagesh M., 1996. Evaluation of plant based formulations of *Trichoderma harzianum* for the management of *Meloidogyne incognita* on eggplant. *Nematologia Mediterranea, 26*: 59-62.

Saifullah and Thomas B.J., 1996. Studies on the parasitism of *Globodera rostochiensis* by *Trichoderma harzianum* using low temperature scanning electron microscopy. *Afro-Asian Journal of Nematology, 6*: 117-122.

Sharon E., Bar-Eyal M., Chet I., Herrera-Estrella A., Kleifeld O. and Spiegel Y., 2001. Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. *Phytopahology*, *91*: 687-693.

Sikora R.A., 1992. Management of the antagonistic potential in agricultural ecosystem for the biological control of plant parasitic nematode. *Annual Review of Phytopathology, 32*: 245-270.

Sikora R.A. and Sitaramaiah K., 1995. Antagonistic effect of the endomycorrhizal fungus, *Glomus fasciculatum* on *Rotylenchulus reniformis* penetration and population development on cotton. *Indian Journal of Nematol*ogy, *25*: 60-65.

Spiegel Y. and Chet I., 1998. Evaluation of *Trichoderma* spp. as a biocontrol agent against soilborne fungi and plant-parasitic nematodes in Israel. *Integrated Pest management Review, 3*: 169-494.

Southey J.F., 1986. Laboratory Methods for Work with Plant and Soil Nematodes. Ministry of Agriculture, Fishery and Food, HMSO, London, U.K., 202 pp.

Suarez B., Rey M., Castillo P., Monte E. and Llobello A., 2004. Isolation and characterization of PRA1, a trypsin-like protease from the biocontrol agent *Trichoderma harzianum* CECT 2413 displaying nematicidal activity. *Applied Microbiology and Biotechnology, 65*: 46-55.

Tanu, Prakash A. and Adholeya A., 2004. Effect of different organic manures/composts on the herbage and essential oil yield of *Cymbopogon winterianus* and their influence on the native AM population in a marginal alfisol. *Bioresource Technology, 92*: 311-319.

Windham G.L., Windham M.T. and Pederson G.A., 1993. Interaction of *Trichoderma harzianum*, *Meloidogyne incognita*, and *Meloidogyne arenaria* on *Trifolium repens*. *Nematropica, 23*: 99-103.

Accepted for publication on 4 March 2005.