# EFFICACY OF PESTICIDES, NEEM SEED POWDER AND BIO-CONTROL AGENTS ON *MELOIDOGYNE INCOGNITA* AND GROWTH AND OIL YIELD OF *MENTHA ARVENSIS*

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**Summary.** The pesticides carbofuran (1.5 mg a.i./kg soil) and carbendazim (1.0 mg a.i./kg soil), neem seed powder (50 mg/kg soil), the fungi *Paecilomyces lilacinus* and *Trichoderma harzianum* (50 mg culture/kg soil) and the bacterium *Pseudomonas fluorescens* (50 mg/kg soil), alone and in various combinations (at half the standard dose) were tested for their effects on the root-knot nematode *Meloidogyne incognita* and growth and yield of *Mentha arvensis* cv. Gomti. All treatments improved plant growth, controlled the nematode and increased oil content and oil yield of *M. arvensis*. The greatest performance was achieved by applying carbofuran or neem seed powder, singly or in combination, followed by combinations of these nematicides with the two fungi or the bacterium. The effects of the three bio-agents were least when used singly and increased only a little when they were used in combinations.

**Key words:** Control, nematicides, *Paecilomyces lilacinus*, *Pseudomonas fluorescens*, root-knot nematode, *Trichoderma harzianum*.

Menthol mint, *Mentha arvensis* L., a crop well known for its high quality of menthol, has shown a continuous increase in area of cultivation and production in India due to its high yield potential and the ever-increasing price and demand for it in national and international markets. The root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw. is the most important pest of menthol mint (Haseeb and Pandey, 1989; Haseeb and Shukla, 2000, 2001). Although encouraging success has been obtained in efforts to develop high yielding cultivars, no cultivar of menthol mint resistant to *M. incognita* is yet available. Therefore, the management of plant parasitic nematodes on this crop relies mainly on application of high rates of the nematicide carbofuran, which is increasing the cost of cultivation.

In this investigation, several management strategies were tested in order to find eco-friendly and cost-effective treatments for the management of this root-knot nematode in menthol mint. Neem (*Azadirachta indica* Juss.) seed powder was used because of its proven efficacy against *M. incognita*, compatibility with bio-control agents and plentiful availability. The bio-control agents *Trichoderma harzianum* Rifai, *Paecilomyces lilacinus* Thom. and *Pseudomonas fluorescens* Migula were included because they had been found effective in previous studies (Jatala *et al.*, 1979; Kloepper *et al.*, 1980; Morgan and Rodriguez-Kabana, 1984; Oostendorp and Sikora, 1989; Khan and Saxena, 1997). The nematicide carbofuran was included as a treated control and the fungicide carbendazim was also used because it is a popular treatment in mint cultivation in India. This fungicide is mostly applied as a sucker dip treatment (in 0.5% solution) before planting and a soil application after the first harvest (at the rate of 2 kg a.i./ha) to minimize fungal infection in underground plant parts. Its effect on *M. incognita* on mint had not been tested before.

## MATERIALS AND METHODS

*Fungal isolates*. *Trichoderma harzianum* TH-16b and *P. lilacinus* Pl-3m are maintained at the Nematology Laboratory, Department of Plant Protection, Aligarh Muslim University, Aligarh. They were cultured using sorghum seeds soaked in 5% sucrose solution for 16 hours. The seeds were transferred into 500 ml conical flasks (200 $cm^3$ of sorghum seeds/flask) and sterilized by autoclaving for 30 minutes at 1 $kg/cm^2$ pressure and 120 °C. After cooling, they were inoculated with 1-cm-diameter potato dextrose agar (PDA) discs removed from the edge of 5-day-old fungal cultures. The flasks were then incubated at 27 ± 1 °C, for at least 10 days, with periodic shaking of the flasks, and examined for fungal colonization until the surfaces of all sorghum seeds were colonized and more than $10^8$ colony forming units (cfu)/g culture were present.

*Bacterial isolate*. A pure culture of *Pseudomonas fluorescens* PF-17bs, maintained at the Nematology Laboratory, Department of Plant Protection, Aligarh Muslim University, Aligarh, was cultured in tubes containing 10 ml King's medium B (Broth) (King *et al.*, 1954) that had been autoclaved for 30 minutes at 1 $kg/cm^2$ pressure and 120 °C. After cooling, each tube was inoculated with a single colony of *P. fluorescens* strain PF-17bs. The tubes

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were placed in a BOD incubator for 48 hours at 30 ± 1 °C to allow multiplication of the *P. fluorescens*. For mass production, one-litre conical flasks containing 500 ml of autoclaved King's medium B were inoculated with 1 ml of the *P. fluorescens* culture medium and kept at 30 ± 1 °C in the BOD incubator for 120 h; they were shaken twice a day. The culture was mixed with talc in the ratio of approximately 1:4, to form an inoculum with the amount of talc adjusted to obtain $2\times10^8$ cfu/cm$^3$ of inoculum.

*Neem seed powder*. Oven dried (at 55 °C for 72 h) clean neem seeds were ground and sieved through a 25-mesh sieve to obtain fine powder for use in the experiment.

*Nematode isolate*. The population of *M. incognita* was maintained on brinjal (*Solanum melongena* L.) grown in sterilized loamy-sand soil in earthen pots under glasshouse conditions. Mature egg masses were hand-picked from the roots and incubated in Baermann trays (Southey, 1986). Hatched juveniles were collected each 24 h and stored at 20 °C temperature for 15 days before inoculation.

*Treatments*. The treatments were made in a glasshouse at 30 ± 6 °C and 13 h day length. Clay pots of 30 cm diameter were filled with 5 dm$^3$ of autoclaved soil and farmyard manure (5:1 v/v) mixture and this was thoroughly mixed with pesticides, neem seed powder and bio-agent cultures at the rates and combinations reported in Table I.

The neem seed powder and bio-control agents were applied a week before transplanting the suckers, while the pesticides were applied a day before sucker transplanting. The treated pots were irrigated as needed to maintain soil moisture. Four inoculated pots were left untreated and four pots were left uninoculated.

Five-cm-long healthy suckers of *M. arvensis* cv. Gomti were transplanted singly into each clay pot and the pots were placed on concrete platforms. Plants were irrigated as needed. At the fourth leaf stage, 5,000 15-day-old second stage juveniles ($J_2$) of *M. incognita* were poured into four 5-cm-deep holes around the base of the plants and covered with soil. There were four replicates per treatment, and the pots were arranged in a completely randomized block design.

One hundred days after inoculation, the plants were carefully uprooted from the pots and roots/suckers were washed in running tap water and blotted dry. Plant growth was determined by measuring dry weights of shoots and roots/suckers and the percentage reduction in plant growth over the uninoculated control was cal-

**Table I.** Nematicides and bio-agents used, their combination and rates of application.

| Treatment | Rate/ha | Rate/kg soil |
|---|---|---|
| Non-treated and non inoculated | | |
| Non- treated inoculated | | |
| Carbofuran (Furadan 3G) | 3.0 kg a.i. | 1.5 mg a.i. |
| Carbendazim (Bavistin 50%) | 2.0 kg a.i. | 1.0 mg a.i. |
| Neem seed powder | 100 kg | 50 mg |
| *Pseudomonas fluorescens* ($10^8$cfu/g) | 100 kg | 50 mg |
| *Paecilomyces lilacinus* ($10^8$cfu/g culture) | 100 kg | 50 mg |
| *Trichoderma harzianum* ($10^8$cfu/g culture) | 100 kg | 50 mg |
| Neem seed powder + Carbofuran | * | * |
| Neem seed powder + Carbendazim | * | * |
| Neem seed powder + *P. fluorescens* | * | * |
| Neem seed powder + *P. lilacinus* | * | * |
| Neem seed powder + *T. harzianum* | * | * |
| *P. lilacinus* + *P. fluorescens* | * | * |
| *P. fluorescens* + *T. harzianum* | * | * |
| *P. lilacinus* + *T. harzianum* | * | * |
| Carbofuran + Carbendazim | * | * |

* In all combined treatments, the rate of application of each nematicide/bio-control agent was half of the standard (single application) rate.

culated. Roots were rated for galling severity on 0 to 4 scale (Barker, 1985), where 0 = no galling (0%), 1 = light galling (1% - 25%), 2 = moderate galling (26% - 50%), 3 = heavy galling (51% - 75%), 4 = severe galling (76% - 100% galled roots).

Nematode populations ($J_2$s and other developing stages) in roots/suckers were determined by maceration of 5 g samples, obtained after chopping up the entire root/sucker system, in a Waring blender. The suspension was passed through 25- and 400-mesh sieves and the catch on the 400-mesh sieve was collected in a beaker. The volume of suspension was made up to 100 ml and nematodes in three 1 ml aliquots were counted. Also, the soil of each pot was thoroughly mixed and $J_2$ were extracted from a sub-sample of 250 $cm^3$ by Cobb's sieving and decanting and Baermann funnel methods (Southey, 1986).

*Determination of oil content*. The essential oil content of the plants was determined by hydro-distillation of fresh herbage using a Clevenger apparatus (Clevenger, 1928). All aerial parts, including leaves and stems, of each replicate were cut into 2-3 cm long pieces and placed, with 400 ml of water, in 1-litre capacity round bottom flasks of the Clevenger apparatus. Distillation was conducted at a 90 °C heating mantle temperature for 1 h and the amount of condensed essential oil was recorded on the scale in the apparatus. The percentage reduction in yield relative to the uninoculated control was calculated.

*Statistical analysis*. The data were subjected to ANOVA and least significant difference (L.S.D.) at $P< 0.05$ and $P < 0.01$ was used to compare the treatments.

**Table II.** Effect of pesticides, neem seed powder and bio-control agents on the root-knot *Meloidogyne incognita* and growth and oil yield of *Mentha arvensis* cv.Gomti.[a]

| Treatment | Plant dry weight (g) | | Oil yield (ml/100 g fresh herb) | Final nematode population | | | Nematode reproduction factor[b] | Nematode gall index[c] |
|---|---|---|---|---|---|---|---|---|
| | Shoot | Roots+ suckers | | Roots+ suckers | Soil (5 kg) | Total | | |
| Non-treated non-inoculated control | 38.0 | 27.7 | 0.75 | - | - | - | - | - |
| Non-treated inoculated | 28.2 (25.8)[d] | 19.8 (28.5) | 0.58 (22.70) | 47518 | 50000 | 97518 | 19.50 | 1.87 |
| Carbofuran | 37.7 (0.8) | 27.2 (1.8) | 0.72 (4.00) | 5640 | 6000 | 11640 | 2.33 | 0.37 |
| Carbendazim | 29.8 (21.6) | 21.0 (24.2) | 0.62 (17.33) | 40330 | 46000 | 86330 | 17.27 | 1.62 |
| Neem seed powder | 37.6 (1.0) | 27.0 (2.5) | 0.72 (4.00) | 5632 | 6000 | 11632 | 2.33 | 0.37 |
| *Pseudomonas fluorescens* | 31.9 (16.0) | 23.2 (16.2) | 0.64 (14.67) | 21330 | 23000 | 44330 | 8.87 | 0.95 |
| *Paecilomyces lilacinus* | 32.7 (13.9) | 23.6 (14.8) | 0.65 (13.33) | 18375 | 22000 | 40375 | 8.07 | 0.90 |
| *Trichoderma harzianum* | 32.6 (14.2) | 23.0 (17.0) | 0.64 (14.67) | 19200 | 22000 | 41200 | 8.24 | 0.90 |
| Neem seed powder + Carbofuran | 37.8 (0.5) | 27.6 (0.4) | 0.75 (0.0) | 4260 | 5000 | 9260 | 1.85 | 0.25 |
| Neem seed powder + Carbendazim | 35.0 (7.9) | 25.0 (9.7) | 0.68 (9.30) | 15564 | 17000 | 32564 | 6.51 | 0.75 |
| Neem seed powder + *P. fluorescens* | 36.0 (5.3) | 26.0 (6.1) | 0.68 (9.30) | 14652 | 16000 | 30652 | 6.13 | 0.70 |
| Neem seed powder + *P. lilacinus* | 36.4 (4.5) | 26.2 (5.4) | 0.71 (4.00) | 12195 | 13000 | 25195 | 5.04 | 0.62 |
| Neem seed Powder + *T. harzianum* | 36.1 (5.0) | 26.0 (6.1) | 0.70 (6.67) | 13500 | 14000 | 27500 | 5.50 | 0.65 |
| *P. fluorescens* + *P. lilacinus* | 33.8 (11.0) | 24.3 (12.6) | 0.66 (12.00) | 16276 | 20000 | 36276 | 7.25 | 0.87 |
| *P. fluorescens* + *T. harzianum* | 33.2 (12.6) | 24.0 (13.3) | 0.66 (12.00) | 17360 | 20000 | 37360 | 7.47 | 0.87 |
| *P. lilacinus* + *T. harzianum* | 34.3 (9.7) | 24.6 (11.2) | 0.60 (12.00) | 15300 | 18000 | 33300 | 6.66 | 0.75 |
| Carbofuran + Carbendazim | 36.9 (2.9) | 26.5 (4.3) | 0.71 (4.00) | 11000 | 12000 | 22000 | 4.4 | 0.50 |
| $L.S.D._{0.05}$ | 0.8 | 0.7 | 0.03 | 74.00 | 2658.0 | 3113.5 | 0.17 | 0.06 |
| $L.S.D._{0.01}$ | 1.1 | 0.9 | 0.04 | 101.74 | 3587.7 | 4202.5 | 0.23 | 0.09 |

[a]Data are means of four replicates.

[b]Reproduction factor: Pf/Pi, where Pi and Pf are the initial (5,000 $J_2$) and final population densities, respectively.

[c]Gall index : 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.

[d]Figures in parentheses are percent reduction over non-treated non-inoculated control.

## RESULTS

The nematode significantly reduced plant growth (25.8-28.5%), oil content of fresh tops (22.7%) and oil yield per pot (51.2%) when the non-inoculated and non-treated controls were compared (Table II). The least reductions in shoot weight, root/sucker weight and oil yield were observed in plants treated with carbofuran alone, neem seed powder alone and neem seed powder + carbofuran. The application of the various treatments and their combinations significantly increased plant growth and oil yield of *M. arvensis* and suppressed nematode soil population and root gall index when compared to non-treated inoculated plants (Table II).

Among the single treatments, carbofuran and neem seed powder were the most effective and carbendazim the least effective. *Trichoderma harzianum*, *P. lilacinus* and *P. fluorescens* also significantly ($P < 0.01$) increased plant growth and oil content and reduced nematode population and root gall index, but they were significantly less effective then carbofuran and neem seed powder. The treatments containing one or a combination of bio-agents greatly increased plant growth and oil yield and suppressed the nematode significantly. However, while the combination of carbofuran + neem seed powder completely counteracted the negative impact of the nematode, the combination of neem seed powder with the bio-agents did not eliminate reductions in plant growth (4.5 to 5.3 %), oil content of the plant tops (4.0 to 9.3%) or oil yield per pot (10.8 to 15.1%).

The effects of the treatments on the nematode were similar to those on plant growth and yield. Compared to a total population per pot of 97,518 nematodes, a reproduction rate of 19.5 and a gall index of 1.9 of the non-treated plants, pots treated with carbofuran or neem seed powder and their combination showed significantly lower values for these parameters, in the ranges 9,260-11,640, 1.8-2.3, and 0.2-0.3, respectively. With the other treatments, there were 25,195-44,330 nematodes per pot, reproduction rates of 4.4-8.9, and gall indices of 0.5-1.6.

## DISCUSSION

It has been suggested that nematode control by neem seed powder might be due to the toxicity of decomposition products, such as ammonia, phenolics, etc., to changed physical and chemical properties of the soil, or to host resistance (Alam *et al.,* 1979; Singh *et al*., 1980; Haseeb, 1983 ) due to the increase of phenolics in roots.

In this investigation, the addition of bio-control organisms to neem seed powder did not improve plant growth, increase oil yield or further suppress *M incognita* compared to the neem seed powder alone. However, the results clearly indicated the effectiveness of *P. lilacinus* in reducing plant damage caused by the nematode. In previous studies, this fungus reduced reproduction of root-knot nematodes (*M. incognita* and *M. javanica*) through colonization, penetration and rupturing of egg cuticles, and by complete colonization of developing embryos and females (Jatala *et al.,* 1979; Morgan-Jones and Rodriguez-Kabana, 1984).

*Trichoderma harzianum*, alone or in combination with the other two bio-control organisms, also significantly increased plant growth and oil yield and reduced root-galling severity and reproduction of *M. incognita*. It is likely that this fungus acts through its proteolytic activity. Saifullah and Thomas (1996), in an *in vitro* study, demonstrated that *T. harzianum* penetrated cysts and eggs of *Globodera rostochiensis* and caused the death of $J_2$. Khan and Saxena (1997) found the metabolites of *T. viride* effective against nematodes.

*Pseudomonas fluorescens* also was effective against *M. incognita* and improved plant growth. The mechanism responsible for the reduction of the nematode population may be related to the ability of the bacterium to envelop or bind the root surface with carbohydrate lectin, thereby interfering with normal host recognition by the nematode (Oostendorp and Sikora, 1989).

The experiment clearly demonstrated that *M. incognita* reduces the oil content of menthol mint. In addition, as the nematode greatly suppressed the plant top weight, the oil yield per pot was affected much more than oil content. In India, the average yield of herbaceous plant tops per hectare is 187.3 quintals. Therefore, a loss of more than 100 litres of oil would occur in fields infested with a nematode population of 1 $J_2/cm^3$ soil. Considering the average price of menthol oil to farmers, this loss would be of 80,000 Rupees (= 1,455 US$ or 1,110 €). Therefore, the use of a combination of carbofuran and neem seed powder can be an economically sound strategy to control the nematode and increase oil yield of menthol mint.

## LITERATURE CITED

Alam M.M., Khan A.M. and Saxena S.K., 1979. Mechanism of control of plant parasitic nematodes as result of the application of organic amendments to the soil, V- Role of phenolic compounds. *Indian Journal of Nematology, 9*: 136-142.

Barker K.R., 1985. Nematode extraction and bioassay. Pp. 19-38. *In*: An Advanced Treatise on *Meloidogyne*. Vol. II, Methodology (Barker K.R., Carter C.C. and Sasser J.N., eds). North Carolina State University Graphics, Raleigh, USA.

Clevenger J.F., 1928. Apparatus for the determination of volatile oils. *Journal of American Pharmaceutical Association, 17*: 346.

Haseeb A., 1983. Studies on the changes in the roots of eggplant (*Solanum melongena* L.) resulting from infection of root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood-*Rhizoctonia solani* Kuhn complex. Ph.D. Thesis, Aligarh Muslim University, Aligarh, India, pp. 132.

Haseeb A. and Pandey R., 1989. Observation on *Meloidogyne* species affecting Japanese mint - new disease record. *Nematropica, 19*: 93-97.

Haseeb A. and Shukla P.K., 2000. Effect of *Pratylenchus thornei* and *Meloidogyne incognita*, alone and in combinations, on the growth and oil yield of *Mentha arvensis. Nematologia Mediterranea, 28*: 151-155.

Haseeb A. and Shukla P.K. 2001. Nematode diseases of *Mentha* and their management. Pp. 60-77. *In*: Role of Resistance in Intensive Agriculture (Nagarajan S. and Singh D.P., eds). Kalyani Publication, Ludhiana, India.

Jatala P., Kaltenbach R. and Bocangel M., 1979. Biological control of *Meloidogyne incognita acrita* and *Globodera pallida* on potatoes. *Journal of Nematology, 11*: 303 (Abstr.).

Khan T.A. and Saxena S.K., 1997. Effect of root-dip treatment with fungal filtrates on root penetration, development and reproduction of *Meloidogyne javanica* on tomato. *International Journal of Nematology, 7*: 85-88.

King E.O., Ward M.K. and Randey D.E., 1954. Two simple media for the demonstration of pycocynin and fluorescein. *Journal of Laboratory and Clinical Medicine, 44*: 301-307.

Kloepper J.W., Leong J., Teintze M. and Schroth M.N., 1980. Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. *Nature,* 286: 884-886.

Morgan-Jones G. and Rodriguez-Kabana R., 1984. Species of *Verticillium* and *Paecilomyces* as parasites of cyst and root-knot nematodes. *Phytopathology, 74*: 83.

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

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.

Singh S.P., Khan A.M. and Saxena S.K., 1980. Effect of nematicides and oil-cakes separately and in mixture on plant parasitic nematodes. *Geobios, 7*: 111-113.

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

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