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CONTROL OF *MELOIDOGYNE INCOGNITA* ON EGG PLANT USING *GLOMUS MOSSEAE* INTEGRATED WITH *PAECILOMYCES LILACINUS* AND NEEM CAKE

by

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Summary. An experiment was conducted to standardise a strategy of integrated management of the root-knot nematode, *Meloidogyne incognita*, on egg plant under field conditions by a combination of the endomycorrhiza *Glomus mosseae*, the fungus *Paecilomyces lilacinus* and neem cake (*Azadirachta indica*). Nursery beds infested with *M. incognita* were amended with neem cake two weeks before the incorporation of *G. mosseae* or *P. lilacinus*, or both. Healthy and vigorous seedlings colonised with endomycorrhiza as well as the biocontrol fungus were obtained for transplanting. Transplants obtained from the nursery beds treated with neem cake + *G. mosseae* + *P. lilacinus* were least infected in the field. The parasitization of eggs of root-knot nematode was significantly increased by *P. lilacinus* and the transplants yielded significantly more fruit. Neem cake amendment in the nursery beds played a positive role in increasing the colonization of endomycorrhiza and the biocontrol fungus on the roots of transplants before and after transplanting. The combined effect of these three components facilitated the sustainable management of *M. incognita* on egg plant under field conditions.

The role of endomycorrhizal fungi in reducing the root-knot nematode infestation on crop plants is well established (Sikora, 1979; Rao *et al.*, 1995). Similarly various researchers have reported the efficacy of *Paecilomyces lilacinus* against root-knot nematodes (Jatala, 1986; Rao *et al.*, 1997). Soil amendment with neem cake has been found to be effective in reducing root-knot nematode attack on various vegetable crops (Rao *et al.*, 1995).

This paper presents the results of integration of *Glomus mosseae* and *P. lilacinus* in neem cake amended nursery beds on the management of *M. incognita* (Kofoid *et* White) Chitw. infecting egg plant (*Solanum melongena* L. cv. Pusa Round) under field conditions.

Materials and methods

The starter culture of *G. mosseae* (Nicol. *et* Gerard) was obtained from Raukura Soil and Plant Research Station, Hamilton, New Zealand and multiplied on Rhodes grass (*Chloris gayana* Kunth.) in a glasshouse. A local isolate (IIHR-2) of *P. lilacinus* (Thom.) Samson was grown on autoclaved rice grain. Spore suspensions of *P. lilacinus* were prepared by washing cultures grown on the rice grain with sterile tap water containing 0.01% Triton X-100. The suspension was passed through a muslin cloth and the spore concentration was adjusted to 6×10^5 spores/ml.

The experiment was conducted at the Indian Institute of Horticultural Research farm in 40

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raised nursery beds each 1 m^2, and infested with *M. incognita* at the rate of 124±11 J_2 per 100 g of soil. Twenty nursery beds were amended with neem cake (*Azadirachta indica* Juss) at the rate of 400 g per nursery bed and were watered for 15 days to allow decomposition of the neem cake. Five of the treated beds were inoculated with 500 g of soil containing *G. mosseae* chlamydospores (26-32/g) by placing it 2 cm below the soil surface and another five treated beds were inoculated with *P. lilacinus* by adding 2 l of spore suspension (containing $6x10^5$ spores/ml) to each bed. A further five beds were treated with *P. lilacinus* + *G. mosseae* inocula at the same dosage levels mentioned above. The remaining five beds were treated with neem cake only, to separately evaluate the effect of this amendment. The other 20 beds, in groups of five, were treated with either *P. lilacinus* spore suspension or with *G. mosseae* only or with *G. mosseae* + *P. lilacinus*; the remaining five were maintained as non-inoculated controls.

Nematode population densities were assessed in the nursery beds 15 days after the amendment with neem cake, but before the other treatments, by sampling 100 g of soil from each bed. Nematodes were extracted from each sample by Cobb's decanting and sieving method with final separation using Baermann funnels.

All of the beds were sown with seeds of egg plant in rows. In each bed, forty seedlings were retained in five rows (10 seedlings per row) by thinning. After 30 days five seedlings were randomly selected from each bed for recording observations on height and weight of the seedlings and root colonization by endomycorrhiza. Five more seedlings were examined for assessment of root colonization by *P. lilacinus*. An additional ten seedlings were selected at random for assessment of the number of galls on the roots.

Subsequently the seedlings from each of the treatments were transplanted into 2x3 m plots in a field infested with *M. incognita* at the density of 96±17 J_2 per 100 cc soil. Each treatment was replicated five times in a randomised block design. Observations were recorded on the shoot and root weight, the root-knot index, on a 1-10 scale (Bridge and Page, 1980), root and soil nematode population densities and the yield of egg plant at harvest, 80 days after transplanting. Root colonization by *G. mosseae* and *P. lilacinus* was determined 40 and 80 days after transplanting to determine their interaction as the plant grows.

To evaluate colonization by *P. lilacinus*, the root system was carefully washed to remove soil, blotted dry, weighed and cut into small pieces of about 1 cm each. One gram samples of roots were taken at random and crushed in sterile distilled water and plated on to a semi-selective medium developed by Mitchell *et al.* (1987), with pentachloronitrobenzene, NaCl, benomyl, streptomycin sulphate, chlorotetracyclin hydrochloride and turgital NP-10 in potato dextrose agar medium. The Petri plates were incubated at 25-27 °C for seven days. *P. lilacinus* was identified by the hyphal, conidiophore morphology and the characteristic colony colour. To study egg parasitism, ten egg masses from each plant root system were randomly selected and treated with 0.5% sodium hypochlorite and the number of eggs infected was counted. *P. lilacinus* was isolated from adult females and eggs of *M. incognita* by using the semi-selective medium mentioned above.

Observations were made on root colonization by *G. mosseae* by clearing the roots with 10% KOH and then staining with trypan blue (Phillips and Hayman, 1970). The number of nematodes in the roots was estimated by staining 5 g of root samples in acid fuchsin and homogenising them and counting nematodes using a microscope.

The data were analyzed by ANOVA (statistical) design.

Results and discussion

The number of galls on the roots of the seedlings in nursery beds amended with neem

cake + *G. mosseae* or neem cake + *P. lilacinus* or neem cake + *G. mosseae* + *P. lilacinus* were significantly reduced (Table I). This could result from a reduction of nematode population by the treatment of nursery beds with neem cake (Table I). These results show the additive effect of *G. mosseae*, *P. lilacinus* and neem cake amendment on the growth of egg plants in the nursery stage and also on the nematode infestation and production of highly vigorous and mycorrhizal seedlings for transplanting in the field. The seedlings were highly vigorous because there was a significant increase in growth of the seedlings raised on all the nursery beds amended with neem. The seedlings were also highly mycorrhizal because there was a significant increase in root colonization by *G. mosseae* on the seedlings raised in the nursery beds amended with neem cake, with or without *P. lilacinus*. The results also indicate the favourable effects of neem cake amendment on *G. mosseae* and *P. lilacinus* colonization (Table I and II). Bagyaraj *et al.* (1979) found that mycorrhizal seedlings were less infected by *M. incognita* or *M. javanica* after transplanting; in our studies, mycorrhizal transplants were less infected by *M. incognita* under field conditions (Table II). However, the infestation was much less in the transplants obtained from nursery beds treated with neem cake + *G. mosseae*. Further, the nematode infestation was least on the transplants from nursery beds treated with neem cake + *G. mosseae* + *P. lilacinus*; this could be due to the combined effect of all three components of management.

Integration of *P. lilacinus* did not affect the root colonization by *G. mosseae* after transplanting (Table III) and indeed the presence of *G. mosseae* tended to increase the root colonization by *P. lilacinus*.

Integration of the three components not only decreased the root-knot index or the number of

TABLE I - *Effect of integration of* Glomus mosseae *and* Paecilomyces lilacinus *inoculation in nursery beds amended with neem cake on the growth of egg plant seedlings and infestation by* Meloidogyne incognita.

Treatment	Seedling height (cm)	Seedling weight (g)	Root colonization of *G. mosseae* (%)	Root colonization by *P. lilacinus* (CFU/g root)	No. of galls in ten seedlings	No. of J2/100 cc of soil 15 days after amendment in nursery beds
G. mosseae	21	2.3	34	–	55	118
Neem cake	19	2.0	3	–	47	41
P. lilacinus	20	2.2	4	17,575	69	129
Neem cake + *G. mosseae*	23	2.4	46	–	44	37
Neem cake + *P. lilacinus*	24	2.3	2	21,345	48	45
G. mosseae + *P. lilacinus*	24	2.5	43	19,421	52	127
Neem cake + *G. mosseae* + *P. lilacinus*	26	2.7	41	23,789	34	37
Control	17	1.7	2	–	78	135
C.D. 5%	3.56	0.59	6.45	967.21	7.63	

CFU/g = Colony forming units/gram of root.

eggs/egg mass, but also significantly increased growth and fruit yield of egg plants (Table II and IV). However, the final soil population densities of nematodes were reduced by only 60% (Table IV) since all three components of management were used only in the nursery. The field could not be treated because of the high cost of these treatments and practical implications involved in the delivery of these components of management in the larger area of the field.

The main purpose of these studies was to produce egg plant seedlings whose roots are colonized by *P. lilacinus* as well as *G. mosseae* before transplanting so that they could carry these components of management to the field. During this process the field soil would be enriched with

TABLE II - *Effect of integration of* G. mosseae, P. lilacinus *and neem cake on the growth of transplants of egg plant and* M. incognita *infestation in the field.*

Treatment	Shoot weight (g)	Root weight (g)	Root-knot index (1-10)	No. of egg masses per 5 grams of root
G. mosseae	326	95	7.2	24
Neem cake	285	83	6.6	18
P. lilacinus	282	87	7.0	21
Neem cake + *G. mosseae*	342	104	5.9	15
Neem cake + *P. lilacinus*	312	96	5.7	10
G. mosseae + *P. lilacinus*	351	112	5.4	13
Neem cake + *G. mosseae* + *P. lilacinus*	374	119	4.8	7
Control	243	97	8.7	38
C.D. 5%	36.32	18.48	0.49	6.35

TABLE III - *Effect of integration of* G. mosseae, P. lilacinus *and neem cake on the root colonization by endomycorrhiza and biocontrol fungus.*

Treatment	40 days after transplanting		80 days after transplanting	
	Colonization of *P. lilacinus* (CFU/g root)	Root colonization of *G. mosseae* (%)	Colonization of *P. lilacinus* (CFU/g root)	Root colonization of *G. mosseae* (%)
G. mosseae	–	47	–	26
Neem cake	–	5	–	3
P. lilacinus	32650	3	41660	2
Neem cake + *G. mosseae*	–	58	–	32
Neem cake + *P. lilacinus*	42565	5	59600	4
G. mosseae + *P. lilacinus*	33672	56	44760	29
Neem cake + *G. mosseae* + *P. lilacinus*	43249	50	51382	30
Control	–	4	–	3
C.D. 5%	1286.88	6.49	1377.21	2.76

TABLE IV - *Effect of integration of* G. mosseae, P. lilacinus *and neem cake on the % of* M. incognita *eggs infected by* P. lilacinus.

Treatment	No. of eggs/egg mass	Infection of eggs by *P. lilacinus* (%)	No. of nematodes in 5 g of root	No. of nematodes in 100 cc of soil	Fruit yield (Kg/3x2 m plot)
G. mosseae	363	–	107	134	6.2
Neem cake	455	–	92	128	5.6
P. lilacinus	465	31	100	102	5.3
Neem cake + *G. mosseae*	326	–	64	106	7.3
Neem cake + *P. lilacinus*	427	36	52	76	6.9
G. mosseae + *P. lilacinus*	342	40	60	85	6.5
Neem cake + *G. mosseae* + *P. lilacinus*	289	51	38	63	7.8
Control	520	–	162	152	5.3
C.D. 5%	42.76	4.52	21.34	16.32	0.89

the propagules of eco-friendly components of management in two or three seasons. Data generated by repeating the trial in the same piece of land and different areas and growing seasons reveal that the similar treatments with the above mentioned components of management reduce the final populations of nematode in soil by 60%, 74% and 86% in first, second and third season respectively in the same area.

The role of endomycorrhizal fungi in the improvement of plant growth has been documented (Sikora, 1979; Rao *et al.*, 1996). Some colonization by endomycorrhizal fungi was observed in the treatment where *G. mosseae* was not added and this was because of a residual, negligible population (not identified) in the soil. Transplants raised from the nursery beds which were separately treated with *G. mosseae* alone, neem cake alone or spore suspension of *P. lilacinus* were found to be infested by *M. incognita* in the field (Table II and IV). Individual treatments were not effective in the management of this nematode in the field. Hence, rational integration of all these components reported here would help in the sustainable management of root-knot nematode in field conditions.

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