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INTEGRATION OF *PAECILOMYCES LILACINUS* WITH NEEM LEAF SUSPENSION FOR THE MANAGEMENT OF ROOT-KNOT NEMATODES ON EGG PLANT*

by

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Summary. Integration of the bio-control fungus, *Paecilomyces lilacinus* with neem (*Azadirachta indica*) leaf aqueous suspensions was attempted for the management of root-knot nematode, *Meloidogyne incognita* on egg plant (*Solanum melongena*). Results revealed the suitability of neem leaf suspensions (5 and 10%) for mass multiplication of *P. lilacinus*. Further, significant reduction in root-knot index and final population of *M. incognita* were observed in the egg plant seedlings which were given root-dip treatment in neem leaf suspensions mixed with *P. lilacinus* spores. In addition, significant increases were observed on the colonization of *P. lilacinus* on the roots of egg plant and parasitisation of eggs of *M. incognita*, indicating the complementary interaction between these two components for the sustainable management of root-knot nematodes on egg plant.

Paecilomyces lilacinus (Thoms.) Samson has been reported as an effective biolgical control agent of root-knot nematodes (Jatala, 1986; Rodriguez Kabana *et al.*, 1987; Rao and Parvatha Reddy, 1994). Neem leaf aqueous suspension was also used as a root-dip treatment for the management of root-knot and reniform nematodes on tomato (Siddiqi and Alam, 1988). Hence, investigations were carried out on the use of neem leaf aqueous suspensions mixed with the spores of *P. lilacinus* as a root-dip treatment for the management of *Meloidogyne incognita* (Kofoid *et* White) Chitw. on egg plant (*Solanum melongena* L.) cv. Pusa Purple Round.

Materials and methods

Five and ten per cent (W:V) aqueous suspensions of neem leaf, *Azadirachta indica* A. Juss, were prepared by blending the fresh (washed) leaves of neem in distilled water and filtering through a muslin cloth. Thirty ml of these suspensions of each concentration were taken in conical flask for autoclaving at 15 lbs pressure for 30 min. The suspension in each conical flask was inoculated with 4 mm disk of *P. lilacinus* culture grown on potato dextrose agar medium and incubated at 25 °C for 25 days. Five replicates were maintained for each treatment. The number of

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spores per ml at different concentrations of the neem leaf suspensions were recorded.

The neem leaf aqueous suspensions of ten and 20 per cent concentrations were prepared and mixed with equal quantity of spore suspension in sterile tap water so as to get the concentration of $4x10^5$ spores per ml. These suspensions were used for the root-dip treatment of egg plant seedlings (30 day old seedlings grown in sterilised soil) for 30 minutes. Each seedling was transplanted in separate pots containing 2 kg steam sterilised soil. To evaluate the individual effects, the seedlings were given the root-dip treatment either in neem leaf suspensions (5 or 10%) or *P. lilacinus* spore suspension containing $4x10^5$ spores per ml of water. The seedling roots were dipped in sterile tap water as a control treatment. Each treatment was replicated five times. Five days after transplanting, each seedling was inoculated with 2500 J_2 of *M. incognita*. Two months after nematode inoculation, the experiment was terminated and observations were made on the plant height, root weight, root length, root-knot index, final (root + soil) population of nematodes, percentage parasitism of *M. incognita* eggs by *P. lilacinus*, colonisation of *P. lilacinus* on the roots and propagule density of *P. lilacinus* in the soil.

For assessing the colonization of *P. lilacinus*, root system was carefully washed to remove soil, blotted dry, weighed and cut into small pieces of about 1 cm each. One g sample of roots were taken at random and crushed with a sterile pestle and mortar. Serial dilutions were made in sterile distilled water and plating was done by using the semi-selective medium developed by Mitchell *et al.* (1987). For estimating *P. lilacinus* spore density in soil, the same semi-selective medium was used for soil dilution plating. *P. lilacinus* was identified by the hyphae, conidiophore morphology and colony colour characteristic to *P. lilacinus*. For making observations on egg mass infection, ten egg masses were picked up at random from each plant root system and treated with 1% sodium hypochlorite solution and the infected eggs counted using a microscope (50x magnification). *P. lilacinus* was reisolated from the adult females and eggs of *M. incognita*, by placing surface sterilized adult females on the same semi-selective medium. Adult females were surface sterilized with 0.1 per cent sodium hypochlorite solution for ten seconds. Egg masses were washed in sterile tap water and eggs were dispersed in 1 ml of water using an homogenizer (Jensons) and plating them on the semi-selective medium for re-isolation of *P. lilacinus*. Similarly, nematode females, eggs and soil from control treatment were analyzed for the presence of *P. lilacinus* which could not be found in these samples (Table II). Nematodes from 200 cc of soil samples were extracted using modified Baermann funnel technique (Schindler, 1961). Nematodes in five ml samples (five replicates) were counted and total nematode population was estimated. The roots from each plant were stained with acid fuchsin in lactophenol (Franklin and Goodey, 1949). Nematodes in five ml of samples (five replicates) were counted and nematode population was estimated.

Results and discussion

The aqueous neem leaf suspensions supported the growth of *P. lilacinus*. The number of spores per ml of neem leaf suspensions of 5 and 10% was $17.4x10^6$ and $22.7x10^6$, respectively. Mani and Anandan (1989) have reported the suitability of neem leaf as a substrate for the mass multiplication of *P. lilacinus*. This is the first report on the suitability of neem leaf aqueous suspensions as substrates for the growth of *P. lilacinus*.

Significant increases in plant growth parameters, reductions in the root-knot index and final populations of *M. incognita* were observed in the treatments where egg plant seedlings were given the root-dip treatment in the neem leaf suspensions mixed with *P. lilacinus* spores (Table I). The reduction in root-knot index was observed in *P. lilacinus* treatment (Table I) which may be due

to less invasion of the first generation juveniles. The nematode completed two generations during the experiment. Further, neem leaf suspensions also reduced the root-knot index (Table I). Siddiqi and Alam (1988) reported that root-dip treatment in neem leaf suspension was found effective against root-knot nematodes on tomato. In the present studies also, it was observed that these components individually are effective to some extent in reducing the root-knot nematode infestation (Table I). However, integration of both the components (neem leaf suspension + *P. lilacinus*) maximized their effects in reducing the root-knot index. Further, the complementary interactive effect of these components resulted in a significant increase in the colonization of *P. lilacinus* on the roots, increased the percentage parasitization of eggs of *M. incognita* by *P. lilacinus* and the density of propagules of *P. lilacinus* in the soil (Table II). From the above results, it may be concluded that the successful integrated management of *M. incognita* on egg plant was achieved by a root-dip treatment in neem leaf suspension mixed with *P. lilacinus* spores.

TABLE I - *Effect of root-dip treatment with* Paecilomyces lilacinus *and neem leaf suspension on the growth of egg plant and population of* Meloidogyne incognita.

Treatments	Plant height (cm)	Shoot weight (g)	Root weght (g)	Length of root/gram (cm)	Root-knot index	Final nema. population (Root + soil)
P. lilacinus	62	18.3	13.0	195	2.8	3832
Neem leaf suspension - 5%	64	17.9	13.5	176	3.1	5346
Neem leaf suspension - 10%	65	18.8	14.0	182	2.7	4056
P. lilacinus + Neem leaf suspension - 5%	79	21.5	15.0	225	2.4	2964
P. lilacinus + Neem leaf suspension - 10%	81	22.7	15.2	230	2.1	2356
Control	57	13.5	11.2	156	4.2	8258
C.D. at 5%	8.75	2.64	1.87	31.35	0.46	785.32

TABLE II - *Effect of root-dip treatment with* P. lilacinus *and neem leaf suspension on the biocontrol fungus root colonization, spore density in soil and parasitization of eggs of* M. incognita.

Treatments	Colonization of *P. lilacinus* (CFU/g of root)	*P. lilacinus* spore density (CFU/g of soil)	% parasitization of eggs by *P. lilacinus*
P. lilacinus	32,540	20,560	53
Neem leaf suspension - 5%	–	–	–
Neem leaf suspension - 10%	–	–	–
P. lilacinus + Neem leaf suspension - 5%	36,680	25,640	59
P. lilacinus + Neem leaf suspension - 10%	39,460	27,690	64
Control	–	–	–
C.D. at 5%	3050.76	2850.78	4.59

CFU - Colony Forming Units.

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