Nematol. medit. (1991), *19*: 247-249

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EFFECT OF *PAECILOMYCES LILACINUS* APPLICATION TIME AND METHOD IN CONTROLLING *MELOIDOGYNE JAVANICA* ON OKRA

by
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Summary. In a pot experiment, *Paecilomyces lilacinus* cultured on wheat bran (WBF) was applied either as soil treatment 10 days before/after sowing 1 g/kg soil (5 x 10^8 spores); as seed treatment (3 x 10^7 spores per seed); or in different combinations, to sandy soil infested with *Meloidogyne javanica*. Fungus application, in general, resulted in better top growth. The methods (soil/seed treatment) or time (pre-/post- sowing) of fungus application were equally effective. Wheat bran alone (WB) also promoted shoot length and fresh shoot weight to a lesser extent. Root galling was significantly reduced in all fungus treatments. Application of WB alone failed to reduce root galling. The time and method of fungus application were at par as far as gall reduction was concerned. WBF proved better than carbofuran (1 kg a.i/ha) in gall suppression.

Paecilomyces lilacinus (Thom.) Samson, initially discovered as an egg parasite of root-knot nematode, *Meloidogyne incognita* infesting potato in Peru (Jatala *et al.*, 1979), has subsequently been found to parasitise a wide range of economically important nematode species (Jatala, 1986). Conflicting reports of its efficacy against nematodes (Jatala, 1986), may be attributed to varying agro-climatic conditions. The greatest nematode suppression of 70% was obtained with 80 g of fungus-infested rice kernels per 15-cm diam pot against *Meloidogyne incognita* on tomato (Adiko, 1984).

Previously, we reported that *P. lilacinus* can alternatively be cultured on wheat bran which is a relatively cheaper and equally efficient substrate (Bansal *et al.*, 1988) compared with wheat/rice kernels (Jatala, 1983). Further, we found that *P. lilacinus* grown on wheat bran gave satisfactory control of *M. javanica* when applied at 1 g/kg soil (unpublished). The objective of this study was to ascertain the most suitable time and method of application of *P. lilacinus* against *M. javanica* (Treub) Chitw. on okra (*Abelmoschus esculentus* Moench.).

Materials and methods

A 10-day-old culture of *P. lilacinus* (obtained from CIP, Lima, Peru) grown on PDA in a 10 cm diam petri-plate was flushed with 5 ml sterile water. The surface of the medium was gently rubbed to collect a spore suspension. 20 g wheat bran mixed with distilled water (1:1 w/v) was transferred to each 250 ml Erlenmeyer flask and autoclaved for 30 min. at 15 p.s.i. One ml spore suspension was poured into each flask and the contents thoroughly mixed and incubated at 28 ± 1°C. The flasks were manually shaken every day to allow a uniform growth of the fungus. After 7 days the spore load per g substrate was estimated by a serial-dilution method.

The fungus was applied by two methods of soil and seed treatment. For soil treatment, 1 g of fungus infected wheat bran (WBF) per pot was manually mixed in the upper 2-3 cm soil layer 10 days before or after sowing. For seed treatment 10 g of okra seeds were uniformly coated with a sticker (arabic gum) and mixed with 10 g of WBF in a covered petri-plate till maximum coverage of seeds by WBF was obtained. Ten such seeds were used to estimate the spore load per seed as described earlier. To elucidate the influence of medium alone, wheat bran without fungus was processed similarly, and applied in the same manner to serve as appropriate controls.

The soil mixture (sand-90.4%, silt-2.0%, clay-7.6%, organic matter-0.77%, pH-7) used in the experiment was obtained from the culture pots of *M. javanica* and mixed with steamed soil to obtain an initial nematode level of one juvenile per g soil. 15 cm clay pots of 1 kg capacity were completely randomised on a green-house bench previously treated with 4% formalin. To study the efficacy of *P. lilacinus* vis-a-vis nematicide, in one treatment, carbofuran (Furadan 3G) 1 kg a.i./ha was mixed in soil at sowing time. Two seeds (uncoated/coated with WBF or WB) of okra cv. Pusa Sawani were sown per pot and upon germination one plant was retained. The atmospheric temperature during the course of experiment (30.8.1989 to 30.10.1989) ranged between 16-35°C. Each treatment was replicated five times.

At the termination of experiment, the plants were gently uprooted from the pots, and observations on growth characteristics recorded. Root gall index was measured visually on 1-10 scale (1 = no galling, 10 = 100% root area galled). Soil from each pot was mixed thoroughly and processed by dilution- plating technique for isolation of *P. lilacinus*. The basal medium was PDA amended with Penicillin, Streptomycin sulphate and Karathane at 50, 50 and 8 ppm, respectively. One g soil sample was diluted (10^{-4}) in sterile water, and 0.1 ml aliquot poured over each of the petri-plates. The petri-plates were incubated at 28 ± 1°C and fungal colonies counted after 5 days.

Ten eggmasses from each root system were removed gently, surface sterilized with 0.5% sodium hypochlorite for 30 sec., rinsed thrice in sterile water and placed over medium (as described above) in petri-plates. Microscopic observation were also taken to confirm the presence of fungal hyphae inside the nematode eggs.

The statistical analysis consisted of an analysis of variance of data obtained for plant growth, nematode and fungus population. Treatment means were compared by linear contrasts.

Results

Data indicate that the fungus application, irrespective of its time and method of application, significantly enhanced shoot growth (length, fresh and dry weight), but not the root growth, when compared to the treatment of nematode alone (Table I). Wheat bran alone (WB) also promoted shoot length and fresh shoot weight.

Soil treatment (pre- or post-sowing) or seed coating with WBF did not differ significantly in respect of plant growth (except shoot length which was better in soil treatment). Similar was the case with soil treatment alone and soil + seed treatment. Similarly, pre- or post-sowing soil application of fungus did not differ significantly from each other.

Fungus application, in general, proved more effective than nematicide in promoting shoot length and dry shoot

TABLE I - *Efficacy of the fungus* Paecilomyces lilacinus *at different times of application in the soil, and as seed treatment to control* Meloidogyne javanica *on okra.*

Treatment	Shoot			Root		Gall index (1-10)	Fungus recovery (no. of spores/ g soil)	Bioassay (%eggmasses infected)
	Length (cm)	fresh wt. (g)	dry wt. (g)	fresh wt. (g)	dry wt. (g)			
1 WBF' in soil, 10 days before sowing	41.4	18.0	4.5	8.2	0.8	3.0	4.5×10^4	30
2 WB in soil, 10 days before sowing	40.2	16.8	3.3	6.5	0.6	5.6	0	0
3 Seed treatment'' with WBF + 1	47.5	21.9	6.1	9.9	1.0	2.6	2.5×10^4	60
4 Seed treatment with WB ÷ 2	43.7	20.1	4.9	9.0	0.9	6.2	0	0
5 Seed tratment with WBF	48.0	18.9	4.8	10.1	0.9	5.6	0.3×10^4	30
6 Seed tratment with WB	37.0	13.8	3.3	6.1	0.6	6.4	0	0
7 WBF in soil, 10 days after sowing + 5	42.8	16.0	3.8	8.4	0.7	7.2	0.6×10^4	20
8 WB in soil, 10 days after sowing + 6	40.8	15.8	3.7	7.0	0.7	5.0	0	0
9 WBF in soil, 10 days after sowing	40.2	15.3	4.0	6.8	0.7	3.0	2.3×10^4	40
10 WB in soil, 10 days after sowing	36.0	14.0	3.1	6.9	0.6	6.4	0	0
11 Nematode alone, no WBF, no WB	32.7	10.2	2.2	6.4	0.6	7.8	0	—
12 Nematode + Carbofuran 1 kg a.i./ha	38.1	18.6	2.9	10.8	0.9	8.4	0	—
13 Steamed soil, no WBF, no WB	50.2	20.7	6.0	12.5	1.5	—	0	—
14 Steamed soil + WBF, 10 days before sowing	46.4	12.8	5.2	10.9	0.9	—	1.0×10^4	—
CV (%)	20.4	32.7	44.3	36.9	43.7	54.8		
Linear contrasts								
1,3,5,7,9 vs 11	*	*	*	NS	NS	*		
1,3,5,7,9 vs 12	*	NS	*	NS	NS	*		
2,4,6,8,10 vs 11	*	*	NS	NS	NS	NS		
1,3,5,7,9 vs 2,4,6,8,10	*	NS	*	*	NS	*		
1,9 vs 5	*	NS	NS	NS	NS	NS		
1,9 vs 3,7	*	NS	NS	NS	NS	NS		
1 vs 9	NS	NS	NS	NS	NS	NS		
13 vs 14	NS	NS	NS	NS	*	—		

WBF = Fungus cultured on wheat bran; WB = Wheat bran alone; ' WBF mixed in soil 1 g/kg soil (5×10^8 spores, approx.); '' Fungus spore load = 3×10^7 spores/seed (2 seeds sown/pot); * indicates no significant difference at P = 0.05.

weight. The fungus when applied in steamed soil (without nematodes) did not adversely affect plant growth, thus confirming its non- pathogenicity to plants.

In all the treatments where fungus was applied (irrespective of time and method of application), there were significant reductions in root galling as compared to treatment of nematode alone. Application of WB alone did not suppress root galling. The efficacy of fungus in reducing root galling was better than nematicide. However, the method (soil/seed) or time (pre-, post- sowing) of fungus application were at par in reducing severity of root galling.

4.5×10^4 and 0.3×10^4 spores/g soil were recovered at the end of experiment from soils where the fungus was applied 10 days before sowing and as seed treatment, respectively. However, 60% of the eggmasses were infected when pre-sowing soil and seed treatment were combined.

Discussion

The efficacy of *P. lilacinus* in suppressing *M. javanica* was evident in all the treatments where fungus was applied. *P. lilacinus* being an egg parasite, though it has been reported to occasionally parasitise females of *M. incognita* as well (Jatala, 1986), suppresses the nematode population only at the time of egg formation. The impact of the fungus, therefore, is not evident till the completion of first egg to egg generation by the nematode. Since under Haryana conditions, *M. javanica* is known to complete two generations during August-September, the results achieved can, therefore, be attributed to the reduction in secondary infection by the second-generation juveniles due to fungal colonisation of eggs. Time of fungus introduction is, therefore, an important factor in governing its efficacy. Cabanillas and Barker (1989) have also reported that *P. lilacinus* is more effective in protecting tomatoes against *M. incognita* when it is delivered before transplanting and (or) at transplanting than at mid-season.

The method of fungus introduction apparently may not be very important as long as it is delivered in optimum quantity (pathogenic level), which will vary according to the prevailing ecological conditions. This is perhaps one of the major reasons leading to the variability in results achieved by different workers.

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Accepted for publication on 2 June 1991.