

MANAGEMENT OF *MELOIDOGYNE JAVANICA* ON ACID LIME NURSERY SEEDLINGS BY USING FORMULATIONS OF *POCHONIA CHLAMYDOSPORIA* AND *PAECILOMYCES LILACINUS*

M.S. Rao

Nematology Laboratory, Division of Entomology and Nematology, Indian Institute of Horticultural Research, Hessaraghatta Lake P.O., Bangalore 560 089, India

Summary. Bio-efficacy and compatibility of formulations of *Pochonia chlamydosporia* (2×10^6 cfu/g) and *Paecilomyces lilacinus* (2×10^6 cfu/g) on root-knot nematode, *Meloidogyne javanica*, infecting nursery seedlings of acid lime were evaluated. Application of 5 or 10 g of each bio-agent formulation per kg of soil significantly reduced root-galling index and the number of nematodes in the roots. The combined use of *P. lilacinus* and *P. chlamydosporia*, each at 10 g/kg, significantly reduced rhizosphere colonization and the soil propagule density of *P. chlamydosporia* compared to the densities achieved when the fungus was applied alone. However, the root colonization and soil propagule density of *P. lilacinus* were not affected when it was used at both the dosages (5 and 10 g) of *P. chlamydosporia*. The seedling roots were colonised by both bio-agents. These data will be useful for the development of a combined formulation of the two bio-agents.

Meloidogyne javanica (Treb.) Chitw. is reported as one of the important factors affecting the production of acid lime (*Citrus aurantifolia* Christm et Panz) in India (Mani, 1986; McSorley, 1981; Reddy and Rao, 2001). During surveys of acid lime orchards in southern states of India, very high incidences of *M. javanica* were observed (Reddy and Rao, 2001). Mani (1986) also reported that *M. javanica* caused severe crop loss of acid lime in south India. Since the use of chemical nematicides has proved to be hazardous, it was thought to develop a method of management using a formulation of the fungus *Pochonia chlamydosporia* Zare et al., which was reported to be a very effective bio-control agent against root-knot nematodes (De Leij and Kerry, 1991; Kerry et al., 1993; Rao et al., 1997c, 1998a, 2003). *Paecilomyces lilacinus* (Thom.) Samson was also reported to be effective against root-knot nematodes (Jatala, 1986; Rao and Reddy, 1994; Reddy et al., 1999; Rao et al., 1997a, 1997b, 1998b, 1999). However, there are no reports on the combined use of these two promising bio-agents for the management of root-knot nematodes on acid lime nursery seedlings. Hence, the present investigations were carried out.

MATERIALS AND METHODS

Local isolates of *P. lilacinus* (IIHR-PL 2) and *P. chlamydosporia* (IIHR-VC 3) were mass produced separately through liquid and solid fermentation processes (the details of the fermentation process are not revealed here for patent considerations). The identification of *P. chlamydosporia* was confirmed by Prof. Brian Kerry,

Rothamsted Research, United Kingdom (personal communication, 2000), by a diagnostic test based on specific primers from the β tubulin gene used in PCR (Arora et al., 1996). In the field experiments, products (formulated at Indian Institute of Horticultural Research, Bangalore, India) of *P. lilacinus* (2×10^6 spores/g) and *P. chlamydosporia* (2×10^6 chlamydospores/g) were evaluated, each at dosages of 5 and 10 g per kg of soil.

The experiment was conducted at the Indian Institute of Horticultural Research, Bangalore, for two seasons during 2002 and 2003 (June to November). The nursery soil (a mixture of soil, farm yard manure and sand in the ratio of 3:2:1) was infested with 89 ± 5 hatched juveniles of *M. javanica* per 100 g of soil. The soil was mixed with the bio-agent formulations and 1 kg of mixture was added to polythene bags (10×15 cm). One seed of acid lime was sown in each bag. The treatments were: a) soil mixed with *P. lilacinus* at 5 g/kg, b) soil mixed with *P. lilacinus* at 10 g/kg, c) soil mixed with *P. chlamydosporia* at 5 g/kg, d) soil mixed with *P. chlamydosporia* at 10 g/kg, e) soil mixed with *P. lilacinus* and *P. chlamydosporia* at 5 g each/kg, f) soil mixed with *P. lilacinus* and *P. chlamydosporia* at 10 g each/kg, and g) control with no treatment. Each treatment was replicated ten times in a completely randomized block design.

Observations on length of seedlings, seedling weight, root-galling index on a 1-10 scale (Bridge and Page, 1980), number of eggs/egg mass, root colonisation by *P. lilacinus* and *P. chlamydosporia*, soil propagule densities of both bio-agents, and soil nematode population densities were recorded 150 days after sowing.

To evaluate root colonisation by *P. lilacinus* and *P. chlamydosporia*, five seedlings were uprooted. Each root system was carefully washed to remove soil and excess water was removed using blotting paper. Root systems

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were weighed and cut into small pieces about 1 cm long. Two grams of root pieces were picked at random from each seedling to make a composite sample of 10 grams from the five plants taken from the ten replicates (the remaining five replicates were used for other observations). Out of this composite sample, 1-g samples of roots were taken for estimation of root colonization by *P. lilacinus* and *P. chlamydosporia* separately, using the semi-selective media developed by Mitchell *et al.* (1987) and Kerry *et al.* (1993), respectively. Petri plates were incubated in an incubator at 25-27 °C for 15 days. Soil propagule densities of both bio-agents were estimated by following a serial dilution technique and using the above-mentioned semi-selective media.

To determine the number of eggs per egg mass, two egg masses of *M. javanica* were randomly selected from each plant. The ten egg masses collected from five plants were dissolved in 0.05% sodium hypochlorite solution (Hussey and Barker, 1973) and the number of eggs was counted. To investigate parasitism of the eggs by *P. lilacinus* or *P. chlamydosporia*, two egg masses were again randomly selected from each of the five plants and the ten egg masses were treated with 0.01% sodium hypochlorite in a Petri plate for 60 seconds, for surface sterilization. The eggs from these egg masses were dispersed in 3 ml of sterile water using a blender (Jencons) and plated on the semi-selective medium developed by Mitchell *et al.* (1987) and Kerry *et al.* (1993). Petri plates were incubated at 25-27 °C for 4 days and infected eggs were readily identified. The proportions of the eggs infected by the fungi were determined by examination of 100 eggs on each Petri plate.

The fungi, *P. lilacinus* and *P. chlamydosporia*, were isolated from adult females of *M. javanica* by using the semi-selective media mentioned above. Females were incubated in Petri plates at 25-27 °C for 15 days in the dark and, on the basis of morphological features of *P. lilacinus* and *P. chlamydosporia*, parasitism of adult females was confirmed. Root populations of the nematodes were estimated from 10-g composite samples of roots, collected

from five replicates at 2 g/replicate. The root samples were stained using acid fuchsin following the method of Bridge *et al.* (1982), homogenised, and the numbers of nematodes (J₂ and adults) counted. To estimate the reduction in the soil nematode density due to the application of *P. chlamydosporia*, *P. lilacinus* or both organisms, the infective stage juveniles of *M. javanica* were extracted from 100 cm³ soil per replicate by Cobb's sieving and decanting method (Cobb, 1918) and counted.

The data were analyzed using ANOVA.

RESULTS AND DISCUSSION

Soil treatment with the combination of *P. lilacinus* and *P. chlamydosporia* significantly reduced the root galling index to 3.0 and 3.5 at dosages of 5 and 10 g/kg, respectively, in season 2 (Table I); untreated plants had a galling index of 8.4 (Table I). *Paecilomyces lilacinus* was also found effective against *M. javanica* on potato and tomato, *Globodera rostochiensis* (Wollenweber) Skarbilovich on potato, *Tylenchulus semipenetrans* Cobb on citrus, and *Rotylenchulus reniformis* Linford *et* Oliveira on tomato and egg plant (Reddy and Khan, 1988, 1989; Rao *et al.*, 2001).

When the efficacy of individual treatments with these bio-agents was compared, *P. lilacinus* was found to be comparatively more effective than *P. chlamydosporia* in reducing the galling index, the number of nematodes in roots and soil, and the number of eggs per egg mass (Tables I and II). Moreover, this treatment significantly decreased the root galling index, the number of eggs per egg mass and total number of nematodes in roots when compared with treatments of individual bio-agents (Tables I and II).

The combined use of *P. lilacinus* and *P. chlamydosporia*, each at 10 g/kg soil, significantly reduced root colonisation by and soil propagule density of *P. chlamydosporia* in both seasons (Table IV) but, when both bio-agents were used at 5 g/kg, neither affected the colonisation of the other (Table IV). Further, root colonisation and soil

Table I. Effects of *Pochonia chlamydosporia* and *Paecilomyces lilacinus*, singly or in combination, on the growth of acid lime seedlings and their infestation by *Meloidogyne javanica*.

Treatment	Seedling height (cm)		Seedling weight (g)		Root-galling index (1-10)	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
<i>P. lilacinus</i> at 5 g/kg (Pl-5)	34.6	32.8	7.2	7.1	5.5	5.6
<i>P. lilacinus</i> at 10 g/kg (Pl-10)	36.4	37.6	7.9	8.1	5.1	5.0
<i>P. chlamydosporia</i> at 5 g/kg (Pc-5)	33.1	32.2	6.9	6.7	6.2	6.3
<i>P. chlamydosporia</i> at 10 g/kg (Pc-10)	34.6	35.4	7.1	6.8	5.9	5.4
Pl-5 + Pc-5	36.5	37.2	7.1	8.0	3.2	3.0
Pl-10 + Pc-10	36.2	35.3	6.9	7.2	3.8	3.5
Untreated	26.8	28.2	5.2	5.8	7.9	8.4
C.D. (P = 0.05)	4.56	5.69	1.67	1.34	0.54	0.68

C.D. = Critical Difference

propagule density of *P. lilacinus* were not affected when it was used at either dose of *P. chlamydosporia* (Table IV).

Treatment of nursery soil with formulations of both *P. lilacinus* and *P. chlamydosporia*, each at the rate of 5 g/kg of soil, increased the growth of acid lime seedlings (Tables I). The data also indicate that these rates are suffi-

cient to control *M. javanica* and that they avoid the competition between the bio-agents observed when both fungi were used at 10 g/kg of soil (Tables III). Root colonisation is an important criterion for assessing the bio-efficacy of any formulated product, as the transplanted seedlings would carry the bio-agents to the field.

Table II. Effects of combinations of *P. chlamydosporia* and *P. lilacinus* on root and soil populations of *M. javanica*.

Treatment	Nematodes (J ₂ and adults) in 10 g roots		Nematodes (J ₂) in 100 cm ³ of soil		Eggs per egg-mass	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
<i>P. lilacinus</i> at 5 g/kg (Pl-5)	76	72	85	80	342	331
<i>P. lilacinus</i> at 10 g/kg (Pl-10)	64	67	72	78	320	315
<i>P. chlamydosporia</i> at 5 g/kg (Pc-5)	82	85	92	90	389	360
<i>P. chlamydosporia</i> at 10 g/kg (Pc-10)	74	70	81	85	363	349
Pl-5 + Pc-5	47	49	58	52	274	269
Pl-10 + Pc-10	65	60	69	74	289	301
Untreated	97	96	142	134	476	459
C.D. (P = 0.05)	6.8	7.4	9.3	8.4	35.9	32.8

C.D. = Critical Difference.

Table III. Effects of a combination of *P. chlamydosporia* and *P. lilacinus* on the parasitisation of eggs of *M. javanica*.

Treatment	% eggs parasitised by <i>P. lilacinus</i>		% eggs parasitised by <i>P. chlamydosporia</i>	
	Season 1	Season 2	Season 1	Season 2
<i>P. lilacinus</i> at 5 g/kg (Pl-5)	43.9	45.9	0.0	0.0
<i>P. lilacinus</i> at 10 g/kg (Pl-10)	57.4	66.5	0.0	0.0
<i>P. chlamydosporia</i> at 5 g/kg (Pc-5)	0.0	0.0	51.9	55.8
<i>P. chlamydosporia</i> at 10 g/kg (Pc-10)	0.0	0.0	58.9	55.4
Pl-5 + Pc-5	40.7	42.7	40.8	38.1
Pl-10 + Pc-10	56.9	53.9	30.8	32.9
Untreated	0.0	0.0	0.0	0.0
C.D. (P = 0.05)	9.6	8.4	7.6	8.5

C.D. = Critical Difference.

Table IV. Effect of a combination of *P. chlamydosporia* and *P. lilacinus* on the colonisation of roots of acid lime and propagule density of bio-agents in the soil.

Treatment	Root colonization by <i>P. chlamydosporia</i> (CFU/g)		<i>P. chlamydosporia</i> propagule density in the soil (CFU/g)		Root colonization by <i>P. lilacinus</i> (CFU/g)		<i>P. lilacinus</i> propagule density in the soil (CFU/g)	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
<i>P. lilacinus</i> at 5 g/kg (Pl-5)	0	0	0	0	31678	30176	27975	25432
<i>P. lilacinus</i> at 10 g/kg (Pl-10)	0	0	0	0	34578	35287	29874	27935
<i>P. chlamydosporia</i> at 5 g/kg (Pc-5)	28765	26754	25439	24573	0	0	0	0
<i>P. chlamydosporia</i> at 10 g/kg (Pc-10)	32674	31642	27896	25693	0	0	0	0
Pl-5 + Pc-5	27347	27643	25873	25128	30785	31568	28753	27185
Pl-10 + Pc-10	23879	25678	21784	20864	35687	36843	28796	28549
Untreated	0	0	0	0	0	0	0	0
C.D. (P = 0.05)	2145.9	2268.0	1987.5	1864.9	2598.4	2694.8	2566.6	2388.0

C.D. = Critical Difference.

CFU = Colony Forming Units.

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