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Department of Nematology, CCS Haryana Agricultural University,
Hisar - 125 004, India

NEMATICIDAL EFFICACY OF PLANT LEAVES AND *PAECILOMYCES LILACINUS*, ALONE OR IN COMBINATION, IN CONTROLLING *MELOIDOGYNE INCOGNITA* ON OKRA AND TOMATO

by
R. K. WALIA, S. N. NANDAL and D. S. BHATTI

Summary. In a pot experiment, the addition of chopped fresh leaves of castor, eucalyptus or neem enhanced growth of okra with or without *Meloidogyne incognita* and castor was the best. The addition of *Paecilomyces lilacinus* without plant leaves increased plant dry weight and reduced root galling. However, the effect of the fungus was not discernible in pots in which plant leaves were also incorporated. The impact of the fungus was evident by increased dry shoot weight in the presence of castor leaves only. Among the plant leaves, castor was most effective in suppressing root galling. With eggs as inoculum, plant leaves alone failed to reduce the final juvenile population in soil; however, in combination with the fungus, it was significantly reduced. When juveniles were used as inoculum, the fungus was not effective, while plant leaves reduced their final population to below a detectable level. Fungus recovery at the end of experiment ranged between $1\text{-}3\text{x}10^3$ CFU per g soil when used in combination with castor leaves. In other cases, it was below the detectable level. *P. lilacinus* was not recovered from egg masses in any of the treatments. In a field trial involving the use of castor leaves and *P. lilacinus* on tomato, maximum increase (120% over untreated control) in yield was recorded with the addition of leaves at 6 kg/m^2. The fungus population stabilised around $3\text{x}10^3$ CFU per g soil, but it did not contribute towards enhanced yields either alone or in combination with castor leaves.

The use of *Paecilomyces lilacinus* (Thom) Samson as a biocontrol agent of root-knot nematodes has given successful results in India (Walia *et al.*, 1991). Suppression of *Meloidogyne* species through addition of plant parts or extracts of them is also very effective (Bhatti and Nandal, 1994).

Therefore, it was thought useful to test the effect of nematicidal plant leaves through reduced dosages and integrate them with *P. lilacinus* so that the former could provide the initial organic substrate for the establishment and build-up of the fungus in the soil.

Materials and methods

In a pot experiment conducted at 28±4 °C, fresh leaves of castor (*Ricinus communis* L.), eucalyptus (*Eucalyptus citriodora* Hook.) and neem (*Azadirachta indica* A. Juss.) were chopped and mixed with steam-sterilized sandy soil, 20 g per kg soil. About 1 kg of the amended and unamended soil was filled into 15 cm diam clay pots. About 1800 eggs (obtained by NaOCl treatment, viability 55.5%) or 1000 J2 of *Meloidogyne incognita* (Kofoid *et* White) Chitw. were pipetted into the centre of each pot and

covered with soil. All the pots were watered lightly every day. Wood charcoal powder containing $1x10^9$ spores of *P. lilacinus* (Bansal *et al.*, 1992) was coated onto okra [*Abelmoschus esculentus* (L.) Moench], cv. Pusa Sawani, seeds 20% w/w using gum arabic as a sticker. The coated seeds were dried in sterile air. Ten seeds picked at random were used to estimate the number of spores per seed by a dilution-plating technique on potato dextrose agar. Three coated or uncoated seeds were sown per pot ten days after the addition of leaves and nematodes. Upon germination, only one plant was retained in each pot. There were five replicates for each treatment.

The experiment was terminated 60 days after sowing. Observations were recorded on plant growth parameters and root gall index, 1-10 scale (Walia *et al.*, 1991). The soil from each pot was mixed thoroughly and 100 cm^3 used for the extraction of J2 by wet screening. One g soil sample was collected from each pot separately in a sterile glass vial for the estimation of *P. lilacinus* CFU by a dilution-plating method (Walia *et al.*, 1991). Five egg masses from each plant were removed at random and used for assessing the extent of fungus colonisation (Walia *et al.*, 1991).

Another experiment was conducted at the village of Surjanwas (District Mahendergarh, Haryana) in a field with a sandy soil (sand = 89.2%, silt = 1.6%, clay = 9.2%, pH = 8.8) and initial population of 2.3 J2 of *M. incognita* per g soil. The experiment was arranged in 2x2 m plots in a randomised block design. Treatments in the experiment were: chopped castor leaves alone, 3 or 6 kg/m^2; fungus alone (multiplied on wheat bran as described by Bansal *et al.* (1988), 0.1 or 0.2 kg of wheat bran + fungus/m^2; or the two in combination at lower doses (Table II). Appropriate controls i.e., wheat bran alone, carbofuran (Furadan 3G) at 1 kg a.i. per ha, and untreated plots were also maintained. There were four replicates for each treatment. Required quantities of leaves, wheat bran + fungus or wheat bran alone were mixed manually down to plough depth and all the plots were irrigated lightly. Carbofuran was applied at the time of seedling transplanting.

Six-week-old healthy tomato, *Lycopersicon esculentum* Mill., cv. Pusa Ruby, seedlings were transplanted 15 days after the application of leaves/fungus, 16 seedlings per plot (row to row 45 cms, plant to plant 30 cms) in November. Nitrogen at 30 kg/ha and potash (K_2O_5) at 60 kg/ha were applied at transplanting with the application of nitrogen repeated at 30 and 50 days after transplanting. Data were recorded on yield per plot and after the last picking five plants were randomly uprooted from each plot for recording root gall index (1-10 scale). About 1 kg composite soil sample was collected from each plot and after thorough mixing, 100 cc and 1 g samples were processed for the estimation of final J2 and fungus population in the soil, respectively, as described earlier. Five egg masses from each uprooted plant were detached and assayed for fungus parasitism (Walia *et al.*, 1991).

Results

The results of the pot experiment are presented in Table I. In general, the addition of chopped fresh leaves enhanced growth of okra in the absence (treatment 1 v/s 7, 12, 17; see Table I) or presence of nematodes, whether the nematode inoculum comprised eggs (treatment 2 v/s 8, 13, 18) or juveniles (3 v/s 9, 14, 19). Castor was better than neem (7-11 v/s 12-16) and eucalyptus (12-16 v/s 17-21) in terms of increase in plant dry matter. Neem and eucalyptus were similar as far as shoot growth is concerned, but the latter resulted in better root growth (7-11 v/s 17-21). All the three plant leaf treatments enhanced root development when nematode eggs were inoculated; however, shoot growth was increased only by castor (2 v/s 8, 13 and 18). When juveniles were used as

TABLE I - *Integration of chopped plant leaves and* Paecilomyces lilacinus *for the control of* Meloidogyne incognita *infecting okra.*

Sr. No.	Treatment codes	Dry wt. (g)		Gall Index (1-10 scale)	Final No. J2/100 cm^3 soil
		Shoot	Root		
1	SS only	0.76	0.15	1.0	–
2	NE	0.46	0.14	5.4	710
3	NJ	0.90	0.15	3.2	170
4	NE + PL	1.34	0.23	3.6	1180
5	NJ + PL	1.84	0.31	2.2	250
6	NJ + Sticker	0.60	0.16	4.0	370
7	NL only	1.36	0.26	1.0	–
8	NE + NL	1.08	0.27	3.4	1190
9	NJ + NL	1.20	0.32	1.8	0
10	NE + NL + PL	1.30	0.24	4.6	570
11	NJ + NL + PL	1.94	0.27	1.6	0
12	CL only	2.40	0.33	1.0	–
13	NE + CL	1.80	0.44	2.2	650
14	NJ + CL	1.78	0.27	2.0	0
15	NE + CL + PL	2.34	0.32	2.4	170
16	NJ + CL + PL	2.40	0.34	1.6	0
17	EL only	1.82	0.35	1.0	–
18	NE + EL	1.08	0.32	4.2	710
19	NJ + EL	1.22	0.33	1.6	0
20	NE + EL + PL	1.28	0.34	3.2	490
21	NJ + EL + PL	1.40	0.37	1.6	0
	CV (%)	26	25	25	–
Linear Contrasts (P = 0.05)					
1 v/s Rest		*	*	–	–
1 v/s 7, 12, 17		*	*	–	–
2 v/s 8, 13, 18		*	*	*	NS
3 v/s 9, 14, 19		*	*	*	*
2 v/s 4		*	NS	*	*
3 v/s 5		*	*	*	NS
8, 13, 18 v/s 10, 15, 20		NS	NS	NS	*
9, 14, 19 v/s 11, 16, 21		NS	NS	NS	NS
7-11 v/s 12-16		*	*	NS	*
12-16 v/s 17-21		*	*	NS	*
17-21 v/s 7-11		NS	*	NS	*
8, 9 v/s 10, 11		NS	NS	*	*
13, 14 v/s 15, 16		*	NS	NS	*
18, 19 v/s 20, 21		NS	NS	NS	*
2 v/s 8		NS	*	*	*
2 v/s 13		*	*	*	NS
2 v/s 18		NS	*	*	NS
3 v/s 9		NS	*	*	*
3 v/s 14		NS	*	*	*
3 v/s 19		NS	*	*	*

SS = sterilized soil; NE = nematode eggs; NJ = nematode juveniles; CL = castor leaves; EL = eucalyptus leaves; NL = neem leaves; PL = *Paecilomyces lilacinus*; * significant for P = 0.05.

inoculum, all the three plant leaves enhanced only root growth (3 v/s 9, 14 and 19). Addition of fungus without plant leaves resulted in increased shoot and root growth when inoculated with eggs (2 v/s 4) or juveniles (3 v/s 5). However, the effect of fungus alone was not discernible when plant leaves were also incorporated. This was true for both types of nematode inocula (8, 13, 18 v/s 10, 15, 20 and 9, 14, 19 v/s 11, 16, 21). In combination with individual plant leaves, the impact of the fungus was evident by increased shoot weight in the presence of castor only (13, 14 v/s 15, 16), while with neem (8, 9 v/s 10, 11) and eucalyptus (18, 19 v/s 20, 21), no significant effect was observed.

All the three plant leaf treatments significantly reduced root galling irrespective of the type of nematode inoculum (2 v/s 8, 13, 18 and 3 v/s 9, 14, 19). When eggs were used as inoculum, the root gall index was reduced from 5.4 to 2.2 by castor (13 v/s 2), 3.4 by neem (8 v/s 2) and 4.2 by eucalyptus (18 v/s 2). However, a comparison between the plant leaves revealed no significant differences (7-11 v/s 12-16, 12-16 v/s 17-21, 17-21 v/s 7-11). Fungus alone, without leaves, significantly reduced the root gall index, whether the nematode inoculum was eggs (2 v/s 4) or juveniles (3 v/s 5). However, the effect of fungus on root galling was offset in the presence of plant leaves (8, 13, 18 v/s 10, 15, 20; 9, 14, 19 v/s 11, 16, 21).

The type of the nematode inoculum resulted in contrasting differences in the final juvenile population in soil. Among the treatments where eggs were inoculated, addition of fungus reduced the final nematode population in soil (8, 13, 18 v/s 10, 15, 20). Among the treatments where juveniles were used, the final nematode population was very small even in the absence of plant leaves or fungus. While incorporation of the fungus alone did not reduce it further (3 v/s 5), addition of plant leaves resulted in no recovery of juveniles from the soil (3 v/s 9, 14, 19), thus reducing the final juvenile population to below detectable level (Table I).

Recovery of *P. lilacinus* CFU at the end of experiment was generally below detectable level, except in the treatment where it was used in combination with castor leaves. In this case the fungus CFU ranged between $1\text{-}3 \times 10^3$ per g soil. Egg masses infected with fungus were not detected in any of the treatments.

In the field experiment (Table II), maximum yields were obtained in the treatments with castor leaves, irrespective of the dose and whether used with or without fungus. These treatments were statistically ($P = 0.05$) similar, though maximum yield was obtained with the application of castor leaves, 6 kg/m^2; it amounted to 120% increase over the untreated control. *P. lilacinus* did not enhance yield, either alone or in combination with castor leaves. There were no differences between the treatments with regard to gall index and final nematode population. The final fungus population (CFU per g soil) stabilized around 3×10^3 per g soil.

Discussion

Earlier experiments conducted in our laboratory revealed that the aqueous leaf extracts of neem, eucalyptus and castor are not inhibitory to the growth of *P. lilacinus*. On the contrary, the leaf extracts of them supported the mycelial growth and sporulation of *P. lilacinus in vitro* which augurs well for the integration of these two components in nematode management.

Addition of fresh chopped leaves of the three plant species enhanced plant growth also in the absence of nematodes, which may be attributed to the manurial effect of the organic matter; comparatively, castor was better than the others. Though neem was best in suppressing nematode hatching and causing juvenile mortality, castor was better in the suppression of galling. It indicates that besides water soluble components, the degradation products of castor leaves may be more nematoxic than those of neem and eucalyptus.

Nematode inoculum in field soils may be present as eggs in roots or as free juveniles. The effect of chopped leaves and their degradation products appear to be more detrimental to nematode juveniles than eggs. In our earlier laboratory experiments (unpublished) it was observed that leaf extracts caused more juvenile immobilisation than mortality, and that the immobilised juveniles generally revived after exposure to leaf extracts for 24-48 hrs. In contrast, in the soil system, the concentration of leaf degradation products is expected to increase and maintain itself for several days. Thus longer exposure to leaf extracts in soil might have contributed to greater juvenile mortality. Conversely, when eggs were used as inoculum, the effect of leaf extracts during their 'active' period might have only temporarily arrested hatching, which presumably upon revival led to more root infection and egg mass production.

In the pot experiment, *P. lilacinus* was applied on the seed coat in an inert base i.e., wood charcoal powder. The application of chopped leaves was intended to serve as an organic base to assist in the establishment of the fungus in the soil. However, the density of the fungus at the end of the experiment was below the detectable level in soils treated with neem or eucalyptus. It was only when used in combination with castor, that *P. lilacinus* could be detected at densities of $1\text{-}3\text{x}10^3$ CFU per g soil. The water insoluble products and/or degradation products of castor leaves may be nutritionally better for the growth of *P. lilacinus* than those of neem and eucalyptus. However, despite the incorporation of leaves, egg parasitism was not observed in this experiment. It was, therefore, concluded that an energy source in the form of wheat bran is essential for the initial establishment of the fungus in soil, since *P. lilacinus* has proved effective in previous experiments when applied on wheat bran (Walia *et al.*, 1991) or gram seeds (Zaki and Bhatti, 1990).

TABLE II - *Effect of chopped castor leaves and* P. lilacinus, *alone or in combination, in controlling* M. incognita *on tomato.*

Treatment	Yield (kg/4 m^2)	Gall Index (1-10 scale)	Final J2 population (100 cm^3 soil)	Final fungus population (CFU/g soil)
Castor leaves (3 kg/m^2)	20.2	7.8	312	$0.66\text{x}10^3$
Castor leaves (6 kg/m^2)	29.3	8.5	110	$0.33\text{x}10^3$
WBF (0.1 kg/m^2)	10.8	9.7	173	$3.33\text{x}10^3$
WBF (0.2 kg/m^2)	11.0	10.0	112	$2.66\text{x}10^3$
WB (0.1 kg/m^2)	16.3	9.0	130	0
WB (0.2 kg/m^2)	15.7	8.8	100	$1.33\text{x}10^3$
Castor leaves + WBF (3 + 0.1 kg/m^2)	21.0	8.3	120	$2.33\text{x}10^3$
Castor leaves + WB (3 + 0.1 kg/m^2)	26.7	6.3	148	$0.33\text{x}10^3$
Carbofuran (Furadan 3G) (1 kg a.i./ha)	17.8	10.0	180	$0.66\text{x}10^3$
Untreated control	13.3	10.0	220	$0.66\text{x}10^3$
C.D. (P = 0.05)	10.16	NS	NS	–

WBF = Wheat bran colonised with fungus; WB = Wheat bran alone.

In the field experiment, the fungus was introduced on a wheat bran base, and only castor leaves were used as a soil amendment. The field experiment clearly revealed the manurial effect of castor leaves and a 120% increase in tomato yield was achieved in soil amended with them at 6 kg/m^2. The *P. lilacinus* population stabilized at approximately $3x10^3$ CFU per g soil but nematode population reduction or gall suppression could not be attributed to the fungus. Owino *et al.* (1993) concluded from pot experiments that it is unlikely that the organic additions (castor, marigold, neem) used in their study could stimulate egg parasitism in the field. Our study reflects that *P. lilacinus* is a poor competitor in the natural soil eco-system and perhaps survived as a saprophytic fungus. In the pot experiment steam-sterilized soil was used which failed to elicit the competitive potential of *P. lilacinus*. The fungus may, therefore, require repeated applications to augment its population in order to achieve significant nematode suppression.

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