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BARE ROOT DIP TREATMENT OF TOMATO SEEDLINGS IN CALOPTROPIS OR CASTOR LEAF EXTRACTS MIXED WITH *PAECILOMYCES LILACINUS* SPORES FOR THE MANAGEMENT OF *MELOIDOGYNE INCOGNITA**

by

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Summary. Experiments were conducted for the management of *Meloidogyne incognita* by a bare root dip treatment of tomato seedlings in plant leaf extracts mixed with *Paecilomyces lilacinus* spores. The bare root-dip treatment of tomato seedlings in castor leaf aqueous extract (5 or 10%) mixed with *P. lilacinus* spores significantly increased the plant growth parameters and reduced the root-knot index and nematode population in comparison to the calotropis leaf extract (5 or 10%) + *P. lilacinus* and *P. lilacinus* alone treatments. Castor leaf extract both at 5 and 10% significantly increased mycelial growth, sporulation and propagule density of *P. lilacinus* on roots resulting in increased colonisation of the bio-agent on the roots of tomato offering enhanced biological protection with the consequent effect of increased parasitisation of eggs of *M. incognita.*

Paecilomyces lilacinus has been reported to be an effective biological control agent against the root-knot nematodes (Jatala, 1986; Zaki and Bhatti, 1990). *P. lilacinus* was found to colonise the roots of tomato as an epiphyte (Cabanillas *et al.*, 1988). Recently, Zaki and Bhatti (1990) tried soil amendment with castor leaves and *P. lilacinus* for the management of *M. javanica* on tomato. But large quantities of leaf material and bio-agent inoculum of the fungus are required for acceptable nematode control. The present study explores the possibility of using a bare root dip treatment on tomato seedlings in castor and calotropis leaf extracts mixed with the spores of *P. lilacinus* for the management of *Meloidogyne incognita* under screenhouse conditions.

Materials and methods

Five and 10% extracts of castor (*Ricinus communis* L.) and calotropis [*Calotropis procera* (Ait.) R.Br.] leaves were prepared by blending the fresh (washed) leaves in distilled water and filtering through Whatman filter paper No.1. A fifteen day old culture of *P. lilacinus* (Thom.) Samson (Peruvian isolate) grown on standard potato dextrose agar medium was scraped and comminuted in distilled water with 0.01% Triton X-100 in a Warring blender and the spore concentration was adjusted to 3×10^8 spores/ml of water. Equal quantities of spore suspension and leaf extract were thoroughly mixed to provide a spore density of 1.5×10^8 spores/ml. Roots of 30 day old tomato (*Lyco-*

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persicon esculentum Mill. cv. Arka Vikas) seedlings raised in steriized soil were washed and dipped for 20 minutes in castor/calotropis leaf extracts at 5 and 10% concentrations with or without *P. lilacinus* spores. These seedlings were transplanted in pots containing 2 kg sterilised soil mixed with optimum fertilizer dose. To study the individual effect, roots of tomato seedlings were dipped for 20 minutes in distilled water containing only *P. lilacinus* spores (1.5×10^8 spores/ml). Seedling roots dipped for 20 minutes in distilled water served as a control. Each treatment was replicated ten times. Five days after transplanting, each pot was inoculated with 2000 J_2 of *M. incognita* (Kofoid *et* White) Chitw.

Observations on length and fresh weights of shoot and root, gall index (1-5 scale), nematode population (root+soil), per cent egg masses infected, per cent eggs parasitised and root colonization were recorded 60 days after nematode inoculation. Nematodes from 200 ml soil samples were extracted using a modified Baermann funnel technique (Schindler, 1961). Nematodes in five ml samples (ten replicates) were counted and total nematode population density was estimated. The roots from each plant were stained with acid fuchsin in lactophenol. Nematodes in five g samples were counted and nematode population per plant was estimated. Ten egg masses, collected at random from infected roots, were treated with 4% sodium hypochlorite solution for 2-3 minutes and egg masses were observed for the presence of *P. lilacinus* mycelium to confirm its infection. To assess the colonization of *P. lilacinus*, the root system was carefully washed to remove soil, blotted dry, weighed and cut into small pieces of about 1 cm each. A one g sample of roots was taken at random and crushed in a sterile pestle and mortar. Serial dilutions were made with sterile distilled water and plating was done by using the semi-selective medium developed by Mitchell *et al.* (1987). Fungus was reisolated from the egg masses and eggs and identified on the basis of hyphae, conidiophore morphology and colony colour characteristic to *P. lilacinus*.

A further 10 ml of fresh leaf extracts of castor or calotropis (5 or 10%) were added to 90 ml of PDA medium in a flask and poured into 10 cm diam sterile Petri dishes after autoclaving at 15 lb for 30 minutes. PDA without any leaf extract served as the control. *P. lilacinus* disks (grown on PDA) were kept in the centre of all the Petri dishes. Each treatment was replicated ten times. The Petri dishes were incubated at 26 °C±1 for 20 days and then fungal colony diameter was measured.

Another experiment was conducted to ascertain whether these extracts could support the growth of *P. lilacinus*. Twenty ml of fresh castor or calotropis leaf extracts (5 and 10%) were poured separately into conical flasks and autoclaved at 15 lb for 30 minutes. Leaf extracts in the flasks were inoculated with *P. lilacinus* and incubated at 26 °C±1 for 20 days. Observations on spore density were recorded 20 days after inoculation.

Results and discussion

The data presented in Table I indicate that all treatments were significantly effective in improving plant growth parameters and reducing the root-knot index on tomato when compared to the control. Root dips in leaf extracts alone indicated that calotropis leaf extacts (5 and 10%) were comparatively better than castor leaf extracts in increasing shoot length of tomato and reducing the population of *M. incognita* in root and soil. Recently, Akhtar and Alam (1990) reported bare root-dip treatment of tomato with aqueous extracts of castor leaf to be an effective treatment for the management of root-knot nematodes. However, the effects of these extracts mixed with *P. lilacinus* spores in the present study indicated that 10% castor leaf extract in combination with *P. lilacinus* was significantly more effective compared to all other

TABLE I - *Effect of root-dip in calotropis leaf extract (CaLE) or castor leaf extract (CLE) mixed with* Paecilomyces lilacinus (PL) *spores on growth of tomato infested with* Meloidogyne incognita.

Treatment	Shoot length (cm)	Fresh shoot weight (g)	Root length (cm)	Fresh root weight (g)
CaLE-5%	47.2 bcd	22.7 bc	12.9 d	5.6 cd
CaLE-10%	50.5 bc	23.6 bc	13.2 d	5.9 cd
CLE-5%	44.3 d	20.4 c	14.3 cd	5.2 de
CLE-10%	45.7 cd	22.8 bc	13.4 d	6.0 cd
PL	41.5 e	20.2 c	15.9 bc	5.0 e
CaLE-5%+PL	50.0 bcd	21.2 c	14.3 cd	6.2 bc
CaLE-10%+PL	52.3 b	21.8 c	15.6 bcd	6.0 cd
CLE-5%+PL	53.2 ab	26.7 ab	17.6 b	7.1 b
CLE-5%+PL	59.3 a	28.7 a	20.8 a	8.7 a
Control	32.1 f	14.7 d	7.5 e	4.0 f
C.D. at 5%	5.36	3.56	2.08	0.91

Figures in a column followed by a common letter do not differ significantly according to Duncan's Multiple Range Test.

treatments in increasing shoot length, shoot weight and reducing the gall index.

Different effects of leaf extracts with and without *P. lilacinus* spores were due to differences in the interactive effect of a bioagent with leaf extracts. Studies on the interaction between leaf extracts and the bioagent clearly revealed the additive effect between castor leaf extracts and *P. lilacinus*. Mycelial growth of *P. lilacinus* in PDA + castor leaf extract was significantly more than PDA + calotropis leaf extract and control (Table II). Water soluble compounds of castor leaves incorporated in Czepak Dox medium were reported to enhance the growth of *P. lilacinus* (Anon, 1988). *P. lilacinus* spore production was significantly greater in castor leaf extracts than in calotropis leaf extracts (Table II). These data show that castor leaf extracts enhance the growth of *P. lilacinus*, while calotropis extracts support the growth only to a limited extent. The combined effect of leaf extracts and *P. lilacinus* affected *M. incognita* and significantly reduced the final population densities of nematodes (Table III). Further, significant increase in parasitisation of eggs in castor leaf extract + *P. lilacinus* treatments was due to increased colonisation of the bioagent on tomato roots which appeared as dense growth on the root surface (viewed by microscope) in contrast to the sparse growth of *P. lilacinus* on roots treated with calotropis leaf extract + *P. lilacinus*. There were significantly higher densities of spores (cfu/g root) of *P. lilacinus* on the root surface of tomato treated with castor leaf extract (5 or 10%) + *P. lilacinus* spores in comparison with

TABLE II - *Effect of calatropis leaf extract (CaLE) or castor leaf extract (CLE) on mycelial growth and spore production of* P. lilacinus (PL).

Treatment	Mycelial growth (cm)	Spore production (x 10^6/ml)
P.D.A.	3.16 b	–
CaLE-5%	3.22 b	2.2 c
CaLE-10%	3.42 b	2.4 c
CLE-5%	6.71 a	10.2 b
CLE-10%	7.22 a	13.9 a
C.D. at 5%	0.774	2.54

Figures in a column followed by a common letter do not differ significantly according to Duncan's Multiple Range Test.

TABLE III - *Effect of root-dip in calotropis leaf extract (CaLE) or castor leaf extract (CLE) mixed with* P. lilacinus (PL) *spores on* M. incognita *infesting tomato.*

Treatment	Gall index	Final nematode population (soil+root)	% egg masses parasitised	% eggs infected	Propagule density of *P. lilacinus* (Cfu/g root) (x 10^1)
CaLE-5%	2.6 cd	2478 cd	–	–	–
CaLE-10%	2.3 cde	2247 d	–	–	–
CLE-5%	3.4 b	2849 b	–	–	–
CLE-10%	2.9 c	2683 bc	–	–	–
PL	2.8 cd	2349 d	51 b	54 b	4122 c
CaLE-5%+PL	2.3 cd	1932 e	54 b	55 b	4430 c
CaLE-10% + PL	2.2 ef	1738 ef	55 b	57 b	4497 c
CLE-5%+PL	2.5 cd	1637 f	63 a	65 a	7126 bc
CLE-10%+PL	1.8 f	1396 g	66 a	67 a	7834 a
Control	4.4 a	6749 a	–	–	–
C.D. at 5%	0.32	265.89	6.94	5.7	531.78

Figures in a column followed by a common letter do not differ significantly according to Duncan's Multiple Range Test.

the calotropis leaf extract (5 or 10%) + *P. lilacinus* or *P. lilacinus* alone treatments (Table II).

The root-dip method requires small quantities of castor leaves and *P. lilacinus* inoculum. These studies described the potential utilisation of bare root-dip treatment of tomato seedlings in castor leaf extract mixed with *P. lilacinus* spores for the effective management of *M. incognita* on tomato.

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