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Comparative efficacy of *Paecilomyces lilacinus* (Thom.) Samson and *Verticillium lecanii* (A. Zimmerman) Viegas in combination with botanicals against *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 infecting *Crossandra undulaefolia* L.

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ABSTRACT : Investigations were conducted on the management of the root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 infecting crossandra, by integrating the use of two biocontrol agents *viz.*, *Paecilomyces lilacinus* (Thom.) Samson and *Verticillium lecanii* (A. Zimmerman) Viegas with leaf extracts of castor and neem (5% each) as bare root dip and soil drench. Combinations of *V. lecanii* and *P. lilacinus* (2 x 10^4 spores/ml each) with 5% neem leaf extract resulted in significantly higher plant growth parameters and flower yield. Root gall index was least under *V. lecanii* plus neem leaf extract (5%) combination followed by *V. lecanii* plus castor leaf extract (5%). The per cent egg and egg mass parasitization were higher in *P. lilacinus* than in *V. lecanii* when they were employed singly without any leaf extract combinations. However, integration with castor leaf extract enhanced the fungal parasitization by *P. lilacinus* more than that by *V. lecanii*. Integration with neem leaf extract improved the parasitization by *V. lecanii* more than that by *P. lilacinus*.

KEY WORDS : Bare root dip, castor, crossandra, leaf extracts, *Meloidogyne incognita*, neem, *Paecilomyces lilacinus*, soil drench, *Verticillium lecanii*

A major biotic factor limiting the commercial cultivation of crossandra (*Crossandra undulaefolia* L.) in the four southern states of India *viz.*, Andhra Pradesh, Karnataka, Kerala and Tamil Nadu is root parasitization by the root-knot nematode, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 causing about 25-30% decline in flower yield (Rajendran *et al.*, 1978; Khan and Reddy, 1991). Further concomitant occurrence of root-knot nematode and *Fusarium oxysporum* Schlecht. on crossandra was reported to result in death of plants in the nursery and in the main field (Srinivasan and Muthukrishnan, 1976; Anon., 1990) warranting an eco-friendly and cost effective control technology. Keeping these facts in view, an attempt was made to test the efficacy of two antagonistic fungi, *Paecilomyces lilacinus* (Thom.) Samson and *Verticillium lecanii* (A. Zimmermann) Viegas in combination with castor and neem leaf extracts as bare root dip and soil drench to manage *M. incognita* infecting crossandra.

MATERIALS AND METHODS

Isolates of *P. lilacinus* and *V. lecanii* were separately cultured on paddy grain in 500 ml bottles and incubated at 22 °C. Thirty days after incubation, cultures were washed through 325 mesh sieve with water and the fungal propagules were collected in separate containers. The spores were counted using a haemocytometer. The inoculum was prepared by adjusting the spore concentration to 2 x 10^4 spores/ml of water through serial dilution.

Fresh mature leaves of castor (*Ricinus communis* L.) and neem (*Azadirachta indica* L.) were weighed separately, homogenised in waring blender with water (5 : 100 w/v) and strained through muslin cloth to obtain 5% leaf extract. Similarly, stock solution of fungal spores were mixed with concentrated leaf extracts to make the final spore concentration of 2 x 10^4 spores/ml and leaf extract concentration 5% (w/v).

Crossandra undulaefolia nursery was raised in seed pan containing autoclaved soil and compost mixture (3:1 v/v). A set of earthen pots (30 cm diameter) were prepared for conducting the experiment. Two kg of autoclaved soil-compost mixture (3:1 v/v) was filled in each pot followed by the inoculation of two freshly hatched 2nd stage juveniles (J_2) of *Meloidogyne incognita*/g soil. Controls consisted of pots containing (i) no nematode inoculation and (ii) nematode inoculation.

After forty five days, the seedlings were taken out from the seed pan (nursery), roots were cleared of soil and given a root dip for 30 minutes in different treatments. The root dip treatments included castor leaf extract (CLS); neem leaf extract (NLS); aqueous spore suspension of *P. lilacinus*; aqueous spore suspension of *V. lecanii*; CLS plus *P. lilacinus* spore suspension; CLS plus *V. lecanii* spore suspension; NLS plus *P. lilacinus* spore suspension and NLS plus *V. lecanii* spore suspension.

Observations on plant growth parameters such as plant height, root length, shoot dry weight and root gall index were recorded 90 days after transplantation. The roots were collected from the plants treated with *P. lilacinus, V. lecanii* and their combinations with leaf extracts separately and the number of parasitized and healthy egg masses counted for calculating the per cent egg masses parasitized. Further, the parasitized egg masses from these treatments were treated in 0.2 per cent NaOCl for 2 minutes and the number of eggs parasitized and the total number of eggs/egg mass were counted in order to arrive at percentage of eggs parasitized. The number of juveniles were also recorded per 100 cc soil from each treatment. The data were subjected to analysis of variance according to modified Duncan's multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The application of antagonistic fungi, *P. lilacinus* and *V. lecanii* and leaf extracts individually and in combination as bare root-dip followed by soil-drench, significantly improved the growth characters and flower yield of crossandra compared to *M. incognita* inoculated check (Table 1). Maximum increase in plant height, shoot dry weight, root length and flower yield were recorded under *V. lecanii* plus neem leaf extract treatment. The results indicated that integration of neem leaf extract with *V. lecanii* or *P. lilacinus* enhanced plant growth and flower yield significantly compared to the application of antagonistic fungi individually. However, integration of castor leaf extracts with *P. lilacinus* significantly enhanced root length and flower yield (23.0 and 32.0, respectively) compared to its integration with *V. lecanii* (21.0 and 28.0, respectively). Considering the most stable growth parameter, namely, shoot dry weight, there was 29.4% and 50.5% increase over the individual application of *P. lilacinus* when *P. lilacinus* was integrated with castor and neem leaf extracts, respectively. On the other hand, the shoot dry weight increased by 44.4% and 61.1%, respectively, when *V. lecanii* was integrated with castor and neem leaf extracts, than when they were applied individually. Plant growth was distinctly better when *V. lecanii* was integrated with leaf extracts compared to the integration of *P. lilacinus* and leaf extracts.

The host root infection by *M. incognita* as expressed by root galls and the nematode multiplication rate were significantly reduced by the application of antagonistic fungi and leaf extracts and their combination (Table 2). Root gall index and nematode multiplication rates were least (2.0 and 1.6, respectively) when *V. lecanii* and neem leaf extracts were applied together followed by *V. lecanii* plus castor leaf extract (2.5 and 2.0, respectively) and *P. lilacinus* plus neem leaf extract (2.8 and 2.0, respectively). Although, both the antagonistic fungi and leaf extracts individually reduced the root galls and nematode multiplication rate significantly compared to the inoculated check, the combined applications showed additive effect in reducing the root damage (in terms of root galls) and the nematode multiplication rate. *Paecilomyces lilacinus* performed better than *V. lecanii* in terms of reducing root galls and nematode multiplication rate when applied individually. However, *V. lecanii* when integrated with leaf extracts reduced the root galls and nematode multiplication more effectively compared to the combined application of *P. lilacinus* and leaf extracts.

The per cent fungal parasitization of egg masses and eggs of *M. incognita* was observed to be significantly enhanced by integrating leaf extracts with both the fungi (Table 2). Higher percentage of egg masses and egg parasitization by *P. lilacinus* compared

to *V. lecanii* was observed when they were applied individually and also in combination with castor leaf extract. However, neem leaf extract plus *V. lecanii* enhanced the parasitization of egg masses and eggs more than that by neem leaf extract plus *P. lilacinus*.

Tomato root-dip in aqueous spore suspension of *P. lilacinus* (2-4 x 10^5 spores/ml) for five minutes was reported to significantly control *M. incognita* (Sivakumar and Vidhyasekharan, 1989). The nematode egg parasitization by the fungus was to the tune of 16.59 per cent. Treatment of planting material with spores of *P. lilacinus* has also been reported to have given control of *Radopholus similis* (Cobb, 1893) Thorne, 1949 on banana (Tandingan and Davide, 1986) and *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 on potato (Davide and Zorilla, 1987). Further, *M. incognita* on tomato was managed effectively by integrating botanicals (oil cakes) with *P. lilacinus* as soil amendment (Rao and Reddy, 1992).

Results of the present study indicated that bare root-dip followed by soil drench with a combination of leaf extracts and the antagonistic fungi, *P. lilacinus* and *V. lecanii* gave an additive effect in terms of crossandra plant growth and flower yield and, synergistic effect against *M. incognita* in terms of reduced multiplication rate and enhanced fungal parasitization. Further, *P. lilacinus* was more effective against *M. incognita* compared to *V. lecanii* when employed individually or integrated with castor leaf extract. On the other hand, *V. lecanii* performed better than *P. lilacinus* when integrated with neem leaf extract.

Table 1. Effect of *P. lilacinus* and *V. lecanii* in combinations with botanicals on growth of *C. undulaefolia* infected with *M. incognita*

Treatment	Dose/plant	Shoot length (cm)	Shoot dry wt (g)	Root length (cm)	Flower yield (g/plant)
Check (Inoculated)	-	26.5a	6.0a	17.4a	18.0a
Check (Uninoculated)	-	28.0a	10.0d	20.0b	28.0c
Castor Leaf Extract (CLE)	5%	29.8b	8.0b	20.0b	24.0b
Neem Leaf Extract (NLE)	5%	34.4c	10.0d	23.0c	28.0c
P. lilacinus (PL)	10^4 spores/ml	31.6b	8.5b	21.0b	25.0b
V. lecanii (VL)	10^4 spores/ml	32.1b	9.0c	24.0c	24.0b
PL + CLE	10^4 spores/ml + 5%	39.5d	11.0e	23.0c	32.0d
PL + NLE	10^4 spores/ml + 5%	40.4e	12.8f	24.0c	33.0d
VL + CLE	10^4 spores/ml + 5%	37.4d	13.0f	21.0b	28.0c
VL + NLE	10^4 spores/ml + 5%	43.6f	14.5g	24.4c	38.0e

Data in columns followed by a common letter are not statistically different (P = 0.05) by DMRT

Table 2. Comparative effect of *P. lilacinus* and *V. lecanii* in combination with botanicals on *M. incognita*

Treatment	Dose/plant	RGI*	MF**	Fungal parasitisation (%)	
				Egg masses	Eggs
Check (Inoculated)	-	4.2f	3.8e	-	-
Check (Uninoculated)	-	-	-	-	-
Castor Leaf Extract (CLE)	5%	3.8e	3.2d	-	-
Neem Leaf Extract (NLE)	5%	3.0c	2.8c	-	-
P. lilacinus (PL)	10^4 spores/ml	3.0c	2.6b	13.0b	16.2a
V. lecanii (VL)	10^4 spores/ml	3.4d	3.0c	8.0a	15.4a
PL + CLE	10^4 spores/ml + 5%	2.8c	2.5b	16.2e	20.4c
PL + NLE	10^4 spores/ml + 5%	2.8c	2.0b	15.0d	19.6c
VL + CLE	10^4 spores/ml + 5%	2.5b	2.0b	14.0c	18.0b
VL + NLE	10^4 spores/ml + 5%	2.0a	1.6a	18.0f	29.6d

Data in columns followed by a common letter are not statistically different (P = 0.05) by DMRT

* RGI = Root Gall Index ; ** MF = Multiplication Factor

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