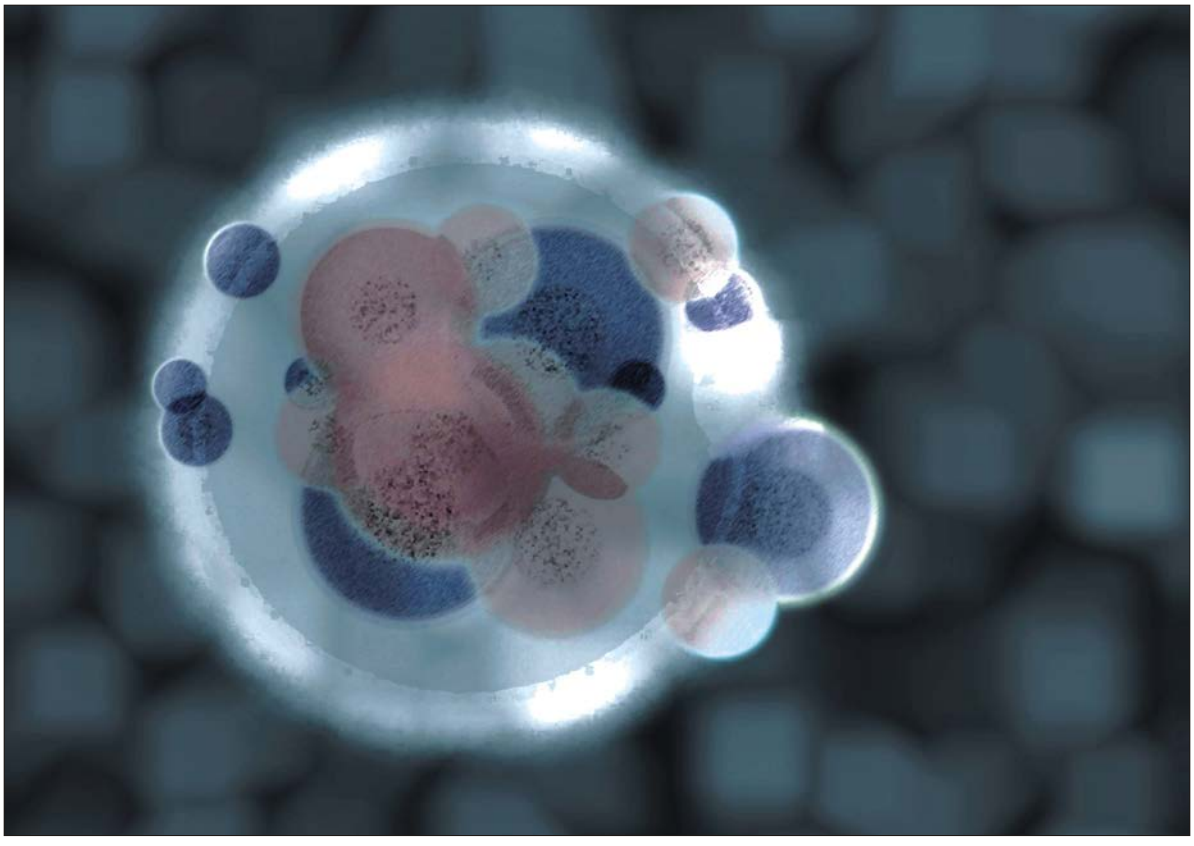

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Evaluation of the Combined Effects of *Paecilomyces lilacinus* and *Trichoderma harzianum* Against Root-knot Disease of Tomato

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Abstract: The addition of *Paecilomyces lilacinus* and *Trichoderma harzianum* as nematophagous fungi separately along with organic substrate to the infested soil, sufficiently retarded the pathogenic activity of *Meloidogyne incognita*. Addition of *Paecilomyces lilacinus and Trichoderma harzianum* in combination amended with organic substrate gave the effective control of root-knot nematodes population thus reduced root-knot disease and increased plant vigour.

Key words: *Paecilomyces lilacinus*, *Trichoderma harzianum*, root-knot, tomato

Introduction

Controlling the soil inhabitant pathogens in a crop are difficult and root-knot disease caused by *Meloidogyne* spp. is no exception to it. Biological control of plant pathogens is a distinct possibility for future and it can be successfully exploited in modern agriculture. Biological control of plant diseases aims at reduction in inoculum density or pathogen activity. The discovery of new biocontrol agents and the demonstration of their value in reducing disease incidence and severity have opened new promising avenues for practical applications in agriculture and for promoting environmental safety (Boland, 1990).

Antibiotic production by bacteria, antagonistic to fungi is well recognized but there have been a limited number of observations of antibiotic production by *P. lilacinus* and *T. harzianum*. Dennis and Webster (1971) and Elad *et al*. (1982) reported that *Trichoderma* spp. are capable of producing either antibiotics and or intracellular lytic enzymes are responsible for antagonism. *Trichoderma harzianum* could antagonize *Meloidogyne incognita* eggs by producing antinematodal compounds that directly affect nematodes or make the roots less attractive and thus limit nematode penetration. Different species of *Aspergillus, penicillium* and *Trichoderma* are known to produce toxins and antibiotics like malformin, hadacidine, gliotoxin, viridin and penicillin (Subramanian, 1964). Shukla and Swarup (1971) obtained lethal effects of culture filtrates of *Sclerotium rolfsii* on *M. incognita larvae*. They visualized the presence of oxalic acid and other inhibitory substances synthesized by *S. rolfsii* in culture filtrate.

Vicente and Acosta (1992) compared the effect of fungus *Paecilomyces lilacinus* (added I week before planting or at planting) with Carbofurn (1x or 2x) on yields of Pepper and on the population levels of *M. incognita* and *Rotylenchulus reniformis*. They reported that more and heavier fruits were obtained from the plants where fungus *P. Lilacinus* was added 1 week before planting as compared to Carbofuran (2x) treated plots than from the check.

Zaki (1994) established the optimum/ effective dose of the biocontrol fungus, *P. lilacinus* against *M. javanica* in tomato. He found that four gram of fungus per kg soil was the optimum dose for effective reduction in the gall index (69%) and second stage juveniles (86%) of *M. javanica* in tomato with an optimum egg mass infection (58%) and egg destruction (66%).

Materials and Methods

The effect of *P. lilacinus* and *T. harzianum* as biocontrol agent against *Meloidogyne incognita* was evaluated on tomato cv. Moneymaker. One-month-old seedlings were used in the experiment. Culture of *P. lilacinus* and *T. harzianum* multiplied on wheat grains were mixed at 6 gm/kg soil with formalin sterilized soil containing 1% organic substrate (wheat bhoosa). The infested soil was filled into earthen pots (20×22 cm). *P. lilacinus* and *T. harzianum* was allowed to establish on the substrate for 15 days. Then one tomato seedling per pot was planted. One week after transplanting, approximately 6000 freshly hatched second stage juveniles of *M. incognita* were pipetted through holes made in the soil around the base of the plant. Each treatment had five replicates. The inoculated and non treated/non inoculated pots served as control. The pots were placed in a completely randomized block design (CRBD) in a glass house, where the temperature during the growth period was kept between 27-34°C. Plants were irrigated regularly. Plant growth and symptoms were observed regularly and recorded. After six week of inoculation, plants with soil were gently removed from pots and their roots were carefully washed in running water. Data on number of leaves/plant, fresh and dry weight of shoots and roots, length of shoot and roots and number of galls and egg masses/plant were recorded. The analysis of variance and individual comparison between treatments was done.

Results and Discussion

It was found that *P. lilacinus* and *T. harzianum* when applied along with organic substrate to the infested soil, suppressed the activity of the pathogen. The maximum reduction in the activity of the pathogen was obtained where antagonistic organisms were used in combination along with substrate. The effect of these antagonistic organisms on different parameters of the plants was also observed. Inoculation of tomato plants with root-knot nematode alone reduced the fresh weight of shoots, dry weight of shoots, fresh and dry weight of roots, height of plants and number of leaves as compared to uninoculated plants.

P. lilacinus alone and in combination with *T. harzianum* resulted into 26.08 and 33.76% increase in plant height respectively over the inoculated plants while 11.77 and

Table 1: Effect of *P. lilacinus and T. harzianum* on the plant height (cm) and number of leaves of tomato plants infected with *M. incognita*

Treatments	Plant height			Number of leaves		
	Mean Plant height (cm)	% increase in plant height over control-I	% incr/decr in plant height over control-II	Mean no. of leaves	% increase in no. of of leaves over control-I	% increase in no. of leaves over control-II
P. lilacinus	67.49a	26.08	11.77	168.20a	42.78	29.18
T. harzianum	59.18bc	10.55	1.99	143.80b	22.07	10.44
P. lilacinus + *T. harzianum*	71.60a	33.76	18.58	179.60a	52.46	37.94
Substrate	56.81bc	6.13	5.91	136.40b	15.79	4.76
Nematode alone (Control-I)	53.53c	-	11.34	117.80c	-	9.52
Uninoculated (Control-II)	60.38b	12.80	-	130.20bc	10.52	-

Figures having same letters do not differ significantly at 5% level of significance.

Table 2: Effect of *P. lilacinus and T. harzianum* on the fresh and dry weight (gm) of shoot of tomato plants infected with *M.incognita*

Treatments	Fresh shoot weight			Dry shoot weight		
	Mean fresh root weight (gm)	% decrease in freash shootwt. Over control-I	% incr/decr in freash shootwt. over control-II	Mean dry roog weight (gm)	% decrease in dry shootover control-I	% incr/decr in dry shootwt. control-II
P. lilacinus	54.52ab	28.86	9.17	18.19b	30.58	4.78
T. harzianum	51.68ab	22.15	3.48	16.25bc	16.65	6.39
P. lilacinus + *T. harzianum*	59.38a	40.35	18.90	23.65a	69.78	36.23
Substrate	47.54bc	12.36	4.80	14.50bc	4.09	16.47
M. incognita alone (Control-I)	42.31c	-	15.28	13.93c	-	19.76
Uninoculated (Control-II)	49.94bc	18.03	-	17.36bc	24.62	

Figures having same letters do not differ significantly at 5% level of significance

Table 3: Effect of *P. lilacinus and T. harzianum* on the fresh and dry weight(gm) of roots of tomato plants infected with *M. incognita*

Treatments	Fresh root weight			Dry root weight		
	Mean fresh root weight (gm)	% decrease in freash root wt. Over control-I	% incr/decr in freash root wt. over control-II	Mean dry roog weight (gm)	% decrease in dry root over control-I	% incr/decr in dry root wt. control-II
P. lilacinus	6.66d	49.51	16.54	2.18d	56.49	30.35
T. harzianum	7.52cd	42.99	5.76	2.92c	41.72	6.71
P. lilacinus + *T. harzianum*	4.60e	65.13	42.35	1.17e	76.65	62.62
Substrate	10.19b	22.74	27.69	4.11b	17.96	31.31
M. incognita alone(Control-I)	13.19a	-	65.29	5.01a	-	60.06
Uninoculated (Control-II)	7.98c	39.50	-	3.13c	37.52	-

Figures having same letters do not differ significantly at 5% level of significance

Table 4: Effect of *P. lilacinus and T. harzianum* on the root length(cm), number of galls and number of egg masses/plant of tomato seedlings infected with *M.incognita*

Treatments	Root length			Number of galls			Number of egg masses		
	Mean	% incre./over control-I	control-II	Mean	% decre/over control-I	control-II	Mean	% decre/over Cont.I	Cont.II
P. lilacinus	35.96a	93.54	62.27	134.60c	62.38	-	97.4d	67.85	-
T. harzianum	30.16b	62.33	36.10	157.60c	55.95	-	130.40c	56.96	-
P. lilacinus + *T. harzianum*	39.13a	110.60	76.58	101.00d	71.77	-	76.00e	74.92	-
Substrate	25.46bc	37.03	14.89	205.80b	42.48	-	185.20b	38.88	-
M. incognita alone (Control-I)	18.58d	-	16.15	357.80a	-	-	303.00a	-	-
Uninoculated (Control-II)	22.16cd	19.27	-	0.00e	-	-	0.00f	-	-

Figures having same letters do not differ significantly at 5% level of significance

18.58% increase respectively over the uninoculated plants. The minimum increase 6.31% in plant height over inoculated plants was achieved where only organic substrate was applied (Table 1). Combination of *P. lilacinus* and *T. harzianum* gave maximum number of leaves (179.6) which is a 52.46% increase in number of leaves over inoculated plants and 37.94% increase over uninoculated plants. The organic substrate had comparatively less effect on the number of leaves as it gave 15.79% increase in number of leaves over inoculated plants (Table 1).

Evaluation of the combined effects with *P. lilacinus* and *T. harzianum* on the fresh weight of shoots of tomato plants revealed that it gave maximum fresh shoot weight over the inoculated plants and caused 18.90% increase in fresh shoot weight over the inoculated plants and caused 18.90% increase over the uninoculated plants. Similarly *P. lilacinus, T. harzianum* and organic substrate also gave an increase in fresh shoot weight by 28.86, 22.15 and 12.36% respectively as compared with inoculated plants (Table 2). Combination of fungi (*P. lilacinus, T. harzianum*) gave minimum fresh root weight (4.60 g) which was 65.13% less as compared with inoculated plants and 42.35% less as compared with uninoculated plants. It was followed by *P. lilacinus* which gave 49.51% less fresh root weight compared to inoculated plants. The organic substrate proved to be the least effective among all the treatments as it gave 22.74% decrease over the inoculated plants. Evaluation of the combined effects of fungi (*P. lilacinus, T. harzianum*) on the dry root weight of tomato plants was similar to that on fresh weight of roots (Table 3). Combination of fungi (*P. lilacinus, T. harzianum*) significantly increased 110.60% root length over the inoculated plants and 76.58 percent increase in root length over the uninoculated plants. It was followed by *P. lilacinus* as it gave 93.54% increase in root length over the inoculated plants. Among the treatments organic substrate proved to be the least effective as it gave 37.03% increase over the inoculated plants.

Combination of fungi (*P. lilacinus, T. harzianum*) significantly decreased 71.77% galls over the inoculated plants while *P. lilacinus* and *T. harzianum* both were effective in reducing number of galls over the inoculated plants and these caused 62.38 and 55.95% reduction in number of galls, respectively.

Evaluation of the effects of *P. lilacinus* and *T. harzianum* on the number of egg masses of tomato plant roots showed that in uninoculated plants where sterilized soil was used, no gall formation and egg masses were observed. The comparison of treatment means indicated that inoculated plants had maximum egg masses and minimum number of egg masses (76.00) were found in combination of fungi (*P. lilacinus* + *T. harzianum*), it gave 74.92 reduction over inoculated plants. Among the treatments organic substrate proved to be the least effective as it gave 38.88% reduction in number of egg masses over inoculated plants (Table 4).

P. lilacinus (Thom) Samson has been reported to be a potential biological control agent against root-knot and other plant parasitic nematodes (Adiko, 1984; Franco *et al*., 1981; Jatala, 1982; Jatala *et al*., 1979). *P. lilacinus* is a common soil Hyphomycete, closely related to *Penicillium* (Samson, 1974). It parasitizes eggs of *Meloidogyne* spp. and *Globodera pallida* (Stone) Behrens. (Dunn *et al*., 1982; Jatala, 1986). This fungus also invades the females or cysts of a number of nematode species (Franco *et al*., 1981; Gintis *et al*., 1983; Jatala, 1982; 1986). It exhibits chitinase activity when grown on chitin agar medium (Gintis *et al*., 1983) and produces a peptidal antibiotic which has wide antimicrobial activity against fungi, yeast and gram-positive bacteria (Isogai *et al*.,1980; 1981). *Paecilomyces lilacinus* colonizes *M. incognita* eggs, preventing them from hatching and leaving fewer Juveniles to penetrate root tissues (Dunn *et al.,* 1982; Jatala, 1986).

It may be indicated on the basis of findings by various workers that nematophagous fungi are capable of producing toxic substances in the presence of suitable substrate in rhizosphere of plants. The presence of high population of such fungi in the rhizosphere where nematode population is also high may help in reducing the deleterious effects of nematodes on plants by suppressing their population.

In our studies where *P. lilacinus* and *T. harzianum* were used separately suppressed the activity of the *M.incognita.* Similar results were reported by Jansson (1980), Cayrol (1978), Kerry (1980) and Mankau (1969). They found that the agents which attack the reproducing adults and their egg masses were more likely to be effective in reducing nematode population than those that attack Juveniles. In treatment where both nematophagous fungi applied in combination gave best results and significantly suppressed the activity and nematode population. These results are in conformity with Cayrol and Frankowski (1979) Matskevich *et al.* (1971), Melendez and Powell (1969), Patel *et al.* (1992), Vicente and Acosta (1992), Pandey and Trivedi (1992), Khan and Khan (1992), Persoon and Friman (1993), Zaki (1994), Zuckerman *et al.,* (1994).

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