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Biological Potential of *Paecilomyces lilacinus* on Pathogenesis of *Meloidogyne javanica* Infecting Tomato Plant

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Abstract: The biocontrol potential of bioagent *viz., Paecilomyces lilacinus* was evaluated *in vitro* conditions against the *Lycopersicon esculentum* root knot nematode *Meloidogyne javanica*. The parameters measured were plant length, fresh weight, dry weight and number of leaves per plant. The number of galls, number of egg masses, infection of eggs and final nematode population was also evaluated. Inoculation of 2000 juveniles of *M. javanica* caused a significant reduction in various plant growth parameters. *Paecilomyces lilacinus* significantly improved the growth of tomato plants inoculated with 2000 juveniles of *M. javanica*. The plant length, fresh weight, dry weight and number of leaves per plant significantly improved whereas, number of galls, egg masses, eggs per eggmass and final nematode population greatly reduced on simultaneous and sequential inoculation of *P. lilacinus* and *M. javanica*. In the present study it was observed that efficacy of *P. lilacinus* significantly varied with inoculation time. The simultaneous inoculation of *P. lilacinus* and *M. javanica* significantly improved plant growth parameters. However, sequential inoculation of *P. lilacinus* ten days prior to *M. javanica* was more effective than sequential inoculation of *M. javanica* ten days prior to *P. lilacinus*. A good percentage of eggs were parasitized by bioagent thereby inhibiting the development of nematodes.

Key words: Biocontrol potential・*P. lilacinus*・*M. javanica*・Egg parasitism・Root galling・Tomato

INTRODUCTION

Tomato (*Lycopersicon esculentum*, Mill.) is of tropical American origin and belongs to family solanaceae. It is grown all over the world because of it's high nutritive value and an excellent source of vitamin C. Like other vegetable crops, tomato supports a complex of pests including nematodes, pathogens, insects and other orthapods. All the 4 major species of *Meloidogyne* and their known races readily attack tomato crop in indoor and outdoor cultivation. Root knot nematodes cause as high as 85% suppression in the yield of tomato [1]. Short life cycle of six to eight weeks enables them to survive well in the presence of a suitable host. In susceptible plants, the nematode population build up to a maximum usually as crop reach maturity [2] and in some cases the plants die even before reaching maturity [3]. *Meloidogyne javanica* is found worldwide in tropical and temperate climates. The root knot nematodes cause extensive damage to a wide range of crops, including many vegetables [4]. Yield losses in tomato caused by root -knot nematode in India range from 40 to 46% [5, 6]. The yield of okra, tomato and brinjal suffered 90.9%, 96.2% and 27.3% loss respectively due to *M. incognita* infestation @3-4 larvae per gram soil under field conditions in India [7]. Losses caused by root knot nematode on chickpea are 31-37% in Pakistan [8].

The control of plant parasitic nematodes is a difficult task, has mainly depended on chemical nematicides for decades and remarkable reduction of nematode population has been achieved [9]. Nematicides have been too expensive for use in developing countries, were their application has been limited to few crops [3]. Due to it's hazardous effects it has led to an increased interest in biological control in it's widest sense, in order to achieve environmentally safe method of reducing the nematode damage [2]. Biocontrol seems to be the most relevant and practically damaging approach for the control of root knot nematodes. Some of the opportunistic bio-control agents like soil hyphomysetes have shown great promise

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[10-14]. *Paecilomyces lilacinus* has been reported to reduce nematode population densities and is considered as one of the most promising and practicable biocontrol agent for the management of plant parasitic nematodes [12, 15]. *Paecilomyces lilacinus* species are listed by Hawaii state quarantine Branch as non restricted microorganism [16]. Data obtained from several countries indicated that this fungus adapts well in varied climatic conditions and is effective in controlling root knot nematodes [17, 9]. Increasing awareness of humankind towards the ecosystem and environment has made a marked shift from synthetic materials to bio-products. Fungi constitute a major group of bioagents against various kinds of pests. A good number of fungi such as *Trichoderma, Gliocladium* and *Paecilomyces* can suppress the parasitism of root knot nematode. In view of the damage caused to crop yield, food value and commercial potential of tomato, which is being grown as crash crop in certain areas of india, the present study was undertaken.

In the present study the efficacy of *Paecilomyces lilacinus* against root knot nematode *Meloidogyne javanica* on tomato under green house conditions has been investigated.

MATERIALS AND METHODS

Fifteen day old tomato (*Lycopersicon esculentum,* Mill.) seedlings raised in sterilized soil were transplanted in six inch clay pots containing autoclaved sandy loam soil. The seedlings were later inoculated with the infective stage of root knot nematode *M. javanica* and bioagent *P. lilacinus. Meloidogyne javanica* were collected from an infected tomato field and multiplied one tomato plant using a single egg mass. Egg masses were handpicked using sterilized forceps and placed in 9 cm diameter sieves of 1 mm pore size, which had been lined with cross-layered tissue paper. The sieves were placed in Petridishes with distilled water for hatching and incubated at 27°C. The bioagent used in the present study were obtained from Division of Mycology, IARI, New Delhi, India. One week old cultures of bioagent maintained on PDA slants at 28±1 were used for the present study. The bioagent was grown on Richard's liquid medium [18] in order to obtain mycelium mat for inoculation purposes. 2000 freshly hatched *M. javanica* juveniles and 2g of *P. lilacinus* mycelium per pot were inoculated. Inoculation of the nematodes and the fungus was done simultaneously as well as sequentially, according to the following experimental design.

1 → Control (without any inoculation).
2 → Inoculated with *M. javanica* alone.
3 → Inoculated with *P. lilacinus* alone.
4 → Inoculated with *M. javanica* and *P. lilacinus* simultaneously.
5 → Inoculated with *M. javanica* followed by *P. lilacinus* after 10 days.
6 → Inoculated with *P. lilacinus* followed by *M. javanica* after 10 days.

All the treatments were replicated three times. After inoculation the pots were left undisturbed in green house. The crop was terminated after 90 days and further study was carried out.

Statistical Analysis: The data obtained in the present study was statistically analyzed by using SPSS software version 16. The treatment means were compared by Duncan's One-Way ANOVA test at appropriate level of significance.

RESULTS AND DISCUSSION

In the present study it was observed that tomato seedling inoculated with 2000 J_2 of *M. javanica* showed significant reduction in plant growth parameters in comparison to control. A significant reduction in plant length, fresh weigh, dry weight and number of leaves was observed. Simultaneous and sequential inoculation of *M. javanica* and *P. lilacinus* resulted in improved plant growth parameters. A significant increase in shoot length, root length, fresh weight of root and shoot, dry weight of shoot and root and number of leaves was observed when compared t o *M. javanica* inoculated plants. Greater increase in root growth of the tomato plants was observed on inoculation of *P. lilacinus* as compared to shoot. Improved plant growth characters by application of *P. lilacinus* in controlling root-knot nematodes was also reported earlier by Walia *et al.* [19], Khan and Goswami [20], Hasan [21]. However, inoculation of *P. lilacinus* alone did not cause significant differences in various plant growth parameters as compared to un-inoculated one. This is because *P. lilacinus* only causes short term disturbances to the soil biota and does not have any long term effect as other bio-agents do [14, 22]. The simultaneous and sequential inoculation of *M. javanica* and *P. lilacinus* significantly reduced the number of galls, egg masses and final nematode population. The simultaneous inoculation of *M. incognita* and *P. lilacinus* showed significant results.

Table 1: Effect of *Paecilomyces lilacinus* on different plant growth parameters of tomato

Treatment	Plant length(cm)		Plant fresh weight (g)		Plant dry weight (g)		
	Shoot	Root	Shoot	Root	Shoot	Root	No. of leaves/plant
Control	58.4 a	26.6 b	37.5 a	15.2 b	6.3 a	3.4 a	67.3 a
M. javanica	37.3 c (36.13)	16.2 e (39.09)	25.7 d (31.46)	8.4 e (44.7)	3.2 d (49.20)	1.2 e (64.70)	43.5 d (35.36)
P. lilacinus	60.2 a (+3.08)	29.5 a (+10.90)	36.5 a (2.66)	16.5 a (+8.55)	6.3 a (0.0)	3.5 a (+2.94)	72.6 a (+7.87)
M. javanica + P. lilacinus	46.6 b (20.2)	18.7 cd (29.6)	31.2b c (16.80)	12.3 d (19.07)	3.9 c (38.09)	2.4 c (29.41)	52.3 c (22.28)
M. javanica → *P lilacinus*	40.7 c (30.3)	17.5 de (34.21)	28.3 cd (24.5)	9.2 e (39.4)	3.7 c (41.26)	1.7 d (50.0)	48.4 cd (28.08)
P. lilacinus → *M. javanica*	49.5 b (15.23)	20.3 c (23.6)	33.4 b (10.93)	13.6 c (10.52)	5.6 b (11.11)	3.0 b (11.76)	58.2 b (13.52)
LSD(P=0.05)	4058	2.04	3.00	1.18	0.46	0.24	5.34
LSD(p=0.01)	6.51	2.90	4.26	1.68	0.65	0.34	7.60

*Figures in parenthesis are percentage reduction over control

Figures in parenthesis are per cent increase/decrease over control

Means not followed by same letters are significantly different according to SPSS one way ANOVA test

Table 2: Effect of *Paecilomyces lilacinus* on nematode index of tomato plant

Treatment	Number of Galls/root	No. of egg masses/root	No of eggs/ egg mass	%. of eggs infected	Nematode population/ pot	Reproduction Factor (Rf=Pf/Pi)
Control	0.0 d	0.0 d	0.0 e	0.0 d	0.0 d	0.0 d
M. javanica	178.7 a	110.5 a	165.3 a	0.0 d	6545.50 a	3.27 a
P. lilacinus	0.0 d	0.0 d	0.0 e	0.0 d	0.0 d	0.0 d
M. javanica + P. lilacinus	75.5 c (57.75)	42.2 c (61.80)	75.6 c (54.26)	65.0 b	2850.20 c (56.45)	1.42 c
M. javanica → *P. lilacinus*	122.5 b (31.44)	73.6 b (33.39)	88.6 b (46.40)	47.0 c	3460.45 b (47.13)	1.73 b
P. lilacinus → *M. javanica*	70.3 c (60.66)	36.5 c (66.96)	61.3 d (62.9)	76.0 a	2535.40 c (61.26)	1.26 c
LSD(P=0.05)	9.76	5.86	8.59	4.05	338.71	0.17
LSD(p=0.01)	13.88	8.33	12.22	5.75	481.76	0.24

Figures in parenthesis are per cent increase/decrease over *M. javanica* inoculated plants

Means not followed by same letters are significantly different according to SPSS one way ANOVA test

However the effect of *P. lilacinus* ten days prior to *M. javanica* was most effective as compared to *P. lilacinus* ten days after the inoculation of *M. javanic*a. The average number of galls and egg masses per plant in case of *M. javanica* alone was 178.7 and 110.5 respectively. On simultaneous inoculation of *M. javanica* and *P. lilacinus* the number of galls and egg masses was reduced to 75.5 and 42.2 per plant respectively. The highest reduction in number of galls and egg masses was 70.3 and 36.5 per plant respectively on sequential inoculation of *P. lilacinus* 10 days prior to *M. javanica.* Therefore, it is right to expect that presence of *P. lilacinus* before the nematode inoculation would offer greater protection to plant. Association of *P. lilacinus* along with *M. incognita* resulted in a significant egg parasitism. 65% eggs were foun parasitized on simultaneous inoculation of *M. javanica* and *P. lilacinus.* However, the infection was more severe (76% egg parasitism) when fungus preceded the nematodes. Our results are in agreement with earlier findings of Santos *et al.* [23] and Carneiro and Gomes [24] who observed the variations of *P. lilacinus* for egg parasitism of *M. incognita*. The plants inoculated with *M. javanica* alone were found to contain a final nematode population of 6545.50 per pot. All the treatments inoculated with *P. lilacinus* significantly reduced the final nematode population. Simultaneous inoculation of *M. javanica* and *P. lilacinus* resulted in 56.45 reduction in final nematode population. The highest reduction of 61.26% in final nematode population was observed in treatments in which *P. lilacinus* preceded *M. javanica* by ten days. However, when *M. javanica* preceded *P. lilacinus* by ten days a reduction of only 47.13% was observed in final nematode population.

The present study concludes that fungus *P. lilacinus* is a potential bio-control agent causing reduction in the number of root knot nematode *M. javanica* and thereby improving the various plant growth parameters. The present study also supports the earlier fact that *Paecilomyces lilacinus* colonizes nematode eggs preventing them from hatching and leaving fewer juveniles to penetrate root tissues [25, 9, 12].

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