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Effect of *Paecilomyces lilacinus* on tomato plants and the management of root knot nematodes

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Abstract

Effect of *Paecilomyces lilacinus* on tomato plant growth and the management of root knot nematodes in tomato was studied. The research work was conducted in Microbiology & Bio-control Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh and in Net-house of Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, during the period from July, 2016 to October, 2017. In net-house pot culture experiment, four different treatments were used viz., T_1: Inoculation of egg masses (10 egg masses/plant) of Nematodes (*Meloidogyne* spp.), T_2: Application of *P. lilacinus,* T_3: Application of egg masses of Nematodes and *P. lilacinus* simultaneously, and T_4: Control (non-treated). Spore suspension (10×10^5 Conc.) of *P. lilacinus* was mixed with the soil before transplantation and *Meloidogyne* spp. was inoculated on three days after transplantation. Application of *P. lilacinus* in soil enhanced the plant growth parameters of tomato plants. Inoculation of *Meloidogyne* spp. reduced plant growth and the reduction was increased with the increase of inoculum density of *Meloidogyne* spp. Maximum plant growth reduction was recorded when *Meloidogyne* spp. was inoculated alone. The maximum plant growth was recorded in case of application of *P. lilacinus* to soil. A high percentage (85%) of egg masses of *Meloidogyne* spp. was infected by *P. lilacinus* when applied together.

Introduction

The tomato belongs to the family *Solanaceae* and the species *Solanum lycopersicum* is a premier vegetable in Bangladesh and are widely cultivated (Lucioli *et al.*, 2014). Regular outbreaks of root-knot nematode disease caused by *Meloidogyne* spp. have occurred in recent years, impacting considerably on both tomato crop yield and quality, and are increasing problem in global tomato production (Wesemael *et. al.*, 2011; Seid *et. al.*, 2015). Management of Root knot nematodes is difficult due to its wide host range. Once the root knot nematode establishes a feeding site, a group of cells known as giant cells, it remains permanently attached at that location within the plant root. An increase in production of plant growth regulators occurs due to esophageal gland secretions from the nematodes, which cause an increase in cell size and division, resulting in gall formation. Nutrient deficiency symptoms may also appear on plants; chlorosis and stunting frequently occur in root knot nematode infested fields. If high populations of root knot nematode occur early in the growing season, the host plant can be killed (Rahim *et al*. 2016). Among more than 80 species of the genus *Meloidogyne*, four important species viz., *M. incognita, M. Javanica*, *M. arenaria and M. hapla* were responsible for at least 90% of all damages as root parasites (Castagnone-Sereno, 2002). Tomato is highly susceptible to infection by the species *M. incognita* and *M. javanica* (Khan *et al.* 1984). Some chemical nematicides (insecticides) are reported to control effectively the root-knot nematodes of tomato. But for killing the pests, nematicide exerts adverse effects on human beings, livestock and other living things, which come in contact directly or indirectly. In this condition, there is a need to use bio-pesticides that are pest specific, nontoxic to humans, less expensive, and safe for the environment. Environmental side effect associated with chemical control and the loss of methyl bromide as a multipurpose soil fumigant have spurred research into nematode control alternatives (Nico *et al*., 2004). Researchers all over the world are engaged in standardizing nematode management strategies by following non-chemical and eco-friendly approaches such as biological control agents to stabilize crop production (Sumathi *et al*., 2006). Several attempts have been made to use antagonistic fungi to control root-knot nematodes.

Biological control is gaining popularity in nematode control, predominantly utilizing the microorganism groups like the fungi and bacteria already present in the soil biota (Crawford and Clardy, 2011). Many microorganisms reveal the capacity to parasitize the egg and juvenile forms and sometimes even adult nematodes. *Paecilomyces lilacinus* is a soil-inhabiting fungus with a wide range of activity against the most important plant parasitic nematodes (Vasanthi & Kumaraswamy, 1999; Brand et. al., 2010)**.** Siddiqui et al. (2000) reported that *P. lilacinus* significantly reduced *Meloidogyne* spp. infection on tomato. Thus, the present investigation was designed to evaluate the effect of *P. lilacinus* on tomato plants and the management of tomato root knot caused by nematodes.

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Materials and Methods

The laboratory work was carried out in Microbiology and Bio-control Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. Host pathogen interaction and management of pathogen studies were conducted in net-house of Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. The experiment was performed during the period from July, 2016 to October, 2017. Tomato seedlings (variety Ratan) were collected from Horticulture Center, Department of Agriculture Extension (DAE), Kewatkhali, Mymensingh.

Culturing *Paecilomyces lilacinus*

P. lilacinus was collected from Microbiology and Bio-control Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh that previously isolated from commercial bio-nematicide supplied by Haychem (Bangladesh) Limited. Potato Dextrose Agar (PDA) media was used to isolate *P. lilacinus* (Monjil and Ahmed, 2017). Purified cultures (from seven days old fungal culture) of the isolated fungi were examined microscopically in order to identify the strain on the basis of their morphological traits and cultural characteristics of the fungi such as mycelium growth, colony texture, spores production and other characteristics (Abubakar *et al*., 2005; Ahmad and Jairajpuri, 1993).

Collection of root knot diseased materials

Possible nematode infested tomato plants were selected by observing above ground symptoms. Selected plants were uprooted by digging with spade and sickle. Caution was taken to get intact root system without leaving any root parts or gall produced by nematodes. Nematode eggs were separated from previously collected heavily galled (knotted) tomato roots in the laboratory. Only well developed light brown and live egg masses were selected for the research work. Nematode identification was performed by using photographic microscope (Carl Zeiss Microscope GmbH, Model Stemi 508), at Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. *Melodogyne* spp. were identified by observing their identifying characters as described by Eisenback and Hirschmann (1981).

Net-house experiment

The soil of the experiment was collected and sun dried for two days. Then the soil was grounded, and large particles and plant debris were removed. Compost was collected from the BAU-farm, Bangladesh Agricultural University, Mymensingh. Soil and compost were composited and mixed well in a ratio of 4:2. The mixture of soil sterilized with formalin 5% solution @ 200 ml /cft soil. Then the formaldehyde added soil was covered with polyethylene sheets and kept for 2 days. After two days the soil was uncovered to release the gas of formalin by spreading the soil mixture. The prepared soil was poured into perforated plastic pot (22 cm height and 18 cm diameter) at the rate of 3 kg soil per pot. Tomato seedlings of 11-12 cm height (28 days old) were planted in each pot previously filled with sterilized soil mixture. Different intercultural operations were done and the crop was monitored regularly.

In net-house pot culture experiment, four different treatments were used as follows:

T_1: Inoculation of egg masses (10 egg-masses/plant) of Nematodes (*Meloidogyne* spp.)

T_2: Application of *Paecilomyces lilacinus* (10×10^5 spores/g dry weight of sample)

T_3: Application of egg masses of Nematodes (*Meloidogyne* spp.) and *Paecilomyces lilacinus* simultaneously.

T_4: Control (non-treated)

Parameters of data collection

Data on different parameters were collected viz., plant height, number of leaves, number of flowers, number of effective flowers (flowers converted to fruits), length of roots, number of gall and dry weight of roots. Plant height and umber of leaves per seedlings were measured after 15, 30, 45 and 60 days of planting. Number of flowers and Number of effective flowers were counted after 30, 45 and 60 days of planting. Flower which produced fruit was considered as effective flower. Two months after potting of seedling, plants were harvested and the length of the roots (cm) was measured. Number of galls in root was counted in order to compare the efficacy of *P. lilacinus* against *Meloidogyne* spp. Dry weight of roots was measured.

Statistical analysis: The experiment was done following Completely Randomized Design (CRD). The data were subjected to analysis of variance (ANOVA) and mean comparison was conducted using the least significant difference (LSD) test at 5% level of probability. Pearson's correlation coefficients were calculated on the plant parameters and nematode indexes. Differences between means were compared.

Results

Effect of *Paecilomyces lilacinus* on plant height and number of leaves per plant in tomato

Tomato plants inoculated with egg masses of *Meloidogyne* spp. (T_1) highly reduced the Plant height (62.67 cm) than Control treatment (76.5 cm) as shown in Table 1. Highest plant height was recorded by *P. lilacinus* (78.16 cm) application followed by simultaneous application of egg masses of *Meloidogyne* spp. and *P. lilacinus* (77.00 cm). Significant difference of plant height was not obtained in the treatments T_2 (*P. lilacinus*), T_3 (Application of egg masses of *Meloidogyne* spp. and *P. lilacinus,* simultaneously) and T_4 (Control). Percent decrease of plant height over control was 18.08% when inoculated with egg masses of *Meloidogyne* spp. (Table 1).

Table 1. Effect of *Paecilomyces lilacinus* on Plant height and Number of leaves per plant

Treatments	Plant height (cm)	% increase(+) or decrease(-) over control	Number of Leaves per plant	% increase(+) or decrease(-) over control
T_1 =Egg masses of Nematodes	62.67 a	-18.08	1.83 a	-59.33
T_2 = *Paecilomyces lilacinus*	78.16 b	+2.17	6.83 d	+51.78
T_3 = Nematodes and *Paecilomyces lilacinus*	77 b	+0.65	6 c	+33.33
T_4 = Control	76.5 b	-	4.5 b	-
Level of significance	**		*	

Figures in a column with same letters do not differ significantly.
* = Significant at 5% level of probability, ** = Significant at 1% level of probability

In case of number of leaves per plant, highest number (6.83) was found by treatment T_2 (Application of *P. lilacinus*) followed by T_3 (Application of egg masses of *Meloidogyne* spp. and *P. lilacinus,* simultaneously) and T_4 (Control). Number of leaves per plant increased over control was 51.78% in T_2 (Application of *P. lilacinus*) and 33.33% in T_3 (Application of egg masses of *Meloidogyne* spp. and *P. lilacinus,* simultaneously). In case of treatment T_1 (Inoculation of egg masses of *Meloidogyne* spp.), 59.33% reduction of number of leaves per plant over control was recorded.

Effect of *P. lilacinus* on number of flowers per plant and number of effective flowers per plant in tomato

Significantly different effect was observed among the treatments (Table 2). Highest number of flowers per plant (25.26) was found in T_2 followed by T_3 (20.32). Application of *P. lilacinus* increased number of flowers per plant over control by 84.92%, and application of egg masses of *Meloidogyne* spp. and *P. lilacinus* simultaneously increased number of flowers per plant over control (48.76%). On the other hand, inoculation of egg masses of *Meloidogyne* spp. (T_1) highly decreased number of flowers per plant over control (37.45%). Almost similar results was obtained for the parameter on number of effective flowers per plant in tomato. Highest number of effective flowers per plant (14.66) was found in T_2 followed by T_3 (12.66). Application of *P. lilacinus* increased number of flowers per plant over control by 83.25%, and application of egg masses of *Meloidogyne* spp. and *P. lilacinus* simultaneously increased number of flowers per plant over control by 58.25%. On the other hand, inoculation of egg masses of *Meloidogyne* spp. (T_1) highly decreased number of flowers per plant over control (41.75%).

Table 2. Effect of *Paecilomyces lilacinus* on number of flowers per plant and number of effective flowers per plant in tomato

Treatments	Number of flowers per plant	% increase(+) or decrease(-) over control	Number of effective flowers per plant	% increase(+) or decrease(-) over control
T_1 =Egg masses of Nematodes	8.49 **a**	-37.45	4.66 **a**	-41.75
T_2 = *Paecilomyces lilacinus*	25.26 **d**	84.92	14.66 **d**	83.25
T_3 = Nematodes and *Paecilomyces lilacinus*	20.32 **c**	48.76	12.66 **c**	58.25
T_4 = Control	13.66 **b**	-	8 **b**	-
Level of significance	*		*	

Figures in a column with same letters do not differ significantly.
* = Significant at 5% level of probability

Effect of *P. lilacinus* and other treatments on dry weight of root per plant and root length per plant in tomato

Comparative efficacy of *P. lilacinus* and other treatments on dry weight of root per plant and root length per plant in tomato was recorded (Table 3). Highest amount of dry weight of root per plant (1.04 g) was found in treatment T_2 followed by T_3 (0.93 g). Application of *P. lilacinus* increased amount of dry weight of root per plant over control was 85.71%, and application of egg masses of *Meloidogyne* spp. and *P. lilacinus* simultaneously increased amount of dry weight of root per plant over control was 66.07%. On the other hand, Inoculation of egg masses of *Meloidogyne* spp. (T_1) highly decreased Number of flowers per plant over control (23.21%).

In case of root length per plant, highest root length per plant (7.00 cm) was recorded in T_2 followed by T_3 (6.00). Application of *P. lilacinus* increased root length per plant over control by 55.55%, and application of egg masses of *Meloidogyne* spp. and *P. lilacinus* simultaneously increased root length per plant over control by 33.33%. On the other hand, inoculation of egg masses of *Meloidogyne* spp. (T_1) highly decreased number of flowers per plant over control (11.11%).

Table 3. Effect of *Paecilomyces lilacinus* on dry weight of root per plant and root length per plant

Treatments	Dry weight of root per plant (g)	% increase(+) or decrease(-) over control	Root length per plant (cm)	% increase(+) or decrease(-) over control
T_1 =Egg masses of Nematodes	0.43	-23.21	4.00	-11.11
T_2 = *Paecilomyces lilacinus*	1.04	+85.71	7.00	+55.55
T_3 = Nematodes and *Paecilomyces lilacinus*	0.93	+66.07	6.00	+33.33
T_4 = Control	0.56	-	4.50	-

Effect of *P. lilacinus* and other treatments on number of rootlets per plant and number of galls per plant in tomato

Comparative efficacy of *P. lilacinus* and other treatments on number of rootlets per plant and number of galls per plant in tomato was recorded (Table 4). Highest amount of number of rootlets per plant (95) was found in treatment T_1 followed by T_3 (75). Application of *Meloidogyne* spp. increased number of rootlets per plant over control was 42.31%, and application of egg masses of *Meloidogyne* spp. and *P. lilacinus* simultaneously increased number of rootlets per plant over control by 44.23%. On the other hand, inoculation of egg masses of *P. lilacinus* (T_2) decreased number of rootlets per plant over control (23.21%). In case of number of galls per plant, highest number of galls per plant (54.00) was observed in T_1 and lowest Number of galls per plant (54.00) was recorded in T_3. Gall formation in root system was not found in T_2 and T_4 treatments.

Table 4. Effect of *Paecilomyces lilacinus* on number of rootlets per plant and number of galls per plant

Treatments	Number of rootlets per plant	% increase(+) or decrease (-) over control	Number of galls per plant
T_1 =Egg masses of Nematodes	95	+82.69	54
T_2 = *Paecilomyces lilacinus*	30	-42.31	0
T_3 = Nematodes and *Paecilomyces lilacinus*	75	+44.23	8
T_4 = Control	52	-	0

Discussion

The results of the present experiment indicated that application of *P. lilacinus* suppressed *Meloidogyne* spp. and increased plant growth parameters. In the present study, it was observed that tomato plants treated with *P. lilacinus* showed significant variation in plant growth parameters in comparison to control. A significant increase in plant shoots and root length, number of flowers and effective flowers, dry weight of roots was observed (Table 1~3). Improved plant growth characters by application of *P. lilacinus* in controlling root knot nematodes were also reported earlier by Khan and Goswami (2000). They tested the effects of *P. lilacinus* on *Meloidogyne* spp. of tomato and observed that highest root length and shoot length, fresh weight and dry weight of root and shoot were achieved when plants were inoculated with *P. lilacinus* to control root knot nematode. Banana plants treated with *P. lilacinus* significantly increase plant height, pseudostem girth, number of leaves, leaf area, shoot weight, root length and weight (Jonathan and Rajendran, 2000).

It was observed that *Meloidogyne* spp. retarded the growth and reduced the fresh and dry weight of the tomato plants. Apparently *P. lilacinus* showed effectiveness in suppressing *Meloidogyne* spp.. Khan *et al.* (2012) recorded an enhancement in growth and yield of eggplant with bio-control agents *Pochonia chlamydosporia, Paecilomyces lilacinus,* and *Trichoderma harzianum* by the suppression of galls formation. Jonathan and Rajendran (2000) reported that in banana significant reduction was observed in root gall index, egg masses, eggs per egg mass, females and soil population of the nematode treated with *P. lilacinus* inoculated neem cake. *P lilacinus* treated root samples showed many empty eggshells and an abundance of hypae were present endogenously in the eggs. Similar observations were reported on potato and betel vine due to *P. lilacinus* against *M. incognita* (Jonathan *et. al*., 1995).

Although the isolates of *P. lilacinus* show some potential as a bio-control agent, the study must be continued to increase its efficacy. Monjil and Ahmed (2017) found the fungus attacked eggs of *Meloidogyne* spp. and inhibit the nematode hatching from the egg masses. *P. lilacinus* caused substantial egg deformation in *M. incognita*, these deformed eggs never matured or hatched (Jatala *et al*., 1985). The serine protease produced by *P. lilacinus* might play a role in penetration of the fungus through the egg shell of the nematode (Bonants *et al*., 1995). Therefore, application of *P. lilacinus* before the nematode attack would offer greater protection to plants without damaging plant roots. *P. lilacinus* attacked and destroyed egg masses and juveniles of *Meloidogyne* spp. resulting improved plant growth.

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