IMPACT OF *PAECILOMYCES LILACINUS* APPLICATION TIME ON PLANT GROWTH AND SUPPRESSION OF ROOT KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) IN SOME SELECTED VEGETABLES

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CERTIFICATE

This is to certify that thesis entitled, **"IMPACT OF *Paecilomyces lilacinus* APPLICATION TIME ON PLANT GROWTH AND SUPPRESSION OF ROOT KNOT NEMATODE (*Meloidogyne incognita*) IN SOME SELECTED VEGETABLES"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY,** embodies the result of a piece of *bona fide* research work carried out by **Afroga Islam Mitu, Registration No. 06-02150** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

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Dated: 05.05.2013

Place: Dhaka, Bangladesh

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The Author

Impact of *Paecilomyces lilacinus* application time on plant growth and suppression of root knot nematode *(Meloidogyne incognita*) in some selected vegetables

BY

Afroga Islam Mitu

ABSTRACT

Experiments were carried out to study the effect of *Paecilomyces lilacinus* application time on plant growth and suppression of root knot nematode, *Meloidogyne incognita* in brinjal (var. Singnath and Khotkhotia), tomato (var. BARI Tomato 14) and cucumber (var. Kashinda) in shade house condition. There were seven treatments used in the experiment which were- BC (Blank control), M_P (Inoculation of *M. incognita* at planting), PL_P (Application of *P. lilacinus* at planting), PL_P+M_P (Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting), M_PPL_{7DAP} (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting), PL_PM_{7DAP} (Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting), $PL_{7DBP}M_P$ (Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting). *P. lilacinus* was applied @ 36×10^7spore/plant by mixing with the pot soil where *M. incognita* was inoculated @ 10,000 eggs/plant. Two months after transplantation, the data on plant growth parameters, root knot index (0-10 scale), nematode reproduction and fungus colonization was recorded. In brinjal variety Singnath, the shoot length (45.35cm) and dry weight of shoot (6.58g) were highest in PL_P+M_P. The fresh weight of root (21.44g), dry weight of root (10.4g) were highest in PL_PM_{7DAP}. The shoot length (34.13cm), fresh weight of shoot (23.79g), dry weight of shoot (3.95g), root length (19.16cm), fresh weight of root (10.60g), dry weight of root (7.55g) were lowest in M_P. The gall index, egg masses/root, eggs/egg mass and reproduction factor of the treatment PL_P+M_P were reduced 77.38, 88.29, 68.73 and 86.29% over M_P in brinjal variety singnath. Similar type of results were also recorded in brinjal variety khotkhotia, tomato variety BARI Tomato 14 and cucumber variety Kashinda. The treatment $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting) was not as good as simultaneous inoculation and sequential inoculation. Soil colonization ability of *P. lilacinus* is greatly reduced in absence of plant or nematode host during the pre-planting application time.

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LIST OF ABBREVIATED TERMS

ABBREVIATION	FULL WORD
et al.	And others
BARI	Bangladesh Agricultural Research Institute
BADC	Bangladesh Agricultural Development Corporation
Cm^3	Centimeter cube
CV.	Cultivar
^0C	Degree centigrade
Etc.	Etcetera
Ed.	Edited
Eds.	Edition
G	Gram
J.	Journal
No.	Number
PDA	Potato Dextrose Agar
LSD	Least Significant Difference
DMRT	Duncan's New Multiple Range Test
%	Percent
RCBD	Randomized Completely Block Design
R	Replication
Res.	Research
SAU	Sher-e-Bangla Agricultural University
Viz.	Namely
Var.	Variety

INTRODUCTION

Brinjal, tomato and cucumber are considered as most favorable vegetables in our food habit. These are important and most common popular vegetable grown in Bangladesh and being consumed as cooked vegetable in various ways. It is largely cultivated in almost all district of Bangladesh. But incidence of diseases and insect pests greatly hampered the production of these vegetables in Bangladesh.

Among the disease root knot caused by *Meloidogyne javanica* and *Meloidogyne incognita* is a destructive disease of brinjal, cucumber and tomato in Bangladesh causing enormous crop loss. The yield loss of brinjal caused by root knot disease is estimated as 27.2% in the country (Bari, 2001). Estimation of crop loss in the tropics due to root knot nematodes ranged from 17-20% on brinjal, 18-33% on melon and 24-38% on tomato (Subarshan and Chakraborty, 2001).

Among the plant-parasitic nematodes, root knot nematodes (*Meloidogyne* spp.) are worldwide in distribution and attack many economically important crops (Sasser, 1979). The damage caused by root knot nematode is much higher in tropical and subtropical countries (Taylor and Sasser, 1978). Although over 90 species of *Meloidogyne* have been described todate, four species, *viz. M. incognita, M. javanica, M. arenaria* and *M. hapla* are of particular economic importance to vegetable production (Taylor and Sasser, 1978). *M. incognita* is more dominant, accounting for approximately 64 per cent of the total population of the root knot nematodes occurring in tropical countries (Sasser, 1979).

Control of root-knot nematodes has been primarily accomplished through chemical nematicides (Widmer and Abawi, 2000). Chemical control methods have been successful, but have initiated problems related to the pollution hazards involved in their manufacturing and residues left in the consumable parts of the treated plants (Fassuliotis, 1979).

However, due to the significant drawbacks of the chemical control including threats to human health and the environment, biological control has become one of the promising alternatives (Stirling, 1991). Bio-control seems to be the most relevant and practically demanding approach for the control of root knot nematodes. 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. Among these fungi *Paecilomyces lilacinus* shows the most opportunistic performance against root knot nematode.

Some of the opportunistic bio control agents like soil hyphomycetes have shown great promise (Alamgir *et al.*, 1997; Hooper and Evans, 1993; Jatala, 1985; Jatala *et al.*, 1979; Kerry and De Leij, 1992). *Paecilomyces lilacinus* has been reported to reduce nematode population densities and is considered as one of the most promising and practicable bio control agent for the management of plant parasitic nematodes (Jatala, 1985). *Paecilomyces lilacinus* species are listed by Hawaii state quarantine branch as non restricted microorganisms (Schenck, 2004). Data obtained from several countries indicated that this fungus adapts well in varied climatic conditions and is effective in controlling root knot nematodes (Holland *et al.*, 2001; Jalata, 1986).

Various mechanisms of action have been suggested for the biological activity of *P. lilacinus* against plant-parasitic nematodes. The main mechanism of action is direct infection of sedentary stages in particular the egg stage. The production of leucinotoxins, chitinases, proteases and acetic acid by *P. lilacinus* has been associated with the infection process (Djian *et al.*, 1991; Khan *et al.*, 2003; Khan *et al.*, 2004; Park *et al.*, 2004).

Paecilomyces lilacinus is an opportunistic bio control agent and a facultative pathogen of eggs of root knot and cyst nematodes (Kiewnick, 2009). The effectiveness of formulated product containing spores of the naturally occurring fungus *Paecilomyces lilacinus*, strain 251, was evaluated against root-knot nematodes in pot and green house and decrease of second-stage juveniles hatching from eggs was recorded by using the bio-nematicide. This fungus was most effective when the fungus and the nematode were inoculated simultaneously or the fungus preceded the nematode in sequential inoculation (Esfahani and Ansaripour, 2006). The efficacy of bionematicide *Paecilomyces lilacinus* strain 251 toward the burrowing nematode *Radopholus similis* in banana was determined and the highest level of disease suppression was obtained with $6x10^6$ cfu/g dry soil of *P. lilacinus* applied to the soil three times; 6 days before planting, at planting and a plantlet drench. Therefore time of application of *P. lilacinus* has got more attention to obtain sufficient biocontrol efficacy of bioagent against plant parasitic nematodes.

In the present study, the efficacy of *Paecilomyces lilacinus* application in different time against root knot nematode *Meloidogyne incognita* on these vegetables under shade house conditions was investigated with the following objectives

1. To examine the effect of application time of *Paecilomyces lilacinus* on root knot (*Meloidogyne incognita*) and growth parameters of brinjal.
2. To evaluate the effect of application time of *Paecilomyces lilacinus* on root knot (*Meloidogyne incognita*) and growth parameters of tomato.
3. To determine the effect of application time of *Paecilomyces lilacinus* on root knot (*Meloidogyne incognita*) and growth parameters of cucumber.

REVIEW OF LITERATURE

For the control of phytonematodes, chemical control still remains as one of the most practical methods in terms of immediate results. Thomsason (1987) has pointed out that the environmental risks with nematicides warrant the development of safe options as many of these chemicals are proven to be carcinogenous, build up residues in food plants and infiltrate into ground water (Zukerman and Esnard, 1994). Some of these chemicals are equally hazardous to livestock, plant and also to the beneficial fauna and flora of the soil.

More than 100 bioagents belonging to fungi, bacteria, viruses, nematodes, protozoans, etc. are reported world over in last five decades. Among them bio-control fungus are effectively control root knot nematode. Jatala *et al.* (1979) claimed the most practical and promising results for the fungus *Paecilomyces lilacinus*.

2.1. Effect of *Paecilomyces lilacinus* application time on brinjal

Zaki and Maqbool (1990) conducted an experiment to find out the effect of *Pasteuria penetrans* and *Paecilomyces lilacinus* on the control of root-knot nematodes of brinjal and mung. Application of *Pasteuria penetrans* and *Paecilomyces lilacinus* enhanced plant growth parameters such as shoot and root weight and length in brinjal and significantly reduced root-knot indices both on brinjal and mung when these organisms were used individually or in combination with each other. Weight of brinjal shoots was increased by 94% with *Pasteuria penetrans*, 170% with *Paecilomyces lilacinus* alone and by 230% where *P. penetrans* was used in combination with *P. lilacinus*.

Rao and Reddy (2001) conducted an experiment to standardise a strategy of integrated management of the root-knot nematode, *Meloidogyne incognita,* on egg plant under field conditions by a combination of the endomycorrhiza *Glomus mossaea,* the fungus

Paecilomyces lilacinus and neem cake *(Azadirachta indica).* Nursery beds infested with *M. incognita* were amended with neem cake two weeks before the incorporation of *G. mosseae* or *P. lilacinus,* or both. Healthy and vigorous seedlings colonised with endomycorrhiza as well as the biocontrol fungus were obtained for transplanting. Transplants obtained from the nursery beds treated with neem cake + *G. mosseae* + *P. lilacinus* were least infected in the field. The parasitization of eggs of root-knot nematode was significantly increased by *P. lilacinus* and the transplants yield significantly more fruit. Neem cake amendrnent in the nursery beds played a positive role in increasing the colonization of endomycorrhiza and the biocontrol fungus on the roots of transplants before and after transplanting. The combined effect of these three components facilitated the sustainable management of *M. incognita* on egg plant under field conditions.

Vyas *et al.* (2009) planned an integrated experiment to manage the root-knot nematode economically. Result of three years trials indicated that application of *Paecilomyces lilacinus* @25 kg spore dust with carrier/ha (10^9 conidia/g) at the time of transplanting+ poultry manure @10 tons/ha (a week prior to transplanting) or mustard cake @ 2 tons/ha (a week prior to transplanting) or *P. lilacinus* @ 25 kg spore dust with carrier/ha (10^9 conidia/g) at the time of transplanting + neem cake @ 2 tons/ha, a week prior to transplanting or *P. lilacinus* @ 25 kg spore dust with carrier/ha (10^9 conidia/g) at the time of transplanting + carbofuran 3G @ 2 kg/ha in two equal splits one at the time of transplanting and the other after 2.5 months improved plant growth and considerably reduced gall index and also gave higher brinjal fruit yield over control.

Abbas *et al.* (2011) assessed the efficacy of bioagent (*Paecilomyces lilacinus*) and the bioproduct (Radiant) in various combinations on the reproduction of *Meloidogyne incognita* on eggplant. The influence of *P. lilacinus* and Radiant was determined on egg hatching and second stage juvenile (J_2) mortality under in vitro conditions. The concentrations of 1% and 100% of Radiant and *P. lilacinus* respectively both alone and in combined application caused significant mortality and reduction in egg hatching at all

time intervals. The interaction of *P. lilacinus* and Radiant was determined individually, concomitantly, and sequentially on reproduction of *M. incognita* on eggplant under greenhouse. The reproduction of *M. incognita* was significantly reduced in the concomitant treatment consisting of both *P. lilacinus* and Radiant followed by sequential and individual treatment of Radiant and the plant growth parameters incresed significantly. Their findings suggest that *P. lilacinus* and Radiant have the ability to regulate nematode population and may serve as nematicides.

Usman and Siddiqui (2012) conducted a glasshouse experiment to control root-knot nematode, *M. incognita* of eggplant. Two biocontrol fungal strains of *Trichoderma harzianum* and *Paecilomyces lilacinus* were used at 1g/pot and 2g/pot. Inoculation of fungus was done simultaneously along with 1000 second stage juveniles (J_2) of *M. incognita.* Strains of *T. harzianum* were found to be most effective when treated at 2g/pot. *P. lilacinus* also gave almost similar results and enhanced all plant growth characters with the reduction in the root- knot infestation.

2.2. Effect of *Paecilomyces lilacinus* application time on tomato

Ibrahim *et al.* (1987) examined the effectiveness of the fungus *P. lilacinus* and the nematicide aldicarb (Temik 10G) against *M. incognita* on tomato. *P. lilacinus* reduced root galling and egg masses by 66 and 81%, respectively, whereas aldicarb reduced root galling and egg masses by 68 and 60%, respectively.

Cabanillas and Barker (1989) was conducted a microplot trial to evaluate the effects of inoculum level and time of application of *Paecilomyces lilacinus* on the protection of tomato against *Meloidogyne incognita*. The best protection against *M. incognita* was attained with 10 and 20 g of fungus-infested wheat kernels per microplot which resulted in a threefold and fourfold increase in tomato yield. Greatest protection against this

pathogen was attained when *P. lilacinus* was delivered into soil 10 days before planting and again at planting.

Regina *et al*. (1991) applied five dose of a commercial product of *Paecilomyces lilacinus* from eggs of *Meloidogyne incognita* isolated in a powder formulation (10^{11} spores/g of product) in a glasshouse pot experiment against large infestations of *Meloidogyne arenaria*. The trial was conducted over eleven months on three successive tomato crops, cv. Saint pierre. Results showed that the number of fungal propagules in the soil was correlated to the initial dose applied and decreased progressively through the time with increased dose. Populations of *M. arenaria* were significantly reduced by the fungus at 10 and 100 g of spores/m^2 in the second and third generations.

Al-Raddad (1995) tested the effects of *Glomus mosseae* and *Paecilomyces lilacinus* on *Meloidogyne javanica* of tomato in a greenhouse experiment. Chicken layer manure was used as a carrier substrate for the inoculum of *P. lilacinus*. The following parameters were used: gall index, average number of galls per root system, plant height, shoot and root weights. Inoculation of tomato plants with *G. mosseae* did not markedly increase the growth of infected plants with *M. javanica*. Inoculation of plants with *G. mosseae* and *P. lilacinus* together or separately resulted in similar shoots and plant heights. The highest root development was achieved when mycorrhizal plants were inoculated with *P. lilacinus* to control root-knot nematode. Inoculation of tomato plants with *G. mosseae* suppressed gall index and the average number of galls per root system by 52 and 66%, respectively, compared with seedlings inoculated with *M. javanica* alone. Biological control with both *G. mosseae* and *P. lilacinus* together or separately in the presence of layer manure completely inhibited root infection by *M. javanica*. Mycorrhizal colonization was not affected by the layer manure treatment or by root inoculation with *P. lilacinus*.

Kiewnick and Sikora (2003) conducted a dose response experiments with the root-knot nematode *Meloidogyne incognita* on tomatoes using the new WDG formulation of *Paecilomyces lilacinus*. The results revealed a clear correlation between rate applied and the degree of control concerning the reduction in damage to the root and multiplication of the nematode. Best control was achieved by applying the biological nematicide at rates of 2 to 4 times 10^9 conidia per plant as a soil treatment one week before planting. Monitoring the *P. lilacinus* population in the rhizosphere showed a decline after 2 to 3 month which can lead to insufficient control over a full growing season. Repeated application to maintain the antagonist population at a sufficient level could be used to secure long term control of root-knot nematodes.

Kiewnick and Sikora (2004) conducted a greenhouse experiments with the root knot nematodes *Meloidogyne incognita* and *M. hapla* on tomato. *P. lilacinus*, formulated as WG (BIOACT WG), was incorporated into soil inoculated with root-knot nematode eggs prior to transplanting the susceptible tomato cultivar "Hellfrucht". Furthermore, soil treatments were combined with seedling treatments 24 hours before transplanting and a soil drench two weeks after planting, respectively. Seedling and post planting treatment was also combined with a soil treatment at planting. All single or combination treatments tested decreased the gall index and the number of egg masses compared to the untreated control 12 weeks after planting. It could be demonstrated that the above mentioned combination of pre-planting application plus the seedling and one post plant drench gave the best control and resulted in a significant fruit yield increase in concurrence with a decrease in number of galls per root.

Alamgir *et al.* (2006) assayed the common soil inhabiting nematophagous fungus *Paecilomyces lilacinus* (Thom) Samson and the nematode trapping fungus *Monacrosporium lysipagum* (Drechsler) Subram for their ability to reduce the populations of three economically important plant-parasitic nematodes in pot trials. The fungi were tested individually and in combination against the root-knot nematode *Meloidogyne javanica* (Treub) Chitwood, cereal cyst nematode *Heterodera avenae*

Wollenweber, or burrowing nematode *Radopholus similis* (Cobb) Thorne on tomato, barley and tissue cultured banana plants, respectively. In all cases, nematode populations were controlled substantially by both individual and combined applications of the fungi. Combined application of *P. lilacinus* and *M. lysipagum* reduced 62% of galls and 94% of *M. javanica* juveniles on tomato when compared to the experiment with no fungi added. Sixty five percent of *H. avenae* cysts were reduced on barley by combined application of fungi. Control of *R. similis* on banana, both in the roots and in the soil, was greatest when *M. lysipagum* was applied alone (86%) or in combination with *P. lilacinus* (96%), using a strategy where the fungi were inoculated twice in 18 weeks growth period. Overall, combined application of *P. lilacinus* and *M. lysipagum* was the most effective treatment in controlling nematode populations, although in some cases *M. lysipagum* alone was as effective as the combined application of fungi, particularly against *M. javanica*.

Bijoy *et al.* (2006) were carried out an experiments to study the effect of two fungal bioagents along with mustard oil cake and furadan against root knot nematode *Meloidogyne incognita* infecting tomato under greenhouse condition. Bioagents viz., *Paecilomyces lilacinus* and *Trichoderma viride* alone or in combination with mustard oil cake and furadan promoted plant growth, reduced number of galls/plant, egg masses/root system and eggs/egg mass. The fungal bioagents along with mustard oil cake and nematicide showed least nematodes reproduction factor as compared to untreated infested soil.

Esfahani and Ansaripour (2006) evaluated *Paecilomyces lilacinus* against root-knot nematode, *Meloidogyne javanica* of tomato in greenhouse condition. *P. lilacinus*, effectively promoted the growth of plants inoculated with *M. javanica* by suppressing its pathogenesis as root galling by the nematode and egg mass production was greatly reduced. Simultaneous inoculation or sequential inoculation in which the fungus was

added prior to the nematode was more effective in controlling the nematode than when nematodes preceded the fungus.

Kiewnick and Sikora (2006) evaluated the fungal biocontrol agent, *Paecilomyces lilacinus* strain 251 (PL251), for its potential to control the root-knot nematode *Meloidogyne incognita* on tomato. In growth chamber experiments, a pre-planting soil treatment reduced root galling by 66%, number of egg masses by 74% and the final nematode population in the roots by 71% compared to the inoculated control. Significant dose-response relationships were established when conidia were applied to soil either with or without the glucose-based formulation. They demonstrated that a single pre-plant application at a concentration of 1×10^6 CFU/g soil is needed for sufficient bio-control of *M. incognita* by PL251.

Goswami *et al.* (2006) carried out an experiment to study the effect of two fungal bioagents along with mustard oil cake and furadan against root knot nematode *Meloidogyne incognita* infecting tomato under greenhouse condition. Bioagents viz., *Paecilomyces lilacinus* and *Trichoderma viride* alone or in combination with mustard cake and furadan promoted plant growth, reduced number of galls/plant, egg masses/root system and eggs/egg mass. The fungal bioagents along with mustard cake and nematicide showed least nematodes reproduction factor as compared to untreated control.

Mucksood and Tabreiz (2010) evaluated the biocontrol potential of bioagent *Paecilomyces lilacinus* 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. 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.

Oclarit and Cumagon (2010) conducted an experiment to evaluate the efficacy of *Paecilomyces lilacinus* strain UP1 as biological control agent of *Meloidogyne incognita* attacking tomato under greenhouse condition pot experiments. Root weight and gall index ratings were significantly higher in untreated plants than those with *P. lilacinus* and with the commercial fungicide Nemacur. Number of galls, nematodes and egg masses per gram root sample were significantly reduced by the application of *P. lilacinus* at all levels and this was comparable with Nemacur. However, egg mass count in plants treated with the lowest concentration of the biocontrol agent was not significantly different from the uninoculated control. Per cent reduction in gall number was the highest at treatment with 7.92×10^6 spores per ml of *P. lilacinus*.

Kiewnick *et al.* (2011) evaluated the fungal biocontrol agent, *Paecilomyces lilacinus* strain 251 (PL251), for its potential to control the root-knot nematode *Meloidogyne incognita* on tomato at varying application rates and inoculum densities. A pre-planting soil treatment with the lowest dose of commercially formulated PL251 (2×10^5 CFU/g soil) was already sufficient to reduce root galling by 45% and number of egg masses by 69% when averaged over inoculum densities of 100 to 1,600 eggs and infective juveniles per 100 cm^3 of soil.

Khalil *et al.* (2012a) conducted a greenhouse experiment to evaluate the efficacy of certain microbial agents against *Meloidogyne incognita* infesting tomato plants (cv. super strain B). The treatments were the antagonistic bacteria *Bacillus subtilis* and *Bacillus thuringiensis*, the antagonistic fungus *Paecilomyces lilacinus* and mycorrhizal fungi *Glomus intraradices* and *Glomus macrocarpium* which were compared with the synthesis nematicides Oxamyl and Cadusafos. The *Paecilomyces lilacinus* product was the best

treatment in suppressing 85.2% the root-knot populations in the soil, followed by *B. subtilis* and *B. thuringiensis* with 82.6 and 80.5% reduction, respectively. *P. lilacinus* also increased the shoot length and fresh weight of the root system by 229.0 and 476.46%, respectively.

Aminuzzaman *et al.* (2013) isolated fungi from *Meloidogyne* spp. eggs and females on 102 field-collected root samples in China. Of the 235 fungi isolated, the predominant fungi were *Fusarium* spp., *F. oxysporum*, *P. lilacinus* and *P. chlamydosporia*.The most promising fungi included five *Paecilomyces* isolates, 10 *Fusarium* isolates, 10 *Pochonia* isolates and one *Acremonium* isolate. *Paecilomyces lilacinus* Yes-2 and *P. chlamydosporia* HDZ-9 selected from the in vitro tests were formulated in alginate pellets and evaluated for *M. incognita* control on tomato. *P. lilacinus* at the highest rate (1.6%) reduced root galling by 66.7%. *P. chlamydosporia* pellets at the highest rate reduced the final nematode density by 90%.

2.3. Effect of *Paecilomyces lilacinus* application time on cucumber

Schenck (2004) tested the efficacy of a commercial product of *Paecilomyces lilacinus* strain 251 for control of nematodes on tomato and cucumber in Hawaii. There were three treatments namely (1) MeloCon, (2) Vapam, and (3) untreated check. The means were consistent for every total in that the MeloCon treatment fruit yield was greatest, vapam a close second, and the untreated check lower. However, the consistency of the results indicates that there was a very real effect of MeloCon in protecting tomato plants against nematode damage. The overall results indicate that MeloCon was as effective as Vapam soil fumigant and significantly better than no treatment for control of nematodes in tomato.

Kalele *et al.* (2010) tested *Paecilomyces lilacinus* (PL251) and *Arthrobotrys conoides* for their efficacy against *Meloidogyne* spp. in tomato and cucumber under greenhouse

conditions. The study aimed at determining the application rates and timing of application of the fungi. Both pre-planting and at planting application of PL251 were found to reduce nematode populations and root galling in both tomato and cucumber. Pre-planting soil treatment (0.4g/10 L of soil) reduced final nematode populations by 69 and 73% in the roots and soil, respectively, compared to the non-inoculated control in tomato. However, soil treatment at planting reduced nematode level at 54 and 74% in the roots and soil, respectively over control.

Yan *et al.* (2011) tested seed treatment with endophytic fungi as an effective method for plant parasitic nematode control. Endophytic fungi from cucumber seedlings were isolated and screened for their potential to be used as seed treatment agents against *Meloidogyne incognita*. Among the 294 isolates screened, 23 significantly reduced galls formed by *M. incognita* in greenhouse test. The 10 most effective isolates were *Fusarium* (5), *Trichoderma* (1), *Chaetomium* (1), *Acremonium* (1), *Paecilomyces*), and *Phyllosticta* (1). *Trichoderma* Tr882, *Paecilomyces* Pa972, and *Acremonium* Ac985 had low colonizations on both the roots and the aboveground parts. *Acremonium* Ac985, *Chaetomium* Ch1001, *Paecilomyces* Pa972, and *Phyllosticta* Ph511 produced compounds affecting motility of the second stage juveniles of *M. incognita*.

2.4. Effect of *Paecilomyces lilacinus* application time on other vegetables

Amit and Trivedi (1989) conducted an experiment to control *Meloidogyne incognita* infecting *Trigonella foneum-graecum* using *Paecilomyces lilacinus* raised on goat dung and sesame oil cake. Of the four treatments used relatively better reduction in nematode population was observed on substrate+ fungus+ nematode treatment compared with substrate + nematode alone. The fungus penetrated the eggs and fed upon their contents leaving empty shell. Invaded eggs were swollen in comparison with uncolonized ones.

Cabanillas *et al.* (1989) isolated 13 *Paecilomyces lilacinus* isolates from various geographic regions as biocontrol agents against *Meloidogyne incognita*. The best control of *M. incognita* was attained from Peru or a mixture of isolates of *P. lilacinus*. As soil temperatures increased from 16 to 28^0 c, both root-knot damage caused by *M. incognita* and percentage of egg masses infected by *P. lilacinus* increased.

Zaki and Irshad (1996) conducted experiments about biological control of plant parasitic nematodes by fungi. A large number of fungi known to trap or pray on nematodes but the most important genera include *Paecilomyces*, *Verticillium*, *Hirsutella*, *Nematophthora*, *Arthrobotrys*, *Drechmeria*, *Fusarium* and *Monacrosporium*. Application of some of these fungi has given very interesting results. There is a urgent need to develop some easy technologies for formulation and mass production of fungi at a commercial scale for field application. Some of these fungi may be used in integrated nematode management programmes despite some obstacles.

Khan *et al.* (2001) conducted an experiment where 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 with amended organic substrate gave the effective control of root-knot nematodes population thus reduced root-knot disease and increased plant vigor.

Abd El-Raheem *et al.* (2005) investigated the nematophagous fungi *Pochonia chlamydoporia*, *Paecilomyces lilacinus* and *Arthrobotrys dactyloidae* as biological control agents for *Meloidogyne incognita* under greenhouse conditions. Experiments confirmed the effectiveness of these predatory and parasitic fungi that actively reduced the number of infective larvae of *M. incognita*. The killing effect of these fungi is significantly better than the commercial preparation of bioagent Nameless. The fungi

under consideration have the potentiality to reduce population density of *M. incognita* along the growing season of faba bean plant to 95.4 to 98.9%. These nematophagous fungi enhanced shoot and root growth of faba bean.

El-Shanshoury *et al.* (2006) evaluated the nematophagous fungi *Pochonia chlamydosporia* (*Verticillium chlamydosporium*), *Paecilomyces lilacinus* and *Arthrobotrys dactyloides* as biological control agents against *Meloidogyne incognita* under greenhouse conditions. The nematicidal effects of these fungi are comparable to those of the synthetic nematicide Furadan and significantly better than the commercial preparation of the biocontrol agent Nameless®. The fungi tested have the potential to reduce the population density of *M. incognita* associated with faba bean by 95.4 to 98.9%. In addition, the application of these nematophagous fungi resulted in enhanced shoot and root growth of faba bean.

Mohd Yaqub Bhat *et al.* (2009) conducted an experiment where *Momordica charantia* roots were histologically examined for the interaction of *Meloidogyne incognita* and the fungus *Paecilomyces lilacinus* which was applied at different time intervals. The fungus *P. lilacinus* soon after the application, entered the roots and spread through the lumen of the vessel elements. The plants that were treated with fungus either one weak before nematode inoculation or simultaneously, produced significantly (P=0.01) small sized galls in comparison to untreated plants. The size of galls remained unchanged after completion of one life cycle by the nematode. In fungus treated plants the giant cells were small sized and the abnormality of vascular plants was less. *Paecilomyces lilacinus* entered the giant cells and also into the body of mature females. It destroyed the eggs and egg masses in and outside females.

Khan *et al.* (2012) conducted an experiment where treatments involving neem leaves, *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, *Trichoderma harzianum*, or aldicarb reduced the suppressive effect of the nematode, leading to a significant increase in the

dry matter production and yield of inoculated plants compared to the un-inoculated control. Neem leaves induced a 19% increase in the weight of fruits/plant of inoculated plants; similar results were obtained using *P. chlamydosporia*, *P. lilacinus*, *T. harzianum*, and aldicarb treatments, with increases over the inoculated control of 11, 14, 6 and 8%, respectively. Declines in galling, egg mass production, and fecundity were found to be greater with aldicarb and lower with the neem leaf treatment. The incorporation of neem leaves into biocontrol treatments increased the efficiency of the treatment and resulted in a 17, 21 and 14% increase in the yield with *P. chlamydosporia*, *P. lilacinus*, and *T. harzianum* treatments, respectively. Decreases in galling and egg mass production were also greater in the presence of neem leaves than in methods using the biocontrol agents alone. The percentages of infection in adult nematode females and egg masses with *P. chlamydosporia*, *P. lilacinus*, and *T. harzianum* applied to plants were considerably greater in the presence of neem leaves (77-92% and 43-57%) than in their absence (69-87% and 33-47%).

Pau *et al.* (2012) isolated ten indigenous isolates of *Paecilomyces lilacinus* (PL) from two black pepper farms in Sarawak heavily infested with root-knot nematodes (RKN) as an initiative to control RKN problem. All isolates showed varying degree in colonizing female nematodes. In the female nematode bioassay on water agar, both indigenous strains of PL namely PLA, PLB, and a commercial strain, PLM (as positive control) demonstrated highly significant colonization (>90%, $P \leq 0.01$) on female. In egg parasitism test, spore suspension (10^5 spore/ml) of the strains PLA, PLB and PLM exhibited 78.8, 66.0 and 73.4% parasitism on eggs, respectively. Meanwhile, hatching of nematode eggs incubated in spore suspension of PLA, PLB and PLM for seven days were significantly reduced; 88-89% of eggs were hatch-inhibited as compared to control (26%). This illustrated both local isolates, PLA and PLB are comparable with PLM as biological control agents for managing RKN infestation on black pepper vines.

Kannan and Veeravel (2012) evaluated the biocontrol potential of *Paecilomyces lilacinus* in field conditions in two seasons during 2005-2008. In two field trials of okra at two locations, shoot length, shoot weight and root length were significantly increased in mixture treatments compared to individual treatments, principally combination of seedling treatment (10g/l water) + soil application treatment (5.0 kg/acre) documented maximum shoot length (60 and 90 DAS), shoot weight (90 DAS) and root length (90 DAS) and they were positively correlated with fruit yield of okra.

MATERIALS AND METHODS

Pot experiments were conducted to study the effect of application time of *Paecilomyces lilacinus* on root knot nematode *(Meloidogyne incognita*) and growth parameters of some selected crops. In these study brinjal, tomato and cucumber were used as selected crops. The materials used and the methods followed in the study are presented in this chapter.

3.1. Experimental site and experimental period

The present investigation was carried out during the period from May 2011 to June 2012 in the Laboratory and shade house of the Department of Plant Pathology, Sher-e-Bangla Agricultural university, Sher-e-Bangla Nagar, Dhaka-1207.

3.2. Environment of experiments

Most of the experimental plants were kept in the shade house where the temperature was 30 ± 2^0 C during the "day" and 23 ± 2^0 C during "night" with an average temperature of 28 ± 2^0 C.

3.3 Pot Experiment

3.3.1. Crops variety used

There were three different crops used in this experiment Viz: Brinjal (Var. Singnath and Khotkhotia), Tomato (Var. BARI Tomato 14) and Cucumber (Var. Kashinda).

3.3.2. Collection of seeds

All the collected seeds were healthy, mature, same size and disease and offtype seeds free. Brinjal seeds (Singnath) and tomato seeds (BARI Tomato 14) were collected from Bangladesh Agricultural Research Institute (BARI). Brinjal seeds (Khotkhotia) were

collected from BADC. Cucumber seeds (Kashinda) were collected from Lalteer, seed company, Bangladesh.

3.3.3. Soil collection and sterilization

Required soils were collected from agricultural farm of Sher-e-Bangla Agricultural university. Sand and decomposed cowdung also collected with soil. Then soil, sand and cowdung mixed properly in a ratio of 6:2:1. For raising seedlings in plastic trays. The mixture was autoclaved at 121^0C for 15 minutes on two successive days. The sterilized soil was allowed to cool to room temperature and was later used to fill the plastic trays for raising seedlings. For final experiment set up after mixing soil was sterilized with formalin. Formalin was mixed with soil (5% formalin @ 400ml/cft soil) properly and covered with polythene sheet for three days. After that the polythene was removed and soil was kept open in sun for next three days.

3.3.4. Seedling raising

Several plastic trays were filled with sterilized and fertile soil. Seeds of brinjal, tomato and cucumber cultivars were soaked in water for one night in different intervals and treated with NaOCl for one minute and washed with distilled water for three times. After that the seeds were sown in plastic trays and covered with a thin layer of soil and watered. Then the trays were covered with polythene sheet and kept in sunlight for raising seedlings. Seedlings were observed regularly and watering was done as per necessity up to transplanting in poly bag (Plate-1+2). Cucumber seeds were directly sown in polybag (1 seed/polybag) for raising seedling.

(a)

(b)

Plate -1. Raising and transplanting of brinjal seedlings
(a) Brinjal (Var. Singnath)
(b) Brinjal (Var. Khotkhotia)

(a)

(b)

Plate-2. Raising of seedlings
(a) Tomato (Var. BARI Tomato 14) in plastic tray
(b) Cucumber (Var. Kashinda) in polybags

3.4. Preparation of pots

Plastic pots of 1000 cm^3 were cleaned, washed, dried up and sterilized by ethanol properly. Sterilized and fertile soil was filled in required amount into each pot. Each pot contains 800 g soil. Then the pots were arranged according to experimental design.

3.5. Treatments of the experiment

There were seven treatments used in the experiment are given below-

BC = Blank control (without any inoculation)

M_p = Inoculation of *Meloidogyne incognita* at planting (Negative control)

PL_p = Application of *P. lilacinus* at planting

PL_P+M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP}= Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP} = Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$ = Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting

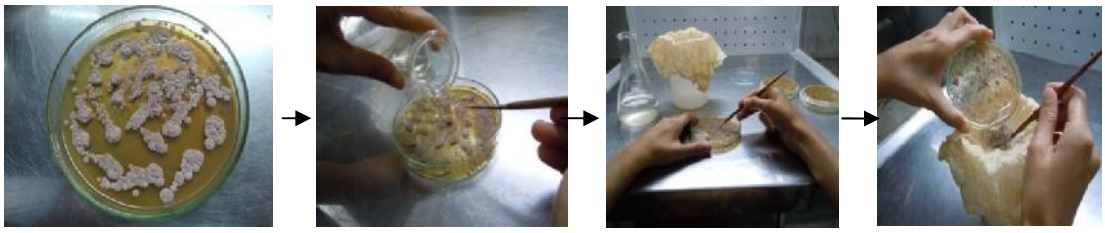
3.6. Culture and application of *Paecilomyces lilacinus*

Paecilomyces lilacinus was grown on Potato Drextose Agar (PDA) medium for 15 days. The pure culture of fungus was collected from the Department of Plant Pathology, Sher-e-Bangla Agricultural University. After complete sporulation (15 days) the plates were put into the laminar air flow chamber in a stelized condition. Then sterile water was added and the spore masses scraped away with sterile brush. The harvested spores were filtered through sterilized cheesecloth. The spore was harvested from each fungus plate more than two times. The spore suspension was collected and spore was counted with a haemacytometer and adjusted to a concentration of 10×10^7 spore/ml solution. Then the

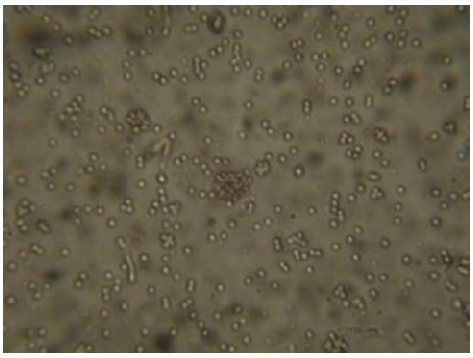
inoculation was done @ 36×10^7 spore/plant in each pot with micropipette. Spores were mixed thoroughly to the soil. It was done on the time of final experiment set up and transplanting the plant. (Plate-3)

3.7. Transplanting of seedlings

After preparation of pot in the shade house, 30 days old seedlings were uprooted carefully from the poly bag and transplanted in the experimental pot. Initial root and shoot weight measured before transplanting. Only one plant was transplanted to each pot. Sufficient irrigation was given just after transplantation. Watering was continued till seedlings were established.



(a) (b) (c) (d)



(e)

Plate-3. *Paecilomyces lilacinus*

(a) Pure culture

(b) Harvesting of spore

(c)+(d) Sieving of spore

(e) Spore of *P. lilacinus* under microscope (400X)

3.8. Culturing *Meloidogyne incognita*, inoculum preparation and inoculation

Meloidogyne incognita was cultured and maintained in susceptible tomato plants grown in plastic pots containing sterilized soil for two months. For culturing nematodes, egg masses were handpicked, sterilized with NaOCl for 1 min and rinsed with water for three times and inoculated in young seedlings of tomato. Sub-culturing were done subsequently by inoculating new tomato seedlings with egg masses. Mature eggmasses of (*Meloidogyne incognita*) was collected from severely galled roots of tomato. The number of eggs/egg mass were counted with the help of compound microscope. Three holes of five cm depth around the plants were made with the help of metallic rod. Twenty egg masses containing approximately 10,000 eggs were inoculated in these holes. The holes were covered with soil to prevent drying. After few days of inoculation these pots were watered carefully to prevent loss of nematodes through leaching or excessive drying. (Plate-4)

3.9. Intercultural operations

After transplantation of seedling and final experiment set up weeding and irrigation were regularly done as per necessity. General sanitation was maintained throughout the growing period. Insecticide named marshal was sprayed @ 1.5ml/litre in 4 times at 15 days interval.

3.10. Harvesting and data recording

After two months of transplanting, plants were harvested and data was recorded. The following parameters were considered

Shoot length (cm)

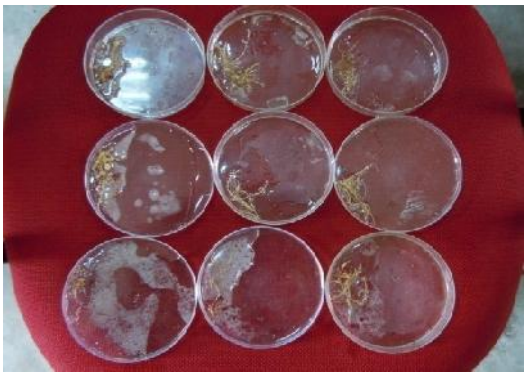
Root length (cm)

Shoot fresh and dry weight (g)

Root fresh and dry weight (g)

(a)

(b)



(c)

Plate- 4. (a)+(b)+(c) Collection of egg masses for inoculation of *Meloidogyne incognita*

Gall index (0-10 scale)

Number of egg masses per root

Number of eggs per egg mass

Number of eggs per root system

Number of juveniles per g soil

Reproduction factor (RF)

% Egg masses colonized by *P. lilacinus*

Soil colonization by *Paecilomyces lilacinus* (CFUg-^{1} soil)

3.11. Design and layout of the experiment

The experiment was laid out in a Randomized Complete Block Design (RCBD) with eight replications per treatment.

3.12. Data recorded

3.12.1. Plant data

Shoot length was measured before harvest. The shoot height (cm) was measured from the base of the plant to the growing point of the youngest leaf with a measuring scale. Then the roots are harvested by cutting with a anti-cutter. Roots are carefully separated from soil, cleaned gently with water and collected in different polybag that were leveled according to different treatments. Finally the root length (cm) was taken. The length of root was measured from the growing point of root to the longest available lateral root apex. For fresh weight (g) of root and shoot was blotted dry and the weight was recorded. For dry weight (g), the shoot and root were sun dried for three days and then kept in drier machine for 4-6 hours at 70^{0} C temperature. And after complete drying the weight was recorded.

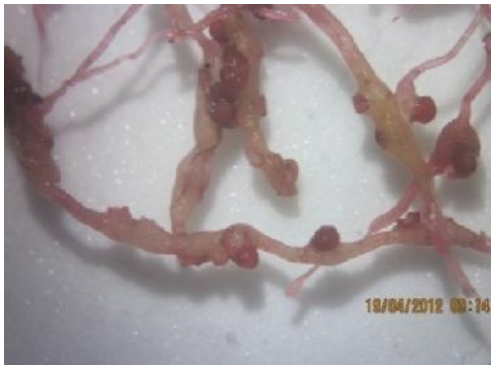
3.12.2. Counting of nematode egg masses and eggs/egg mass

Number of egg masses/root system was counted following Holbrook *et al.* (1983). The roots were soaked in Phloxine-B (2mg/l) for 15 minutes (Hartman and Sasser, 1985). The roots were observed and eggmasses/root were counted with a magnifying glass. Then egg masses were picked with forcep treated with Naocl for three minutes to dissolve gelatinous materials. After subsequent washing with water eggs were counted under compound microscope. (Plate-5).

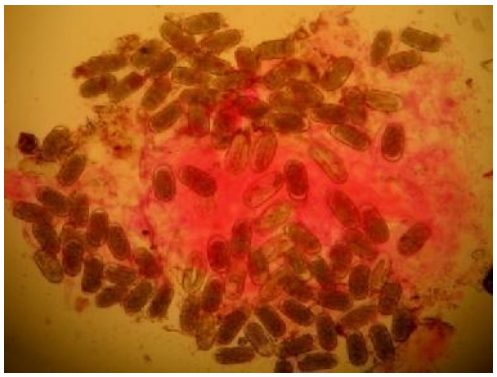
3.12.3. Slide preparation and counting of eggs/egg mass

Heavily galled roots were collected and properly washed with water. Care was taken so that an egg mass does not washed with water. Then the roots were soaked in Phloxine-B (2mg/l) solution for 15 minutes (Hartman and Sasser, 1985). Then water was soaked by placing the root in tissue paper for one minute. A clean slide was prepared. Three drops of glycerin was placed on the slide. Then egg masses was collected from the root with the help of fine forcep and placed on the slide and also crashed with the help of bottom side of needle. Then after placing cover slip the slide was examined under microscope and counting the eggs/egg mass.

(a)



(b)



(c)

Plate-5. (a) Highly galled roots treated by Phloxine- B

(b) Phloxine-B treated egg masses

(c) Phloxine-B treated eggs

3.12.4. Extraction of nematode from soil and counting of juveniles

The extraction of nematodes from soil was done by using a Whitehead and Hemming tray method (1965) as follows: Pot soil was mixed thoroughly and different samples of 100 g soil was weighted and put it on the sieve that was on a bowl filled with water. The upper portion of sieve was lined with three layers of kitchen tissue paper. After 5 days the nematode suspension was collected in a beaker and left for a day, excess water was discarded leaving 100 ml suspension and 5 ml sub sample was taken and put into a counting dish. Juveniles counting were done by using a compound microscope. (Plate- 6)

(a)

(b)

Plate-6. (a) Extraction of *Meloidogyne incognita* from soil by Bangladeshi Plate method (Modified White Head and Hemming Method, (1965)

(b) Micrographs showing second stage juveniles and eggs of *Meloidogyne incognita*

3.12.5. Gall index

Root galls were indexed on a 0-10 scale of Bridge and Page (1980), which were as follows:

Scales	Specification
0	No galls
1	Few small gall, difficult to find
2	Small gall only, clearly visible, main root clean
3	Some larger galls visible, main root clean
4	Larger galls predominant but main root clean
5	50% of the roots infected, galling on some main roots, reduced root system
6	Galling on main roots
7	Majority of the main roots galled
8	All main roots including tap roots galled, few clean roots visible
9	All roots severely galled, plants usually dying
10	All roots severely galled, no root system

3.12.6. % Eggmasses colonization by *Paecilomyces lilacinus*

Eggmasses were collected as per treatment from the brinjal, tomato and cucumber plant roots, washed with water and disinfected with a solution of Clorox and put on a Potato Dextrose Agar (PDA) media in petridish. Randomly five eggmasses/root was collected so that 40 eggmasses per treatments was collected. The number of colonized eggmasses was determined after 5 days of incubation. The presence of *P. lilacinus* with egg mass was confirmed by preparating slides from the culture grown on PDA.

3.12.7. Soil colonization by *Paecilomyces lilacinus* ($CFUg^{-1}$ soil)

Samples of 1g soil from each treatment were collected after harvest of the crop around the root zone. The number of spores ($CFUg^{-1}$ soil) per gram soil was determined using the soil dilution plate method (Plate-7).

3.13. Analysis of data

The data were statistically analyzed using analysis of variance to find out the variation of results from experimental treatments. Treatment Means were compared by DMRT. Data were analyzed by MSTAT software.

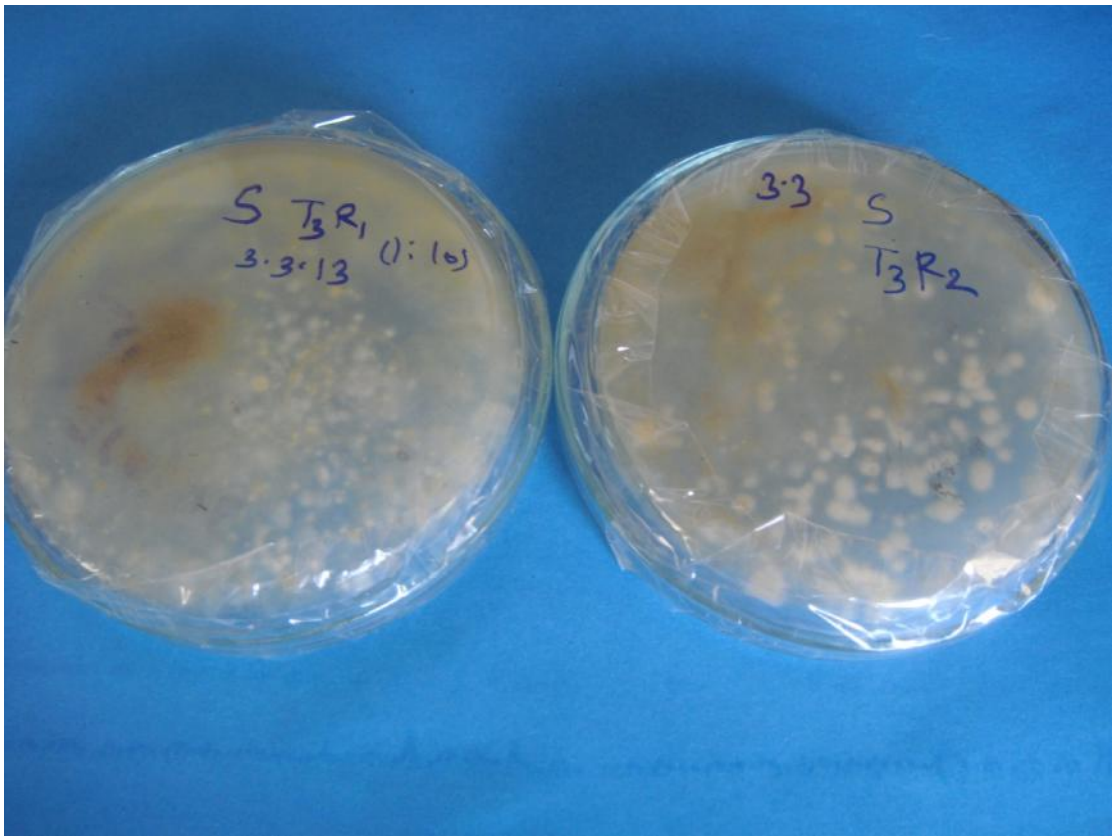


Plate-7: Colony growth of *P. lilacinus* on PDA (Soil dilution plate technique)

RESULTS

In the present study the fungus *P. lilacinus* was tested for its beneficial effect against root-knot nematode suppression and plant growth promotion of brinjal (Var. Singnath and Khotkhotia), tomato (Var. BARI Tomato 14) and cucumber (Var. Kashinda). The ability of fungus to suppress root knot disase of these crops were tested in pot experiments under shade house conditions. The results obtained on these aspects are presented here.

4.1.1. Effect of *Paecilomyces lilacinus* application time on growth parameters of brinjal variety singnath.

Brinjal plants inoculated with *Meloidogyne incognita* showed significant reduction in their growth (Table 1). Shoot length, fresh and dry weights were significantly poor in nematode inoculated plant in compare to un-inoculated control. When brinjal plants were inoculated with *P. lilacinus* at planting, there was no significant difference in length (43.47 cm), fresh weight (34.50 g) and dry weight (7.10 g) of the plants in compare to un-inoculated control treatment of the plants length (37.75 cm), fresh weight (26.75 g) and dry weight (6.83 g). In simultaneous application of *P. lilacinus* and inoculation of *Meloidogyne incognita* at planting, plant length (45.35 cm) significantly differed ($P \geq 0.01$) from the blank control (37.75 cm). When compared to plants inoculated with *Meloidogyne incognita* at planting (34.13 cm), plant length was significantly greater. When *P. lilacinus* applied at planting and *Meloidogyne incognita* at 7 days after planting, plant length (40.79 cm) differed significantly from the control. However, when *M. incognita* was inoculated at planting and *P. lilacinus* at 7 days after planting, significant reduction was observed in plant length (38.34 cm) as compared to blank control (37.75 cm) and plant length differed from plants inoculated with *M. incognita* at planting (Table 1). Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting showed a reduction in plant length (34.45 cm) as compared to blank control. (Plate-8)

Plate- 8: Photograph showing the effect of *Paecilomyces lilacinus* application time on shoot growth of brinjal var. Singnath in comparison to control

BC = Blank control (Without any inoculation)

M_P = Inoculation of *Meloidogyne incognita* at planting (Negative control)

PL_P= Application of *P. lilacinus* at planting

PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP}= Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP}= Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$= Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting.

The fresh and dry weights of plants in various treatments showed a similar trend as length. Significant reduction occurred in fresh weight (23.79 g) and dry weight (3.95 g) due to the infection of *M. incognita* at planting. Application of *P. lilacinus* at planting did not cause an adverse effect on fresh weight (34.50 g) and dry weight (7.10 g) of plants. When fungus and nematode inoculated simultaneously at planting, fresh weight (32.56 g) and dry weight (6.58 g) of plants were significantly higher than plants inoculated with *M. incognita* at planting and the weights differed significantly from the blank control. In sequential inoculations, when nematode was inoculated at planting and *P. lilacinus* at 7 days after planting, fresh weight (28.60 g) and dry weight (3.96 g) of plants did not differ from plants inoculated with *M. incognita* at planting. On the other hand, when *P. lilacinus* applied at planting and *M. incognita* at 7 days after planting, fresh weight (34.00 g) and dry weight (6.11 g) were significantly higher than plants inoculated with *M. incognita* at planting and the plant inoculated with nematode at planting and bioagent at 7 days after planting (Table 1). But application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting showed a reduction in fresh weight (26.06 g) and dry weight (3.78 g) of the plant as compared to blank control but increase in fresh and dry weight of shoot in compare to *M. incognita* inoculated control.

Brinjal plants inoculated with *M. incognita* at planting showed significant reduction in their root growth (Table 1). Root length and fresh and dry weights of roots were significantly lower in negative control (*M. incognita* inoculated at planting) plant in compare to blank control (Plate- 9). When brinjal plants were inoculated with *P. lilacinus* at planting, there was no significant difference in root length (25.73 cm), fresh weight (21.60 g) and dry weight (9.55 g) of the plants in compare to blank control treatment of the root length (21.19 cm), fresh weight (17.02 g) and dry weight (9.11g). In simultaneous application of bioagent and nematode at planting, root length (23.02 cm) significantly higher

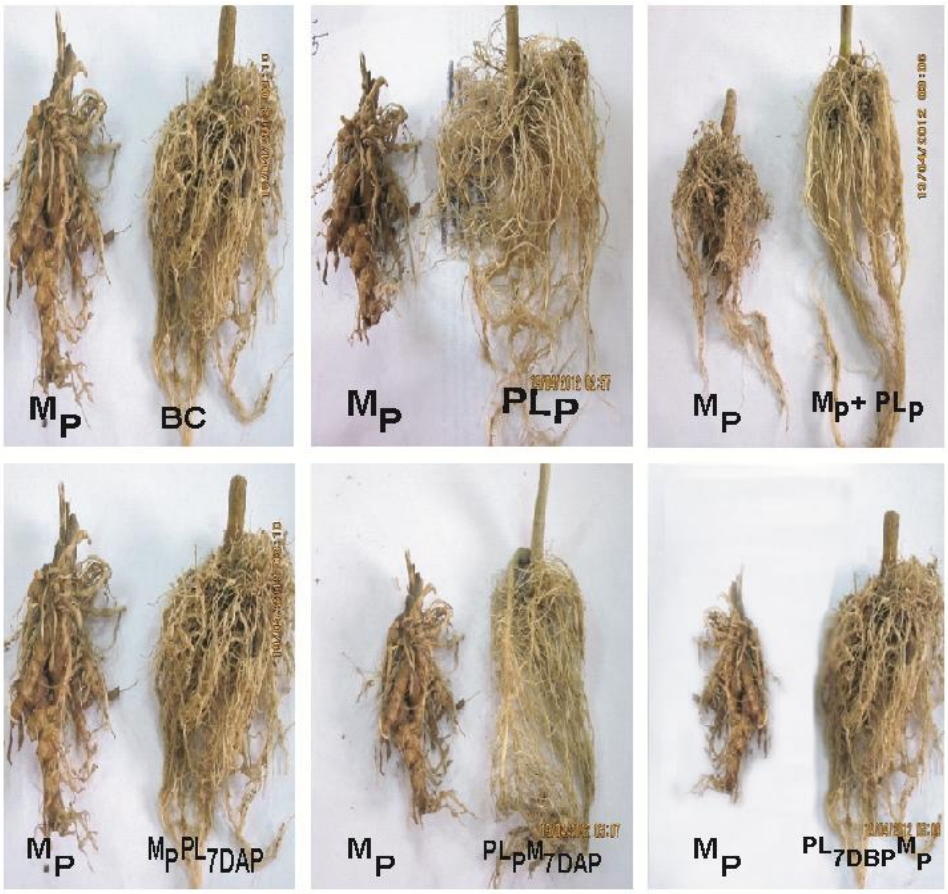


Plate- 9: Photograph showing the effect of *Paecilomyces lilacinus* application time on root growth of brinjal var. Singnath in comparison to control

BC = Blank control (Without any inoculation)

M_P = Inoculation of *Meloidogyne incognita* at planting (Negative control)

PL_P= Application of *P. lilacinus* at planting

PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP}= Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP}= Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$= Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting.

(P≥0.01) from the negative control (19.16 cm). When *P. lilacinus* applied at planting and *M. incognita* at 7 days after planting, root length (23.51 cm) differed significantly from the blank control. However, significant reduction was observed in root length (15.44 cm) as compared to blank control (21.19 cm) and root length differed from plants inoculated with nematode at planting and when nematode was inoculated at planting and *P. lilacinus* at 7 days after planting (Table 1). Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting showed a reduction in root length (16.73 cm) as compared to blank control.

The fresh and dry weights of roots in various treatments showed a similar trend as length. Significant reduction occurred in fresh weight (10.60 g) and dry weight (7.55 g) of root due to the infection of *M. incognita* at planting. Application of *P. lilacinus* at planting increased fresh weight (21.60 g) and dry root weight (9.55 g) of plants. When *P. lilacinus* and *M. incognita* inoculated simultaneously at planting, fresh root weight (20.35 g) and dry root weight (9.90 g) of plants were significantly higher than plants inoculated with *M. incognita* at planting and the root weights differed significantly from the blank control. In sequential inoculations, when *Meloidogyne incognita* was inoculated at planting and *P. lilacinus* at 7 days after planting, fresh weight (18.35 g) and dry root weight (7.33 g) of plants did not differ from plants inoculated with *M. incognita* at planting. On the other hand, when *P. lilacinus* was applied at planting and *M. incognita* at 7 days after planting, fresh root weight (21.44 g) and dry root weight (10.4 g) were significantly higher than plants inoculated with *M. incognita* at planting and *M. incognita* was inoculated at planting and *P. lilacinus* at 7 days after planting (Table 1). Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting showed a reduction in fresh root weight (16.46 g) and dry root weight (7.57 g) of plants as compared to blank control.

Table 1. Effect of *Paecilomyces lilacinus* application time on the growth parameters of brinjal var. singnath

Treatments	Shoot length (cm)	Shoot weight (g)		Root length (cm)	Root weight (g)	
		Fresh weight (g)	Dry weight (g)		Fresh weight (g)	Dry weight (g)
BC	37.75 bc	26.75 abc	6.83 a	21.19 abc	17.02 ab	9.11 ab
M_P	34.13 c	23.79 c	3.95 b	19.16 bc	10.60 b	7.55 ab
PL_P	43.47 ab	34.50 a	7.10 a	25.73 a	21.60 a	9.55 ab
PL_P+M_P	45.35 a	32.56 ab	6.58 a	23.02 ab	20.35 a	9.90 ab
M_PPL_{7DAP}	38.34 bc	28.60 abc	3.96 b	15.44 c	18.35 ab	7.33 b
PL_PM_{7DAP}	40.79 abc	34.00 ab	6.11 a	23.51 ab	21.44 a	10.4 a
$PL_{7DBP}M_P$	34.45 c	26.06 bc	3.78 b	16.73 c	16.46 ab	7.57 ab
LSD(P≥0.01)	6.45	-	1.61	5.75	8.05	-
0.05	-	7.21	-	-	-	-
0.10	-	-	-	-	-	2.65

BC = Blank control (Without any inoculation), M_P = Inoculation of *Meloidogyne incognita* at planting, PL_P = Application of *P. lilacinus* at planting, PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting, M_PPL_{7DAP}=Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting, PL_PM_{7DAP} = Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting, $PL_{7DBP}M_P$ = Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting.

4.1.2. Effect of *P. lilacinus* application time on gall index, nematode population and reproduction factor of *Meloidogyne incognita* in brinjal cultivar Singnath

4.1.2.1. Root-galling and egg mass production: The application of *P. lilacinus* at planting reduced root-galling and egg mass production of the nematode in compared to plants inoculated with *M. incognita* at planting (Table 2). In simultaneous application of *P. lilacinus* and *M. incognita* at planting, gall index was 1.50 and number of egg masses/root was 97.50 in comparison to 6.63 and 832.5, respectively in *M. incognita* inoculated plants respectively. Similar reduction was observed, when *P. lilacinus* was applied at planting and *M. incognita* at 7 days after planting, where gall index was 1.63 and number of egg masses/root was 168.8. In other sequential inoculations, when nematode was inoculated at planting and bioagent at 7 days after planting, the gall index (3.13) and number of egg masses/root (270.8) were slightly reduced. In application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting, gall index was 1.88 and number of egg masses/root was 204.6 (Table 2).

4.1.2.2. Number of juveniles/g soil: The application of *P. lilacinus* at planting reduced number of juveniles/g soil as compared to plants inoculated with nematode at planting. In simultaneous application of *P. lilacinus* and *M. incognita* at planting, J_2/g soil was 105.6 in compare to 737.5 in *M. incognita* inoculated plants. Similar reduction was observed, when *P. lilacinus* was applied at planting and *M. incognita* at 7 days after planting sequentially, where J_2/g soil was 90.25. In other sequential inoculations, when nematode was inoculated at planting and bioagent at 7 days after planting, J_2/g soil was 578.8 which were slightly reduced. In application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting J_2/g soil was 280.1 (Table 2).

4.1.2.3. Reproduction factor: The application of *P. lilacinus* at planting reduced reproduction factor (RF) as compared to plants inoculated with *M. incognita* at planting. In simultaneous application of *P. lilacinus* and *M. incognita* at planting RF was 9.81 in comparison to 71.58 in *M. incognita* inoculated plants. Similar reduction was observed, when *P. lilacinus* was applied at planting and *M. incognita* at 7 days after planting in sequential inoculation where RF was 9.76. In other sequential inoculations, when *M. incognita* was inoculated at planting and *P. lilacinus* at 7 days after planting, RF was 53.56. In application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting RF was 27.08 (Table 2).

Table 2. Effect of *P. lilacinus* application time on gall index, nematode population and reproduction factor of *Meloidogyne incognita* on brinjal cv. Singnath

Treatments	Gall Index (0-10 scale)	Number of egg masses/root	Number of eggs/Egg mass	Number of J_2/g soil	Reproduction Factor
BC	0.00 c	0.00 c	0.00 d	0.00 d	0.00 d
M_P	6.63 a	832.5 a	317.8 a	737.5 a	71.58 a
PL_P	0.00 c	0.00 c	0.00 d	0.00 d	0.00 d
PL_P+ M_P	1.50 bc	97.50 bc	99.38 c	105.6 d	9.81 d
M_PPL_{7DAP}	3.13 b	270.8 b	295.9 ab	578.8 b	53.56 b
$PL_{7DAP}M_P$	1.63 bc	168.8 bc	103.1 c	90.25 d	9.76 cd
$PL_{7DBP}M_P$	1.88 b	204.6 b	214.6 b	280.1 c	27.08 c
LSD (P≥.01)	1.69	169.8	93.88	156.0	16.94

BC = Blank control (Without any inoculation), M_P = Inoculation of *Meloidogyne incognita* at planting, PL_P = Application of *P. lilacinus* at planting, PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting, M_PPL_{7DAP}=Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting, PL_PM_{7DAP} = Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting, $PL_{7DBP}M_P$ = Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting.

4.1.3. Effect of *P. lilacinus* application time on egg masses colonization and soil colonization by *P. lilacinus* in pot soil of brinjal var. Singnath at harvest

4.1.3.1. Egg masses colonization by *P. lilacinus* at harvest: In simultaneous application of *P. lilacinus* and *M. incognita* at planting egg masses colonization by fungus was 56.25% (Figure 1). Similar trend was observed in sequential inoculation when *P. lilacinus* was applied at planting and *M. incognita* at 7 days after planting, where egg masses colonization by fungus were 56.75%. In other sequential inoculations, when *M. incognita* was inoculated at planting and *P. lilacinus* at 7 days after planting, egg masses colonization by fungus was 37.50% which were reduced. In application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting, egg masses colonization was 44.13% by fungus.

4.1.3.2. Soil colonization by *P. lilacinus* ($CFUg^{-1}$ soil): Soil colonization by the biocontrol fungus was measured ($CFUg^{-1}$ soil) at harvest (Figure 2). In *P. lilacinus* inoculated soil the fungal density was $13.96x10^3$ $CFUg^{-1}$ soil. In simultaneous application of *P. lilacinus* and *M. incognita* at planting, soil colonization by fungus was higher ($17.95x10^3$ $CFUg^{-1}$ soil). Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting, results a soil colonization of $32.96x10^3$ $CFUg^{-1}$ by fungus. When *M. incognita* was inoculated at planting and *P. lilacinus* at 7 days after planting, soil colonization by fungus was $7.53x10^3$ $CFUg^{-1}$ soil. In application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting, soil colonization by fungus was $13.20\ x10^3$ $CFUg^{-1}$ soil.

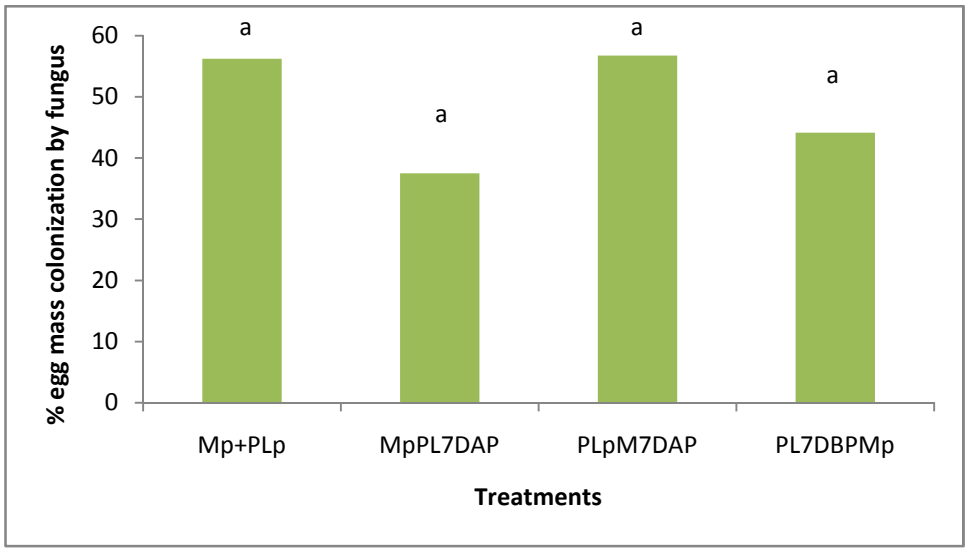


Figure 1: Effect of *P. lilacinus* application time on % egg masses colonization by *P. lilacinus* in brinjal var. Singnath. Bars headed by same letters are not significantly different

Here,

PL_P+M_p = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP} = Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP} = Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$ = Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting

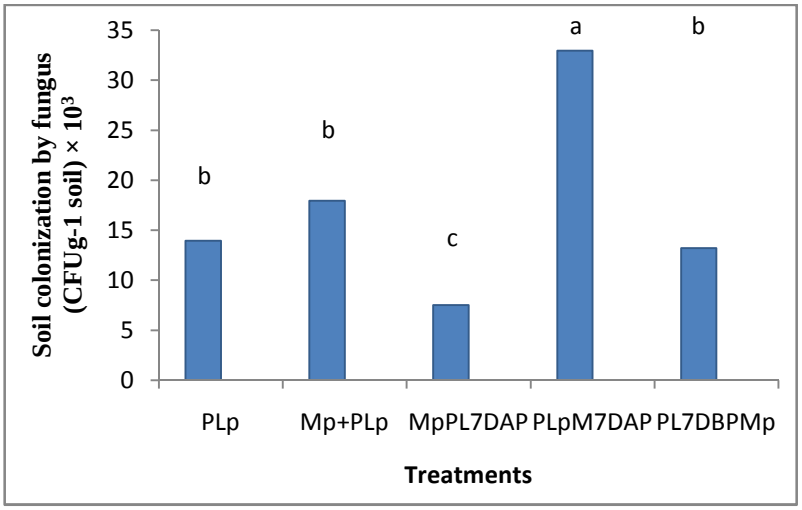


Figure 2: Effect of *P. lilacinus* application time on soil colonization by fungus ($CFUg^{-1}$) in pot soil of brinjal var. Singnath. Bars headed by different letters are significantly different

Here,

PL_P = Application of *P. lilacinus* at planning

PL_P+M_p = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP} = Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP} = Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$ = Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting

4.2.1. Effect of *Paecilomyces lilacinus* application time on growth parameters of brinjal variety Khotkhotia

Inoculation of *Paecilomyces lilacinus* in different time of application provided different results. Application of *P. lilacinus* reduced the damage caused by *M. incognita* and the effect of the treatments on growth characterstics of brinjal plant viz. shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight and root dry weight studied are presented in Table 3.

The highest shoot length (25.98 cm) was recorded in the treatment PL_PM_{7DAP} (Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting) which was statistically similar to PL_P+M_P (Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting), PL_P (Application of *P. lilacinus* at planting) and BC (blank control). The lowest shoot length (18.10 cm) was observed in M_P (Inoculation of *M. incognita* at planting) which was statistically similar to M_PPL_{7DAP} (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting) (Table 3) and (Plate 10).

The highest fresh weight of shoot (10.84 g) was recorded in the treatment PL_PM_{7DAP} (Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting) which was statistically similar to PL_P+M_P (Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting), PL_P (Application of *P. lilacinus* at planting) and BC (blank control). The lowest result (4.01 g) was observed in M_P (Inoculation of *M. incognita* at planting) which was statistically similar to M_PPL_{7DAP} (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting).

Plate- 10: Photograph showing the effect of *Paecilomyces lilacinus* application time on shoot growth of brinjal var. Khotkhotia in comparison to control

BC = Blank control (Without any inoculation)

M_P = Inoculation of *Meloidogyne incognita* at planting (Negative control)

PL_P= Application of *P. lilacinus* at planting

PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP}= Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP}= Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$= Application of *P. lilacinus* at 7 days before planting and Inoculation of *M. incognita* at planting.

Among different treatments, the highest dry weight of shoot (4.18 g) was recorded in the treatment PL_PM_{7DAP} (Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting) which was statistically similar to PL_P+M_P (Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting), PL_P (Application of *P. lilacinus* at planting) and BC (blank control). But the lowest dry weight of shoot (1.24 g) was observed in M_P (Inoculation of *M. incognita* at planting) which was statistically similar to M_PPL_{7DAP} (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting).

It was observed that inoculation of *P. lilacinus* followed by *Meloidogyne incognita* after one week and simultaneous inoculation of *P. lilacinus* and *Meloidogyne incognita* showed good results among the treatments (Table 3). In terms of length of root, treatments effect differed significantly among them selves (Plate 11). Maximum root length (15.81cm) was observed in PL_P+M_P (Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) which was statistically similar to PL_PM_{7DAP} (Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting), PL_P (Application of *P. lilacinus* at planting) and BC (blank control). The lowest result (10.81 cm) was observed in M_P (Inoculation of *M. incognita* at planting) which was statistically similar to M_PPL_{7DAP} (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting).

Considering fresh weight of root (Plate-11), the highest weight (12.96 g) was recorded in PL_P+M_P (Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) which was statistically similar to PL_PM_{7DAP} (Application of *P. lilacinus* at

planting and *M. incognita* at 7 days after planting), PL_P (Application of *P. lilacinus* at planting) and BC (blank

Plate- 11: Photograph showing the effect of *Paecilomyces lilacinus* application time on root growth of brinjal var. Khotkhotia in comparison to control

BC = Blank control (Without any inoculation)

M_P = Inoculation of *Meloidogyne incognita* at planting (Negative control)

PL_P= Application of *P. lilacinus* at planting

PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP}= Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP}= Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$= Application of *P. lilacinus* at 7 days before planting and Inoculation of *M. incognita* at planting.

control). The lowest result (4.13 g) was observed in M_P (Inoculation of *M. incognita* at planting) which was statistically similar to M_PPL_{7DAP} (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting).

Among different treatments, the highest dry weight of root (4.56 g) was recorded in PL_P+M_P (Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) which was statistically similar to PL_PM_{7DAP} (Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting), PL_P (Application of *P. lilacinus* at planting) and BC (blank control). But the lowest dry weight of root (0.76 g) was observed in M_P (Inoculation of *M. incognita* at planting) which was statistically similar to M_PPL_{7DAP} (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting).

Table 3. Effect of *Paecilomyces lilacinus* application time on the growth parameters of brinjal var. Khotkhotia

Treatments	Shoot Length (cm)	Shoot weight (g)		Root length (cm)	Root Weight (g)	
		Fresh Weight (g)	Dry Weight (g)		Fresh Weight (g)	Dry Weight (g)
BC	22.92 ab	9.59 a	3.09 bc	14.56 ab	10.65 a	4.18 a
M_P	18.10 b	4.01 b	1.24 e	10.81 c	4.13 b	0.76 b
PL_P	23.4 ab	10.31 a	3.34 ab	14.75 ab	11.39 a	4.25 a
PL_P+ M_P	25.49 a	10.20 a	3.78 ab	15.81 a	12.96 a	4.56 a
M_PPL_{7DAP}	18.32 b	4.59 b	2.24 cd	13.66 ab	6.25 b	1.10 b
PL_PM_{7DAP}	25.98 a	10.84 a	4.18 a	15.25 ab	12.13 a	4.26 a
$PL_{7DBP}M_P$	19.71 b	4.10 b	1.60 de	12.74 bc	4.88 b	1.36 b
LSD (P≥.10)	5.11	2.29	0.93	2.59	2.69	1.18

BC = Blank control (Without any inoculation), M_P = Inoculation of *Meloidogyne incognita* at planting, PL_P = Application of *P. lilacinus* at planting, PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting, M_PPL_{7DAP}=Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting, PL_PM_{7DAP} = Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting, $PL_{7DBP}M_P$ = Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting.

4.2.2. Effect of *P. lilacinus* application time on gall index, nematode population and reproduction factor of *Meloidogyne incognita* in brinjal cultivar Khotkhotia

The treatment effects against gall formation, egg masses, eggs, juveniles production and reproduction factor was presented in Table 4. Significant variations are observed among different treatments.

Lowest gall index (0.63) was recorded in treatment PL_P+M_P (Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) and PL_PM_{7DAP} (Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting) which was statistically similar to PL_P (Application of *P. lilacinus* at planting) and BC (blank control). The highest gall index (5.25) was recorded in M_P (Inoculation of *M. incognita* at planting). High gall index was also observed in M_PPL_{7DAP} (2.38) (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (3.63) (Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting).

In terms of number of egg masses per root, treatments effect differed significantly among them. The lowest number of egg masses per root (5.63) was observed in PL_P+M_P (Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting). This was statistically similar to PL_PM_{7DAP} (Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting), PL_P (Application of *P. lilacinus* at planting) and BC (blank control).

Considering number of eggs per egg mass, the highest effect of treatment was (121.9) recorded in in PL_P+M_P (Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting). This was statistically similar to PL_PM_{7DAP} (Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting), PL_P (Application of *P.*

lilacinus at planting) and BC (blank control). The lowest effect was (305.6) observed in M_P (Inoculation of *M. incognita* at planting). The lowest effect also observed in M_PPL_{7DAP} (213.1) (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (201.3) (Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting).

In terms of number of juveniles/g soil, treatments effect differed significantly among them. Maximum number of juveniles (17.50) was observed in M_P (Inoculation of *M. incognita* at planting) which was not statistically similar to all other treatments. The lowest number of juveniles (3.00) was observed in PL_P+M_P (Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) which was statistically similar to PL_PM_{7DAP} (Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting), PL_P (Application of *P. lilacinus* at planting) and BC (blank control).

Reproductions of *Meloidogyne incognita* were suppressed most by the application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting in PL_P+M_P (0.42) which was statistically similar to PL_PM_{7DAP} (Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting), PL_P(Application of *P. lilacinus* at planting) and BC (blank control). The highest reproduction factor (3.89) was recorded in M_P (Inoculation of *M. incognita* at planting) which was followed by M_PPL_{7DAP} (1.97) (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (2.07) (Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting).

Table 4: Effect of *P. lilacinus* application time on gall index, nematode population and reproduction factor of *Meloidogyne incognita* on brinjal cultivar Khotkhotia

Treatments	Gall Index (0-10 scale)	Number of egg masses/root	Number of eggs/egg mass	Number of J_2/g soil	Reproduction factor
BC	0.00 d	0.00 c	0.00 c	0.00 c	0.00 c
M_P	5.25 a	78.75 a	305.6 a	17.50 a	3.89 a
PL_P	0.00 d	0.00 c	0.00 c	0.00 c	0.00 c
$M_P + PL_P$	0.63 d	5.63 c	121.9 b	3.00 c	0.42 c
M_PPL_{7DAP}	2.38 c	40.00 b	213.1 ab	11.29 b	1.97 b
PL_PM_{7DAP}	0.63 d	7.75 c	183.8 b	3.18 c	0.48 c
	3.63 b	47.50 b	201.3 ab	10.94 b	2.07 b
LSD (P≥.10)	1.04	22.34	101.3	3.60	0.85

BC = Blank control (Without any inoculation), M_P = Inoculation of *Meloidogyne incognita* at planting, PL_P = Application of *P. lilacinus* at planting, PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting, M_PPL_{7DAP}=Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting, PL_PM_{7DAP} = Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting, $PL_{7DBP}M_P$ = Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting.

4.2.3. Effect of *P. lilacinus* application time on egg masses colonization and soil colonization by *P. lilacinus* in pot soil of brinjal var. Khotkhotia at harvest

Among different treatments the % of egg masses colonized by fungus (15.63) was recorded in PL_P+M_P (Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) which was statistically similar to PL_PM_{7DAP} (Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting). The lowest egg masses colonized by M_PPL_{7DAP} (12.50%) (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (9.38%) (Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting) (Figure 3).

Soil colonization ($CFUg^{-1}$ soil) was observed higher ($15.70x10^3$) in PL_P (Application of *P. lilacinus* at planting). Soil colonization ($CFUg^{-1}$ soil) was $4.18x10^3$ in PL_P+M_P (Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting), $3.39x10^3$ in M_PPL_{7DAP} (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting), $3.81x10^3$ in PL_PM_{7DAP} (Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting) and $3.00x10^3$ in $PL_{7DBP}M_P$ (Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting). (Figure 4)

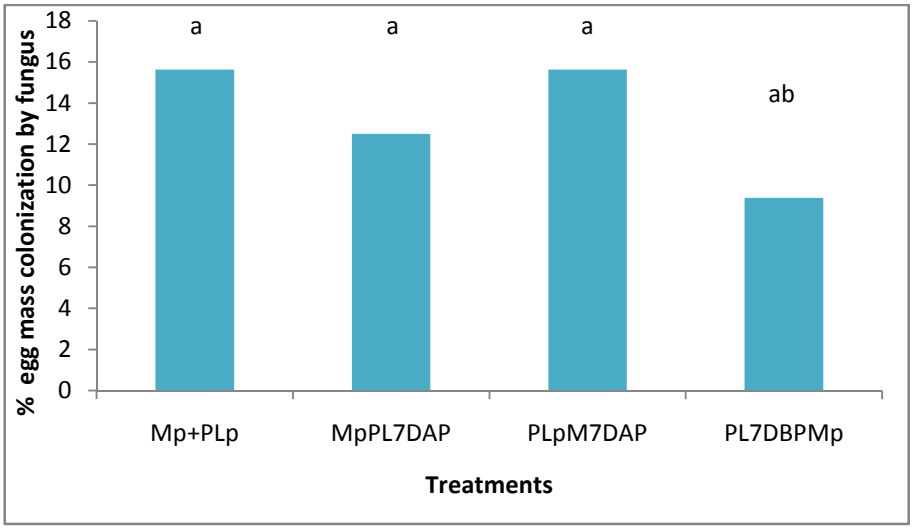


Figure 3: Effect of *P. lilacinus* application time on % egg masses colonization by *P. lilacinus* in brinjal var. Khotkhotia. Bars headed by different letters are significantly different

Here,

PL_P+M_p = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP} = Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP} = Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$ = Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting

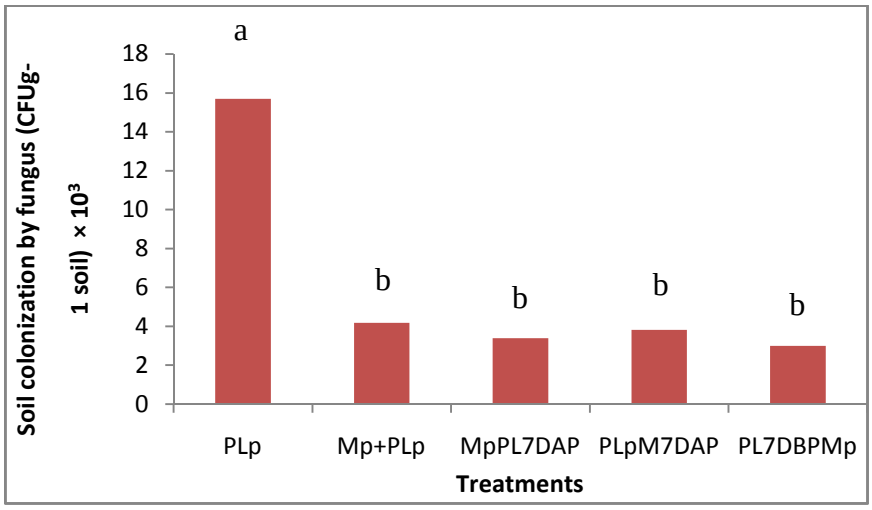


Figure 4. Effect of *P. lilacinus* application time on soil colonization by fungus($CFUg^{-1}$) in pot soil of brinja (var. Khotkhotia). Bars headed by different letters are significantly different

Here,

PL_P = Application of *P. lilacinus* at planting

PL_P+M_p = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP} = Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP} = Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$ = Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting

4.3.1. Effect of *Paecilomyces lilacinus* application time on growth parameters of BARI Tomato 14

Tomato plants inoculated with *M. incognita* showed significant reduction in their growth (Table 5). Shoot length, fresh weight and dry weights were significantly poor in nematode inoculated plant in compare to un-inoculated control. When tomato plants were inoculated with *P. lilacinus* at planting, there was no significant difference in length (24.11cm), fresh weight (8.69g) and dry weight (1.43g) of the plants in compare to un-inoculated control treatment of the plants length (23.42cm), fresh weight (7.73g) and dry weight (1.24g). In simultaneous application of *P. lilacinus* and inoculation of *M. incognita* at planting, plant length (24.48cm) significantly differed ($P \geq 0.10$) from the blank control (23.42cm). When compared to plants inoculated with *M. incognita* at planting (16.67cm), shoot length was significantly greater. When *P. lilacinus* was applied at planting and *M. incognita* at 7 days after planting, shoot length (20.31cm) differed significantly from the blank control. However, when *M. incognita* was inoculated at planting and *P. lilacinus* at 7 days after after planting, significant reduction was observed in shoot length (15.60cm) as compared to blank control (23.42cm) and shoot length differed from plants inoculated with nematode at planting (Table 5). Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting showed a reduction in shoot length (17.91cm) as compared to blank control. (Plate-12)

The fresh weight and dry weight of plants in various treatments showed a similar trend as length. Significant reduction occurred in fresh weight (5.09g) and dry weight (0.49g) due to the infection of *M. incognita* at planting. Application of *P. lilacinus* at planting did not cause an adverse effect on fresh weight (8.69g) and dry weight (1.43g) of plants. When fungus and nematode

Plate- 12: Photograph showing the effect of *Paecilomyces lilacinus* application time on shoot length of BARI Tomato 14 in comparison to control

BC = Blank control (Without any inoculation)

M_P = Inoculation of *Meloidogyne incognita* at planting (Negative control)

PL_P= Application of *P. lilacinus* at planting

PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP}= Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP}= Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$= Application of *P. lilacinus* at 7 days before planting and Inoculation of *M. incognita* at planting.

inoculated simultaneously at planting, fresh weight (10.09g) and dry weight (1.50g) of plants were significantly higher than plants inoculated with *M. incognita* at planting and the weights differed significantly from the blank control. In sequential inoculations, when nematode was inoculated at planting and *P. lilacinus* at 7 days after planting, fresh shoot weight (6.23g) and dry shoot weight (0.53g) of plants did not differ from plants inoculated with *M. incognita* at planting. On the other hand, when *P. lilacinus* was applied at planting and *M. incognita* at 7 days after planting, fresh weight (8.36g) and dry weight (1.41g) of shoot were significantly higher than plants inoculated with *M. incognita* at planting and the plants inoculated with nematode at planting and bioagent at 7 days after planting (Table 5). But application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting showed a reduction in fresh weight (7.63g) and dry weight (0.48g) of the shoot as compared to blank control but increase in fresh weight and dry weight of shoot in compare to *M. incognita* inoculated control.

Tomato plants inoculated with *M. incognita* showed significant reduction in their root growth (Table 5) and (Plate-13). Root length, fresh weight and dry weight of roots were significantly lower in negative control (*M. incognita* inoculated at planting) plant in compare to blank control. When tomato plants were applied with *P. lilacinus* at planting, there was no significant difference in root length (8.69cm), fresh weight (2.88g) and dry weight (0.63g) of the roots in compare to blank control treatment of the root length (7.73cm), fresh root weight (2.66g) and dry root weight (0.53g). In simultaneous application of fungus and inoculation of nematode at planting, the root length (10.09 cm) significantly higher (P=0.10) from the *M. incognita* control (5.09cm). When *P. lilacinus* was applied at planting and *M. incognita* at 7 days after planting, root length (8.36cm) differed significantly from the blank control. However, significant reduction was observed in root length in M_P (inoculation of *M. incognita* at planting) (5.09cm) as compared to blank control (7.73cm) and root length was 6.23cm, when nematode was inoculated at planting and *P.*

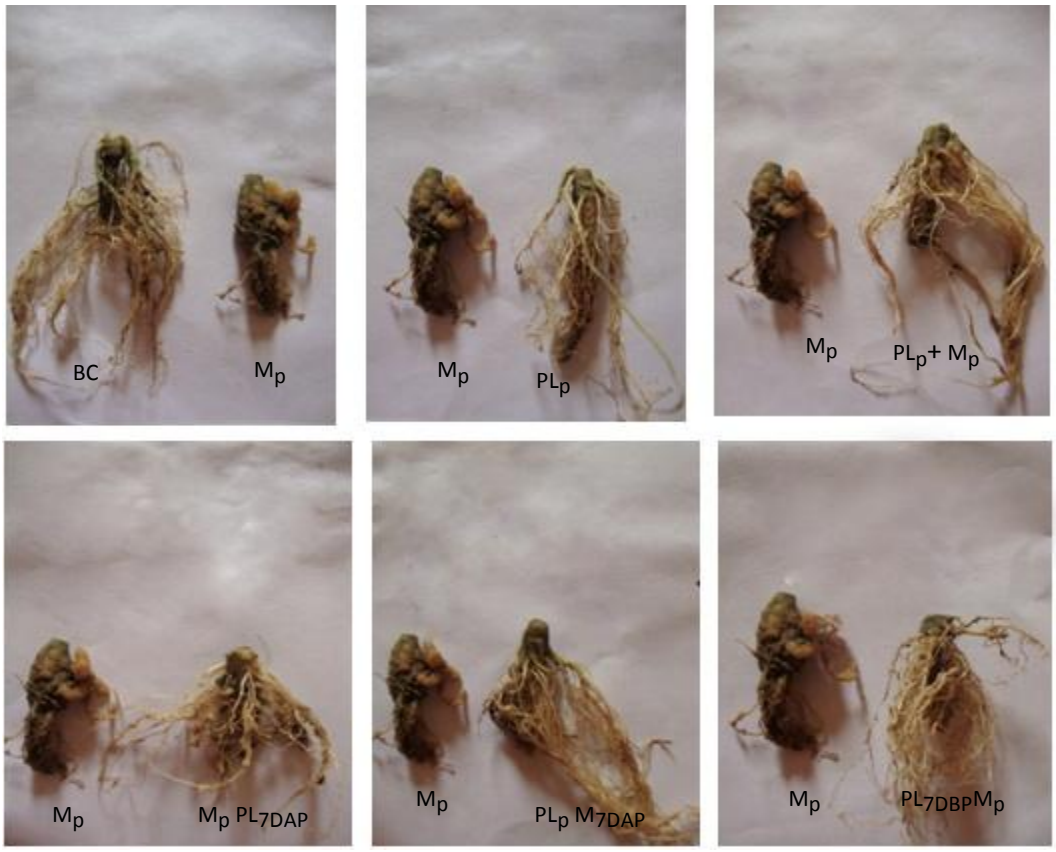


Plate- 13: Photograph showing the effect of *Paecilomyces lilacinus* application time on root growth of BARI Tomao 14 in comparison to control

BC = Blank control (Without any inoculation)

M_P = Inoculation of *Meloidogyne incognita* at planting (Negative control)

PL_P= Application of *P. lilacinus* at planting

PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP}= Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP}= Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$= Application of *P. lilacinus* at 7 days before planting and Inoculation of *M. incognita* at planting.

lilacinus at 7 days after planting (Table 5). Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting showed a reduction in root length (7.63cm) as compared to blank control but increased in fresh and dry weight of root in compare to *M. incognita* inoculated control.

The fresh and dry weights of roots in various treatments showed a similar trend as length. Significant reduction occurred in fresh weight (0.65 g) and dry weight (0.23 g) due to the inoculation of *M. incognita* at planting. Application of *P. lilacinus* at planting increased in fresh root weight (2.88 g) and dry root weight (0.63 g) of plants. When application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting, fresh root weight (3.29 g) and dry root weight (1.09 g) of plants were significantly higher than plants inoculated with *M. incognita* at planting and the root weights differed significantly from the blank control. In sequential inoculations, when *Meloidogyne incognita* was inoculated at planting and *P. lilacinus* at 7 days after planting, fresh root weight (0.89 g) and dry root weight (0.30 g) of plants did not differ from plants inoculated with *M. incognita* at planting. On the other hand, when *P. lilacinus* was applied at planting and *M. incognita* at 7 days after planting, fresh root weight (3.04 g) and dry root weight (1.20 g) were significantly higher than plants inoculated with *M. incognita* at planting and *M. incognita* was inoculated at planting and *P. lilacinus* at 7 days after planting (Table-5). Application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting showed a reduction in fresh root weight (1.46 g) and dry root weight (0.28 g) of the plant as compared to blank control.

Table 5. Effect of *Paecilomyces lilacinus* application time on the growth parameters of BARI Tomato 14

Treatments	Shoot Length (cm)	Shoot weight (g)		Root length (cm)	Root Weight (g)	
		Fresh Weight (g)	Dry Weight (g)		Fresh Weight (g)	Dry Weight (g)
BC	23.42 ab	7.73 abc	1.24 a	7.73 abc	2.66 a	0.53 bc
M_P	16.67 d	5.09 c	0.49 b	5.09 c	0.65 c	0.23 c
PL_P	24.11 a	8.69 ab	1.43 a	8.69 ab	2.88 a	0.63 b
PL_P+ M_P	24.48 a	10.09 a	1.50 a	10.09 a	3.29 a	1.09 a
M_PPL_{7DAP}	15.60 d	6.23 bc	0.53 b	6.23 bc	0.89 bc	0.30 bc
PL_PM_{7DAP}	20.31 bc	8.36 ab	1.41 a	8.36 ab	3.04 a	1.20 a
$PL_{7DBP}M_P$	17.91 cd	7.63 abc	0.48 b	7.63 abc	1.46 b	0.28 bc
LSD (P≥.10)	3.13	2.45	0.45	2.45	0.67	0.33

BC = Blank control (Without any inoculation), M_P = Inoculation of *Meloidogyne incognita* at planting, PL_P = Application of *P. lilacinus* at planting, PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting, M_PPL_{7DAP}=Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting, PL_PM_{7DAP} = Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting, $PL_{7DBP}M_P$ = Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting.

4.3.2. Effect of *P. lilacinus* application time on gall index, nematode population and reproduction factor of *Meloidogyne incognita* in BARI Tomato 14

4.3.2.1. Root-galling and egg mass production: The inoculation of *P. lilacinus* reduced root-galling and egg mass production of the nematode in compared to plants inoculated with *M. incognita* alone (Table 6). In application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting gall index was 0.50 and number of egg masses/root was 3.25 in comparison to 5.63 and 43.13, respectively in *M. incognita* inoculated plants. Similar reduction was observed, when *P. lilacinus* applied at planting and *M. incognita* at 7 days after planting where gall index was 0.25 and number of egg masses/root was 1.00. In other sequential inoculations, when nematode was inoculated at planting and bioagent at 7 days after planting, the gall index (2.38) and number of egg masses/root (9.25) was slightly reduced. In application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting, gall index was 1.38 and number of egg masses/root was 6.38 (Table 6).

4.3.2.2. Number of Juveniles/g soil: The application of *P. lilacinus* reduced number of juveniles/g soil as compared to plants inoculated with nematode alone. In application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting J_2/g soil were 104.4 in compare to 438.8 in *M. incognita* inoculated plants. Similar reduction was observed, when *P. lilacinus* was applied at planting and *M. incognita* at 7days after planting sequentially, where J_2/g soil was 71.88. In other sequential inoculations, inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting, J_2/g soil was 349.4 which was slightly reduced. In application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting J_2/g soil was 235.6 (Table 6).

4.3.2.3. Reproduction factor: The inoculation of *P. lilacinus* reduced reproduction factor (RF) as compared to plants inoculated with *Meloidogyne incognita* alone. In application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting RF was 8.41 in comparison to 36.46 in *Meloidogyne incognita* inoculated plants. Similar reduction was observed, when *P. lilacinus* followed by *Meloidogyne incognita* in sequential inoculation where RF (5.77). In other sequential inoculations, inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting RF (28.21). In application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting RF was 18.87.

Table 6. Effect of *P. lilacinus* application time on gall index, nematode population and reproduction factor of *Meloidogyne incognita* in BARI Tomato 14

Treatments	Gall Index (0-10 scale)	Number of egg masses/ Root	Number of eggs/egg mass	Number of J_2/g soil	Reproduction factor
BC	0.00 d	0.00 b	0.00 c	0.00 c	0.00 c
M_P	5.63 a	43.13 a	316.9 a	438.8 a	36.46 a
PL_P	0.00 d	0.00 b	0.00 c	0.00 c	0.00 c
$M_P + PL_P$	0.50 cd	3.25 b	62.50 c	104.4 c	8.41 c
M_PPL_{7DAP}	2.38 b	9.25 b	251.9 a	349.4 a	28.21 a
PL_PM_{7DAP}	0.25 d	1.00 b	28.75 c	71.88 c	5.77 c
$PL_{7DBP}M_P$	1.38 bc	6.38 b	155.0 b	235.6 b	18.87 b
LSD (P≥.10)	1.06	9.80	74.61	112.0	9.02

BC = Blank control (Without any inoculation), M_P = Inoculation of *Meloidogyne incognita* at planting, PL_P = Application of *P. lilacinus* at planting, PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting, M_PPL_{7DAP}=Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting, PL_PM_{7DAP} = Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting, $PL_{7DBP}M_P$ = Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting.

4.3.3. Effect of *P. lilacinus* application time on egg masses colonization and soil colonization by *P. lilacinus* in pot soil of BARI Tomato 14 at harvest

4.3.3.1. Egg masses colonization by *P. lilacinus* at harvest: In application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting egg masses colonization by fungus was 28.13% (Figure 5). Similar trend was observed in sequential inoculation when application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting where egg masses colonization by fungus was 25.00%. In other sequential inoculations, when inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting egg masses colonization by fungus was 21.88% which was reduced. In application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting egg masses colonization by fungus was 21.88% (Figure 5).

4.3.3.2. Soil colonization by fungus: Soil colonization by the biocontrol fungus was measured ($CFUg^{-1}$ soil) at harvest (Figure 6). In application of *P. lilacinus* at planting the fungal density was $2.18x10^3$ $CFUg^{-1}$ soil. In application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting soil colonization by fungus was higher ($2.35x10^3$ $CFUg^{-1}$ soil). Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting results a soil colonization of $2.18x10^3$ $CFUg^{-1}$ soil by fungus. When inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting soil colonization by fungus was $1.18x10^3$ $CFUg^{-1}$ soil. In application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting soil colonization by fungus was $1.53x10^3$ $CFUg^{-1}$ soil (Figure 6).

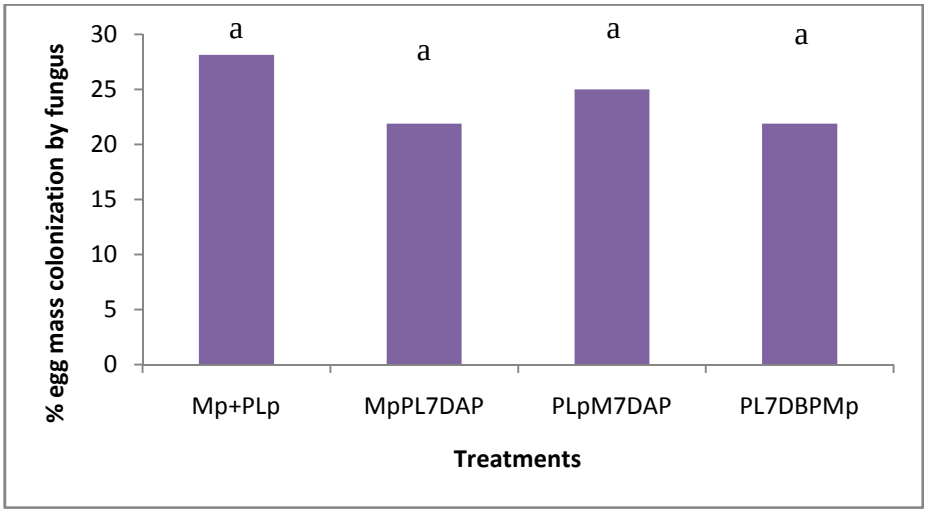


Figure 5. Effect of *P. lilacinus* application time on % egg masses colonization by *P. lilacinus* in BARI Tomato 14. Bars headed by similar letters are not significantly different

Here,

PL_P+M_p = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP} = Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP} = Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$ = Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting.

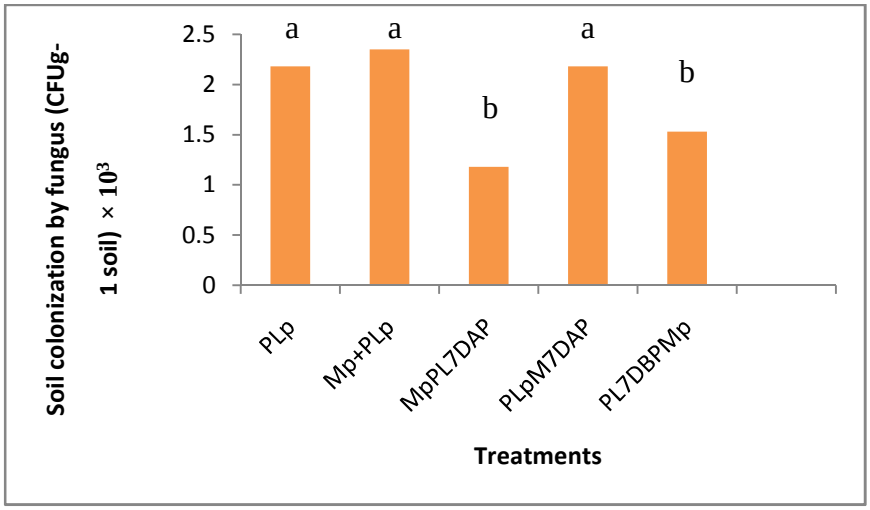


Figure 6. Effect of *P. lilacinus* application time on soil colonization by fungus (CFUg^{-1}) in pot soil of BARI Tomato 14. Bars headed by different letters are significantly different

Here,

PL_P = Inoculation of *P. lilacinus* at planting

PL_P+M_p = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP} = Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP} = Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$ = Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting.

4.4.1. Effect of *Paecilomyces lilacinus* application time on growth parameters of cucumber variety Kashinda

Inoculation of *Paecilomyces lilacinus* in different time of application provided different result. Inoculation of *P. lilacinus* reduced the damage caused by *Meloidogyne incognita* and the effect of the treatments on growth characterstics of cucumber plant viz. shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight and root dry weight are presented in Table 7.

The highest shoot length (77.40 cm) was recorded in the treatment PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting) which was statistically similar to PL_P+M_p (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting), PL_P (application of *P. lilacinus* at planting) and BC (blank control). The lowest shoot length (28.42 cm) was observed in M_P (inoculation of *M. incognita* at planting) which was statistically similar to M_PPL_{7DAP} (inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting) (Table 7 and Plate 14).

The highest fresh weight of shoot (25.84 g) was recorded in the treatment PL_P+M_p (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) which was statistically similar to PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting), PL_P (application of *P. lilacinus* at planting) and BC (blank control). The lowest result (7.21 g) was observed in M_P (inoculation of *M. incognita* at planting) which was statistically similar to M_PPL_{7DAP} (inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at

Plate- 14: Photograph showing the effect of *Paecilomyces lilacinus* application timeon shoot length of cucumber var. Kashinda in comparison to control

BC = Blank control (Without any inoculation)

M_P = Inoculation of *Meloidogyne incognita* at planting (Negative control)

PL_P= Application of *P. lilacinus* at planting

PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP}= Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP}= Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$= Application of *P. lilacinus* at 7 days before planting and Inoculation of *M. incognita* at planting.

planting). Among the different treatments, the highest dry weight of shoot (12.57 g) was recorded in the treatment PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting) which was statistically similar to PL_P+M_p (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting), PL_P (application of *P. lilacinus* at planting) and BC (blank control). But the lowest dry weight of shoot (2.88 g) was observed in M_P (inoculation of *M. incognita* at planting) which was statistically similar to M_PPL_{7DAP} (inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting).

It was observed that application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting and simultaneous application of *P. lilacinus* and *M. incognita* at planting showed good results among the treatments (Table 7). In terms of length of root, treatments effect differed significantly among themselves (Plate 15). Maximum root length (30.13 cm) was observed in PL_P+M_p (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) which was statistically similar to PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting), PL_P (application of *P. lilacinus* at planting) (12.44 cm) was observed in M_P (inoculation of *M. incognita* at planting) which was statistically similar to M_PPL_{7DAP} (inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting). The root length was 20.33 cm in $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting).

Plate- 15: Photograph showing the effect of *Paecilomyces lilacinus* application time on root growth of cucumber var. Kashinda in comparison to control

BC = Blank control (Without any inoculation)

M_P = Inoculation of *Meloidogyne incognita* at planting (Negative control)

PL_P= Application of *P. lilacinus* at planting

PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP}= Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP}= Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$= Application of *P. lilacinus* at 7 days before planting and Inoculation of *M. incognita* at planting.

Considering fresh weight of root, the highest weight (15.94 g) was recorded in PL_P (application of *P. lilacinus* at planting) which was statistically similar to PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting), PL_P+M_p (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) and BC (blank control). The lowest result (3.99 g) was observed in M_P (inoculation of *M. incognita* at planting). The fresh weight of root was 6.61 g in M_PPL_{7DAP} (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) which was statistically similar to $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting).

Among the different treatments, the highest dry weight of root (8.00 g) was recorded in PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting) which was statistically similar to PL_P+M_p (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting). The dry weight of root was 6.49 g in PL_P (application of *P. lilacinus* at planting) which was statistically similar to BC (blank control). But the lowest dry weight of root (1.43 g) was observed in M_P (inoculation of *M. incognita* at planting). The dry weight of root was 2.59 g in M_PPL_{7DAP} (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and 3.63 g in $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting).

Table 7. Effect of *Paecilomyces lilacinus* application time on the growth parameters of cucumber var. Kashinda

Treatments	Shoot Length (cm)	Shoot weight (g)		Root length (cm)	Root Weight (g)	
		Fresh Weight (g)	Dry Weight (g)		Fresh Weight (g)	Dry Weight (g)
BC	61.71 a	22.42 a	10.86 a	26.50 b	12.13 b	6.13 b
M_P	28.42 b	7.21 c	2.88 b	12.44 d	3.99 d	1.43 e
PL_P	69.88 a	24.23 a	11.73 a	27.31 ab	15.94 a	6.49 b
M_P + PL_P	76.11 a	25.84 a	12.27 a	30.13 a	14.40 a	7.58 a
M_PPL_{7DAP}	36.84 b	9.88 bc	5.45 b	13.48 d	6.61 c	2.59 d
PL_PM_{7DAP}	77.40 a	25.71 a	12.57 a	29.26 ab	15.27 a	8.00 a
$PL_{7DBP}M_P$	34.28 b	13.14 b	5.43 b	20.33 c	7.13 c	3.63 c
LSD (P≥.10)	17.15	4.18	2.69	3.15	1.95	0.96

BC = Blank control (Without any inoculation), M_P = Inoculation of *M. incognita* at planting, PL_P = Application of *P. lilacinus* at planting, PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting, M_PPL_{7DAP}=Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting, PL_PM_{7DAP} = Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting, $PL_{7DBP}M_P$ = Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting.

4.4.2. Effect of *P. lilacinus* application time on gall index, nematode population and reproduction factor of *Meloidogyne incognita* in cucumber cultivar Kashinda

The treatment effects against gall formation, egg masses, eggs, juveniles production and reproduction factor are presented in Table 8. Significant variations were observed among different treatments.

Lowest gall index (1.25) was recorded in treatment PL_P+M_p (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting). This was statistically similar to PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting), PL_P (Inoculation of *P. lilacinus* at planting) and BC (blank control). The highest gall index (6.50) was recorded in M_P (Inoculation of *M. incognita* at planting). High gall index (3.88) was also observed in M_PPL_{7DAP} (inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) which was statistically similar to $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting).

In terms of number of egg masses per root, treatments effect differed significantly among them. The lowest number of egg masses per root (24.38) was observed in PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting). This was statistically similar to PL_P+M_p (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting), PL_P (Inoculation of *P. lilacinus* at planting) and BC (blank control).

Considering number of eggs per egg mass, the highest effect of treatment was (75.00) recorded in PL_P+M_p (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting). This was statistically similar to PL_PM_{7DAP} (application of *P.*

lilacinus at planting and *M. incognita* at 7 days after planting), PL_P (Inoculation of *P. lilacinus* at planting) and BC (blank control). The lowest effect was (212.5) observed in M_P (Inoculation of *M. incognita* at planting) which was statistically similar to M_PPL_{7DAP} (inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting).

In terms of number of juveniles/g soil, treatments effect differed significantly among them. Maximum number of J_2/g soil (16.88) was observed in M_P (Inoculation of *M. incognita* at planting) which was statistically similar to M_PPL_{7DAP} (inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting). The lowest number of juvenile (3.00) was observed in PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting). This was statistically similar to PL_P+M_p (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting), PL_P (Application of *P. lilacinus* at planting) and BC (blank control).

Reproductions of *Meloidogyne incognita* were suppressed most by the application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting in PL_PM_{7DAP} (0.63) which was statistically similar to PL_P+M_p (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting), PL_P (Application of *P. lilacinus* at planting) and BC (blank control). The highest reproduction factor (3.34) was recorded in M_P (Inoculation of *M. incognita* at planting). The highest reproduction factor (2.70) was also recorded in M_PPL_{7DAP} (inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and also (1.87) in $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting).

Table 8: Effect of *P. lilacinus* application time on gall index, nematode population and reproduction factor of *Meloidogyne incognita* in cucumber cultivar Kashinda

Treatments	Gall Index (0-10 scale)	Number of egg masses/root	Number of eggs/egg mass	Number of J_2/g soil	Reproduction factor
BC	0.00 d	0.00 d	0.00 b	0.00 c	0.00 c
M_P	6.50 a	109.4 a	212.5 a	16.88 a	3.34 a
PL_P	0.00 d	0.00 d	0.00 b	0.00 c	0.00 c
$M_P + PL_P$	1.25 cd	28.13 cd	75.00 b	3.25 c	0.72 c
M_PPL_{7DAP}	3.88 b	78.75 ab	184.1 a	13.38 ab	2.70 ab
PL_PM_{7DAP}	1.75 c	24.38 cd	78.75 b	3.00 c	0.63 c
$PL_{7DBP}M_P$	3.38 b	50.63 bc	161.0 a	10.50 b	1.87 b
LSD (P≥.10)	1.43	37.32	78.52	3.53	0.89

BC = Blank control (Without any inoculation), M_P = Inoculation of *Meloidogyne incognita* at planting, PL_P = Application of *P. lilacinus* at planting, PL_P+ M_P = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting, M_PPL_{7DAP}=Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting, PL_PM_{7DAP} = Application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting, $PL_{7DBP}M_P$ = Application of *P. lilacinus* at 7 days before planting and inoculation of *M. incognita* at planting.

4.4.3. Effect of *P. lilacinus* application time on egg masses colonization and soil colonization by *P. lilacinus* in pot soil of cucumber var. Kashinda at harvest

Among different treatments the % of egg masses colonized by fungus (31.25) was recorded in PL_P+M_p (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) which was statistically similar to PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting). The lowest egg masses (18.75%) colonized by M_PPL_{7DAP} (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) which was statistically similar to $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting). (Figure 7)

Soil colonization ($CFUg^{-1}$ soil) was observed higher (5.53×10^3) in PL_P (Application of *P. lilacinus* at planting). Soil colonization ($CFUg^{-1}$ soil) was 2.71×10^3 in PL_P+M_p (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting), 1.75×10^3 in M_PPL_{7DAP} (Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting), 3.36×10^3 in PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting) and 1.58×10^3 in $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting). (Figure 8)

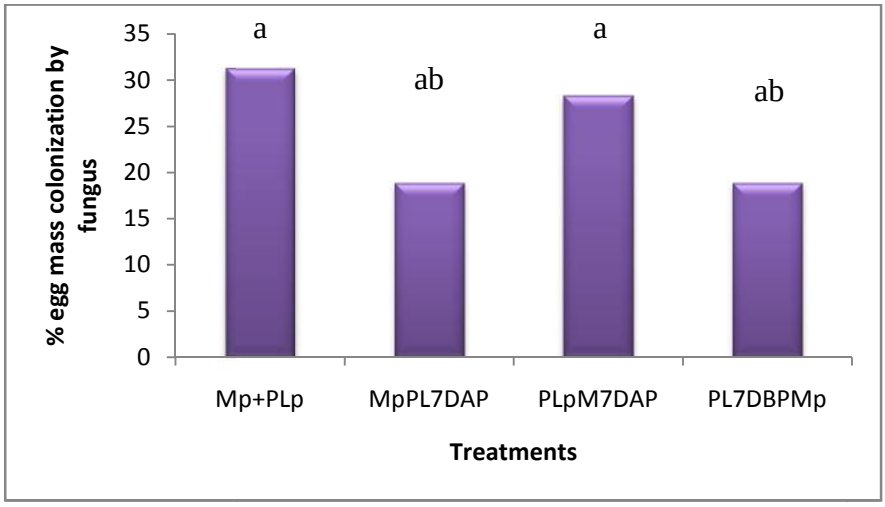


Figure 7. Effect of *P. lilacinus* application time on % egg masses colonization by *P. lilacinus* in cucumber var. Kashinda.Bars headed by different letters are significantly different

Here

PL_P+M_p = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP} = Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP} = Application *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$ = application of *P. lilacinus* at 7 days before plantig and inoculation of *M. incognita* at planting.

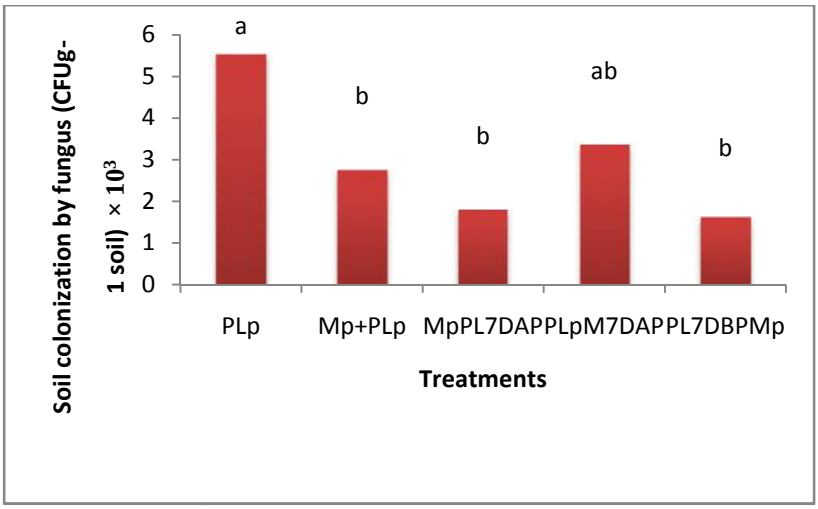


Figure 8. Effect of *P. lilacinus* application time on soil colonization by fungus ($CFUg^{-1}$) in pot soil of cucumber var. Kashinda.Bars headed by different letters are significantly different

Here,

PL_P = Application of *P. lilacinus* at planting

PL_P+M_p = Application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting

M_PPL_{7DAP} = Inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting

PL_PM_{7DAP} = Application *P. lilacinus* at planting and *M. incognita* at 7 days after planting

$PL_{7DBP}M_P$ = application of *P. lilacinus* at 7 days before plantig and inoculation of *M. incognita* at planting.

DISCUSSION

The experiment was conducted to study the effect of application time of nematophagous fungus *Paecilomyces lilacinus* as a bio control agent against root-knot nematode (*Meloidogyne incognita*) on brinjal, tomato and cucumber in pot condition. Our results indicated that using microbial agents suppressed the root-knot nematodes and resulted in positive changes in plant growth.

In the experiment, it was found that due to the infection of nematode (*Meloidogyne incognita*) root knot occoured and reduced plant growth. In the present study it was observed that brinjal, tomato and cucumber plants inoculated with 10,000 eggs of *M. incognita* showed significant reduction in plant growth parameters in comparison to un-inoculated control. A significant reduction in plant length of shoot and root, fresh weight of shoot and root, dry weight of shoot and root was observed. Reduced plant growth characters by inoculation of *M. incognita* was also reported earlier by Esfahani and Ansaripour (2006), Mucksood and Tabreiz (2010) and Kiewnick and Sikora (2006).

The results of the present experiment indicated that application of *P. lilacinus* suppressed root knot and increased plant growth parameters. In the present study it was observed that brinjal, tomato and cucumber plants inoculated with 36×10^7 spore of *P. lilacinus* showed significant increase in plant growth parameters in comparison to un-inoculated control. A significant increase in plant length of shoot and root, fresh weight of shoot and root, dry weight of shoot and root was observed. Improved plant growth characters by application of *P. lilacinus* in controlling root knot nematodes was also reported earlier by Walia *et al.* (1999), Khan and Goswami (2000), and Hasan (2004). *Paecilomyces lilacinus* was the best treatment which reduced root galls and egg masses in tomato plants under greenhouse conditions (Khalil *et al.*, 2012b). Moreover, the activity of *P. lilacinus* attributed to ability to infect eggs, juveniles and females of *M. javanica* by direct hyphal

penetration (Khan *et al*., 2006). Moreover, *P. lilacinus* contains protease and chitinase enzyme which play an important role in the degradation of the egg shell (Khan *et al*., 2004). Meanwhile, Khan *et al*. (2012) recorded an enhancement in growth and yield of eggplants with biocontrol agents *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma harzianum* as a result to suppress galls formation and egg masses. Also, the using of bioproducts of *Bacillus megaterium*, *Trichoderma album*, *T. harzianum* and *Ascophyllum nodosum* showed decreasing in the second stage juveniles and root galls on tomatoes (Radwan *et al.*, 2012).

Kalele *et al.* (2010) obtained that PL251 have some suppressive effect as a nematode biocontrol agent. This was explained by the reduced *Meloidogyne* spp. population densities both in the soil and roots in both tomato and cucumber.

Results of pot experiment demonstrated the efficacy of biocontrol fungus *P. lilacinus* in controlling the root-knot nematode *Meloidogyne incognita* with reduction in galling and nematode population. *P. lilacinus* enhanced plant growth and reduced galling index and nematode population. In similar experiment, Aminuzzaman *et al.* (2011) reported that pellets of *P. lilacinus* enhanced plant growth, reduced galling index and nematode population. They also mentioned that root galling index and final nematode population decreased up to 40.7 and 73%, respectively for tomato and 55.6 and 66.9%, respectively for brinjal.

Kiewnick and Sikora (2006) conducted a growth chamber experiments using tomato, where they found that PL251 reduced 66% root galling and 71% population of *M. incognita*. Sun *et al.* (2006) observed that *P. lilacinus* was highly pathogenic to root-knot nematode and it reduced root gall index by 13.4-58.9%.

The fungus was effective in reducing the resulting population of *Meloidogyn*e *incognita*. This proved the ability of *P. lilacinus* as a bio-control agent of *Meloidogyne incognita*.

The performance of bioagent in relation to reduced root galling and egg mass production was significantly better in simultaneous inoculation or in inoculation, where *P. lilacinus* preceeded *Meloidogyne* i*ncognita* after seven days.

In this exmeriment, it is observed that *Meloidogyne incognita* readily infected brinjal (Var. Singnath and Khotkhotia), tomato (Var. BARI Tomato 14) and cucumber (Var. Kashinda), retarded its growth, and reduced the fresh and dry weight of the plants. Apparently, *P. lilacinus* was effective in suppressing *Meloidogyne incognita*, but time of application was important. In simultaneous inoculations, adverse effects of *Meloidogyne incognita* were greatly reduced and plant growth was as good as un-inoculated plants. Similar better result was also found in sequential inoculation, when *P. lilacinus* was applied at planting and *M. incognita* at 7 days after planting.

In the present study, it was found that *P. lilacinus* penetrated the eggs and developed profusely inside and over the eggs completely inhibiting juvenile development. Some juveniles were attacked and deformed. This happened mostly when simultaneous inoculations occoured and when *P. lilacinus* followed by *Meloidogyne incognita* after one week. In simultaneous inoculation a high percentage of egg masses (31.25) was infected by fungus, the root galling (1.25) and egg mass production (28.13) were also poor. Similarly, good result was also obtained in sequential inoculation, when *P. lilacinus* followed by *M. incognita* after one week. But the negative results was obtained when *Meloidogyne incognita* followed by *P. lilacinus* after one week in sequential inoculation. A low percentage of egg masses (18.75) was infected, the root galling (3.88) and egg mass production (78.75) were also high. This was primarily because the fungus was not present in the soil at the time the juvenile penetrated the roots. Consequently, plant growth was suffered and the population growth of the nematode was increased.

Similar result was also obtained earlier by Kalele *et al.* (2010) in their study. The results showed PL251 to have some suppressive effect as a nematode biocontrol agent. In

addition, reduction of nematode damage parameter i.e. root galling intensity as well as nematode reproduction rate was attained when the application was done at planting as well as at pre-planting. *P. lilacinus* strain 251 showed promising results as a biological control agent for root-knot nematode. After 10 weeks, there was reduction of 54 to 74% of J_2 in both soil and tomato roots, respectively. The findings confirmed the results of Lara *et al.* (1996), who reported that *P. lilacinus* significantly reduced soil and root population of *M. incognita* and increased yield of tomato. The results are in agreement with earlier findings of Santos *et al.* (1992) and Carneiro and Gomes (1993), who observed the variations of *P. lilacinus* for egg parasitism of *M. incognita*.

Ganaie and Khan (2010) reported that the growth parameters were improved by biological control agent *P. lilacinus* while it also reduced *M. javanica* reproduction on simultaneous and sequential inoculation. Walia *et al.* (1999) observed improved tomato plant growth parameter with simultaneous and sequential inoculations of *P. lilacinus* and *Meloidogyne* spp.

However, prior inoculation of *Meloidogyne incognita* followed by *P. lilacinus* after seven days was not that effective. This variation was in favor of the application of timing of *P. lilacinus* which is an egg parasite (Jatala, 1986). Its presence in the rhizosphere of roots at the time of penetration may reduce the number of juveniles that could ingress the roots. This finding is in agreement with Holland *et al.* (2001), who stated that *P. lilacinus* colonized the root and protects its surface from root knot nematode attacks. It also reduced the number of viable eggs and juveniles of the second generation during the experimental period.

Therefore, it is plausible to expect that the presence of *P. lilacinus* before the nematode attack would offer greater protection to plants. *P. lilacinus*, a saprophytic soil-inhabitant is not expected to cause any harm to plant roots in general and is not a plant endophyte, as was true in these trials too. But, when *Meloidogyne incognita* eggs, egg masses and

juveniles were present, it attacked and destroyed them to a great extent, thereby improving plant growth. It is clear that, fungal hyphae of *P. lilacinus* penetrate eggshells of *Meloidogyne incognita* with enzymes and pressure following the formation of a simple appressorium. The entire contents of the egg are then used as a food resource by the fungus, completely destroying the embryo or larva in the process. Eggs containing embryoes or larvae can then become infected by the fungus (Alamgir *et al.*, 1997).

It is also important to know what will happen to a bio-control agent after it has been applied to the soil. The persistence of *P. lilacinus* after application to the soil has been estimated and results indicated that, levels fall after application and after a few months, it is difficult to isolate the fungus from the soil. This suggests that *P. lilacinus* will only cause short-term disturbances to the soil biota and will not have any long-term effect as other bio-control agents do (Lacky and De Leij, 1992).

In my experiment it was also found that application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting was not as good as simultaneous inoculation and sequential inoculation. Probably *P. lilacinus* can't survive in the soil without its plant host or nematode host. That results a rapid reduction of $CFUg^{-1}$ of soil after application. This was also proved by a comparatively higher root galling, nematode reproduction factor and lower egg masses and soil colonization by the fungus.

Therefore plant hosts have some influence to colonize *P. lilacinus* in root zone and soil. Without the presence of host the proliferation and colonization of *P. lilacinus* is lower. This was also proved when Kiewnick *et al.* (2011) applied *P. lilacinus* with nematode 7 days before transplantation where nematodes were first infected by the bioagent resulted a better protection of tomato plant against root knot nematode.

But in other work which was done by Kalele *et al.* (2010) where pre-planting application *P. lilacinus* without any host results better protection of tomato plants against root knot

nematode. This results indicated that soil proliferation and colonization of *P. lilacinus* may vary from species to species and different isolates of *P. lilacinus* of different geographic origin have different bioefficacy to adopt in the soil and controlling the enemy. Soil properties might have some role that also influence *P. lilacinus* population to decline in soil over time. So not only time of application of *P. lilacinus* is important but it is also important to know about the nature of the bioagent and its adaptability in different soil environment with or without hosts.

SUMMARY AND CONCLUSION

The pot experiment was conducted in shade house of the Department of Plant Pathology, Sher-e-Bangla agricultural University, Dhaka. Pot experiment were conducted to study the effect of *P. lilacinus* application time in controlling root-knot nematode (*Meloidogyne incognita*) and growth parameters of some selected vegetables viz. brinjal, tomato and cucumber.

In the pot experiment the treatments were blank control (BC), inoculation of *M. incognita* at planting (M_P), application of *P. lilacinus* at planting (PL_P), application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting (PL_P+M_P), inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting (M_PPL_{7DAP}), application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting (PL_PM_{7DAP}) and application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting ($PL_{7DBP}M_P$). Brinjal varieties, Singnath and khotkhotia, tomato variety BARI-14 and cucumber variety Kashinda was used in this study. The experiment was laid out in Randomized Complete Block Design (RCBD) with 8 replications. Inoculation was done in pot soil by drenching of spore suspension @ 36×10^7 spore/plant with the help of micropipette. Spore suspension was mixed with the pot soil carefully. Mature eggmass of nematode (*Meloidogyne incognita*) was collected from severely galled roots of tomato plants. Then the egg masses were picked and eggs per egg mass was counted. Each plant was inoculated with 20 egg masses equivalent to approximately 10,000 eggs on the time of transplanting of plant. Data was recorded at 60 days after transplanting (DAT).

In brinjal variety Singnath, the highest shoot length (45.35cm) and the highest dry weight of shoot (6.58g) were recorded in PL_P+M_P (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) which were statistically similar to PL_PM_{7DAP}

(application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting). The highest fresh weight of shoot (34.00g), maximum root length (23.51cm), the highest fresh weight of root (21.44g), the highest dry weight of root (10.4g) were recorded in PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting) which were statistically similar to PL_P+M_P (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting). The lowest shoot length (34.13cm), the lowest fresh weight of shoot (23.79g), lowest dry weight of shoot (3.95g), lowest root length (19.16cm), lowest fresh weight of root (10.60g), lowest dry weight of root (7.55g) were recorded in M_P (inoculation of *M. incognita* at planting) which were statistically similar to M_PPL_{7DAP} (inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting). The gall index (1.50), egg masses per root (97.50), eggs per egg mass (99.38) were lowest in PL_P+M_P (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) and number of juveniles per gram soil (90.25), reproduction factor (9.76) were lowest in PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting) compared to M_P (inoculation of *M. incognita* at planting).

In brinjal variety Khotkhotia, the highest shoot length (25.98cm), the highest fresh weight of shoot (10.84g) and the highest dry weight of shoot (4.18g) were recorded in PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting) which were statistically similar to PL_P+M_P (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting). The maximum root length (15.81cm), the highest fresh weight of root (12.96g), the highest dry weight of root (4.56g) were recorded in PL_P+M_P (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting)which were statistically similar to PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting). The lowest shoot length (18.10cm), the lowest fresh weight of shoot (4.01g), lowest dry weight of shoot (1.24 g), lowest root

length (10.81cm), lowest fresh weight of root (4.13g), lowest dry weight of root (0.76g) were recorded in M_P (inoculation of *M. incognita* at planting) which were statistically similar to M_PPL_{7DAP} (inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting). The gall index (0.63), egg masses per root (5.63), eggs per egg mass (121.9), number of juveniles per gram soil (3.00) and reproduction factor (0.42) were lowest in PL_P+M_P (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) compared to M_P (inoculation of *M. incognita* at planting).

In BARI Tomato 14, the highest shoot length (24.48cm), the highest fresh weight of shoot (10.09g), the highest dry weight of shoot (1.50g), the maximum root length (10.09cm), the highest fresh weight of root (3.29g) and the highest dry weight of root (1.09g) were recorded in PL_P+M_P (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) which were statistically similar to PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting). The shoot length (16.67cm), fresh weight of shoot (5.09g), dry weight of shoot (0.49g), root length (5.09cm), fresh weight of root (0.65g), dry weight of root (0.23g) were lowest in M_P (inoculation of *M. incognita* at planting) which were statistically similar to M_PPL_{7DAP} (inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting). The gall index (0.25), egg masses per root (1.00), eggs per egg mass (28.75), number of juveniles per gram soil (71.88) and reproduction factor (5.77) were lowest in PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting) compared to M_P (inoculation of *M. incognita* at planting).

In cucumber variety Kashinda, the shoot length (77.40cm), dry weight of shoot (12.57g), fresh weight of root (15.27g) and dry weight of root (8.00g) were highest in PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting) which were statistically similar to PL_P+M_P (application of *P. lilacinus* and inoculation of *M.*

incognita simultaneously at planting). The highest fresh weight of shoot (25.84g) and the maximum root length (30.13cm) were recorded in PL_P+M_P (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) which were statistically similar to PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting). The shoot length (28.42cm), fresh weight of shoot (7.21g), dry weight of shoot (2.88g), root length (12.44cm), fresh weight of root (3.99g), dry weight of root (1.43g) were lowest in M_P (inoculation of *M. incognita* at planting) which were statistically similar to M_PPL_{7DAP} (inoculation of *M. incognita* at planting and *P. lilacinus* at 7 days after planting) and $PL_{7DBP}M_P$ (application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting). The gall index (1.25) and eggs per egg mass (75.00) were lowest in PL_P+M_P (application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting) and egg masses per root (24.38), number of juveniles per gram soil (3.00) and reproduction factor (0.63) were lowest in PL_PM_{7DAP} (application of *P. lilacinus* at planting and *M. incognita* at 7 days after planting) compared to M_P (inoculation of *M. incognita* at planting).

Considering the overall results it is concluded that most of parameters the effect of simultaneous application of *P. lilacinus* and inoculation of *M. incognita* at planting and sequential inoculation where *P. lilacinus* was applied at planting and *M. incognita* at 7 days after planting, the results were statistically similar. So, *P. lilacinus* might be most useful either simultaneous inoculation or sequential inoculation where *P. lilacinus* was applied at planting and *M. incognita* at 7 days after planting in controlling root knot nematode (*M. incognita*) with increasing growth parameters of brinjal, tomato and cucumber plants. However, further experiment need to be conduct including more vegetables available in the country at different agro-ecological zone in order to evaluate and timely use of bio-control fungus *P. lilacinus* in controlling root knot nematode (*M. incognita*).

In the present study it was also found that application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting was not as good as simultaneous inoculation and sequential inoculation. Probably soil colonization ability of *P. lilacinus* is greatly reduced in absence of plant or nematode host during the pre-planting application time.

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