EFFECTS OF *Meloidogyne incognita* INOCULUM DENSITY AND APPLICATION RATE OF *Paecilomyces lilacinus* ON BIOCONTROL EFFICACY OF BIOAGENT AGAINST ROOT KNOT OF BRINJAL

MOST. SINTHIA SARVEN

DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA 1207

JUNE, 2013

EFFECTS OF *Meloidogyne incognita* INOCULUM DENSITY AND APPLICATION RATE OF *Paecilomyces lilacinus* ON BIOCONTROL EFFICACY OF BIOAGENT AGAINST ROOT KNOT OF BRINJAL

By

MOST. SINTHIA SARVEN

Registration No. 06-01870

A Thesis
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

SEMESTER: JANUARY-JUNE, 2013

Approved By:

.....	.....
(Dr. F. M. Aminuzzaman)	**(Dr. M. Salahuddin M. Chowdhury)**
Associate Professor	**Professor**
Supervisor	**Co-Supervisor**

.....
Dr. F. M. Aminuzzaman
Associate Professor
Chairman
Examination Committee
Department of Plant Pathology
Sher-e-Bangla Agricultural University, Dhaka

DEDICATED
TO
MY BELOVED PARENTS

Department of Plant Pathology
Sher-e-Bangla Agricultural University
Dhaka-1207, Bangladesh

Fax: +88029112649
Web site: www.sau.edu.bd

CERTIFICATE

This is to certify that the thesis entitled, *"EFFECTS OF Meloidogyne incognita INOCULUM DENSITY AND APPLICATION RATE OF Paecilomyces lilacinus ON BIOCONTROL EFFICACY OF BIOAGENT AGAINST ROOT KNOT OF BRINJAL"* submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in the partial fulfillment of the requirements for the degree of *MASTER OF SCIENCE (M.S.) IN PLANT PATHOLOGY*, embodies the result of a piece of *bona fide* research work carried out by *MOST. SINTHIA SARVEN* **bearing** *Registration No. 06-01870* 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.

Dated: 31st December, 2013
Place: Dhaka, Bangladesh

(Dr. F. M. Aminuzzaman)
Associate Professor
Department of Plant Pathology
Supervisor

ACKNOWLEDGEMENTS

First of all I would like to thank to ***Almighty Allah****, the most merciful and compassionate. The most gracious and beneficent to whom every praise is due and his* ***Prophet Muhammad*** *(****SM****) who is forever a Torch of knowledge and guidance for humanity as a whole with whose delighting the present endeavor has been beautiful.*

Now I would like to express my earnest respect, sincere appreciation and enormous indebtedness to my reverend supervisor, ***Dr. F. M. Aminuzzaman****, Associate Professor and Chairman, Department of Plant Pathology, Faculty of Agriculture, Sher-e- Bangla Agricultural University, Dhaka. I am grateful to his ever inspiring guidance, keen interest, helpful commentary, scholastic and constructive suggestions and polite behavior throughout the course of my study.*

I wish to express my gratitude and best regards to my respected Co-Supervisor, ***Dr. M. Salahuddin M. Chowdhury****, Professor, Department of Plant Pathology, Faculty of Agriculture, Sher-e- Bangla Agricultural University, Dhaka, for his continuous direction, constructive criticism, encouragement and valuable suggestions in carrying out the research work and preparation of this thesis.*

I am highly grateful to my honorable teachers Prof. Dr. Md. Rafiqul Islam, Professor Mrs. Nasim Akhtar, Prof. Nazneen Sultana, Khadija Akhter, Associate Professor, Abu Noman Faruq Ahmmed, Assistant Professor, Dr. Nazmoon Naher Tonu, Assistant Professor, Md. Belal Hossain, Assistant Professor, Sukti Rani Chowdhury, Assistant Professor, Md. Ziaur Rahman Bhuiya, Lecturer, Department of Plant Pathology, and Professor Dr. Md. Razzab Ali, Department of Entomology, Faculty of Agriculture, Sher-e –Bangla Agricultural University, for their valuable teaching, direct and indirect advice, and encouragement and co-operation during the whole study period.

I am pleased to thank all staffs and workers of Plant Pathology Department of Sher-e-Bangla Agricultural University, Dhaka. for their valuable and sincere help in carrying out the research work.

I also thanks to my friends Sanzida Hoque, Roksana Akter, Kohinur Begum, Enamul Huq , Faria and my roommates for their help, support, inspiration and patience during my work.

I found no words to thanks my parents, my sisters, my sister in low, my brother- in- low, brothers especially Md. Atiar Rohman for their unquantifiable love and continuous support, their never ending affection, immense strength and untiring efforts for bringing my dream to proper shape. They were constant source of inspiration, zeal and enthusiasm in the critical moment of my studies.

The Author

EFFECTS OF *Meloidogyne incognita* INOCULUM DENSITY AND APPLICATION RATE OF *Paecilomyces lilacinus* ON BIOCONTROL EFFICACY OF BIOAGENT AGAINST ROOT KNOT OF BRINJAL

By

MOST. SINTHIA SARVEN

ABSTRACT

The experiment was conducted in shade house of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, during the period from October 2011 to May 2013 to study effects of *Meloidogyne incognita* inoculum density and application rate of *Paecilomyces lilacinus* on biocontrol efficacy of bioagent against root knot nematode of brinjal. Four doses of *Paecilomyces lilacinus*, viz., 0 CFU/g soil, 1×10^5 CFU/g soil, 5×10^5 CFU/g soil, 1×10^6 CFU/g soil and five inoculum level of *M. incognita*, viz., 0 eggs/100 cm^3 of soil, 400 eggs/100 cm^3 of soil, 800 eggs/100 cm^3 of soil, 1600 eggs/100 cm^3 of soil, and 3200 eggs/100 $cm^3$of soil were used to conduct this experiment. The experiment was laid out in Randomized Complete Block Design (RCBD) having two factors and replicated eight times. Spore suspension of *P. lilacinus* was mixed into the soil before transplantation and *Meloidogyne incognita* was inoculated on three days after transplantation. Crop was harvested carefully after eight weeks of transplantation. The application of *Paecilomyces lilacinus* @ 1×10^5 CFU/g soil enhance plant growth parameters. Inoculation of *Meloidogyne incognita* reduced plant growth and the reduction was increased with the increase of inoculum density of *Meloidogyne incognita*. Maximum plant growth reduction was recorded when 3200 eggs/100 $cm^3$of soil was inoculated. The maximum plant growth was recorded in a combined application of *Paecilomyces lilacinus* 1×10^5 CFU/g soil with 400 eggs of *Meloidogyne incognita*/100 cm^3 of soil. Minimum gall index (2.13) and number of eggs per root system (3.16×10^3) were recorded in 1×10^6 CFU/g soil when crop was challenged with *Meloidogyne incognita* @ 400 eggs/100 cm^3 of soil. The highest reproduction factor (31.71) was found in inoculum density of 400 eggs/100 cm^3 of soil. Application of 5×10^5 CFU/g soil of *Paecilomyces lilacinus* resulted lowest reproduction factor (7.42) when the crop was challenged with *Meloidogyne incognita* @ 800 eggs per 100 cm^3 of soil. The application of *P. lilacinus* @ 1×10^6 CFU per g soil reduced máximum 72% gall index and 84% egg masses when the crop was challenged with 800 eggs/100 cm^3 soil. The dose 5×10^5 CFU/g soil of *P. lilacinus* showed effectiveness to reduce reproduction factor of *M. incognita* upto 89.6 and 85% when the crop was challenged with 800 eggs and 3200 eggs of the pest/100 cm^3 soil, respectively.

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATION

SYMBOLS AND ABBREVIATIONS	FULL WORD
%	Percent
et al.	And others
J.	Journal
No.	Number
Cm^3	Centimeter cube
Var.	Variety
^oC	Degree centigrade
Etc.	Etcetera
Ed.	Edited
Eds.	Edition
BARI	Bangladesh Agricultural Research Institute
LSD	Least Significant Difference
PDA	Potato Dextrose Agar
RCBD	Randomized Completely Block Design
Res.	Research
SAU	Sher-e-Bangla Agricultural University
Viz.	Namely
@	At the rate of
CFU	Colony Forming Unit
Naocl	Sodium hypochloride
i.e.	That is
BBS	Bangladesh Bureau of Statistics
CV%	Percentages of Co-efficient of Varience
g	Gram
BAU	Bangladesh Agricultural University

INTRODUCTION

Brinjal (*Solanum melongena L.*) is one of the major solanaceous crops and second most important vegetable crop, next to potato in Bangladesh in respect of acreage and production (BBS, 2005). It is one of the most common popular and principle vegetable crops grown in Bangladesh and others parts of the world due to its diversified uses. It is cultivated as a populous and commercial vegetable throughout the tropical and sub tropical regions of the world. Brinjal is nutritious vegetable and has multifarious use as a dish item (Bose and Som, 1986 and Rashid, 1993). It contains higher calorie, iron, phosphorus and riboflavin than tomato (Shaha, 1989). In rabi 2009-2010 the total area covered by brinjal cultivation was 28.75 thousand hectares with the production of 216 thousand metric tons and in kharif (summer), the hectares and production was 10.93 thousand and 92 thousand metric tons, respectively (BBS, 2011).

Incidence of insects, pests and diseases generally hampered the production of eggplant. This crop suffers from the various diseases; about 13 different diseases so far recorded in Bangladesh (Das *et al.*, 2000 and Rashid, 2000). Among those diseases, root knot has been treated as one of the major constrains in eggplant cultivation in the country. Root knot caused by *Melodoigyne* spp. is widely distributed in all eggplant growing area of Bangladesh, with moderate incidence (27.2%) (Talukder, 1974; Mian, 1986 and Bari, 2001). Infection of roots by nematodes alter uptake of loss water and nutrient, and interferes with the translocation of minerals and photosynthates (Williamason and Hussey, 1996). Such alterations change the shoot; root ratio (Anwar and Van Gundy, 1989) and expose the plants to other pathogens infection. For example, nematode root infection increases the incidence and severity of *Fusarium* wilt diseases on a variety of crops (Martin *et al.,* 1994) which can negatively influence yield (Orr and Robison, 1984). The yield reductions have reached as high as 30% for susceptible genotypes in the presence of plant parasitic nematodes in some production areas (Anwar *et al.,* 2009).

To control the plant parasitic nematode, is a difficult task. To date and farmers are depended on chemical nematicide for reduction of nematode population density (Jatala, 1985). But nematicide has been too expensive for use in developing countries,

where their application has been limited to a few crops (Hague and Gowen, 1987). Due to its hazardous effects it has led to an increased interest in biological control in its widest sense, in order to achieve environmentally safe method of reducing the nematode damage (Davise *et al*., 1991; Kiewnick and Sikora, 2004). Biocontrol seems to be the most relevant and practically damaging approach for controlling of root knot nematodes. *Paecilomyces lilacinus* is an excellent biocontrol agent in tropical and subtropical agricultural soils, has been reported to reduce nematode population density. It is considered as one of the most promising and practicable biocontrol agents for the management of plant parasite nematodes (Jatala, 1986; Kiewnick *et al.*, 2011). Studies from several countries indicated that this fungus adapts well in varied climatic conditions and in effective in controlling root knot nematodes (Usman and Siddiqui, 2012). In the past, *Paecilomyces lilacinus* applied to soil using various organic materials such as oil cakes, leaf residue, wheat bran and gram seeds as carrier (Cannayane and Sivakumar, 2001). *Paecilomyces lilacinus* YES-2 strain is an important nematophagus egg parasitic fungus, that can isolate from root-knot nematode egg and applied to soil in alginate pellet form with various doses. Various mechanisms of action have been suggested for the biological activity of *Paecilomyces lilacinus* against plant parasitic nematodes. The main mechanism is direct infection of sedentary stages in particular the egg stage. The production of leucinotoxins, chitinases, proteases and acetic acid by *Paecilomyces lilacinus* has been associated with the infection process (Djian *et al.*, 1991; Anastasiadis *et al.*, 2007). The fungus directly penetrated all stages of the nematode after formation of aspersoria.

The use of chemicals to control the root-knot nematode is very costly for the growers. Thus, the experiment was undertaken with the target to replace the use of chemical nematicide by the nematophagous fungus *Paecilomyces lilacinus* to establish an eco-friendly management of root-knot nematode of various density levels with the following objective:

1. To determine the biocontrol efficacy of *Paecilomyces lilacinus* against different levels of root knot nematode, *Meloidogyne incognita* in eggplant.

REVIEW OF LITERATURE

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) conducted a microplot experiment to evaluate the inoculum level and time of application of *P. lilacinus* on the protection of tomato against *M. incognita*. They observed that *P. lilacinus* applied into soil 10 days before planting and again in planting resulted increased yield with the improvement of plants compared with the nematode treated plots.

Cabanillas *et al*. (1989) isolated 13 *P. lilacinus* isolates from various geographic regions as biocontrol agents *Meloidogyne incognita*. The best control of *M. incognita* was provided by an isolate from Peru or a mixture of isolates of *P. lilacinus.* As soil temperatures increased from 16 to 28° C, both root knot damage caused by *M. incognita* and percentage of egg masses infected by *P. lilacinus* increased. The greatest residual *P. lilacinus* activity on *M. incognita* was attained with a mixture of fungal isolates. These isolates effected lower root-galling and necrosis, egg development, and enhanced shoot growth compared with plants inoculated with *M. incognita* alone.

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.

Sivakumar and Vidyasekaran (1990) reported that performance of *Paecilomyces lilacinus* @ 2g/kg in combination with farmyard manure @ 4% (W/W) for control of *Meloidogyne incognita* on *C. forskohlii*. Minimum egg masses were observed in treatment with FYM + *P. lilacinus*, followed by treatment with FYM alone.

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.

Mittal *et al.* (1995) evaluated *P. lilacinus,* a rhizosphere inhabiting nematophagous fungus, along with chitin in sterilized soil for the suppression of *Meloidogyne incognita*, causal agent of root knot disease in *Solanum melongena, Lycopersicon esculentum* and *Cicer arietinum*. The plant growth after 30, 60, 90 days was assessed in terms of shoot and root length, shoot and root fresh and dry weight and number of galls/g root fresh weight. Combination of fungus with chitin enhanced suppression of *Meloidogyne incognita* more than using them alone.

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.

Oduor and Waudo (1996) evaluated *P. lilacinus*, *Phoma herbarum* and three isolates of *Fusarium oxysporum* in controlling root knot (*M. javanica*) in eggplant. *P. lilacinus* and *Fusarium oxysporum*-1 significantly ($P<0.05$) parasitzed more than 70% eggs and female while *Fusarium oxysporum*-3 parasitized less than 20%, control of *Meloidogyne incognita* on eggplant.

Zaki and Irshad (1996) conducted an 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.

Paecilomyces lilacinus has been reported to be very effective in controlling root-knot nematode, *Meloidogyne* spp. on several crops (Zaki and Maqbool, 1992 ; Sosamma and Koshy, 1997).

Siddiqui, (2000) studied the efficacy of *Pseudomonas aeruginosa* alone or in combination with *P. lilacinus* control of root knot nematode and root infection fungi under laboratory and field conditions. Ethyl acetate extract (1 Mg/ml) of *P. lilacinus* and *P. aeruginosa,* respectively, caused 100 and 64% mortality of *Meloidogyne javanica* larvae after 24 h. in field experiments, biocontrol fungus and bacterium significantly suppressed soilborne root-infecting fungi including *Macrophomina phaseolina, Fusarium oxysporum, Fusarium solani, Rhizoctonia solani* and the root knot nematode *Meloidogyne javanica, P. lilacinus* parasitized eggs and female of *M. Javanica*.

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.

Rao and Reddy (2001) used *Glomus mosseae* in combination with *P. lilacinus* and neem cake to control root knot nematode of eggplant. The parasitization of eggs of root knot nematode was significantly increased by *P. lilacinus* and the transplants yielded significantly more fruit. Neeem cake amendment 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 condition.

Oduor and Waudo (2003) used agrochemicals, organic matter and the antagonistic fungus *P. lilacinus* in natural field soil in controlling root knot nematode. He found that the smallest galling index, number of galls and nematode population were in soil treated with aldicarb in combination with *P. lilacinus.*

Three applications of *Trichoderma viride* along with oil cake at quarterly intervals was found second best in reducing the nematode population and leaf yield in AAU centre after carbofuran treatment compared to control. However, both the treatments,

carbofuran and three applications of *T. viride* were on per in leaf yield at Jawaharlal Nehru krishi Vishwa Vidyalaya (Anon., 2003).

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 to control root-knot nematodes *Meloidogyne incognita* and *M. hapla* on tomato using *P. lilacinus* 251. 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. However, the combination of the seedling treatment with a pre or at planting application of *P. lilacinus* was necessary to achieve higher levels of control. They found 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.

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.

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.

In India Eapen *et al*. (2005) used 73 freshly collected fungal isolates and 76 isolates obtained from other sources. Fifty-nine isolates showed 50-90% inhibition in egg hatch of *Meloidogyne* spp. *Pochonia chlamydosporia*, *Verticillium lecanii*, *P. lilacinus* and few isolates of *Trichoderma* spp. showed>25% parasitism on root knot nematode eggs.

A total 455 fungul isolates belonging to 24 genera and 52 isolates of actinomycetes were obtained from 28 samples by Sun *et al.* (2006) and they observed that *P. lilacinus* was highly pathogenic in controlling root knot nematode and it reduced tomato root gall index by 13.4-58.9% compared to the no treatment control.

Bijoy *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 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.

Esphahani and Pour (2006) observed that *P. lilacinus* was effective in controlling root knot nematode on tomato and suppressing its population growth and effectively promoted the growth of plant.

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.

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*.

Nisha and Sheela (2006) reported that integration of soil solarization in the nursery for 15 days and application of either *P. lilacinus*, *B. mucerans* or neem cake in the main field are the better treatments in improving biometric characters and reducing the nematode population in soil and root on Coleus (*Solenostemon rotundifolius*).

Khan *et al*. (2006) described the mode and severity of infection of nematodes by a soil saprophyte *P. Lilacinus.* Infection of stationary stages of nematodes by *P. lilacinus*

was studied with three plant parasitic nematodes *Meloidogyne javanica*, *Heterodera avenae* and *Radopholus similis*. *P. lilacinus* infected eggs, juveniles and females of *M. javanica* by direct hyphal penetration. The early developed eggs were more susceptible than the eggs containing fully developed juveniles. *P. lilacinus* also infected immature cysts of *H. avenae* including eggs in the cysts and the eggs of *R. similis* and the fungus was shown to infect mobile stages of all the plant-parasitic nematodes.

Kiewnick and Sikora (2006) mentioned that successful biocontrol of RKN depends on initialy low nematode density in the soil. They used fungul biocontrol agent, *P. lilacinus* strain 251 (PL251), and evaluated for its potential to control the root-knot nematode *Meloidogyne incognita* on tomato. In growth chamber experiment, 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. They also mentioned that a single pre-plant application at a concentration of 1×10^6 CFU/g soil is needed for sufficient biocontrol of *Meloidogyne incognita* by PL251.

Senthamarai *et al.* (2006) conducted two pot experiments to study the pathogenicity of *Meloidogyne incognita* (at 10, 100, 1000, 10000 and 100,000 J2 per plant containing 5 kg of soil) on (*C. forskohlii*). A pot without inoculate was included as the control. The inoculum level of 100 or more J2 per 5 kg of soil was pathogenic causing significant reduction in shoot length, root length and shoot weight. At the initial levels of 10,000 and 100,000 J2, the reduction in root weight was 22.22 and 43.05 per cent, respectively over the untreated control. A significant reduction in tuber yield was observed at and above 10 J2 per plant with maximum reduction at highest inoculum level of 100,000 J2 per plant. The galling decreased at highest inoculum level of 10,000 or 100,000 J2 per plant. The nematode population in soil increased with increasing inoculum levels upto 1000 J2 per plant.

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.

Tripati and Singh (2006) reported that effect of compatible biocontorl agent along with mustard cake and furadan against *Meloidogyne incognita* in tomato. A significant increase in length and weight of root and shoot and considerable reduction in root galling as well as nematode population in soil and root was observed when plants were treated with *P. lilacinus* and *T. viride* in combination with mustard cake and furadan.

Anastasiadis *et al.* (2007) evaluated a formulated product (BioAct) of the naturally occurring fungus *P. lilacinus*, strain 251, against root knot nematodes in pot and green house experiments. They observed that application of *P. lilacinus* and the bacteria *Bacillus fitmus*, significantly or together in pot experiments, provided effective control of second stage juveniles, eggs or egg masses of root-knot nematodes.

Singh and Khurma (2007) examined root galls of rice caused by *Meloidogyne graminicola* for natural colonization by nematophagous fungi and observed that application of inocula of *A. dactyloides* and *D. brachophaga* in soil infested with *Meloidogyne graminicola*, respectively, reduced the number of root galls by 86%, and females by 94% and eggs and juveniles by 94%. The application of these fungi to soil increased plant growth.

The pesticides carbofuran (1.5 mg a.i./kg soil) and carbendazim (1.0 mg a.i./kg soil), neem seed powder (50 mg/kg soil), the fungi *Paecilomyces lilacinus* and *Trichoderma harzianum* (50 mg culture/kg soil) and the bacterium *Pseudomonas fluorescens* (50 mg/kg soil), alone and in various combinations (at half the standard dose) were tested for their effects on the root-knot nematode *Meloidogyne incognita* and growth and yield of *Mentha arvensis* cv. gomti. All treatments improved plant growth, controlled the nematode and increased oil content and oil yield of *M. arvensis*. The greatest performance was achieved by applying carbofuran or neem seed powder, singly or in combination, followed by combinations of these nematicides with the two fungi or the

bacterium. The effects of the three bio-agents were least when used singly and increased only a little when they were used in combinations (Perveen *et al*., 2007**)**.

Lopez Llorca *et al*. (2008) observed the mode of action and interactions of nematophagous fungi and discussed types of recognition phenomena (e.g. chemotaxis and adhesion), signaling and differentiation , penetration of the nematode cuticle/eggshell using mechanical, as well as enzymatic (protease and chitinase) means. They observed that *P. lilacinus* is an egg and female parasitic fungus and it infects nematode by it's appressoria. It produced chitinases enzymes and damages the eggshell and destroyed nematode.

Aminuzzaman (2009) used fungal pellet containing spores of nematophagous fungus *P. lilacinus* YES-2 in green house condition to assess its biocontrol potency against root knot of tomato and observed *P. lilacinus* significantly reduced the number of nematode population in soil and root and increased 20.75% tomato yield over untreated control.

Bhat *et al*. (2009) observed the interaction of fungus *P. lilacinus* and *Meloidogyne incognita* in bitter gourd at different time intervals. They found that *Meloidogyne incognita* induced large sized galls on the plants. The xylem and the pholeam exhibited abnormalities in structure near the giant cells. Abnormal vessel elements were occupying larger area near giant cells. The plants that were treated with fungus either one week before nematode inoculation or simultaneously, produced significantly ($p=0.01$) small sized galls in comparison to untreated plants.

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.

Castillo *et al.* (2010) conducted to evaluate the biological control potential of *Drechslerella dactyloides*, *Drechslerella brochopaga*, and *Paecilomyces lilacinus* against the reniform nematode, *Rotylenchulus reniformis* under *in vito,* and in greenhouse conditions. Pathogenicity tests *in vitro* with *Drechslerella dactyloides and Drechslerella brochopaga* revealed conidial germination at 14 hours and at 72 hours trappings had formed and ensnared deiform nematodes. *Paecilomyces lilacinus* conidia germinated in 12 hours and parasitized nematode eggs within 24 hours after the initial exposure. Pathogenicity of the fungi was examined in the greenhouse in autoclaved and non-autoclaved soil. In autoclaved soil, all three fungi reduced ($P \leq 0.05$) the number of vermiform *R. reniformis* nematodes in soil 60 days after planting. *Paecilomyces lilacinus* also reduced ($P \leq 0.05$) numbers of eggs extracted from the cotton roots. In non-autoclaved soil, *D. dactyloides* reduced *R. reniformis* vermiform life stages, but none of the fungal isolates affected the number of *R. reniformis* eggs extracted from the roots. *Drechslerella dactyloides, Drechslerella brochopaga,* and *Paecilomyces lilacinus* parasitize of *R. reniformis in vitro* and in the greenhouse using autoclaved soil. However, the fungi did not reduce numbers of *R. reniformis* in non-autoclaved soil. These results illustrate these fungi are parasites of *R. reniformis;* however they need to have an advantage to compete with native soil microorganisms.

Kalele *et al.* (2010) worked with antagonistic fungus *P. lilacinus* stain 251 in controlling root knot nematodes in tomato and cucumber. He applied *P. lilacinus* inoculums at different rates and different times. He found that pre-planting soil treatment 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 recorded reduction level of 54 and 74% in the roots and soil respectively. He described that PL251 was a promising potential bioagent that could be exploited in the management of *Meloidogyne* spp. in vegetable production systems.

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*.

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*.

Aminuzzaman *et al.* (2011) first reported the isolation and evaluation of biocontrol fugus *P. lilacinus* recorded in Bangladesh. They collected galled roots of eggplant infected with *Meloidogyne* spp. from farmer's fields of Mymensingh district, Bangladesh. Eggs were collected and placed and smeared on PDA plates and incubated at 25° C. After detailed morphological examination, the fungal hybphae grown from eggs were identified as *P. lilacinus.* They mentioned that the fungus showed more than 80% egg parasitism and 52% juvenile mortality of *Meloidagyne* spp. They also mentioned that the fungus increased shoot height, fresh shoot weight, root length and fresh root weight and also reduced root galling up to 63% and number of egg mass per root system up to 40% when compared with control treatment.

Hashem and Abo-Elyousr (2011) tested the nematicidal effect of *Pseudomonas fluorescens, P. lilacinus, Pichia guilliermondii* and *Calothrix parietina* singly or in comination against root knot nematode, *Meloidogyne incognita*. Treatment with *P. fluorescens* and *P. lilacinus* caused mortality of *M. incognita* as 45 and 30% of

juveniles after 48h of exposures, respectively compared to water control in vitro. Under greenhouse conditions, all treatments reduced the disease severity and enhanced plant growth compared to untreated control. Application of *P. fluorescens, P. lilacinus* and *P. guilliermondii* was more effective compared to *C. Parietina*. Fresh and dry weight of shoots and roots of plants were significantly reduced as a result of infection with M. *incognita*, however application of biocontrol agents singly or in mix recovered this reduction. The results proved that application of different biocontrol agents (*P. fluorexcens, P. lilacinus and P. guilliermondii*) not only has a lethal effect on nematode, but also enhances the plant growth, supplying many nutritional elements and induction the systemic resistance in plants.

Kiewnick *et al.* (2011) evaluated the fungal biocontrol agent; *P. lilacinus* strain 251 for its potential to control the root-knot nematode *Meloidogyne incognita* on tomato at varying application rate and inoculums densities. He demonstrated that 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 inoculums densities of 100 to 1600 eggs and infective juveniles per 100 cm^3 of soil. These results demonstrate that rihzosphere competence in not the key mode of action for PL251 in controlling *M. incognita* on tomato.

P. liacinus enhanced plant growth and reduced galling index and nematode population which was supported by Aminuzzaman *et al*. (2011). They mentioned that root galling index and final nematode population decreased up to 40.7 and 73.8%, respectively for tomato and 55.6 and 66.9%, respectively for brinjal by application of the biocontrol fungus. They also mentioned that *P. lilacinus* enhanced plant growth and reduced galling index with its increased doses.

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 nematicide. Khalil *et al.* (2012) 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.

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%).

The biocontrol potential of *Paecilomyces lilacinus* was evaluated in field conditions in two seasons during 2005-2008 at Annamalainagar and Vallambadugai of Cuddalore district of Tamilnadu, India. In the field evaluation of different doses and application methods of *P. lilacinus viz*., seed treatment, seedling treatments, soil application treatments and the integration treatments produced mixed results on the growth of okra in both Annamalainagar and Vallambadugai. 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 (Kannan and Veeravel, 2012).

Shammi (2012) conducted two experiments to study the effects of biocontrol fungus *Paecilomyces lilacinus* and fosthiazate on root knot *Meloidogyne* spp. and yield of eggplant. She mentioned that *Paecilomyces lilacinus*, Fosthiazate and their combination reduced 53.86, 82.05 and 92.28% gall index, respectively over control in variety Singnath and 63.86, 71.99 and 91.88% gall index, respectively over control in variety Khotkhotia. In the field experiment those combination reduced 44.56, 69.7 and 81.25% gall index, respectively over control in variety Islampuri.

Mitu (2012) carried out an experiment 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. kashindo) in shade house condition. She reported that the highest shoot length and shoot dry weight was found in simultaneous inoculation. She also mentioned the gall index, egg mass/root, eggs/egg mass and reproduction factor were reduced upto 77.38, 88.29. 68.73 and 86.29% ,respectively over control in

brinjal variety singnath in simultaneous inoculation of *Paecilomyces lilacinus* and *Meloidogyne incognita*.

Usman and Siddiqui (2012) observed the effect of some fungal strains for the management of root knot nematode (*Meloidogyne incognita*) on eggplant (*Solanum melongena*). They used two biocontrol fungal strains of *Trichodema harzianum* and *Paecilomyces liacinus* at 1 g/pot and 2 g/pot. Inoculation of fungus was done simultaneously along with 1000 second stage juveniles (J2) 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 enhance all plant growth characters with the reduction in the root knot infestation.

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%.

MATERIALS AND METHODS

Pot experiment was done to study effects of *Meloidogyne incognita* inoculum density and application rate of *Paecilomyces lilacinus* on biocontrol efficacy of bioagent against root knot of brinjal. The materials were used and the methods were followed in the study are presented in this chapter.

3.1. Experimental site and period

The experiment was conducted in shade house of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The experiment was carried out during the period from October 2011 to May 2013.

3.1.1. Environment of experiments

The experimental plants were kept in the shade house where the temperature was 30 ± 2º C during the day and 21±2º C during night with an average temperature of 28 ± 2º C.

3.1.2. Brinjal variety used

Brinjal cv singnath was used for the experiment.

3.1.3. Collection of seeds

Healthy, mature and disease free seeds of singnath variety were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

3. 1. 4. Soil collection and sterilization

Required soil sand and decomposed cowdung were collected from agronomy farm of Sher e-Bangla Agricultural University. Then soil, sand and cowdung were mixed properly in a ratio of 6:2:1. The mixture was autoclaved at 121ºC for 15 minutes at 15 PSI. The sterilized soil was allowed to room temperature followed by filled in the plastic trays for raising seedlings.

3.1.5. Raising of seedlings

Plastic trays were filled with sterilized and fertile soil. Seeds of brinjal cv singnath were soaked in sterile water for better germination. Then the seeds were sown in the plastic tray. The trays were covered with polyethylene 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. After establishment of seedlings, they are transfer to polybag containing same soil (Plate- 1).

3.1.6. Preparations of pots

Plastic pots of 1000 cm^3 were cleaned, washed, dried up properly and sterilized with 70% ethanol properly. Sterilized and fertile soil was filled in required amount into each pot. Each pot contain 800 g soil. Then the pots were arranged according to experimental design.

3.1.7. Nematode culture

Nematode (*Meloidogyne incognita*) samples were collected from nematode infected brinjal roots. Egg masses were picked up and inoculated in young seedlings of brinjal. Sub-culturing was done subsequently by inoculating new brinjal seedling with egg masses.

3.1.8. Fungus culture

Paecilomyces lilacinus was grown on Potato Dextrose Agar (PDA) media at 25°C temperatures for 15 days. The fungus was collected from stock culture of the Department of Plant Pathology, Sher-e-Bangla Agricultural University (Plate-2).


a b

Plate 1. Photograph showing raising and transplanting of seedlings of brinjal cv singnath (a) Plastic tray with seedlings (b) transplanted plantlets in polybag

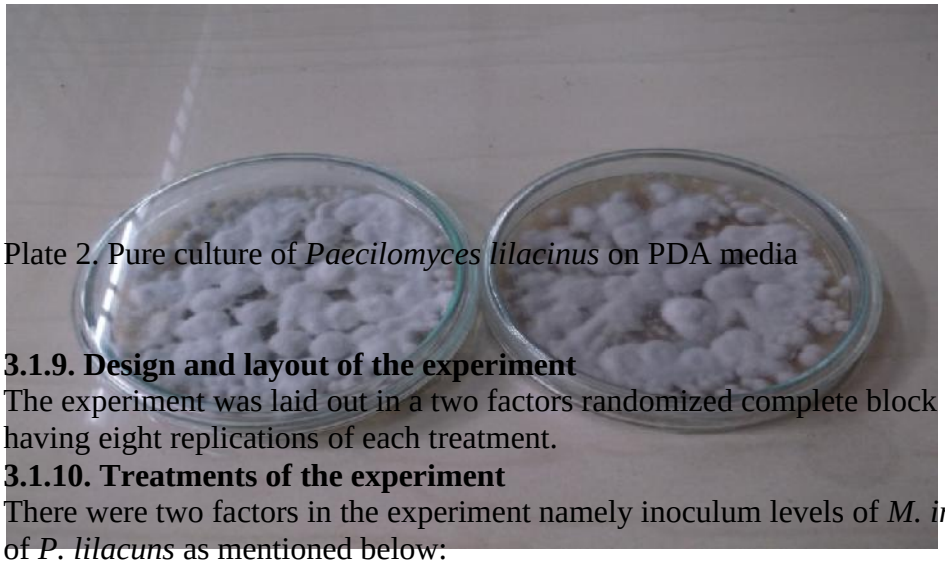


Plate 2. Pure culture of *Paecilomyces lilacinus* on PDA media

3.1.9. Design and layout of the experiment

The experiment was laid out in a two factors randomized complete block design (RCBD) having eight replications of each treatment.

3.1.10. Treatments of the experiment

There were two factors in the experiment namely inoculum levels of *M. incognita* and doses of *P. lilacuns* as mentioned below:

Factor A: Four doses of *Paecilomyces lilacinus*

P_0 = 0 CFU/g soil
P_1 = 1×10^5 CFU/g soil
P_2 = 5×10^5 CFU/g soil
P_3 = 1×10^6 CFU/g soil

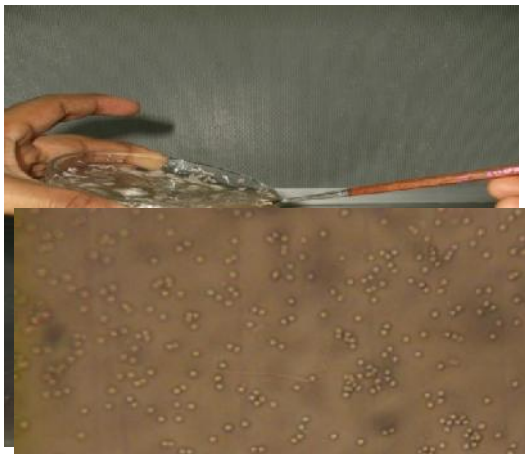
Factor B: Five inoculum level of *M. incognita*

M_0 = 0 eggs/100 cm^3 of soil
M_1 = 400 eggs/100 cm^3 of soil
M_2 = 800 eggs/100 $cm^3$of soil
M_3 = 1600 eggs/100 $cm^3$of soil
M_4 = 3200 eggs/100 cm^3 of soil

3.1.11. Application of *Paecilomyces lilacinus*

After sporulation (15 days old) sterile water was added in the petridishes and the spore masses scraped away with sterile brush. Then the harvested spores were filtered through sterilized cheesecloth. The spore suspension was collected and were counted with a haemacytometer and adjusted to a required concentration. The inoculation was done with required number of spores/plant in each pot with micropipette. Spores were mixed thoroughly to the soil. It was done before transplanting (Plate-3).

A



B

C

Plate 3. Hervesting (A) and Sieving (B) of *Paecilomyces lilacinus* spore. Spore of *P. lilacinus* under microscope (400X) (C)

3.1.12. Determination of *Paecilomyces lilacinus* application rate (CFU /g soil)

To confirm the correct concentration of *Paecilomyces lilacinus* in soil, samples from individual pot replicates of each treatment were taken and pooled, and the number of CFU/g of soil was determined by dilution plating (Kiewnick and Sikora, 2006; Rumbos and Kiewnick, 2006).

3.1.13. Transplanting of seedlings

After preparation of pot in the shade house, 30 days old seedlings were uprooted carefully from the polybag and transplanted in the experimental pot. Each pot contains 800 g sterilized soil and only one plant was transplanted to each pot. Sufficient irrigation was given just after transplantation. Watering was continued till the seedlings were established.

3.1.14. Inoculation of *Meloidogyne incognita*

Mature egg mass of nematode (*Meloidogyne incognita*) was collected from severely galled roots of brinjal. The number of eggs per egg mass was counted. Each plant was inoculated with 10000 eggs next day of transplanting, by using micropipette making three holes surrounding the transplanted seedlings (Plate-4).

3.1.15. Intercultural operations

After transplantation of seedling into pots weeding and irrigation were done as necessary. The plants were observed regularly. General sanitation was maintained throughout the growing period.

3.1.16. Harvesting and data recording

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

I. Root length
II. Shoot length
III. Root weight (fresh and dry)
IV. Shoot weight (fresh and dry)
V. Gall index (0-10) scale
VI. Number of egg mass per root
VII. Number of egg per egg mass
VIII. Number of egg per root system
IX. Number of juvenile per g soil
X. Reproduction factor
XI. % egg mass colonized by *P. lilacinus*
XII. Soil colonization by *P. lilacinus* (CFU/g soil)

Plate 4. Photograph showing inoculation of egg suspension of nematode (*Meloidogyne incognita*)

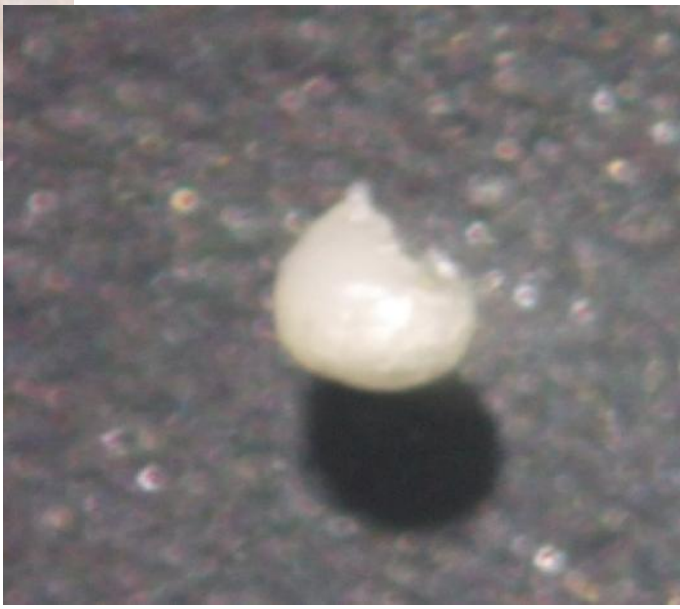


Plate 5. Photograph showing female of *Meloidogyne incognita*

3.1.17. Recording on plant growth parameters

Shoot length was measured before harvest. The shoot length was measured from the base of the plant to the growing point of the youngest leaf. Then the roots are harvested. Roots were carefully separated from soil and collected in different polybag that were lebelled according to different treatment. Then the roots were cleaned gently with water and length was taken. The length of root was measured from the growing point of root to the longest available lateral root apex. For fresh weight of root and shoot the portion was blotted dry and the weight was recorded. The shoot and root was oven dried for 2 days at 40° C and their weight was recorded.

3.1.18. Counting of nematode egg masses/ root system

Number of egg mass/root system was counted following Holbrook *et al.* (1983). The roots were soaked in Phloxine-B (2mg/L) solution (Plate-6) for 15 min (Hartman and Sasser, 1985). The roots were observed and egg masses/root were counted with a magnifying glass (Plate-7).

3.1.19. 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 dose not washed with water. Then the roots were soaked in Phloxine- B (2mg/L) solution for 15 minutes (Hartmann 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 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 over slide was examined under microscope and counting the eggs/egg mass (Plate-8-9).

galled

Plate 6. Photograph showing roots treated by Phloxine-B

egg

Plate 7. Phloxine –B treated mass in root system

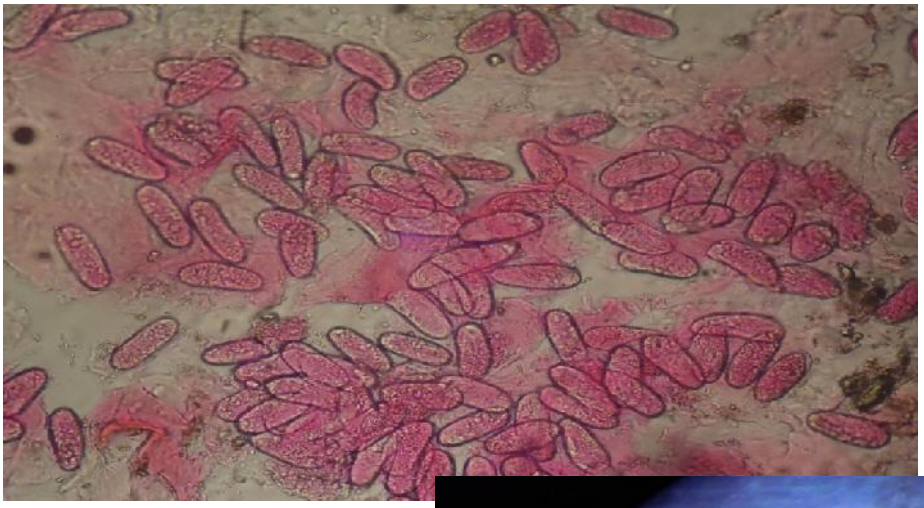


Plate 8. Microphotograph showing phloxine – B treated eggs of *M. incognita*

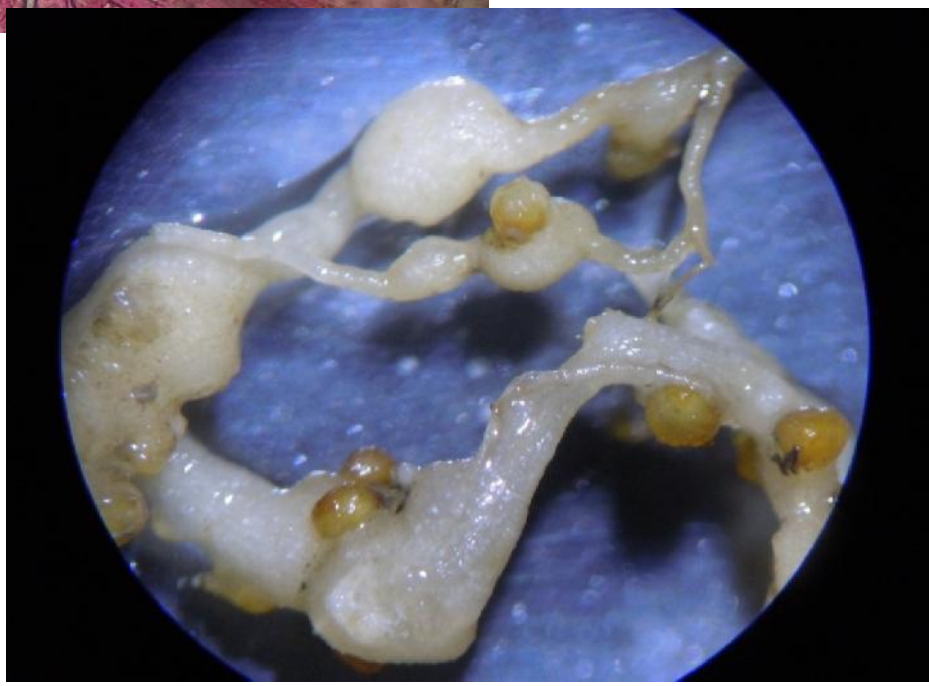


Plate 9. Light photograph showing egg masses of *Meloidogyne incognita*

3. 1.20. 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 (Whitehead and Hemming; 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 (Plate- 10). 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 5ml sub sample was taken and put into a counting dish. Juveniles counting were done by using a compound microscope (Plate-11).

3.1.21. Gall index

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

Scales	Specification
0	No gall
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

Plate 10. Extraction of *Meloidogyne incognita* from soil by Bangladeshi plate method (Modified White Head and Hemming method)

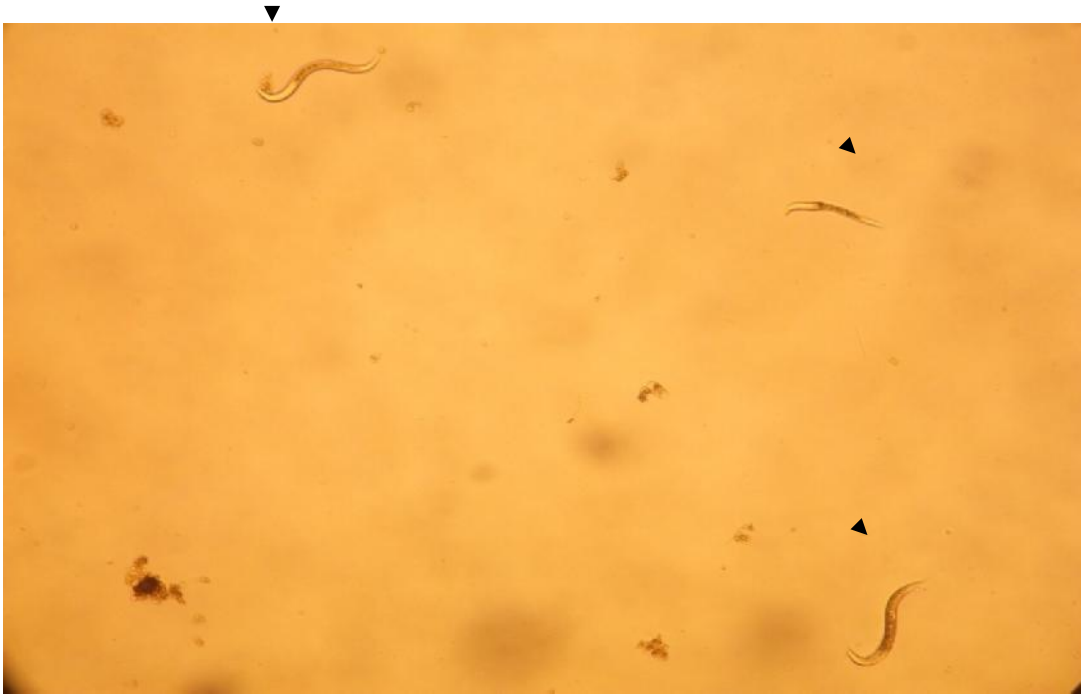


Plate 11. Microphotograp showing second stage larve of *Meloidogyne incognita*

3.1.22. % Egg mass colonization by *Paecilomyces lilacinus*

Five egg masses/plant were randomly collected from the eggplant roots, washed with sterile water and disinfected with a solution of Clorox and put on PDA plate in Petridis. The number of colonized egg masses was determined after 5 days of incubation (Plate 12-13).

3.1.23. Soil colonization by *Paecilomyces lilacinus* (CFU/g soil)

Samples of 100 g soil were collected on harvesting time from around the root zone. The number of spores per g soil was determined following soil dilution plate technique (Plate 14-15).

3.1.24. Analysis of data

The recorded data on various parameters were statistically analyzed by using MSTAT-C statistical package programme. Difference between treatment means were determined by Duncan`s new Multiple Range Test (DMRT) according to Gomez and Gomes, (1984).

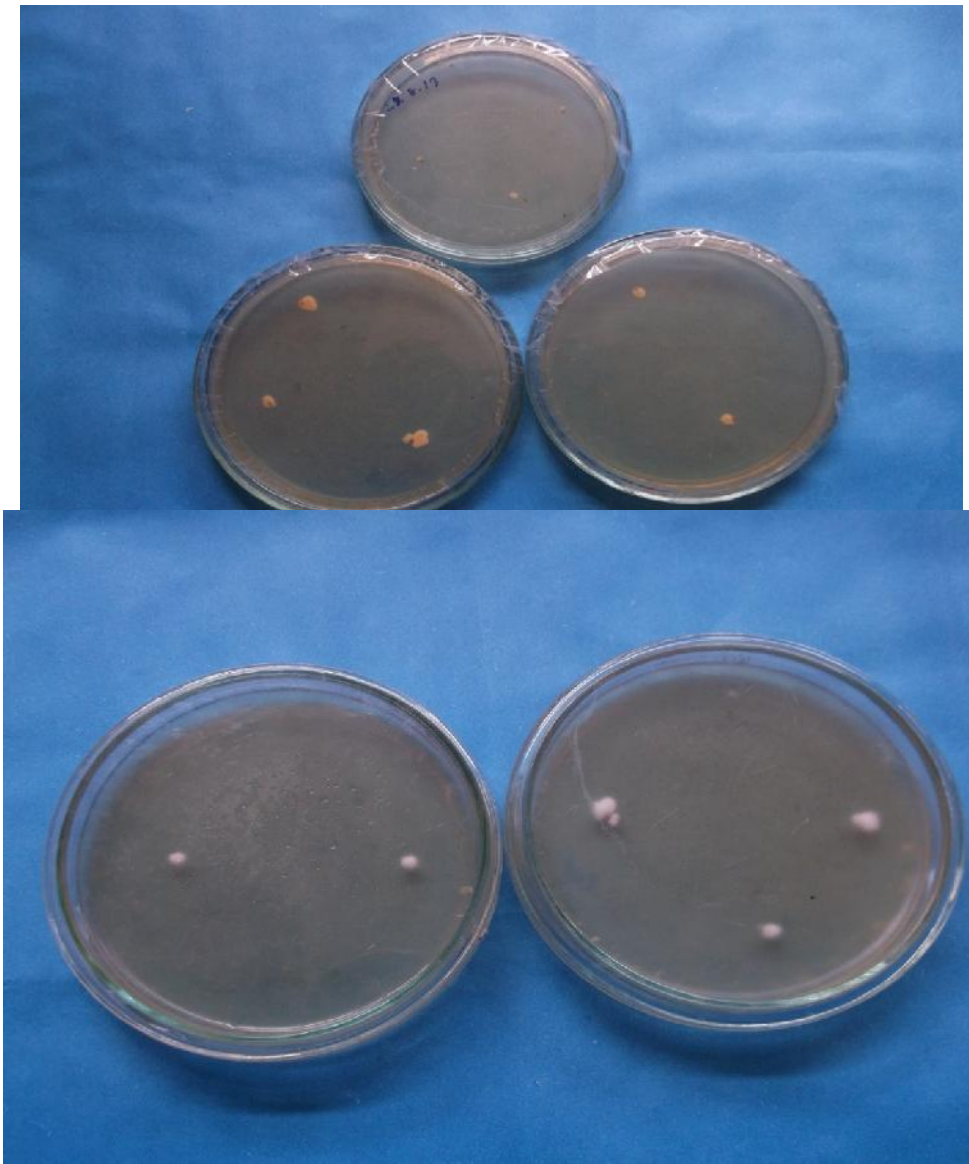


Plate 12. Photograph showing egg mass colonization by *P. lilacinus* on PDA media (opposite view)

Plate 13. Photograph showing egg mass colonization by *P. lilacinus* on PDA media (front view)

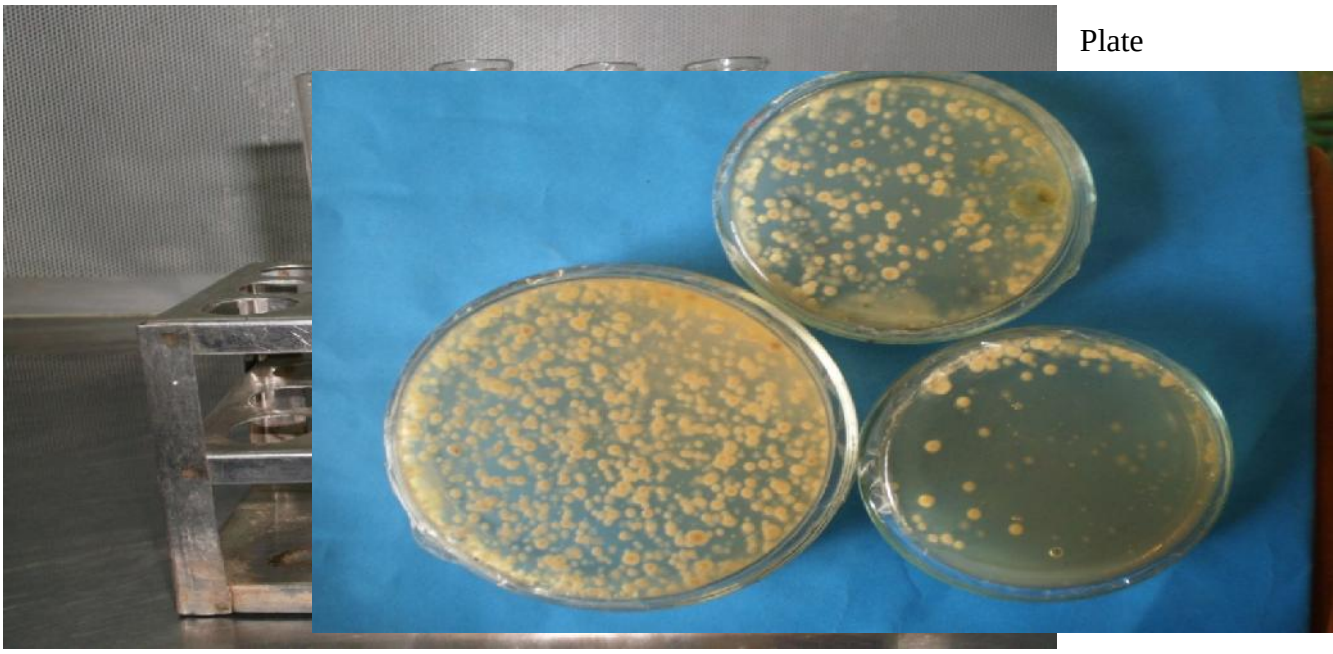


Plate 14. Stock solution and several soil dilution for soil dilution plate

:100

1:1000

Plate 15. Colony growth of *P. lilacinus* on PDA (soil dilution plate technique)

RESULTS

The experiment was conducted to investigate the effects of *Meloidogyne incognita* inoculum density and application rate of *Paecilomyces lilacinus (P.l)* on biocontrol efficacy of bioagent against root knot of brinjal. Data of the different parameters analyzed statistically and the results have been presented in this chapter under the following headings.

4.1 Main effect of *Paecilomyces lilacinus* application rate on growth Parameters

The shoot length varied significantly due to the different application rate of *Paecilomyces lilacinus* (Table 1). The maximum shoot length (25.12 cm) was recorded in application rate of 1×10^5 CFU/g soil. On the other hand minimum shoot length (20.89 cm) was found in control treatment.

Applications of *Paecilomyces lilacinus* significantly influenced on shoot fresh weight (Table 1). The maximum shoot fresh weight (12.78 g) was recorded in where 1×10^5 CFU/g soil of *P. lilacinus* applied and the minimum shoot fresh weight (10.87 g) was found where no bioagent used.

The shoot dry weight also varied significantly due to the application of *Paecilomyces lilacinus*. The maximum shoot dry weight (5.06 g) was obtainedin application rate of 1×10^5 CFU/g soil and the minimum shoot dry weight, after control (4.57g) was recorded where *P. lilacinus* was applied @ 5×10^5 CFU/g soil (Table 1).

The root length varied significantly due to *Paecilomyces lilacinus* (Table 1). The maximum root length (15.24 cm) was recorded in application rate of 1×10^6 CFU/g soil. On the other hand minimum root length (12.38 cm) was found in application rate of 1×10^5 CFU of bioagent/g of soil after control value (10.18 cm).

Applications of *Paecilomyces lilacinus* significantly influenced on root fresh weight (Table 1). The highest root fresh weight (7.74 g) was got in application rate 1×10^6 CFU of *P. lilacinus* per g of soil and the lowest root fresh weight (5.49 g) was obtained in 5×10^5 CFU of bioagent /g soil.

The maximum root dry weight (3.38 g) was recorded in control treatment, and the minimum root dry weight (2.73) was recorded in application rate 5×10^5 CFU soil (Table 1).

Table 1. Main effect of *Paecilomyces lilacinus* application rate on growth parameters of brinjal

Application rate	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
0 CFU/g soil	20.89 c	10.87 b	4.34 c	10.18 c	6.12 b	3.38 a
1×10^5 CFU/g soil	25.12 a	12.78 a	5.06 a	12.38 b	6.03 b	2.95 b
5×10^5 CFU/g soil	23.39 ab	11.53 b	4.57 b	12.52 b	5.49 b	2.73 b
1×10^6 CFU/g soil	22.19 bc	10.97 b	5.02 a	15.24 a	7.74 a	2.80 b
$LSD_{(0.05)}$	2.315	0.9583	0.2193	1.933	1.035	0.427
CV (%)	13.47	13.47	14.84	18.6	10.25	14

In a column means having, similar letter(s) do not differ significantly at 5% level of probability.

4.2 Main effect of *Meloidogyne incognita* inoculum level on growth parameters of brinjal

Significant variation was found in shoot length of brinjal due to *Meloidogyne incognita* inoculum level. The maximum shoot length (27.33 cm) was recorded in control treatment (0 eggs /100 g of soil). The minimum shoot length (18.98 cm) was found in where *M. incognita* was inoculated @ 3200 eggs/100 g of soil (Table 2 and Plate-16).

Significant variation was found in shoot fresh weight due to the effect of *Meloidogyne incognita* (Table 2). The maximum shoot fresh weight (13.81 g) was recorded in control treatment, while minimum shoot fresh weight (8.71 g) was found in the treatment of 3200 eggs/100 g of soil and showing significant different result from other treatments.

The shoot dry weight also varied significantly due to the effect of different inoculum level of *M. incognita*. The highest shoot dry weight (4.96 g) was recorded in the

control treatment (0 eggs/100 g of soil) and minimum (4.59 g) was in treatment of 3200 eggs/100 g of soil (Table 2 and Plate-17).

Significant variation was shown in root length of brinjal due to the effect of *Meloidogyne incognita*. The maximum root length (14.14 cm) was found in control treatment (0 eggs/100 g of soil) and the minimum root length (11.32 cm) was recorded in treatment of 3200 eggs/100 g of soil (Table 2).

Significant variation was found in case of root fresh weight due to the effect of *Meloidogyne incognita* (Table 2). The maximum root fresh weight (6.53 g) was found in the control treatment (0 eggs/100 g of soil). The treatment 3200 eggs/100 g of soil, gave minimum root fresh weight (5.00 g) and showing significantly different result from other treatments.

The root dry weight varied significantly due to the effect of different inoculum level of *M. incognita*. The highest root dry weight (3.09 g) was recorded in the control treatment (0 eggs/100 g of soil) and minimum (2.84 g) was in the treatment of 1600 eggs/100 g of soil (Table 2).

Table 2. Main effect of *Meloidogyne incognita* inoculum level on growth parameters of brinjal

Inoculum level*	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Control	27.33 a	13.81 a	4.96 a	14.14 a	6.53 a	3.09 a
400	24.45 b	12.75 ab	4.73 bc	13.53 ab	6.09 b	2.86 b
800	22.95 b	11.92 bc	4.63 bc	11.69 c	5.50 c	3.08 a
1600	20.78 c	10.5 cd	4.82 ab	12.22 bc	5.50 c	2.84 b
3200	18.98 c	8.711 d	4.59 c	11.32 c	5.00 d	2.96 ab
$LSD_{(0.05)}$	2.02	1.808	0.19	1.69	0.49	0.21
CV (%)	13.47	13.47	14.84	18.6	10.25	14.00

*Number of eggs/100g soil

In a column means having, similar letter(s) do not differ significantly at 5% level of probability.

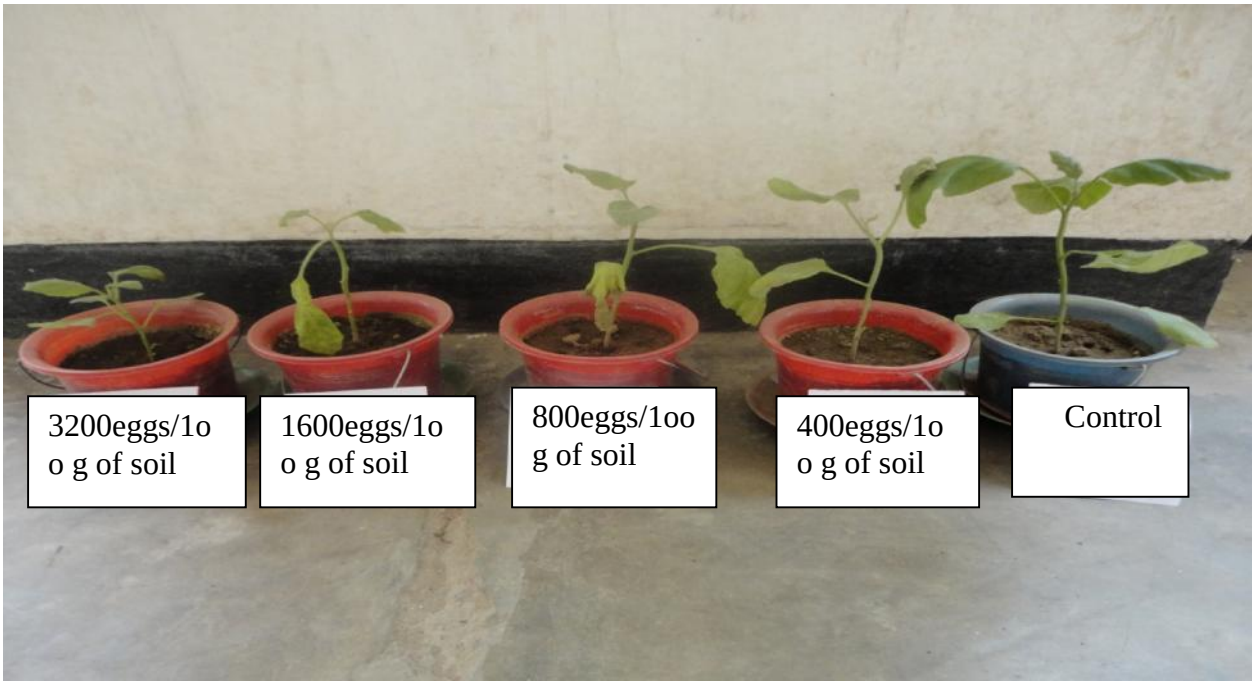


Plate 16 . Photograph showing the effect of *Meloidogyne incognita* on plant growth of eggplant in comparison to control

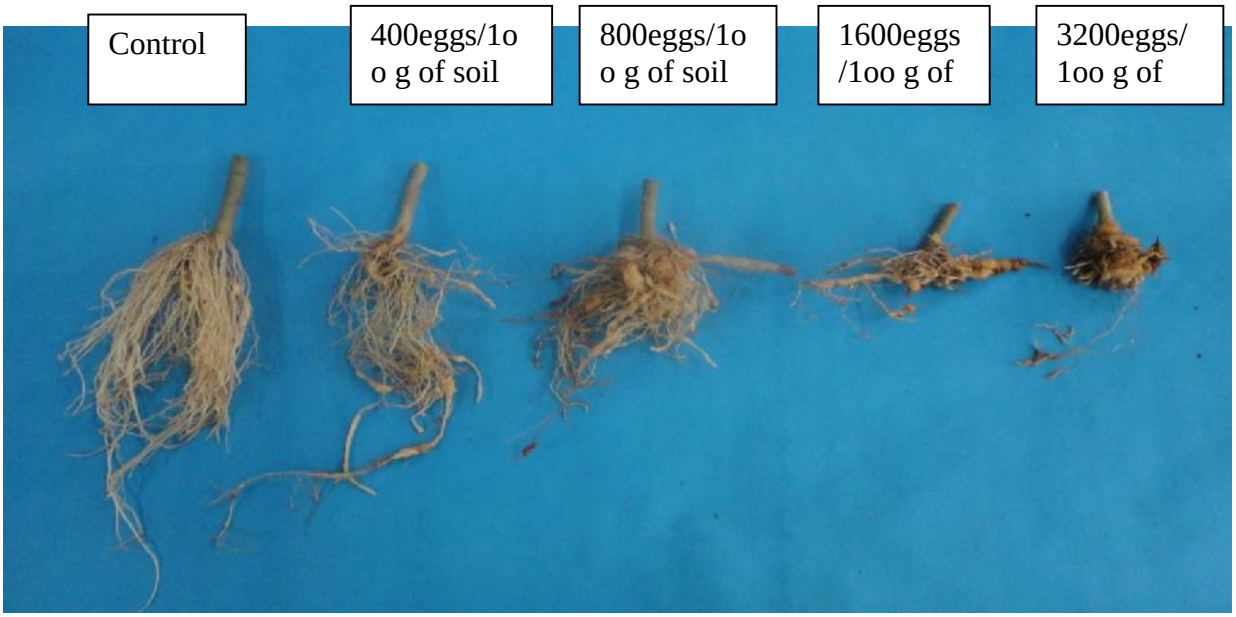


Plate 17. Photograph showing the effect of inoculum level of *M. incognita* on root growth in comparison to control

4.3. Combined effect of *Paecilomyces lilacinus* application rate and *Meloidogyne incognita* inoculum density on growth parameters

The shoot length was significantly influenced by the interaction effect of *Paecilomyces lilacinus* application rate *and Meloidogyne incognita* inoculum level. The maximum shoot length (30.34 cm) was recorded in a combination of 5×10^5 CFU/g soil of *P. lilacinus* with 0 eggs/100 g of soil and the minimum shoot length (17.93 cm) was recorded from 1×10^6 CFU/g soil with 3200 eggs/100 g of soil of *M. incognita* (Fig. 1 and Plate 18-19).

Shoot fresh weight was significantly influenced by the interaction effect of *Paecilomyces lilacinus* and *M. incognita*. The highest shoot fresh weight (14.78 g) was recorded in combination of 5×10^5 CFU/g soil with 0 eggs/100 g of soil. The lowest shoot fresh weigh (8.07 g) was found in 0 CFU/g soil of *P. lilacinus* with 3200 eggs/100 g of soil of *M. incognita* (Fig. 2).

The shoot dry weight was also significantly influenced by the interaction effect of *Paecilomyces lilacinus* and *M. incognita*. The highest shoot dry weight (5.65 g) was found from 1×10^6 CFU/g soil of *P. lilacinus* with 1600 eggs/100 g of soil of *M. incognita* and the lowest shoot dry weight (4.29 g) was found in 5×10^5 CFU/g soil with 3200 eggs of *M. incognita*/100 g of soil (Fig. 3).

The root length was significantly influenced by the interaction effect of *Paecilomyces lilacinus and Meloidogyne incognita*. The maximum root length (15.41 cm) was recorded in a application rate 1×10^5 CFU /g soil of *P. lilacinus* where no *M. incognita* was inoculated and the minimum root length (9 cm) was recorded in 1600 eggs of *M. incognita*/100 g of soil where no bioagent was applied (Fig. 4).

The root fresh weight was also significantly influenced by the interaction effect of *Paecilomyces lilacinus* and *M. incognita*. The highest root fresh weight (6.89 g) was recorded in a combination of 1×10^5 CFU/g soil with control. The lowest root fresh weight (5.40 g) after control weight (4.9 g) was found in combination of a rate application 5×10^5 CFU/g soil of *P. lilacinus* with 400 eggs/100 g of soil of *M. incognita* (Fig. 5).

The root dry weight was also significantly influenced by the interaction effect of *Paecilomyces lilacinus* and *M. incognita*. The highest root dry weight (3.35 g), after control weight (3.81 g), was recorded in a combination of application rate of 1×10^6 CFU/g soil of *P. lilacinus* with 400 eggs of *M. incognita*/100 g of soil. The lowest root dry weight (2.66 g) was found in 1×10^6 CFU/g soil with 1600 eggs/100 g of soil (Fig. 6).

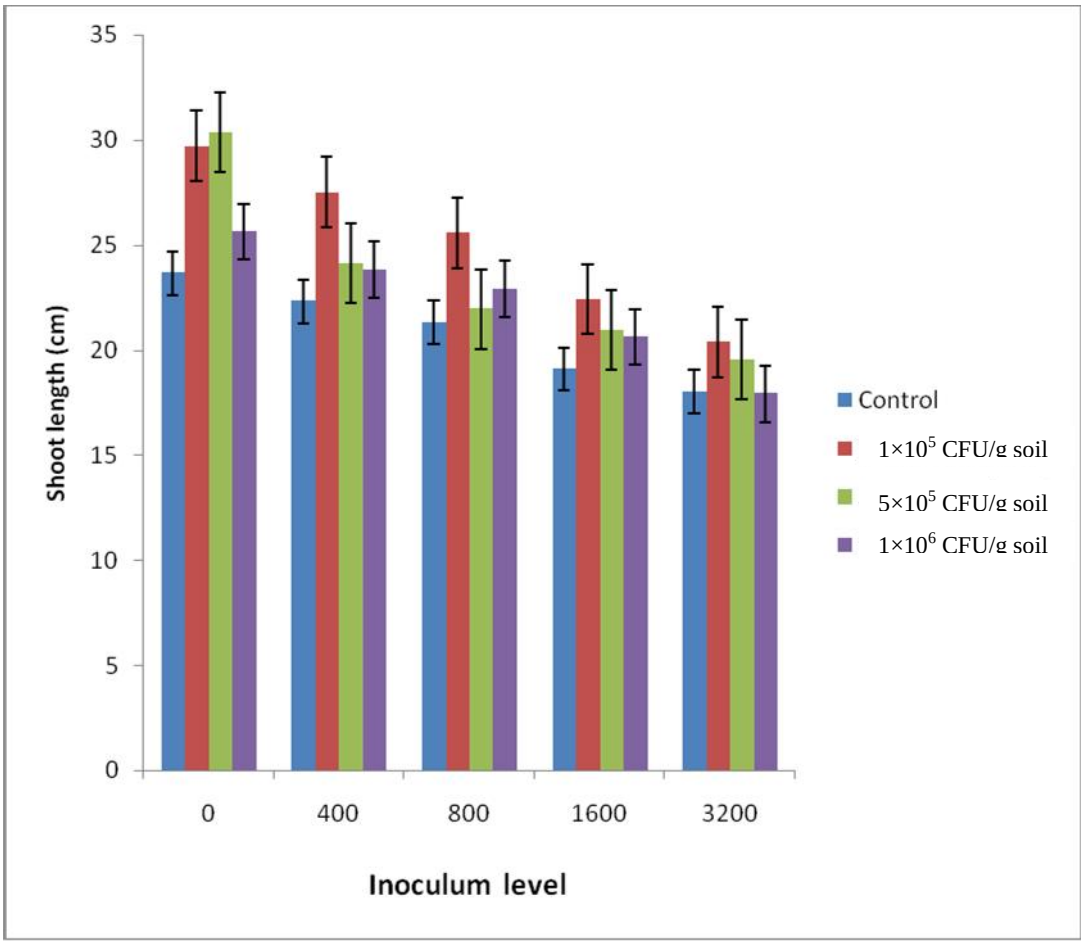


Fig. 1. Effect of *Meloidogyne incognita* inoculum level and application rate of *Paecilomyces lilacinus* to the soil on shoot length of brinjal. Vertical bars represent mean ± standard error (SE)

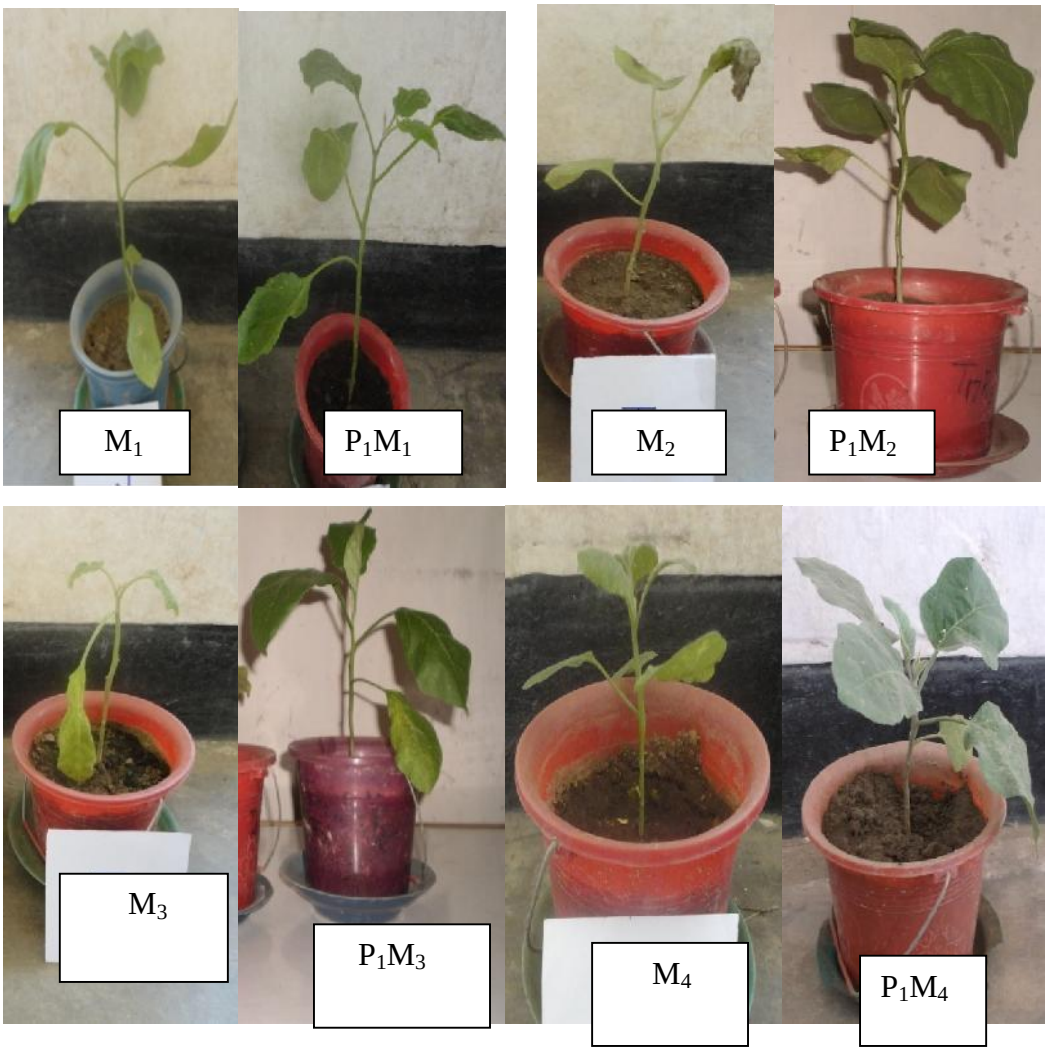


Plate 18. Photograph showing the effect of dose of *Paecilomyces lilacinus* (1×105 CFU/g of soil) on shoot growth of brinjal cv singnath in comparison to control

M_1 = Inoculation of *M. incognita* @ 400 eggs/ 100 cm^3 of soil
P_1M_1 = Inoculation of *M. incognita* @ 400 eggs/100 cm^3 of soil + *P. lilacinus* @ 1×10^5 CFU/ g of soil
M_2 = Inoculation of *M. incognita* @ 800 eggs/ 100 cm^3 of soil
P_1M_2 = Inoculation of *M. incognita* @ 800 eggs/ 100 cm^3 of soil + *P. lilacinus* @ 1×10^5 CFU/ g of soil
M_3 = Inoculation of *M. incognita* @ 1600 eggs/ 100 cm^3 of soil
P_1M_3 = Inoculation of *M. incognita* @ 1600 eggs/ 100 cm_3 of soil + *P. lilacinus*@ 1×10^5 CFU/ g of soil
P_1M_4= Inoculation of *M. incognita* @3200 eggs/ 100 cm^3 of soil + dose of *P. lilacinus* 1×10^5 CFU/ g of soil

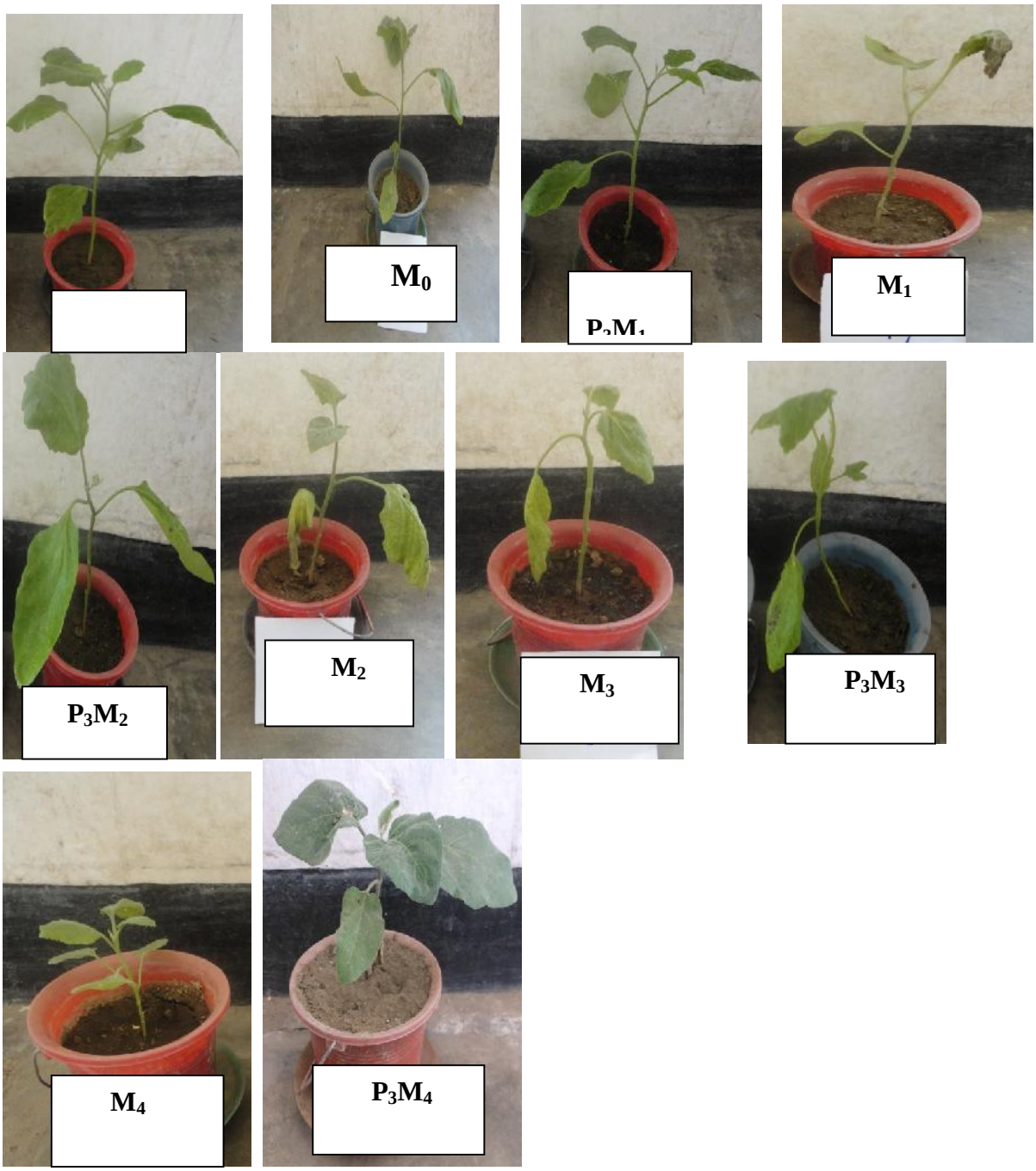


Plate 19. Photograph showing the combined effect of *P. lilacinus* and *M. incognita* on shoot growth in comparison to control

M_0 = No inoculation of *Meloidogyne*
P_3M_0 = No inoculation of *Meloidogyne* + *Paecilomyces lilacinus* @ 1×10^6 CFU/g of soil
M_1 = Inoculation of *M. incognita* @ 400 eggs/ 100 cm^3 of soil
P_3M_1 = Inoculation of *M. incognita* @ 400 eggs/ 100 cm^3 of soil + *Paecilomyces lilacinus* @ 1×10^6 CFU/g of soil
M_2 = Inoculation of *M. incognita* @ 800 eggs/ 100 cm^3 of soil
P_3M_2 = Inoculation of *M. incognita* @ 800 eggs/ 100 cm^3 of soil + *Paecilomyces lilacinus* @ 1×10^6 CFU/g of soil
M_3 = Inoculation of *M. incognita* @ 1600 eggs/ 100 cm^3 of soil
P_3M_3 = Inoculation of *M. incognita* @ 1600 eggs/ 100 cm^3 of soil + *P. lilacinus* @ 1×10^6 CFU/ g of soil
M_4 = Inoculation of *M. incognita* @ 3200 eggs/ 100 cm^3 of soil
P_3M_4 = Inoculation of *M. incognita* @ 3200 eggs/ 100 cm^3 of soil + dose of *P. lilacinus* @ 1×10^6 CFU/ g of soil

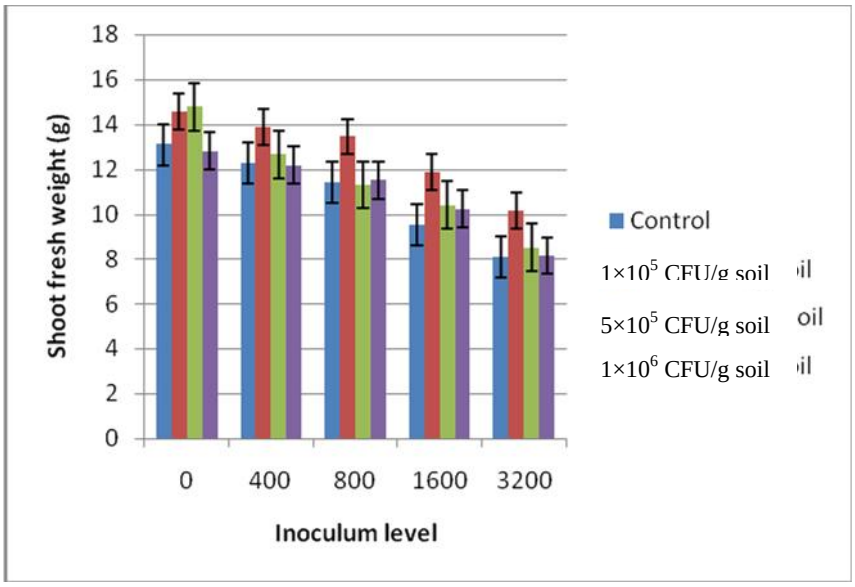


Fig. 2. Effect of *Meloidogyne incognita* inoculum level and application rate of *Paecilomyces lilacinus* to the soil on shoot fresh weight of brinjal. Vertical bars represent mean ± standard error (SE)

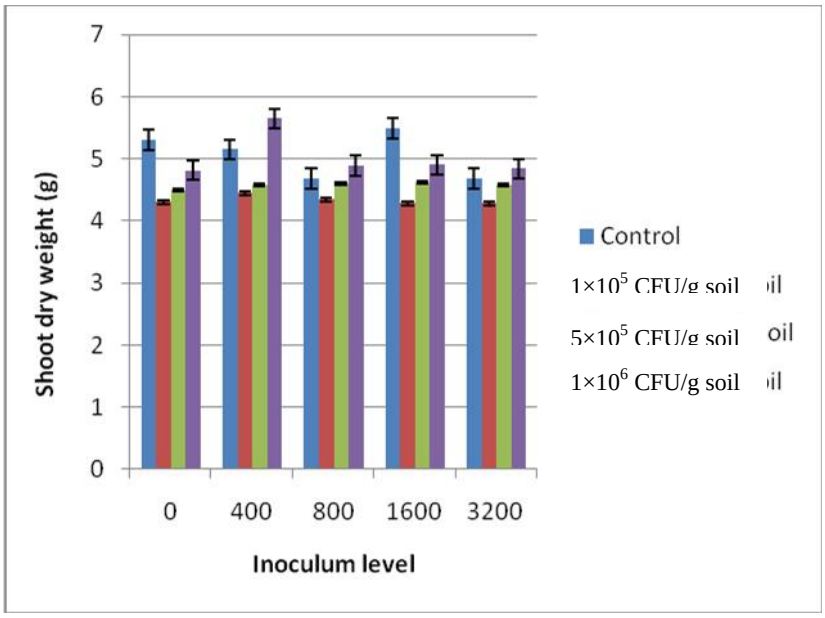


Fig. 3. Effect of *Meloidogyne incognita* inoculum level and application rate of *Paecilomyces lilacinus* to the soil on shoot dry weight of brinjal. Vertical bars represent mean ± standard error (SE)

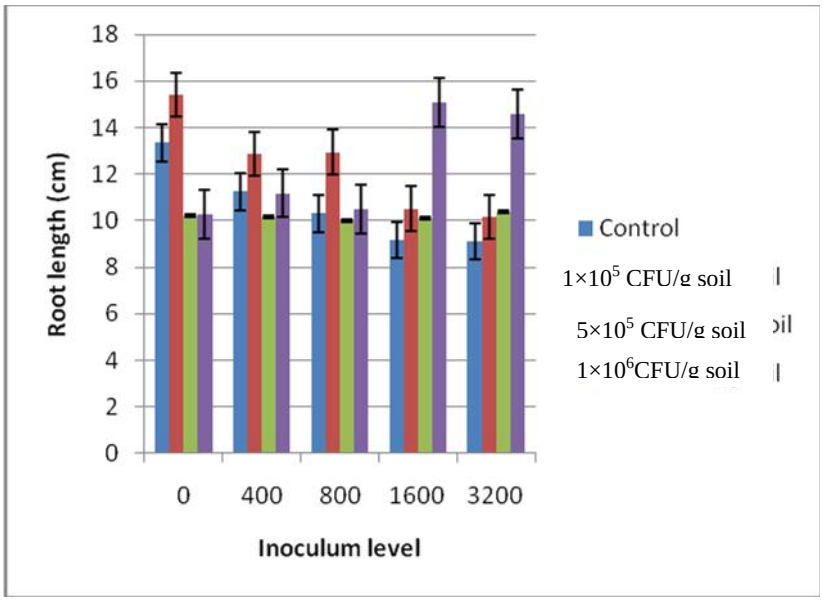


Fig. 4. Effect of *Meloidogyne incognita* inoculum level and application rate of *Paecilomyces lilacinus* to the soil on root length of brinjal. Vertical bars represent mean ± standard error (SE)

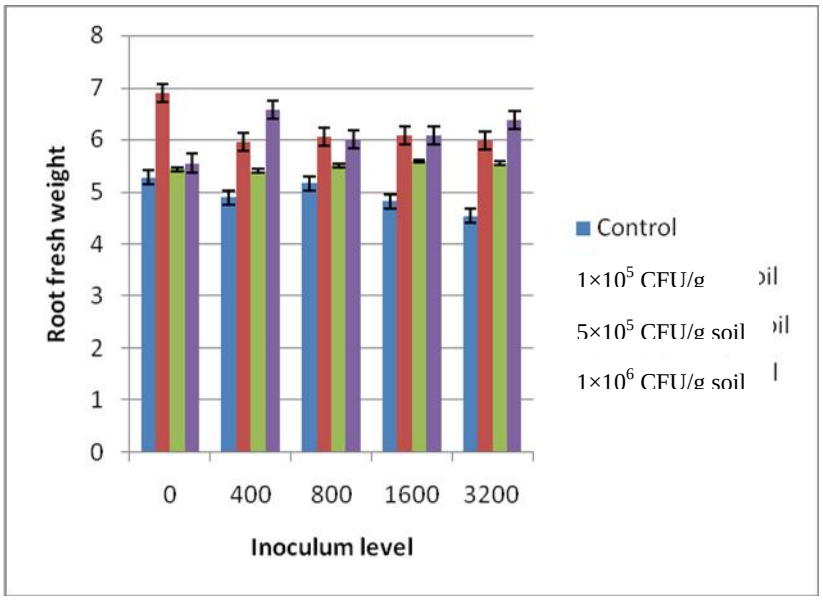


Fig. 5. Effect of *Meloidogyne incognita* inoculum level and application rate of *Paecilomyces lilacinus* to the soil on root fresh weight of brinjal. Vertical bars represent mean ± standard error (SE)

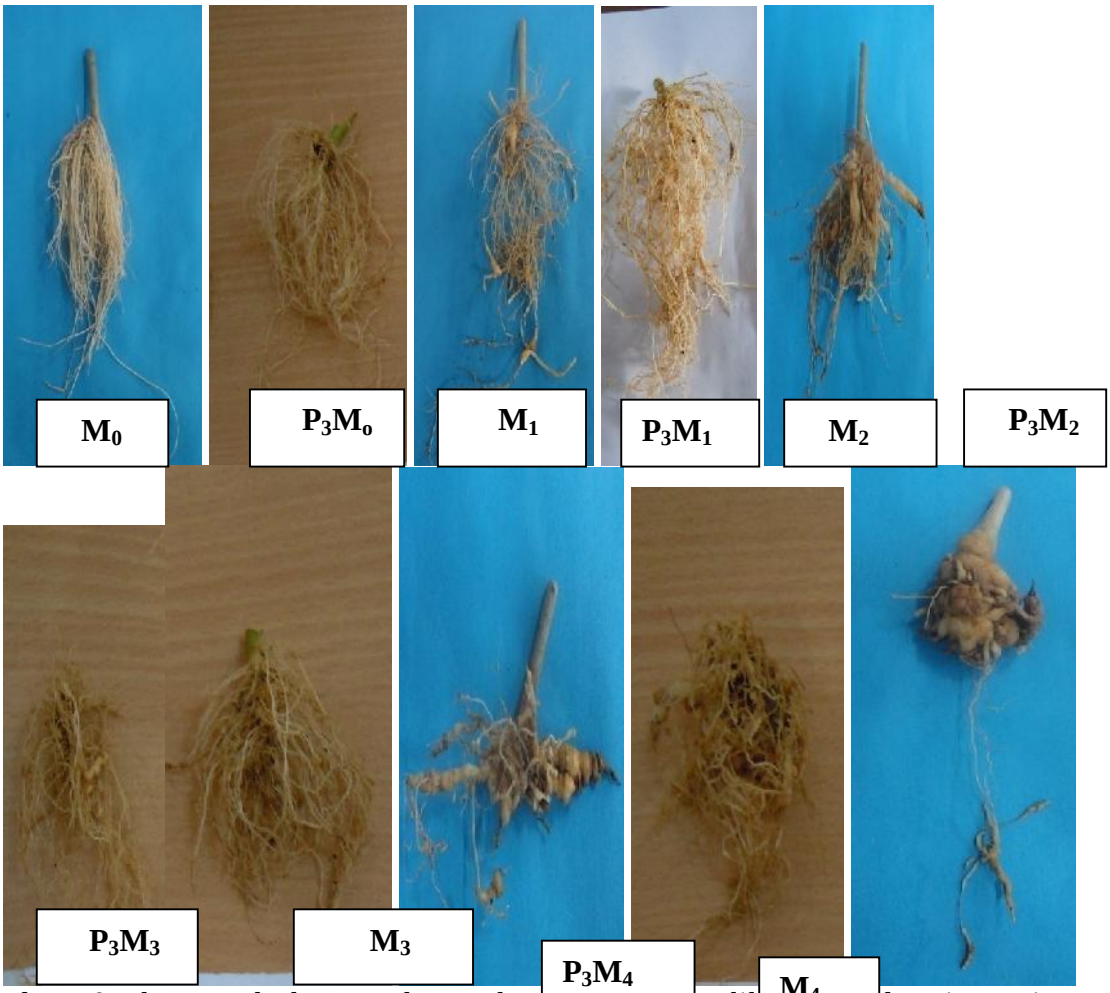


Plate 20. Photograph showing the combined effect of *P. lil* d *M. incognita* on root length in comparison to control

M_0 = No inoculation of *M. incognita*
P_3M_0 = No *Meloidogyne* + *Paecilomyces lilacinus* @1×10^6 CFU/g of soil
M_1= Inoculation of *M. incognita* @400 eggs/ 100 cm^3 of soil
P_3M_1= Inoculation of *M. incognita*@ 400 eggs/ 100 cm^3 of soil + *P. lilacinus* @1×10^6 CFU/g of soil
M_2= Inoculation of *M. incognita* @ 800 eggs/ 100 cm^3 of soil
P_3M_2= Inoculation of *M. incognita* @ 800 eggs/ 100 cm^3 of soil + *P. lilacinus* @1×10^6 CFU/g of soil
M_3= Inoculation of *M. incognita* @ 1600 eggs/ 100 cm^3 of soil
P_3M_3 = Inoculation of *M. incognita* @ 1600 eggs/ 100 cm^3 of soil + *P. lilacinus* @ 1×10^6 CFU/ g of soil
M_4 = Inoculation of *M. incognita* @ 3200 eggs/ 100 cm^3 of soil
P_3M_4= Inoculation of *M. incognita* @ 3200 eggs/ 100 cm^3 of soil + *P. lilacinus* @ 1×10^6 CFU/ g of soil

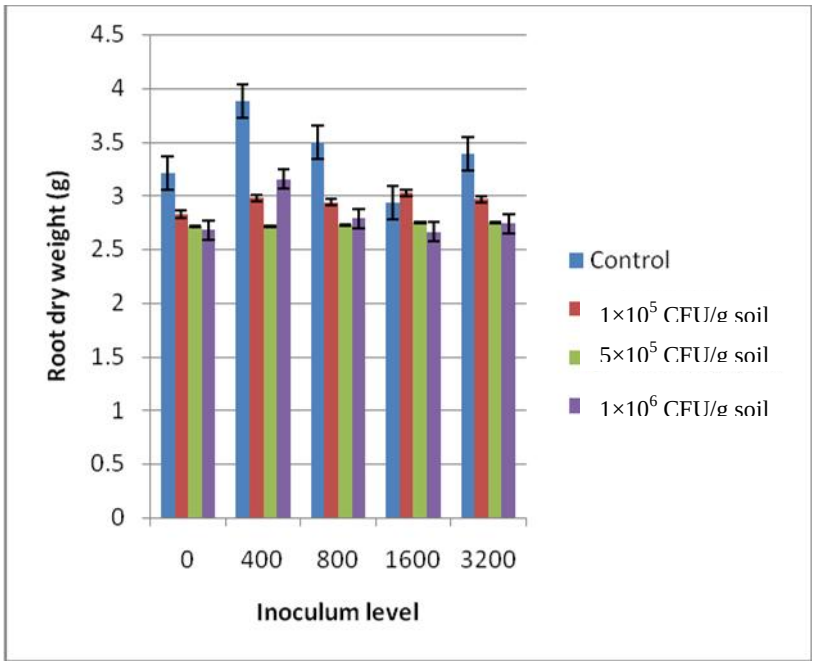


Fig. 6. Effect of *Meloidogyne incognita* inoculum level and application rate of *Paecilomyces lilacinus* to the soil on root dry weight of brinjal. Vertical bars represent mean ± standard error (SE)

4.4 Main effect of *Paecilomyces lilacinus* application rate on gall index and number of egg mass per root of *M. incognita* in brinjal

The results showed that *Paecilomyces lilacinus* had significant effect on gall index. The control treatment gave the maximum gall index (6.73). The application of bioagent *P. lilacinus* @ 1×10^6 CFU/g soil treatment gave minimum (2.55) gall index (Table 3).

The number of egg mass per root varied significantly due to the application of *Paecilomyces lilacinus*. The maximum number of egg mass per root (65.03) was

found in control, and the minimum number of egg mass per root (16.95) was recorded in where *P. lilacinus* was applied @ 1×10^6 CFU/g soil (Table 3).

Table 3. Main effect of *Paecilomyces lilacinus* application rate on gall index and number of egg mass per root of *M. incognita* in brinjal

Application rate	Gall index	Number of egg mass/root
0 CFU/g soil	6.73 a	65.03 a
1×10^5 CFU/g soil	4.38 b	34.40 b
5×10^5 CFU/g soil	3.88 b	19.30 c
1×10^6 CFU/g soil	2.55 c	16.95 c
$LSD_{(0.05)}$	1.05	10.46
CV (%)	15.35	19.36

In a column means having, similar letter(s) do not differ significantly at 5% level of probability.

4.5 Main effect of *Meloidogyne incognita* inoculum level on gall index, number of egg mass per root of *M. incognita* in brinjal

The gall index was significantly influenced by different inoculum level of *M. incognita*. Inoculation of 3200 eggs of *M. incognita*/100 g of soil produced maximum gall index (6.81) and the minimum (4.00) gall index was recorded in 400 eggs/100 g of soil (Table 4).

The number of eggmass per root varied significantly due to the different inoculum level of *M. incognita*. The highest number of egg mass per root (48.94) was recorded in 3200 eggs of *M. incognita*/100 g of soil, which was statistically similar to 800 and 1600 eggs/100 g of soil. The minimum number of egg mass per root (27.03) was found in where M. *incognita* applied @ 400 eggs/100 g of soil (Table 4).

Table 4. Main effect of *Meloidogyne incognita* inoculum level on gall index and number of egg mass per root of *M. incognita.* in brinjal

Inoculum level*	Gall index	Number of egg mass/root
Control	0.00 d	0.00 c
400	4.00 c	27.03 b
800	5.31 b	44.88 a
1600	5.78 b	48.94 a
3200	6.81 a	48.75 a
$LSD_{(0.05)}$	0.919	9.122
CV (%)	15.35	19.36

*Nubmer of eggs/100 g soil
In a column means having, similar letter(s) do not differ significantly at 5% level of probability.

4.6 Combined effect of *Paecilomyces lilacinus* application rate and *Meloidogyne incognita* inoculum density to the soil on gall index and number of egg mass per root of brinjal

Interaction effect of *Paecilomyces lilacinus* and inoculum level of *M. incognita* had a significant variation on gall index (Fig. 7). The maximum gall index (9.25) was recorded in 0 CFU of *P. lilacinus*/g soil with 3200 eggs/100 g of soil, which was statistically similar to 0 CFU of *P. lilacinus*/g soil with 1600 eggs/100 g of soil, 0 CFU of *P. lilacinus*/g soil with 800 eggs/100 g of soil, while the minimum gall index (2.13) was recorded in where *P. lilacinus* applied @1×10^6 CFU/g soil with 400 eggs of *M. incognita*/100 g of soil.

The number of egg mass per root was significantly influenced by the interaction effect of *Paecilomyces lilacinus* and *M. incognita* (Fig. 8). The highest number of egg mass per root (71.77) was found in 0 CFU of *P. lilacinus*/g soil with 800 eggs/100 g of soil, which was statistically similar to 0 CFU of *P. lilacinus*/g soil with 400 eggs/100 g of soil, 0 CFU of *P. lilacinus*/g soil with 1600 eggs/100 g of soil, 0 CFU of *P. lilacinus*/g soil with 3200 eggs/100 g of soil. The lowest number of egg mass per root (8.50) was

recorded in 1×10^6 CFU/g soil of *P. lilacinus* with 400 eggs of *M. incognita*/100 g of soil.

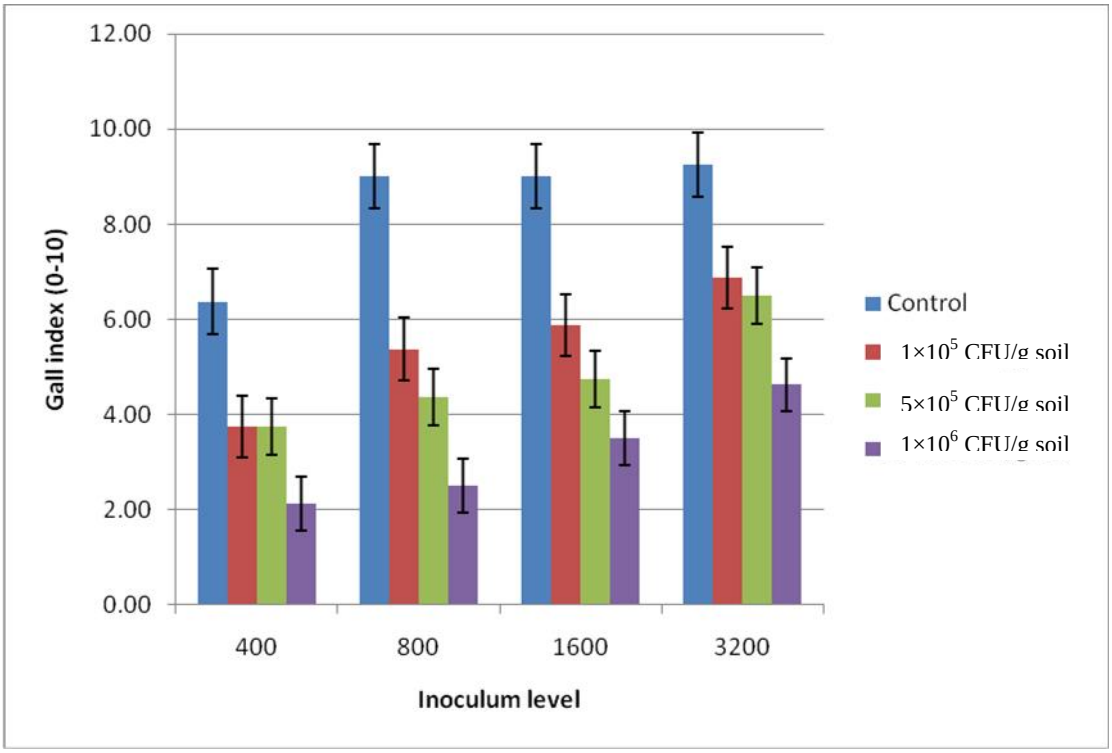


Fig. 7. Effect of *Meloidogyne incognita* inoculum level and application rate of *Paecilomyces lilacinus* to the soil on gall index of brinjal. Vertical bars represent mean ± standard error (SE)

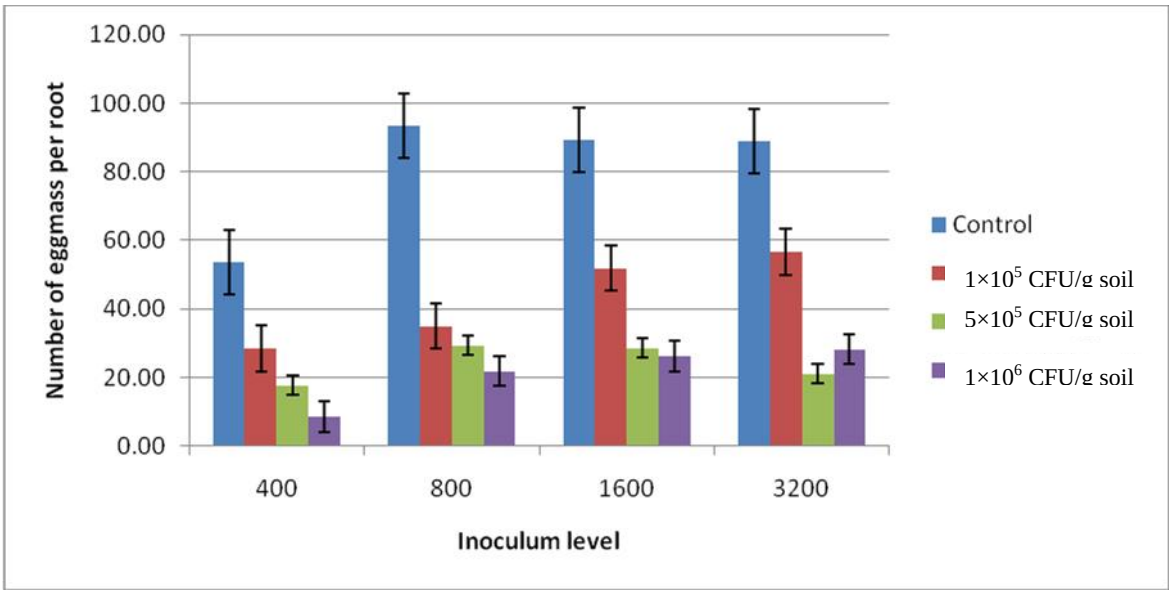


Fig. 8. Effect of *Meloidogyne incognita* inoculum level and application rate of *Paecilomyces lilacinus* to the soil on number of egg mass/root of brinjal . Vertical bars represent mean ± standard error (SE)

4.7. Main effect of *Paecilomyces lilacinus* application rate on nematode population of *M. incognita* in brinjal

The number of egg per egg mass varied significantly due to the application of *Paecilomyces lilacinus*. The maximum number of egg per egg mass (418.00) was recorded in control, which was statistically similar to 1×10^5 CFU/g soil. The minimum number of egg per egg mass (240.10) was recorded in 1×10^6 CFU of *P. lilacinus*/g soil, which was statistically similar to 5×10^5 CFU/g soil (Table 5).

The number of egg per root system varied significantly due to the application of *Paecilomyces lilacinus*. The maximum number of egg per root system (3.66×10^3) was found in control, which was statistically similar to 1×10^5 CFU/g soil. The minimum number of egg per root system (2.96×10^3) was recorded in where *P. lilacinus* applied @ 1×10^6 CFU/g soil, which was statistically similar to 5×10^5 CFU/g soil (Table 5).

The number of juveniles/800g pot varied significantly due to the application of *Paecilomyces lilacinus*. The maximum number of juveniles/800g soil/pot (4.58×10^5) was recorded in control and the minimum number of juveniles/800g soil/ pot

(3.86×10^5) was recorded in 5×10^5 CFU/g soil, which was statistically similar to 1×10^5 CFU/g soil and 1×10^6 CFU/g soil (Table 5).

The total nematode population varied significantly due to the application of *Paecilomyces lilacinus*. The maximum total nematode population (4.61×10^5) was observed in where no *Meloidogy*ne applied, and the minimum total nematode population (3.90×10^5) was recorded in 5×10^5 CFU of *P. lilacinus*/g soil, which was statistically similar to 1×10^6 CFU/g soil and 1×10^5 CFU/g soil (Table 5).

The reproduction factor varied significantly due to the application of *Paecilomyces lilacinus*. The maximum reproduction factor (54.86) was observed in control, and the minimum reproduction factor (7.19) was recorded in 5×10^5 CFU/g soil, which was statistically similar to1×10^5 CFU/g soil and 1×10^6 CFU/g soil (Table 5).

Table 5. Main effect of *Paecilomyces lilacinus* application rate on nematode population in brinjal

Application rate	Number of egg/egg mass	Number of egg/root system (10^3)	No of J2/800gm soil/plot (10^5)	Total nematode population (10^5)	Reproduction factor Pf/Pi
0 CFU/g soil	418.00 a	3.66 a	4.58 a	4.61 a	54.86 a
1×10^5 CFU/g soil	369.10 a	3.39 a	4.05 b	4.06 b	12.64 b
5×10^5 CFU/g soil	260.90 b	3.02 b	3.86 b	3.90 b	7.19 b
1×10^6 CFU/g soil	240.10 b	2.96 b	3.97 b	3.97 b	11.65 B
$LSD_{(0.05)}$	102.20	0.31	0.21	0.30	15.64

In a column means having, similar letter(s) do not differ significantly at 5% level of probability.

4.8. Main effect of inoculum level of *Meloidogyne incognita* on nematode population in brinjal

A significant variation was observed in number of egg per egg mass due to the different inoculum level of *M. incognita*. The highest number of egg per egg mass

(461.80) was found in 3200 eggs/100 g of soil. The minimum number of egg per egg mass (326.10) was recorded in 400 eggs/100 g of soil (Table 6).

A significant variation was observed in number of egg per root system due to the different inoculum level of *M. incognita*. The highest number of egg per root system (4.22×10^3) was recorded in 3200 eggs/100 g of soil, which was statistically similar to 800 eggs/100 g of soil and 1600 eggs/100 g of soil. The minimum number of egg per root system ($3.80\times10^{3)}$ was found in 400 eggs/100 g of soil (Table 6).

A significant variation was observed in number of juveniles/800g soil/pot due to the different inoculum level of *M. incognita*. The highest number of juveniles/800g soil/pot (5.54×10^5) was observed in 3200 eggs/100 g of soil. The minimum number of juveniles/800g soil/pot (4.74×10^5) was found in 400 eggs/100 g of soil (Table 6).

The total nematode population varied significantly due to the different inoculum level of *M. incognita*. The highest nematode population (5.57×10^5) was found in 3200 eggs/100 g of soil, which was statistically similar to 1600 eggs/100 g of soil. The minimum nematode population (4.77×10^5) was recorded in 400 eggs/100 g of soil (Table 6).

The reproduction factor varied significantly due to the effect of different inoculum level of *M. incognita*. The highest reproduction factor (31.71) was recorded in 400 eggs/100 g of soil. The minimum reproduction factor (24.16) was found in 3200 eggs/100 g of soil (Table 6).

Table 6. Effect of inoculum level of *Meloidogyne incognita* on nematode population in brinjal

Inoculum level *	Number of egg/egg mass	Number of egg/root system (10^3)	No of J2/800gm soil/plot (10^5)	Total nematode population (10^5)	Reproduction factor Pf/Pi
Control	0.00 c	0.00 c	0.00 e	0.00 c	0.00 b
400	326.10 b	3.80 b	4.74 d	4.77 b	31.71 a
800	413.30 ab	4.09 a	4.98 c	5.02 b	26.78 a

1600	409.00	ab	4.17	a	5.31	b	5.32	a	25.27	a
3200	461.80	a	4.22	a	5.54		5.57	a	24.16	a
$LSD_{(0.05)}$	89.140		0.271		0.191		0.263		13.640	

In a column means having, similar letter(s) do not differ significantly at 5% level of probability.

4.9. Combined effect of *Paecilomyces lilacinus* application rate and *Meloidogyne incognita* inoculum density to the soil on nematode population in brinjal

The number of egg per egg mass was significantly influenced by the interaction effect of *Paecilomyces lilacinus* and *M. incognita* (Fig. 9). The highest number of egg per egg mass (638.80) was found in 0 CFU of *P. lilacinus*/g soil with 3200 eggs/100 g of soil. The lowest number of egg per egg mass (190.40) was recorded in 1×10^6 CFU of *P. lilacinus*/g soil with 400 eggs/100 g of soil.

The number of egg per root system was significantly influenced by the interaction effect of *Paecilomyces lilacinus* and *M. incognita* (Fig. 10). The highest number of egg per root system (4.66×10^3) was found in 0 CFU of *P. lilacinus*/g soil with 3200 eggs/100 g of soil. The lowest number of egg per root system (3.16×10^3) was recorded in 1×10^6 CFU of *P. lilacinus*/g soil with 400 eggs of *M. incognita*/100 g of soil.

The total nematode population was significantly influenced by the interaction effect of *Paecilomyces lilacinus* and *M. incognita* (Fig. 11). The highest total nematode population (6.10×10^5) was found in 0 CFU of *P. lilacinus*/g soil with 3200 eggs/100 g of soil, which was statistically similar to 0 CFU of *P. lilacinus*/g soil with 1600 eggs/100 g of soil. The lowest total nematode population (4.46×10^5) was recorded in 1×10^6 CFU/ of *P. lilacinus* g soil with 400 eggs of *M. incognita*/100 g of soil.

The number of juveniles/800g soil/pot was significantly influenced by the interaction effect of *Paecilomyces lilacinus* and *M. incognita* (Fig. 12). The highest number of juveniles/800g soil/ pot (6.08×10^5) was found in 0 CFU of *P. lilacinus*/g soil with 3200 eggs/100 g of soil. The lowest number of juveniles/800g soil/ pot (1.66×10^5) was recorded in 1×10^6 CFU/g soil with 400 eggs/100 g of soil.

The reproduction factor was significantly influenced by the interaction effect of *Paecilomyces lilacinus* and *M. incognita* (Fig. 13). The highest reproduction factor

(71.77) was recorded in where no bioagent applied in soil with 800 eggs/100 g of soil, which was statistically similar to 0 CFU of *P. lilacinus*/g soil with 400 eggs/100 g of soil. The lowest reproduction factor (7.42) was recorded in 5×10^5 CFU soil with 800 eggs/100 g of soil.

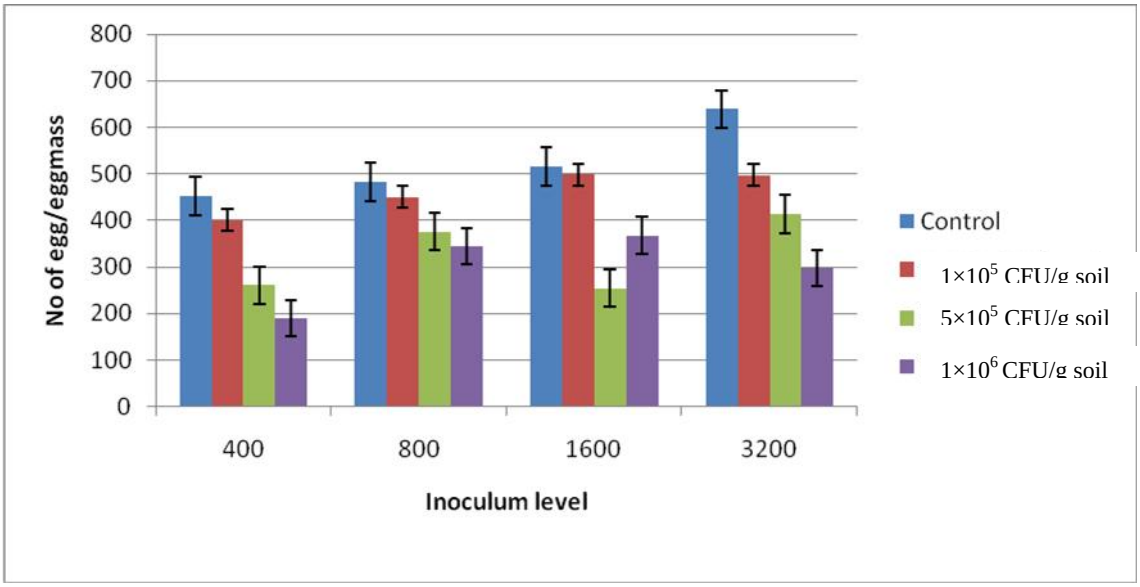


Fig. 9. Effect of *Meloidogyne incognita* inoculum level and application rate of *Paecilomyces lilacinus* to the soil on number of egg/egg mass of brinjal. Vertical bars represent mean ± standard error (SE)

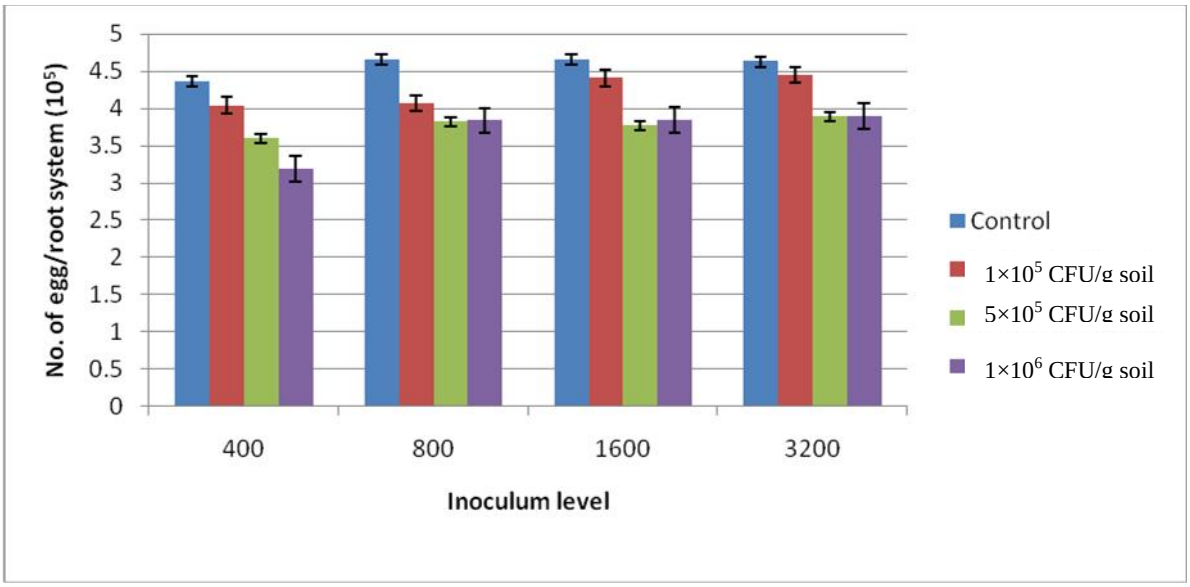


Fig. 10. Effect of *Meloidogyne incognita* inoculum level and application rate of *Paecilomyces lilacinus* to the soil on number of egg/root system of brinjal. Vertical bars represent mean ± standard error (SE)

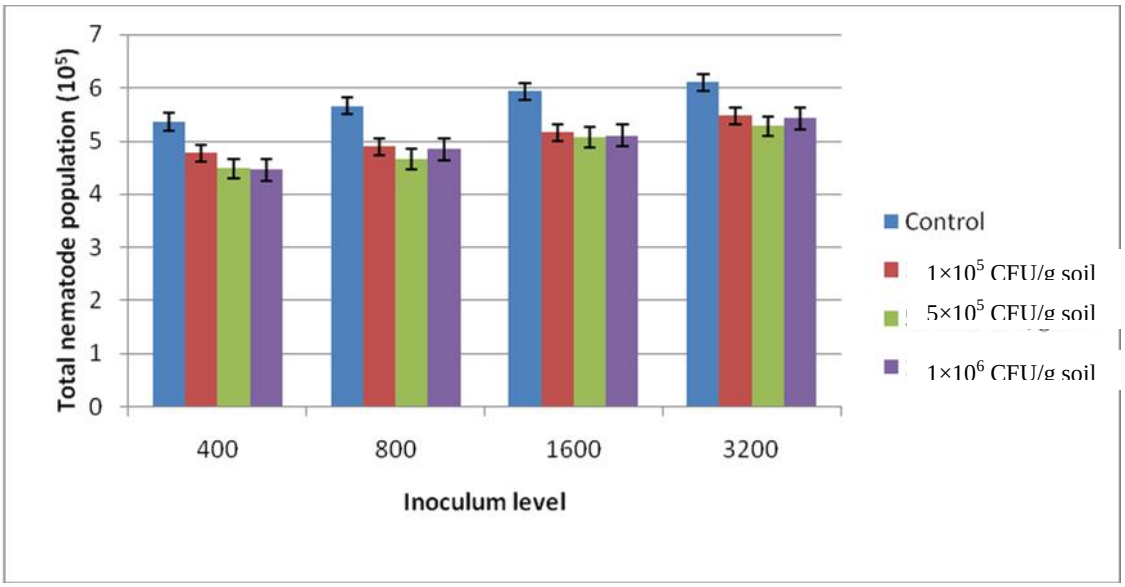


Fig. 11. Effect of *Meloidogyne incognita* inoculum level and application rate of *Paecilomyces lilacinus* to the soil on total nematode population of brinjal. Vertical bars represent mean ± standard error (SE)

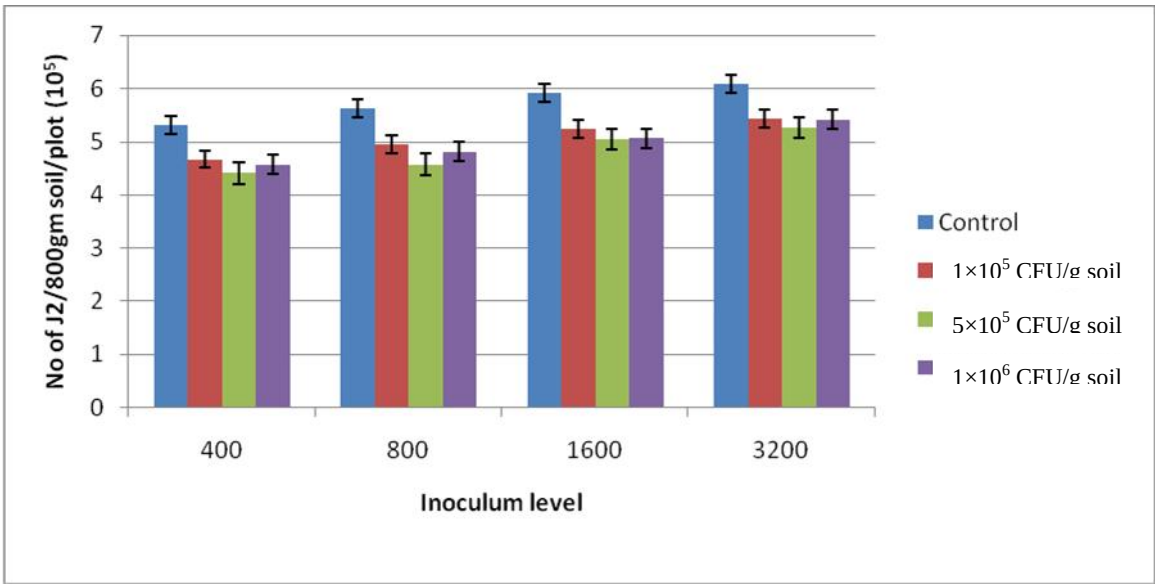


Fig. 12. Effect of *Meloidogyne incognita* inoculum level and application rate of *Paecilomyces lilacinus* to the soil on number of J2/800g plot of brinjal. Vertical bars represent mean ± standard error (SE)

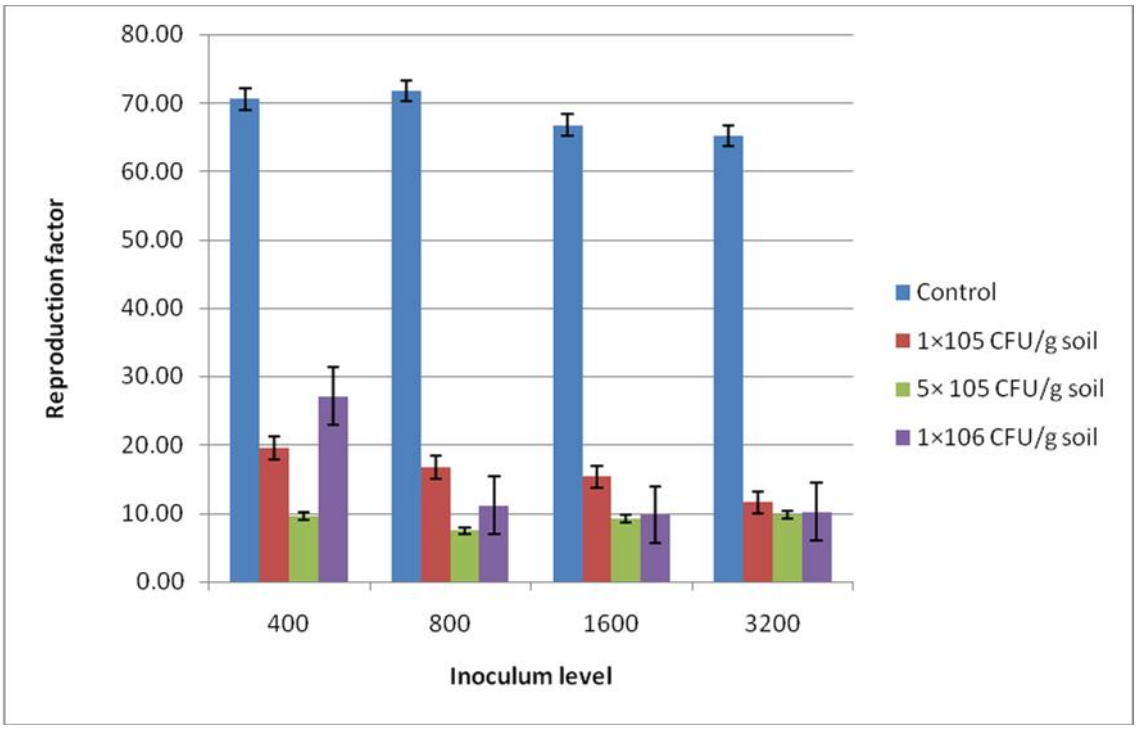


Fig. 13. Effect of *Meloidogyne incognita* inoculum level and application rate of *Paecilomyces lilacinus* to the soil on reproduction factor of brinjal. Vertical bars represent mean ± standard error (SE)

4.10 Main effect of *Paecilomyces lilacinus* application rate on soil colonization and egg mass colonization of *M. incognita*

The percent of egg mass colonization by fungus varied no significantly due to the application of *Paecilomyces lilacinus*. The maximum percent of egg mass colonization by fungus (36.88) was found in 1×10^5 CFU/g soil and the minimum percent of egg mass colonization by fungus (29.38) was recorded in 1×10^6 CFU/g soil (Table 7).

The colonization of fungus/g soil varied significantly due to the application of *Paecilomyces lilacinus*. The maximum soil colonization by *P. lilacinus* (1735.00 CFU/g soil) was found in where 1×10^6 CFU of *P. lilacinus*/g soil was applied and the minimum soil colonization by *P. lilacinus* (1391.00 CFU/g soil) was recorded in a application rate of 1×10^5 CFU/ g soil (Table 7).

Table 7. Main effect of *Paecilomyces lilacinus* application rate on soil colonization and egg mass colonization of *Meloidogyne incognita*

Application rate of *P . lilacinus*	% Egg mass colonized by *P. lilacinus*	Soil colonization by
0 CFU/g soil	0 b	0
1×10^5 CFU/g soil	36.88 a	1391.00
5×10^5 CFU/g soil	35.17 a	1655.00
1×10^6 CFU/g soil	29.38 a	1735.00
$LSD_{(0.05)}$	10.78	61.88
CV (%)	9.95	13.85

In a column means having, similar letter(s) do not differ significantly at 5% level of probability.

4.11. Main effect of *Meloidogyne incognita* inoculum level on soil colonization and egg mass colonization of *M. incognita*

A significant variation was found in percent of egg mass colonization by fungus due to the different inoculum level of *M. incognita*. The highest percent of egg mass colonization by fungus (45.31) was recorded in 800 eggs/100 g of soil and 3200 eggs/100 g of soil. The minimum percent of egg mass colonization by fungus (34.59) was recorded in 400 eggs/100 g of soil (Table 8).

The colonization of fungus/g soil varied significantly due to the effect of different inoculum level of *M. incognita*. The highest soil colonization by *P. lilacinus* (1740.00

CFU/g soil) was found in 3200 eggs/100 g of soil. The minimum soil colonization by *P. lilacinus* (1506.00 CFU/g soil) was recor in 800 eggs/100 g of soil (Table 8).

Table 8. Main effect of *Meloidogyne incognita* inoculum level on soil colonization and egg mass colonization of *M. incognita*

Inoculum level*	**% Egg mass colonized by *P. lilacinus***	**Soil colonization by *P. lilacinus* (CFU/g soil)**
Control	0 c	0 d
400	34.59 b	1580.00 b
800	45.31 a	1506.00 c
1600	41.41 ab	1617.00 b
3200	45.31 a	1740.00 a
$LSD_{(0.05)}$	9.40	53.99
CV (%)	9.95	13.85

In a column means having, similar letter(s) do not differ significantly at 5% level of probability.

***** Number of eggs/100g soil

4.12 Combined effect of inoculum level of *Meloidogyne incognita* and *Paecilomyces lilacinus* on number of eggmass colonization, colonization of fungus/g soil in brinjal

The percent of egg mass colonization by fungus was significantly influenced by the interaction effect of *Paecilomyces lilacinus* and *M. incognita*. The highest percent of egg mass colonization by fungus (59.38) was found in 1×10^5 CFU/g soil with 800 eggs/100 g of soil. The lowest percent of egg mass colonization by fungus (28.13) was recorded in 1×10^5 CFU/g soil with 400 eggs/100 g of soil and 1×10^6 CFU/g soil with 400 eggs/100 g of soil (Fig. 14).

The soil colonization of the fungus was significantly influenced by the interaction effect of *Paecilomyces lilacinus* and *M. incognita*. The highest soil colonization by *P. lilacinus* (2443.00 CFU/g soil) was found in 1×10^5 CFU/g soil with 400 eggs/100 g of soil. The lowest soil colonization by *P. lilacinus* (1108.00 CFU/g soil) was recorded in 1×10^6 CFU/g soil with 800 eggs/100 g of soil (Fig. 15).

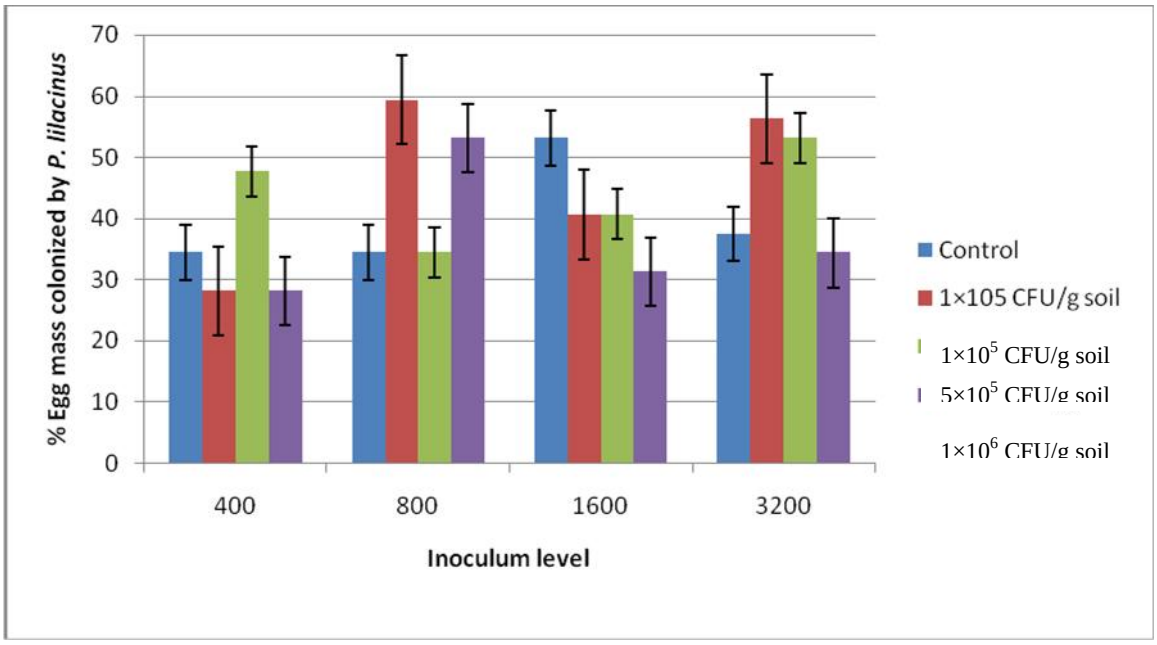


Fig. 14. Effect of *Meloidogyne incognita* inoculum level and application rate of *Paecilomyces lilacinus* to the soil on %egg mass colonization of brinjal. Vertical bars represent mean ± standard error (SE)

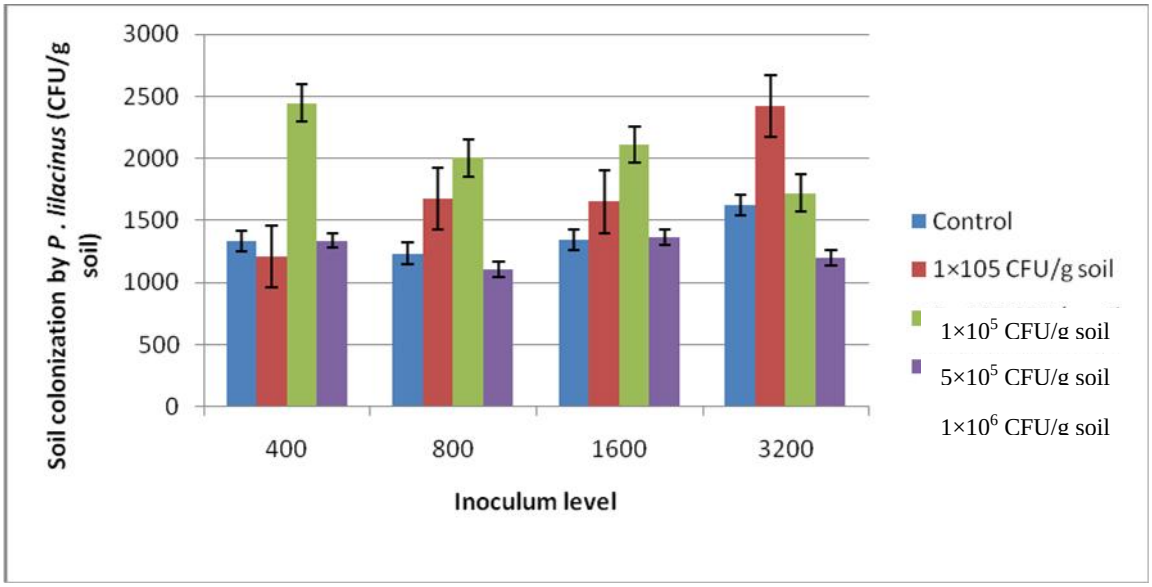


Fig. 15. Effect of *Meloidogyne incognita* inoculum level and application rate of *Paecilomyces lilacinus* to the soil on soil colonization of brinjal. Vertical bars represent mean ± standard error (SE)

DISCUSSION

The experiment was conducted to study effects of *Meloidogyne incognita* inoculum density and application rate of a biocontrol fungus *Paecilomyces lilacinus* into the soil on biocontrol efficacy of bioagent against root knot of brinjal. Our results indicated that using microbial agents suppressed the root-knot nematodes and resulted in positive changes in plant growth.

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 plants treated with 1×10^5 CFU/g soil of *P. lilacinus* showed significant variation in plant growth parameter in comparison to control. A significant increase in plant shoots and root length, 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); Hassan (2004) and Al-Raddad (1995). They were tested the effects of *Glomus mosseae* and *P. lilacinus* on *Meloidogyne javanica* of tomato in a greenhouse experiment. Inoculation of tomato plants with *G. mosseae* did not markedly increase the growth of inoculated 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 length and shoot length, fresh weight and dry weight of root and shoot was achieved when plants were inoculated with *P. lilacinus* to control root knot nematode.

The present experiment demonstrated that soil treated with the lowest dose of *P. lilacinus* (1×10^5CFU/g soil) reduced root galling by 41 and 25% and number of egg masses by 46 and 36% when 400 and 3200 eggs of *M. incognita* per 100 cm^3 of soil was inoculated. This result is supported by Kiewnick *et al*. (2011). They reported that a preplanting soil treatment with the lowest dose *P. lilacinus* (2×10^5CFU/g of soil) was already sufficient to reduce root galling by 45% and number of egg masses by 69% when averaged over inoculum densitiy of 100 to 1600 eggs and infective juvenile per 100 cm^3 of soil.

In this experiment, it was revealed a significant interaction between the dose of *P. lilacinus* applied to soil and the inoculum level for the parameters gall index egg

masses per root. A similar effect was demonstrated by Bourne and Kerry (1999). He also mentioned that sufficient biocontrol efficacy was achieved with the dose of PL251 (5×10^5CFU/g of soil) and this result was supported by Kiewnick *et al.* (2011).

In the present study, a drastical reduction in the galling index upto 72% and number of egg masses per root system upto 84% was found after *P. lilacinus* application @ 1×10^6 CFU/g of soil where the treatment was challenged by an inoculum level upto 800 eggs/100 cm^3 of soil. Similar result was also obtained earlier by Gerco and De Vito (2009) in their study. They reported a strong reduction in the galling index and the number of egg masses per root system after PL251 soil treatment @ 2×10^5 CFU/g soil at inoculum density upto 800 eggs/100 cm^3 of soil.

It was observed that *Meloidogyne incognita* readily infected brinjal (cv. singnath), retarded its growth and reduced the fresh and dry weight of the plants. Apparently *P. lilacinus* was showed effectiveness in suppressing *Meloidogyne incognita* , but rate of application and inoculum density was important. This experiment showed that it reduced 72% root galling of brinjal when the application rate of *P. lilacinus* and inoculum densities was 1×10^6 CFU/g of soil and 800eggs/100 cm^3 of soil, respectively. In case of number of egg masses it reduced 84 % egg masses when the application dose of *P. lilacinus* and inoculum densitiy was 1×10^6CFU/g of soil and 400eggs/100 cm^3 of soil, respectively. Moreover, the activity of *P. lilacinus* attributed to ability to infect eggs, juveniles and female of *M. javanica* by direct hypal penetration (Khan *et al.,* 2006). *P. lilacinus* contains protease and chitinase enzyme which play an imprtant 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 eggplant with biocontrol agents *Pochonia chlamydosporia, P. lilacinus,* and *Trichoderma harzianum* as a result to suppress galls formation and egg masses.

Results of pot experiment demonstrated the efficacy of biocontrol fungus, *P. lilacinus*, in controlling the root-knot nematode *M. incognita* with reduction in galling and nematode population. *P. lilacinus* enhanced plant growth and reduced galling index upto 72% and number egg masses upto 84%. In similar experiment, Aminuzzaman *et al.* (2011) reported that pellets of *P. lilacinus* enhanced plant growth, reduced galling index and nematode population. They also mentined that root galling index and final

nematode population decreased upto 40.70 and 73%, for tomato and 55.6 and 66.9%, respectively for brinjal.

In the experiment, it was found that due to the infection of nematode (*Meloidogyne incognita)* root knot occoured and reduced plant growth. It was observed that brinjal plants inoculated with 3200 eggs of *M. incognita* showed significant reduction in shoot and root length, fresh weight of shoot and root and dry weight of shoot and root. Reduced plant growth characters by inoculation of *M. incognita* was also reported earlier by Esfahani and Ansaripour (2006), Muchsood and Tabreiz (2010) and Kiewnick and Sikora (2006).

In the present study, a high percentages (84%) egg masses were infected with 1×10^6CFU/g soil of *P. lilacinus* and infected eggs contained mycelium of *P. lilacinus*. This result was supported by Esfahani and Ansaripour (2006). They reported that 55% egg masses were infected with P. *lilacinus*. They also observed that some juveniles of infected eggs showed various degrees of deformity and abnormal development and a number of juveniles that emerged from eggs were infected and showed mycelia growth over their body (Ganaie and Khan, 2010 and Jatala, 1985).

It has been found that *P. lilacinus* has significant effect on *Meloidogyne incognita* reproduction factor. It reduced 72% egg msss against 800 eggs/100 $cm^3$of soil when the soil treated with the lowest dose of 1×10^5CFU/g of soil. The comparative higher dose of *P. lilacinus* (5×10^5CFU and 1×10^6CFU/g of soil) gave more or less similar result. This experiment also found that 5×10^5CFU/g soil application rate of *Paecilomyces lilacinus* reduced reproduction factor upto 89.6 and 85% against 800 eggs and 3200 eggs of *Meloidogyne incognita* per 100 cm^3 soil. 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. But, when *M. 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 into eggshells of *M. incognita* with enzymes and pressure following the formation of a simple aspersorium. 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 embryos or larvae can then become infected by the fungus (Alamgir *et al.*,1997).

SUMMARY AND CONCLUSION

Pot experiment was done to study effects of *Meloidogyne incognita* inoculum density and application rate of *Paecilomyces lilacinus* on biocontrol efficacy of bioagent against root knot of brinjal.

Four doses of *Paecilomyces lilacinus*, viz., 0 CFU/g soil, 1×10^5 CFU/g soil, 5×10^5 CFU/g soil, 1×10^6 CFU/g soil and five inoculum level of *M. incognita*, viz., 0 eggs/100 $cm^3$of soil, 400 eggs/100 cm^3 of soil, 800 eggs/100 cm^3 of soil, 1600 eggs/100 $cm^3$of soil and 3200 eggs/100 $cm^3$of soil were used to conduct this experiment. The experiment was laid out in Randomized Complete Block Design (RCBD) having two factors and replicated eight times. The summary of the results has been described in this chapter. Bioagent was applied to the pot soil before transplantation and *Meloidogyne incognita* was inoculated three days after transplantation. The crop was harvested at 8 weeks of transplantation.

All parameter varied significantly due to *Paecilomyces lilacinus*. The maximum shoot length (25.12 cm) and shoot fresh weight (12.78 g) was recorded in 1×10^5 CFU/g soil treatment. The maximum shoot dry weight (5.06 g) was recorded in where *P. lilacinus* applied @ 1×10^5 CFU/g soil. The maximum root length (15.24 cm) and root fresh weight (7.74 g) were found in 1×10^6 CFU/g soil. The maximum root dry weight (3.38 g) were observed in control treatment The results on effects of *Paecilomyces lilacinus* showed that *Paecilomyces lilacinus* had significant effect on gall index. The 1×10^6 CFU/g soil gave minimum (2.55) gall index and number of egg mass per root (16.95). The lowest number of egg per egg mass (240.10) and number of egg per root system (2.96×10^3) were recorded in 1×10^6 CFU/g soil. The minimum total nematode population (3.90×10^5) and number of juveniles/800g pot (3.86×10^5) were recorded in 5×10^5 CFU soil. The minimum reproduction factor (7.19) was recorded in 5×10^5 CFU soil. The maximum percent of egg mass colonization by fungus (36.88) was found in where 1×10^5 CFU of *P. lilacinus*/g soil was applied. The maximum soil colonization by *P. lilacinus* (1735.00 CFU/g soil) was recorded in where 1×10^6 CFU of *P. lilacinus* /g soil was applied.

Significant variation on shoot length of brinjal was shown due to the effect of different inoculum level of *Meloidogyne incognita*. The maximum shoot length (27.33 cm) was

recorded in under the treatment where no *M. incognita* was inoculated. The maximum shoot fresh weight (13.81 g) was obtained from the same treatment. The highest shoot dry weight (4.96 g), root length (14.14 cm), root fresh weight (6.53 g) and root dry weight (3.09 g) were recorded in the control treatment (0 eggs/100 g of soil). The minimum gall index (4.00), number of egg mass per root (27.03), number of egg per egg mass (326.10), total nematode population (4.77×10^5) and number of juveniles/800g soil/ pot (4.74×10^5) was observed in 400 eggs/100 g of soil after the control treatment value (o.oo). The highest reproduction factor (31.71 was recorded in 400 eggs/100 g of soil. The minimum percent of egg mass colonization by fungus (34.59) was recorded in 400 eggs/100 g of soil. The minimum soil colonization by *P. lilacinus* (1506.00 CFU/g soil) was found in 800 eggs/100 g of soil.

All parameter was significantly influenced by the interaction effect of *Paecilomyces lilacinus* and *M. incognita*. The maximum shoot length (30.34 cm) was recorded in a combination of 5×10^5 CFU/g soil of *P. lilacinus* with 0 eggs/100 g of soil. The shoot fresh weight was recorded to be the highest (14.78 g) from 5×10^5 CFU/g soil in where no *M. incognita* was inoculated. The highest shoot dry weight (5.65 g) was found from in 1×10^6 CFU/g soil with 1600 eggs/100 g of soil. The maximum root length (15.41 cm) was recorded in a application rate 1×10^5 CFU/g soil of *P. lilacinus* where no *M. incognita* was inoculated. The root fresh weight was recorded to be the highest ((6.89 g) from 1×10^5 CFU/g soil where no *Meloidogyne* inoculated. The highest root dry weight (3.5 g) after control was recorded in a combination of application rate of 1×10^6 CFU /g soil of *P. lilacinus* with 400 eggs of *M. incognita* /100 g of soil. The minimum gall index (2.13) was recorded in 1×10^6 CFU/g soil of bioagent with 400 eggs/100 g of soil. The lowest number of egg mass per root (8.50) was found in 1×10^6 CFU/g soil of *P. lilacinus* with 400 eggs/100 g of soil. The lowest number of egg per egg mass (190.40), number of egg per root system (3.16×10^3), total nematode population (4.46×10^5) and number of juveniles/800g soil/pot (1.66×10^5) was recorded from application rate of 1×10^6 CFU/g soil with 400 eggs/100 g of soil. The lowest reproduction factor (7.42) was found in 5×10^5 CFU soil with 800 eggs/100 g of soil. The highest percent of egg mass colonization by fungus (59.38) was found from 1×10^5

CFU/g soil with 800 eggs/100 g of soil. The highest soil colonization by *P. lilacinus* (2443.00 CFU/g soil) was found from 1×10^5 CFU/g soil with 400 eggs/100 g of soil. Considering the overall results it is concluded that the application rate of *P. lilacinus* 1×10^6 CFU/g soil whice was showed effectiveness to reduce 72% gall index and 84% egg mass when challenged with 800 eggs/100 cm^3 soil and 5×10^5CFU/g soil application rate of *Paecilomyces lilacinus* reduced reproduction factor upto 89.6 and 85% against 800 and 3200 eggs of *Meloidogyne incognita* per 100 cm^3 soil, respectively.

However, further experiment need to conduct including more vegetables available in the country at different agro- ecological zone in order to evaluate and use of biocontrol fungus *Paecilomyces lilacinus* as supplementation of chemical nematicide.

REFERENCES

Al Raddad, A. M. (1995). Interaction of *Glomus mosseae* and *Paecilomyces lilacinus* on *Meloidogyne javanica* of tomato. *Mycorrhiza.* **5**(3): 233-236.

Alamgir, K. M. M., Holland, R. J. and Williams, K. L. (1997). Recent studies on *Paecilomyces lilacinus* as a bionematicice. Suppression of *Heterodera avenue* populations, infection of *Meloidogyne* sp. eggs, females and juveniles in a pot trial and *Radopholus similes* eggs in laboratory studies. *Australian Nematology Newsletter*. **8**:2

Alamgir, K., Keith, L. W. and Helena, K. M. N. (2006). Comtrol of plant parasitic nematodes by *Paecilomyces lilacinus* and *Monacrosporium lysipagium* in pot trails. *Biocontrol*. **51**(5): 643-658.

Abd El-Raheem, R. E., El-Sayed, S. A., Mahmoud, Y. A. G. and Khalefa, D. M. (2005). Evaluation of *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Arthrobotrys dacctyloides* as biocontrol agents for *Meloidogyne incognita* under green house conditions. *Pakistan J. Biol. Sci.* **8**(11):1511-1516.

Aminuzzaman, F. M. (2009). Biolgical control of root knot nematode. Post doctoral dissertation, Key laboratory of systematic Mycology and Lichenology, Institute of Microbiology, Chinese Academy of Science, Beijing, P. R. China. P.63.

Aminuzzaman, F. M., Shammi, J. and Liu, X. Z. (2011). Dose response of granular formulation of biocontrol agent *Paecilomyces lilacinus* against root knot (*Meloidogyne* spp.) of brinjal and tomato. TWAS-ROESEAP-UB symposium on industrial biotechnology towards a bio-based economy of developing countries, August, 26-30, 2011, Beijing, China. p.72.

Abbas, H., Javed, N., Khan, S. A., Haq, I. U., Ali, M. A. and Safder, A. (2011). Integretion of bioagent and bioproduct for the management of root –knot

nematode, *Meliodogyne incognita* on eggplant. *Journal of Biology Agriculture and Healthcare.* **1(**4**):** 31-36.

Amit, S. A. and Trivedi, P. C. (1989). Control of root- knot nematodes on *Trigonella foenum graecum* by *Paecilomyces lilacinus. Nematol. Medit.* **17**: 131-133.

Aminuzzaman, F. M., Xie, H. Y., Duan, W. J., Sun, B. D. and Liu, X. Z. (2013). Isolation of nematophagous fungi from eggs and females of *Meloidogyne* spp. and evaluation of their biological control potential. *Biocontrol Sci. and Technology*. **23** (2): 170-182.

Anastasiadis, I. A., Giannakou, I. O., Prophatou-Athansiadou, D. A. and Gowen, S. R. (2007). The combined effect of a boicontrol agent *Paecilomyces lilacinus* with various practices for the control of root-knot nematodes. *Crop. Prot*. **27**: 352-361.

Anonymous, (2003). *Annual Report* 2002, Central Institute of Medicinal and Aromatic Plants, Lucknow, pp. 38-43.

Anwar, S. A. and Van Gundy, S. D. (1989). Influence of four nematodes on root and shoot growth parameters in grapes. *J. Nematol.* **21:** 276-283.

Anwar, S. A., Mckenty M. V. and Legari A. U. (2009). Host suitability of sixteen vegetable crop genotypes for *Meloidogyne incognita. J. Nemtol.* **41:** 64-65.

Bari, M. A. (2001). Biological control of soil borne diseases of vegetables, contract Research Project, Plant Pathology Division, Bangladesh Agril. Res. Inst., Joydebpur, Gazipur. pp. 21- 49.

BBS. (2005). Year book of Agricultural Statistic of Bangladesh. Bangladesh Bureau of Statistics, Ministry of Planning, Govt. of the People's Republic of Bangladesh. pp. 100.

BBS. (2011).Year book of Agricultural Statistic of Bangladesh. Bangladesh Bureau of Statistics, Ministry of Planning, Govt. of the People's Republic of Bangladesh. pp. 120.

Bhat, M. Y., Muddin, H. and Bhat, N. A. (2009). Histological interection of *Paecilomyces lilacinus* and *Meloidogyne incognita* on bittergourd. *J. Ame. Sci*. **5**(1): 8-12.

Bijoy, K. G., Rajesh, K. P., Kabindra, S. R., Chaitali, B. and Lokendra, S. (2006). Integrated application of some compatible biocontrol agents along with mustard oil seed cake and furadan on *Meloidogyne incognita* infecting tomato plants. *J. Zhejiang Univ. Sci.* **7**(11):873-875.

Bose, T. K. and Som, M. G. (1986). Vegetable crops in India. B. Mitra, Naya prokash, 206, Bidhansarani, Kalkata. pp. 262 - 264.

Bourne, J. M. and Kerry, B. R. (1999). Effect of the host on the efficacy of *Verticillium chlamydosporium* as a biological control agent of root-knot nematodes at different nematode densities and fungal application rates. *Soil Biol. Biochem*. **31**:75-84.

Bridge, J. and Page, S. L. J. (1980). Estimation of root-knot nematode infestation levels on roots using a rating chart. *Tropical Pest Management.* **26**:296-298.

Cabanillas, E. and Barker, K. R. (1989). Impact of *Paecilomyces lilacinus* inoculu level and application on control of *Meloidogyne incognita* on Tomato. *J. Nematol.* **21:** 115-120.

Cabanillas, E., Barker, K. R. and Nelson, L. A. (1989). Growth of isolates of *Paecilomyces lilacinus* and their efficacy in biocontrol of *Meloidogyne incognita* on Tomato. *J. Nematol.* **21 (**2): 164-72.

Cannayane, I and Sivakumar, C. V. (2001). Nematode egg parasitic funugus 1: *Paecilomyces lilacinus* - A. review. *Agric. Rev*. **22**: 115-120.

Castillo, J. D., Lawrence, K. S., Kloepper, J. W. and Santen, E. van. (2010). Evaluation of *Drechslerella dactyloides, Drechslerella brochopaga,* and *Paecilomyces lilacinus* for the biocontrol of *Rotylenchulus reniformis. Nematropica.* **40:**71-85.

Das, G. P., Ramaswamy, S. and Bari, M. A. (2000). Integrated crop management practices for the control of the brinjal shoot and fruit borer in Bangladesh, DAE-DANIDA Strengthening Plant Protection Services (SPPS) Project. Dept. of Agril. Extension, Khamarbari, Dhaka. 12p.

Davise, K. G., De F. A., Leij, A. M. and Kerry, B. R. (1991). TPM Special Review No. 4. Microbial agents for the biocontrol of plant parasitic nematodes in tropical agriculture. *Tropical Pest Management.* **34**: 303-320.

Djian, C. Pijarowski, L., Ponchet, M., Arpin, N. and Favre-Bonvon, J. (1991). Acetic acid: a selective nematicidal metabolite from culture filtrates of *Paecilomyces lilacinus* (Thom) Samson and *Trichoderma longibrachitum* Rifai. *J. Nematol.* **37**: 101-112.

Eapen, S. J., Beena, B. and Ramana, K. V. (2005). Tropical soil microflora of Spice-based cropping system as potential antagonists of root-knot nematodes. *J. Invert. Pathol.* **88**: 218-225.

El- Shanshoury, A. R., El-Sayed, S. A., Mahmoud, Y. A. G .and Khalefa, D .M. (2006). Biological control root –knot nematode *Meloidogyne incognita* on faba bean by selected predatory and parasitic fungi. *International Journal of Scientic Research.* **15**: 81-90.

Esphahani, M. N. and Anaspour, B. (2006). The effects of *Paecilomyces lilacinus* on the pathogenesis of *Meloidogyne javanica* and tomato plant growth parameters. *Iran Agricultural Research.* **24**(2) and **25**(1): 67-76.

Ganaie, M. A. and Khan, T. B. (2010). Biological potential of *Paecilomyces lilacinus* on pathogenesis of *Meloidogyne javanica* infecting tomato plants. *Euro. J. Appl. Sci.* **2** (2):80-84

Gerco. N., and De Vito, M. (2009). Population dynamics and damage levels. Pages 246-274 in: Root knot Nematodes Perry, R. N.; Moens, M. and Starr, J. L. eds. CAB International. Wallingford. UK.

Gomez, K. A. and Gomes, A. A. (1984). Statistical Procedure of Agricultural Research. John Wiley and Sons. p. 28.

Goswami, B. K., Pandey, R. K., Rathour, K. S., Bhattacharya, C. and Singh, L. (2006). Integrated application of some compatible biocontrol agents along with mustard oil seed cake and furadan on *Meloidogyne incognita* infecting tomato plants. *J. Zhejiang Univ. Sci*. B 7(11):873-875.

Hague, N. M. H. and Gowen, S. R. (1987). Chemical control of nematodes. In *Principles and Practices of Nematodes control in crops,* Academic Press, Sydney, pp. 131-178.

Hartman, K. M. and Sasser, J. N. (1985). Identification of *Meloidogyne* species on the basis of differential host test and perinial pattern morphology. In Barker, K. R., Carter C. C. and Sasser, J. N., eds. An Advanced Treaties on *Meloidogyne*. Methodology. North Carabian State of University Graphics, Raleigh, NC. **2**: 67-77.

Hashem, M. and Abo-Elyousr, K. A. (2011). Management of the root-knot nematode *Meloidogyne incognita* on tomato with combinations of different biocontrol organisms. Botany Department, Faculty of Science, Assiut University, Assiut 71516, Egypt. **3**. pp. 285-292.

Hassan, N. (2004). Evaluation of native strain of *Paecilomyces liacinus* against *Meloidogyne incognita* in cowpea followed by Lucerne. *Ann. Pl. Protec. Sci.* **3:**145-148.

Holbrook, C. C., Knauft, D. A. and Dickson, D. W. (1983). A technique for screening peanut for resistance to *Meloidogyne arenaria. J. Pl. Dis.* **67:**957-958.

Ibrahim, I. K. A., Rezk, M. A., El-Saedy, M. A. and Ibrahim, A. A. M. (1987). Control of *Meloidogyne incognita* on corn, tomato and okra with *paecilomyces lilacinus* and nematicide aldicord, *Nematol. Medit*. **15**: 265-268.

Jalata, P. (1985). Biological control of Nematodes, In an Advached Treatise on *Meloidogyne* spp. Sasser J. N. and Carter, C. C. (eds), North carolina State University Graphicd: Raleigh, NC. USA. pp. 303-308.

Jalata, P. (1986). Biological control of plant parsitic Nematodes. *Annual Rev. Phytopathol.* **24:** 453.489.

Kalele, D. N., Affokpon, A., Coosemans, J. and Kimenju, J. W. (2010). Suppression of root-knot nematodes in tomato and cucumber using biological control agents. *Afr. J. Hort. Sci*. **3**:72-80.

Kannan, R. and Veeravel, R. (2012). Effect of different dose and application methods of *Paecilomyces lilacinus* (Thom) samson against root knot nematode, *Meloidogyne incognita* (Kofoidand White) chitwood in okra. *J. Agril. Sci.* **4**(11):119.

Khalil, M. S. H., Allam, A. F. G. and Barakat, A. S. T. (2012). Nematicidal activity of some biopesticide agents and microorganisms against root-knot nematode on tomato plants under greenhouse conditions. *Journal of Plant Protection Research*. **52**(1): 47-52.

Khan, A., Williams, K.L. and Nevaiainen, H. K. M. (2004). Effects of *Paecilomyces lilacinus* protease and chitinase on the eggshell structures and hatching of *Meloidogyne javanica* juveniles. *Biol. Control*. **31**:346-352.

Khan, H. U., Ahmad, R., Ahmed, W., Khan, S. M. and Khan, M. A. (2001). Evaluation of the combined effects of *Paecilomyces lilacinus* and *Trichoderma harzianum* against root-knot disease of tomato. *J. Biol. Sci*. **1**(3): 139-142.

Khan, A., Williams, K. L. and Nevalainen, H. K. M. (2006). Infection of plant parasitic nematodes by *Paecilomyces lilacinus* and *Monacrosporium lysipagum*. *Biological Control.* **51**: 659-678.

Khan, M. R., Mohiddin, F. A., Ejaz, M. N. and Khan, M. M. (2012). Management of root-knot disease in eggplant through the application of biocontrol fungi and dry neem leaves. *Turkish Journal of Biology*. **36**: 161-169.

Khan, M. R. and Goswami, B. K. (2000). Effect of culture filtrates of *Paecilomyces liacinus* isolates on hatching of *Meloidogyne incognita* eggs. *Ann. Pl. Protec. Sci.* **8:**62-65**.**

Kiewnick, S. and Sikora, R. A. (2003). Efficacy of *Paecilomyces lilacinus* (strain 251) for the control of root- knot nematodes. *Commun Agric Appl Biol Sci.* **68(**4): 123-128.

Kiewnick, S. and Sikora, R. (2004). Optimizing the efficacy of *Paecilomyces lilacinus (*strain 251) for the control of the root-knot nematode. *Comm. Agric. Appl. Bio. Sci.* **69 (3)**: 373-80.

Kiewnick, S. and Sikora, R. (2006). Biological control of the root- knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. *Biol. Control*. **38**:179-187.

Kiewnick, S., Neumann, S. Sikora, R. A. and Frey, J. E. (2011). Efect of *Meloidogyne incognita* inoculum density and application rate of *Paecilomyces lilacinus* strain 251 on biocontrol of egg masses analyzed by real-time quantitative PCR. *Phytopathol.* **101** (1): 105-112.

Lopez-Llorca, L.V., Macia-Viente, J. G. and Jasson, H. B. (2008). Mode of action and interactions of nematophagous fungi. In: Ciancio A, Mukerji, K. G. (eds) Integrated mangement and biocontrol of vegetable and grain crops nematology. J. *Springer NL*. pp. 49-74.

Martin, S. B., Mueller, J. D., Saunders, J. A. and Jones, W. I. (1994). A survey of South Carolina cotton fields for plant parsitic nematodes. *Pl. Dis*. **78**: 717-719.

Mian, I. H. (1986). Plant parasitic nematodes associated with some crop species in Bangladesh. Bangladesh. *J. Plant Path*. **2** (1): 7-13.

Mittal, N., Saxena, G. and Mukerji, K. G. (1995). Integrated control of root knot disease in three crop plants using chitin and *Paecilomyces lilacinus*. *Crop Protec*. **14**: 647-651.

Mitu, A. I. (2012). Impact of *Paecilomyces lilacinus* application time on plant growth and suppression of root knot nematode (*Meloidogyne incognita*) in some selected vegetables. M. S. Thesis. Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka.

Muschsood, A. G. and Tabreiz, A. K. (2010). Biological potential of *Paecilomyces liacinus* on pathogenesis of *Meloidogyne javanica* infecting tomato plants. *European J. Appl. Sci.* **2**(2): 80-84.

Nisha, M. S. and Sheela, M. S. (2006). Bio management of *Meloidogyne incognita* on Coleus, *Solenostemon rotundifolius* by integrating solanization, *Paecilomyces lilacinus*, *Bacillus muceraus* and neem cake. *Indian J. Nematol.* **36**: 136-138.

Oclarit, E. and Cumagun, C. (2010). Evaluation of efficacy of *Paecilomyces lilacinus* as biological control agent of *Meloidogyne incognita* attacking tomato. *J. Plant Protection Res.* **49**(4):337-340.

Oduor, O. P. and Waudo, S. W. (2003). Integrated mangement of root-knot nematode using agro chemicals, organic matter and the antagonistic fungus, *Paecilomyces lilacinus* in natural field soil. *Nematol. Medit.* **31**: 121-123.

Oduor, O. P. and Waudo, S. W. (1996). Effect of five fungul isolates on hatching and parasitism of root knot nematodes. *Nematol. Medit.* **24**: 189-194.

Orr, C. C and Robinson, A. F. (1984). Assessment of cotton losses in western Texas caused by *Meloidogyne incognita. Pl. Dis.* **68:** 284-292**.**

Perveen, K., Haseeb, A. and Shukla, P. K. (2007). Efficacy of pesticides, neem seed powder and bio-control agents on *Meloidogyne incognita* and growth and oil yield of *mentha arvensis*. *Nematol. Medit. **35***: 75-79

Rao, M. S. and Reddy, P. R. (2001). Control of *Meloidogyne incognita* plant using *Glomus mosseae* integrated with *Paecilomyces liacinus* and neem cake. *Nematol. Medit.* **29**:153-157.

Rashid, M. (1993). Sabjee bighan. Published by Bangla Academy, Dhaka. pp 137-144.

Rashid, M. M. (2000). A Guidebook of plant pathology, Dept. of Plant Pathology. HSTU, Dinajpur. pp: 58.

Rumbos, C., and Kiewnick, S. (2006). Effect of plant species on persistence of *Paecilomyces lilacinus* strain 251 and on root colonization by the fungus. *Plant soil*. 28:25- 31.

Regina, M. D., Carneiro, G. and Cayrol, J. C. (1991). Relationship between inoculum density of the nematophagous fungus *Paecilomyces lilacinus* and control of *Meloidogyne arenaria* on tomato. *Revue Nematol.* **14**(4): 629-634.

Senthamarai, M., Poornima, K. and Subramanian, S. (2006). Pathogenicity of *Meloidogyne incognita* on *Coleus forskohlii* Briq. *Indian J. Nematol.* **36** (1) : 123-125.

Schenck, S. (2004). Control of nematodes in tomato with *Paecilomyces lilacinus* strain 251. *Vegetable Reports***. 5**: 1-5.

Shaha, M. G. (1989). Combining ability estiamates and hybrid vigour in eggplant (*Solanum melongena* L.). M.Sc. (Ag) Thesis. Department of Horticulture. BAU, Mymensingh.

Siddiqui, M. R. (2000). Tylenchida Parasites of Plants and Insects. Second edition, CAB International,Wallingford, UK, 833 p.

Singh, S. K. and Khurma, U. R. (2007). Susceptibility of six tomato cultivars to the root-knot nematode, *Meloidogyne incognita. The south pacific J. Nat. Sci.* **13**: 73-77.

Sivakumar, C. V. and Vidyasekaran, P. (1990). Control of *Meloidogyne incognita* on *Coleus forskohlii* with *Paecilomyces lilacinus* in farmyard manure amended and nonamended soil. *Biol. Control.* **4**(1): 68-69.

Sosamma, V. K. and Koshy, P. K., (1997). Biological contorl of *Meloidogyne incognita* on black pepper by *Pasteuria penatrans* and *Paecilomyces lilacinus*. *J. Plantation Crops.* **25**: 72-76.

Sun, M. H. Gao, Li., Shi, Y. X., Li, B. J. and Liu, X. Z. (2006). Fungi and actinomycetes associated with *Meloidogyne* spp. eggs and females in China and their biocontrol potential. *J. Inver. Pathol*. **93:** 22-28**.**

Shammi, J. (2012). The effects of biocontrol fungus, *Paecilomyces lilacinus* and fosthiazate on root- knot *Meloidogyne* spp. and yield of eggplant. M.S. Thesis. Department of Plant Pathology. Sher-e-Bangla Agricultural University, Dhaka.

Talukdar, M. J. (1974). Plant diseases in Bangladesh. *J. Agric. Res.* **1**: 61-86.

Tripati, P. K. and Singh, C. S. (2006), Effect of some compatible biocontrol agents along with mustard cake and furadon on *Meloidogyne incognita* infesting tomato plant. *Indian J. Nematol.* **36**(2): 309-310.

Usman, A. and Siddiqui, M. A. (2012). Effect of some fungul strains for the mangement of root-knot nematode (*Meloidogyne incognita)* on eggplant (*Solanum melongen*), *Agril. Tech*. **1**: 213-218.

Williamson,V. M. and Hussey, R. S. (1996). Nematode pathogenesis and resistance in plants. *Plant Cell.* **8:** 1735-1745.

Walia, R. K., Nandal, S. N. and Bhatti, D. S. (1999). Nematicidal efficacy of plant leaves and *Paecilomyces lilacinus* alone or in combination in controlling *Meloidogyne incognita* on okra and tomato. *J. Nematol. Medit*. **27**:3-8.

Whitehead, A. D. and Hemming, A. K. (1965). Comparison of quantitative methods of extracting small vermification nematodes from soil. *Annals of Applied Biology.* **55**: 25-38.

Yan, X., Sikora, R. A. and Zheng, J. (2011). Potential use of cucumber (*Cucumis sativus* L.*)* endophyttic fungi as seed treatment agents against root- knot nematode *Meloidogyne incognita. J. Zhejiang Univ. Sci.B.* **12**(3): 219-225.

Zaki, A. S. and Irshad, M. (1996). Biological control of plant parasitic nematodes by fungi. *Bioresource Technology*. **58**: 229-239.

Zaki, M. J. and Maqbool, M. A. (1992). Effect of *Pasteuria penatrans* and *Paecilomyces lilacinus* on the control of root-knot nematodes on brinjal. *Pakistan J. Nematol.* **10** : 75-79.