EFFICACY OF DIFFERENT APPLICATION RATE OF *PURPUREOCILLIUM LILACINUM* AGAINST DIFFERENT INOCULUM DENSITY OF ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) OF TOMATO

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CERTIFICATE

This is to certify that the thesis entitled, **"EFFICACY OF DIFFERENT APPLICATION RATE OF *PURPUREOCILLIUM LILACINUM* AGAINST DIFFERENT INOCULUM DENSITY OF ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) OF TOMATO"** 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 **AFRIN AKTER FARIA** bearing **Registration No. 07-02424** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

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ABSTRACT

The effectiveness of different dose of *Purpureocillium lilacinum* was evaluated against tomato root knot pathogen *Meloidogyne incognita* at different level. The pot experiment was conducted over two months in shade house on tomato crops, cv. BARI Tomato-14. Pots treated with different doses (0, 1×10^4, 5×10^4, 1×10^5 and 5×10^5 CFU/g soil) of *P. lilacinum* during transplanting of tomato. After three days different inoculum density (0, 250, 500, 1000 and 2000 eggs per 100 g soil) of *M. incognita* inoculated against each doses. The optimum dose against nematode protection was considered at concentration of 5×10^4 CFU/g soil where other doses gave statistically similar result. A significant correlation was recorded between doses of *P. lilacinum* and inoculum level of *M. incognita*. *P. lilacinum* at lower rate (5×10^4 CFU/g soil) reduced root galling by 76.53% when 250 eggs of *M. incognita* were inoculated per 100 g soil. The highest reduction of number of egg masses per root and reproduction factor (Rf) by 91.13 and 79.32%, respectively was observed when 2000 eggs per 100 g soil was challenged by 5×10^4 CFU/g soil of *P. lilacinum*. However, highest colonization rate of egg masses by bioagent was observed at 5×10^5 CFU/g soil with about 45.19% when pots inoculated with 1000 eggs per 100 g soil. The experiment demonstrated that *P. lilacinum* was effective in suppression of root knot nematode on BARI Tomato-14 and can be an important component of eco-friendly management strategies

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LIST OF ABBREVIATED TERMS

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

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) belongs to solanaceae family and originated from the highlands of the West coast of South America (Smith, 1994). It is an important vegetable crop and plays a vital role in maintaining health (Myers and Croll, 1921; Saywell and Lane, 1933 and Conn and Stumpy, 1970). In Bangladesh tomato production was about 143000 M. tons in 2007-2008 (BBS, 2008). At present 6.10% (BBS, 2005) area is under tomato cultivation both in winter and summer. It is the most consumable vegetable crop after potato and sweet potato occupying the top of the list of canned vegetable (Chowdhury, 1979). Tomato has a significant role in human nutrition because of its rich source of lycopene and vitamins such as ascorbic acid and β-carotene which are anti-oxidants and promote good health (Wilcox *et al*., 2003). As compared to others vegetables, it is rich in nutritional value and available comparatively at low prices.

Tomato is cultivated all over the country due to its adaptability to wide range of soil and climate (Ahmed, 1976). However, the yield of the crop is very low compared to those obtained in some advanced country (Sharfuddin and Siddique, 1985). There can be several reasons for this low yield such as changing climates conditions, conventional methods of cultivation, uncertified low yielding varieties and biotic factors. The biotic factors that affect the productivity and quality of tomato are fungi, bacteria, viruses, nematodes, weeds and insects. Among these biotic factors root knot nematodes are known to cause severe damage to tomato in different parts of the world (Dropkin, 1989) and tomato is considered as the most favorable host for root-knot nematodes (Lamberti, 1979). Among the nematodes, causing different plant diseases, root-knot nematodes (*Meloidogyne* spp.) are the most devastating and damaging. Yield loss of tomato due to root-knot disease is

estimated to be 40% in the country and 20.6% in the world (Faruk and Momotaz, 2009). Amongst all the nematode genera, *Meloidogyne* genus ranked first and considered very important plant pathogen (Dropkin, 1989).

The root knot nematodes are sedentary endoparasites and are among the most destructive agricultural pests attacking a broad range of crops and cause high levels of economic losses (Mai and Abawi, 1987). They are found in tropical and subtropical areas of the world and infect more than 2000 plant species including almost all cultivated plants (Hussey, 1985). More than 60 species of root-knot nematodes have been reported so far but *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* are relatively more destructive accounting for 95 percent of all root-knot nematode infestations in field crops. The proportion of *Meloidogyne incognita* in the agriculture soils is 52%, *M. javanica* 31%, *M. arenaria* 8%, *M. hapla* 7% and other species are about 27% (Hussey and Janssen, 2002).

Among the species, *M. incognita* is widely prevalent, common. The infection starts when second stage juveniles (J_2) penetrate in roots. These nematodes establish feeding sites within the roots where they induce roots galls or knots deprive plants from nutrients and cause cellular, metabolic and structural changes within plant tissues. These physiological and physical changes to the plant can reduce crop yield and quality drastically. Nematodes attacking susceptible plants at seedling stage cause heavy losses and may result in complete destruction of the crop. But infections in older plants may show only minor effects on yield or may reduce yields noticeably (Agrios, 2005).

The control of plant parasitic nematodes is a difficult task and mainly depends on chemical nematicides for remarkable reduction of nematode population (Jatala, 1985). The most effective method of nematode disease control is the use of synthetic chemical nematicides. However, health hazards, and the attendant adverse effects of these chemicals on the beneficial non-target organisms and the environment are serious constraints. Of late, alternative nematode management options have been sought by many researchers to reverse this ugly trend. Some microorganisms that colonize the root rhizosphere provide an initial barrier against pathogens, including nematodes (Weller, 1988). Papavizas (1985) suggested that antagonistic fungi in the rhizosphere possess excellent potential to suppress plant pathogens. One such fungus, *Paecilomyces lilacinus*, is primarily a saprophyte that is able to infect the eggs and females of root-knot nematodes, destroying the embryo within 5 days (Jatala, 1986). This fungus is found in most agricultural soils (Samson, 1974) also may infect adult stage of plant parasitic nematodes (Jatala, 1986). *Paecilomyces lilacinus* grows well temperature ranges between 15 & 30°C, but optimal growth occurs between 25 & 30°C, similar to its hosts (Jatala, 1986).

Paecilomyces lilacinus is a common soil hyphomycete, reported from numerous part of the world, but more frequently from warm regions (Samson, 1974 and Domsch *et al*., 1980). *Paecilomyces lilacinus* attacks mainly sedentary stages and to a lesser extent juveniles of root knot and cyst nematodes. There are also reports of control of other nematode species by fungus (Walters and Barker, 1994). During infection the fungal hyphae grow and form a mycelial network around the nematode egg. Appersoria are formed, which enable the penetration of the fungal hyphae into the egg (Dunn *et al*., 1982; Holland *et al.*, 1999 and Morton *et al*., 2004). Penetration of eggshell is a result of mechanical as well as enzymatic activities.

The first report of *Paecilomyces lilacinus* as an effective parasite of *Meloidogyne incognita* and *Globodera pallida* was by Jatala *et al*., (1979). Science then many glasshouse and field studies have been conducted in a number of countries with different isolates of *Paecilomyces lilacinus* that have demonstrated the potential of the fungus to suppress populations of root knot and cyst nematodes (Dube and Smart, 1987; Cabanillas and Barker, 1989; Gomes Carneiro and Cayrol, 1991 and Mittal *et al*., 1995). The fungus is applied as spores in large quantities to the soil, where it parasitizes the nematode eggs reducing the nematode multiplication and providing population control.

Several studies on antagonistic dose-response relationships in biological control systems have been reported (Larkin and Fravel, 1999; Montesions and Bonaterra, 1996 and Smith *et al*., 1997). To fully evaluate the potential of a biological control agent, a dose-response relationship between the concentration of antagonist applied and the reduction of plant damage is needed to be established. This trial was conducted with an objective to evaluate the efficacy of *Purpureocillium lilacinum* at varying application rate for controlling different level of root knot nematode, *Meloidogyne incognita* of tomato.

REVIEW OF LITERATURE

Dube and Smart (1987) used *Paecilomyces lilacinus* and *Pasteuria penetrans* for controlling root knot nematode on tomato, tobacco and pepper plant. The root-knot nematode *Meloidogyne incognita* was controlled more effectively and yields of host plants were greater when *P. lilacinus* and *P. penetrans* were applied together in field microplots than when either was applied alone.

Ibrahim *et al.* (1987) observed the efficacy of *Paecilomyces lilacinus* and chemical nematicide aldicarb for controlling the root knot nematode (*Meloidogyne incognita*) on corn, tomato and okra. In the tomato experiment treatment with *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. The root and shoot dry weights and fruit weight were significantly decreased by nematode infestation. On the other hand, the growth and yield of plants in fungal and aldicarb treatments showed no significant differences from the control.

Cabanillas *et al.* (1988) examined histological interactions of the fungus *Paecilomyces lilacinus* and *Meloidogyne incognita* race 1 on tomato roots. Few to no galls and no giant-cell formation were found in roots dipped in a spore suspension of *P. lilacinus* and inoculated with *M. incognita*. Numerous large galls and giant cells were present in roots inoculated only with *M. incognita*. *P. lilacinus* colonized the surface of epidermal cells as well as the internal cells of epidermis and cortex and gives protection of plant surfaces against root knot nematodes.

Cabanillas and Barker (1989) used *Paecilomyces lilacinus* to evaluate the effects of inoculum density and time of application on the protection of tomato against *MeIoidogyne incognita.* The best protection against 5000 eggs of *M. incognita* was attained with 10 and 20 g of fungus-infested wheat kernels per microplot which resulted in a threefold and fourfold increase in tomato yield, respectively, compared to tomato plants treated with this nematode alone. Greatest protection against this pathogen was attained when *P. lilacinus* was delivered into soil 10 days before planting and again at planting.

Cabanillas *et al*. (1989) identified 13 *Paecilomyces lilacinus* isolates from various geographic regions as biocontrol agents against *Meloidogyne incognita,* the effects of temperature on their growth, and the characterization of the impact of soil temperature on their efficacy for controlling this nematode were investigated. 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 ºC, 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.

Gaspard *et al*. (1990) measured the suppressive effect of *Paecilomyces lilacinus*, *Verticillium chlamydosporium,* and other naturally occurring antagonists into sterilized soil or unsterilized soil collected from 20 California *Meloidogyne incognita*-infected tomato fields. Three months after infected seedlings were transplanted to unsterilized or sterilized soil, unsterilized soils K, L, and Q had 97, 62 and 86% fewer *M. incognita* second-stage juveniles (J2) than the corresponding sterilized soils. *Paecilomyces lilacinus* and *V. chlamydosporium*

increased in colony forming units in unsterilized soil of all bioassays, but they were not associated with lower numbers of juveniles (J2).

Gomes Carneiro and Cayrol (1991) studied relationship between inoculums density of *Paecilomyces lilacinus* against *Meloidogyne arenana* on tomato. They incorporated five doses (0.01 - 0.1 - 1 - 10 and 100 g/m^2) of a commercial product of *Paecilomyces lilacinus* isolated from eggs of *Meloidogyne incognita* that applied in a powder formulation (10^{11} spore/g of product) in a glasshouse pot experiment against large infestations of *Meloidogyne arenana.* 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 nematode generations. The number of colonized egg masses and the number of non-viable eggs increased with fungal inoculum and the fungus was most effective at a density of 10^6 spore/g of soil. In the highest level of control (100% colonized egg masses) only 50% of the eggs were parasitized. Twenty three percent of the larvae remained which constitutes an important residual inoculum potential. This fact and a rapid decrease in fungal density in soil below the acceptable control levels, limit the use of this fungus as a biological control agent.

Mao-Song *et al*. (1993) applied *Paecilomyces lilacinus*, a parasitic fungus on tomato root-knot nematode, *Meloidgyne incognita* in southern China. Results of the laboratory tests showed a 54% parasitization of the nematode eggs by the fungus. Application of 4 and 8 grams of rice kernel infected with *P. lilacinus* in green house pot condition, resulted in 35.3 and 41.2% of egg parasitization 50 days after the treatment. The average growth height of the tomato plants treated with wheat bran and sand infected fungus resulted 4-5 cm higher plant than that in

a non-treated control plot, while the number of flowers and fruits, number of nematode infected root and number of nematode larvae were about 1/3 to 1/4 of those in the control.

Zaki (1994) established the optimum or effective dose of the biocontrol fungus *Paecilomyces lilacinus* against *Meloidogyne javanica* in tomato. He found that 4g of fungus per kg soil was the optimum dose for the effective reduction in gall index (69%) and second stage juvenile (86%) of *Meloidogyne javanica* in tomato with an optimum egg mass infection (58%) and egg destruction (66%).

Al-Raddad (1995) examined interaction 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 inoculums of *P. lilacinus*. Inoculation of tomato plant 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 average number of galls per root system with about 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*. Addition of layer manure had a beneficial effect on plant growth and reduced *M. javanica.*

Mittal *et al.* (1995) examined suppressive ability of a rhizosphere inhabiting nematophagous fungus, *Paecilomyces lilacinus*, along with chitin in sterilized soil against *Meloidogyne incognita*, causal agent of root-knot disease in *Solanum melongena, Lycopersicon esculentum* and *Cicer arietinum*. Combination of fungus with chitin enhanced suppression of *Meloidogyne incognita* more than using them alone.

Oduor-owino and Waudo (1996) conducted a test to evaluate the ability of five fungal isolates *Paecilomyces lilacinus, Phoma herharum* and three isolates of *Fusarium oxysporum* to parasitize eggs and females of *Meloidogyne javanica. P. lilacinus* and *F. oxysporum-l* significantly ($P<0.05$) parasitized more than 70% of eggs and females while *F. oxysporum-3* parasitized less than 20%. Also, *P. lilacinus* and *F. oxysporum-1* had the greatest suppressive effect on hatching. In general, control Petri-dishes and those treated with *F. oxysporum-3* had the highest proportions of hatched eggs, but exhibited the least levels of egg parasitism. The fungus *P. lilacinus* significantly ($P<0.05$) parasitized eggs of *M. javanica, M. incognita* and *M. arenaria* but no significant differences were detected in the levels of parasitism.

Khan and Saxena (1997) used organic materials and *Paecilomyces lilacinus* to increase tomato-plant growth and reduced the nematode multiplication. Highest improvement in plant growth and reduced reproduction factor and root galling were recorded in neem-cake amended soil. The least effect was with sesame-cake. The integration of oil-cakes (except mahua-cake), bone and horn meals with *P. lilacinus*, resulted in increased plant growth and reduced population build up of nematodes and root galling. The groundnut-cake with *P. lilacinus* was most effective. The organic amendments increased the parasitism of *P. lilacinus* on root-knot nematodes.

Laifa *et al.* (1998) examined the parasitic rates of *Paecilomyces lilacinus, Verticillium chlamydosporium* isolated on the eggs of *M. incognita* were 90%, 85% in laboratory tests respectively. When a mixed preparation of the fungi *P. lilacinus* and *V. chlamydosporium* with meadow land was applied in the tomato plots and fields, it was found that the average height of the tomato plants in the treated plots and fields were 20～35cm higher than that in non-treat control plots and fields, while root-knot index was 30～46% of those in the control.

Bhat and Mahmood (2000) controlled the root knot nematode *Meloidogyne incognita* more effectively when *P. lilacinus* and *G. mosseae* were applied together in a pot experiment than either was applied alone. Inoculation of tomato plant with *G. mosseae* did not markedly increase the growth of plant infected with *M. incognita.* Inoculation of plant with *G. mosseae* and *P. lilacinus* together or alone resulted in a similar shoot and plant height. The highest root development was achieved when mycorrhizal plants were inoculated with *P. lilacinus* to combat root knot nematode. Inoculation of tomato plant with *P. lilacinus* suppressed galls/root system and eggs/egg masses, compared to seedling inoculated with *M. incognita* alone. The mycorrhizal colonization was not affected by inoculation of P. *lilacinus.*

Khan and Goswami (2000) observed the efficacy of different levels (2, 4, 6, 8 and 10 g/kg soil) of *P. lilacinus* (isolate 6) on twenty-day-old tomato cv. Pusa Ruby seedlings, inoculated at about 2000 J_2 of *M. incognita*/kg soil one week after transplanting. All treatments receiving *P. lilacinus* exhibited higher plant growth parameter values compared to those treated with the nematode alone. Increasing the dose of *P. lilacincus* was accompanied by an increase in plant height and root length. The percentage egg infection increased from 30.4% at 2 g *P. lilacinus*/kg soil with higher concentrations and the gall index, number of eggs per egg mass

and the final soil population of nematodes decreased with increasing inoculum concentrations. The results indicate that 8 g *P. lilacinus*/kg soil is the optimum dose for the suppression of *M. incognita.*

The experiment was conducted by Siddiqui *et al.* (2000) under laboratory and field conditions to evaluate the efficacy of *Pseudomonas aeruginosa* alone or in combination with *Paecilomyces lilacinus* in the control of root-knot nematode and root-infecting fungi. 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 *Meloidogyne javanica*, the root-knot nematode. *P. lilacinus* parasitized eggs and female of *M. javanica* and this parasitism was not significantly influenced in the presence of *P. aeruginosa*. *P. aeruginosa* was reisolated from the inner root tissues of tomato, whereas *P. lilacinus* did not colonize tomato roots.

Khan *et al*. (2001) used biocontrol fungus *Paecilomyces lilacinus* and *Trichoderma harzianum* for controlling *Meloidogyne incognita* on tomato. Culture of *P. lilacinus* and *T. harzianum* multiplied on wheat grain were mixed into soil @ 6g/kg, inoculated with approx. 6000 freshly hatched second stage juveniles of *M. incognita*. Addition of *P. lilacinus* and *T. harzianum* in combination amended with organic substance significantly decrease 71.77% galls over the inoculated plants, while both *P. lilacinus* and *T. harzianum* effectively reduced number of galls over the inoculated plants and these caused 62.38 and 55.95% reduction in number of galls, respectively.

A dose response experiments was conducted to suppress the root-knot nematode *Meloidogyne incognita* on tomatoes by using the new water dispersible granule (WDG) formulation of *Paecilomyces lilacinus* Kiewnick and Sikora, (2003). The results revealed a clear correlation was observed between rate applied and the degree of control concerning the reduction in damage to the root and multiplication of the nematode. Application of bio-nematicide into soil one week before planting at the rate of 2 to 4 times 10^9 conidia per plant shown best control of nematode. 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.

Agrochemicals, organic matter and the antagonistic fungus, *Paecilomyces lilacinus* applied in natural field soil in controlling root knot nematode on tomato cv. Moneymaker Oduor-Owino, (2003). He found that the smallest galling index, number of galls and nematode population were in soils treated with aldicarb in combination with *P. lilacinus.*

Kiewnick and Sikora (2004) incorporated *P. lilacinus*, formulated as WG (BIOACT WG), into soil inoculated with root knot nemaotdes (*Meloidogyne incognita* and *M. hapla*) eggs prior to transplanting the susceptible tomato cultivar "Hellfrucht". Furthermore, soil treatments were combined with seedling treatments 24 hours before transplanting and a soil drench 2 weeks after planting, respectively. Seedling and post planting treatment was also combined with a soil treatment at planting. All single or combination treatments tested decreased the gall index and the number of egg masses compared to the untreated control 12 weeks after planting. It could be demonstrated that the above mentioned combination of pre-planting application plus the seedling and one post plant

drench gave the best control and resulted in a significant fruit yield increase in concurrence with a decrease in number of galls per root.

Two fungal bioagents *Paecilomyces lilacinus* and *Trichoderma viride* were incorporated into soil along with mustard oil cake and furadan against root knot nematode *Meloidogyne incognita* infecting tomato under greenhouse condition. Bioagents viz., *P. lilacinus* and *T. 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 infested soil Goswami *et al.*, (2006).

The effect of *Paecilomyces lilacinus* on the pathogenesis of *Meloidogyne javanica* as well as plant growth parameters was examined under greenhouse condition on tomato. *Paecilomyces lilacinus* (1g mycelium/pot), effectively promoted the growth of plants inoculated with 2000 freshly hatched J_2/pot of *Meloidogyne javanica*. Root galling by the nematode and egg mass production was greatly reduced. The fungus was most effective when the fungus and nematode were inoculated simultaneously or the fungus preceded the nematode in sequential inoculation Esfahani and Pour (2006).

Khan *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. 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*.

Kiewnick and Sikora (2006 a) established significant dose–response relationships, when conidia of *Paecilomyces lilacinus* strain 251 were applied to soil either with or without the glucose-based formulation. The effective concentration 50 (EC50) values for the commercially formulated product ranged between 0.097 g and 0.08 g/500 cm^3 soil, equivalent to an EC50 of 1.29×10^6 and 9.88×10^5 colony forming units (CFU)/g soil for the parameters gall index and final population per root, respectively. For the number of egg masses per root the EC50 was 0.007 g product or 2.64×10^5 CFU/g soil. Similarly, EC50 values for conidia applied without formulation were 0.068 g or 0.103 g/500 cm^3 soil (EC50 of 8.10×10^5–1.40×10^6 CFU/g soil) for gall index and final population per root. In contrast, the EC50 was 0.096 g (EC50 of 1.28×10^6 CFU/g soil) for the number of egg masses per root. They demonstrated that a single pre-plant application at a concentration of 1×10^6 CFU/g soil is needed for sufficient biocontrol of *M. incognita* by PL251.

Kiewnick and Sikora (2006 b) used egg-pathogenic fungus *Paecilomyces lilacinus* strain 251 (PL251) under different temperature regimes for biocontrol activity against the northern root-knot nematode *Meloidogyne hapla*. Biocontrol activity by PL251 was affected by temperature and initial nematode inoculum density. At 20/23°C (night/day) control efficacy reached 71% at a high inoculum density of 10000 eggs and juveniles (E + J) per 500 cm^3 compared to 65% control at the lower inoculum density (5000 E + J/500 cm^3). By contrast, average efficacy

was 5 and 46% at low and high inoculum densities, respectively, when temperatures were between 19°C and 21°C (night/day). A significant interaction between the factors inoculum density and treatment with PL251 was observed at low temperatures, when only few *M. hapla* galls and egg masses developed after inoculation with 5000 E + J/500 cm^3. When the temperature was favorable for the fungal parasite (25°C), efficacy reached 90%. In glasshouse experiments with unfavorable temperatures for *M. hapla* development (25°C-33°C), tomato plants repeatedly treated with PL251 showed significantly higher fruit yield (23-102%).

Rumbos *et al.* (2006) observed the interactions of *Paecilomyces lilacinus* strain 251 with the arbuscular mycorrhizal fungus *Glomus intraradices* against *Meloidogyne incognita* on tomato in greenhouse experiments. Application of *P. lilacinus* had no effect on the frequency and intensity of tomato root colonization by *G. intraradices*. Likewise, the decline of the nematophagous fungus densities after single application in soil was not affected by the presence of the mycorrhizal fungus. Single application of *P. lilacinus*, as pre-planting soil treatment, resulted in significant reduction of nematode damage. In contrast, mycorrhizal inoculation did not provide sufficient biocontrol. Combined application of the two agents did not enhance root protection compared to single treatments. Double treatment of mycorrhized seedlings with *P. lilacinus*, as seedling drench and pre-planting soil treatment, 4 and 1 week before transplanting, respectively, resulted in the highest reduction of the nematode damage. These results indicate the potential of the commercial *P. lilacinus* strain 251 and mycorrhiza for integration in nematode control strategies.

Shanmuga and Kumar (2006) conducted an experiment under glass house conditions to establish the optimum dose of the fungus, *Paecilomyces lilacinus* against *Meloidogyne incognita* in tomato. The fungal suspension was inoculated a $1x10^6$, $2x10^6$, $3x10^6$, $4x10^6$, $5x10^6$, $6x10^6$, $7x10^6$ and $8x10^6$ spores per 3 kg soil. Four ml of fungal suspension ($8x10^6$ spores) of *P. lilacinus* per 3 kg soil was found to be the optimum dose for effective reduction of *M. incognita* population in soil, egg masses and number of galls and increased the plant growth parameters.

Sun *et al.* (2006) obtained a total of 455 fungal isolates belonging to 24 genera and 52 isolates of actinomycetes from plant roots and infested soil in China 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 no treatment control.

Kiewnick (2007) examined the efficacy of *Paecilomyces lilacinus* 251 (PL251) for controlling root knot nematode. In addition, it was found that rhizosphere competence is not the key factor for the efficacy of PL251. Co-application of PL251 with other soil antagonists increased biocontrol efficacy against root-knot nematodes. The efficacy of PL251 depended strongly on the ratio between application rate and inoculum density in soil. As pre-plant treatment, a rate from 1.5 to 7.5×10^5 CFU/g soil resulted in significant control at inoculum densities of 100 to 400 root-knot nematode eggs/100 ml soil. However, due to the rapid decline of PL251 in soil, repeated applications are needed to maintain a sufficient density of PL251 for season-long protection.

Anastasiadis *et al.* (2008) evaluated the effectiveness of a formulated product containing spores of the naturally occurring fungus *Paecilomyces lilacinus*, strain 251, against root-knot nematodes in pot and greenhouse experiments. Decrease of second-stage juveniles hatching from eggs was recorded by using the bio-nematicide at a dose of 4 kg ha^{-1}, while a further decrease was recorded by doubling the dose. However, the mortality rate decreased by increasing the inoculum density. Application of *P. lilacinus* and *Bacillus firmus*, singly or together in pot experiments, provided effective control of second-stage juveniles, eggs or egg masses of root-knot nematodes. In a greenhouse experiment, the bio-nematicide was evaluated for its potential to control root-knot nematodes either as a stand-alone method or in combination with soil solarization. Soil solarization for 15 d either alone or combined with the use of *P. lilacinus* did not provide satisfactory control of root-knot nematodes. The use of oxamyl, which was applied 2 weeks before and during transplanting, gave results similar to the commercial product containing *P. lilacinus* but superior to soil solarization.

Aminuzzaman (2009) observed the egg and juvenile parasitism of *Meloidogyne* spp. by nematophagous fungus *Paecilomyces lilacinus* in soil tube test and it has been found that egg and juvenile parasitism depends on the fungal population density but not on nematode population density.

Bo *et al.* (2009) studied the antagonistic characters of two biocontrol agents *Paecilomyces lilacinus* (colonize the eggs of *Meloidogyne* spp.) and Actinomycetes spp. (kill the second stage juveniles of root knot nematodes) and their combinations for controlling *M. incognita*. In vitro bioassays, the effects of inhibiting eggs hatching and killing second stage juveniles of *M. incognita* by four combined *P. lilacinus* and Actinomycetes spp. treatments were significantly higher than those with single strain treatments. The maximum inhibition rate to egg hatching and mortality of the second stage juveniles were 66.6 and 78.0%,

respectively by which treatment. In greenhouse pot experiments, the combination of *P. lilacinus* strain PL1 with Actinomycetes spp. strain LH117 produced the highest biocontrol effects, with 68.7% of root knots and 58.4% of egg masses on tomato plants reduced when compared to the control test.

Oclarit and Cumagun (2009) 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, gall index rating, number of galls, egg masses and nematodes per one gram root sample were determined and per cent reduction in gall number was computed. 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 one 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 un-inoculated control. Per cent reduction in gall number was the highest at treatment with 7.92×10^6 spores per ml of *P. lilacinus*.

Siddiqui and Akhtar (2009) conducted a glasshouse experiment to identify best biocontrol agents on the growth of tomato and on the reproduction of the nematode *Meloidogyne incognita*. They used antagonistic fungi (*Paecilomyces lilacinus*, *Pochonia chlamydosporia* and *Trichoderma harzianum*) and plant growth-promoting rhizobacteria (PGPR), namely *Bacillus subtilis*, *Paenibacillus polymyxa* and *Burkholderia cepacia* alone and in combination. Application of antagonistic fungi and PGPR caused a significant ($P < 0.05$) increase in tomato growth (based on shoot dry weight) both with and without nematodes. *P. lilacinus* was more effective in reducing galling and improving the growth of nematode-inoculated plants than *T. harzianum*, while *P. polymyxa* was more effective than *B.*

subtilis. The greatest increase in growth of nematode-inoculated plants and reduction in nematode galling was observed when *P. polymyxa* was used with *P. lilacinus* or *P. chlamydosporia. P. lilacinus* parasitized more females and eggs than the other fungi tested.

Singh *et al.* (2009) used 1, 2 and 3 g substrate of *P. lilacinus* (19.59×10^8 spores/g substrate) per kg soil in pot experiments on plant growth and nematode multiplication on tomato. There was a significant increase in growth characters and reduction in nematode population in the treatments receiving 1 g of *P. lilacinus* and this effect increased further with the increase in *P. lilacinus* levels. A significant enhancement in all the plant growth parameters and reduction in nematode populations were recorded in the treatments where *P. lilacinus* was used in combination with carbofuran. The eggs parasitized by *P. lilacinus* were found to be fungus density dependent and parasitization increased with the increase in level of fungus in the soil.

The biocontrol potentiality of bioagent *Paecilomyces lilacinus* was examined in vitro conditions against the *Lycopersicon esculentum* root knot nematode *Meloidogyne javanica*. The parameters were measured on plant height, 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. *Paecilomyces lilacinus* significantly improved the growth of tomato plants inoculated with 2000 juveniles of *Meloidogyne javanica*. The plant length, fresh weight, dry weight and number of leaves per plant significantly improved whereas, number of galls, egg masses, eggs per egg mass and final nematode population greatly reduced on simultaneous and sequential inoculation of *P. lilacinus* and *M. javanica* (Ganate and Khan, 2010).

Kalele *et al.* (2010) used three rates (0.1, 0.2 and 0.4 g/10 L of soil) of fungal strain 251 of *P. lilacinus* (PL251) and *Arthrobotrys conoides* for their efficacy against *Meloidogyne* spp. in tomato and cucumber under greenhouse conditions. Both pre-planting and at planting application of PL251 were found to reduce nematode populations and root galling in both tomato and cucumber. Pre-planting soil treatment (0.4 g/10 L of soil) reduced final nematode populations by 69 and 73% in the roots and soil, respectively, compared to the non-inoculated control in tomato. At planting soil treatment resulted reduction level of 54 and 74% in the roots and soil, respectively. Only 28 and 21% reduction levels were recorded in the roots and soil when the fungus was applied at planting, respectively. This study has demonstrated that PL251 has a promising potential that could be exploited in the management of *Meloidogyne* spp. in vegetable production systems.

Aminuzzaman *et al*. (2011) used 0.0, 0.2, 0.4, 0.8 and 1.6% (w/w) fungal dried pellets of *Paecilomyces lilacinus* YES-2 on tomato (variety BARI tomato-14) and brinjal (variety BARI Begun-5) inoculated with 10,000 fresh eggs of *Meloidogyne incognita*. The result showed that the pellets of *Paecilomyces lilacinus* YES-2 enhanced plant growth and reduced galling index and nematode population in all treatments of different dosages applied. The biological control efficiency of the fungus against root knot nematode was significantly higher along with the increase of dosages applied for both of the crops. 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 at the highest rate (1.6%) of application of the biocontrol fungus.

Aminuzzaman and Liu (2011) observed in greenhouse study, each pot containing 4.0×10^7 spore/ml. On 30 days tomato seedling (variety BARI tomato-14) was transplanted in the centre of each pot and 6000 fresh eggs of *Meloidogyne* sp. were inoculated after three days of transplanting. Plants were harvested at 60 days and the growth of the plants and root knot galling index were measured. It was found that the fungus increased shoot height, fresh shoot weight, root length and fresh root weight and also reduced root galling index up to 63% and number of egg mass per root system up to 40% when compared with control treatment. This was a new record of *Meloidogyne* egg parasitic fungus reported in Bangladesh and the evaluation of the biocontrol potential *Paecilomyces lilacinus* against root knot nematodes.

Khan *et al.* (2011) used potential fungi *Trichoderma harzainum, Paecilomyces lilacinus* and *Arthrobotrys oligospora* along with natural organic compound (neem compound mix) to control the nematodes *M. incognita*. The three test fungi grown on Maize Sand medium were separately mixed with the soil of experimental pots, at concentrations of 10^9 CFU/g of medium and approx. 1000 nematodes per pot were inoculated after two days of transplanting. Also there potential to control Nematode was compared with that achieved by using the chemical control agent; carbofuran. The fungal agent significantly controlled nematode population and enhance the plant growth.

Kiewnick *et al*. (2011) observed the potentiality of the fungal biocontrol agent, *Paecilomyces lilacinus* strain 251 (PL 251) to control the root knot nematode *Meloidogyne incognita* on tomato at varying application rates and inoculum densities. They used six inoculum densities of *Meloidogyne incognita* (0, 100, 200, 400, 800, 1600 eggs and juveniles/100 cm^3 of soil) and six doses of PL251 applied at 0, 0.01, 0.025, 0.05, 0.1 and 0.2 g of water-dispersible granule formulation per 500 cm^3 of soil which were corresponded to a concentration of 0,

2×10^5, 5×10^5, 1×10^6, 2×10^6 and 4×10^6 CFU of PL251 per gram of soil. They 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 60% when averaged over inoculums densities of 100 to 1600 eggs and infective juveniles per 100 cm^3 of soil.

Luambano-Nyoni *et al.* (2011) conducted to assess the efficacy of six isolates (10, 126, 144, 147, 177 and 392) of *Pochonia chlamydosporia* and two isolates (Pl-Rothamsted and Pl-plus™) of *Paecilomyces lilacinus*. Isolates 10 and 392 were significantly ($P<0.001$) more effective in parasitizing more eggs compared to other isolates. A significant ($P<0.001$) reduction in the numbers of second-stage juveniles compared to the untreated control was achieved with Pl-plus in sterilized and non-sterilized soil and with isolate 10 in non-sterilized soil.

Khalil *et al.* (2012 a) carried out a pot experiment under greenhouse conditions to study the impact of the evaluated treatments namely abamectin, azadirachtin 0.15%, azadirachtin 0.03%, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Paecilomyces lilacinus* and oxamyl on the tomato plants cv. Super strain B inoculated with 5000 eggs of *Meloidogyne incognita* per pot. The results indicated that the most of the tested treatments obviously reduced root galls and remarkably increase tomato plant growth characters significantly and egg masses on root system, as well as, juvenile's numbers in the soil. *P. lilacinus* was the most effective treatment on both galls and egg masses achieving 88.23 and 76.94% reduction, respectively.

A greenhouse experiment was carried on to evaluate the efficacy of certain microbial agents against *Meloidogyne incognita* infesting tomato plants (cv. super strain B). The antagonistic bacteria *Bacillus subtilis* and *Bacillus thuringiensis*, antagonistic fungus *Paecilomyces lilacinus* and mycorrhizal fungi *Glomus intraradices* as well as *Glomus macrocarpium* that were compared with the synthesis nematicides Oxamyl and Cadusafos. The *Paecilomyces lilacinus* product was the best treatment in suppressing the root knot populations in the soil with (85.2%), followed by those with *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 Khalil *et al.*, (2012 b).

Mitu (2012) evaluated impact of *Paecilomyces lilacinus* application time on plant growth and suppression of root knot nematode (*Meloidogyne incognita*) in some selected vegetables. The application of *P. lilacinus* at the rate of 36×10^7spore of *P. lilacinus*/plant reduced number root galling, egg mass production, number of juvenile (J_2)/g soil and reproduction factor (Rf) as compared to plants inoculated with nematode alone. In application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting gall index, egg mass/root, J_2/g soil and Rf were 0.50, 3.25, 104.4 and 8.41 in comparison to 5.63, 43.31, 438.8 and 36.46 in *M. incognita* inoculated plants. Similar reduction was observed, when *P. lilacinus* was applied at planting and *M. incognita* at 7 days after planting sequentially, where gall index, egg mass/root, J_2/g soil and Rf were 0.25, 1.00, 71.88 and 5.77. In other sequential inoculations, when nematode was inoculated at planting and bioagent at 7 days after planting, the gall index, egg mass/root, J_2/g soil and Rf were 2.38, 9.25, 349.4 and 28.21. In application of *P. lilacinus* at 7 days before planting and *M. incognita* at planting, gall index, egg mass/root, J_2/g soil and Rf were 1.38, 6.38, 235.6 and 18.87.

Sabet *et al.* (2012) examined antagonistic effects of four isolates of *Paecilomyces lilacinus* and an isolate of *Isaria farinosa* on root-knot nematode *Meloidogyne javanica* under *in vitro* and greenhouse conditions. In vitro tested results indicated that nematode eggs parasitization percent by various fungal isolates and J_2 mortality as well as egg hatching inhibition of the culture filtrate of these fungi have variable effects on target nematode. *P. lilacinus* (isolate P3) showed more efficiency than the other isolates. In greenhouse experiment, results showed that *P. lilacinus* isolate P3, P1, P4,and P2 and *I. farinosa* had 65, 44, 42, 29 and 23% nematode control, respectively, when tomato plants inoculated with 4000 eggs and juveniles (J_2).

Rao *et al.* (2012) evaluated bio-efficacy of a bio-nematicide, IIHR - *Paecilomyces lilacinus* (*Purpureocillium lilacinum* Luangsa-Ard) for the management of *Meloidogyne incognita* (Chitw.) on tomato (*Solanum lycopersicum* L.). The trials were conducted in two different agro-climatic regions in India using Farm Yard Manure (FYM) enriched with bio-nematicide containing culture of *P. lilacinus* (CFU of 2×10^6/g) (1%) w. p. against 136-154 J_2 of *M. incognita*/100 g of soil. The seed of tomato treated at the rate of 20 g/kg, nursery bed treated at the rate of 50 g *P. lilacinus*/m^2 and application of FYM (5 tons) enriched with 5 kg of *P. lilacinus*/ha proved to be significantly effective in the management of *M. incognita* and these treatments increased the yield of tomato significantly. The results of these trials revealed the potential of this bio-nematicide in the management of *M. incognita* on tomato in two different agro-climatic regions.

Aminuzzaman *et al*. (2013) used alginate pellets of *Paecilomyces lilacinus* YES-2 and *Pochonia chlamydosporia* HDZ-9 for controlling of *M. incognita* on tomato in a greenhouse by adding them into a soil with sand mixture at rates of 0.2, 0.4, 0.8 and 1.6% (w/w). *P. lilacinus* pellets 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%. The results indicate that *P. lilacinus* and *P. chlamydosporia* as pellet formulation can effectively control root-knot nematodes.

Azam *et al*. (2013) conducted a glass house experiment to assess the effect of *Paecilomyces lilacinus* on the reproduction of root-knot nematode and on the growth of tomato. The results indicated that the use of *P. lilacinus* one week before nematode inoculation caused an increase in growth and yield characteristics of tomato, and also reduced the reproduction of nematode as compared to other treatments. The results of histological studies indicated that *P. lilacinus* parasitized on the *M. incognita* eggs through the formation of fungal hyphae and conidiophores and caused the disintegration of the eggshells, egg masses and juveniles of *M. incognita*.

Khalil (2013) conducted a pot experiment to select potentially useful eco-biorational product that could be used to reduce the reproduction of root knot nematode. The experiment was carried out in pots under net house. The results revealed that the bio-product Dipel® (*Bacillus thuringiensis*) proved to be the most effective treatment that reduced the root galls and egg masses by 71.60 and 77.78% respectively. Also, Dipel® (*Bacillus thuringiensis*) and Bio-nematon® (*Paecilomyces lilacinus*) showed their superiority among all treatments on the shoot, root length and root weight.

Udo *et al*. (2013) conducted a greenhouse experiment to investigate the single and combined effects of different arbuscular mycorrhizal fungi (AMF) and bio-formulated *Paecilomyces lilacinus* against *Meloidogyne incognita* race 1 on tomato. Three applications of the bionematicide were combined with five species of AMF plus an un-inoculated control. The results indicated that AMF species differed significantly ($p \leq 0.05$) in their efficacy of gall and egg mass inhibition, tomato root colonization rate as well as growth and fresh fruit yield enhancement. *Glomus etunicatum* and *G. deserticola* were the most efficient species. Two applications of the bio-nematicide more significantly ($p \leq 0.05$) reduced galling and egg production than a single application. Individual combinations of two AMF (*G. etunicatum* and *G. deserticola*) with a double application of the bio-nematicide, resulted in the greatest gall and egg mass inhibition and consequently the greatest growth and fresh fruit yield enhancement.

Liu *et al.* (2014) conducted to evaluate the efficacy of two nematicides (Fosthiazate and Dazomet), a biocontrol agent (*Purpureocillium lilacinum* (= *Paecilomyces lilacinus*) YES-2), their combination on controlling root-knot nematodes on tomato plants and their effects on the rhizosphere microbial community in long-term glasshouse experiments. The gall index and numbers of second-stage juveniles (J_2) were significantly reduced by the individual treatments of Fosthiazate, Dazomet or *P. lilacinum* YES-2. This study suggested a synergistic effect on the control of root-knot nematode by *P. lilacinum* YES 2 combined with Fosthiazate, and the contribution of these two treatments to the microbial communities in the soil.

MATERIALS AND METHODS

Pot experiments were conducted to study the effect of *Meloidogyne incognita* inoculum density and application rate of *Purpureocillium lilacinum* on biocontrol efficacy against root-knot disease of tomato. The materials used and the methods followed in the study are presented in this chapter.

3.1. Experimental site and experimental period

The present investigation was carried out during the period from November 2012 to March 2013 and November 2013 to March 2014 in the Laboratory and in the shade house of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka -1207.

3.2. Environment of experiments

All the experimental plants were kept in the shade house where the temperature was 28 ± 2° C during the "day" and 23 ± 2° C during "night" with an average temperature of 26± 2° C.

3.3 Pot Experiment

3.3.1. Crop variety used

In this experiment BARI Tomato-14 was used as selected crop.

3.3.2. Collection of seeds

BARI Tomato-14 seed was collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

3.3.3. Soil collection and sterilization

Required soils were collected from agricultural farm of Sher-e-Bangla Agricultural University. Sand, decomposed cow dung and compost were also collected with soil. Then soil, sand, cow dung and compost mixed properly in a ratio of 6:3:1. For raising seedlings in plastic trays and for final experiment set up the mixture was autoclaved at 121°C, 15 psi for 15 minutes on two successive days. The sterilized soil was allowed to cool to room temperature and was later used to fill the plastic trays for raising seedlings and pot for seedling transplanting.

3.3.4. Raising of Seedling

Several plastic trays were filled with sterilized and fertile soil. Seeds of BARI Tomato-14 was soaked in water for one night and treated with NaOCl for one minute and washed with distilled water for three times. After that the seeds were sown in plastic trays and covered with a thin layer of soil and watered. Then the trays were covered with polythene sheet and kept in sunlight for raising seedlings. Seedlings were observed regularly and watering was done as per necessity up to hardening the seedling in plastic pot.

3.4. Preparation of pots

Plastic pots of 1000 cm^3 were cleaned, washed and dried up. Sterilized and fertile soil was filled in required amount into each pot. Each pot contained 800 g soil. Then the pots were arranged according to selected experimental design. Detailed of soil properties presented in Table 1.

Table 1. Physicochemical characteristics of pot soil

Soil type	P^H	Organic matter	Total N	Particle size (%)			P	
		(%)		Sand	Silt	Clay	µg/g soil	
Loam	5.2	2.29	0.114	48	41	11	210.88	
Exchangeable cations (meq/100g soil)			B	S	Mn	Fe	Cu	Zn
Ca	Mg	K	µg/g soil					
17.50	21.25	3.76	10.40	523.70	8.60	23.82	1.68	8.05

3.5. Treatments of the experiment

The experiment was conducted according to the following treatment scheme:

Factor A: Nematode (*Meloidogyne incognita*) inoculum density

N_1= 0 eggs of *M. incognita*/100 g soil

N_2= 250 eggs of *M. incognita*/100 g soil

N_3= 500 eggs of *M. incognita*/100 g soil

N_4= 1000 eggs of *M. incognita*/100 g soil

N_5= 2000 eggs of *M. incognita*/100 g soil

Factor B: Application rate of *Purpureocillium lilacinum*

F_1= 0 CFU of *P. lilacinum*/g of soil

F_2= 1×10^4 CFU of *P. lilacinum*/g of soil

F_3= 5×10^4 CFU of *P. lilacinum*/g of soil

F_4= 1×10^5 CFU of *P. lilacinum*/g of soil

F_5= 5×10^5 CFU of *P. lilacinum*/g of soil

All the treatment was replicated eight times.

3.6. Culture, mass production and harvesting of *Purpureocillium lilacinum*

Purpureocillium lilacinum was grown on Potato Dextrose Agar (PDA) medium for 8-10 days (Aminuzzaman and Liu, 2011). Within 8-10 days the fungus was transferred on chick pea for mass production. For mass production one hundred grams of chick pea seed free of any pesticide treatment was placed in 250-ml conical flasks and soaked in lukewarm water for 3-4 hours. Then the water was drained off, and each flask was closed with a cotton plug and covered with brown paper in two layer paper. Then flasks were placed in an autoclave for 15 minutes at 15 psi. After the flasks and contents cooled, *P. lilacinum* as a mycelial mat growing on PDA was added aseptically to one flask and shaken for better distribute of the fungus; the other flask served as an un-inoculated control. The flasks were incubated at 25-30° C for 20 days. After incubation the sterile water was added into the conical flask and the spore masses scraped away with sterile brush within laminar air flow chamber. The harvested spores were filtered through sterilized cheesecloth. The spore was harvested from each conical flask and spore was counted with a haemacytometer (Plate 1& Plate 2).

A

B

C

D

E

Plate 1. A: Pure culture of *P. lilacinum* on PDA media, B: Sterilized chick pea grain without inocula, C: Mass production of *P. lilacinum* on chick pea, D: Harvesting of spore from conical flask, E: Sieving of spore

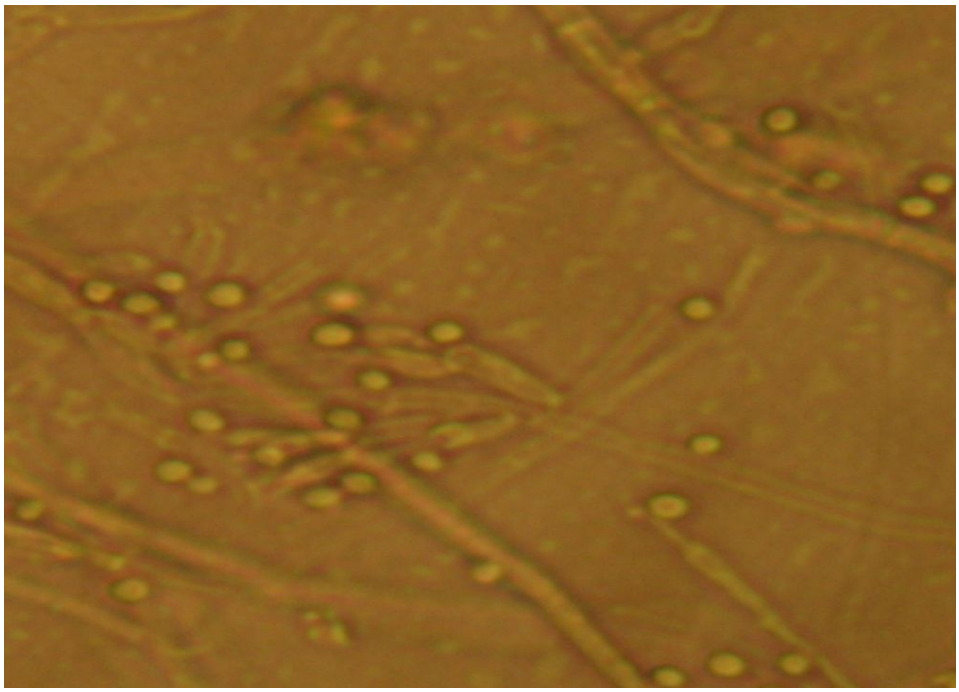


Plate 2. Conidiophore and Conidia of *P. lilacinum* under compound microscope (400X)

3.7. Culturing and inoculum preparation of *Meloidogyne incognita*

Inoculum of *Meloidogyne incognita* was collected from Bangladesh Agricultural Research Institute and cultured and maintained in BARI Tomato-14 plants grown in plastic pots containing sterilized soil for two months. For culturing nematodes, egg masses were handpicked, sterilized with NaOCl for 1 min and rinsed with water for three times and inoculated in young seedlings of tomato. Sub-culturing was done subsequently by inoculating new tomato seedlings with egg masses. Mature egg masses of *Meloidogyne incognita* was collected from severely galled roots of tomato with the help of fine forceps. The numbers of egg/egg mass were counted with the help of compound microscope. Then eggs suspension was prepared with sterile water.

3.8. Transplanting of seedlings and inoculation of *P. lilacinum* and *M. incognita*

After preparation of pot in the shade house, spore of *P. lilacinum* carefully mixed into soil @ 0, 2.15×10^{10}, 1.075×10^{11}, 2.15×10^{11} and 1.075×10^{12} spore/plant for 0, 1×10^{4}, 5×10^{4}, 1×10^{5} and 5×10^{5} CFU of *P. lilacinum*/g of soil in defined pot with micropipette. 30 days old seedlings were uprooted carefully from the plastic pot and transplanted in the fungal treated pot. Initial root and shoot weight measured before transplanting. Only one plant was transplanted to each pot. Sufficient irrigation was given just after transplantation. Then after 4 days nematode was inoculated @ 0, 2000, 4000, 8000 & 16000 eggs/plant. Four holes of five cm depth around the plants were made with the help of metallic rod and eggs suspension inoculated into holes with micropipette. The holes were covered with soil to prevent drying. After few days of inoculation these pots were watered carefully to prevent loss of nematodes through leaching or excessive drying (Plate 3).

A

B

C

Plate 3. A: Photograph showing raising of tomato seedlings in plastic tray, B: Inoculation of eggs suspension of *Meloidogyne incognita*, C: Pots in the shade house condition

3.9. Intercultural operations

After transplantation of seedling and final experiment set up weeding and irrigation were regularly done as per necessity. General sanitation was maintained throughout the growing period.

3.10. Harvesting and data recording

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

Shoot length (cm)

Root length (cm)

Shoot fresh and dry weight (g)

Root fresh and dry weight (g)

Gall index (0-10 scale)

Number of egg masses per root

Number of eggs per egg mass

Number of eggs per root system

Number of juveniles per 800 g soil

Total number of nematode population/plant (J_2+ eggs)

Reproduction factor (RF)

% Egg masses colonized by *P. lilacinum*

Soil colonization by *P. lilacinum* ($CFUg-^{1}$ soil)

3.11. Design and layout of the experiment

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

3.12. Data recorded

3.12.1. Plant data

Shoot and root length were measured before harvest. The shoot height (cm) was measured from the base of the plant to the growing point of the youngest leaf with a measuring scale. Then the roots are harvested by cutting with an anti-cutter. Roots are carefully separated from soil, cleaned gently with water and collected in different polybag that were leveled according to different treatments. Finally the root length (cm) was taken. The length of root was measured from the growing point of root to the longest available lateral root apex. For fresh weight (g) of root and shoot was blotted dry and the weight was recorded. For dry weight (g), the shoot and root were sun dried for three days and then kept in drier machine for 4-6 hours at 40° C temperatures. And after complete drying the weight was recorded.

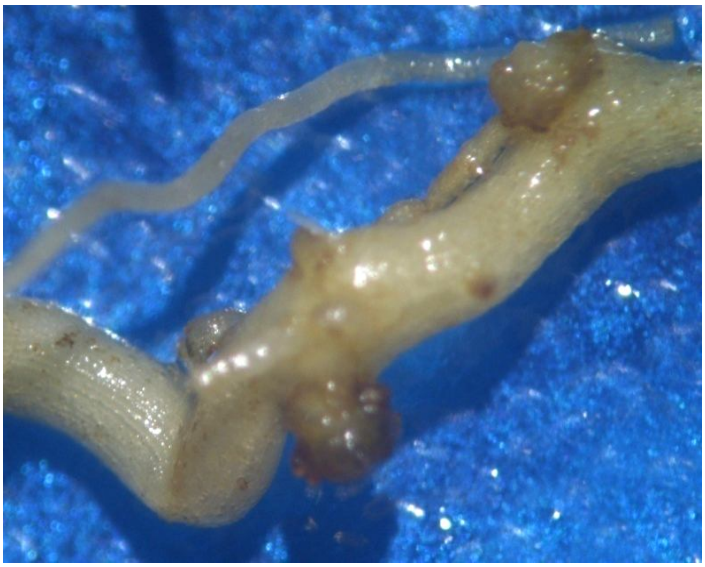
3.12.2. Counting of nematode egg masses/root system

Number of egg masses/root system was counted following Holbrook *et al.*, (1983). The roots were soaked in Phloxine-B (2mg/l) for 15 minutes (Hartman and Sasser, 1985). The roots were observed and egg masses/root was counted with a magnifying glass. Then egg masses were picked with forceps treated with NaOCl for three minutes to dissolve gelatinous materials. After subsequent washing with water eggs were counted under compound microscope (Plate 4 and Plate 5).

A

B

Plate 4. A: Heavily galled root treated with Phloxine-B solution, B: Phloxine-B treated root



A

B

Plate 5. A: Phloxine-B treated egg masses, B: Phloxine-B treated stained female of *M. incognita*

3.12.3. Slide preparation and counting of eggs/egg mass

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

3.12.4. Extraction of nematode from soil and counting of juveniles

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

A

B

Plate 6. A: Extraction of nematode by Bangladeshi plate method (modified White Head and Heaming method, 1965), B: Second stage juveniles of *Meloidogyne incognita*

3.12.5. Gall index

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

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

3.12.6. % Egg masses colonization by *Purpureocillium lilacinum*

Egg masses were collected as per treatment from the tomato plant roots, washed with water and disinfected with a solution of 10% Clorox, rinsed with sterile water and put on a Potato Dextrose Agar (PDA) media in petridish. Randomly ten egg masses/root was collected so that 80 egg masses per treatments were collected. The number of colonized egg masses was determined after 5 days of incubation. The presence of *P. lilacinum* with egg mass of *M. incognita* was confirmed by preparation of slides from the culture grown on PDA (Plate 7 and Plate 8).

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

Samples of 1g soil from each treatment were collected after harvest of the crop around the root zone. The number of colony forming unit ($CFUg^{-1}$ soil) per gram soil was determined using the soil dilution plate method (Plate 9).

3.13. Analysis of data

The data were statistically analyzed using analysis of variance to find out the variation of results from experimental treatments. Treatment means were compared by Duncan's New Multiple Range Test (DMRT) according to Gomez and Gomes, (1984). Data were analyzed by MSTAT-C statistical package programmed.

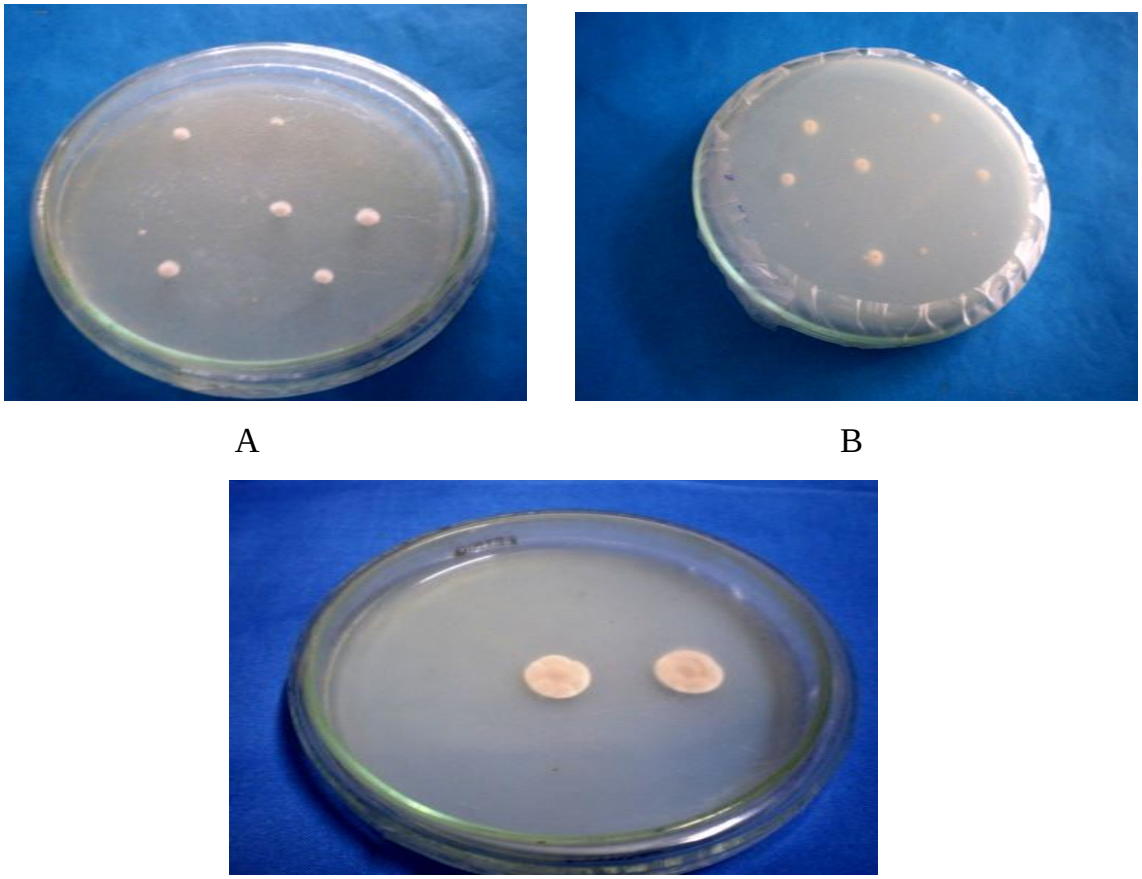
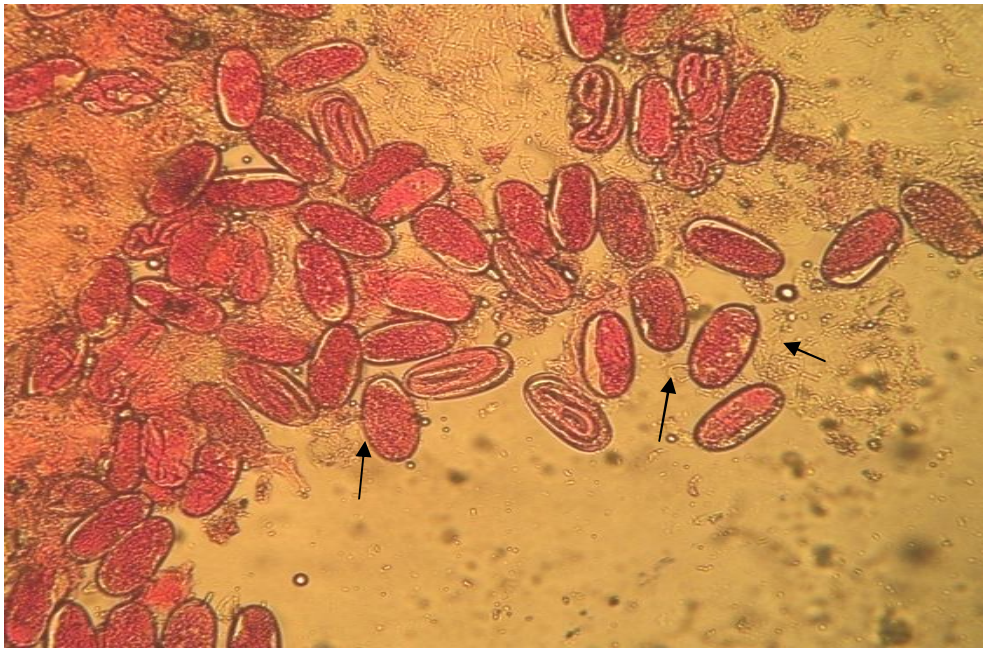
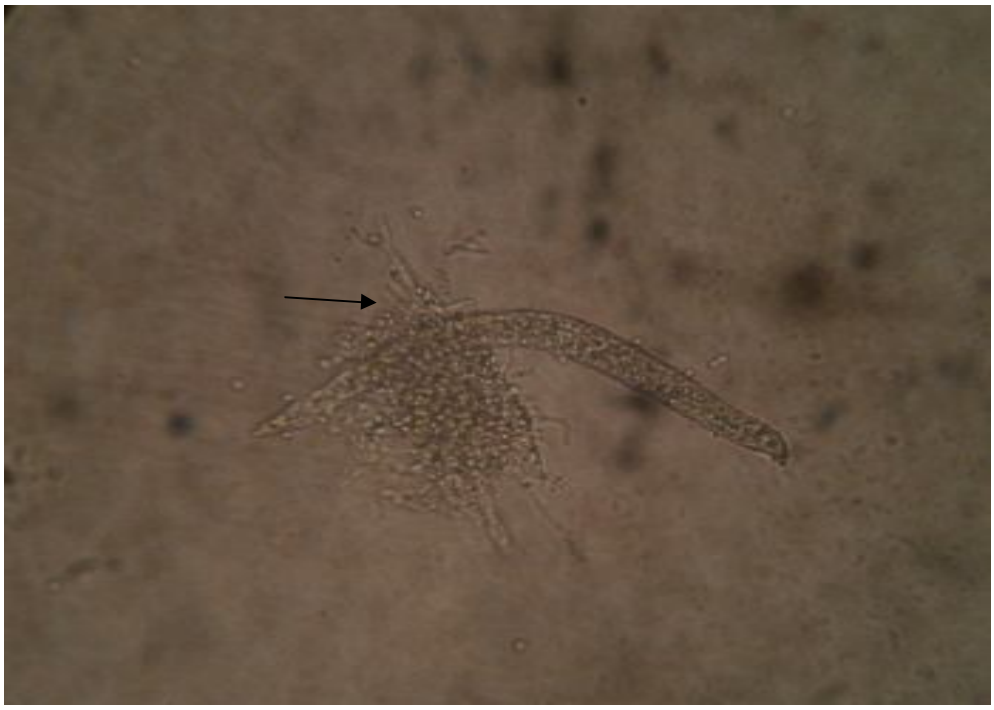


Plate 7. A: Egg masses colonization by *P. lilacinum* on PDA media after 4 days later (front view), B: Egg masses colonization by *P. lilacinum* on PDA media after 4 days later (opposite view), C: After 10 days later culture of *P. lilacinum* from colonized egg masses



A



B

Plate 8. A: Colonization of eggs by *P. lilacinum*, B: Colonization of second stage juvenile by *P. lilacinum*

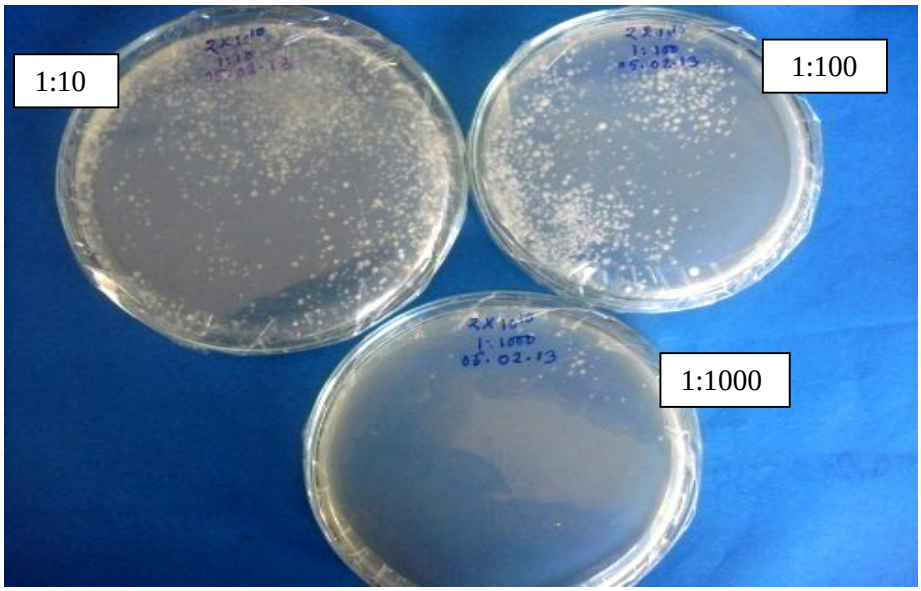


Plate 9: Colony growth of *P. lilacinum* on PDA media by soil dilution plate technique

RESULTS

4.1 Effect of doses of *Purpureocillium lilacinum* on growth parameters of tomato

The shoot length of tomato varied significantly due to the different application rate of *Purpureocillium lilacinum* (Table 2). The maximum shoot length (51.90 cm) was measured in application rate of 5×10^4 CFU/g soil. The shoot length 48.91 cm was observed when soil treated with 1×10^4 CFU/g soil which shown statistically similar to 5×10^5 CFU/g soil. On the other hand, the minimum shoot length (43.71 cm) was observed in untreated control (0 CFU of *P. lilacinum*/g soil) treatment which was shown statistically similar result to the dose of 1×10^5 CFU/g soil.

Application of *Purpureocillium lilacinum* significantly influenced shoot fresh weight (Table 2). The maximum shoot fresh weight (22.41 g) was recorded when using 5×10^4 CFU of *P. lilacinum* per g soil, which was statistically similar to all other doses (1×10^4, 5×10^5 and 1×10^5 CFU/g soil) of *P. lilacinum* used in this experiment. The minimum shoot fresh weight (15.02 g) was statistically differed from other treatment that found where only *M. incognita* inoculum applied.

The shoot dry weight of tomato plant significantly influenced by different dose of *P. lilacinum* (Table 2). The maximum shoot dry weight (4.32 g) was found in application rate of 1×10^4 CFU of *P. lilacinum*/g soil which was statistically similar to 5×10^4 CFU of *P. lilacinum*/g soil. The shoot dry weight 3.35 g was observed in 5×10^5 CFU/g soil which was statistically similar to 1×10^5 CFU of *P. lilacinum*/g soil. On the other hand, the minimum shoot dry weight (1.71g) was obtained when no bioagent used but only pathogenic organism inoculated.

The root length also varied significantly due to the different application rate of *Purpureocillium lilacinum* (Table 2). The maximum root length (23.20 cm) was examined in application rate of 1×10^4 CFU/g soil, which was statistically different from all other fungal doses. The root length 16.67 cm was recorded in 1×10^5 CFU/g soil which was statistically similar to 5×10^4 CFU/g soil and 5×10^5 CFU/g soil. The minimum root length (13.19 cm) was observed in untreated inoculated treatment which was statistically similar to 5×10^5 CFU/g soil.

Table 2. Effect of *Purpureocillium lilacinum* application rate on growth parameters of tomato

Fungal doses	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	43.71 b	15.02 b	1.71 c	13.19 c	3.29 c	0.31 c
1×10^4 CFU/g soil	48.91 ab	22.21 a	4.32 a	23.20 a	10.71 a	0.68 a
5×10^4 CFU/g soil	51.90 a	22.41 a	4.27 a	16.54 b	4.54 bc	0.33 c
1×10^5 CFU/g soil	46.55 b	20.52 a	3.12 b	16.67 b	4.92 b	0.47 b
5×10^5 CFU/g soil	47.94 ab	21.58 a	3.35 b	15.31 bc	3.52 bc	0.35 bc
$LSD_{(0.05)}$	5.33	3.78	0.69	2.73	1.46	0.12
CV (%)	11.29	18.82	22.37	16.31	27.54	27.76

In a column treatments mean followed by different letter is statistically different

The root fresh weight of tomato significantly influenced by different doses of *Purpureocillium lilacinum* (Table 2). The maximum root fresh weight (10.71 g) was got by using 1×10^4 CFU/g soil of *P. lilacinum*. The root fresh weight 4.92 g was recorded when pots treated with 1×10^5 CFU/g soil. The root fresh weight 4.54 g was examined when pot soil mixed with 5×10^4 CFU of *P. lilacinum*/g soil which was statistically similar to 5×10^5 CFU/g soil. The minimum root fresh weight (3.29 g) was found where applied only different inoculum density of *Meloidogyne incognita*.

Application of different dose of *Purpureocillium lilacinum* significantly influenced the root dry weight of tomato plant (Table 2). The maximum root dry weight (0.68 g) was obtained in application rate of 1×10^4 CFU/g soil. The root dry weight 0.47 g was recorded when pots treated with 1×10^5 CFU of *P. lilacinum*/g soil. The minimum root dry weight (0.31 g) was observed when inoculation done by only *Meloidogyne incognita* which was statistically similar to 5×10^4 and 5×10^5 CFU/g soil. The root dry weight 0.35 g was recorded when applied 5×10^5 CFU of *P. lilacinum*/g soil which was statistically similar to 1×10^5 CFU of *P. lilacinum*/g soil.

4.2 Effect of different inoculum density of *Meloidogyne incognita* on growth parameters of tomato

A significant variation was found in shoot length of tomato because of using different *Meloidogyne incognita* inoculum density (Table 3). The maximum shoot length (52.29 cm) was examined in control treatment (0 eggs/100 g of soil) which was statistically similar to 250 eggs/100 g soil and 500 eggs/100 g soil. The minimum shoot length (43.77 cm) was observed when soil inoculated with highest level (2000 eggs/100 g of soil) of *Meloidogyne incognita* and showing significant similar result with 1000 eggs/100 g soil and 500 eggs/100 g soil (Plate 10).

The shoot fresh weight of tomato varied significantly due to the effect of different *Meloidogyne incognita* inoculum density. The maximum shoot fresh weight (22.74 g) was obtained in control treatment (0 eggs/100 g of soil). The shoot fresh weight 21.19 g was observed when plant inoculated with 250 eggs/100 g soil which was statistically similar to 500 eggs *M. incognita*/100 g soil and 1000 eggs *M. incognita*/100 g soil. The minimum shoot fresh weight (18.64 g) was found in where *Meloidogyne incognita* was inoculated @ 2000 eggs/100 g of soil which was statistically similar to all other treatments except control (Table 3).

A significant variation of shoot dry weight was found because of application of different *Meloidogyne incognita* inoculum density in soil. The maximum shoot dry weight (4.99 g) was observed in treatment where no pathogenic organism used which was shown statistically similar result to 250 eggs/100 g soil. The minimum shoot dry weight (2.60 g) was got when used large number of nematode population (2000 eggs/100 g of soil) in limited soil which was statistically similar to 1000 eggs/100 g soil and 500 eggs/100 g soil (Table 3).

A significant variation was found in root length of tomato because of using different *Meloidogyne incognita* inoculum density (Table 3). The maximum root length (19.41g) was examined in controlled treatment (0 eggs/100 g of soil). The root length 17.77 g was recorded when plant suffered infestation of 250 eggs of *M. incognita*/100 g soil which was statistically similar to 500 eggs/100 g soil. The minimum root length (15.47 g) was observed when soil inoculated with highest inoculum level (2000 eggs/100 g of soil) of *Meloidogyne incognita* which was statistically similar to all other treatments except control.

Table 3. Effect of different *M. incognita* inoculum density on growth parameters of tomato

Inoculum density*	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Control	52.29 a	22.74 a	4.99 a	19.41 a	4.8	0.37 b
250	49.22 ab	21.19 ab	3.76 ab	17.77 ab	5.02	0.39 ab
500	47.93 abc	20.25 ab	3.24 bc	16.70 ab	5.39	0.43 ab
1000	45.81 bc	18.92 ab	2.83 c	15.56 b	5.75	0.44 ab
2000	43.77 c	18.64 b	2.60 c	15.47 b	6.0	0.51 a
LSD $_{(0.05)}$	5.33	3.78	0.69	2.73	NS	0.12
CV (%)	11.29	18.82	22.37	16.31	27.54	27.76

In a column treatments mean followed by different letter is statistically different

*Number of eggs per 100 g soil

NS= Not significant

The root fresh weight of tomato was not varied significantly due to the effect of different *Meloidogyne incognita* inoculum density. The maximum root fresh weight (6.0 g) was obtained when pots infested with 2000 eggs per 100 g soil which showed statistically similar result to other inoculum density. The minimum root fresh weight (4.8 g) was found in where *Meloidogyne incognita* was inoculated @ 0 eggs of *M. incognita*/100 g of soil followed by 250 eggs of *M. incognita*/100 g soil (5.02 g), 500 eggs of *M. incognita*/100 g soil (5.42 g) and 1000 eggs of *M. incognita*/100 g of soil (5.75 g) (Table 3).

A significant variation of root dry weight was found because of application of different inoculum density of *Meloidogyne incognita* in soil. The maximum root dry weight (0.51 g) was observed when pots treated with 2000 eggs/100 g of soil. The root dry weight 0.44 g was recorded when plants suffered 1000 eggs of *M. incognita*/100 g soil which was statistically similar with 500 eggs of *M. incognita*/100 g soil and 250 eggs of *M. incognita*/100 g soil. The minimum root dry weight (0.37 g) was got in treatment where no pathogenic organism used (Table 3).

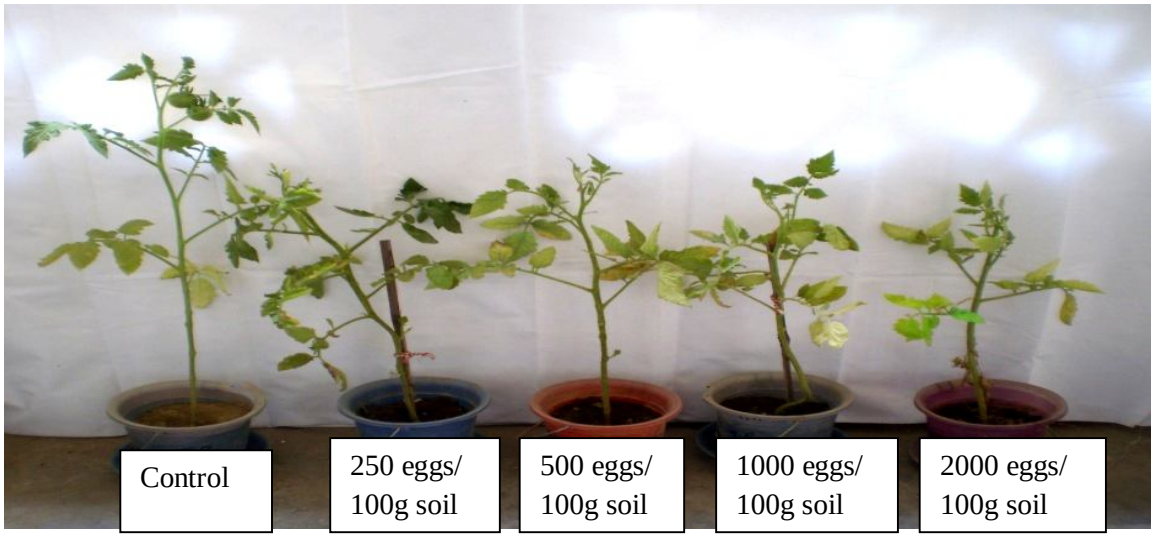


Plate 10. Photograph showing the effect of *Meloidogyne incognita* inoculum density on plant growth of tomato in comparison to control

4.3 Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on growth parameters of tomato

The shoot length of tomato was significantly affected by the combined effect of *Purpureocillium lilacinum* application rate and *Meloidogyne incognita* inoculum density (Fig 1). The maximum shoot length (54.39 cm) was observed in a combination of 5×10^4 CFU/g soil of *P. lilacinum* with 0 eggs/100 g of soil which was statistically similar to combination of 5×10^4 CFU/g soil of *P. lilacinum* with 250 eggs/100 g of soil and 1×10^5 CFU/g soil of *P. lilacinum* with 0 eggs/100 g of soil. The minimum shoot length (38.2 cm) was recorded from the combination where only 2000 eggs of *M. incognita* used per 100 g of soil which shown statistically similar to combination of 1×10^5 CFU/g soil of *P. lilacinum* with 2000 eggs/100 g of soil (Plate 11 &Plate 12).

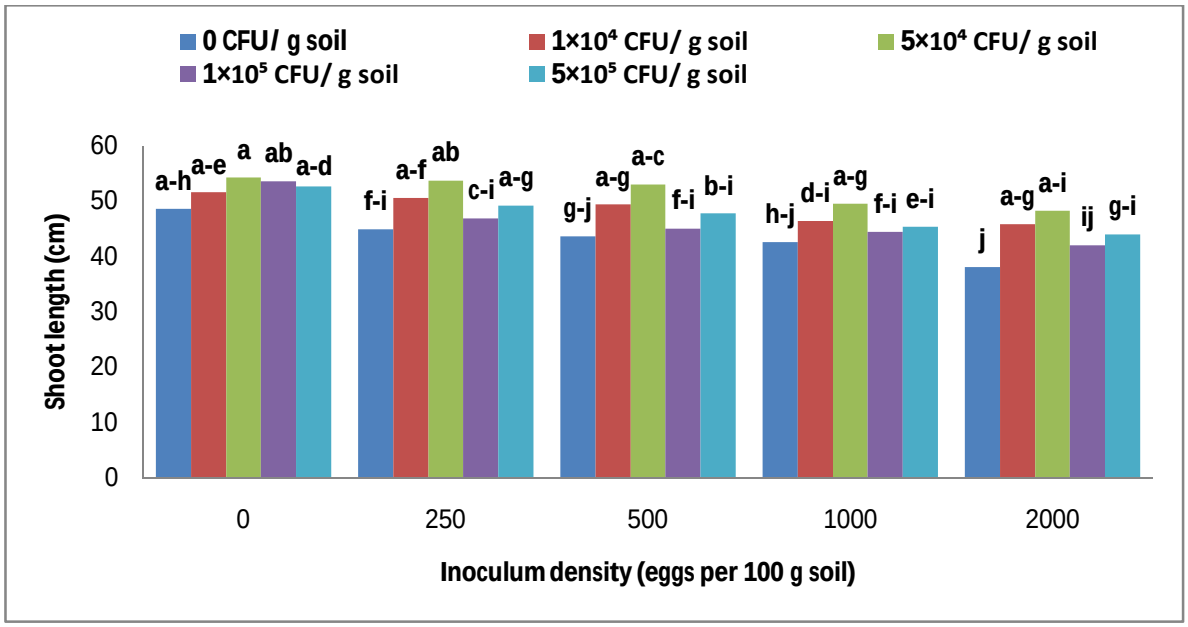


Fig 1. Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on shoot length of tomato. Bars headed by different letters are significantly different

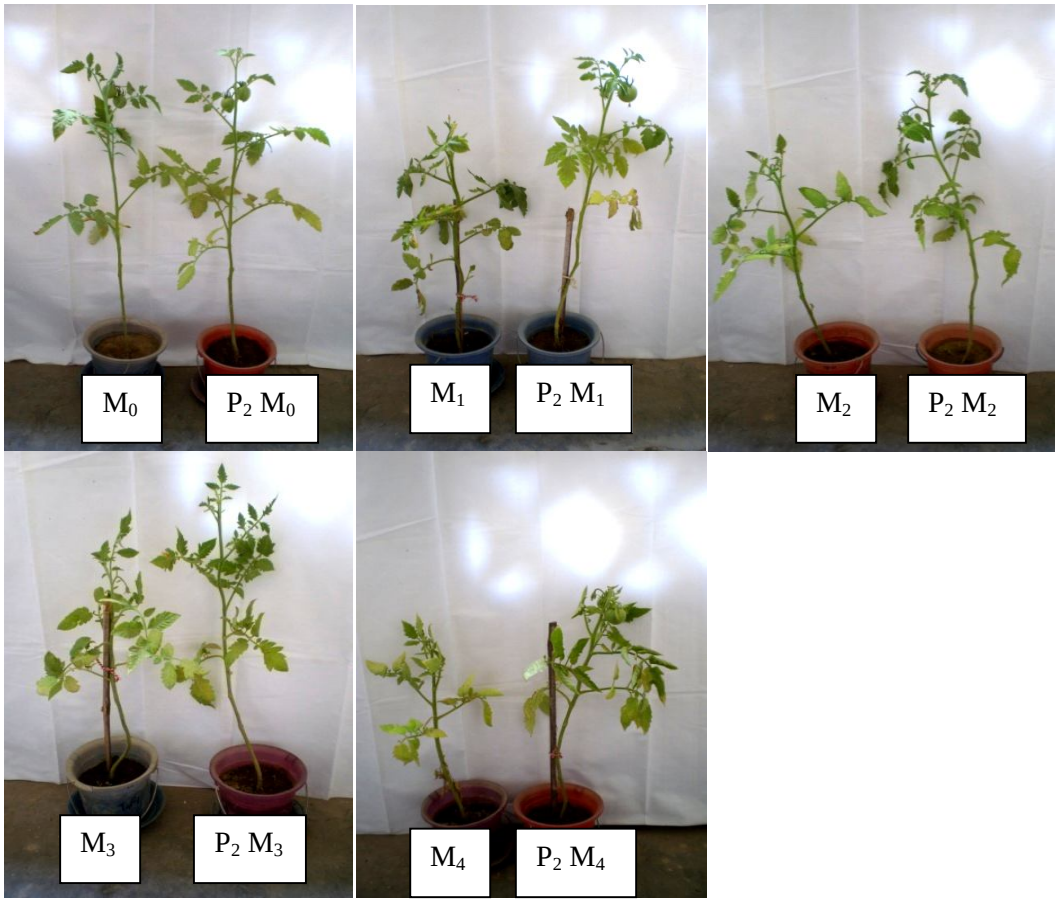


Plate 11. Photograph showing the effect of dose of *P. lilacinum* (5×10^4 CFU/g soil) against different inoculum density of *M. incognita* on shoot growth of tomato cv. BARI Tomato-14 in comparison to control

M_0= Control (without pathogenic organism)

P_2M_0= Inoculation of *M. incognita* @ 0 eggs/100 g soil + *P. lilacinum* @ 5×10^4 CFU/g soil

M_1= Inoculation of *M. incognita* @ 250 eggs/100 g soil

P_2M_1= Inoculation of *M. incognita* @ 250 eggs/100 g soil + *P. lilacinum* @ 5×10^4 CFU/g soil

M_2= Inoculation of *M. incognita* @ 500 eggs/100 g soil

P_2M_2= Inoculation of *M. incognita* @ 500 eggs/100 g soil + *P. lilacinum* @ 5×10^4 CFU/g soil

M_3= Inoculation of *M. incognita* @ 1000 eggs/100 g soil

P_2M_3= Inoculation of *M. incognita* @ 1000 eggs/100 g soil + *P. lilacinum* @ 5×10^4 CFU/g soil

M_4= Inoculation of *M. incognita* @ 2000 eggs/100 g soil

P_2M_4= Inoculation of *M. incognita* @ 2000 eggs/100 g soil + *P. lilacinum* @ 5×10^4 CFU/g soil

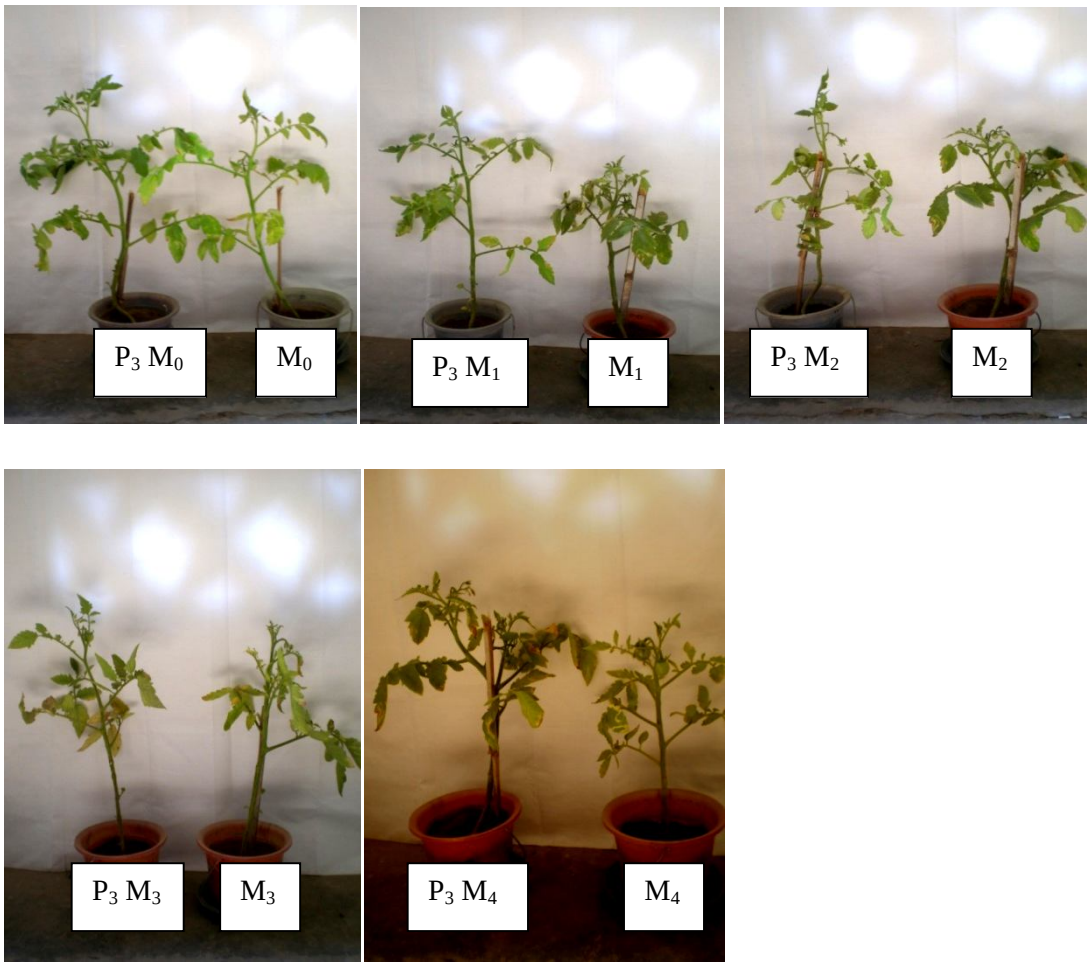


Plate 12. Photograph showing the effect of dose of *P. lilacinum* (1×10^5 CFU/g soil) against different inoculum density of *M. incognita* on shoot growth of tomato cv. BARI Tomato-14 in comparison to control

M_0= Control (without pathogenic organism)

P_2M_0= Inoculation of *M. incognita* @ 0 eggs/100 g soil + *P. lilacinum* @ 1×10^5 CFU/g soil

M_1= Inoculation of *M. incognita* @ 250 eggs/100 g soil

P_2M_1= Inoculation of *M. incognita* @ 250 eggs/100 g soil + *P. lilacinum* @ 1×10^5 CFU/g soil

M_2= Inoculation of *M. incognita* @ 500 eggs/100 g soil

P_2M_2= Inoculation of *M. incognita* @ 500 eggs/100 g soil + *P. lilacinum* @ 1×10^5 CFU/g soil

M_3= Inoculation of *M. incognita* @ 1000 eggs/100 g soil

P_2M_3= Inoculation of *M. incognita* @ 1000 eggs/100 g soil + *P. lilacinum* @ 1×10^5 CFU/g soil

M_4= Inoculation of *M. incognita* @ 2000 eggs/100 g soil

P_2M_4= Inoculation of *M. incognita* @ 2000 eggs/100 g soil + *P. lilacinum* @ 1×10^5 CFU/g soil

The shoot fresh weight of tomato was significantly responding with the impact of application rate of *Purpureocillium lilacinum* on *Meloidogyne incognita* inoculum density (Fig 2). The maximum shoot fresh weight (25.42 g) was observed in combination of 5×10^4 CFU/g soil of *P. lilacinum* with 0 eggs/100 g of soil which was statistically similar to combination of 1×10^4 CFU/g soil of *P. lilacinum* with 0 eggs/100 g of soil. The minimum shoot length (12.96 g) was recorded when pots treated with only 2000 eggs of *M. incognita* per 100 g soil which was statistically similar to combination of 0 CFU/g soil of *P. lilacinum* with 1000 eggs/100 g of soil and 0 CFU/g soil of *P. lilacinum* with 500 eggs/100 g of soil.

The shoot dry weight of tomato was significantly affected by the combined effect of *Purpureocillium lilacinum* and *Meloidogyne incognita*. The maximum shoot dry weight (5.7 g) was observed in a combination of 5×10^4 CFU/g soil of *P. lilacinum* with 0 eggs/100 g of soil which was statistically similar to combination of 1×10^4 CFU/g soil of *P. lilacinum* with 0 eggs/100 g of soil and 5×10^4 CFU/g soil of *P. lilacinum* with 250 eggs/100 g of soil. The minimum shoot dry weight (1.45 g) was recorded from 0 CFU/g soil with 2000 eggs of *M. incognita*/100 g of soil which shown statistically similarity to interaction between 0 CFU/g soil with 1000 eggs/100 g soil, 0 CFU/g soil with 500 eggs/100 g soil and 0 CFU/g soil with 250 eggs/100 g soil (Fig 3).

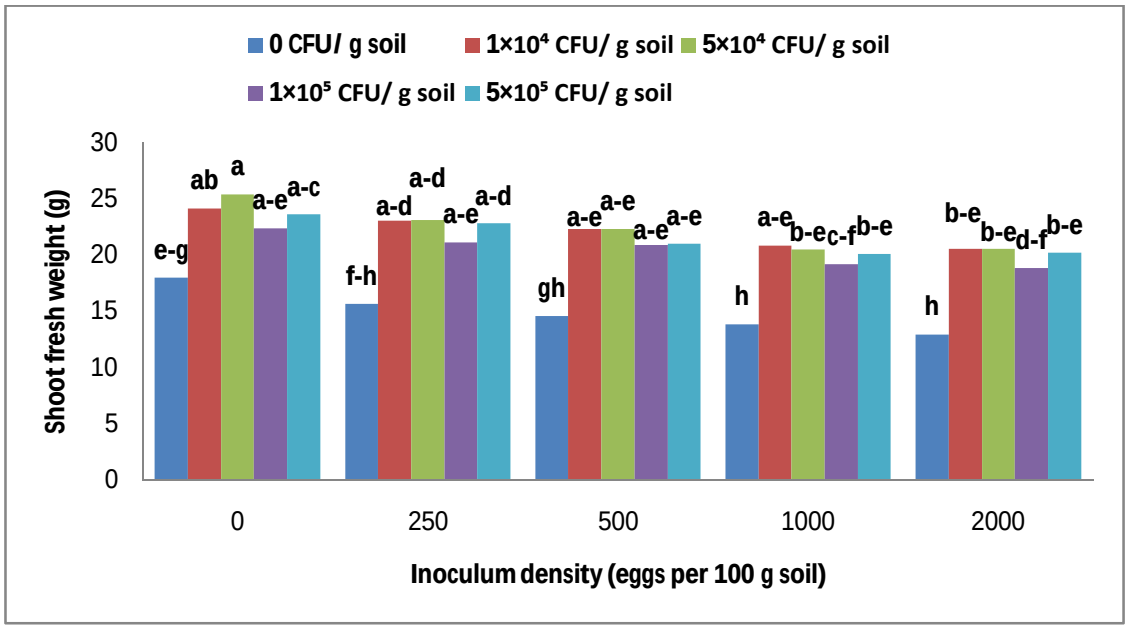


Fig 2. Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on shoot fresh weight of tomato. Bars headed by different letters are significantly different

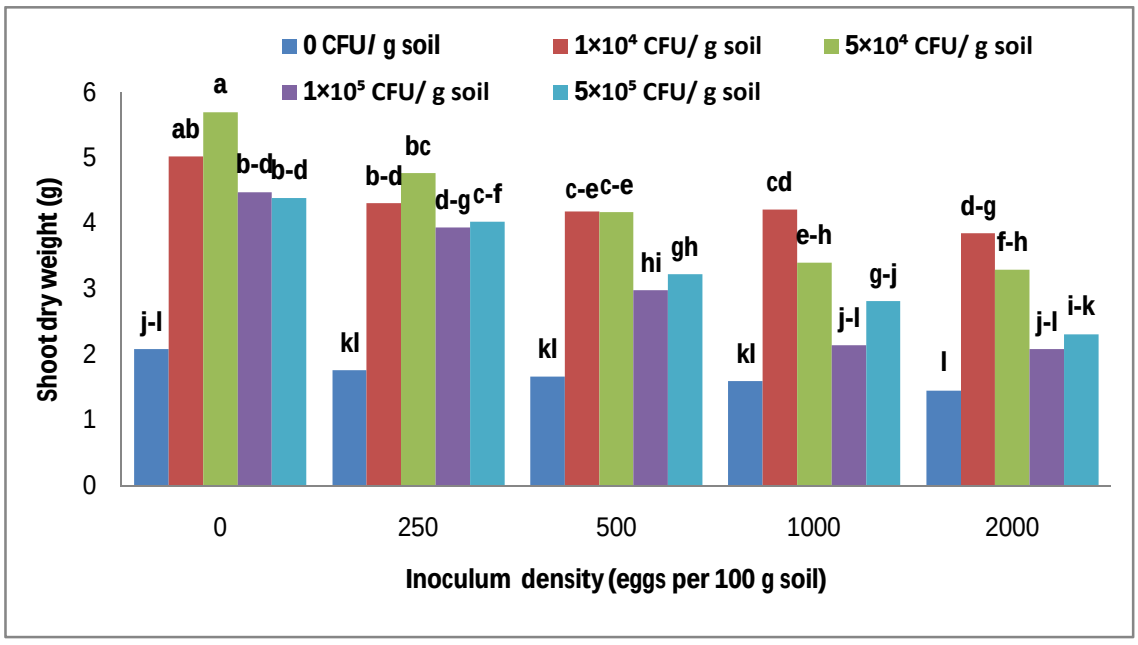


Fig 3. Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on shoot dry weight of tomato. Bars headed by different letters are significantly different

The root length of tomato was significantly affected by the combined effect of *Purpureocillium lilacinum* and *Meloidogyne incognita* (Fig 4). The maximum root length (25.94 cm) was observed in a combination of 1×10^4 CFU/g soil of *P. lilacinum* with 0 eggs/100 g of soil which shown statistically similarity to interaction between1×10^4 CFU/g soil of *P. lilacinum* with 250 eggs/100 g of soil. The minimum root length (10.55 cm) was recorded from the combination where plant suffered only 2000 eggs per 100 g soil which was statistically similar to combination of 0 CFU/g soil of *P. lilacinum* with 1000 eggs/100 g of soil.

The root fresh weight of tomato was significantly affected by the combined effect of *Purpureocillium lilacinum* application rate and *Meloidogyne incognita* inoculum density. The maximum root fresh weight (11.52 g) was observed in a combination of 1×10^4 CFU/g soil of *P. lilacinum* with 0 eggs/100 g of soil which shown statistically similarity to interaction between 1×10^4 CFU/g soil of *P. lilacinum* with 2000 eggs/100 g of soil, 250 eggs/100 g of soil and 500 eggs/100 g of soil. The minimum root fresh weight (2.38 g) was recorded when pots served as blank control (no fungal dose and no nematode inoculum) which was statistically similar to combination of 0 CFU/g soil of *P. lilacinum* with 250 eggs/100 g of soil and 5×10^5 CFU/g soil of *P. lilacinum* with 0 eggs/100 g of soil (Fig 5).

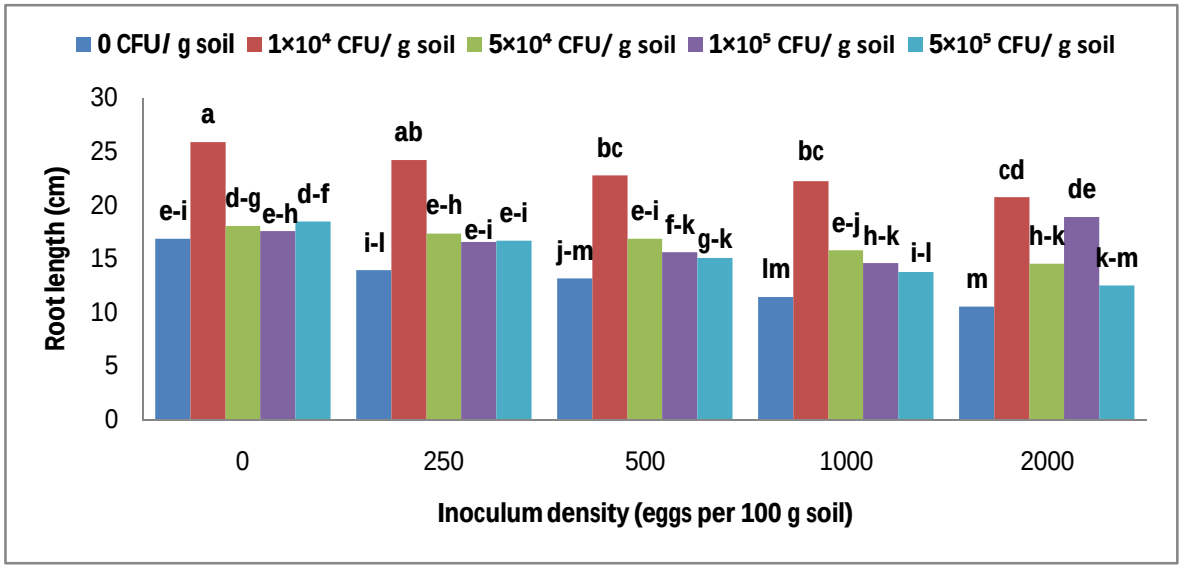


Fig 4. Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on root length of tomato. Bars headed by different letters are significantly different

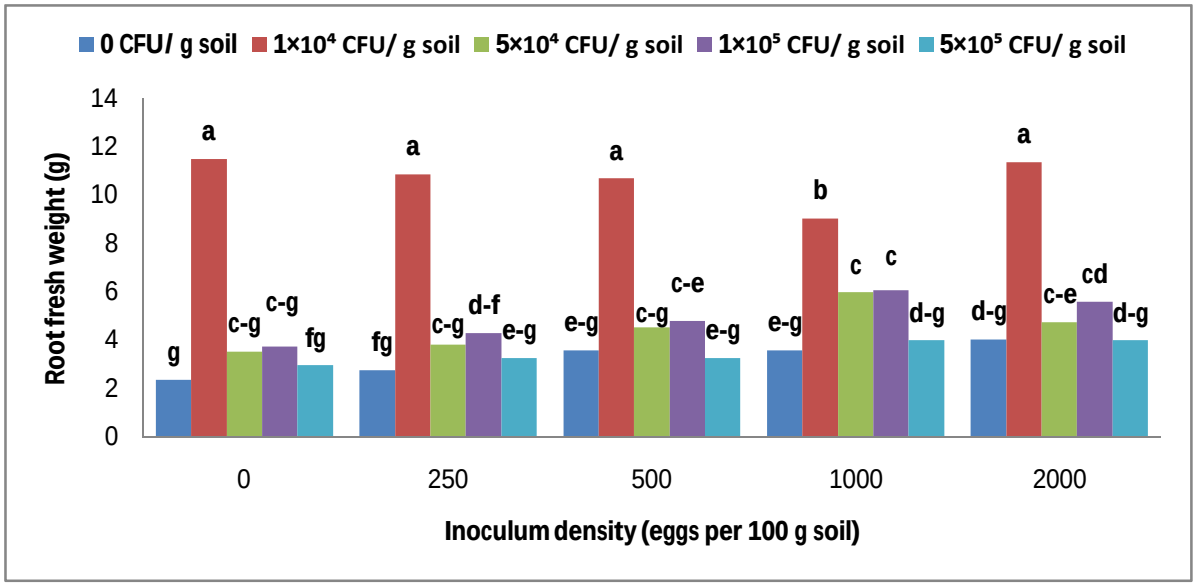


Fig 5. Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on root fresh weight of tomato. Bars headed by different letters are significantly different

The root dry weight of tomato was significantly affected by the combined effect of *Purpureocillium lilacinum* and *Meloidogyne incognita*. The maximum root dry weight (0.77 g) was observed interaction between 1×10^4 CFU of *P. lilacinum*/g soil with 0 eggs/100 g of soil which shown statistically similarity to interaction between 1×10^4 CFU/g soil of *P. lilacinum* with 2000 eggs/100 g of soil and 1×10^4 CFU/g soil of *P. lilacinum* with 250 eggs/100 g of soil. The minimum root dry weight (0.23 g) was recorded from when no fungal dose and inoculum density used in pots, which was statistically similar to combination of 0 CFU/g soil of *P. lilacinum* with 1000 eggs/100 g of soil and 5×10^4 CFU/g soil of *P. lilacinum* over 250 eggs/100 g of soil (Fig 6).

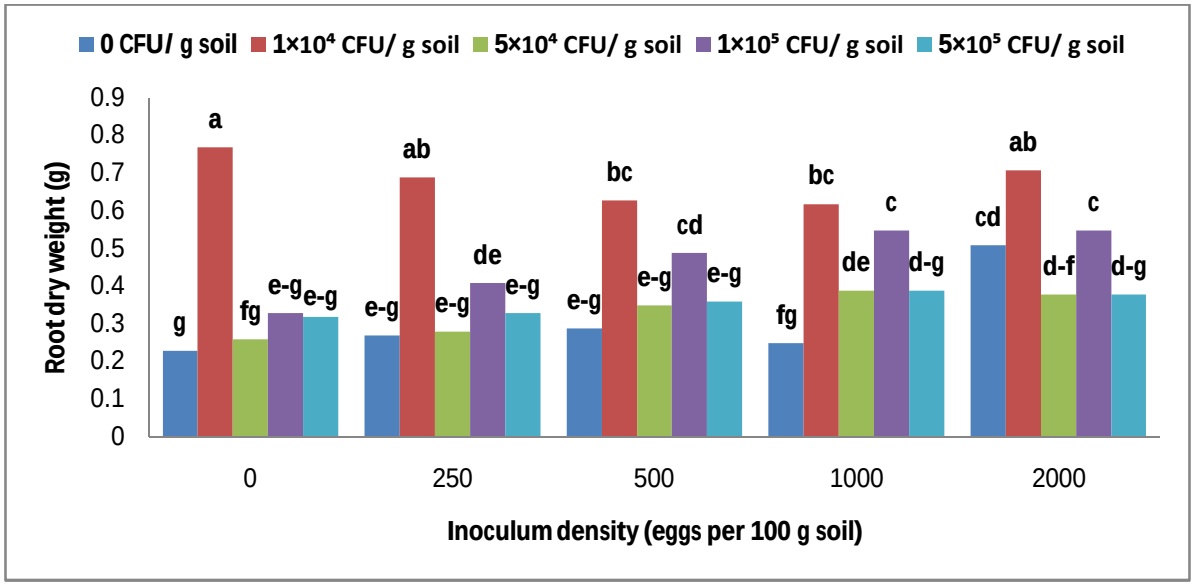


Fig 6. Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on root dry weight of tomato. Bars headed by different letters are significantly different

4.4 Effect of *Purpureocillium lilacinum* application rate on gall index and number of egg masses per root of *M. incognita* in tomato

The gall index caused by *M. incognita* of tomato was significantly reduced by application of *Purpureocillium lilacinum.* The maximum gall index (5.23) was found where only nematode population present which shown statistically different from all doses of *P. lilacinum*. But reducing of galling on root was not correlated to use of increasing doses of *P. lilacinum*. The lower galling index (1.93) on root examined in the dose of 5×10^4 CFU of *P. lilacinum*/g soil than other doses followed by 5×10^5 CFU of *P. lilacinum*/g soil, 1×10^5 CFU of *P. lilacinum*/g soil and 1×10^4 CFU of *P. lilacinum*/g soil (Table 4).

Positive response by using nematophagous fungus *P. lilacinum* was found in case of number of egg masses per root system. Maximum number of egg masses per root system (44.58) observed where no bioagent used (Table 4). The number of egg masses/root 35.08 was recorded in 1×10^4 CFU/g soil, which was statistically similar to 5×10^5 CFU/g soil. In case of doses of *P. lilacinum*, result shown the lower number of egg masses than control treatment. The lowest number of egg masses per root system (5.40) examined by using the dose of 5×10^4 CFU of *P. lilacinum*/g soil.

Table 4. Effect of *Purpureocillium lilacinum* application rate on gall index and number of egg masses per root of *M. incognita* in tomato

Fungal doses	Gall index (0-10 scale)	Number of egg mass/root
0 CFU/g soil	5.23 a	44.58 a
1×10^4 CFU/g soil	3.75 b	35.08 ab
5×10^4 CFU/g soil	1.93 c	5.40 d
1×10^5 CFU/g soil	3.45 b	24.38 c
5×10^5 CFU/g soil	3.43 b	25.80 bc
$LSD_{(0.05)}$	1.13	9.65
CV (%)	32.15	36.14

In a column treatments mean followed by different letter is statistically different

4.5 Effect of different inoculum density of *Meloidogyne incognita* on gall index and number of egg masses per root of tomato

Gall index of tomato caused by nematode significantly response with inoculums level of *M. incognita* (Table 5). The highest gall index (5.85) was found where plant infested with highest inoculum (2000 eggs per 100 g soil) of *M. incognita* and their decreasing rate recorded with decreasing the inoculum density consequently the lowest gall index (2.93) was observed where lowest number of nematode population (250 eggs per 100 g soil) was used. The gall index 4.90 recorded in 1000 eggs per 100 g soil which shown statistically similarity to 2000 eggs per 100 g soil and 500 eggs per 100 g soil (Plate 13).

Significant variation was found among different inoculum density of nematode in case of number of egg masses per root system. Highest number of egg masses (51.88) observed when applied highest inoculum density (2000 eggs per 100 g soil) of *M. incognita*. The number of egg masses 38.88 per root recorded when pots challenged with 1000 eggs/100 g soil. The lowest number of egg masses (18.92) examined in case of 250 eggs per 100 g soil which shown statistically similarity to 500 eggs per 100 g soil (Table 5).

Table 5. Effect of different inoculum density of *Meloidogyne incognita* on gall index and number of egg masses per root of tomato

Inoculum density*	Gall index (0-10 scale)	Number of egg mass/root
Control	0.00 d	0.00 d
250	2.93 c	18.92 c
500	4.10 b	25.55 c
1000	4.90 ab	38.88 b
2000	5.85 a	51.88 a
$LSD_{(0.05)}$	1.13	9.65
CV (%)	32.15	36.14

In a column treatments mean followed by different letter is statistically different

*Number of eggs per 100g soil

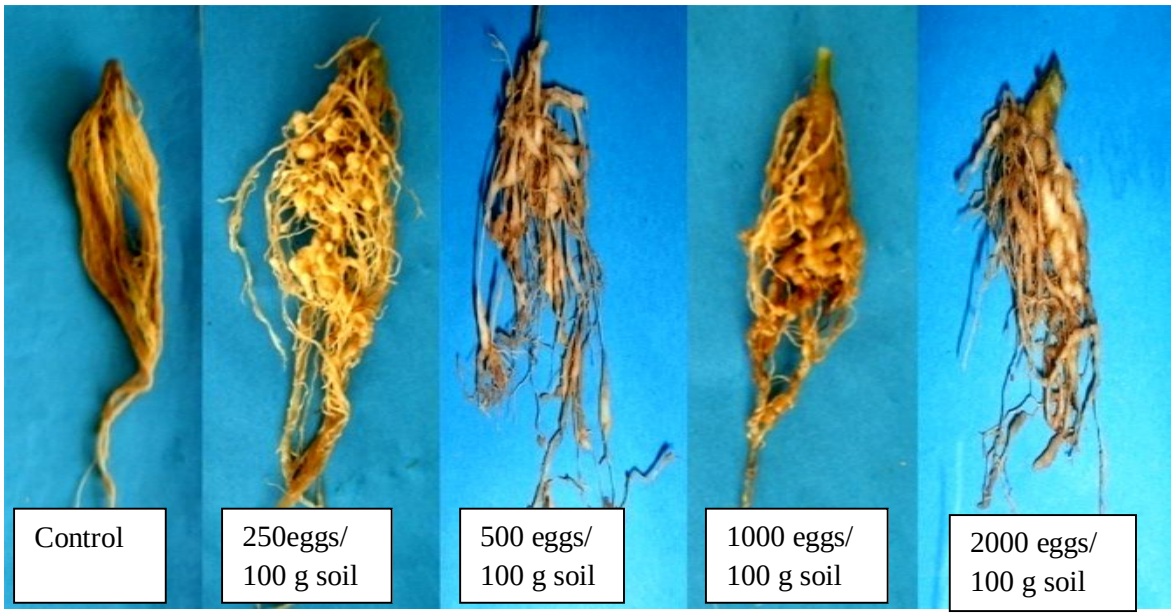


Plate 13. Photograph showing effect of different inoculum density of *Meloidogyne incognita* on root damage by galling on tomato cv. BARI Tomato-14 in comparison to control

4.6 Combined effect of *Purpureocillium lilacinum* and inoculum density of *Meloidogyne incognita* on gall index and number of egg masses per root system of tomato

Positive effect was shown between two factors on the development of nematode damage parameter in case of gall index (Fig 7) and (Plate 14). The lower gall index rating found for all four doses of *P. lilacinum* compared with untreated, inoculated control treatment but highly significant suppression of galling index on root recorded in case of 5×10^4 CFU/g soil over 250 to 2000 eggs/100 g soil. The maximum gall index (7.88) was observed in combination of 0 CFU of *P. lilacinum* per g soil and highest inoculum (2000 eggs/100 g soil) level. The minimum gall index (1.38) observed in a combination of 5×10^4 CFU/g soil of *P. lilacinum* with 250 eggs/100 g soil which was statistically similar to combination of 5×10^4 CFU/g soil with 500 to 1000 eggs/100 g soil, 5×10^5 CFU/g soil with 250 eggs/100 g soil and 1×10^4 CFU/g soil with 250 eggs/100 g soil.

Number of egg masses of *M. incognita* significantly responds with all doses of *P. lilacinum*. From the experiment shown number of egg masses per root system increased with increasing inoculum density in spite of using fungal doses. In case of four fungal doses, highest reduction of egg mass numbers shown at the dose of 5×10^4 CFU of *P. lilacinum* per g soil compared with other doses. The highest number of egg masses (80.38) per root system was observed where inoculated only 2000 eggs/100 g soil and lowest number of egg masses (4.75) per root system found from the highest reductive doses in combination with 250 eggs of *M. incognita*/100 g soil that dose shown statistically similar result to 500 eggs of *M. incognita*/100 g soil, 1000 eggs of *M. incognita*/100 g soil and 2000 eggs of *M. incognita*/100 g soil (Fig 8).

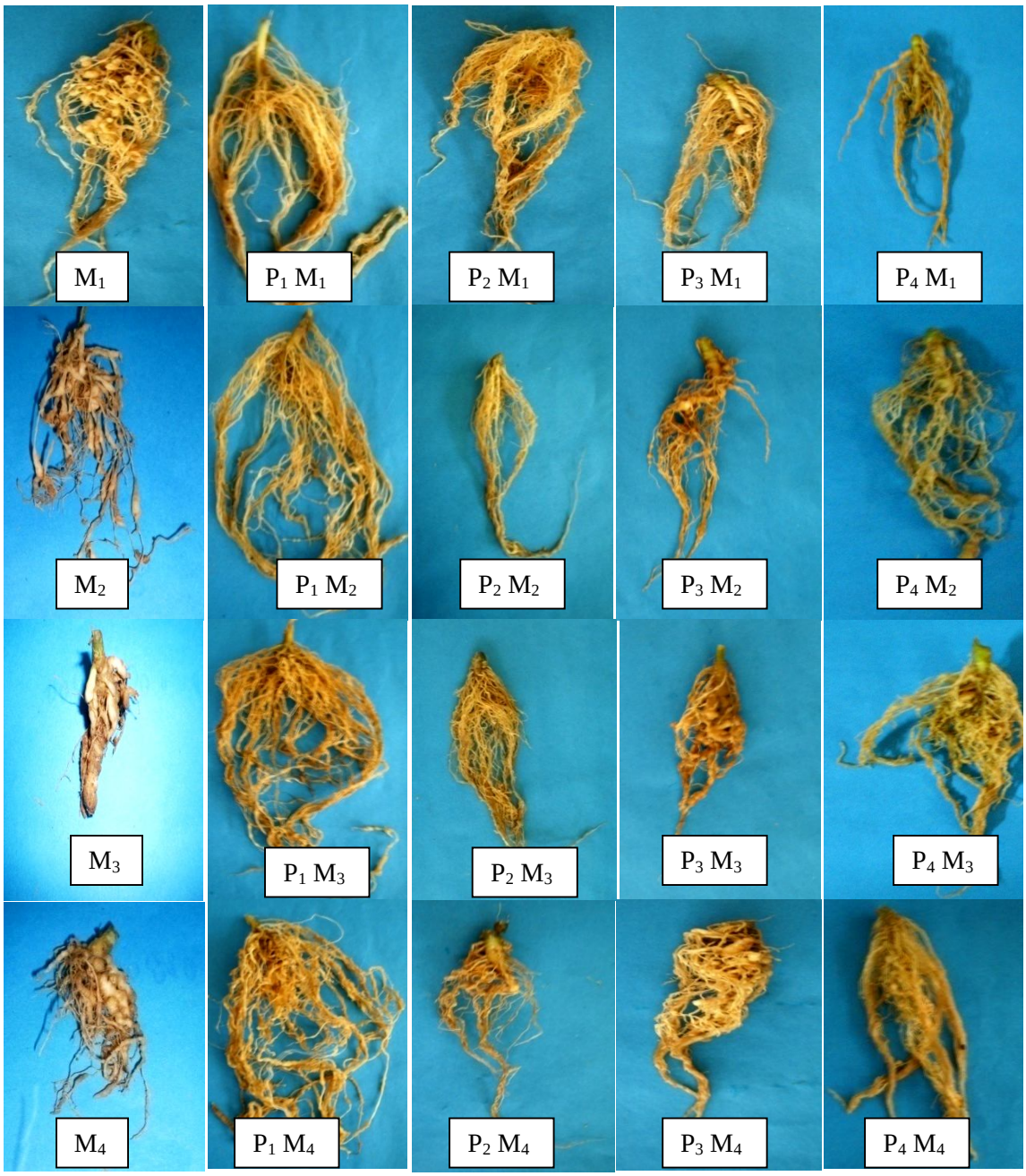


Plate 14. Photograph showing combined effect of different application rate of *P. lilacinum* against different inoculum density of *M. incognita* on root damage by galling on tomato cv. BARI Tomato-14 in comparison to control

M_1 = 250 eggs/100 g soil, M_2 = 500 eggs/100 g soil, M_3 = 1000 eggs/100 g soil, M_4 = 2000 eggs/100 g soil, P_1 = 1×10^4 CFU/g soil, P_2 = 5×10^4 CFU/g soil, P_3 = 1×10^5 CFU/g soil, P_4 = 5×10^5 CFU/g soil

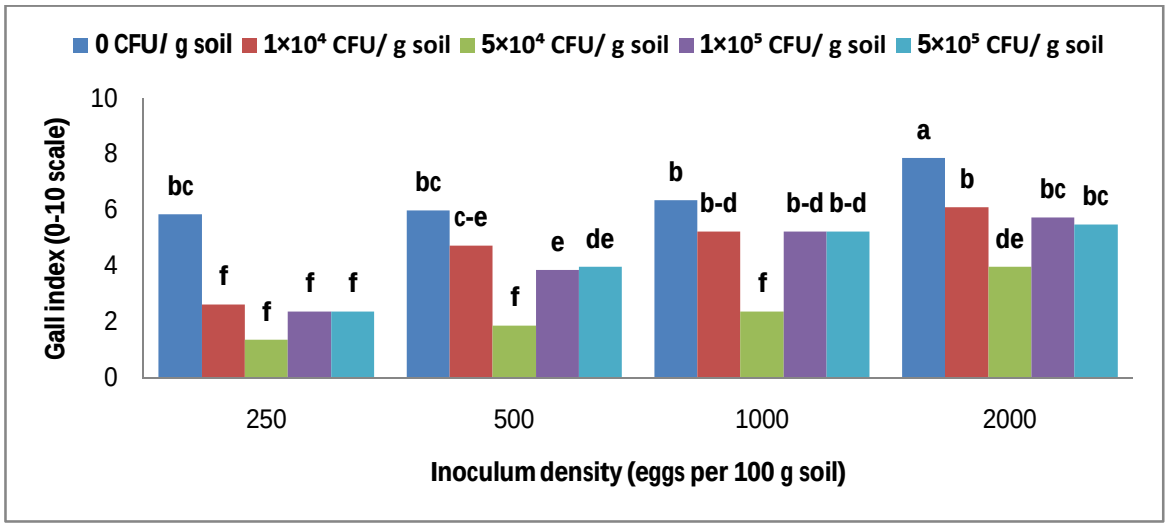


Fig 7. Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on gall index of tomato. Bars headed by different letters are significantly different

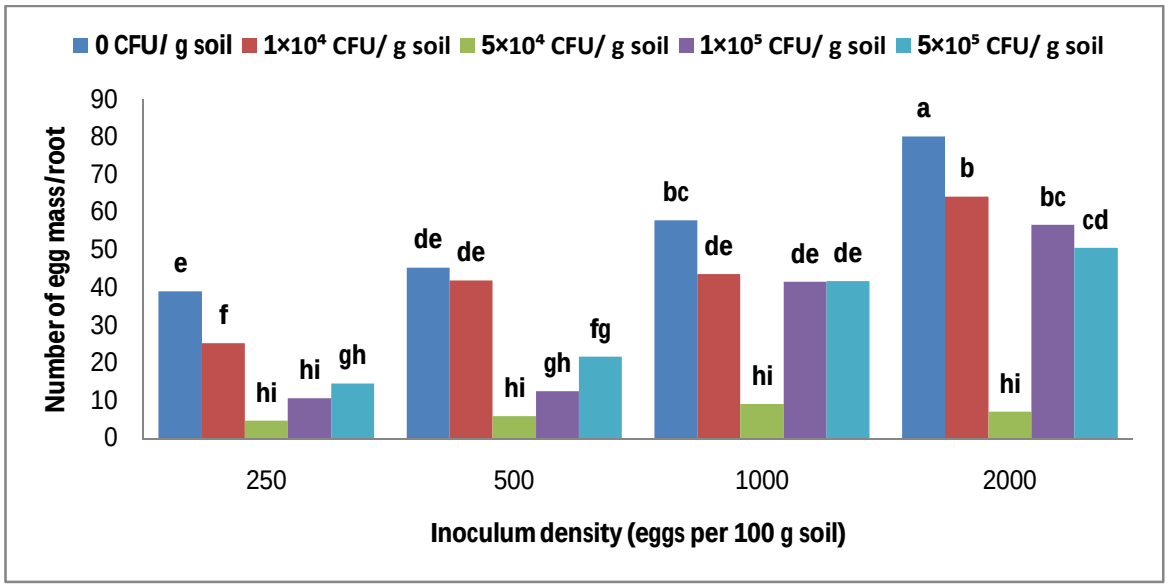


Fig 8. Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on number of egg masses/root of tomato. Bars headed by different letters are significantly different

4.7 Effect of *Purpureocillium lilacinum* doses on nematode population of *Meloidogyne incognita* in tomato

A significant variation of number of eggs per egg mass was observed between untreated inoculated and treated inoculated treatments. Size of egg masses greatly reduced by using bioagent *P. lilacinum* compared to untreated inoculated treatment. The highest number of eggs (421.9) per egg mass identified when no bioagent used which was statistically different from all fungal doses used in this experiment. The lowest number of eggs (84.55) per egg mass observed at the dose of 5×10^4 CFU of *P. lilacinum*/g soil followed by 5×10^5 CFU of *P. lilacinum*/g soil, 1×10^5 CFU of *P. lilacinum*/g soil and 1×10^4 CFU of *P. lilacinum*/g soil (Table 6).

The number of eggs per root system was significantly varied with the application of different doses of *P. lilacinum* (Table 6). The highest number of eggs (3.54×10^4) per root system examined where only nematode population used. The statistically lower number of eggs found in all four fungal doses compared to untreated inoculated treatment but the lowest number of eggs (2.23×10^4) per root system identified by the fungal dose 5×10^4 CFU/g soil followed by 1×10^5, 5×10^5 and 1×10^4 CFU/g soil.

A significant variation of juvenile numbers per 800 g of soil was recorded by using nematode destroying fungus *P. lilacinum*. The highest juvenile numbers (4.10×10^4) per 800 g soil recorded when only nematode population presents (Table 6). But the fungal dose 1×10^4 CFU per g showed statistically similar result to untreated inoculated control. The lowest number of juvenile (3.61×10^4) per 800 g soil was found in the fungal dose 5×10^5, which was statistically similar to 5×10^4 and 1×10^5 CFU per g soil.

The total number of nematode population (egg + j_2) was significantly differed by the application of *P. lilacinum*. The highest number of nematode population (4.17×10^5) was observed in negative control which was statistically different from all fungal doses. The lowest number of nematode population (3.70×10^5) was found in the fungal dose 5×10^4 which was statistically different from 0 CFU/g soil and 1×10^4 CFU/g soil. But both $1\times10^5$and 5×10^5 CFU/g soil showed statistically similar result to 5×10^4 CFU/g soil (Table 6).

The multiplication rate of *M. incognita* significantly reduced by using bioagent *P. lilacinum* (Table 6). The highest reproduction rate (31.99) was observed in case of untreated inoculated treatment, which was statistically different from all other treatment. The lowest multiplication rate (7.63) of nematode population was found in case of fungal dose 5×10^4 CFU/g soil followed by 1×10^5 CFU/g soil, 5×10^5 CFU/g soil and 1×10^4 CFU/g soil.

Table 6. Effect of *Purpureocillium lilacinum* doses on nematode population of *Meloidogyne incognita* in tomato

Fungal doses	Number of eggs/egg mass	Number of eggs/root system (10^4)	Number of J_2/800 g soil (10^4)	Total number of nematodes (no. of egg + J_2) (10^5)	Reproduction factor (Rf) pi/pf
0 CFU/g soil	421.9 a	3.54 a	4.10 a	4.17 a	31.99 a
1×10^4 CFU/g soil	122.5 b	3.01 b	3.84 ab	3.87 b	12.93 b
5×10^4 CFU/g soil	84.55 b	2.23 d	3.69 b	3.70 c	7.63 b
1×10^5 CFU/g soil	95.53 b	2.74 c	3.75 b	3.77 bc	9.75 b
5×10^5 CFU/g soil	95.53 b	2.84 bc	3.61 b	3.76 bc	9.85 b
LSD $_{(0.05)}$	49.81	0.21	0.34	0.14	8.79
CV (%)	30.77	7.29	9.03	3.79	61.77

In a column treatments mean followed by different letter is statistically different

Pi= Initial population

Pf= Final population

4.8 Effect of different inoculum density of *Meloidogyne incognita* on nematode population of *Meloidogyne incognita* in tomato

The number of eggs per egg mass was significantly similar among four nematode inoculums level of *M. incognita* (Table 7). Number of eggs per egg mass gradually increased with the increasing inoculum density. The highest number of eggs (221.1) per egg mass examined where 1000 eggs of nematode used in 100 g soil. The lowest numbers of egg (181.7) per egg mass was observed by the using of 250 eggs of nematode in 100 g soil followed by 500 eggs/100 g soil and 2000 eggs/100 g soil.

The number of eggs per root system was significantly varied among four nematode inoculum density of *M. incognita*. The highest number of eggs (3.82×10^4) per root system was found when 2000 eggs used in 100 g soil which was statistically similar in the inoculum density of 1000 eggs per 100 g soil. The lower number of eggs (3.34×10^4) per root system identified when used the inoculum density of 250 eggs per 100 g soil which was statistically similar in the inoculum density of 500 eggs per 100 g soil (Table 7).

The juvenile numbers per 800 g of soil was shown significantly similar results among four nematode inoculums level of *M. incognita*. The highest number of juveniles (4.82×10^4) per 800 g soil found when 2000 eggs used in 100 g soil. The lower juvenile number (4.68×10^4) per 800 g soil observed when used the inoculum density of 1000 eggs per 100 g soil followed by 250 eggs of *M. incognita*/100 g soil, 500 eggs of *M. incognita*/100 g soil and 2000 eggs of *M. incognita*/100 g soil (Table 7).

A significant variation was found among different inoculum density of nematode in case of total number of nematode population (egg + j_2). The highest nematode population (4.88×10^5) was observed when 2000 eggs inoculated in 100 g soil which was shown statistically similar report in case of other inoculum density. The lowest nematode population (4.77×10^5) was identified where 250 eggs used in 100 g soil (Table 7).

A significant reduction of nematode reproduction factor (Rf) was examined in increasing *M. incognita* inoculum density (Table 7). The highest nematode multiplication rate (35.98) was observed when 250 eggs inoculated in 100 g soil and the lowest reproduction factor (Rf) (6.25) was found where 2000 eggs used in 100 g soil. But inoculum density 1000 eggs/100 g soil and 500 eggs/100 g soils showed statistically similar results.

Table 7. Effect of different inoculum density of *Meloidogyne incognita* on nematode population of *Meloidogyne incognita* in tomato

Inoculum density*	Number of eggs/egg mass	Number of eggs/root system (10^4)	Number of J_2/800 g soil (10^4)	Total number of nematodes (no. of egg + J_2) (10^5)	Reproduction factor (Rf) pi/pf
Control	0.00 b	0.00 c	0.00 b	0.00 b	0.00 d
250	181.7 a	3.34 b	4.73 a	4.77 a	35.98 a
500	203.1 a	3.48 b	4.75 a	4.78 a	18.07 b
1000	221.1 a	3. 73 a	4.68 a	4.85 a	11.85 bc
2000	214.2 a	3.82 a	4.82 a	4.88 a	6.25 cd
LSD $_{(0.05)}$	49.81	0.21	0.34	0.14	8.79
CV (%)	30.77	7.29	9.03	3.79	61.77

In a column treatments mean followed by different letter is statistically different

* Number of eggs per 100g soil

Pi= Initial population

Pf= Final population

4.9 Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on nematode population of *Meloidogyne incognita* in tomato

Highly significant effect of doses of *P. lilacinum* was studied against on number of eggs per egg mass of different *M. incognita* inoculum density. The size of egg masses greatly reduced by using bioagent *P. lilacinum* compared to untreated inoculated treatment. The highest number of eggs (582.9) per egg mass was found when only 1000 eggs inoculated/100 g soil. The lowest result (103.1eggs/egg mass) was identified in combination of 5×10^4 CFU of *P. lilacinum* per g soil with 1000 eggs/100 g soil which was statistically similar to the combination of 5×10^4 CFU of *P. lilacinum* per g soil over 250-2000 eggs per 100 g soil and 1×10^5, 1×10^4 and 5×10^5 CFU of *P. lilacinum* per g soil over 250 eggs of *M. incognita* per 100 g soil (Fig 9).

The number of eggs per root system was significantly varied with different combination of biocontrol fungus *P. lilacinum* and parasitic organism *M. incognita* (Fig 10). For all four inoculum number the higher number of eggs was observed when biocontrol agent absent and in case of doses of *P. lilacinum*, 5×10^4 CFU per g soil shown lower number of eggs compared to other fungal doses. The highest number of eggs (4.6×10^4) was recorded in the combination of no bioagent with 2000 eggs per 100 g soil which shown statistically similar result to the combination of 1000 eggs per 100 g soil with no bioagent. The lowest number of eggs (2.65×10^4) was found in interaction between 5×10^4 CFU per g soil and 250 eggs per 100 g soil which was statistically similar to combination of 5×10^4 CFU of *P. lilacinum* per g soil over 500 eggs of *M. incognita* per 100 g soil.

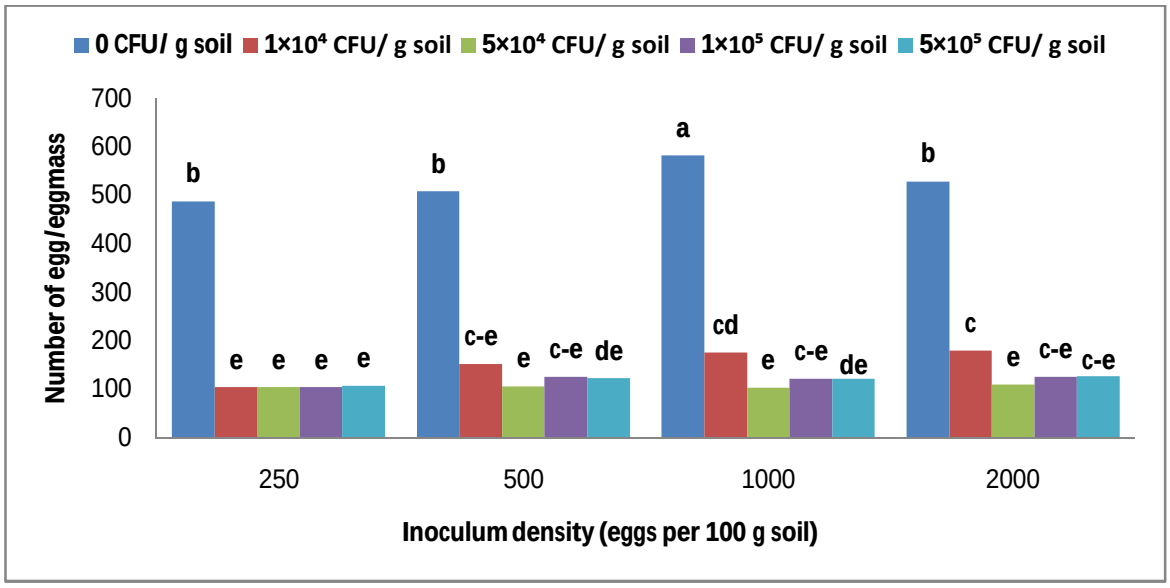


Fig 9. Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on number of eggs/egg mass of tomato. Bars headed by different letters are significantly different

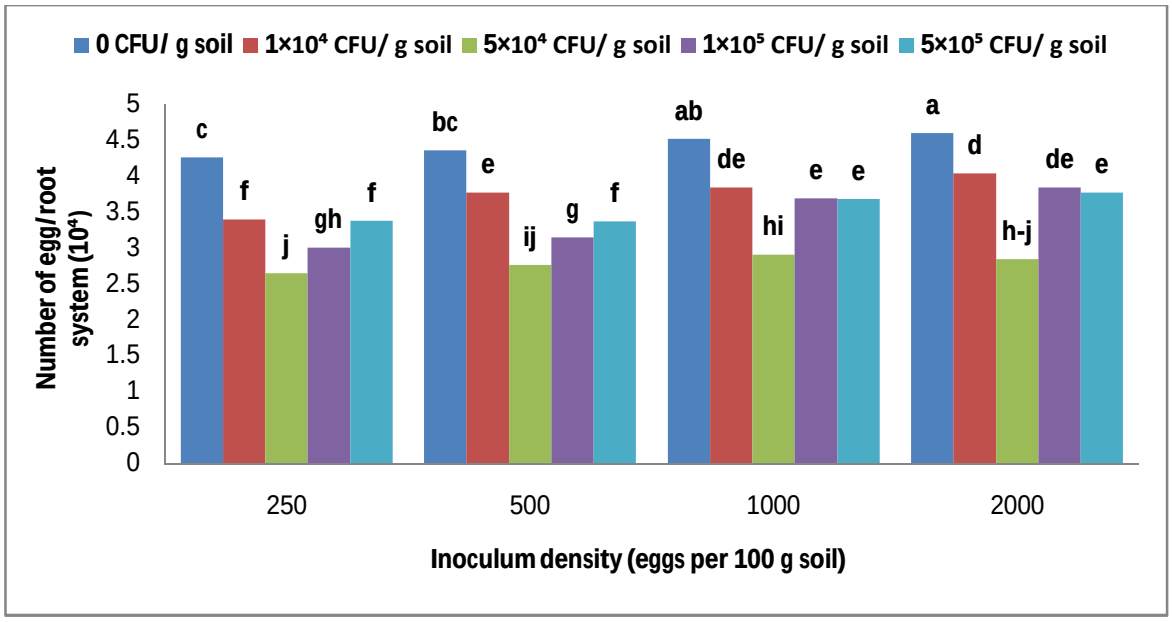


Fig10. Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on number of eggs/root of tomato. Bars headed by different letters are significantly different

The juvenile numbers per 800 g of soil was significantly influenced by the interaction of *P. lilacinum* application rate and *M. incognita* inoculum density. The maximum number of juveniles per pot was obtained in all four nematode inoculum density compared with fungal doses. The highest number of juveniles (5.22×10^4) per 800 g soil was found when soil only inoculated with nematode population @ 2000 eggs/100 g soil which was statistically similar to the combination of 0 CFU/g soil and 500 eggs/100 g soil. The lowest juvenile numbers (4.18×10^4) per 800 g soil was observed by the interaction between 5×10^5 CFU per g soil over1000 eggs per 100 g soil (Fig 11).

The total number of nematode population (egg + j_2) was significantly correlated with the interaction of nematophagous fungus *P. lilacinum* and pathogenic organism *M. incognita* (Fig 12). The lower number of nematode population was observed in fungus treated pot compared to nematode alone. The lowest number of final nematode population (4.53×10^5) was identified in relationship between 5×10^4 CFU per g soil and 250 eggs per 100 g soil. The 5 and 10 times increased in the fungal doses did not show lower result than when used 5×10^4 CFU of *P. lilacinum* per g soil. The highest number of final nematode population (5.32×10^5) was observed in combination between 0 CFU per g soil and 2000 eggs per 100 g soil which was statistically similar to the combination of 0 CFU/g soil and 1000 eggs/100 g soil, 0 CFU/g soil and 500 eggs/100 g soil and 0 CFU/g soil and 250 eggs/100 g soil.

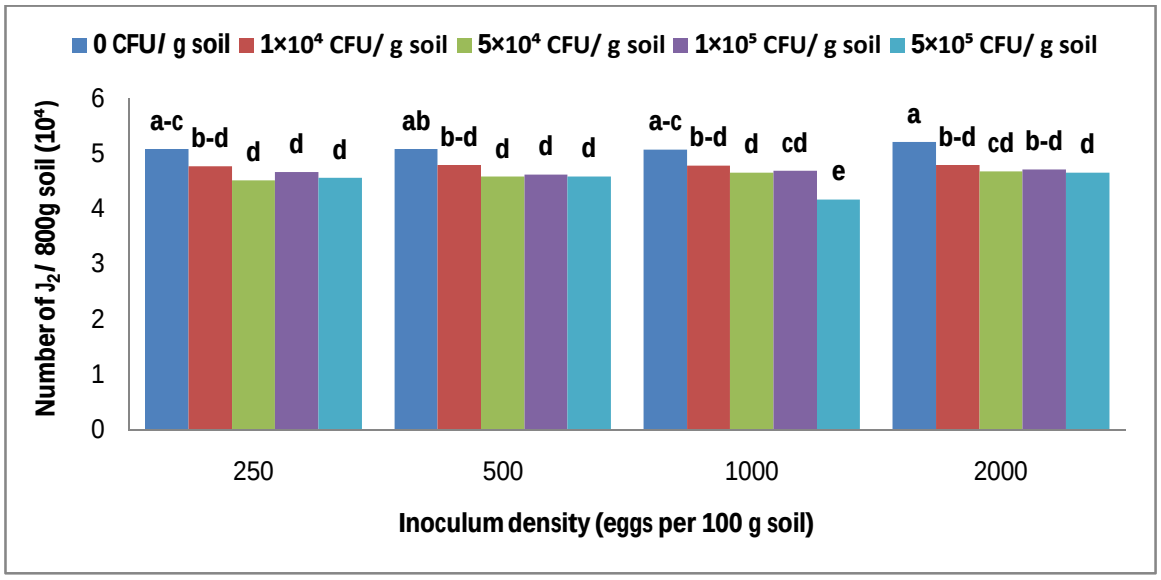


Fig 11. Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on number of J_2/800 g soil of tomato. Bars headed by different letters are significantly different

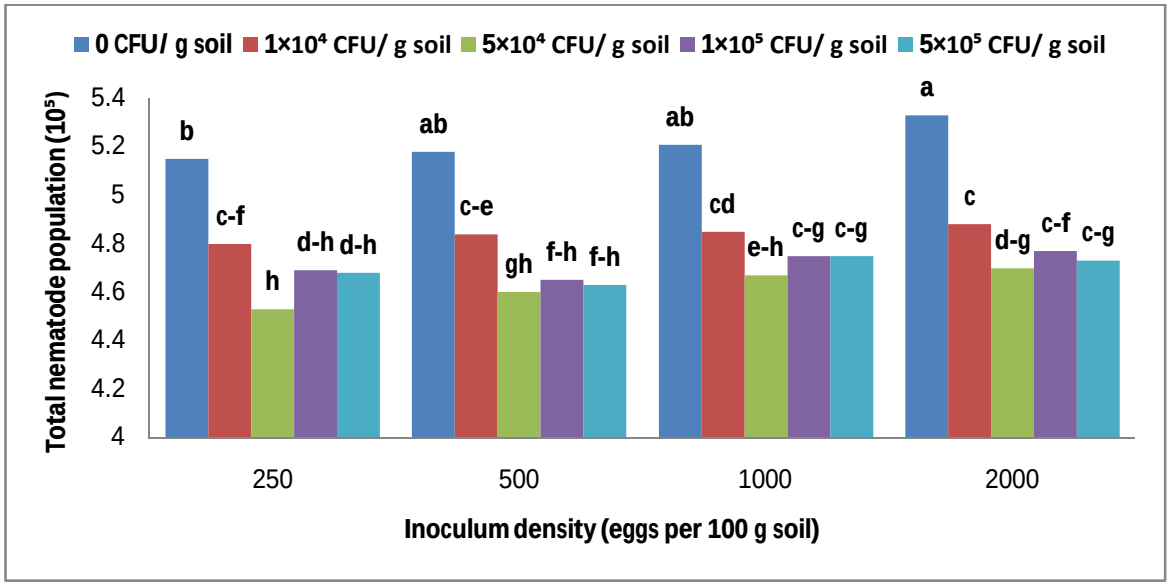


Fig 12. Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on total number of nematode of tomato. Bars headed by different letters are significantly different

The reproduction factor (Rf) of nematode population was significantly correlated with the impact of *P. lilacinum* against *M. incognita*. After two months later highest multiplication rate of nematode population both treated and untreated treatment was observed when lowest number of nematode inoculum used in fixed amount of soil and their multiplication rate gradually decreased with increasing nematode population. The highest reproduction factor (75.56) was found when only 250 eggs per 100 g soil used, which was significantly differed from other treatment. The lowest reproduction factor (3.25) was observed in case of relationship between 5×10^4 CFU per g soil and 2000 eggs per 100 g soil. Which was shown statistically similar result at fungal doses 5×10^5 CFU per g soil, 1×10^5 CFU per g soil and 1×10^4 CFU per g soil over inoculum density 2000 eggs per 100 g soil (Fig 13).

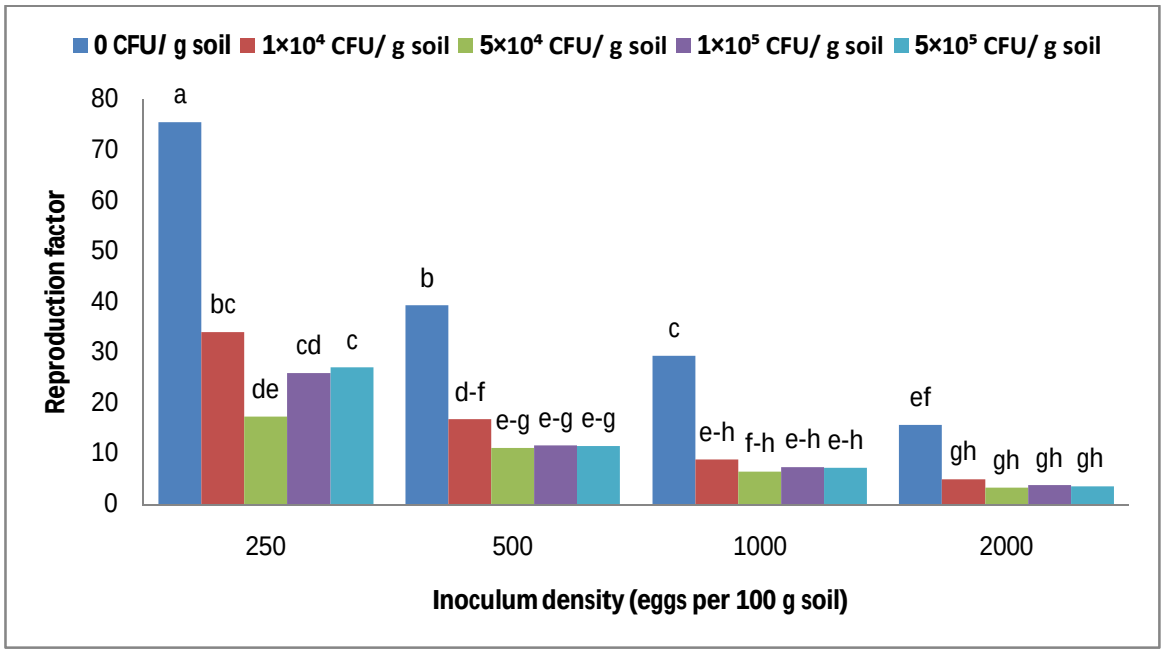


Fig 13. Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on reproduction factor (Rf) of tomato. Bars headed by different letters are significantly different

4.10 Effect of *Purpureocillium lilacinum* doses on soil and egg masses colonization of *Meloidogyne incognita* in tomato

The percent of egg masses colonization of *M. incognita* was significantly affected by the application of fungal doses. The highest percent of egg parasitism (30.28) was observed when plants treated with 5×10^5 CFU per g soil which showed statistically similar report to other fungal doses. The lowest percent of egg parasitism (19.32) was observed when plants treated with 10 fold lower dose i.e. 5×10^4 CFU per g soil (Table 8).

The presence of fungal propagules in soil after harvesting was depended by the application rate of fungal doses (Table 8). The highest soil colonization by fungus (2002 CFU/g soil) was identified by the application of 5×10^5 CFU per g soil which was statistically similar to 1×10^5 CFU per g soil. The 1675 CFU/g soil was found when pots treated with fungal dose @ 5×10^4 CFU per g soil. The least number of fungus (1288 CFU/g soil) present in soil when pots treated with 1×10^4 CFU per g soil.

Table 8. Effect of *Purpureocillium lilacinum* doses on soil and egg masses colonization of *Meloidogyne incognita* in tomato

Fungal doses	% of egg masses colonized by fungus	Soil colonized by fungus (CFU/g soil)
0 CFU/g soil	0.00 c	0.00 d
1×10^4 CFU/g soil	24.09 a	1288 c
5×10^4 CFU/g soil	19.32 a	1675 b
1×10^5 CFU/g soil	22.88 a	1915 a
5×10^5 CFU/g soil	30.28 a	2002 a
LSD (0.05)	12.01	215.1
CV (%)	47.60	12.65

In a column treatments mean followed by different letter is statistically different

4.11 Effect of different inoculum density of *Meloidogyne incognita* egg masses colonization and soil colonization by fungus

A significant influence of inoculum density of *M. incognita* was observed on percent egg masses colonized by fungus. The highest percent of egg parasitism (33.94) was observed when plants suffered with 1000 eggs per 100 g soil. This was followed by 2000 eggs per 100 g soil and 500 eggs per 100 g soil. The lowest percent of egg parasitism (27.61) was found when pots inoculated with 250 eggs per 100 g soil (Table 9).

Soil colonized by fungus was influenced by different level of nematode population (Table 9). The highest number of fungal propagules (1943 CFU/g soil) present in soil when soil inoculated by 1000 eggs per 100 g soil. The presence of 1846 CFU/g soil was recorded at inoculums level 2000 eggs/100 g soil which was statistically similar to 250 eggs/100 g soil and 500 eggs/100 g soil. The lower number of fungus (1376 CFU/g soil) was found when pots treated with only bioagent.

Table 9. Effect of different inoculum density of *Meloidogyne incognita* egg masses colonization and soil colonization by fungus

Inoculum density*	% of egg masses colonized by fungus	Soil colonized by fungus (CFU/g soil)
Control	0.00 b	1376 c
250	27.61 a	1723 b
500	27.62 a	1711 b
1000	33.94 a	1943 a
2000	31.55 a	1846 ab
LSD $_{(0.05)}$	12.01	215.1
CV (%)	47.60	12.65

In a column treatments mean followed by different letter is statistically different

*Number of egg per 100 g soil

4.12 Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on soil and egg masses colonization in tomato

The colonization of egg masses of *M. incognita* was correlated with the effect of doses of *P. lilacinum* and inoculum density of pathogenic organism (Fig 14). The highest egg masses parasitism rate (45.19 %) was examined in combination of 5×10^5 CFU per g soil over 1000 eggs of *M. incognita* per 100 g soil which was statistically similar when pots treated with the combination of 5×10^5 CFU per g soil against 2000 eggs/100 g soil. The lowest colonization rate of egg masses (22.91%) was observed when 1×10^5 CFU of *P. lilacinum* used against 250 eggs per 100 g of soil which was statistically similar in combination of 5×10^4 CFU per g soil over 500 eggs of *M. incognita* per 100 g soil and 5×10^4 CFU per g soil over 250 eggs of *M. incognita* per 100 g soil.

A significant correlation was observed between inoculum density of *M. incognita* and application rate of *P. lilacinum* in case of presence of fungal population in soil during harvesting (Fig 15). The highest number of fungal propagules per g soil (2165) was present in combination of 5×10^5 CFU per g soil and 1000 eggs per 100 g soil which was statistically similar to interaction between concentration at 5×10^5 CFU per g soil over 2000 eggs per 100 g soil and 1×10^5 CFU per g soil over 1000 eggs per 100 g soil. The lowest number of fungus per g soil (1087) was present when no nematode population present only pots treated with 1×10^4 CFU per g soil.

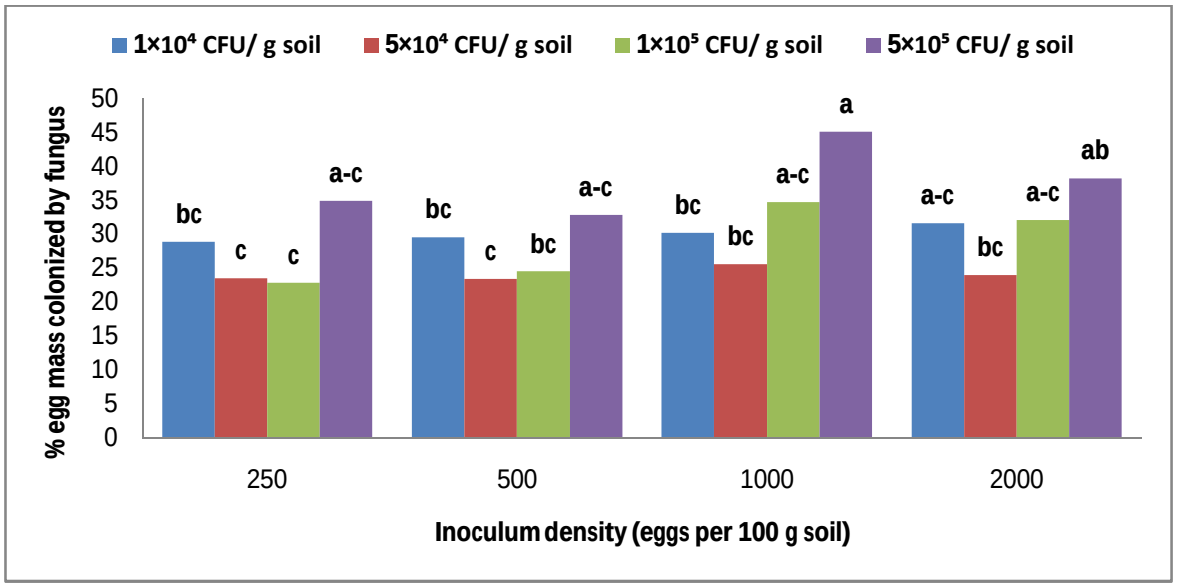


Fig 14. Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on % egg masses colonized by fungus of tomato. Bars headed by different letters are significantly different

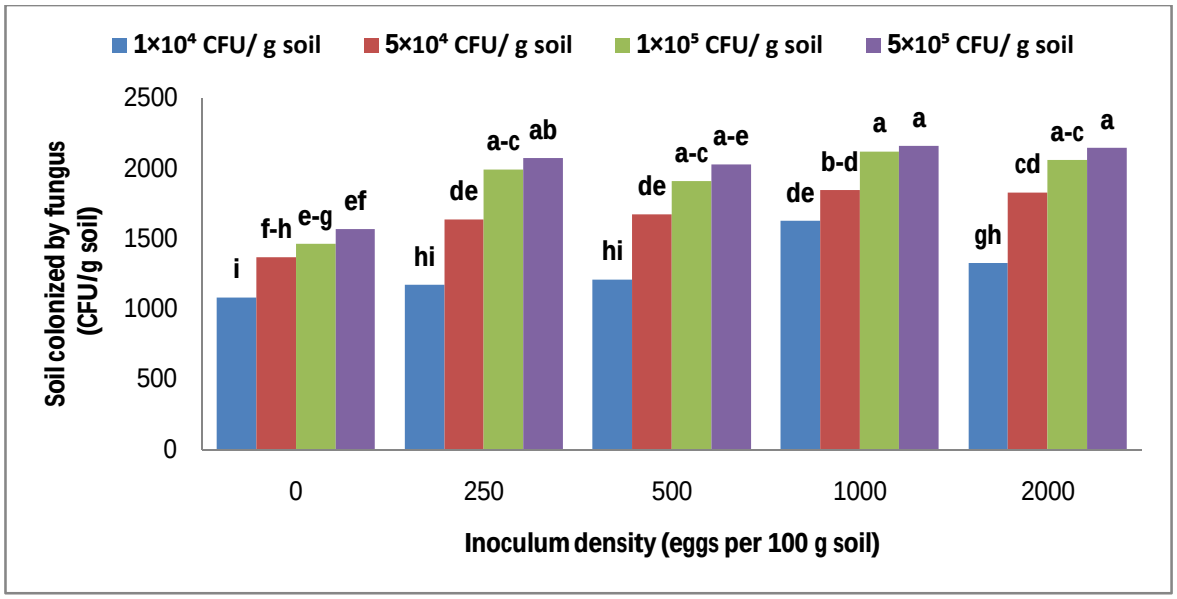


Fig 15. Combined effect of *Purpureocillium lilacinum* application rate and inoculum density of *Meloidogyne incognita* on soil colonized by fungus (CFU/g soil) of tomato. Bars headed by different letters are significantly different

DISCUSSION

The experiment was carried on to study the efficacy of different application rate of *Purpureocillium lilacinum* against different inoculum density of root-knot nematode (*Meloidogyne incognita*) of tomato in shade house pot condition. In the present study results confirmed the suppressive effect of *Purpureocillium lilacinum* on inoculum density of *Meloidogyne incognita* and increased the plant growth parameters.

In this experiment, it was observed that *P. lilacinum* controlled the root-knot nematode, *M. incognita* efficiently and increased the plant growth characters. To identify the appropriate dose of *P. lilacinum* against different level of nematode damage five fungal doses namely 0, 1×10^4, 5×10^4 1×10^5 and 5×10^5 CFU/g soil were applied. Among different fungal doses the identified optimum fungal dose was 5×10^4 CFU/g soil where highest nematode suppression and improved plant growth was observed. Due to increasing rate of fungal propagules in limited ecological environment their suppressiveness did not similar in lower one. *P. lilacinus* propagules in the soil were correlated to the initial dose applied and decreased progressively through time with increased dosages (El-Shanshoury *et al*., 2005).

This result validates the report of Kiewnick *et al.* (2011). They found that a preplanting soil treatment with the lowest dose of commercially formulated *P. lilacinus* (2×10^5 CFU/g soil) was sufficient to reduce root galling by 45% and number of egg masses by 69% when averaged over inoculum densities of 100 to 1600 eggs and infective juveniles per 100 cm^3 of soil.

But our report was contradictory in several studies because of result indicated that the better nematode suppression was obtained by increasing doses of bioagent. Shanmuga and Kumar (2006) and Kiewnick and Sikora (2006 a) reported that application at a high concentration of *P. lilacinus* per g soil was needed for sufficient biocontrol of root knot nematode of *M. incognita* in tomato. Oclarit and Cumagun (2009) and Khalil *et al*. (2012 a) reported that *P. lilacinus* is an effective biocontrol agent of *M. incognita* on tomato. The efficacy of the fungus was greater with a double application than that of a single application. This finding confirms the report by Cabanillas and Barker (1989) and Rumbos *et al.* (2006) where they reported that in a pre-plant application and repeated applications of *P. lilacinus* at planting and the later growth stage of the crop were more effective in reducing the damage and reproductive potential of root-knot nematodes than a single application. But Jatala *et al*. (1981) reported that *P. lilacinus* had the ability to reduce population densities of *M. incognita* progressively with succeeding generations and without reapplication of the fungus. However, Mendoza *et al.* (2007) reported a significant correlation between dosages of *P. lilacinus* and the level of *Radopholus similis* suppression where they found highest level of suppression with 6×10^6 CFU per g dry soil of *P. lilacinus* applied to the soil three times: 6 days before planting, at-planting and as a plantlet drench.

In present study the plant growth characters of tomato were significantly affected by application of bioagent. Plant growth parameters (shoot and root length, shoot and root fresh and dry weight) were improved by using all doses of *P. lilacinum* compared to nematode alone. This result validated the report of Cabanillas *et al.* (1989), Kiewnick and Sikora (2004) and Esfahani and Pour (2006) where they reported that plant growth parameters and yield promoted by using the bioagent *P. lilacinus* compared with plants treated with this nematode alone. However, application of *P. lilacinus* with other antagonistic organism or chemical

nematicides shown better suppression of nematode population and increased plant growth (Lafia *et al*., 1998; Bhat and Mahmood, 2000 and Goswami *et al*., 2006).

The findings of the present study revealed that the highest shoot length and weight was observed when plant treated with the fungal dose of 5×10^4 CFU of *P. lilacinum*/g soil against at inoculum densities 250 to 2000 eggs per 100 g soil but in case of root parameters which was 1×10^4 CFU of *P. lilacinum*/g soil over 250 to 2000 eggs per 100 g soil. This was contradictory with the report of Khan and Goswami (2000), they reported that all treatments receiving *P. lilacinus* exhibited higher plant growth parameter values compared to those treated with the nematode alone. Increasing the dose of *P. lilacinus* was accompanied by an increase in plant height and root length.

The results of the present experiment indicated that plant growth parameters badly affected by inoculum density of *M. incognita*. The result indicated that plant growth parameters were decreased with increasing nematode population and lower plant growth characters observed when plants inoculated 1000 and 2000 eggs per 100 g soil. But root weight (fresh and dry) of tomato increased with increasing root damage i.e. galling on root. Similar result observed from Charegani *et al.*, (2012). They reported that inoculation of tomato with inoculums level (4 eggs and J_2) of *M. incognita* or *M. javanica* per g of soil reduced shoot length and fresh dry weights to similar extents. Ibrahim *et al.* (1987) reported that the root and shoot dry weights and fruit weight were significantly decreased by nematode infestation.

Suppressive effect of *P. lilacinum* against different inoculum density of *M. incognita* was reestablished in this experiment. All doses of *P. lilacinum* reduced root galling and number of egg masses per root were obtained compared to that nematode alone. Amongst four doses of *P. lilacinum* the optimum dose for maximum reduction of root galling and number of egg masses per root was

observed at low fungal dose (5×10^4 CFU/g soil) with about 63.1 and 87.88%, respectively. The highest reduction level of root galling was recorded in combination of application of dose of 5×10^4 CFU/g soil on 250 eggs of *M. incognita* with about 76.53% and 91.13% reduction of egg masses number was observed in combined application of dose of 5×10^4 CFU/g soil on 2000 eggs of *M. incognita*. Rao (2005) reported that soil treated with formulation both *P. lilacinus* and *P. chlamydosporia*, each at the rate of 5 g/kg of soil are sufficient to control *M. javanica* and that they avoid the competition between the bio-agents observed when both fungi were used at 10 g/kg of soil.

This was contradictory with the report of Khan and Goswami, (2000). They demonstrated that a good level of reduction of root galling and nematode population was observed by using high dose @ 8 g (57.92×10^8 spores) fungus infected rice per kg soil. This report confirms the result of Aminuzzaman *et al*. (2013) they reported that *P. lilacinus* pellets at the highest rate (1.6%) reduced root galling by 66.7%. But 0.2% *P. lilacinus* pellets at a density of 1.3×10^5 CFU/g soil significantly suppressed the development of root-knot nematodes. But the suppression was increased with increasing the rate of application.

Various mechanisms of action have been suggested by different scientist for the biological activity of *Purpureocillium lilacinum* 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; Khan *et al*., 2003, 2004 and Park *et al*., 2004). Azam *et al*. (2013) reported that *P. lilacinus* parasitized on the *M. incognita* eggs through the formation of fungal hyphae and conidiophores and caused the disintegration of the eggshells, egg masses and juveniles of *M. incognita*.

The biocontrol fungus *Paaecilomyces lilacinus* reduced 72% root galling when the application rate of *P. lilacinus* and inoculum densities was 1×10^6 CFU/g soil over 800 eggs/100 g soil, respectively and reduced 84% egg masses when the application rate of *P. lilacinus* and inoculum densities was 1×10^6 CFU/g soil against 400 eggs/100 g soil, respectively of brinjal cv. Singnath reported by Sarven, (2013).

This present experiment demonstrated that the size of egg mass was significantly correlated with the treated bioagent. The highly nematode infected plant (1000 and 2000 eggs per 100 g soil) was observed when no bioagent used resulted larger size of egg masses. But their size was greatly reduced in case of all four fungal doses. The minute egg masses was observed at 5×10^4 CFU/g soil and in that dose number of egg masses per root as well as gall index was very poor in comparison with other doses.

The present experiment indicated that the reduction of *M. incognita* per plant was depended on the doses of *P. lilacinum*. The highest level of nematode reduction was observed by the dose of 5×10^4 CFU/g soil with about 71 to 79% at inoculum densities of 250 to 2000 eggs per 100 g soil. At the end of the study total nematode population was reduced 79.32% by using the dose 5×10^4 CFU of *P. lilacinum*/g soil against 2000 eggs per 100 g soil. In contrast to the present study Kalele *et al*. (2010) observed that the pre-planting soil treatment with highest dose (0.4g/10 L soil) reduced 69 and 73% of nematode population in the roots and soil, respectively, compared to the non-inoculated control in tomato.

The biological control efficiency of fungal dried pellets of *Paecilomyces lilacinus* YES-2 against root knot nematode was significantly higher along with the increase of dosages reported by Aminuzzaman and Liu, (2011). Root galling index and final nematode population decreased up to 40.7 and 73.8%, respectively for tomato cv. BARI tomato-14 and 55.6 and 66.9%, respectively for brinjal cv. BARI Begun-5 at the highest rate (1.6%) of application of the biocontrol fungus.

The fungus significantly reduced the multiplication rate of *M. incognita* in comparison with untreated inoculated control. This proved the ability of *P. lilacinum* as a biocontrol agent against *M. incognita.* In present study highest reduction of reproduction factor (76.15%) was observed when plant treated with 5×10^4 CFU of P. *lilacinum*/g soil over inoculum densities of 250 to 2000 eggs per 100 g soil. The highest (79.32%) reduction of nematode multiplication rate was observed when antagonist used @ 5×10^4 CFU of *P. lilacinum*/g soil against 2000 eggs per 100 g soil. In general, the reproduction rate of the nematode decreased with the increase of the initial densities, thus agreeing with previous studies (Lindsey and Clayshulte, 1982; Di Vito *et al*., 1985; Korayem, 2006 and El-Sherif *et al*., 2007). This was probably due to the reduced amount of food available per nematode at higher nematode densities and the greater competition for food and space in the roots (Seinhorst, 1970).

In previous report of Kiewnick (2007) shown significant control over low inoculum densities @ 100 to 400 root knot nematode eggs/100 ml soil was observed when pots treated with 1.5 to 7.5×10^5 CFU of *P. lilacinus* 251/g soil. In contrast, present findings indicated that low fungal dose surprisingly suppressed nematode damage from 250 to 2000 nematode eggs per 100 g soil. This interaction might be due to the fact that disease suppression by *P. lilacinum* was correlated with presence of level of nematode inoculum density in soil.

In this experiment it was proved that inoculum density of *M. incognita* had adverse effect on plant growth characters due to higher nematode damage. The highest level of nematode damage i.e. gall index, egg mass numbers and final nematode population was recorded when no bioagent was applied, plants only challenged with highest level of nematode inoculum (2000 eggs per 100 g soil).

The present study demonstrated that the percentage of egg masses colonized by fungus depended directly on the fungal density in the soil. The maximum colonization of egg masses (30.28%) was observed when pots treated with highest dose @ 5×10^5 CFU/g soil. In that dose our result indicated that maximum colonization of egg masses occurred due to maximum number of egg masses per root present. The lower egg masses parasitism rate was observed in the dose (5×10^4 CFU of *P. lilacinum*/g soil) which shown better suppression of root galling as well as nematode population. It happened because of lowest number of egg masses recorded in that dose. So the present study indicated that egg parasitism rate of *P. lilacinum* depend on the presence of number of egg masses per root. The highest 45.19% egg masses colonization was recorded interaction between 5×10^5 CFU/g soil over 1000 eggs of *M. incognita* per 100 g soil. Due to higher parasitism rate of egg masses lower number of second stage juvenile per pot was observed which indicated lower number of egg hatching rate. Similar result was observed from Gomes Carneiro and Cayrol, (1991) experiment. They observed the number of colonized egg masses and the number of non-viable eggs increased with fungal inoculum and the fungus was most effective at a density of 10^6 spores/g of soil.

The isolation of *P. lilacinum* from soil two months after application of fungal doses indicated that the fungus survived throughout the growing season, and compatible with the environmental conditions. Although result reported that the CFUs were lower than those added in the beginning of the experiment. The highest soil colonization by fungus (2165 CFU/g soil) was recorded at concentration of 5×10^5 CFU per g soil against 1000 eggs per 100 g soil. Lower variation observed among different fungal doses in case of presence of fungal propagules in soil. Cabanillas and Barker (1989) recovered 6000 and 7000 spores/g soil from infested soil when pots treated 10 and 20 g fungus-infested wheat, respectively. However, the decline in CFU numbers 2-3 weeks after the initial application has been reported by several researchers (Gomes Carneiro and Cayrol, 1991; Hewlett *et al*. 1988; Kiewnick *et al*., 2004 and Kiewnick and Sikora, 2006 a).

SUMMARY AND CONCLUSION

Experiments were conducted to evaluate the efficacy of a biocontrol agent *P. lilacinum* against different level of root-knot nematode, *Meloidogyne incognita* on tomto cv. BARI Tomato-14. Five doses of *Purpureocillium lilacinum*, viz., 0, 1×10^4, 5×10^4, 1×10^5 and 5×10^5 CFU per g soil and five inoculum level of *Meloidogyne incognita*, viz., 0, 250, 500, 1000 and 2000 eggs per 100g soil were used in this experiment. The experiment was laid out in Randomized Completely Block Design (RCBD) having two factors and replicated eight times. Bioagent was applied to the pot soil just before transplanting and nematode eggs were inoculated 3 days after transplanting. Data was recorded 8 weeks after transplanting. After analysis of result it has been proved *P. lilacinum* has the ability to suppress root-knot (*Meloidogyne incognita*) damage in infected tomato roots. At planting application of different level of bioagent *P. lilacinum* significantly reduced nematode population of tomato cv. BARI Tomato-14 in shade house pot conditions. But their reduction rate of nematode damage was dependent presence of nematode population in that environment.

Suppression of nematode damage in tomato was related to presence of amount of fungal propagules and also initial nematode population. Amongst different rate of fungal propagules higher shoot length was observed in case of the dose of fungal propagules 5×10^4 CFU/g soil compared to other doses. In that dose shoot length was 51.90 cm and shoot fresh weight as well as shoot dry weight were 22.41 g and 4.32 g, respectively. Whereas increased root parameters i.e. root length (23.20 cm), root fresh weight (10.71 g) and root dry weight (0.68 g) were observe at 1×10^4 CFU/g soil compared with other doses. The maximum reduction of gall index (63.1%) and number of egg masses (87.88%) per root were found when

plants were treated by bioagent @ 5×10^4 CFU/g soil. The maximum reduction of egg mass size (80%) and total number of nematode population (76.15%) were observed in case of 5×10^4 CFU of *P. lilacinum*/g soil. The maximum reduction of nematode multiplication rate (76.15%) was found when pots treated with @ 5×10^4 CFU/g soil compared to other doses. The maximum colonization of egg masses (30.28%) was observed at 5×10^5 CFU/g soil. The maximum soil colonization (2002 CFU/g soil) was reduced when plants were treated with 5×10^5 CFU/g soil.

The plant growths adversely affected by different inoculum density of *M. incognita* compared to un-inoculated control. The highest plant growths i.e. shoot length (52.29 cm), shoot fresh weight (22.74 g) as well as shoot dry weight (4.99) and higher root length (19.41 cm) were observed in pots where no nematode populations present. But the root fresh weight (6.0 g) and root dry weight (0.51 g) was recorded when plant suffered highest level (2000 eggs per 100 g soil) of nematode inoculation. Compared to that the lowest plant growths were examined when plant was inoculated with 1000 and 2000 eggs per 100 g soil which was inverse in case of root weight (fresh and dry). The maximum gall index (5.85), number of egg masses (51.88) per root and final nematode population (4.88×10^5) were conducted when pot soil was inoculated with 2000 eggs of pest per 100 g soil. The maximum eggs (221.1) per egg mass were recorded where 1000 eggs per 100 g soil used. But maximum reproduction factor (35.98) was observed in the treatment 250 eggs per 100 g soil. The maximum egg mass colonization (33.94%) was observed when highest number of nematode inoculum (1000 eggs per 100 g soil) was inoculated. The maximum soil colonization (1943 CFU/g soil) was observed when pots inoculated with1000 eggs per 100 g soil.

The plant growths and level of nematode damage was significantly correlated with the interaction effect of *P. lilacinum* and *M. incognita*. The maximum shoot length (54.39 cm), fresh weight of shoots (25.42 g) as well as dry weight of shoots (5.7 g) were recorded when plants treated with only 5×10^4 CFU of *P. lilacinum*/g soil. The maximum root length (25.94 cm) was observed in combination of 1×10^4 CFU of *P. lilacinum*/g soil and 0 eggs per 100 g soil. The maximum fresh weight of roots (11.52 g) as well as dry weight of roots (0.77 g) was recorded when pots treated with 1×10^4 CFU of *P. lilacinum*/g soil and 0 eggs per 100 g soil which shown similarity with when plants root severally affected by nematode damage (i.e. root galling). Whereas the lower shoot health was recorded in combination of 0 CFU/g soil and 2000 eggs per 100 g soil. And the lower root weight was found when plants served as control. The lowest gall index (1.38) was observed when plants treated with 5×10^4 CFU/g soil against 250 eggs per 100 g soil. The lowest number of egg masses (4.75) was recorded interaction effect of 5×10^4 CFU/g soil and 250 eggs per 100 g soil. But highest suppression of number of egg masses were observed when 5×10^4 CFU mixed with per g soil against 2000 eggs per 100 g soil compared with inoculated treatment. In comparison to only inoculated treatment the maximum reduction of nematode population (79.32%) was calculated at 5×10^4 CFU/g soil against 2000 eggs per 100 g soil. The lower multiplication (3.25) of *M. incognita* was observed in treatment of 5×10^4 CFU/g soil and 2000 eggs per 100 g soil. The maximum colonization of egg masses (45.19%) was recorded when applied 5×10^5 CFU of *P. lilacinum*/g soil against 1000 eggs per 100 g soil. The maximum soil colonization (2165 CFU/g soil) was found when pots treated with 5×10^5 CFU/g soil against 2000 eggs per 100 g soil.

Considering all the result an application of 5×10^4 CFU of *P. lilacinum*/g soil efficiently suppressed nematode damage i.e. gall index, number of egg masses per root, size of egg mass, final nematode population and reproduction factor of the pest inoculum up to 2000 eggs per 100 g soil and increased plant growth. But in specific case highest reduction of nematode population, egg masses number and reproduction factor was observed when plants infested with highest inoculum density (2000 eggs per 100 g soil). In case of gall index maximum reduction was possible in that dose when plant inoculated with 250 eggs per 100 g soil. Although maximum egg mass and soil colonization observed at the dose of 5×10^5 CFU/g soil from 250 to 2000 eggs per 100 g soil but in specific 1000 eggs per 100 g soil reported higher egg mass and soil colonization by 5×10^5 CFU of *P. lilacinum*/g soil.

However, further research is needed to observe the histological interaction among *P. lilacinum*, *M. incognita* on the indigenous cultivar of different vegetables. And to produce bio-formulated product based on *P. lilacinum* more advance research will be needed.

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