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Dose-response relations between *Purpureocillium lilacinum* PLSAU-1 and *Meloidogyne incognita* infecting brinjal plant on plant growth and nematode management: a greenhouse study

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Abstract

The efficacy of various doses of *Purpureocillium lilacinum* PLSAU-1 (*P. lilacinum* PLSAU-1) was evaluated against brinjal root-knot pathogen, *Meloidogyne incognita* (*M. incognita*) at different inoculum levels. Experimental pot soil was treated by four doses (0, 1×10^5, 5×10^5, and 1×10^6 CFU/g soil) of *P. lilacinum* PLSAU-1 before transplanting, and 5 inoculum levels of *M. incognita* (0, 100, 400, 800, 1600, and 3200 eggs per 100 cm^3 soil) were used after 3 days of brinjal transplantation. A significant correlation was observed between doses of *P. lilacinum* PLSAU-1 and inoculum densities of *M. incognita*. Among the doses, the rate of *P. lilacinum* PLSAU-1 of 1×10^6 CFU/g soil enhanced highly plant growth parameters. Inoculation of *M. incognita* reduced plant growth significantly, and the reduction increased with the increase of inoculum density of *M. incognita*. The application of *P. lilacinum* PLSAU-1 at 1×10^6 CFU/g soil reduced a maximum of 72% gall index and 84% egg masses when the crop was challenged by 800 and 400 eggs/100 cm^3 soil, respectively. The dose of 1×10^6 CFU/g soil of *P. lilacinum* PLSAU-1 showed effectiveness to reduce reproduction factor of *M. incognita* up to 81% when the crop was inoculated by 800 eggs of the pest/100 cm^3 soil. The study demonstrated that *P. lilacinum* PLSAU-1 is an effective bio-agent for controlling of root-knot nematode on brinjal and can be a key component of environment-friendly management approach.

Keywords: *Purpureocillium lilacinum* PLSAU-1, Biological control, *Meloidogyne incognita*, Inoculum density, Brinjal

Background

Brinjal (*Solanum melongena* L.) is a very common, popular, and second most (next to potato) important vegetable crop, in Bangladesh and other parts of the world, in respect of acreage and production. It contains higher calorie, iron, phosphorus, and riboflavin than tomato (Meherunnahar and Paul 2009).

The incidence of insect pests and diseases generally hampered the production of eggplant. In Bangladesh, this crop suffers from 13 different diseases, recorded (Rashid 2000). Among those, root knot has been considered as one of the key constrains in eggplant cultivation. It is caused by *Melodoigyne* spp., the widely distributed in all eggplant-growing areas of Bangladesh with moderate incidence (27.2%) (Bari 2001) as well as in other countries of the world. *Meloidogyne incognita* (*M. incognita*) is the most vicious species among them, which causes severe harms to a quantity of economically significant agriculture and greenhouse crops (Huang et al. 2014).

Infection of roots by nematodes affects uptake of water and nutrients and interferes with the translocation of minerals and photosynthates, which change the shoot

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to root ratio (Saikia et al. 2013) and expose the plants to other pathogen infection. For example, nematode root infection increases the incidence and severity of wilt and root rot diseases on a variety of crops, which can negatively influence yield (Osman et al. 2018). Controlling of this type of plant-parasitic nematode is essential for effective crop production. Presently, the most proficient chemical nematicides (e.g., methyl bromide) have been constrained due to their hazardous effect on animal and human. Therefore, it has led to an immense interest in biological control in order to achieve an environmentally safe method of reducing nematode damage (Abdelnabby et al. 2011).

Purpureocillium lilacinum is one of the most promising and practicable biocontrol agents for the management of plant parasite nematodes (Yang et al. 2015b). Previous studies indicated that this fungus adapts well in varied climatic conditions and is much effective in controlling root-knot nematodes. This fungus is capable of infecting all life stages of the root-knot nematode. The production of leucinotoxins, chitinases, proteases, and acetic acid by *P. lilacinum* is associated with the infection process (Yang et al. 2015b). Although *P. lilacinum* has been reported as a nematode biocontrol agent on plant crops (Usman and Siddiqui 2012 and Yang et al. 2015b), and recently, it also has been reported as a biocontrol agent for controlling *Sclerotinia sclerotiorum* the rot on oilseed rape (Yang et al. 2015a).

Recently, some works have been done on the effect of fungal strains for the management of root-knot nematode on tomato and cucumber (Kiewnick et al. 2011 and Huang et al. 2014). However, the dose-response relationship and optimum dose for controlling of root knot (*M. incognita*) on brinjal still remain unidentified.

The main objective of this study was to evaluate the dose effect of *P. lilacinum* PLSAU-1 on the plant growth parameters, root galling at different inoculum level of nematode, and nematode population and reproduction factor of the root-knot nematode *M. incognita* at different inoculum levels in brinjal.

Material and methods

Experimental materials and design

The experimental soil was silty loam (13.8% clay, 56.5% silt, and 29.7% sand) with a pH of 5.2 and an organic matter content of 1.5%. Required soil, sand, and decomposed cow-dung were mixed properly in a ratio of 6:2:1. The mixture was autoclaved at 121 °C for 15 min at 15 PSI. Plastic pots of 1000 cm^3 were cleaned, washed, dried up properly, and sterilized with 70% ethanol. Then, they were filled with 800 g soil and arranged according to the experimental design. The experiment was laid out in two factors according to a randomized complete block design (RCBD) having eight replications for each treatment.

Inoculums and fungus preparation

Nematode samples (*M. incognita*) were collected from nematode-infected brinjal roots. Egg masses were picked up and inoculated in young seedlings of brinjal. Sub-culturing was done subsequently by inoculating new brinjal seedling with egg masses. Galled roots of tomato, eggplant, cucumber, and bottle gourd were collected from farmers' field of different districts (Dinajpur, Narayangonj, Mymensingh, Jessore, and Dhaka) in Bangladesh. The samples were kept in double-layered plastic bags at 48 °C, and fungi were isolated within 1 month. After collection, roots were washed by running tap water and placed under a dissecting microscope. Egg masses were picked up manually, using dissecting forceps, and the roots were dissected beneath the egg masses; females were carefully picked up with a dissecting needle. Eggs were extracted from the egg masses, following the method of Hussey and Barker (1973). Egg masses were treated by 1% NaOCl solution for 1 min to release eggs from the egg masses and disinfect the egg surface, whereas females were treated by 0.5% NaOCl solution for 3 min. Treated eggs and females were rinsed by a sterile distilled water to remove residual NaOCl. One hundred eggs (ca.) were plated and smeared on each potato dextrose agar (PDA) plate; five plates were applied for each sample. Five females were crushed by a sterile forceps and smeared on each PDA plate. All plates were incubated at 25 °C for 5 days. The incubated plates were daily examined under an inverted microscope, and hyphae grown from eggs or females' fragments were transferred on to PDA plates for purification and identification. The isolates were identified, following the standard keys of Houbraken et al. (2010). This fungus was isolated at the laboratory of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The fungus was named as *P. lilacinum* PLSAU-1 (Aminuzzaman et al. 2018).

Inoculation of *P. lilacinum* PLSAU-1 and *M. incognita*

P. lilacinum PLSAU-1 spores were mixed carefully into the soil at (1×10^5, 5×10^5, and 1×10^6 CFU) of *P. lilacinum* PLSAU-1/g of soil in defined pots with a micropipette. Four-week-old seedlings were uprooted cautiously from the plastic trays and transplanted in the fungal-treated pots. Sufficient water was given just after transplantation. Then, after 3 days, nematode was inoculated at (0, 400, 800, 1600, and 3200 eggs/100 cm^3) of pot soil. Four holes (2-cm depth) around the plants were made with the help of metallic rod, and egg suspension was inoculated into the holes by a micropipette. Pot soil contained water only untreated control and nematode

only inoculated control. General sanitation was maintained throughout the growing period.

Data recording and analyzing

Plants were harvested after 2 months for collecting data. The shoot length was measured from the base of the plant to the growing point of the youngest leaf. To measure root length, the roots were cleaned gently by water and the length was measured from the growing point of the root to the longest available lateral root apex with measuring tape. After taking root length and fresh weight, nematode infection was determined by rating root galling based on the 0–10 scale (0 = no galls and 10 = dead plant) (Bridge and Page 1980). The number of egg mass/root system was counted by Hartman and Sasser (1985). The roots were soaked in Phloxine-B (2 mg/l) solution for 15 min, and then, the egg masses/roots were counted using a magnifying lens. The extraction of J2 from the soil and root was done, using a Whitehead and Hemming tray method (Whitehead and Hemming 1965). Pot soil was mixed thoroughly, and a 10-g soil from each pot was weighted and placed on a sieve on a bowl filled with water. The upper portion of the sieve was lined by three layers of kitchen tissue paper. After 3 days, the bowl water was collected in a beaker, and then, nematodes were concentrated into 10 ml of water using 500 mesh sieves. The two-milliliter suspension was taken from each sample and placed into a counting dish, and the J2 were counted under a compound microscope. To estimate the eggs per root, roots were gently washed by tap water to remove soil. Then, roots were chopped into 2 cm lengths placed in a 100 ml solution of 0.6% sodium hypochlorite and mixed, using an electric blender. The blended mixture was poured through the sieves from top to bottom (80, 200, and 500 meshes). Eggs were collected from 500 mesh sieve in a beaker and counted using a counting chamber.

For the percentage of egg mass colonization, five egg masses/plant were randomly collected from the eggplant roots, washed by a sterile water, and disinfected by a solution of Clorox rinsed with water and placed on a PDA plate. The number of colonized egg masses was determined after 5 days of incubation at 25 °C. The presence of *P. lilacinum* PLSAU-1 into egg mass of *M. incognita* was confirmed by preparation of slides from the culture grown on PDA. Soil colonization by *P. lilacinum* PLSAU-1 (CFU/g soil) was determined by a soil dilution plate technique. In details, 2 g of soil (wet weight) was transferred to flasks containing 20 ml of sterile demineralized water and shake on a horizontal shaker for 25 min at 170 rpm. After preparation of the serial dilutions, CFU numbers were determined by plating onto OHIO agar (Johnson and Curl 1972). Final nematode population was determined eggs per root plus the 2nd-stage juvenile per pot; reproduction factor = (final nematode population)/(initial nematode population).

The recorded data of various parameters were statistically analyzed using MSTAT-C statistical package program. Differences among treatment means were determined by Duncan's new multiple range test (DMRT).

Results and discussion

Effect of *P. lilacinum* PLSAU-1 and *M. incognita* application rates on plant growth parameters and nematode's population

Results of the present study indicate significant effects of inoculum level and dose applied on the growth parameters. The treated 1×10^5, 5×10^5, and 1×10^6 CFU/g soil of *P. lilacinum* PLSAU-1 shoot fresh weight and shoot length were significantly greater than the untreated control (Table 1). The mechanism of improvement of plant growth by the biocontrol fungi is unknown. Our hypothesis is maybe it enhanced nutrient uptake, incorporate mineral nutrition through mineral solubilization, disease suppression, or involved production of phytohormones. Also, it may be caused when this bio-agent parasitizes other pathogenic fungi and competes with harmful plant microorganisms (Contreras-Cornejo et al. 2009).

For the inoculum level, shoot fresh weight and shoot length were significantly reduced than the un-inoculated control and the reduction increased with the increase of inoculum's density. Due to the application of *P. lilacinum* PLSAU-1, the root length increased than the untreated control and the increased trend was along with increased dose. The highest dose of 1×10^6 CFU/g soil showed the highest root length, where the other two doses showed similar statistical results. Conversely, a significant reduction of root length was found at all inoculated treatments of *M. incognita* compared to the un-inoculated one. In the case of root fresh weight, the highest dose (1×10^6 CFU/g soil) showed the highest root fresh weight. All the tested inoculum levels showed a significant reduction in root fresh weight than the control. The greater decline of plant growth parameters coupled with an elevated rate of nematode development might be due to the more rapid invasion of roots by larger numbers of J2 and the consequent maturity of adult females. This is possible as the developing giant cell systems and disturbance of the developing xylem lead to change in physiological functions such as nutrient uptake, translocation of nutrients and water from root to plant, and subsequent photosynthesis (Davis et al. 2003).

Obtained results also indicated that the plant growth parameters roughly affected by inoculum density of *M. incognita* as they decreased with increasing the nematode population. As well, lower plant growth characters were observed when the plants were inoculated by 1600

Table 1 Dose-effect and *M. incognita* inoculum level of *P. lilacinum* PLSAU-1 on plant growth parameters, gall index, number of egg masses per root, final nematode population, reproduction factor, % egg mass colonized by *P. lilacinum* PLSAU-1, and soil colonization by *P. lilacinum* PLSAU-1 (CFU/g soil) in brinjal

Dose	Shoot length (cm)	Shoot fresh weight (g)	Root length (cm)	Root fresh weight (g)	Gall index	Number of egg mass/root	Final nematode population (10^5)	Reproduction factor Pf/Pi	% Egg mass colonized by *P. lilacinum PLSAU-1*	Soil colonization by *P. lilacinum* PLSAU-1 (CFU/g soil)
Untreated inoculated control	20.89c	10.87b	10.18c	6.12b	6.73a	65.03a	4.61a	54.86a	0b	0d
1×10^5 CFU/g soil	25.12a	12.78a	12.38b	6.03b	4.38b	34.40b	4.06b	12.64b	29.38a	3855.00a
5×10^5 CFU/g soil	23.39ab	11.53b	12.52b	5.49b	3.88b	19.30c	3.97b	11.65b	34.17a	4363.50b
1×10^6 CFU/g soil	22.19bc	10.97b	15.24a	7.74a	2.55c	16.95c	3.90b	9.50b	45.88b	5275.35c
Inoculum level (eggs per 100 cm^3 soil)										
Uninoculated control	27.33a	13.81a	14.89a	6.53a	0.00d	0.00c	0.00c	0.00b	0c	0d
400	24.45b	12.75ab	13.12b	6.09b	4.00c	27.03b	4.77b	31.71a	37.59a	4100.00b
800	22.95b	11.92bc	12.69c	5.50c	5.31b	44.88a	5.02b	26.78a	36.31a	4550.00a
1600	20.78c	10.5cd	12.00c	5.50c	5.78b	48.51a	5.32a	25.27a	41.41a	3617.00c
3200	18.98c	8.711d	10.29d	5.00d	6.81a	48. 94a	5.57a	24.16a	34.31b	2940.00d

In a column, similar letter(s) do not differ significant at 5% level of probability

and 3200 eggs per 100 g soil. Charegani et al. (2012) reported similar results as the inoculation of tomato with inoculum level (40 eggs and J2) of *M. incognita* or *M. javanica* per gram of soil reduced shoot length and fresh dry weights markedly, and the reduction was significantly correlated to its inoculum density.

The analytical results showed that all the tested doses of *P. lilacinum* PLSAU-1 caused significant reductions in gall index rating, number of egg masses per root system, final nematode population, and reproduction factor compared to untreated control (0 CFU/g soil). The highest reduction was observed for the highest (1×10^6 CFU/g soil) dose of bio-agent. In contrast, all the inoculum densities increased the gall index rating and number of egg masses per root system than the un-inoculated control. Inoculating soil with (3200 eggs per 100 cm^3 soil) led to a highest gall index development and a number of egg masses per root system. The final nematode population increased with the increase of inoculum level. The highest reproduction factor was observed when the soil was inoculated by 400 eggs/100 cm^3 of soil. It showed a statistical similarity with other inoculum levels. The percentage of egg mass colonization and soil colonization was significantly influenced by all tested doses of bio-agents as well as inoculum levels of nematode. The highest percentage of egg mass colonization and soil colonization was recorded when tested with the highest dose (1×10^6 CFU/g soil) of *P. lilacinaus*. Conversely, the lowest percentage of egg mass colonization and soil colonization were observed at the highest inoculum level (3200 eggs/100 cm^3 of soil) of *M. incognita* (Table 1). The results of the present experiment indicated that the application of *P. lilacinum* PLSAU-1 suppressed root-knot and increased plant growth parameters.

Dose-response relationship

Two-factor analysis for the plant growth characters (shoot fresh weight, length; root fresh weight, length) showed significant effects on the inoculum level and rates, applied on the brinjal plant. Among the four doses of *P. lilacinum* PLSAU-1, the rate of (1×10^6 CFU/g soil) enhanced plant growth characters over 400 to 3200 eggs per 100 cm^3 soil inoculum level of *M. incognita* than the untreated inoculated control (Table 2).

The major findings of this study revealed that *P. lilacinum* controlled the root-knot nematode and *M. incognita* efficiently increased the plant growth characters. To find out the appropriate dose of *P. lilacinum* PLSAU-1 against various levels of nematode damage, four fungal doses including 0, 1×10^5, 5×10^5, and 1×10^6 CFU/g soil were applied. Among the different fungal doses, the identified optimum fungal dose was 1×10^6 CFU/g soil, where the highest nematode suppression and improved plant growth was recorded (Table 2). Its existence in the rhizosphere of roots at the penetration time might decrease the number of juveniles that could entrance the roots. This finding is in agreement with Holland et al. (1999) who quantified that *P. lilacinum* colonized the root and protected its surface from root-knot nematode attacks. It correspondingly reduced the number of viable eggs and juveniles of the second generation during the experimental period. These results confirm the findings of Kiewnick and Sikora (2006). They reported that a single pre-plant application at a concentration of (1×10^6 CFU/g soil) was needed for sufficient biocontrol of root-knot nematode of *M. incognita* in tomato. In the present study, a significant increase in plant shoots and root length, fresh weight of shoots, and roots was observed by using all doses of *P. lilacinum* PLSAU-1 compared to untreated control and nematode-inoculated control. Improved character of plant growth by application of *P. lilacinum* for controlling root-knot nematodes was also reported earlier (Al-Raddad 1995). They tested the effects of *Glomus mosseae* and *P. lilacinum* on *M. javanica* of tomato in a greenhouse experiment. The highest root and shoot length, and fresh and dry weight of root and shoot were achieved when the plants were inoculated by *P. lilacinum* to control root-knot nematode. Khan et al. (2012) reported that an enhancement in growth and yield of brinjal with biocontrol agents *Pochonia chlamydosporia*, *P. lilacinum*, and *Trichoderma harzianum* owing to suppress gall formation and egg masses. The result of the present study revealed that the brinjal plants inoculated with 3200 eggs of *M. incognita* showed a significant reduction in shoot and root length, and fresh weight of shoot and root. Reduced plant growth characters by the inoculation of *M. incognita* were also reported previously by (Ganaie and Khan 2010). The bio-agent significantly reduced the multiplication rate of *M. incognita* than the control. It proved the potentiality of *P. lilacinum* PLSAU-1 as a biocontrol agent against *M. incognita*.

All four doses of *P. lilacinum* PLSAU-1 were tested, and the lowest gall index development, number of egg masses per root, total nematode population, and reproduction factor were found at 400 and 800 eggs per (100 cm^3 soil). For all combination effect of doses and inoculum level, the highest gall index, number of egg masses per root, total nematode population, and reproduction factor were resulted in combination of (1×10^5 CFU/g soil) dose of *P. lilacinum* PLSAU-1 and 3200 eggs per (100 cm^3 of soil) inoculum level of *M. incognita*. At all the inoculum levels, the gall index, number of egg masses per root, total nematode population, and reproduction factor were significantly reduced by the application of (1×10^6 CFU/g soil) of *P. lilacinum* PLSAU-1 than the control. However, the maximum reduction of gall index (72%) and number of egg masses

Table 2 Combine effect of *P. lilacinum* PLSAU-1 dose and *M. incognita* inoculum level on plant growth parameters, gall index, number of egg masses per root, total nematode population, and reproduction factor in brinjal

Treatment		Shoot length (cm)	Shoot fresh weight (gm)	Root length (cm)	Root fresh weight (gm)	Gall index	% of gall index reduction	No. of egg mass per root	% No. of egg mass reduction	Total nematode population (10^5) reduction	% Total nematode population reduction	Reproduction factor Rf = Pf/Pi	% of reproduction factor reduction
M. incognita[a]	*P. lilacinum PLSAU-1*[b]												
400 eggs	0	20.4ghi	10.2fg	10.5gh	5.86g	6.38bc	0	53.5b	0	5.36c	0	70.58a	0
400 eggs	1×10^5 CFU	21.52fgh	11.3ef	10.8fg	6.06fg	3.75gh	41.2	28.5cde	46.7	3.6e	32.8	21.5bc	69.5
400 eggs	5×10^5 CFU	23.83cd	12.0cd	12.94cde	6.57de	3.75gh	41.2	17.63g	67	2.9f	45.9	19.65bc	72.0
400 eggs	1×10^6 CFU	25.75c	13.45ab	14.59bc	8.8a	2.13i	66.6	8.5h	84	2.5gf	53.3	14.35c	79.6
800 eggs	0	19.55ij	9.5gh	10.11gh	5.4h	9.0a	0	93.38a	0	5.66b	0	71.77a	0
800 eggs	1×10^5 CFU	22.63ef	10.23fg	10.51gh	6.0fg	5.38de	40.2	35.0c	62.5	4.0de	29	23.0bc	68.0
800 eggs	5×10^5 CFU	20.43ghi	12.18cde	12.34cde	6.08fg	4.38fg	51.3	29.25cd	68.67	3.2ef	43.5	15.56c	78.0
800 eggs	1×10^6 CFU	27.5b	12.63bcd	16.08a	8.16b	2.5i	72.2	21.88efg	76.5	2.35g	58.5	13.25c	81.0
1600 eggs	0	18.06kl	8.14hi	10.2hi	5.43h	9.0a	0	89.25a	0	5.93a	0	66.76a	0
1600 eggs	1×10^5 CFU	21.35fgh	10.4fg	10.38ghi	5.7fg	5.88cd	34.6	51.88b	41.87	4.35d	26.6	16.45c	75.3
1600 eggs	5×10^5 CFU	21.96fgh	11.87de	12.16ef	5.96g	4.75ef	47.2	28.5cde	68	3.8e	35.9	17.34c	74.0
1600 eggs	1×10^6 CFU	24.13cd	13.9ab	13.3cd	7.53c	3.5h	61.1	26.13def	70.7	3.15ef	46.8	15.3c	77.0
3200 eggs	0	17.38 l	8.06i	9.2j	5.0i	9.25a	0	89.0a	0	6.1a	0	65.17a	0
3200 eggs	1×10^5 CFU	19.1jkl	9.8gh	10.16hi	5.5h	6.88b	25.6	56.63b	36.37	4.6d	24.5	28.3b	56.0
3200 eggs	5×10^5 CFU	20.95ghi	10.13fg	11.10fgh	5.9g	6.5bc	29.7	28.28cde	68.25	4de	34.4	26.45bc	59.4
3200 eggs	1×10^6 CFU	22.93def	11.5ef	12.88cde	6.81d	4.63f	49.9	27.0de	69.86	3.6e	4.4	23.0bc	64.7

In a column, similar letter(s) do not differ significant at 5% level of probability
% reduction = (value of control − value of treatment)/value of control × 100
[a]Per 100 cm^3 soil
[b]Per gram soil

per root (84%) was recorded at the dose of 1×10^6 CFU/g soil of *P. lilacinum* PLSAU-1 against 800 and 400 eggs per 100 cm^3 of soil inoculum level, respectively. In this experiment, it revealed a significant interaction between the dose of *P. lilacinum* PLSAU-1 applied to soil and the inoculum level for the parameters of gall index and number of egg masses per root. A similar effect was demonstrated by Kiewnick et al. (2011), and they reported a strong reduction in the galling index and the number of egg masses per root system after PL251 soil treatment at 2×10^5 CFU/g soil at inoculum density up to 800 eggs/100 cm^3 of soil. This result also was confirmed by Aminuzzaman et al. (2018) who mentioned that the root galling index and the final nematode population decreased up to 55.6 and 66.9% for brinjal. In addition, the significant reduction of nematode population (58.5%) was also observed by the same dose against 800 eggs per (100 cm^3 of soil) (Table 2). In case of reproduction factor, 81% reduction was found at the 1×10^6 CFU/g soil dose of *P. lilacinum* PLSAU-1, when pot soil was inoculated by 800 inoculum level per 100 cm^3, which was statistically similar to 400 and 1600 inoculum levels, respectively (Table 2). This study indicated that the reduction of *M. incognita* per plant was depended on the doses of *P. lilacinum* PLSAU-1. This result is consistent with the results of Kalele et al. (2010) who reported that the pre-planting soil treatment, with the highest dose (0.4 g/10 L soil) reduced 69 and 73% of nematode population in the roots and soil, respectively, than the inoculated control in a tomato plant.

The colonization increased with the increase of a dose of *P. lilacinum* PLSAU-1 at all the inoculum levels. The highest percentage of egg mass colonization was recorded in 1×10^6 CFU/g soil of *P. lilacinum* PLSAU-1 against 1600 eggs per 100 cm^3 inoculum level, and the highest soil colonization was found at the same doses of bio-agent over 800 eggs per 100 cm^3 inoculum level of *M. incognita* (Fig. 1a, b).

The results of the present study demonstrated that the percentage of egg masses colonized by *P. lilacinum* PLSAU-1 directly influenced by the fungal density in the soil. This result consistent with the result of Carneiro and Cayrol (1991) who reported that the number of colonized egg masses and the number of non-viable eggs increased with the increase of *P. lilacinum* density, and the fungus was most effective at a density of 10^6 spores/g of soil on banana. The *P. lilacinum* PLSAU-1 was isolated from soil after 2 months of application, which indicated that the fungus survived throughout the growing season and it was compatible with the environmental conditions. Although the empirical result showed that the CFUs were lower than those added at the beginning of the experiment. The decline in CFU numbers, 2–3 months, after the initial application was also reported by several researchers (Kiewnick and Sikora 2003). They revealed that the persistence of entomopathogenic fungi in soil and the relocation of conidia from the superficial to deeper layers are dependent on the presence of antagonistic organisms on the soil properties This result also validated by Rumbos and Kiewnick (2006). They found the decline in fungal densities (CFU numbers) compared to initial densities ranged from 72.3 to 83.8% in the first and in the second experiment in a tomato plant, where they inoculated 2×10^6 CFU/g of dry soil.

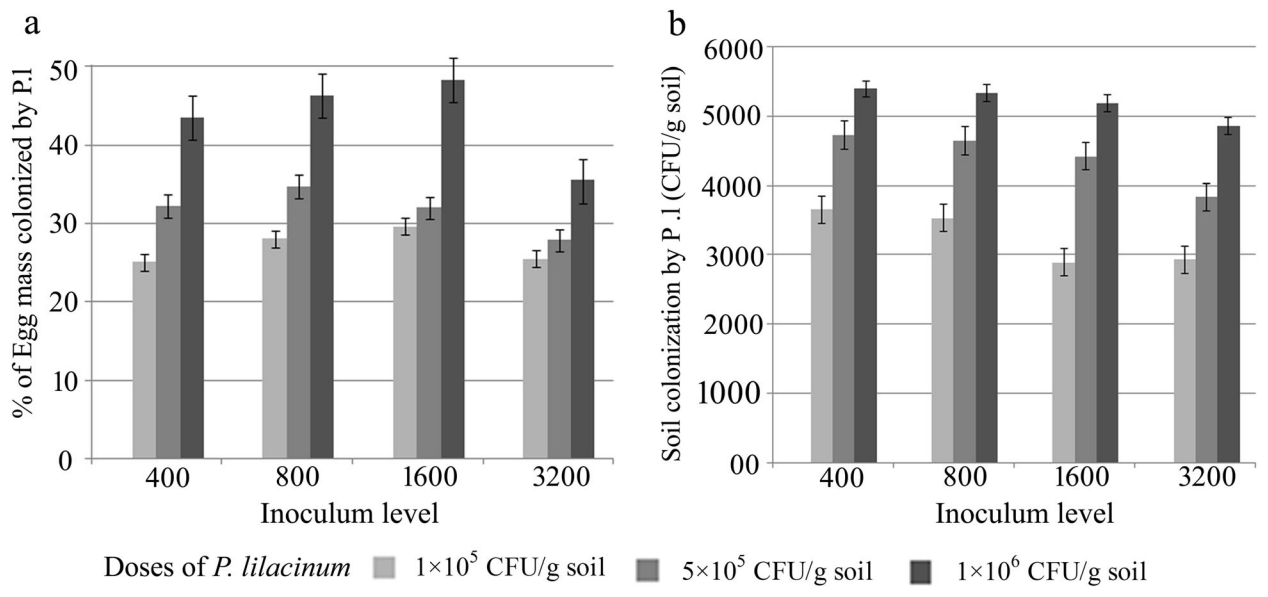

Fig. 1 Dose-response relationship of *M. incognita* and *P. lilacinum* PLSAU-1 on **a** percentage of egg mass colonization and **b** colonization of fungus/g soil in brinjal

Conclusion

The study demonstrated the effects of a dose of *P. lilacinum* PLSAU-1 to suppress root-knot (*M. incognita*) damage in infected brinjal roots. The key findings of the present were the strong relationship between doses of bio-agent and inoculum level of the pest. This study suggests that high (1×10^6 CFU/g soil) dose of *P. lilacinum* PLSAU-1 is necessary for successful control of root-knot nematode *M. incognita* for all tested inoculum level. However, further research is needed to estimate the interactions between inoculum level of *M. incognita*, host plant response, and fungus dose to build up an effective dose-response replica for biocontrol fungus.

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Availability of data and materials

The data and material of this manuscript are available.

Authors' contributions

The first author (MSS) of this manuscript performed the experiments of this study and wrote the introduction, methodology, and results parts. The second author (FMA) wrote the discussion and conclusion parts. The third author (MEH) completed the analysis part. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals.

Consent for publication

The manuscript has not been published in completely or in part elsewhere.

Competing interests

The authors declare that they have no competing interests.

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