



Trends in
**Applied Sciences
Research**

ISSN 1819-3579



Academic
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Research Article

Integrated Management of *Meloidogyne incognita* Infecting *Vigna radiata* L. using Biocontrol Agent *Purpureocillium lilacinum*

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Abstract

Background and Objective: The root-knot nematode, *Meloidogyne incognita* is a sedentary endoparasite and one of the most damaging agricultural pest attacking a wide range of crops including pulses and vegetables and *Purpureocillium lilacinum* is a biocontrol agent used in this study. A pot experiment was conducted to find out the nematocidal potential of a biocontrol agent, *Purpureocillium lilacinum* for the sustainable management of root-knot nematode, *Meloidogyne incognita* infecting *Vigna Radiata* cv. 'PDM-139' under glasshouse conditions. **Materials and Method:** Clay pots (15 cm in diameter) filled with 1 kg autoclaved soil mixed with farmyard manure in the ratio of 4:1 were treated with biocontrol agent, *P. lilacinum* applied at 1.5 g /pot in individual, sequential and concomitant manner. **Results:** All the treatments were found to significantly improve the growth and physiological parameters of mung bean and reduced the pathological parameters as compare to untreated inoculated control. The highest improvement was found in those plants treated with *P. lilacinum* alone. Concomitant and sequential inoculation of *P. lilacinum* with *M. incognita* also showed enhancement in growth parameters of mung bean. Least enhancement in growth parameters were observed in those plants inoculated with nematode alone. **Conclusion:** It may be due to the nematocidal nature of *P. lilacinum* against root-knot nematode, *M. incognita*. Hence, it may be concluded that *P. lilacinum* as biocontrol agent is better alternative against chemical nematocides for the sustainable management of *M. incognita* and reduce environmental hazards.

Key words: Biocontrol agent, mung bean, *M. incognita*, *P. lilacinum*, Sustainable management

Citation: Amir Khan, Mohd. Tariq, Mohd. Asif, Faryad Khan, Taruba Ansari and Mansoor A. Siddiqui, 2019. Integrated management of *Meloidogyne incognita* infecting *Vigna radiata* L. using biocontrol agent *Purpureocillium lilacinum*. Trends Applied Sci. Res., 14: 119-124.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mung bean (*Vigna radiata* L.) commonly known as Moong, belonging to the Family-Fabaceae cultivated for its edible seeds. It has a high source of protein, fiber and other nutrients. Because of their high nutrient density, mung bean considered useful in defending against several chronic, age-related diseases, including heart disease, cancer and diabetes. Mung bean is susceptible to various pathogens such as; fungi, bacteria and plant-parasitic nematodes, which reduces its growth, subsequently the production and productivity. The root-knot nematodes (*Meloidogyne* spp.) are considered as one of the most destructive pest of mung bean limiting its production¹. The root-knot nematode, *Meloidogyne incognita* is a sedentary endoparasite and one of the most damaging agricultural pest attacking a wide range of crops including pulses and vegetables. The pest causing dramatic yield losses mainly in tropical and sub-tropical agriculture². The pathogenicity of *Meloidogyne* spp. had been proved on chickpea, pigeon pea, cowpea, mungbean, pea, lentil, urdbean, rajmash and several other pulses³. These nematodes produce an annual loss of over US\$ 100 billion to world agriculture and an estimated US\$ 500 million were usually spent on nematode control⁴. Current management of root-knot nematodes includes resistant varieties, crop rotation, organic amendments and biocontrol agents. Most important way of nematode control is mainly the use of chemical nematicides. Most of the nematicides are banned due their toxic nature and hazardous effect on the flora and fauna; beside this they disturbed the ecological equilibrium of the soil. Thus, the development of alternative control strategies and long-term integrated approaches is urgently needed in order to replace chemical nematicides. Use of biocontrol agent appears an environmentally and ecologically safer option for the management of root-knot nematode, *M. incognita* with great potential for promoting sustainable agriculture. The *P. lilacinum* had high nematicidal potential for the biological control of nematodes and its successful use against *M. javanica* and *M. incognita* on tomato, vegetables, banana and other crops⁵⁻⁷. Application of two isolates of *Trichoderma* and the two isolates of *P. lilacinum* significantly reduced nematode eggs and eggmasses production and increased the plant growth compare to untreated control^{8,9}. Therefore, a study was undertaken to assess the nematicidal potential of biocontrol, *P. lilacinum* against the root-knot nematode, *M. incognita* infecting mung bean.

MATERIALS AND METHODS

Host plant and pathogen: Mung bean (*Vigna radiata* L.) cv. 'PDM-139' (Family-Fabaceae) was selected as host crop. The root-knot nematode, *Meloidogyne incognita* was selected as test pathogen.

Preparation of fungal biocontrol agents: The pure culture of the fungal biocontrol agent, *Purpureocillium lilacinum* (ITCC No. 6064) was purchased from the Indian Agriculture Research Institute (IARI), New Delhi, India. Fungal culture was maintained on Potato Dextrose Agar (PDA) medium¹⁰.

Nematode management by individual, sequential and concomitant application of *Purpureocillium lilacinum* with *Meloidogyne incognita* on mung bean:

The glasshouse experiments were conducted for evaluating the nematicidal efficacy of biocontrol agent, *P. lilacinum* against the root-knot development caused by the root-knot nematode, *M. incognita* and their potential in enhancing the plant growth parameters of Mung bean cv. 'PDM-139'. Clay pots (15 cm in diameter) filled with 1 kg autoclaved soil mixed with farmyard manure in the ratio of 4:1 were treated with biocontrol agent, *P. lilacinum* at 1.5 g /pot. The experiment was designed as follows:

- PI** : Inoculated with *P. lilacinum* at 1 g/pot alone
- PI→Mi₁₅** : *P. lilacinum* treatment given 15 days prior *M. incognita* inoculation
- PI+Mi** : Inoculated with *P. lilacinum* at 1 g and *Meloidogyne incognita* (1500 J2) simultaneously
- Mi→PI₁₅** : *M. incognita* inoculated 15 days prior *P. lilacinum* treatment
- UIC** : Untreated Inoculated Control (*M. incognita* 1500 J2 alone)
- UUC** : Untreated Uninoculated Control

Observations

Chlorophyll estimation: The chlorophyll content in the fresh leaves was estimated following the method of Mackinney¹¹. One gram of finely cut fresh leaves was grounded to a fine pulp using a mortar and pestle after pouring 20 mL of 80% acetone. The mixture was centrifuged at 5000 rpm for 5 min. The supernatant was collected in 100 mL volumetric flask. The residue was washed three times, using 80% acetone. Each washing was collected in the same volumetric flask and the volume was made up to the mark using 80% acetone. The

absorbance was read at the wavelength of 645 and 663 nm against blank (80% acetone) on spectrophotometer (UV 1700, Shimadzu, Japan). The chlorophyll content present in the extract (mg g^{-1} tissue) was calculated by using the following equation:

$$\text{Total chlorophyll } \text{g}^{-1} \text{ tissue (mg)} = \frac{20.2 (A_{645}) + 8.02 (A_{663})}{1000 \times W} \times V$$

Nitrate reductase activity (NRA): The nitrate reductase activity in fresh leaves was estimated by the following method of Jaworski¹². The leaves were cut into small pieces (1-2 cm). About 200 mg of these chopped leaves were weighed and transferred to plastic vials. To each vial, 2.5 mL of phosphate buffer pH 7.5 and 0.5 mL of potassium nitrate solution was added followed by the addition of 2.5 mL of 5% of isopropanol. These vials were incubated in BOD incubator for 2 h at $30 \pm 2^\circ\text{C}$ in dark. Incubated mixture of 0.4 mL was taken in a test tube to which 0.3 mL each of sulphanilamide solution and NED-HCl were added. The test tubes were left for 20 min for maximum colour development. The mixture was diluted to 5 mL Double Distilled Water (DDW). The absorbance (O.D.) was read at 540 nm using spectrophotometer. A blank was run simultaneously with each sample. Standard curve was plotted by using known graded concentration of NaNO_2 (Sodium nitrite) solution. The absorbance (O.D) of each sample was compared with that of calibration curve and nitrate reductase activity expressed at $\text{nm } (\mu\text{m h}^{-1} \text{g}^{-1})$.

Pathological parameters

Eggmasses per plant: The eggmasses were counted by following the procedure of Daykin and Hussey¹³. The roots were dipped in Phloxine B solution (0.015%) for 20 min and were then washed with running tap water to remove the residual Phloxine B. The eggmasses stained a pink red colour where as the roots remain colorless or stain lightly.

Root-knot index: The degree of root-knot nematode infection was recorded according to rating degree given by Taylor and Sasser¹⁴ as shown in Table 1.

Table 1: Degree of root-Knot nematode infection

Root knot index	Number of galls/root system
0	0
1	1-2
2	3-10
3	11-30
4	31-100
5	>100

Statistical analysis: The data of the experiments were analyzed statistically by using the Statistical Package for the Social Sciences SPSS 12.00 Software (SPSS Inc., Chicago, IL, USA) for analysis of variances (ANOVA). All the values were presented as the mean which were compared according to Least Significant Differences/Critical Differences (C.D) at $p = 0.05$ and $p = 0.01$ level. Duncan's Multiple Range Test was employed to test for significant difference between the treatments.

RESULTS

The present experiment was conducted under glasshouse conditions to evaluate the effect of individual, concomitant and sequential inoculation of *Purpureocillium lilacinum* with *Meloidogyne incognita* against the root-knot development and on growth and physiological parameters of mung bean cv. 'PDM-139'. The data presented in Table 2 and 3 revealed that inoculation of mung bean plant with root-knot nematode, *M. incognita* caused significant reduction in growth and physiological parameters and enhancement in pathological parameters as compared to untreated uninoculated control.

There was significant enhancement in plant growth parameters viz., plant length (cm), fresh weight (g) and dry weight (g) were found when *P. lilacinum* applied alone. Concomitant and sequential inoculation of *P. lilacinum* with *M. incognita* also improve the plant growth parameters. Highest increase in growth parameters of mung bean was observed when *P. lilacinum* was applied 15 days prior the inoculation of *M. incognita* followed by simultaneously inoculation of *P. lilacinum* and *M. incognita* and sequential inoculation of *P. lilacinum* 15 days after the inoculation of *M. incognita* as compare to untreated inoculated control (Table 2).

The highest number of nodules, flowers and pods were found when *P. lilacinum* applied alone. Significant increase in number of nodules, flowers and pods were also observed in sequential inoculation of *P. lilacinum* 15 days prior the *M. incognita* inoculation followed by simultaneously inoculation of *M. incognita* with *P. lilacinum* and sequential inoculation of *P. lilacinum* 15 days after the inoculation of *M. incognita* as compare to untreated inoculated control (Table 3).

When the biocontrol agent *P. lilacinum* applied 15 days prior to *M. incognita* inoculation was found highly effective in enhancing the growth parameters viz., plant length, fresh and dry weight of plant, number of flowers, pods and nodules and least improvement was observed in those pots treated with *P. lilacinum* applied after 15 day to *M. incognita* inoculation as shown in Table 2 and 3.

Table 2: Effect of individual, sequential and concomitant inoculation of *Meloidogyne incognita* and *Purpureocillium lilacinum* on the plant growth of mung bean cv. 'PDM-139' in pots

Treatments	Length (cm)			Fresh weight (g)			Dry weight (g)		
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
<i>Purpureocillium lilacinum</i>	64.8 ^a	30.6 ^a	95.4 ^a	85.94 ^a	24.74 ^a	110.68	21.71 ^a	5.59 ^a	27.30 ^a
PI→Mi ₁₅	50.4 ^c	17.7 ^c	68.1 ^c	65.78 ^c	15.86 ^c	81.64	13.14 ^c	3.17 ^c	16.31 ^c
PI+Mi	45.8 ^d	15.2 ^d	61.0 ^d	57.70 ^d	14.10 ^d	71.80	11.24 ^d	3.05 ^{cd}	14.29 ^d
Mi→PI ₁₅	38.7 ^e	13.5 ^e	52.2 ^e	50.46 ^e	13.05 ^e	63.51	10.11 ^e	2.39 ^e	12.50 ^e
UIC	24.7 ^f	8.2 ^f	32.9 ^f	14.76 ^f	4.86 ^f	19.62 ^f	3.12 ^f	0.91 ^f	4.03 ^f
UUC	47.7 ^b	17.2 ^b	64.9 ^b	30.76 ^b	11.76 ^b	42.52 ^b	6.12 ^b	2.21 ^b	8.33 ^b

Values are mean of five replicates, Means in each column followed by the same letter are not significantly different according to duncan multiple range test ($p \leq 0.05$), Mi→PI₁₅: *Meloidogyne incognita* inoculated 15 days before treatment, PI→Mi₁₅: *Purpureocillium lilacinum* treatment given 15 days prior nematode inoculation, UIC: Untreated inoculated control, UUC: Untreated uninoculated control

Table 3: Effect of individual, sequential and concomitant inoculation of *Meloidogyne incognita* and *Purpureocillium lilacinum* on pathological and physiological parameters of mung bean cv. 'PDM-139' in pots

Treatments	Chlorophyll content (mg g ⁻¹)	NRA ($\mu\text{m g}^{-1}\text{h}^{-1}$)	Number of flowers	Number of pods	Number of nodules	Eggmasses/plant	Root-knot index
<i>Purpureocillium lilacinum</i>	2.84 ^a	0.56 ^a	67 ^a	63 ^a	77 ^a	0 ^e	0.0 ^e
PI→Mi ₁₅	2.05 ^c	0.380 ^c	53 ^c	51 ^c	48 ^c	8 ^d	2.1 ^{cd}
PI+Mi	1.87 ^{cd}	0.349 ^d	46 ^d	42 ^d	42 ^d	90 ^c	2.9 ^{bc}
Mi→PI ₁₅	1.69 ^{de}	0.330 ^{de}	41 ^e	39 ^e	37 ^e	102 ^b	3.5 ^b
UIC	1.21 ^f	0.235 ^f	22 ^f	19 ^f	31 ^f	169 ^a	5.0 ^a
UUC	2.679 ^b	0.389 ^b	48 ^b	43 ^b	58 ^b	0 ^e	0.0 ^e

Values are mean of four replicates, Means in each column followed by the same letter are not significantly different according to duncan multiple range test ($p \leq 0.05$), Mi→PI₁₅: *Meloidogyne incognita* inoculated 15 days before treatment, PI→Mi₁₅: *Purpureocillium lilacinum* treatment given 15 days prior nematode inoculation, NRA: Nitrate reductase activity, UIC: Untreated inoculated control, UUC: Untreated uninoculated control

Highest increase in biochemical parameters viz., chlorophyll content (mg g⁻¹) and nitrate reductase activity ($\mu\text{m h}^{-1}\text{g}^{-1}$) was found when *P. lilacinum* applied alone. Significant improvement in chlorophyll content and nitrate reductase activity were also observed in sequential inoculation of *P. lilacinum* 15 days prior *M. incognita* inoculation followed by simultaneously inoculation of *M. incognita* and *P. lilacinum* and sequential inoculation of *P. lilacinum* 15 days after the inoculation of *M. incognita* as compare to untreated inoculated control (Table 3).

The data presented in Table 2 revealed that highest multiplication of root-knot nematode *M. incognita* was found in case of *M. incognita* alone inoculated pots. The highest reduction in number of eggmasses was observed in sequential application of *P. lilacinum* 15 days prior *M. incognita* inoculation followed by simultaneously inoculation of *M. incognita* with *P. lilacinum* and sequential application of *P. lilacinum* 15 days after *M. incognita* inoculation as compare to untreated inoculated control (Table 3). The least root-knot index was found when pots were treated with the application of *P. lilacinum* 15 days prior *M. incognita* inoculation followed by simultaneously inoculation of *M. incognita* with *P. lilacinum* and sequential application of *P. lilacinum* 15 days after *M. incognita* inoculation as compare to untreated inoculated control (Table 3).

The data present in Table 3 predicted that highest enhancement in physiological parameters viz., chlorophyll content and nitrate reductase activity and highest reduction in pathological parameters like number of egg mass per plant and root knot index was found in those pots when the biocontrol agent *P. lilacinum* applied 15 days prior to *M. incognita* inoculation and least was observed in those pots treated with *P. lilacinum* applied after 15 day to *M. incognita* inoculation.

From the above results, it can be say that biocontrol agent *P. lilacinum* reduced the root knot infestation cause by root knot nematode, *M. incognita* on mung bean in eco-friendly manner.

DISCUSSION

These results indicated that *P. lilacinum* has significant potential as biocontrol agent against the root-knot nematode, *M. incognita* under glasshouse conditions. Our results are in conformity with several scientists¹⁵⁻¹⁷. Inoculation of chickpea with *P. lilacinum* can significantly reduce the population of root-knot nematode and disease severity caused by root-knot nematode. It may be found that biocontrol agent significantly brought down the population of root-knot nematode and some compounds released from bio-agent reduced the nematode infestation. Biocontrol agent, *P. lilacinus*

contained protease and chitinase, play an important role in the degradation of the egg shell³. Ganaie and Khan¹⁸ reported significant reduction in number of galls, eggmasses, eggs/eggmass and final nematode population of *M. javanica* on sequential inoculation of *P. lilacinus* 10 days prior to *M. javanica* treatment. Esfahani and Pour¹⁹ observed that *P. lilacinus*, a biocontrol agent was most effective when the fungus and root-knot nematode, *M. javanica* were inoculated simultaneously or the fungus proceeded the nematode in sequential inoculation on tomato. *Paecilomyces lilacinus* by direct hyphal penetration colonizes the root surface and parasitizes eggs, eggmasses, second stage juveniles and females of *Meloidogyne* spp.²⁰. *Paecilomyces lilacinus* reported as a good biocontrol agent of root-knot nematodes and other plant-parasitic nematodes^{21,22}. Therefore, it was concluded that the severe infection caused by root-knot nematode could be lowered by the use of *P. lilacinus* as bioagent in view of eco-friendly environment. This has an advantage against expensive and hazardous chemical nematicides.

CONCLUSION

From the above study it may be concluded that biocontrol agent, *P. lilacinus* showed nematicidal potential against root-knot nematode, *M. incognita* and showed improvement in growth and physiological parameters of mung bean cv. 'PDM-139' under glasshouse conditions. It may be due to the presence of various secondary metabolites released from biocontrol agent which showed nematicidal effect against root-knot nematode. This method of root-knot nematode control may contribute to minimize toxic effect of chemical nematicides on flora and fauna. Hence, the outcome of the results predict that the use of biocontrol agent *P. lilacinus* may provide safe and environmentally reliable approach for the root-knot nematode management programme for sustainable environment.

SIGNIFICANCE STATEMENT

The root-knot nematode, *Meloidogyne incognita* is a sedentary endoparasite attacking a wide range of crops including pulses, vegetables other ornamental crops worldwide and caused dramatic yield loss. So, that management strategy is urgently needed, chemical nematicides is best way for the management of root knot nematodes, but on the other hand, these chemical nematicides were harmful to flora and fauna and very costly

so, farmers does not applied these chemical nematicides in our field. So, biocontrol agent, *Purpureocillium lilacinum* is better option for the management of *M. incognita* because this method of nematode management is eco-friendly and does not leave any toxic impact on environment.

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