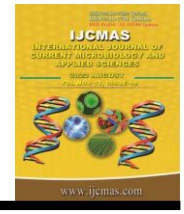
Int.J.Curr.Microbiol.App.Sci (2022) *11*(08): 189-193

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 11 Number 08 (2022)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2022.1108.019

Management of Root-Knot Nematodes (*Meloidogyne* spp.) Using Different Bio Agents in Indian Bean

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Keywords

Management, Bio-agent, Indian bean, *Purpureocillium lilacinum*

Article Info

Received:
05 July 2022
Accepted:
05 August 2022
Available Online:
10 August 2022

A B S T R A C T

Studies on management of root-knot nematodes, *Meloidogyne* spp. on Indian bean using bio-agents under pot condition were carried out at Department of Nematology, Anand Agricultural University, Anand, Gujarat. The pot experiment on efficacy of different bio-agents for the management of root-knot nematodes, *Meloidogyne* spp. in Indian bean implied that application of *Purpureocillium lilacinum* 1% WP (2×10^8 cfu/g) @ 0.1% (w/w) followed by *Pseudomonas fluorescens* 0.5% WP (2×10^8 cfu/g) @ 0.1% (w/w) enriched with FYM reduced root-knot nematode population and increased growth and development of Indian bean.

Introduction

Root-knot nematodes (*Meloidogyne* spp.) are one of the most economically damaging genera of plant-parasitic nematodes on agricultural and horticultural crops globally. Root-knot nematodes are distributed worldwide and are obligate parasites of the roots of thousands of plant species including cereals, oilseeds, herbaceous, fruits, vegetables, pulses, cash crops, ornamental crops, medicinal and aromatic plants, etc. There are more than 75 species of *Meloidogyne*. Indian bean is very important from nutritional view point. Besides, Dolichos is endowed with many medicinal and therapeutic properties. The seeds contain kievitone, which is one of the potential breast cancer fighting flavonoid (Hoffman, 1995).

Numerous plant parasitic nematodes attack pulse crops and the prominent among them are *Meloidogyne* sp., *Heterodera* sp., *Pratylenchus* sp., *Rotylenchulus* sp., *Tylenchorhynchus* sp. and *Helicotylenchus* sp. (Ansari *et al.*, 2017). Indian bean is also infested by root-knot diseases caused by root-knot nematodes. The first detailed investigation of the problem in Sudan was undertaken by Yassin (1974) who referred to 3 species of the root-knot nematodes namely *M. javanica*, *M. incognita* and *M. arenaria*. Estimated annual yield losses in field bean is around 10.9 per cent by root-knot nematodes on world basis (Singh, 2015). Therefore, present investigation was carried out to manage root-knot disease in Indian bean.

Materials and Methods

The present trial was conducted at Department of Nematology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat in 2020-21 to study the effect of various bio-agents for the management of *Meloidogyne* spp. on Indian bean using Completely Randomized Design (CRD) with three repetitions. Earthen pots of 15 cm diameter were washed with tap water and disinfected with 4% formaldehyde (Formalin 40%) solution. Three seeds of Indian bean cv. *Gujarat Papdi* 1 were sown in each pot. After germination, plants were thinned down to one plant/pot.

After drying, pots were filled with nematode infested soil (1 kg/pot) having 288 J_2/200 cc soil using different bio-agents*viz.*,T_1- *Pseudomonas putida* 0.5% WP, T_2-*Pseudomonas fluorescens* 0.5% WP, T_3- *Bacillus amyloliquefaciens* 1.5% AS, T_4- *Bacillus pumilus*1.5% AS, T_5-*Bacillus subtilis* 1.5% AS,T_6- *Bacillus megaterium* 1.5% AS,T_7- *Purpureocilliumlilacinum*1% WP each @ 0.1% (w/w), T_8- Control (Untreated check). Each bio-agents were mixed with FYM @ 10% w/w and this mixture were kept moistened for 15 days. Plants were removed from the pots carefully at 60 DAS and observations were recorded and analysed.

Observations on plant height, fresh shoot and root weight and root-knot index (0-5 scale) were recorded at the time of 1^{st} pulling. Host plant reaction was assessed byusing root-knot galling index followed by Taylor and Sasser (1978) where, 0 = No galling; 0.01-1.0 = 1-2 galls; 1.01-2.0 = 3-10 galls; 2.01-3.0 = 11-30 galls; 3.01-4.0 = 31-100 galls; 4.0-5.0 = >100galls.Roots were cut in to 2-3 cm length and 3g roots were stained in 0.05per centacid fuchsin in lactophenol.

Then roots were washed with tap water to remove excess stain and kept overnight in lactophenol for destaining. Then the roots were examined for nematode population. At the time of termination of experiment final nematode population per 200 cm^3 soil recorded.

Results and Discussion

Maximum plant height was recorded in *Purpureocillium lilacinum* application (T_7). Minimum and significantly lowest plant height was recorded in control. The next to minimum was *Bacillus megaterium* application(T_6).It was followed *by B. pumilus* (T_4) and *Pseudomonas putida* (T_1); all the three being at par with each other. All bio-agents gave significantly more fresh shoot weight as compared to control. Maximum fresh shoot weight was obtained by *P. lilacinum*(T_7) followed by *P. fluorescens* (T_2)application, both being at par with each other. Minimum fresh shoot weight was recorded in control. Fresh root weight was significantly higher in all the treatments over control. The differences for fresh root weight within bioagents were significant. Minimum fresh root weight among bio-agents was obtained by *P. lilacinum*(T_7) followed by *P. fluorescens* (T_2)and *B. subtilis* (T_5) treatments. Maximum fresh root weight was recorded in *B.* megaterium (T_6) application and it was at par with *B. pumilus*(T_4).

Significant reduction in root-knot index was observed in all the treatments compared to control. Lowest root-knot index was recorded in *P. lilacinum* (T_7) application, however, it was at par with *P. fluorescens* (T_2), and *B. subtilis* (T_5). *Bacillus megaterium* (T_6) had maximum root-knot index among the bio-agents followed by *B. pumilus*(T_4)and *B. amyloliquefaciens*(T_3). Regarding root population (no. of females and eggs), *P. lilacinum*(T_7) recorded minimum number of females and eggs per plant followed by *P. fluorescens*(T_2), *B. pumilus* (T_5) and *P. putida* (T_1) and it was maximum among bio-agents in application of *B. megaterium* (T_6). For soil nematode population, all the treatments differed significantly. Minimum soil nematode population was recorded by *P. lilacinum*(T_7) followed by *P. fluorescens*(T_2). Next in order were *B. pumilus* (T_5) and *P. putida* (T_1) applications while maximum soil nematode population was recorded in *B. megaterium* (T_6) followed by *B. pumilus*(T_4), both being at par with each other.

Table.1 Effect of different bio-agents on plant growth and development of Indian bean

Treatments	Plant height (cm)	Fresh weight (g)	
		Shoot	Root
T_1 (*Pseudomonas putida*)	8.6^{cd}	17.43^{c}	3.17^{bc}
T_2 (*Pseudomonas fluorescens*)	11.4^{b}	20.37^{a}	2.97^{bc}
T_3 (*Bacillus amyloliquefaciens*)	9.2^{c}	16.67^{c}	3.23^{b}
T_4 (*Bacillus pumilus*)	7.9^{c}	15.37^{d}	3.80^{a}
T_5 (*Bacillus subtilis*)	10.5^{b}	18.87^{b}	3.03^{bc}
T_6 (*Bacillus megaterium*)	7.4^{d}	13.83^{e}	3.97^{a}
T_7 (*Purpureocillium lilacinum*)	12.7^{a}	21.03^{a}	2.73^{c}
T_8 (Control)	6.0^{e}	10.77^{f}	4.07^{a}
S. Em. ±	0.40	0.38	0.14
CD @ 5%	Sig.	Sig.	Sig.
C.V. %	7.50	3.89	7.06

Figures indicating common letter(s) do not differ significantly from each other at 5% level of significance according to DNMRT.

Table.2 Effect of different bio-agents on multiplication of root-knot nematodes(*Meloidogyne* spp.) on Indian bean

Treatments	RKI (0-5)*	Nematode population/plant				Final nematode population
		Root			Soil	
		No. of females	No. of eggmass	No. of eggs		
T_1 (*Pseudomonas putida*)	1.63^{c} (2.64)**	2.11^{c} (128)***	1.68^{c} (48)***	3.72^{c} (5268)***	2.94^{c} (872)***	3.80^{c} (6318)***
T_2 (*Pseudomonas fluorescens*)	1.00^{d} (1.00)	1.66^{d} (46)	1.19^{e} (15)	3.15^{e} (1409)	2.35^{e} (222)	3.23^{e} (1699)
T_3 (*Bacillus amyloliquefaciens*)	1.73^{bc} (3.00)	2.24^{bc} (175)	1.75^{c} (57)	3.78^{c} (5973)	3.03^{bc} (1060)	3.86^{c} (7271)
T_4 (*Bacillus pumilus*)	1.82^{bc} (3.32)	2.19^{c} (154)	1.93^{b} (86)	3.90^{bc} (7883)	3.15^{ab} (1398)	3.98^{bc} (9526)
T_5 (*Bacillus subtilis*)	1.14^{d} (1.30)	1.80^{d} (63)	1.47^{d} (29)	3.47^{d} (2958)	2.70^{d} (499)	3.55^{d} (3562)
T_6 (*Bacillus megaterium*)	1.91^{b} (3.65)	2.39^{ab} (247)	2.04^{ab} (111)	4.01^{ab} (10319)	3.22^{a} (1666)	4.09^{cb} (12353)
T_7 (*Purpureocillium lilacinum*)	1.00^{d} (1.00)	1.35^{e} (22)	0.54^{f} (3)	2.49^{f} (308)	2.14^{f} (137)	2.68^{f} (474)
T_8 (Control)	2.16^{a} (4.65)	2.55^{a} (356)	2.12^{a} (133)	4.09^{a} (12369)	3.27^{a} (1883)	4.17^{a} (14774)
S. Em. ±	0.08	0.03	0.05	0.04	0.04	0.04
CD @ 5%	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
C.V. %	9.06	2.76	5.15	2.17	2.24	1.69

*0 = Free; 5 = Maximum disease intensity

**Figures in parentheses are re-transformed values of $\sqrt{x}$

***Figures in parentheses are re-transformed values of log

Figures indicating common letter(s) do not differ significantly from each other at 5% level of significance according to DNMRT.

The overall results indicated that among the different bio-agents, *P. lilacinum* and *P. fluorescens* proved to be best in improving the plant growth and development and reducing host infestation than the other treatments. *Purpureocillium lilacinum* proved to be more effective in improving the plant growth characters and reducing the root-knot index and final soil nematode population.

In our studies where *P. lilacinus* and *P. fluorescens* were used separately suppressed the activity of the *Meloidogyne* spp. The effectiveness of bio-agents like, *P. lilacinum* and *P. fluorescens* has been reported by Singh *et al.,* (2011) who observed increase in plant growth characters and reduction in the population of root-knot nematode in chick pea.

Similarly, significant reduction of gall formation and less number of female nematodes in black gram roots due to application of *P. fluorescens* @ 1% (v/w) was reported by Bharali *et al.,* (2019).

Significant reductions in number of galls per root system as compared to the control treatment were observed when isolates of *P. variotii* and *P. lilacinus* were used as reported by Perveen and Shahzad (2013). Siddiqui and Akhtar (2009) reported that *P. lilacinus* KIA caused a greater increase in the growth of nematode inoculated plants than caused by *P. putida* 3604 or *P. alcaligenes* 493 in chick pea to manage *Meloidogyne javanica*. *P. lilacinus* KIA caused a greater reduction in galling and nematode multiplication. Hasan (2004) investigated that seed treatment with the fungus *P. lilacinus* was found to be more effective in reducing the root-knot incidence and increasing the forage yield of cowpea. Vyas *et al.,* (1996) found effective result of *P. lilacinus* @ 1 t/ha based on neemcake treatment for the management of root knot nematodes in chickpea. Kar *et al.,* (2018) reported amendment of soil with bioagents *i.e. P. fluorescens* @ 20 g/m^2 and *P. lilacinum* @ 20 g/m^2 along with neemcake @ 100 g/m^2 has given higher cowpea yield than the solitary application of the bioagents. The final soil nematode populations was significantly found less in number and reduced nematode multiplication.

An experiment was conducted to study the different bio-agents *viz*., *Pseudomonas putida*, *P. fluorescens*, *Bacillus amyloliquefaciens*, *B. pumilus*, *B. subtilis*, *B. megaterium* and *Purpureocillium lilacinum* each @ 0.1% (w/w) enriched with FYM against *Meloidogyne* spp. on Indian bean cv. *Gujarat Papdi* 1. Application of bio agents enhanced growth and reduced root-knot index and root and soil nematode population. Maximum plant growth was recorded in*P. Lilacinum* followed by *P. fluorescens* and *B. subtilis*. Maximum reduction in root-knot index (RKI), number of females, eggmass, eggs, soil and final nematode population/plant and total nematode population observed in the treatment of *P. lilacinum*.

Bio-agent *P. lilacinum* @ 0.1% (w/w) enriched with FYM found most effective to manage root-knot nematodes and increased plant growth with reduction in root-knot index. The second best treatment was *P. fluorescens* @ 0.1% (w/w) enriched with FYM which increased plant growth with reduction in root-knot index.

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How to cite this article:

Himadri S. Patel and Tulika Singh. 2022. Management of Root-Knot Nematodes (*Meloidogyne* spp.) Using Different Bio Agents in Indian Bean. *Int.J.Curr.Microbiol.App.Sci.* 11(08): 189-193.
doi: https://doi.org/10.20546/ijcmas.2022.1108.019