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An Eco-friendly Management option against Root-knot Nematode, (*Meloidogyne incognita*) Infecting Bitter gourd (*Momordica charantia* L.)

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ABSTRACT: Bitter gourd is very important and healthy vegetable crop worldwide. Bitter gourd is greatly affected by several biotic factors and abiotic factors. Among biotic factors fungi, bacteria, insect pests and nematodes cause a heavy loss in quantity and quality of fruits. Among plant parasitic nematode, root-knot nematode (*Meloidogyne incognita*) is the most widely distributed and the destructive nematode. It is considered as one of the major limiting factors in the production of bitter gourd. Due to heavy losses incurred by these ting organisms, it become a tough task to manage them economically. Owing to adverse impacts of chemicals on human health, there is need of some ecofriendly methods of nematode management. Biological nematode control in relation to crop production system is a subject of considerable current interest, because of a perceived urgency to develop and adopt safe, economic and efficient method for managing nematode pest of crops. During present investigation, vermicompost enriched with bio-agents *viz., Beauveria bassiana, Purpureocillium lilacinum* and *Metarhizium anisopliae* were tested as soil treatment @ 2.5 g, 5 g and 10 g/plant for the management of *M. incognita* on bitter gourd. Results showed that *P. lilacinum* @ 10 g/plant was found most effective followed by *Beauveria bassiana* @ 10 g/plant and *Metarhizium anisopliae* @ 10 g/plant which enhanced the plant growth parameters of bitter gourd and also reduced the nematode multiplication factors.

Keywords: Beauveria bassiana, Bio-agents, Bitter gourd, Meloidogyne incognita, Metarhizium anisopliae, Purpureocillium lilacinum and Vermicompost.

INTRODUCTION

Bitter gourd (Momordica charantia L.) is an indigenous vegetable that is grown under tropical regions of Asia. It is also having medicinal properties. Even though whole plant is palatable in nature but it is mainly grown for its fruit part. It is an often-discarded vegetable due to its bitter taste despite the fact that it is a source of several key nutrients such as triterpenes, proteins and steroids. It is also rich in term of minerals such as Cu, Fe, Mg, Zn and Ca. Many fatty acids were identified in M. charantia include lauric, myriaatic, palmitic, stearic and linoleic. The other components of charantin have been shown extremely effective in regulating blood glucose levels in those with insulin resistance. Hence, it is advised to use for diabetes patients in addition, research is undertaken to improve functionality in areas including obesity and colon cancer prevention (Krawinkel et al., 2006). It has been known for its

hypoglycemic effects. It is also very useful to prevent development of gastric and duodenal ulcers. Fruits, leaves and seeds of bitter gourd are traditionally used as medicinal herbs as anti-HIV. anti-ulcer. antiinflammatory, anti-leukemic, antimicrobial, antidiabetic and anti-tumor. The most prominent states in bitter gourd production are Rajasthan, Chhattisgarh, Telangana, Andhra Pradesh, Odisha and Madhya Pradesh. At present, about 1391.80 thousand MT of bitter gourd is produced an area of 111.40 thousand hectare in India. Out of which 2.29 thousand tonnes from 0.63 thousand hectare area are produced in Rajasthan (Anonymous, 2023). However, major problems related to the production of bitter gourd is greatly affected by several biotic and abiotic factors. Among biotic, major influencing factors are fungi, bacteria, insect pests and nematodes. The important plant parasitic nematodes (PPNs) associated with vegetables are Meloidogyne spp., Rotylenchulus

reniformis, Pratylenchus spp., Helicotylenchus spp. and Hoplolaimus spp. Among PPNs, root-knot nematodes (Meloidogyne spp.) are the most widely distributed and the destructive group. Because of its worldwide distribution, extensive host range and destructive nature, these are considered as one of the major devastating factors in the production of vegetable crops. The infected plants show the symptoms of stunting growth and drying of leaves which ultimately result into heavy yield loss. In bitter gourd, it was reported that root-knot nematode could result in a yield loss of 13.50 per cent which reflects the monetary value Rs. 252.82 million (Kumar et al., 2020). Sharma and Baheti (1992) reported losses to the tune of 46 - 56 per cent on okra, tomato and bottle gourd infecting with root-knot nematode, M. incognita and M. javanica under light soil of Rajasthan. Jain et al. (2007) have worked out the monetary loss to the extent of 547.50 million rupees in cucurbits due to M. incognita with an average yield loss to the tune of 18.20 per cent in India. Kaur and Pathak (2011) reported that 38 - 48.2 per cent yield loss had been incurred due to M. incognita on bitter gourd. Root-knot nematode has been found to cause yield losses of 69.2 per cent in cucumber (Krishnaveni and Subramanian, 2003) and 66.84 per cent in cucumber (Bhati and Baheti 2021).

MATERIALS AND METHODS

An experiment was carried out to find out the effect of vermicompost enriched with bio-agents viz., Metarhizium anisopliae, Purpureocillium lilacinum and Beauveria bassiana for the management of rootknot nematode, M. incognita on bitter gourd. Vermicompost enriched with bio-agents (5 kg bioagents in 100 kg vermicompost) were used at three different doses i.e. 2.5g, 5g and 10g/plant along with an untreated check. All the treatments were replicated three times through completely randomized design (C.R.D.). Earthen clay pots were washed, cleaned and disinfected before use by rinsing them through four per cent formalin solution. Then pots were filled with infested soil having an initial inoculum of 420 larvae/100 cc soil with test nematode. Three bitter gourd seeds were sown in each pot. After 12 days of germination, one healthy plant in each pot was maintained and others were uprooted carefully.

Treatments (Plate 1) viz., Vermicompost enriched with anisopliae @2.5g/plant Metarhizium (T1), Vermicompost enriched with Metarhizium anisopliae @5g/plant (T2), Vermicompost enriched with @10g/plant Metarhizium anisopliae (T3), Vermicompost enriched with Purpureocillium lilacinum @2.5g/plant (T4), Vermicompost enriched with Purpureocillium lilacinum @5g/plant (T5), Vermicompost enriched with Purpureocillium lilacinum @10g/plant (T6), Vermicompost enriched with Beauveria bassiana @2.5g/plant (T7). Vermicompost enriched with Beauveria bassiana @5g/plant (T8), Vermicompost enriched with Beauveria bassiana @10g/plant (T9) and Untreated check (T10).

Plants were harvested after 60 days of

germination. Observations on vine length (cm), root length (cm), vine weight (g) and vine weight (g) were recorded at harvest of experiment. The roots were washed carefully under tap water and stained with 0.1 per cent acid fuschin and thereafter, kept in plain lactophenol for at least for 24 hrs for distaining of roots. These roots were examined thoroughly under a stereoscopic binocular microscope for counting number of galls per plant, number of egg masses per plant. After removing the plant from the pot, soil of each treatment was thoroughly mixed and 200 cc soil from each pot were taken and processed by Cobb's sieving and decanting technique followed by Baermann's funnel technique for estimation of nematode population in soil.

RESULTS

A. Plant Growth Parameters

1. Vine length. Results presented in Table 1 and Fig. 1, 3 & 5 showed that vermicompost enriched with different bio-agents found significantly superior over untreated check. Among treatments, maximum vine length was recorded 169.30 cm in vermicompost enriched with *P. lilacinum* @10 g per plant followed by *B. bassiana* (163.06 cm) and *M. anisopliae* (158.16 cm) at same dose. *M. anisopliae* when applied @2.5 g per plant (125.33 cm) was found to be least effective but significantly superior over untreated check (98.16 cm).

Experimental findings indicated that vermicompost enriched with *P. lilacinum* @10 g per plant showed maximum increase in vine length (72.48%) followed by *B. bassiana* @10 g per plant (66.12%) and *M. anisopliae* @10 g per plant (61.13%) over the untreated check. Minimum increase in vine length was obtained with *M. anisopliae* @2.5 g per plant (27.68%).

2. Root length: Result revealed in Table 1 and Fig. 1, 3 & 5 that all the treatments remarkable enhanced the root length of bitter gourd over untreated check. Among treatments, vermicompost enriched with *P. lilacinum* @10 g per plant (32.23 cm) was found to be most effective followed by *B. bassiana* (30.00 cm) and *M. anisopliae* (28.00 cm) @10g over untreated control (17.86 cm). Minimum root length (20.06 cm) was recorded with *M. anisopliae* @2.5 g per. These treatments were significantly differed from each other.

Results illustrated that maximum increase in root length over untreated check was found in the treatment of vermicompost enriched with *P. lilacinum* @10 g per plant(80.46%) subsequently by *B. bassiana* @10 g per plant (67.98%) and *M. anisopliae* @10 g per plant (556.78%). Minimum increase in root length was obtained 12.32 per cent with *M. anisopliae* @2.5 per plant as compared to untreated check.

3. Vine weight: The findings showed that the vine weight of the bitter gourd produced significantly with each treatment of vermicompost enriched with various bio-agents as compared to the untreated control. Among various treatments, maximum vine weight observed 42.33 g with vermicompost enriched with *P. lilacinum* @10 g per plant followed by *B. bassiana*

@10 g per plant (39.40 g) and *M. anisopliae* @10 g per plant (36.93 g). *M. anisopliae* @2.5 g per plant (27.13

g) was found to be least effective but significantly superior over control (23.30 g).

Table 1: Effect of vermicompost enriched with bio-agents against root-knot nematode, Meloidogyne
incognita on plant growth of bitter gourd.

Tr. No.	Detail of Treatments	Vine length (cm)	Root length (cm)	Vine weight (g)	Root Weight (g)	Per cent decrease over untreated check			
						Vine length	Root length	Vine weight	Root Weight
T1	Vermicompost enriched with <i>Metarhizium</i> <i>anisopliae</i> @ 2.5g/plant	125.33	20.06	27.13	5.80	27.68	12.32	16.43	11.54
T2	Vermicompost enriched with Metarhizium anisopliae @5g/plant	143.56	24.30	34.00	6.76	46.26	36.05	45.93	30.00
T3	Vermicompost enriched with <i>Metarhizium</i> <i>anisopliae</i> @10g/plant	158.16	28.00	36.93	8.03	61.13	56.78	58.49	54.43
T4	Vermicompost enriched with Purpureocillium lilacinum @2.5g/plant	127.93	21.53	28.53	6.90	30.33	20.54	22.45	15.92
T5	Vermicompost enriched with Purpureocillium lilacinum @5g/plant	147.56	25.46	34.86	7.00	50.33	42.56	49.62	34.62
T6	Vermicompost enriched with <i>Purpureocillium</i> <i>lilacinum</i> @10g/plant	169.30	32.23	42.33	9.73	72.48	80.46	81.25	87.12
Τ7	Vermicompost enriched with <i>Beauveria bassiana</i> @2.5g/plant	126.40	20.26	27.80	5.90	35.69	13.43	19.32	13.46
Т8	Vermicompost enriched with <i>Beauveria bassiana</i> @5g/plant	145.16	24.66	34.40	6.03	47.89	38.07	47.64	32.69
Т9	Vermicompost enriched with Beauveria bassiana @10 g/plant	163.06	30.00	39.40	9.00	66.12	67.98	69.09	73.08
T10	Untreated check	98.16	17.86	23.30	5.20				
	m±	0.23 3.63	0.54	0.59	0.10				
CD	CD at 5%		1.59	1.75	0.28				



Plate 1. Ecofriendly management of root-knot nematode, *M. incognita* infecting bitter gourd (*Momordica charantia* L.).

Result illustrated in Table 1 and Fig. 1, 3 & 5 revealed that maximum increase (81.25%) in vine weight was observed when vermicompost enriched with *P. lilacinum* @ 10g per plant followed by *B. bassiana* @ 10 g per plant (69.09%) and *M. anisopliae* @10 g per plant (58.49%). However, minimum weight was obtained (16.43%) with *M. anisopliae* @2.5 g per plant over control.

4. Root weight: Data presented in Table 1 and Fig. 1; 3 & 5 clearly showed that vermicompost enriched with bio-agents increased root weight as compared to untreated check. Among various treatments, maximum root weight was recorded with *P. lilacinum* @10 g per plant ((9.73 g) followed by *Beauveria bassiana* (9.00 g) and *M. anisopliae* (8.03 g) @10 g per plant. These treatments found significantly better over untreated check (5.20 g). *M. anisopliae* applied @2.5 g per plant (5.80 g) was found least effective with regards to root weight.

Experimental findings showed maximum increase in root weight with *P. lilacinum* @10 g per plant (87.12) followed by *B. bassiana* @10 g per plant (73.08%) and *M. anisopliae* @10 g per plant (54.43%). Minimum increase in root weight (11.54%) was obtained with *M. anisopliae* @ 2.5 g per plant over untreated check.

B. Nematode Reproduction Parameters

1. Number of galls per plant: It is presented in Table 2 and Fig. 2, 4 & 6 that all treatments significantly reduced the number of galls per plant over untreated control. Among various treatments, lowest numbers of gall per plant was found in vermicompost enriched with *P. lilacinum* @10 g/ plant (28.66) followed by *B. bassiana* @10 g per plant (32.33) and *M. anisopliae* @10 g per plant (35.33) over untreated control (93.33). These treatments were significantly different in case of number of galls per plant from each other. Highest numbers of gall per plant @2.5 g per plant (67.33).

Results showed the maximum reduction in number of galls per plant in the treatment of vermicompost enriched with *P. lilacinum* @ 10 g per plant (69.30%) followed by *B. bassiana* @ 10 g per plant (65.36%) and

M. anisopliae @10 g per plant (62.15%). Minimum reduction was obtained with *M. anisopliae* @2.5 g per plant (27.85%) over the untreated control.

2. Number of egg-masses per plant: Result depicted in Table 2 and Fig. 2, 4 & 6 showed that all the treatments found significantly superior over untreated check to reduce number of egg masses per plant by root-knot nematode on bitter gourd. Among treatments, vermicompost enriched with *P. lilacinum* @10g per plant (23.33) proved to be most effective followed by *B. bassiana* @10 g per plant (29.00) and *M. anisopliae* @10 g per plant (32.33). These treatments significantly differ from each other. *M. anisopliae* applied at 2.5 g per plant (61.33) was found to be least effective over untreated check (84.66).

Experimental findings in Table 2 showed that vermicompost enriched with *P. lilacinum* @10 g per plant reduced galls per plant to the tune of 72.25 per cent while in *B. bassiana* @ 10 g per plant (65.78%) and *M. anisopliae* @10 g per plant (64.82%). It was observed to be minimum (27.56 %) with *M. anisopliae* @2.5 g per plant over the check.

3. Final Nematode Population per 200 cc soil: Data illustrated through Table 2 and Fig. 2, 4 & 6 showed that all the treatments were significantly reduced final nematode population as compared to untreated check. Minimum nematode population was recorded in vermicompost enriched with *P. lilacinum* @10 g per plant (495.00) followed by *B. bassiana* (508.00) and *M. anisopliae* (519.33) at same dose. All treatments were significantly different from each other. *M. anisopliae* was found least effective when applied @2.5 g per plant (770.00) but found significantly superior over untreated check (1090.66).

Experimental results showed that highest reduction in final nematode population per 200 cc soil was in *P. lilacinum* @10 g per plant (54.62%). It was determined 53.39 and 52.38 per cent in *B. bassiana* @ 10 g per plant and *M. anisopliae* @10 g/ plant, respectively. Minimum reduction in nematode population per 200cc soil was noticed in *M. anisopliae* @2.5 g per plant (29.40%) over untreated check.

infecting bitter gourd.											
		No. of	No. of	Final	Per cent o	lecrease over u					
Tr. No.	Detail of Treatments	galls/ plant	egg masses/ plant	Nematode Population/ 200cc soil	No. of galls/plant	No. of egg masses/plant	Final Nematode Population/200cc soil				
T1	Vermicompost enriched with <i>Metarhizium</i> <i>anisopliae</i> @ 2.5g/plant	67.33	61.33	770.00	27.85	27.56	29.40				
T2	Vermicompost enriched with <i>Metarhizium</i> <i>anisopliae</i> @5g/plant	49.33	46.66	682.00	47.15	44.89	37.47				
Т3	Vermicompost enriched with <i>Metarhizium</i> anisopliae @10g/plant	35.33	32.33	519.33	62.15	64.82	52.38				
T4	Vermicompost enriched with <i>Purpureocillium</i> <i>lilacinum</i> @2.5g/plant	63.66	56.00	754.00	31.80	33.86	30.86				
T5	Vermicompost enriched with <i>Purpureocillium</i> <i>lilacinum</i> @5g/plant	44.33	39.66	664.66	52.51	53.16	39.06				
T6	Vermicompost enriched with <i>Purpureocillium</i> <i>lilacinum</i> @10g/plant	28.66	23.33	495.00	69.30	72.25	54.62				
T7	Vermicompost enriched with <i>Beauveria bassiana</i> @2.5g/plant	66.66	60.67	766.66	28.58	28.35	29.66				
T8	Vermicompost enriched with <i>Beauveria bassiana</i> @5g/plant	46.66	43.33	678.00	50.00	49.00	37.84				
T9	Vermicompost enriched with <i>Beauveria bassiana</i> @10 g/plant	32.33	29.00	508.00	65.36	65.78	53.39				
T10	Untreated check	93.33	84.66	1090.66							
	SEm±	1.31	1.16	2.05							
	CD at 5%	2.82	3.44	6.05							

 Table 2: Effect of vermicompost enriched with bio-agents against root-knot nematode, Meloidogyne incognita infecting bitter gourd.

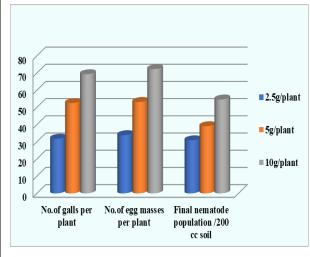
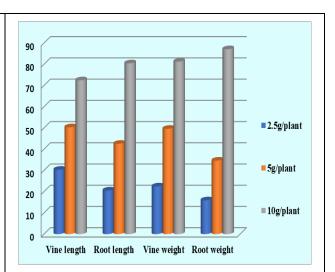
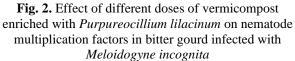
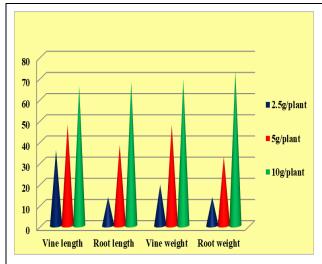
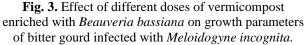


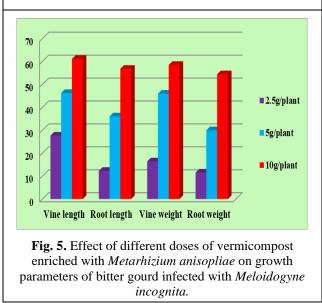
Fig. 1. Effect of different doses of vermicompost enriched with *Purpureocillium lilacinum* on growth parameters of bitter gourd infected with *Meloidogyne incognita*











DISCUSSION

Eco-friendly approaches are the need of the hours as these are safe for human beings and environment. The earliest record of fungi as antagonists of PPNs was reported by Duddington (1954), Mankau (1980) and Jatala (1985). Organic amendments cause relatively rapid decline in nematode population due to decomposition and release of toxic compounds and increase in nematode antagonists. The efficacy of various combination of bio-agents with organic amendments viz., FYM, vermicompost, Poultry manure and oil cakes etc. were also reported by Pandey et al. (2005), Mehta et al. (2016), Pathan et al. (2005), Baheti et al. (2015) and Bhati et al. (2019). In present investigation, efficacy of vermicompost enriched with bio-agents viz. Metarhizium anisopliae, Paecilomyces lilacinum and Beauveria bassiana were tested at 2.5g, 5g and 10g doses per plant as soil application for the management of root-knot nematode, M. incognita on bitter gourd.

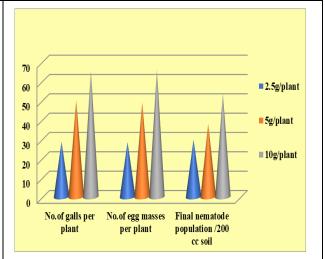
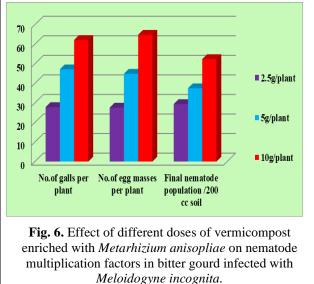


Fig. 4. Effect of different doses of vermicompost enriched with *Beauveria bassiana* on nematode multiplication factors in bitter gourd infected with *Meloidogyne incognita*.



Experimental results exhibited that various combination of different bio-agents with vermicompost were found significantly superior over untreated control. Among the treatments, maximum vine length was recorded in vermicompost enriched with P. lilacinum @10 g per plant followed by B. bassiana and M. anisopliae. M. anisopliae when applied @2.5 g per plant was found to be least effective but better than untreated check. Almost similar trend was noticed pertaining to other plant growth characters viz., root length, vine weight and root weight. Pandey et al. (2005) reported the effect of bio-agent and neem cake against Meloidogyne incognita on chickpea. The treatments comprised of neem cake, Trichoderma harzianum, T. viride, Paecilomyces lilacinus, Aspergillus niger and Verticillum chlamydosporium. The combined application of neem cake and bio-agents significantly increased shoot weight, root weight and chlorophyll content of chickpea. Root and shoot length were highest in integrated application of bio-agents with neem cake. Bhati et al. (2019) tested P. lilacinus,

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Pochonia chlamydosporia and Glomus fasiculatum as seed treatment at 6 and 12 g/kg seed on bitter gourd. Results revealed that P. lilacinus found to be most effective followed by P. chlamydosporia and Glomus fasiculatum at 12 g/kg seed to enhance plant growth and to reduce the infection of M. incognita. In support to our study different bioagents viz T. viride, Pseudomonas fluorescence and Purpureocillium lilacinum were found effective in suppression of rootknot nematode at different dose in cucumber crop when applied as soil applications under field conditions (Patil et al., 2021). The suppression of root-knot nematode with application of vermicompost enriched with bioagents observed due to the fact that organic amendment (vermicompost) decomposes in soil and improve the physical properties of soil, increase the population of natural enemies of plant parasitic nematodes and increase the activity of beneficial microbes in soil. These all factors enhance the growth of bitter gourd infested with root-knot nematode.

In all treatments final nematode population was significantly reduced as compared to untreated check. It was recorded that minimum population was found in vermicompost enriched with bio-agents P. lilacinum @ 10 g per plant followed by B. bassiana and M. anisopliae at same dose. M. anisopliae was found least effective when applied at 2.5 g per plant but found significantly superior over untreated check. Correspondingly, all other nematode reproduction characters viz., number of galls and number of egg masses per plant noticed almost similar effect during present investigation. Results in present investigation are in the accordance with findings of earlier workers (Rao et al., 1997; Krishnaveni and Subramanian, 2004; Sharma et al., 2012; Kumari et al., 2020 and Bhati et al. 2022; Kumar et al., 2022). Rao et al. (1997) observed that seed treatment with Purpureocillium lilacinum have shown lowest root-knot index and final population of Meloidogyne incognita with increased fruit yield in cucumber. Nagesh et al., 2003 reported the combined use of P. lilacinum with neem cake enhanced fungal propagules in rhizosphere, fungal infectivity and yield in chrysanthemum depending on the dose of formulation and reduced M. incognita population. Bhati et al. (2022) observed impact of bioagents against Meloidogyne incognita infecting cucumber in polyhouse. Results exhibited that all the bio-agents significantly reduced nematode reproduction and enhanced the plant growth of cucumber over untreated check. The highest reduction in root galls 60.63%, egg masses 55.46%, egg mass contents 27.54% and nematode population 66.29% recorded with T. viride @ 5.0g per plant over untreated check on cucumber in poly-house followed by application of P. lilacinus and T. harzianum @ 5.0 g per plant.

The studies clearly showed that bio-agents coupled with organic amendments increased the plant growth and decreased nematode population in agrihorticulture crops including bitter gourd. The probable reason may be due to reduction in nematode population by patriotization of eggs and females of M. incognita and competition for food and space for nematode survival. During decomposition vermicompost releases numbers of substances and enhance solubilization of insoluble nutrients in soil phase. They are directly positive correlation with bioagent and increasing population of bioagents. Proliferation of bio-agents are highly antagonistic microorganism resulting in higher antagonistic effect of combined inoculations. The nematode activity may be negatively impacted by nematotoxin generated by P. lilacinum (Paecilotoxin) and Beauveria bassiana (Beauvericin) as well as it damages through appressoria on the egg shell and reducing their fecundity. If eggs are produced, these are colonized on developing juveniles inside egg and destroyed by the rapidly growing hyphae. Many enzymes (proteases & chitinase) also produced by fungus. These enzymes have nematicidal activity and caused degradation of the eggshell and inhibits hatching. Hence, the reduction in the nematode population resulted by a way of fugal attack on larvae or death of females before egg laying.

CONCLUSIONS

Utilization of eco-friendly techniques is a trending current issue and for future research. Due to the wide versatility of this area and synergistic or additive effect with other agricultural inputs, it can be included in eco-friendly management approach. Present investigation focuses on the use of bio-agents and organic amendments in sustainable agriculture system and opens vistas for the use of bio-nematicides which are promising as well as ecologically sound and safe. Hence, efforts in discovering new non-chemical or eco-friendly strategies for nematode management should be continued to overcome future challenges too.

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Conflict of interest. There is no conflict of interest among authors.

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