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Bio-agents: An effective method for suppression of root-knot nematode, *Meloidogyne incognita* infecting bitter gourd (*Momordica charantia* L.) as seed treatment

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Abstract
Bitter gourd is very important and healthy vegetable worldwide. Pest problems related to the production of bitter gourd is greatly affected by several biotic factors *i.e.* fungi, bacteria, insect pests and nematodes. The plant parasitic nematode, root-knot nematode (*Meloidogyne incognita*) is the most widely distributed and the destructive nematode. Because of its worldwide distribution, extensive host range and destructive nature, it is considered as one of the major limiting factor in the production of vegetable crops. 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. Bio-agents *viz*., *Paecilomyces lilacinus, Pochonia chlamydosporia* and *Glomus fasiculatum* were tested as seed treatment @ 6 and 12 g/kg for the management of root-knot nematode, *M. incognita* on bitter gourd. A standard (*Trichoderma viride* at 12 g/kg seed) and untreated check was also maintained for comparison. The results of experiment showed that *Paecilomyces lilacinus* at 12 g/kg seed was found most effective followed by *Pochonia chlamydosporia* at 12 g/kg seed and *Glomus fasiculatum* at 12 g/kg seed to enhancing plant growth of bitter gourd and to reduce the infection of *M. incognita*. Seed treatment with *Trichoderma viride* at 12 g/kg seed was found most effective to enhanced plant growth of bitter gourd and management of *M. incognita*.

Keywords: Bio-agents, seed treatment, *Meloidogyne incognita* and bitter gourd

Introduction
Bitter gourd (*Momordica charantia* L.), an indigenous vegetable to tropical regions of Asia. Taxonomically, bitter gourd is belonging to the Cucurbitaceae. Locally, bitter gourd was known as bitter melon (England), balsam-pear, margose (French), *Balsambirne peria* (Malaysia) and karella (India). These reflect that Bitter gourd globally was recognized as vegetables [18]. Plants contains an active phytochemicals compounds which important for human, such as triterpenes, proteins and steroids. Scholars point out that *M. charantia* rich in term of minerals such as Cu, Fe, Mg, Zn, and Ca. There are also fatty acid were identified in *M. charantia* includes lauric, myriaatic, palmitic, stearic, and linolaic [20]. Scholars point out that Charantin is a potential substance with anti-diabetic properties characteristics [6] and could be used to treat diabetes [14]. Momordicin is the functional components of bitter gourd and it was found in fruits, leaves and roots. Further components of charantin are reported to be highly effective for controlling the blood glucose in relation to insulin resistance. In addition, there is a study on the improvement of the functionality, such as obesity, gourd and colon cancer prevention in progress [11]. In spite of modern medical science and the development of diabetes, hypertension, heart disease and the occurrence of cancer increases steadily. Bitter gourd has been known for its hypoglycemic effects. It’s also identified contributes to prevents development of gastric ulcers and duodenal ulcers. Fruits, leaves and seeds of bitter gourd are traditionally used as medicinal herbs as anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, antimicrobial, anti-diabetic, and anti-tumour to name a few.
Bitter gourd is recognized as a healthy vegetable and becomes one of the important commodities. There are however, pest problems related to the production of bitter gourd is greatly affected by several biotic factors *i.e.* fungi, bacteria, insect pests and nematodes. The plant parasitic nematodes associated with vegetables are *Meloidogyne* spp., *Rotylenchulus reniformis, Heterodera* spp., *Pratylenchus* spp., *Helicotylenchus* spp. and *Hoplolaimus* spp. in

India. Among the plant parasitic nematodes, 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 limiting
factors in the production of vegetable crops. The infected plants show stunting and drying of leaves, which ultimately results in affecting the growth of the plant as well as yield. Losses due to nematodes may approach 100 per cent i.e. complete failure of the crop [3]. Cucurbits are known to be highly susceptible to root-knot nematodes [17]. In bitter gourd, it was reported that root-knot nematode could result in a yield loss of 36.72 per cent [2], in bottle gourd 46.56 per cent [16], in okra 41.30 to 45.50 per cent [1] and in cucumber 69.2 per cent [7].

Materials and Methods

Bio-agents play an important role in management of plant parasitic nematodes. With this view, trial was conducted to test the efficacy of promising bio-agents *viz., Paecilomyces lilacinus*, *Pochonia chlamydosporia* and *Glomus fasiculatum* as seed treatment @ 6 and 12 g/kg for the management of root-knot nematode, *M. incognita* on bitter gourd.

Bitter gourd seeds treated with three different bioagents *viz., Paecilomyces lilacinus*, *Pochonia chlamydosporia* and *Glomus fasiculatum* at the rate of 6 and 12 g/kg seeds. A standard (*Trichoderma viride* @ 12 g/kg seed) and untreated check was also maintained for comparison of experimental results. For treating the seeds, the weighed quantity of seeds for each treatment was taken in a beaker with light moisture and thereafter required amount of bio-agent was added to it, mixed thoroughly to give uniform coating over seeds. Sowing was done with dibbling method in each experimental pot.

All the treatments were replicated four times in completely randomized design. Initial nematode population was estimated prior to sowing, 100 cc soil was taken and processed by using Cobb's sieving and decanting technique followed by Baermann's funnel technique. All agronomical practices viz., weeding, hoeing, irrigation Etc. were performed as and when required throughout the cropping period. The observations on various aspects *viz.* vine length, vine weight, root length, root weight, number of galls/plant, egg masses/plant, eggs and larvae/egg mass and final nematode population/100 cc soil were recorded. After completion of experiments, data were analyzed for interpretation of findings.

Results

Experiment was conducted to study the effect of bio-agents as seed treatment on plant growth characters and reproduction of *M. incognita* on Bitter Gourd. Under this trial, fungal bio-agents *viz., Paecilomyces lilacinus, Pochonia chlamydosporia* and *Glomus fasiculatum* were used as seed treatment @ 6 and 12 g/kg seed. A treated check (*Trichoderma viride* at 12 g/kg seed) and untreated check was also maintained to compare the experimental findings. Observations on plant growth characters and nematode were recorded, analyzed and presented in Table-1 & 2.

Table 1: Effect of bio-agents as seed treatment against root-knot nematode, *Meloidogyne incognita* infecting Bitter Gourd

Treatment		Plant Parameters				Nematode Parameters			
		Vine length (cm)	Root length (cm)	Vine weight (g)	Root weight (g)	No. of galls/Plant	No. of egg masses/plant	No. of eggs & larvae/ egg mass	Nematode population/ 100 cc soil
T_1	*Paecilomyces lilacinus* @ 6 g/Kg Seed	142.25	22.50	244.50	24.25	74.75	68.50	226.00	598.00
T_2	*Paecilomyces lilacinus* @ 12 g/Kg Seed	168.00	28.75	277.25	29.75	36.00	32.25	194.50	476.00
T_3	*Pochonia chlamydosporia* @ 6 g/Kg Seed	138.75	20.25	236.50	22.5	81.25	77.00	238.75	646.00
T_4	*Pochonia chlamydosporia* @12 g/Kg Seed	162.25	26.25	262.50	28.25	45.75	42.75	202.25	518.00
T_5	*Glomus fasiculatum* @ 6 g/Kg Seed	132.50	18.50	225.50	21.5	88.25	83.75	254.50	686.00
T_6	*Glomus fasiculatum* @12 g/Kg Seed	157.25	24.25	258.25	26.75	52.50	47.25	209.50	544.00
T_7	*Trichoderma viride* @12 g/Kg Seed	172.50	30.25	285.25	31.25	28.00	22.50	186.75	436.00
T_8	Control	103.75	15.50	152.50	16.25	164.00	119.50	318.25	1482.00
	SEm ±	2.512	1.470	3.320	1.467	2.180	1.670	1.776	31.216
	CD at 5 %	5.412	4.228	7.582	3.527	4.816	3.871	5.640	82.442

Data are the average value of four replications INP-Initial Nematode Population 620 larvae/100 cc Soil

Table 2: Per cent changes in plant growth and nematode reproduction with the application of bio-agents as seed treatment infecting Bitter Gourd

Treatment		Per cent increase over control				Per cent decrease over control			
		Vine length	Root length	Vine weight	Root weight	No. of galls/Plant	No. of egg masses/plant	No. of eggs & larvae/ egg mass	Nematode population/ 100 cc soil
T_1	*Paecilomyces lilacinus* @ 6 g/Kg Seed	37.37	45.16	60.32	49.23	54.42	42.67	28.98	59.64
T_2	*Paecilomyces lilacinus* @ 12 g/Kg Seed	62.37	85.48	81.80	83.07	78.04	73.01	38.88	67.88
T_3	*Pochonia chlamydosporia* @ 6 g/Kg Seed	33.98	30.64	55.08	38.46	50.45	35.56	24.98	56.41
T_4	*Pochonia chlamydosporia* @12 g/Kg Seed	56.79	69.35	72.13	73.84	72.10	64.22	36.44	65.04
T_5	*Glomus fasiculatum* @ 6 g/Kg Seed	27.91	19.35	47.86	32.30	46.18	29.91	20.03	53.71
T_6	*Glomus fasiculatum* @12 g/Kg Seed	51.94	56.45	69.34	64.61	67.98	60.46	34.17	63.29
T_7	*Trichoderma viride* @12 g/Kg Seed	66.74	95.16	87.04	92.30	82.92	81.17	41.31	70.58
T_8	Control	-	-	-	-	-	-	-	-

1. Plant growth parameters
1.1 Vine length
All the bio-agents significantly improved vine length of Bitter Gourd as compared to untreated check when applied as seed treatment. Among various treatments, maximum vine length (168.00 cm) was recorded with *P. lilacinus* at 12 g/kg seed followed by *P. chlamydosporia* (162.25 cm) at 12 g/kg seed and *G. fasiculatum* at 12 g/kg seed (157.25 cm). Minimum vine length (132.50 cm) was recorded with *G. fasiculatum* at 06 g/kg seed.
Results revealed that seed treatment with *P. lilacinus* at 12 g/kg seed increased vine length to the tune of 62.37% followed by *P. chlamydosporia* (56.79%) at 12 g/kg seed and *G. fasiculatum* at 12 g/kg seed (51.94%) over untreated check.

1.2 Vine weight
Observation on vine weight of plants treated with fungal and bacterial bio-agents significantly increased vine weight as compared to untreated check. The maximum vine weight (277.25 g) was observed with *P. lilacinus* at 12 g/kg seed followed by *P. chlamydosporia* (262.50 g) and *G. fasiculatum* (258.25 g) at 12 g/kg seed. *G. fasiculatum* at 06 g/kg seed (225.50 g) was found least effective. Minimum vine weight (152.50 g) was recorded in untreated check.
Amongst various bio-agents tested, maximum increase in vine weight (81.80%) was observed with *P. lilacinus* at 12 g/kg seed followed by *P. chlamydosporia* (72.13%) and *G. fasiculatum* at 12 g/kg seed (69.34%). Minimum increase in vine weight (47.86%) was observed with *G. fasiculatum* at 06 g/kg seed over untreated check.

1.3 Root length
Results exhibited that root length was increased with bio-agent treatments as compared to untreated check. Among different treatments, maximum root length (28.75 cm) was observed with *P. lilacinus* at 12 g/kg seed followed *P. chlamydosporia* (26.25 cm) and *G. fasiculatum* at 12 g/kg seed (24.25 cm). These treatments found differed significantly from each other with respect to root length. Minimum root length (15.50 cm) was observed in untreated check.
Results showed that maximum increase in root length was recorded with *P. lilacinus* at 12 g/kg seed (85.48%) followed by *P. chlamydosporia* (69.35%) and *G. fasiculatum* at 12 g/kg seed (56.45%) over untreated check. It was observed to be minimum (19.35%) when Bitter Gourd seeds treated with *G. fasiculatum* at 06 g/kg seed.

1.4 Root weight
Results presented in Table-1 exhibited that all bio-agents significantly increased root weight as compared to untreated check (16.25 g). Among all the treatments, maximum root weight (29.75 g) was obtained with *P. lilacinus* at 12 g/kg seed followed by *P. chlamydosporia* (28.25 g) and *G. fasiculatum* at 12 g/kg seed (26.75 g). *G. fasiculatum* at 06 g/kg seed (21.50 g) was found least effective.
Results illustrated through Table-2 revealed that maximum increase in root weight to the tune of 83.07% was observed with *P. lilacinus* at 12 g/kg seed followed by *P. chlamydosporia* (73.84%) and *G. fasiculatum* at 12 g/kg seed (64.61%) over untreated check. Minimum increase (32.30%) in root weight was observed with *G. fasiculatum* at 06 g/kg seed.

2. Nematode parameters
2.1 Galls per plant
The galls produced by *M. incognita* on Bitter Gourd reduced significantly as compared to untreated check. Among different bio-agents, minimum galls per plant (36.00) was observed with *P. lilacinus* at 12 g/kg seed followed by *P. chlamydosporia* (45.75) and *G. fasiculatum* at 12 g/kg seed (52.50). *G. fasiculatum* at 06 g/kg seed (88.25) was found to be least effective. Maximum galls (164.00) were observed with untreated check.
Maximum reduction (78.04%) in galls/plant was observed with *P. lilacinus* when applied at 12 g/kg seed followed by *P. chlamydosporia* (72.10%) and *G. fasiculatum* at 12 g/kg seed (67.98%). Minimum reduction (46.18%) was recorded with *G. fasiculatum* at 06 g/kg seed.

2.2 Egg masses per plant
Data pertaining to egg masses per plant produced by *M. incognita* on Bitter Gourd reduced significantly as compared to untreated check when bio-agents was applied as seed treatment. Among different bio-agents, minimum egg masses per plant (32.25) was observed with *P. lilacinus* at 12 g/kg seed followed by *P. chlamydosporia* (42.75) and *G. fasiculatum* (47.25) at 12 g/kg seed. *G. fasiculatum* at 06 g/kg seed (83.75) was found least effective with respect to egg

masses per plant.

Results revealed that maximum reduction in egg masses per plant (73.01%) was recorded with *P. lilacinus* at 12 g/kg seed followed by *P. chlamydosporia* (64.22%) and *G. fasiculatum* at 12 g/kg seed (60.46%). Minimum reduction (29.91%) was observed with *G. fasiculatum* at 06 g/kg seed.

2.3 Eggs and larvae per egg mass

Experimental data showed that the eggs and larvae per egg mass produced by *M. incognita* on Bitter Gourd reduced significantly as compared to untreated check. Among different bio-agents, minimum eggs and larvae per egg mass (194.50) observed with *P. lilacinus* at 12 g/kg followed by *P. chlamydosporia* (202.25) and *G. fasiculatum* (209.50) at 12 g/kg seed. *G. fasiculatum* at 06 g/kg seed (254.50) was found least effective. Maximum eggs and larvae per egg mass (318.25) were observed with untreated check.

Maximum reduction in eggs and larvae per egg mass (38.88%) was obtained with *P. lilacinus* at 12 g/kg followed by *P. chlamydosporia* (36.44%) and *G. fasiculatum* (34.17%) at 12 g/kg seed. Minimum reduction (20.03%) was obtained with *G. fasiculatum* at 06 g/kg seed.

2.4 Final larvae population per 100 cc soil

Data revealed that the nematode population per 100 cc soil obtained at termination of experiment was found significantly lower in the plants when Bitter Gourd seeds treated with bio-agents. Among bio-agents, minimum larvae population (476 J_2/100cc soil) was recorded with *P. lilacinus* at 12 g/kg followed by *P. chlamydosporia* (518 J_2/100cc soil) and *G. fasiculatum* (544 J_2/100cc soil) at 12 g/kg seed. *G. fasiculatum* (686 J_2/100cc soil) at 06 g/kg seed was found least effective. Maximum larvae population (1482 J_2/100cc soil) was observed in untreated check.

Data showed that maximum reduction in final larvae population per 100cc soil (67.88%) was recorded with the application of *P. lilacinus* at 12 g/kg followed by followed by *P. chlamydosporia* (65.04%) and *G. fasiculatum* (63.29%) at 12 g/kg seed. Minimum reduction (53.71) was recorded when Bitter Gourd seeds treated with *G. fasiculatum* at 06 g/kg seed On the whole, seed treatment with *P. lilacinus* at 12 g/kg was found most effective followed by *P. chlamydosporia* and *G. fasiculatum* at 12 g/kg seed for the management of *M. incognita* and to enhanced plant growth of Bitter Gourd.

Discussion

Results of present investigation are in accordance with the findings of previous workers who reported that application of bio-agents improved plant growth of crops in phyto-nematodes prone areas [5, 21, 19, 10, 5] found that almost all *Heterodera avenae* cysts were parasitized by naturally occurring soil fungi (*Verticillium chlamydosporium* and *Nematophthora gynophila*) and effectively controlled the population of nematodes. Similarly, [21] reported the efficacy of *P. lilacinus*, *T. harzianum* and *T. flavus* as seed treatment and soil drench against root-knot nematode on okra. However, maximum suppression in gall formation and egg mass production was obtained with *P. lilacinus* whereas *T. flavus* and *T. harzianum* were almost equally effective. Conidial suspension of microbial antagonists used as soil amendment also reduced nematode root invasion as well as soil densities [19]. Evaluated the effectiveness of nematophagous fungi, *Arthrobotrys oligospora* and *Paecilomyces lilacinus* against root-knot nematode (*Meloidogyne incognita*) infecting okra and obtained good plant growth of okra in nematode sick pots. [10] evaluated bio-control potential of *T. harzianum*, *T. viride*, *P. chlamydosporia*, *P. lilacinus* and *P. fluorescens* at 20 g/kg seed and they observed significant increase in plant growth and yield of okra infested with *M. incognita* when seeds were treated with bio-agents [15]. Reported that soil application with *T. harzianum* at 3 g per kg soil was found best treatment followed by *T. viride* and *P. fluorescens* 3 g per kg soil to enhanced plant growth and management of *M. incognita* infecting brinjal.

Nematode reproduction parameters were also recorded to interpret the experimental findings. The findings of present investigation are in accordance with the results of previous workers [4, 12, 9, 8, 13] who reported the efficacy of bio-agents against nematodes. The parasitization of eggs and females of *M. incognita* by *P. lilacinus* was reported by [4]. They observed that fungal hyphae enter through natural openings and after five days, eggs and juveniles become infected and population of nematode declined sharply [12]. Carried out an experiment to evaluate the fungal antagonist of nematodes consist of nematode trapping fungi and endo-parasitic fungi. They observed that fungi can be effectively used for management of nematodes and may be employed an alternative to chemicals [9]. Evaluated fungal and bacterial antagonists as seed dressing treatment against root-knot nematode, *Meloidogyne incognita* infecting okra. The seeds of okra variety A-4 were treated with *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescens* at 10 g/ kg seed and carbosulfan 25 (DS) at 3% (w/w). Results revealed that the growth parameters of okra were better and *M. incognita* populations were significantly declined in all the treatments as compared to untreated check. Recently, [13] tested the efficacy of bio-agents *viz.*, *Paecilomyces lilacinus*, *Trichoderma harzianum* and *Pseudomonas fluorescens* at 0.5, 1 and 2% w/w as seed treatment against maize cyst nematode, *Heterodera zeae* infecting maize cv- PEHM-2. Results showed that maximum increased in plant growth of maize and significant reduction in nematode population was observed when seeds were treated with *Paecilomyces lilacinus* at 2 per cent w/w followed by *Trichoderma harzianum* and *Pseudomonas fluorescens* at 2 per cent w/w.

Fig 1: Effect of Bio-agents as seed treatment on plant growth of Bitter Gourd against *Meloidogyne incognita*

Conclusion

These studies clearly showed that seed treatment with bio-agents boost up plant growth parameters in nematode prone areas may be due to reduction in infection of nematodes by attack of fungal bio-agents or release of toxic metabolites by bio-control agents which adversely affect the nematode activity in rhizosphere of crop plants. Results also revealed that seed treatment with bio-agents not only reduced nematode infection due to parasitization of eggs, larvae and females of nematodes by bio-agents but also due to enhancement of competition and space for nematodes survival.

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