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Effect of bio-agents on management of plant parasitic nematodes infecting bittergourd

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Abstract

A pot culture experiment was conducted to assess the efficacy of certain bioagents on management of plant parasitic nematodes infecting bittergourd. Bittergourd seeds of var. Green long were sown in infested soil in 15cm diameter earthen pots. The pre-incubated bioagents *viz*. *Purpureocillium lilacinum*, *Pochonia chlamydosporia, Bacillus pumilus* and *Pseudomonas fluorescens* either alone or in combination; FYM and carbofuran were applied to the pot soil as per treatments. Need based irrigation, weeding and other cultural operations were provided. The effect of treatments on growth parameters of bittergourd plants and population of nematodes were observed 45 days after sowing and application of bioagents. It was revealed that combined application of *Purpureocillium lilacinum* and *Pseudomonas fluorescens* enhanced the vine length, root length, shoot weight and root weight of bittergourd plants to the tune of 73.3%, 62.5%, 58.0% and 82.3% respectively over untreated control. Population buildup of nematodes was also observed to be more affected by this treatment. The population of root knot nematode, lance nematode, spiral nematode and stunt nematode was reduced by 54.0, 78.1, 75.6 and 84.6% respectively by this treatment from their initial population with overall reduction of 63.1%.

Keywords: Efficacy, bioagents, bittergourd and plant parasitic nematode

Introduction

Bittergourd (*Momordica charantia* L.) is an important vegetable crop. It is believed to be originated from Tropical Asia, especially from Eastern India and Southern China- Miniraj *et al*. It is an important commercial cucurbitaceous vegetable with rich source of vitamins, minerals and high dietary fiber. It is widely cultivated throughout India mainly for its tender green fruits. Bitter gourd fruits are rich source of Iron (2mg/100g). It is having disease preventing and health promoting phyto chemical compounds like flavonoids and antioxidants and thus is used in preparation of various herbal medicines. Bittergourd is cultivated in 95,000 ha area with annual production of 10,30,000 MT in India -Horticulture at a glance. The production and productivity of bittergourd seems to be influenced by several pests and diseases including plant parasitic nematodes. Around 13.5% yield loss is attributed to this tiny organism (Walia & Chakrabarty 2018) [18]. The control measures suggested earlier for nematode pests are primarily based on the application of synthetic nematicides which are not only costly, but also having harmful effect on human health and environment. Use of beneficial bioagents is reported to be one of the suitable alternatives to these harmful chemicals. It is not only ecofriendly but also low cost and sustainable approach of nematode management and thus gaining popularity in the recent times. With this backdrop, the present study was undertaken to assess the efficacy of four bioagents alone or in combination along with a chemical check (Carbofuran) and untreated plot (control) for management of parasitic nematodes infecting bittergourd in Odisha condition.

Materials and Methods

The experiment was conducted in net house of the Department of Nematology, College of Agriculture, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar during 2018-2019 following Complete Randomised Design (CRD) with nine treatments, each replicated three times. The treatments are T1- *Purpueocillium lilacinum* @ 10mg/pot with 1kg soil,T2- *Pochonia chlamydosporia* @ 10mg/ pot with 1kg soil,T3- *Pseudomonas fluorescens* @ 10mg/ pot with 1kg soil,T4- *Purpureocillium lilacinum* + *Pseudomonas fluorescens* each @ 5mg/ pot with 1kg soil,T5- *Pochonia chlamydosporia* + *Pseudomonas fluorescens* each @ 5mg/ pot with 1kg soil,T6- FYM @10g/ pot with 1kg soil, T7- *Bacillus pumilus* @ 10mg/ pot with 1kg soil, T8- Carbofuran 3G @ 60mg/pot with 1kg soil and T9- Untreated control. Naturally infested field soil was collected from the experimental plots of AICRP (Nematodes) having bittergourd as previous crop. Nematode species present in the soil were identified and their initial population was estimated taking the composite soil samples and screening through Cobb's sieving and modified Baerman's funnel method in the laboratory of AICRP on Nematodes. Bittergourd seeds (Var. Green long) were sown in that infected soil in 15cm diameter earthen pots. The pre-incubated bioagents, carbofuran and FYM were applied to the pot soil as per treatments during sowing of seeds. One untreated control pot was also maintained along with the treated pots. Need based irrigation, weeding and other cultural operations were provided as per standard recommendations. The effect of the treatments on different plant growth parameters of bittergourd and buildup of nematode population were observed by taking the biometric measurements of different growth parameters and estimating the population of nematodes following standard procedures 45 days after sowing of seeds and application of treatments.

Results and Discussion

Application of pre-incubated bioagents, chemical nematicide carbofuran as well as well decomposed FYM into pot soil during the time of sowing of seeds enhanced the plant growth parameters of bittergourd substantially over untreated control (Table 1). Maximum Vine length (130cm), Fresh shoot weight (128g), Dry shoot weight (42.6g), Root length (26cm), Fresh root weight (23.7g) and Dry root weight (11.8g) were recorded with combined application of fungal bioagent *Purpureocillium lilacinum* and bacterial antagonist *Pseudomonas fluorescens* (T4). The combined application of *Pochonia chlamydosporia* and *Pseudomonas fluorescens* (T5) also indicated better plant growth in terms of Vine length (120cm), Fresh shoot weight (119.0g), Dry shoot weight (39.6g), Fresh root weight (21.5g), Dry root weight (10.7g) and Root length(22cm) next to T4. The vine length and shoot weight of plants in pots treated with bacterial bioagents like *Pseudomonas fluorescens* (T3) or *Bacillus pumilus* (T7) alone are in the increasing order than that from fungal bioagents like *Purpureocillium lilacinum* (T1*)* and *Pochonia chlamydosporia* (T2*)*. On the other hand, the root length and root weight of pots treated with individual bacterial and fungal bioagents are in the same order. The result revealed that the plant growth parameters are mostly positively influenced with application of bacterial bioagents like *Pseudomonas fluorescens* (T3) or *Bacillus pumilus* (T7) either alone or in combination with fungal bioagent (T4& T5). Being Plant Growth Promoting Rhizobacteria (PGPR), their application might have played a greater role in promotion of plant growth parameters. Increased plant vigour similar to the present findings with combined application of *Pseudomonas fluorescens, Purpureocillium lilacinum* and *Bacillus pumilus* has reported earlier by Bhattacharya and Goswami, 1987 [2] in tomato. Vinod kumar and Jain, 2010 [17] in their screen house study evaluated efficacy of some fungal and bacterial antagonists in reduction of *Meloidogyne incognita* population resulting in better growth parameters in Okra. Hasheen and Abo-elyousr, 2011 suggested that application of different biocontrol agents (*Pseudomonas fluorescens* and *Purpureocillium lilacinum*) in combination and alone not only has a lethal effect on nematode and enhances the plant growth, supplying many nutritional elements and induction of the systemic resistance in tomato plants. Kumar *et al.*, 2012 [9] have also reported similar effect of *Pseudomonas fluorescens* used as seed treatment @ 20g/kg of seed in Okra which significantly reduced the soil population density of nematode and improved the plant growth. Majzoob *et al.*, 2012 [11] reported that *Pseudomonas fluorescens* is the most effective bioagent against root knot nematode *Meloidogyne javanica* in cucumber under greenhouse conditions exhibiting maximum increased plant growth. Increased plant growth and enhanced fruit yield in brinjal by soil application of *Pseudomonas fluorescens* with *Trichoderma viride* reported by Pavitra, 2014 [15] is in agreement with the present findings. Nama and Sharma, 2017 [14] have also reported similar result of increased plant vigour by application of *Pseudomonas fluorescens* in cowpea infested by root knot nematode *Meloidogyne incognita.*

The population of four species of plant parasitic nematodes *viz*., Root knot nematode (*Meloidogyne incognita*), Lance nematode (*Hoplolaimus indicus*), Spiral nematode (*Helicotylenchus dihystera*) and Stunt nematode (*Tylenchorynchus mashoodi*) detected from the soils used for experimentation were found to be influenced remarkably 45 days after sowing of bittergourd seeds and application of bioagents. All treatments except untreated control (T9) reduced the population of all plant parasitic nematodes (Fig. 1). The percentage of reduction of root knot nematode was within the range of 4.5% to 54.0% (Table 2). Maximum reduction (54.0%) was observed in T4 with combined application of *Pseudomonas fluorescens* and *Purpureocillium lilacinum* followed by 52.3% in T5 (combined application of *Pseudomonas fluorescens* and *Pochonia chlamydosporia*). Similarly, these two treatments also exhibited maximum reduction of lance nematode (*Hoplolaimus indicus),* spiral nematode (*Helicotylenchus dihystera*) and stunt nematode (*Tylenchorynchus mashoodi*). T4 reduced these nematodes by 78.1%, 75.6% and 84.6% & T5 reduced them by 74.5%, 73.1% and 84.6% respectively. The overall reduction of nematode population by T4 was 63.1% which was maximum and it was followed by T5 with 61.1% reduction in total nematode population. However, the reduction percentage of overall nematode population was within the range of 25.0%-54.6% in other treatments with either one bioagent or FYM or carbofuran. Further, the population of root knot nematode in bittergourd root also indicated the similar trend. This might be due to different mode of action by bacterial and fungal antagonists leading to develop synergistic effect resulting in better suppression of parasitic nematodes in combined application. Different enzymes secreted by egg parasitic fungal bioagents *Purpureocillium lilacinum* or *Pochonia chlamydosporia* might have break down the physical and physiological integrity of nematode cuticle and egg cells facilitating penetration and colonization of both fungal and bacterial antagonists leading to better control of nematodes as reported by Li *et al*., 2015 and Kerry., 2000 [10, 6]. Premature egg hatching, reduction in viability and mortality of juveniles induced by secondary metabolites such as 2,4 Diacetyl-phloroglucinol and lytic enzymes, antibiotics and hydrogen cyanide produced by *Pseudomonas fluorescens* have been reported in case of nematode population reduction by Alh *et al.*, 1986 [1]. Goody *et al.*, 1983 [4] reported about destruction of one third eggs of *Meloidogyne spp* by naturally occurring *Purpureocillium lilacinum* and *Pochonia chlamydosporia* in soil. Reduced galls and nematode egg masses by application of *Purpureocillium* in cotton has also been reported by

Davide and Batine, 1985 [3]. Production of metabolites by *Pseudomonas fluorescens* leading to lysis of nematode eggs and reduced vitality of 2nd stage juvenile resulting in reduced attraction and penetration by *Meloidogyne incognita* in bitter gourd reported by Wetscott and Kluepfel, 1993 [19] is in agreement with the present findings. Mittal and Prasad, 2003 [13] observed the application of bioagents increased the plant growth parameters of soya bean and decreased multiplication of *Meloidogyne incognita.* Siddiqui and Shaukat, 2004 [16] reported that application of *Pseudomonas fluorescens* and *Trichoderma harzianum* to sterilized sandy loam soil greatly reduced population densities of *Meloidogyne incognita* in the roots of tomato. Khan *et al.*, 2004 [7] applied *Purpureocillium lilacinum* against *Meloidogyne incognita* in okra and recorded an increase in plant growth parameters with a significant reduction of nematode population. The combined effect of *Pseudomonas fluorescens* and *Purpureocillium lilacinum* on *Meloidogyne incognita* infecting bittergourd has also been elicited by Kienwick and Sikora, 2006 [8] which is similar to the present findings. Thus, the application of bacterial antagonist *Pseudomonas fluorescens* @5mg/ kg soil (2.5 kg/ha) and fungal antagonists either *Purpureocillium lilacinum* or *Pochonia chlamydosporia* @ 5mg/ kg soil (2.5kg/ha) along with 2.5 ton/ha of FYM can be a recommendation for effective management of parasitic nematodes infecting bittergourd with appreciable enhancement of plant growth characters which ultimately would resulting in higher yield with sustainability and environment safety.

Table 1: Effect of bioagents on plant growth parameters of bittergourd infected by parasitic nematodes.

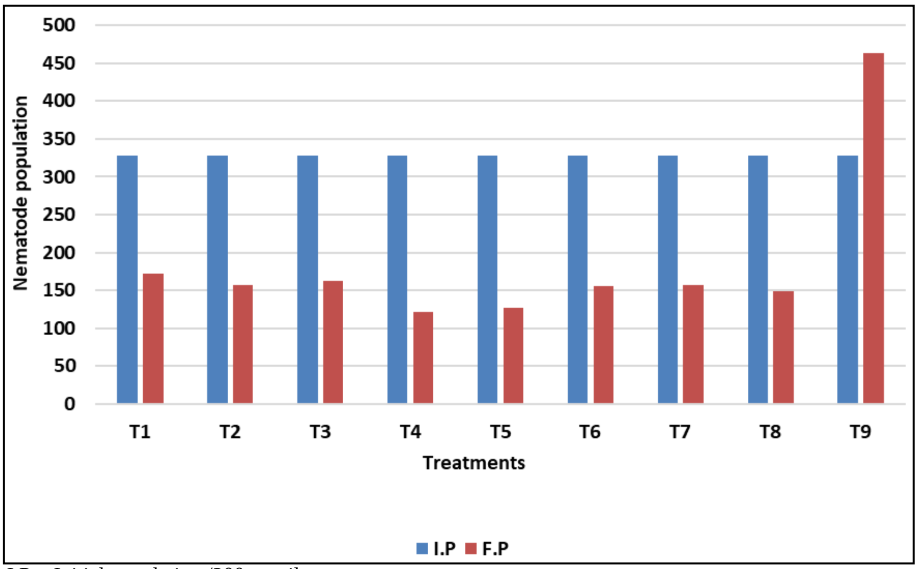
Treatments	Vine length (cm)	% change over control	Fresh shoot weight (g)	% change over control	Dry shoot weight (g)	% change over control	Fresh root weight (g)	% change over control	Dry root weight (g)	% change over control	Root length (cm)	% change over control
T1	89 (9.48)	18.6	90 (9.52)	11.1	29.9 (5.5)	11.1	15 (3.9)	15.3	7.5 (2.9)	17.1	18 (4.3)	12.5
T2	80 (8.97)	6.6	83 (9.16)	2.4	27.6 (5.3)	2.6	13.5 (3.7)	3.8	6.7 (2.7)	4.6	16.5 (4.1)	3.1
T3	107 (10.79)	42.6	100 (10.03)	23.4	33.3 (5.8)	23.7	19 (4.4)	46.1	9.5 (3.2)	48.4	20 (4.5)	25
T4	130 (11.38)	73.3	128 (11.3)	58.0	42.6 (6.5)	58.3	23.7 (4.9)	82.3	11.8 (3.5)	84.3	26 (5.1)	62.5
T5	122 (11.06)	62.6	119 (10.9)	46.9	39.6 (6.3)	47.2	21.5 (4.7)	65.3	10.7 (3.4)	67.1	22 (4.7)	37.5
T6	85 (9.26)	13.3	92 (9.6)	13.5	30.6 (5.6)	13.7	14 (3.8)	7.6	6.9 (2.8)	7.8	19 (4.4)	18.7
T7	90 (9.51)	20	96 (9.8)	18.5	31.9 (5.7)	18.5	16 (4.1)	23.0	7.9 (2.9)	23.4	20 (4.5)	25
T8	82 (9.09)	9.3	89 (9.4)	9.8	29.6 (5.5)	10.0	14.5 (3.9)	11.5	7.2 (2.8)	12.5	18 (4.3)	12.5
T9	75 (8.70)		81 (9.0)		26.9 (5.2)		13.0 (3.7)		6.4 (2.7)		16 (4.1)	
S.Em +	0.45		041		0.23		0.32		0.22		0.19	
CD (0.05)	1.35		1.24		0.71		0.96		0.65		0.57	

T1- *Purpureocillium lilacinum* @ 10mg/pot with 1kg soil
T2- *Pochonia chlamydosporia* @ 10mg/ pot with 1kg soil
T3- *Pseudomonas fluorescens* @ 10mg/ pot with 1kg soil
T4- *Purpureocillium lilacinum* + *Pseudomonas fluorescens* each @ 5mg/ pot with 1kg soil
T5- *Pochonia chlamydosporia* + *Pseudomonas fluorescens* each @ 5mg/ pot with 1kg soil
T6- FYM @10g/ pot with 1kg soil
T7- *Bacillus pumilus* @ 10mg/ pot with 1kg soil
T8- Carbofuran 3G @ 60mg/ pot with 1kg soil
T9- Untreated control

Table 2: Effect of bioagents on population of plant parasitic nematodes infecting bittergourd

Treatment	*Meloidogyne incognita*			*Hoplolaimus indicus*			*Helicotylenchus dihystera*			*Tylenchorynchus mashoodi*			Total population of all nematodes		
	I.P/200cc soil	F.P/200cc soil	% Change	I.P/200cc soil	F.P/200cc soil	% change	I.P/200cc soil	F.P/200cc soil	% change	I.P/200cc soil	F.P/200cc soil	% Change	I.P/200cc soil	F.P/200cc soil	% change
T1	206.5	128.1	-37.9	55	20	-63.6	41	15	-63.4	26	9	-65.3	328.5	172.1	-47.6
T2	206.5	115.0	-44.3	55	21	-61.8	41	13	-68.2	26	8	-69.2	328.5	157	-52.2
T3	206.5	126.5	-38.7	55	18	-67.2	41	12	-70.7	26	6	-76.9	328.5	162.5	-50.5
T4	206.5	94.9	-54.0	55	12	-78.1	41	10	-75.6	26	4	-84.6	328.5	120.9	-63.1
T5	206.5	98.5	-52.3	55	14	-74.5	41	11	-73.1	26	4	-84.6	328.5	127.5	-61.1
T6	206.5	197.2	-4.5	55	25	-54.5	41	16	-60.9	26	8	-69.2	328.5	246.2	-25.0
T7	206.5	112.0	-45.7	55	22	-60.0	41	13	-68.2	26	10	-61.5	328.5	157	-52.2
T8	206.5	111.0	-46.2	55	20	-63.6	41	12	-70.7	26	6	-76.9	328.5	149	-54.6
T9	206.5	285.0	+38.0	55	80	+45.4	41	58	+41.4	26	40	+53.8	328.5	463	+40.9

I.P =Initial population/200cc soil.
F.P = Final population/200cc soil 45 days after sowing.
T1- *Purpureocillium lilacinum* @ 10mg/pot with 1kg soil
T2- *Pochonia chlamydosporia* @ 10mg/ pot with 1kg soil
T3- *Pseudomonas fluorescens* @ 10mg/ pot with 1kg soil
T4- *Purpureocillium lilacinum* + *Pseudomonas fluorescens* each @ 5mg/ pot with 1kg soil
T5- *Pochonia chlamydosporia* + *Pseudomonas fluorescens* each @ 5mg/ pot with 1kg soil
T6- FYM @10g/ pot with 1kg soil
T7- *Bacillus pumilus* @ 10mg/ pot with 1kg soil
T8- Carbofuran 3G @ 60mg/ pot with 1kg soil
T9- Untreated control



I.P = Initial population /200cc soil
F.P = Final population/200cc soil
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T2- *Pochonia chlamydosporia* @10mg/with 1kg soil
T3- *Pseudomonas fluorescens* @10mg/with 1kg soil
T4- *Purpureocillium lilacinum* + *Pseudomonas fluorescens* each @5mg/with 1kg soil
T5- *Pochonia chlamydosporia* + *Pseudomonas fluorescens* each @5mg/with 1kg soil
T6- FYM @10g/with 1kg soil
T7- *Bacillus pumilus* @10mg/with 1kg soil
T8- Carbofuran 3G 60mg/with 1kg soil
T9- Untreated control

Fig 1: Effect of treatments on total nematode population in soil

Conclusion

In conclusions, the study indicated the effect of bioagents in reducing the population of plant parasitic nematodes affecting Bitter gourd. The study revealed that combined application of fungal and bacterial bioagents reduces the population of plant parasitic nematodes *viz*., *Meloidogyne incognita*, *Hoplolaimus indicus*, *Helicotylenchus dihystera*, and *Tylenchorynchus mashoodi* by 54%, 78.1%, 75.6%, 84.6% respectively. The study also provided the increased percentage of plant growth parameters with respect to the application of bioagents when compared with untreated plot.

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