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Effect of *Bacillus pumilus* IIHR Bp-2 1% A.S. and *Pseudomonas putida* IIHR Pp-2 1% A.S. on *Meloidogyne incognita* infecting okra (*Abelmoschus esculentus* (L.) Moench)

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ABSTRACT: Field studies were conducted for three seasons from 2015-16 to 2017-18 on bio-management of root knot nematode, *Meloidogyne incognita* in okra. The study revealed that liquid formulation of BCAs, *Bacillus pumilus* IIHR Bp-2 and *Pseudomonas putida* IIHR Bp-2 were at par with each other in reducing the nematode population and increasing okra yield. It was found that seed treatment with BCAs at 10 ml kg $^{-1}$ followed by soil application of Farm Yard Manure (20 tons ha^{-1}) after enrichment with liquid formulations @ 5 lit recorded 66.51 to 67.57 per cent decrease in nematode population and 29.44 to 30.83 per cent increase in okra yield with cost benefit ratio of 1: 1.91 to 1: 1.93. It was followed by chemical treatment with carbofuran @ 1 kg a.i. ha^{-1} which revealed 50.76 to 62.42 per cent reduction in soil nematode population and 16.19 to 16.38 per cent increase in okra yield. Hence, this study proves that liquid formulations of *Bacillus pumilus* IIHR Bp-2 and *Pseudomonas putida* IIHR Bp-2 can be used as promising components in integrated nematode management packages for successful management of *M. incognita* in okra.

Keywords: *Bacillus pumilus*, *Pseudomonas putida, Meloidogyne incognita,* nematode management, okra

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench), is an important vegetable crop of the tropical and sub-tropical countries including India. It is mainly grown for green tender fruits which are rich in vitamins, proteins, calcium and other minerals (Santos *et al.*, 2013). India is the biggest producer of okra and ranks first in the world contributing 62 per cent of total global production. It occupies nearly 509 thousand hectares area with production of 6095 thousand metric tonnes and productivity of 12 metric tonnes/ ha in India (Anonymous, 2018). However, productivity of okra is hindered due to yield losses caused by several insect pests, diseases and nematodes. In India, Kumar *et al.* (2020) estimated annual crop losses due to plant-parasitic nematodes at 21.3% amounting to Rs. 102,039.79 million (1.58 billion USD), annually. In okra, root-knot nematodes (*Meloidogyne* spp.) are the most serious pests (Hussain *et al.*, 2011; Mukhtar *et al*., 2017) causing yield losses up to 20.4 per cent (Ravichandra, 2012) and monetary yield losses are estimated at Rs. 2480.86 million annually (Kumar *et al.*, 2020). They cause severe growth reductions and damage the roots by formation of characteristic galls. The damage severity is aggravated due to association of nematodes with soil-borne fungal and bacterial pathogens resulting in disease complex situations (Kayani *et al.*, 2018).

Inadvertent use of chemical nematicides is popular among vegetable growers for their ease in application and instantaneous action to mitigate nematode menace. However, owing to their negative impact on environmental and human health, many pesticides are being withdrawn from the market. Hence, biological control agents (BCAs) are steadily gaining importance as an alternative to chemical pesticides due to their capability to antagonize nematodes by different modes of action (Rao *et al.,* 2015a).Many plant growth promoting rhizobacteria (PGPR) including fluorescent pseudomonads and *Bacillus* spp. are reported not only to enhance plant growth by colonizing the plant root system, but also exhibited excellent nematicidal activity against a multitude of phytoparasitic nematodes (Abd-Elgawad, 2016; Zhao *et al.*, 2018).

Pseudomonas spp. serve as ideal candidates for commercial exploitation as they possess many desirable qualities and reportedly increased plant growth and reduced nematode damage in several crops (Tian*et al.*, 2007; Umamaheswari *et al.,* 2020). BCAs belonging to of *Bacillus* group are considered as 'microbial factories' due to their ability to produce a vast spectrum of biologically active molecules and anti-microbial compounds that are antagonistic to a wide range of plant pathogenic microbes and nematodes (Ongena and Jacques, 2008; Stein, 2005). Keeping in view their antagonistic potential, field trials were conducted under All India Coordinated Research Project (Vegetable Crops) to evaluate the efficacy of liquid formulations of *Bacillus pumilus* IIHR Bp-2 (1% A.S.) and *Pseudomonas putida* IIHR Pp-2 (1% A.S.) in the management of root knot nematode, *Meloidogyne incognita* infecting okra.

MATERIALS AND METHODS

Field trials were conducted in nematode sick experimental plots at ICAR-Indian Institute of Horticulture Research, Bengaluru, Karnataka for three consecutive years during 2015-16, 2016-17 and 2017-18. Okra cv. Arka Anamika seeds were sown in plots as per the treatment schedule mentioned in Table 1. Liquid formulations of *Bacillus pumilus* (IIHR Bp-2) 1% A.S. and *Pseudomonas putida* (IIHR Pp-2) 1% A.S prepared in Nematology Laboratory, Division of Crop Protection were evaluated after seed treatment @ 10 ml kg $^{-1}$okra seed, alone and in combination with soil application of 20 tons Farm Yard Manure (FYM) after enrichment with bacterial formulations @ 5 litha^{-1}. For proper enrichment, the liquid formulations were mixed in FYM and maintained under shade for 15 to 21 days at optimum moisture of 25-30%. The entire heap was mixed intermittently to ensure uniform enrichment of BCAs and later applied to soil according to treatments. As standard check, chemical treatment with carbofuran 3 G @ 1 kg a.i. ha^{-1}was evaluated alone and in combination with FYM. The experiment was laid out in a randomized block design with eight treatments and four replications. Regular crop management practices were followed throughout the season as per the package of practices of bhindi (Anonymous, 2020).

Initial root knot nematode population (second stage juveniles –J2) per 100 c.c soil was recorded before sowing and final nematode population per 100 c.c soil was recorded after termination of the experiment in all the three trials. Nematodes were extracted from soil by Cobb's wet sieving and decanting technique and modified Baermann's funnel technique (Southey, 1986). At termination, plants were uprooted and the roots were carefully observed for root knots or galls and recorded for the gall index on 1-5 scale (Heald *et al.*, 1989). After staining the roots with acid fuchsin, number of females in 10 g roots was estimated (Bridge *et al.,* 1982). Cumulative marketable yield per plot was recorded and expressed in tons ha^{-1}. The data of the three seasons were pooled and cost benefit ratio was calculated.

All the data were statistically analyzedusing ANOVA and means separated with the Duncan Multiple Range Test as per Panse and Sukhatme (1989).

RESULTS

Before the start of experiments, initial nematode (J2) population was estimated at 122 ± 3, 114.3 ± 2.1 and 121.3 ± 1.6J2 per 100 cc soil for the three consecutive seasons, respectively. The data from three year's trials showed similar trend and hence pooled analysis was done and treatments were compared. In all the three trials, the

Table 1: Treatment schedule

Treatment	Details
T1	Seed treatment with *Bacillus pumilus* (IIHR Bp-2) 1% A.S. @ 10 mlkg^{-1} seed
T2	Seed treatment with *Pseudomonas putida* (IIHR Pp-2) 1% A.S. @ 10 ml kg^{-1} seed
T3	T1+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Bacillus pumilus*(IIHR Bp-2) 1% A.S.
T4	T2+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Pseudomonas putida*(IIHR Pp-2) 1% A.S.
T5	Application of 20 tons ha^{-1} of FYM
T6	Chemical treatment (carbofuran at 1 kg a.i. ha^{-1})
T7	Chemical treatment (carbofuran at 1 kg a.i.ha^{-1}) +Recommended dose of FYM (20tonsha^{-1})
T8	Control without treatment

Table 2. Effect of *Bacillus pumilus* (IIHR Bp-2) 1% A.S. and *Pseudomonas putida* (IIHR Pp-2)1% A.S. on soil and root population of *M. incognita* infecting okra

Treatment	Final Nematode population (J2) per 100 cc soil				No. of *M. incognita* femalesin 10 g roots			
	2015-16	2016-17	2017-18	Pooled	2015-16	2016-17	2017-18	Pooled
T1	123.50^{b}	124.0^{c}	125.8^{c}	124.42^{c}	20.75^{b}	22.3^{b}	25.8^{c}	23.58^{c}
T2	129.00^{b}	129.3^{c}	131.0^{c}	129.75^{c}	21.75^{b}	21.5^{b}	26.0^{c}	24.33^{c}
T3	93.75^{a}	79.8^{a}	78.3^{a}	83.92^{a}	14.00^{a}	12.5^{a}	13.0^{a}	13.25^{a}
T4	99.00^{a}	81.5^{a}	79.5^{a}	86.67a	14.25^{a}	11.8^{a}	13.3^{a}	13.58^{a}
T5	176.50^{c}	212.8^{d}	216.3^{d}	176.00^{d}	30.25^{c}	37.8^{c}	42.0^{d}	31.92^{d}
T6	101.50^{a}	103.3^{b}	102.5^{b}	127.42^{c}	17.75^{ab}	21.3^{b}	23.5^{bc}	25.25^{c}
T7	96.50^{a}	95.3^{b}	95.0^{b}	97.25^{b}	20.25^{b}	19.0^{b}	21.5^{b}	19.75^{b}
T8	254.00^{d}	259.8^{e}	262.5^{e}	258.75^{e}	36.00^{d}	42.5^{d}	49.8^{e}	43.00^{e}
CD (5%)	8.36	11.68	8.28	6.94	5.07	4.08	3.06	2.87
SE	4.02	5.61	3.98	3.34	2.44	1.96	1.47	1.38

*Numericals followed by same alphabets are not significantly different at P=0.05

[T1-; Seed treatment with *Bacillus pumilus*1% A.S. @ 10 ml kg^{-1} seed; T2 - Seed treatment with *Pseudomonas putida* 1% A.S. @ 10 ml kg^{-1} seed; T3 - T1+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Bacillus pumilus;* T4 - T2+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Pseudomonas putida*; T5 - Application of 20 tons ha^{-1} of FYM; T6 - Chemical treatment (carbofuran at 1 kg a.i. ha^{-1}); T7 - Chemical treatment (carbofuran at 1 kg a.i. ha^{-1}) + Recommended dose of FYM (20tons ha^{-1}); T8 - Control without treatment]

Table 3. Effect of *Bacillus pumilus* (IIHR Bp-2) 1% A.S. and *Pseudomonas putida* (IIHR Pp-2)1% A.S. on nematode gall index and yield of okra

Treatment	Gall index at termination (1 to 5 scale)				Yield (t ha $^{-1}$)				Benefit: Cost ratio
	2015-16	2016-17	2017-18	Pooled	2015-16	2016-17	2017-18	Pooled	
T1	2.45^{b}	2.43^{b}	2.55^{b}	2.44^{b}	30.55^{b}	29.70^{b}	14.9^{b}	25.05^{b}	1.25
T2	2.48^{b}	2.53^{b}	2.60^{b}	2.48^{bc}	30.70^{b}	30.38^{ab}	14.8^{b}	25.28^{b}	1.28
T3	1.78^{a}	1.75^{a}	1.78^{a}	1.75^{a}	34.40^{a}	34.13^{a}	16.1^{a}	28.22^{a}	1.93
T4	1.83^{a}	1.80^{a}	1.80^{a}	1.79^{a}	34.65^{a}	33.10^{a}	16.0^{a}	27.92^{a}	1.91
T5	3.73^{c}	3.93^{c}	3.98^{c}	3.28^{d}	29.10^{bc}	27.33^{c}	13.6^{c}	23.35^{c}	1.12
T6	1.95^{a}	2.15^{ab}	2.05^{a}	2.67^{c}	30.78^{b}	29.53^{b}	14.9^{b}	25.06^{b}	1.54
T7	1.95^{a}	1.88^{a}	1.95^{a}	1.94^{a}	29.78^{b}	30.25^{ab}	15.3^{b}	25.10^{b}	1.61
T8	4.93^{d}	4.90^{d}	4.95^{d}	4.93^{e}	26.78^{c}	25.60^{d}	12.3^{d}	21.57^{d}	
CD (5%)	0.30	0.40	0.30	0.21	2.75	0.71	0.56	0.98	
SE	0.14	0.19	0.14	0.10	1.32	0.34	0.27	0.47	

*Numericals followed by same alphabets are not significantly different at P=0.05

[T1-; Seed treatment with *Bacillus pumilus*1% A.S. @ 10 ml kg^{-1} seed; T2 - Seed treatment with *Pseudomonas putida* 1% A.S. @ 10 ml kg^{-1} seed; T3 - T1+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Bacillus pumilus;* T4 - T2+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Pseudomonas putida*; T5 - Application of 20 tons ha^{-1} of FYM; T6 - Chemical treatment (carbofuran at 1 kg a.i. ha^{-1}); T7 - Chemical treatment (carbofuran at 1 kg a.i. ha^{-1}) + Recommended dose of FYM (20 tons ha^{-1}); T8 - Control without treatment] lowest nematode population in soil (83.92 per 100 c.c soil) and female nematode population in roots (13.25 per 10 g roots) was observed in T3 wherein okra seeds were treated with *Bacillus pumilus* (IIHR Bp-2) 1% A.S. @ 10 ml kg^{-1} followed by soil application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Bacillus pumilus* (IIHR Bp-2) 1% A.S. This was at par with T4. i.e. seed treatment with *Pseudomonas putida* (IIHR Pp-2) 1% A.S.@ 10 ml kg^{-1} followed by soil application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Pseudomonas putida* (IIHR Pp-2) 1% A.S. which recorded 86.67 J2 per 100 c.c soil and 13.58 females per 10 g roots of okra. Chemical treatment with carbofuran 3G recorded 127.42 J2 and 97.25 J2 in 100 CC soil and 25.25 and 19.75 females in 10 g okra roots, when incorporated alone and in combination with FYM, respectively. Seed treatment with *B. pumilus*1% A.S. and *P. putida*1% A.S. also recorded significantly lower nematode population as 124.42 and 129.75 J2 in soil and 23.58 and 24.33 females in okra roots, respectively, as compared to untreated control which recorded the highest nematode population in soil (258.75 nematodes per 100 cc soil) and roots (43 females per 10 g roots) (Table 1).

Pooled analysis of three year's trials revealed that a maximum of 67.57 and 66.51 per cent reduction in soil nematode population was recorded in T3 and T4, respectively (Fig.1). The lowest gall index (1.75) and the highest yield (28.22 tons ha^{-1}) coupled with maximum cost benefit ratio (CBR) of 1:1.93 was recorded in T3. This was at par with T4 which recorded significantly lower gall index (1.79) and 29.44 per cent increase in yield, with CBR as 1:1.91 (Fig.1 and Table 2).

This was followed by chemical treatment with carbofuran (T6) which recorded gall index of 2.67 and yield of 25.06 tons ha^{-1} in okra. When applied together with FYM (T7), there was significantly lesser gall index (1.94) and 16.38 per cent increase in okra yield with CBR of 1:1.61. Seed treatment with *B. pumilus*1% A.S. (T1) and *P. putida*1% A.S. (T2) were at par in recording significantly lower gall index as 2.44 and 2.48 and higher yield as 25.05 and 25.28, respectively, as compared to untreated control which recorded the highest gall index (4.93) and lowest yield (21.57 t ha^{-1}).T2 and T1 caused 49.86 to 51.92 per cent reduction in nematode population and 16.15 to 17.19 per cent increase in okra yield with a CBR of 1:1.25 to 1:1.28 (Table 2; Fig. 1).

Application of FYM alone @ 20 t/ha (T5) revealed 31.98 per cent reduction in nematode population and 8.27 per cent increase in yield, compared to control.It recorded an average of 176 J2 per 100 cc soil and 31.92 females per 10 g roots, in all three trials. Gall indices from 3.73 to 3.98 and yield from 13.6 to 29.1 tons ha^{-1} was recorded in this treatment (Table 1, 2; Fig. 1).

DISCUSSION

In the present study, *B. pumilus* IIHR Bp-2and *P. putida* IIHR Pp-2 applied as seed treatment and soil application were found promising in reducing the nematode population and gall index. This falls in line with the findings of Ali *et al.* (2002) and Elbanna *et al.* (2011) who also reported significant reduction in root knot nematode population and gall index due to *P. putida.* Rao *et al.* (2017a) reported a similar trend by seed treatment with *P. putida* at 20 ml kg^{-1} followed by soil application of 5 tons ha-1 of FYM enriched with 5 lit *P. putida* which caused 61.02 to 61.95 per cent reduction in *M. incognita* population and 77.9-78.5 per cent reduction in disease incidence of *Fusarium oxysporum* f. sp. *vasinfectum* in okra. Similarly Priti *et al.* (2018) revealed the antagonistic potential of *B. pumilus, P. monteilii*and *Trichoderma harzianum* against *M. incognita* and *F. oxysporum* f. sp. *cepae* in onion under field conditions which also increased onion yield by 26.1 to 28.8 per cent. Furthermore, Sowmya and Rao (2012) reported that gladiolus treatment with *P. putida*and *Paecilomyces lilacinus* reduced the disease incidence of *M. incognita* and *F. oxysporium* f. sp.*gladioli* by 66% and 57%, respectively and increased crop yield by 23per cent.

Ann (2013) examined *Bacillus* spp. and confirmed the production of protease enzyme which degraded the nematode cuticle and completely destroyed *M. incognita* juveniles within 12 h. *Bacillus* spp. were also reported to produce a wide range of nematicidal volatile compounds such as benzene acetaldehyde, 2-nonanone, decanal, 2-undecanone and dimethyl disulphide, which exhibited larvicidal and ovicidal action against J2 of *Meloidogyne* spp. (Huang *et al.*, 2010). Lipopeptides of *Bacillus* spp. *viz.* surfactins, iturins and fengycins are well documented biomolecules for their nematicidal and fungicidal action (Kavitha *et al.*, 2012; Sarangi and Ramakrishnan, 2016).

P. fluorescence is capable of altering specific root exudates which control nematode behaviour (Oostendorp and Sikora, 1989). Fluorescent pseudomonads are reported to exhibit nematicidal activity through production of metabolites that reduce egg hatching and cause J2 mortality; alteration of specific root exudates which control nematode behaviour and hinder host finding ability and enhancement of the defence mechanism in plants leading to the induction of systemic resistance (Sikora and Hoffmann-Hergarten, 1993; Hallmann *et al.*, 2001; Siddiqui *et al.*, 2001). Araujo *et al.* (2005) detected phytohormones, IAA and ABA in metabolites of *B. Subtilis* which are responsible for enhanced growth in soybean. These mechanisms exhibited by *Bacillus* spp. and *Pseudomonas* spp. might be responsible for suppression of nematode population and increase in okra yield in our study.

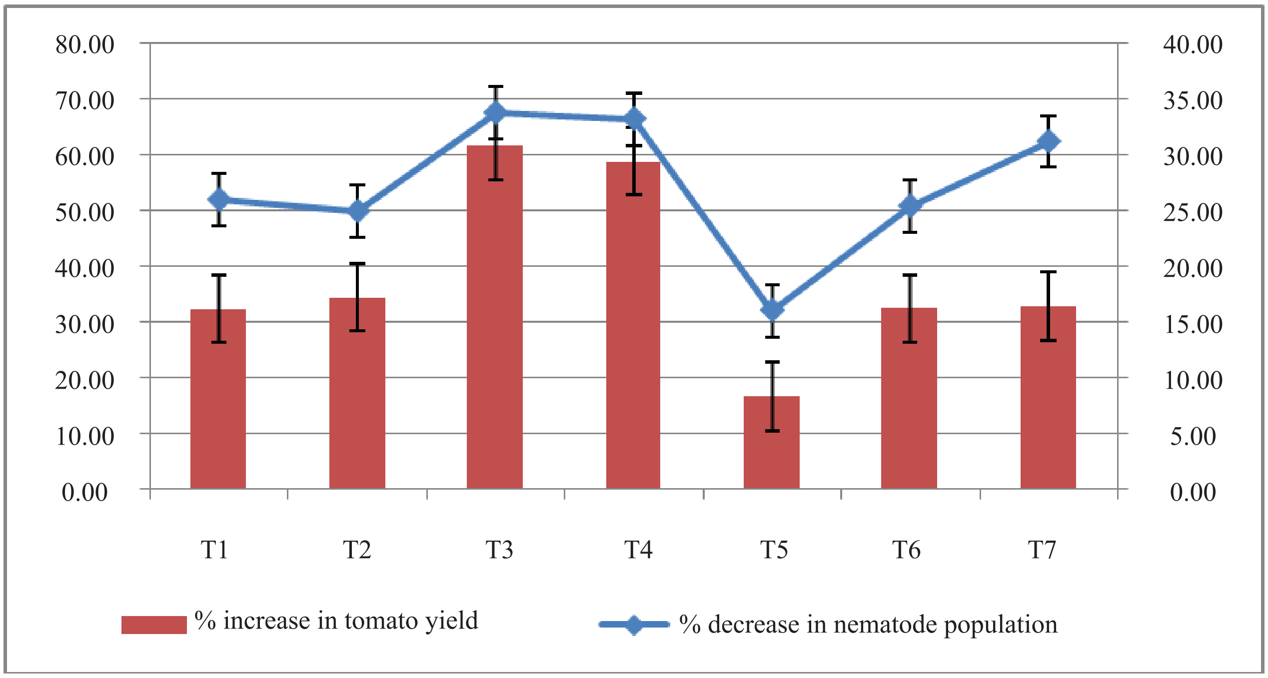

Fig 1. Effect of liquid formulations of *Bacillus* bioagents on nematode gall index and yield of okra (pooled data of three trials)

[T1-; Seed treatment with *Bacillus pumilus*1% A.S. @ 10 ml kg^{-1} seed; T2 - Seed treatment with *Pseudomonas putida* 1% A.S. @ 10 ml kg^{-1} seed; T3 - T1+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Bacillus pumilus;* T4 - T2+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Pseudomonas putida*; T5 - Application of 20 tons ha^{-1} of FYM; T6 - Chemical treatment (carbofuran at 1 kg a.i. ha^{-1}); T7 - Chemical treatment (carbofuran at 1 kg a.i. ha^{-1}) + Recommended dose of FYM (20 tons ha^{-1}); T8 - Control without treatment]

Also in the present study, soil application of BCAs after enrichment in FYM recorded significantly higher reduction in nematode population and increase in yield compared to application of FYM alone. Mass multiplication of BCAs in organic composts is gaining importance among the farming community as it improves soil health, reduces the cost of crop protection and provides sustainable solutions for disease management (Rao *et al.*, 2015b). Application of vermin compost enriched *B. subtilis* IIHR BS-2 liquid formulation increased carrot yield and reduced nematode and soft rot disease complex (Rao *et al.*, 2017b). Sharma (2002) reported that soil application of *P. fluorescens* enriched farm yard manure and vermicompost significantly reduced bacterial wilt in tomato. Similarly, application of *B. cereus* along with organic fertilizers showed enormous nematicidal activity in tomato and muskmelon (Xiao *et al.*, 2013). As reported by Oka (2010), use of manures supports growth of microorganisms, improves soil physiology and decreases nematode population by production of nematicidal compounds through decomposition process. Hence, in the current study, application of FYM as such or after enrichment with biopesticides recorded 31.98 to 67.57 reduction in nematode population.

CONCLUSION

The present study proves the antagonistic potential of liquid formulations of *Bacillus pumilus* IIHR Bp-2 (1% A.S.) and *Pseudomonas putida* IIHR Pp-2 (1% A.S.) against the root knot nematode, *M. incognita* and achieved yield enhancement in okra. As there is growing demand among the public for organic products, there is a large scope for exploiting these microbial biopesticides as safe alternatives for chemical nematicides in conventional and hi-tech horticultural crop production systems.

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