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Bio-efficacy of liquid formulations of *Bacillus subtilis* IIHR Bs-2 (1% A.S.) and *Bacillus amyloliquefaciens* IIHR Ba-2 (1% A.S.) in the management of *Meloidogyne incognita* infecting tomato

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ABSTRACT: Liquid formulations of two beneficial bacteria viz., *Bacillus subtilis* IIHR Bs-2 (1% A.S.) and *Bacillus amyloliquefaciens* IIHR Ba-2 (1% A.S.) were evaluated for their bio-efficacy against *Meloidogyne incognita* in tomato. This study was conducted for three consecutive years from 2015-16 to 2017-18. The bio-inoculants were delivered as substrate treatment (@5 ml kg^{-1} cocopeat) alone and in combination with soil application after enrichment in FYM (@ 5 l ha^{-1}). Pooled analysis of data revealed that the effect of substrate treatment and soil application of *Bacillus subtilis* IIHR Bs-2 1% A.S. was at par with *B. amyloliquefaciens* IIHR Ba-2 1% A.S. in suppressing 70.33 to 71.02 per cent of *M. incognita* population in tomato roots (13.17 to 13.58 females per 10 g roots) and soil (99 to 101.3 J2 per 100 c.c) and reducing the gall index (1.69 to 1.80 out of 5). Maximum yield of 33.01 to 33.46 tons ha^{-1} (+ 29.06 to 30.82 per cent increase over control) was recorded in these treatments with cost benefit ratio of 1:2.10 to 1: 2.12. It was followed by chemical treatment, carbofuran which recorded 63.09 to 64.89 per cent decrease in nematode population and 15.05 to 15.97 per cent increase in yield. Thus, this study proves the potential of liquid formulations of *Bacillus subtilis* IIHR Bs-2 (1% A.S.) and *Bacillus amyloliquefaciens* IIHR Ba-2 (1% A.S.) and hence, can be promising components of INM package for management of *M. incognita* in tomato.

Keywords: *Bacillus subtilis*, biopesticides, root knot nematode, tomato, *Meloidogyne*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), native to South and Central America, has established as one of the most valuable and indispensable vegetables in the world. They are the major dietary source of the antioxidant, lycopene and great source of vitamin C, potassium, folate and vitamin K. In 2019, it was cultivated worldwide in 5.03 x 10^6 ha and yields reached 1.8 x 10^8 metric tons (Food and Agriculture Organization of the United Nations (FAO) Statistics; see URL). In India, tomato is estimated to be grown in 77,800 ha with a production of 1.9 x 10^7 metric tons (National Horticulture Board Statistics; see URL).

Plant parasitic nematodes are one of the major non-insect pests hindering worldwide tomato production with monetary loss estimated at USD 80 billion per year (Nicol *et al.*, 2011). The root knot nematodes, *Meloidogyne* species cause huge economic losses in tomato and its yield loss potential varies from 25 to 100% (Seid *et al.,* 2015). In India, All India Coordinated Research Project on Nematodes estimated 23% yield loss in tomato due to root knot nematodes with monetary loss of Rs. 6035.20 million per annum (Kumar *et al*., 2020).

Though application of chemical nematicides is more popular among the growers for their ease in application and immediate effect on nematode control, their usage is discouraged due to their hazardous effects on biotic life and environment. Hence biological control agents are paid much attention by researchers and policy makers as promising components in integrated nematode management systems (Rao *et al*., 2015a).

Rhizobacteria belonging to *Bacillus* group are much researched upon and often called as 'microbial factories' due to their capability to produce a spectrum of biologically active molecules that are antagonistic to a wide range of phytopathogens (Ongena and Jacques, 2008). The most studied rhizobacterium in this group, *B. subtilis* is reported to produce more than a dozen of diverse antimicrobial compounds (Stein, 2005) and the crude antibiotics secreted by them are reported to be anatagonistic to root knot nematodes (Kavitha *et al.,* 2012). By formation of biofilm, it efficiently colonizes the plant roots and protects against the invading pathogens (Ramyabharathi *et al*., 2020).

B. amyloliquefaciens is a naturally occurring gram positive rhizobacteria that are common in soil and root ecosystems. It exhibits significant antagonistic action against a multitude of phytopathogenic nematodes and microbes by induced systemic resistance and secretion of antimicrobial metabolites (Han *et al.,* 2010; Prabu *et al*., 2019). Presently, many of the beneficial microbial inoculants are formulated mainly in solid carriers like talc, lignite etc. which suffer from major impediments like shorter shelf life, high contamination and difficulty in application though micro-irrigation techniques (Hegde, 2002). Liquid based bio-formulations offer longer shelf life comparatively with high purity and ease in application, storage and transport (Pindi and Satyanarayana, 2012).

Keeping these in view, the present study was conducted under All India Coordinated Research Project (Vegetables) to evaluate the efficacy of liquid formulations of *Bacillus subtilis* IIHR Bs-2 (1% A.S.) and *Bacillus amyloliquefaciens* IIHR Ba-2 (1% A.S.) in the management of root knot nematode, *Meloidogyne incognita* infecting tomato under field conditions.

MATERIALS AND METHODS

The field experiments were conducted in the nematode sick experimental plots at Block VI, ICAR-Indian Institute of Horticulture Research, Bengaluru, Karnataka. The experiments were evaluated for three consecutive years 2015-16, 2016-17 and 2017-18. Tomato var. NS 501 was used and the treatments were applied as per the Table 1. Liquid formulations of *Bacillus subtilis* IIHR Bs-2 (1% A.S.) and *B. amyloliquefaciens* IIHR Ba-2 (1% A.S.) available at Nematology laboratory, Division of Crop Protection were evaluated as substrate treatment (5 ml kg $^{-1}$ of cocopeat) for production of nursery seedlings, alone and in combination with soil application after enrichment in Farm yard manure @ 5 lit ha^{-1}. For enrichment, the liquid formulations of the bioagents were mixed in FYM and kept under shade for 2-3 weeks maintained at optimum moisture of 25 – 30%. Intermittently, the lot was mixed thoroughly from top to bottom to ensure uniform multiplication of bioagents and then applied to field according to the treatments.

Bioagent treatments were compared with chemical nematicide carbofuran @ 1 kg a.i. ha^{-1}. Application of FYM alone and in combination with chemical nematicide was also evaluated. The experiment was laid out in a randomized block design with eight treatments and four replications. Regular crop management practices were followed as per the recommendations throughout the season.

Observations were recorded on nematode population (second stage juveniles –J2) in soil per 100 c.c., both initially before planting and finally at termination of experiment. Soil samples were analysed as per Cobb's decanting and sieving technique followed by modified Baermann's funnel technique for extraction of nematodes (Southey, 1986). Galls caused due to *M. incognita* in roots were indexed on a 1-5 scale after uprooting the plants at termination (Heald *et al*., 1989). Number of females of *M. incognita* in 10g roots was estimated after staining with acid fuchsin (Bridge *et al.,* 1981). The cumulative yield was recorded and expressed as tons ha^{-1} and per cent increase in the yield over control was calculated.

All the data were statistically analysed using analysis of variance and means separated with the Duncan Multiple Range Test as per Panse and Sukhatme (1989). The data of the three seasons were pooled and cost benefit ratio was calculated to evaluate the cost effectiveness of the treatments.

Table 1. Details of treatments used in the study

Treatment code	Details
T1	Treatment of 1 kg cocopeat or substrate with 5 ml of *Bacillus subtilis* IIHR Bs-2 (1% A.S.) for producing seedlings of tomato in protrays
T2	Treatment of 1 kg cocopeat or substrate with 5 ml of *B. amyloliquefaciens* IIHR Ba-2 (1% A.S.) for producing seedlings of tomato in protrays
T3	T1+ soil application of 20 tons of FYM enriched with 5 lit of *Bacillus subtilis* IIHR Bs-2 (1% A.S.) / ha before planting
T4	T2+ soil application of 20 tons of FYM enriched with 5 lit of *B. amyloliquefaciens* IIHR Ba-2 (1% A.S.) / ha before planting
T5	Application of 20 tons of FYM / ha before planting
T6	Chemical treatment (carbofuran at 1 kg a.i./ha) before planitng
T7	Chemical treatment (carbofuran at 1 kg a.i./ha) + Recommended dose of FYM (20t/ha) before planting
T8	Untreated control

RESULTS

Initial population of *M. incognita* was recorded as 118 ± 3, 123.2 ± 1.8, 113.2 ± 3.4 J2/100 c.c soil in the first, second and third year trials, respectively. As similar trend of results was observed in all the three trials, the data were also pooled and analysed. All the treatments with biopesticides and chemicals alone and in combination with FYM recorded significantly lower nematode population and higher yield.

Among all the treatments, T3 - substrate treatment with *B. subtilis* IIHR Bs-2 (1% A.S.) @ 5 ml kg^{-1} cocopeat in protrays and subsequent soil application of 20 tons of FYM enriched with *B. subtilis* IIHR Bs-2 (1% A.S.) at 5 l ha^{-1} recorded the lowest nematode population, both in soil (99 J2 per 100 cc soil) and roots (13.17 females per 10 g root). The effect of this treatment was at par with T4 - substrate treatment with *B. amyloliquefaciens* @ 5 ml kg^{-1} cocopeat in protrays combined with soil application of 5 tons of FYM enriched with *B. amyloliquefaciens* at 5 l ha^{-1} which recorded 101.33 J2 and 13.58 females in soil (100 c.c) and roots (10 g), respectively (Table 2).

Table 2. Bio-efficacy of liquid formulations of *Bacillus* bioagents on soil and root population of *M. incognita* infecting tomato

Treatments	Final Nematode population (J2) per 100 cc soil				No. of *M. incognita* females in 10 g roots			
	2015-16	2016-17	2017-18	Pooled	2015-16	2016-17	2017-18	Pooled
T1	186.5	136.0	139.8	154.08	20.8	24.3	22.3	23.08
T2	183.3	147.5	150.0	160.25	21.8	25.3	23.8	24.08
T3	113.0	90.8	93.3	99.00	14.0	12.8	10.3	13.17
T4	114.5	94.5	95.0	101.33	14.3	13.3	11.8	13.58
T5	244.8	226.0	319.8	263.50	30.3	39.5	41.8	31.83
T6	131.8	127.0	119.5	126.08	17.8	22.0	18.8	19.67
T7	121.3	121.8	116.8	119.92	20.3	20.0	17.0	18.25
T8	317.5	322.3	385.0	341.58	36.0	43.3	49.0	42.75
CD (5%)	19.4	17.51	15.13	10.65	4.86	4.43	2.84	3.10
SE	9.4	8.4	7.27	5.12	2.36	2.13	1.37	1.49

Table 3. Bio-efficacy of liquid formulations of *Bacillus* bioagents on nematode gall index and yield of tomato

Treatments	Gall index at termination (1 to 5 scale)				Yield (ton ha $^{-1}$)				Cost Benefit ratio
	2015-16	2016-17	2017-18	Pooled	2015-16	2016-17	2017-18	Pooled	
T1	2.45	2.33	2.30	2.36	30.6	29.7	28.8	29.68	1:1.37
T2	2.48	2.38	2.35	2.38	30.7	30.4	28.1	29.73	1: 1.41
T3	1.78	1.70	1.60	1.69	34.4	34.1	31.9	33.46	1: 2.12
T4	1.83	1.75	1.83	1.80	34.7	33.1	31.3	33.01	1:2.10
T5	3.73	4.05	4.10	3.33	29.1	27.3	26.8	27.75	1:1.23
T6	1.95	2.23	2.38	2.78	30.8	29.5	28.0	29.43	1:1.81
T7	1.95	1.93	2.00	1.96	29.8	30.3	29.0	29.66	1:1.92
T8	4.93	4.93	4.88	4.91	26.8	25.6	24.4	25.58	
CD (5%)	0.29	0.34	0.33	0.25	2.65	1.06	0.78	1.05	
SE	0.14	0.16	0.16	0.13	1.28	0.51	0.37	0.50	

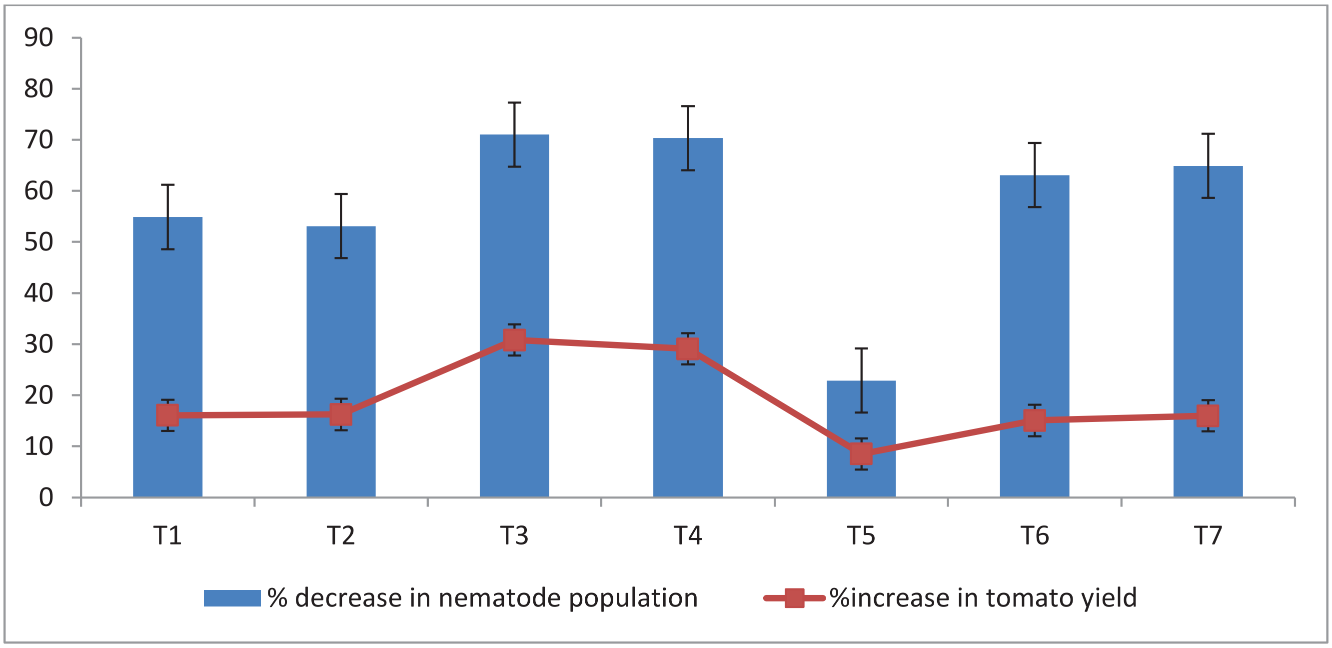

Fig. 1. Bio-efficacy of liquid formulations of *Bacillus* bioagents on nematode gall index and yield of tomato

[T1 - Substrate treatment with 5 ml of *Bacillus subtilis/* kg of cocopeatfor producing seedlings of tomato in portrays; T2 - Substrate treatment with 5 ml of *B. amyloliquefaciens/* kg of cocopeat for producing seedlings of tomato in portrays; T3- T1+ application of 20 tons of FYM enriched with 5 lit of *Bacillus subtilis* / ha; T4 - T2+ application of 20 tons of FYM enriched with 5 lit of *B. amyloliquefasciens* / ha; T5 - Application of 20 tons of FYM/ha; T6 - Chemical treatment (carbofuran at 1 kg a.i./ha); T7 - Chemical treatment (carbofuran at 1 kg a.i./ ha) +Recommended dose of FYM; T8 - Control without treatment] (pooled data of three trials).

A maximum of 71.02 and 70.33 per cent reduction in soil nematode population was recorded in T3 and T4, respectively (Fig. 1). T3 recorded the lowest gall index (GI - 1.69) which was at par with T4 (GI - 1.80) coupled with higher yield of 33.46 and 33.01 t ha^{-1} in T3 and T4, respectively. The maximum cost benefit ratio was recorded as 1:2.12 in T3 and 1:2.10 in T4 (Fig.1; Table 3).

It was followed by treatment with chemical nematicide carbofuran (T6) which revealed 126.08 J2 per 100 cc. soil and 19.67 females per 10 g of tomato roots. When combined with application of FYM (T7), there was a further decrease in nematode population (119.92 J2 per 100 c.c soil; 18.25 per 10g roots) (Table 2). Gall indices in chemical treatments were significantly lower (GI - 1.95 to 2.23) than untreated control (GI - 4.88 to 4.93). Overall for three years, T6 and T7 recorded 63.09 and 64.89 per cent reduction in nematode population and 15.05 and 15.97 per cent increase in tomato yield, resulting in cost benefit ratio was 1: 1.81 to 1:1.92, respectively (Fig. 1; Table 3).

Substrate treatment with *B. subtilis* IIHR Bs-2 1% A.S. and *B. amyloliquefaciens* IIHR Ba-2 1% A.S also significantly reduced 54.89 and 53.09 per cent of nematode population, respectively compared to control (Fig. 1) with cost benefit ratio ranging from 1:1.37 to 1:1.41. Application of FYM alone @ 20 t/ha revealed 22.86 per cent reduction in nematode population compared to control, with an average of 263.5 J2 per 100 cc soil and 31.83 females per 10 g roots, in all the three trials. Gall indices from 3.73 to 4.10 and yield from 26.8 to 29.1 t ha^{-1} was recorded in this treatment (Table 3).

Maximum nematode population in soil (317.5 to 385 J2 per 100 c.c. soil) and roots (36 to 49 females per 10 g roots) and gall index (4.83 to 4.93) was recorded in the untreated control plants. It also recorded the least yield (24.4 to 26.8 t ha^{-1}) in all the three trials (Table &3).

DISCUSSION

In the present study, the effect of substrate treatment and soil application of *Bacillus subtilis* IIHR Bs-2 (1% A.S.) was at par with *B. amyloliquefaciens* IIHR Ba-2 (1% A.S.) in suppressing *M. incognita* population in tomato roots and soil and reducing the gall index. This falls in line with the findings of Roy *et al.* (2015) wherein application of *B. subtilis* @ 50g/m^2 in nursery bed combined with soil application of *B. subtilis* @ 2.5 kg along with 2.5 tons of FYM/ha recorded the lowest gall formation due to *M. incognita* in tomato and the highest yield. Simlilarly, the rate of development of *M. arenaria* was reduced in potato roots treated with *B. subtilis* than untreated control plants (Mohamedova and Samaliev, 2011). Also Rao *et al*. (2014) reported that *B. subtilis* when applied as seed treatment @ 20 g/kg and soil application after enrichment in FYM @ 5 t/ha proved to be effective in bringing down *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* disease complex in okra. Burkett-Cadena *et al*. (2008) reported that application of commercial rhizobacterial products containing *B. subtilis* and *B. amyloliquefaciens* significantly reduced the nematode population, gall index and number of eggs of *M. incognita* in tomato roots.

Bacillus lipopeptides *viz.* surfactins, iturins and fengycins are much documented biomolecules for their antagonistic activity against a multitude of phytopathogenic microbes and nematodes (Gray *et al.,* 2006; Kavitha et al., 2012; Sarangi and Ramakrishnan, 2016). *purL* gene of *B. subtilis* (OKB105 and 69) and *B. amyloliquefaciens* (FZB42 and B3) were reported to exhibit nematicidal property against *M.javanica, Aphelenchoides besseyi*, *Ditylenchus destructor* and *Bursaphelenchus xylophilus* (Xia *et al*., 2011). *B. amyloliquefaciens* FZB42 harbours huge gene clusters involved in synthesis of antimicrobial lipopepetides and polyketides (Chen *et al*., 2006). More recently, Prabu *et al*. (2019) deciphered ovicial and larvicidal acitivity of *B. amyloliquefaciens* IIHR BA2 against *M. incognita* by detecting antibiotic genes viz., iturin A (iturin A synthetase D), iturin C (Iturin A synthetase C), iturin D (Iturin A synthetase D), surfactin (Surfactin synthetase thioesterase), bacillycin BacD (Carboxylate—amine ligase), bacillycin BacAB (Bacilysin biosynthesis protein bacA) and *purL* gene (phosphoribosylformyl - glycinamidine synthase II). Furthermore, Huang *et al*. (2010) reported that *Bacillus* spp. produced many nematicidal volatile compounds such as benzene acetaldehyde, 2-nonanone, decanal, 2-undecanone and dimethyl disulphide, which affected egg hatching of *M. incognita*. Secretion of all these lipopeptides and volatile compounds exhibited antagonistic action against *M. incognita*.

Also in the present study, application of liquid formulations of *Bacillus* spp. was more effective than chemical nematicide carbofuran, in increasing the yield and cost benefit ratio. Similar results were obtained by Rao *et al.* (2015a) wherein liquid *B. subtilis* 1% A.S formulation suppressed *M. incognita* population (-55.5%) and *Fusarium oxysporum* disease incidence (-20.3%) in tomato and increased the yield (+14.9 to 15.2%) and it was more effective than talc based solid formulation and chemical carbofuran. Sarangi *et al.* (2017) observed several *Bacillus* spp. producing plant growth hormones *viz.,* indole acetic acid and gibberlic acid and inducing the defence enzymatic activities of peroxidase, polyphenol oxidase and phenyl alanine lyase against *M. incognita* in tomato. In *B. amyloliquefaciens* SQR9, Shao *et al.* (2015) detected several plant growth promoting factors like extracellular phytase, volatile components (acetoin, 2, 3 - butanediol) and phytohormones (IAA) which made it a promising plant-growth promoting agent to increase crop yield for agricultural application.

The present study also demonstrates that *B. subtilis* IIHR Bs-2 and *B. amyloliquefaciens* IIHR Ba-2 were effectively enriched in FYM and soil application of bioagents enriched in FYM showed more cost benefit ratio. Similar reports were observed by Rao *et al.* (2017) wherein soil application of *B. subtilis* enriched vermicompost successfully decreased the *M. incognita* and *Pectobacterium carotovorum* subsp. *carotovorum* disease complex in carrot. Bioagent activity is directly correlated with organic amendments and enhanced due to accumulation of beneficial microbes and their metabolites in amended soils (Walker, 2004; Wang *et al.,* 2003). Enhancement of bioagent population in organic composts like FYM or vermicompost is readily adopted by farmers as it reduces the cost of production (Rao *et al.*, 2015 b).

CONCLUSION

The present study proves the antagonistic potential of liquid formulations of *Bacillus subtilis* IIHR BS-2 (1% A.S.) and *Bacillus amyloliquefaciens* IIHR Ba-2 (1% A.S.) against the root knot nematode and yield enhancement in tomato. There is a large scope for exploiting these microbes as biopesticides for nematode management in open fields, polyhouses and organic farms across several crops, as safe alternatives for chemical nematicides.

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