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Field efficacy of biocontrol agents for the management of root knot nematode *Meloidogyne incognita* (Kofoid & White) Chitw. and reniform nematode *Rotylenchulus reniformis* (Linford & Oliviera) in tomato

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ABSTRACT: Two field experiments were conducted with tomato Hybrid 5005 at Kuttathottam and Theethipalayam villages of Coimbatore district to investigate the potential of two promising native isolates of plant growth promoting rhizobacteria (PGPR), *viz.*, *Pseudomonas fluorescens* (Pfbv22) and *Bacillus subtilis* (Bbv57) for the management of root knot nematode *Meloidogyne incognita* and reniform nematode *Rotylenchulus reniformis* in tomato. The biocontrol agents were compared with the standard chemical *viz.*, carbofuran. Consortium application of *P. fluorescens* (Pfbv22) and *B. subtilis* (Bbv57) as seed treatment each @ 5 g kg^{-1} seeds and soil application (SA) @ 1.25 kg ha^{-1} significantly reduced the nematode infestation in soil and root. The microbial consortium treatment also significantly enhanced the plant growth parameters such as plant height, shoot weight, root length, root weight and fruit yield.

KEY WORDS: *Bacillus subtilis,* biological control, *Lycopersicon esculentum, Meloidogyne incognita, Pseudomonas fluorescens, Rotylenchulus reniformis*

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important and remunerative vegetable crops grown worldwide for fresh market and food processing industries. It is highly nutritious, rich in vitamin A, C and minerals. The production and productivity of the crop is greatly hampered by plant parasitic nematodes, *viz*., root knot nematode, *M. incognita* and reniform nematode *R. reniformis.* Bhatti and Jain (1977) have reported a yield loss of 46.2 per cent due to *M. incognita* in tomato. Subramanaian *et al*. (1990) reported a yield loss of 42.25 per cent due to *R. reniformis* in tomato. In recent years, use of bioagents is considered as one of the alternative approaches for the management of nematodes since it is safe and eco–friendly. Among the bioagents, plant growth promoting rhizobacteria have emerged as the largest and potentially most promising group against several plant parasitic nematodes. *Pseudomonas fluorescens,* was reported to be effective against *M. incognita* in many crops, *viz*., tomato and brinjal (Anita and Rajendran, 2002)), chickpea (Khan *et al*., 2001), turmeric (Srinivasan *et al*., 2001) and medicinal coleus (*Coleus forskohlii*) (Senthamarai *et al*., 2008). Plant growth promoting rhizobacteria strains, *viz*., *P. fluorescens* and *Bacillus subtilis* induce profuse root development and reduce population of *M. incognita* in banana and tomato (Jonathan *et al*., 2000). Therefore an investigation was carried out to determine the efficacy of two promising native isolates of plant growth promoting rhizobacteria, *viz*., *P. fluorescens* (Pfbv22) and *B. subtilis* (Bbv57) individually and in combination (consortium) for the management of *M. incognita* and *R. reniformis* in tomato.

MATERIALS AND METHODS

The two promising isolates were formulated in purified talc powder (sterilized at 105^{o}C for 12h) with calcium carbonate 15g (to adjust the pH to neutral) and carboxy methyl cellulose (CMC) 10g (adhesive), following the method described by Vidhyasekaran and Muthamilan (1995). The population load of talc formulation was 2.5 – 3 x 10^{8} CFUs g^{-1} and their bioefficacy were compared with that of standard chemical, carbofuran 3% granule. Untreated tomato plants were maintained for comparison.

Two field experiments were conducted during 2007–2008 in tomato Hybrid 5005 at Kuttathottam and Theethipalayam villages of Coimbatore district, Tamil Nadu, India to study the efficacy of two promising native

Table 1. Efficacy of biocontrol agents on *Meloidogyne incognita* infestation in tomato (Hybrid 5005) - pooled data of two field trials at Kuttathottam and Theethipalayam

Treatments	Nematode population /250cm^3 soil				No. of females / 5g root	No. of egg masses / 5g root	No. of eggs / egg mass	Gall index*
	PTP	30 DAA	60 DAA	90 DAA				
Pseudomonas fluorescens (Pfbv22) ST@ 10g kg-1 seeds & SA @ 2.5 kg / ha	195.1 (2.28)	162.3 (2.21)	153.1 (2.18)	137.6 (2.08)	134.8 (2.13)	29.6 (1.47)	186.2	2.2
Bacillus subtilis (Bbv 57) ST@ 10g kg^{-1} seeds & SA @ 2.5 kg ha-1	191.9 (2.28)	166.0 (2.22)	156.0 (2.20)	141.3 (2.12)	190.2 (2.28)	48.6 (1.66)	222.4	2.6
Consortia - *Pseudomonas fluorescens* (Pfbv22) + *Bacillus subtilis* (Bbv 57) each as ST@ 5g kg^{-1} seeds & SA @ 1.25 kg / ha each)	198.4 (2.30)	148.1 (2.17)	134.7 (2.09)	120.8 (2.07)	72.6 (1.86)	9.2 (0.96)	152.8	1.2
Carbofuran SA @ 1kg a.i. ha^{-1}	199.3 (2.30)	170.2 (2.23)	159.9 (2.20)	145.5 (2.12)	197.8 (2.30)	50.4 (1.69)	225.2	2.8
Control	192.8 (2.29)	222.0 (2.35)	249.7 (2.40)	265.8 (2.40)	268.4 (2.43)	75.8 (1.88)	277.8	5.0
S. Ed.	NS	0.005	0.009	0.007	0.010	0.023	1.991	0.36
CD (P=0.05)		0.011	0.019	0.046	0.021	0.048	4.221	0.76

Gall index * 1= 0 galls, 2 = 1-9 galls, 3 = 10-30 galls,4 = 31-100 galls and 5 = more than 100 galls per root system; PTP – Pre treatment population; ST – Seed treatment; SA – Soil application; Figures in the parentheses indicate log transformation

Table 2. Efficacy of biocontrol agents on *Rotylenchulus reniformis* infestation in tomato (Hybrid 5005) - pooled data of two field trials at Kuttathottam and Theethipalayam

Treatments	Nematode population /250cm^3 soil				No. of females / 5g root	No. of egg masses / 5g root	No. of eggs / egg mass
	PTP	30 DAA	60 DAA	90 DAA			
Pseudomonas fluorescens (Pfbv22) ST@ 10g kg-1 seeds & SA @ 2.5 kg / ha	148.1 (2.16)	113.3 (2.05)	99.1 (1.99)	85.5 (1.92)	9.3 (0.97)	7.5 (0.87)	37.8
Bacillus subtilis (Bbv 57) ST@ 10g kg^{-1} seeds & SA @ 2.5 kg ha^{-1}	144.6 (2.16)	117.0 (2.06)	102.3 (2.00)	90.3 (1.95)	13.7 (1.14)	12.3 (1.09)	55.4
Consortia – *Pseudomonas fluorescens* (Pfbv22) + *Bacillus subtilis* (Bbv 57) each as ST@ 5g kg^{-1} seeds & SA @ 1.25 kg / ha each)	143.1 (2.15)	100.0 (1.99)	81.3 (1.90)	73.7 (1.85)	5.6 (0.75)	3.5 (0.54)	21.2
Carbofuran SA @ 1kg a.i. ha^{-1}	150.6 (2.18)	119.6 (2.08)	104.6 (2.01)	93.8 (1.96)	14.6 (1.15)	12.8 (1.11)	56.8
Control	147.7 (2.17)	174.3 (2.23)	202.0 (2.30)	250.4 (2.36)	21.8 (1.34)	19.7 (1.29)	86.4
S. Ed.	0.010	0.009	0.008	0.057	0.010	0.017	0.280
CD (P=0.05)	0.022	0.019	0.017	0.026	0.022	0.360	0.594

PTP – Pre treatment population.; ST – Seed treatment; SA – Soil application; Figures in the parentheses indicate log transformation

Table 3. Efficacy of biocontrol agents on plant growth and yield parameters in tomato (Hybrid 5005) - pooled data of two field trials at Kuttathottam and Theethipalayam

Treatments	Plant height (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Yield / plot (20 m^2) (Kg)	CB Ratio
Pseudomonas fluorescens (Pfbv22) ST@ 10g kg^{-1} seeds & SA @ 2.5kg ha^{-1}	99.3	1177.5	49.8	181.6	222.0	1 : 3.9
Bacillus subtilis (Bbv 57) ST@ 10 g kg^{-1} seeds & SA @ 2.5kg ha^{-1}	92.9	1075.0	42.6	148.0	187.4	1 : 2.6
Consortia - *Pseudomonas fluorescens* (Pfbv22) + *Bacillus subtilis* (Bbv 57) each as (ST@ 5g kg^{-1} seeds & SA @ 1.25kg ha^{-1} each)	109.5	1342.5	57.4	210.2	244.6	1 : 4.8
Carbofuran SA @ 1kg a.i. ha^{-1}	90.0	1040.0	39.4	146.7	181.0	1 : 2.1
Control	60.4	540.0	29.9	71.6	124.0	
S. Ed.	1.80	29.39	1.68	2.78	4.63	
CD (P=0.05)	3.81	62.30	3.56	5.90	9.83	

ST – Seed treatment; SA – Soil application

isolates of plant growth promoting rhizobacteria *viz., P. fluorescens* (Pfbv22) and *B. subtilis* (Bbv57) against root knot nematode *M. incognita* and reniform nematode *R. reniformis* infesting tomato. The experiments were conducted in randomized block design with five treatments replicated five times. Raised nursery beds were prepared (2m length, 1m width and 15cm height) and seeds were treated with the bioagents *viz., P. fluorescens* (Pfbv22) and *B. subtilis* (Bbv57) individually and in combination (consortium) as per the dosages shown in the tables. Seeds were sown in the raised nursery beds and regular watering was done. The seedlings were uprooted carefully 30 days after sowing and transplanted to the main field.

The main field was ploughed to fine tilth and ridges and furrows were formed. The seedlings uprooted from the nursery beds were transplanted in the main field at a spacing of 60 x 45cm. Soil application of bioagents individually and as consortium and standard chemical, carbofuran were applied as per the dosages indicated in the tables at the time of transplanting and one month after transplanting. Pre-treatment soil samples from the respective plots were collected prior to planting at a depth of 10cm at the rate of 5 samples per plot. The collected soil samples were mixed thoroughly and representative sub samples of 250cm^3 were used for the estimation of initial nematode population. The crop was irrigated on third day of planting and subsequently at weekly intervals with underground water and weeding was done regularly. Farm yard manure was applied at the rate of 25 t/ha, at the time of planting. Fertilizer was applied at the rates of 50, 300, 50kg of N, P and K per hectare, respectively as basal dose and N and K each 150 kg ha^{-1} in 3 equal splits at 30, 45 and 60 days after transplanting. Plant growth parameters *viz*., plant height, shoot weight, root length, root weight and yield were recorded at the time of harvest. Post-treatment soil samples were collected on 30 and 60 days after planting (DAP) and at the time of harvest at a depth of 10 cm from 5 spots in each plot and mixed thoroughly to get representative sub-samples of 250cm^3 for nematode estimation. Root samples were collected at the time of harvest from each plot by carefully uprooting the plant. The collected soil samples were processed by Cobb's sieving and decanting method (Cobb, 1918) and modified Baermann funnel technique (Schindler, 1961) to assess the population of root knot nematode infesting tomato. The representative 5g root samples of each plot were washed free of soil and stained with 0.1% acid fuchsin in lactophenol solution to examine the gall index, number of females, egg masses and egg mass per 5g root. Gall indices were graded by rating on a 0 to 5 scale (Taylor and Sasser, 1978). To record *R. reniformis* population, the roots were stained with acid fuchsin lactophenol and examined for the presence of females, egg masses and number of eggs per egg mass. Data were statistically analysed and standard error and critical differences determined (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Significant increase in plant growth parameters *viz*., plant height, shoot weight, root length, root weight and yield were recorded with the consortium application of plant growth promoting rhizobacteria native isolates *viz*., *P. fluorescens* (Pfbv22) and *B. subtilis* (Bbv57) as seed treatment each @ 5g kg^{-1} seeds and SA @ 1.25kg ha^{-1}. Consortium of these two bioagents also significantly suppressed the infestation of root knot nematode in tomato recording lowest gall index, number of females and egg mass per 5g root than that of the standard chemical *viz*., carbofuran (Tables 1 to 3). Control plots recorded the highest soil and root population of nematodes with lowest yield. Application of *P. fluorescens* (Pfbv22) as seed treatment @ 10g kg^{-1} seeds and SA @ 2.5kg ha^{-1} was the next best effective treatment in reducing nematode infestation. *B. subtilis* (Bbv57) @ 10g kg^{-1} seeds and SA @ 2.5kg ha^{-1} and the chemical treatment *viz*., activie ingredient of carbofuran @ 1kg ha $^{-1}$ were equally effective in suppressing root knot and reniform nematode and promoting the plant growth and yield.

P. fluorescens is capable of surviving and colonizing the rhizosphere of all field crops and is reported to promote plant growth by secreting auxins, gibberellins and cytokinins (Vidhyasekaran, 1998). *P. fluorescens* native isolates are reported to be effective in suppressing the population of root knot nematode *M. incognita* (Jonathan *et al*., 2006) in banana. Becker *et al*. (1998) has proved the antagonistic effect of culture filtrates of *P. fluorescens* on eggs and juveniles of *M. incognita.* Initial application of fluorescent pseudomonads prior to invasion protects the crop from the pathogens by strengthening the cell wall structure and causing biochemical and physiological changes in the plant system (Chen *et al*., 2000). Plant growth promoting rhizobacteria *viz., P. fluorescens* and *B. subtilis* were reported to induce systemic resistance (ISR) in banana against lesion nematodes (Shanthi and Rajendran, 2006). The experimental data show the potential of the consortium application of bioagents in suppressing the nematode infestation and promoting plant growth than treated individually. This may be due to the combined effect of both the bioagents. Panneerselvam *et al*. (2008) revealed the superior effect of microbial consortium against root lesion nematode *Pratylenchus coffeae* in coffee plants. Thus the present investigation clearly indicate the combined potential of consortium of *P. fluorescens* (Pfbv22) and *B. subtilis* (Bbv57) in suppressing the root knot nematode infesting tomato.

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