



Bioefficacy of *Bacillus subtilis* against root knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood in tomato

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Abstract: An investigation was conducted for the management of root knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood infesting tomato through the application of bio-control agent like *Bacillus subtilis*, *Trichoderma harzianum* and *Pseudomonas fluorescens*. Experiment result revealed that minimum no. of galls/25seedlings (17.50) and maximum seedling height (27.6cm) were observed in *Bacillus subtilis* @50g/m² in nursery bed + *B. subtilis* @ 5kg along with 2.5 tons of FYM/ ha. The highest weight/25seedlings (69.50g) was noticed in the *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 2.5kg along with 2.5 tons of FYM/ha. The highest growth of the plant at 45 DAT (49.2cm) and at harvest (81.2cm) and maximum fresh (711.3g) and dry weight (265g) was found in *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 2.5kg along with 2.5 tons of FYM/ha. *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 2.5kg along with 2.5 tons of FYM/ha exhibited lowest gall index (1.2/plant) and highest reduction of nematode population and provided highest yield of tomato fruits (335.75q/ha).

Keywords: *Bacillus subtilis*, *Meloidogyne incognita*, Tomato

INTRODUCTION

Tomato, *Solanum lycopersicum* L. is grown worldwide as a protective supplementary food. It is a rich source of vitamins, minerals and organic acid. As it is a short duration crop and gives high yield, it is important from an economic point of view and hence the area under its cultivation is increasing day by day in India. The tomato is attacked by number of nematode pests. Globally, nematodes are common in almost all soils, with their distribution being determined by temperature, degree of moisture and soil particle size, along with the presence of acceptable food source (Bina *et al.*, 2012). *M. incognita* is a serious pathogen hampering the productivity in tomato crop significantly throughout the world (Mucksood and Tabreiz, 2010) and has been found to be very widely distributed with a wide host range and cause very serious damage (specially in vegetables). Root knot nematodes *M. incognita* (Kofoid and White) Chitwood have been found to be very widely distributed with a wide host range and cause very serious damage especially in tomato. In India, annual yield loss due to *M. incognita* has been estimated to as 27.21% in tomato (Jain *et al.*, 2007). Root-knot nematodes are of considerable economic importance (Hussain *et al.*, 2011, Kayani *et al.*, 2012). Plants exhibiting stunted or decline symptoms usually occur in patches of non uniform growth rather than as an overall decline of plants within an entire field.

Symptoms of root galling can in most cases provide positive diagnostic confirmation of nematode presence, infection severity, and potential for crop damage. Moreover, the nematodes not only affect the health of the crop but also reduce its quality and productivity. A number of methods for the management of root-knot nematodes such as chemical control, organic amendments, resistant varieties, soil solarization and biological control have been tried with different levels of successes for the protection of tomato plants (Randhawa *et al.*, 2001; Sakhuja and Jain, 2001). Keeping this background information in mind an approach was made to evaluate the bioefficacy of *Bacillus subtilis* against root knot nematode.

MATERIALS AND METHODS

An investigation was conducted at the Central Research Farm, Bidhan Chandra Krishi Viswavidyalaya, Gayeshpur, Nadia, West Bengal, India, during the year 2012-13. For the management of root knot nematode *M. incognita* (Kofoid and White) Chitwood infests tomato by application of biopesticide. The soil was a typical alluvial soil with a sandy clay loam texture with good drainage, slightly acidic pH and moderate fertility. The experiment was carried out with randomized block design with seven treatments and four replications having plot size 2m × 2.5m. The cultivar Patharkuchi (Local) used for the experiment.

Table 1. Effect of nursery treatments on gall formation, plant height and seedling weight in tomato seedlings during 2013-14.

Treatments	No. of galls/25seedlings	Plant height (cm)	Weight of 25 seedlings (g)
T1: <i>Trichoderma harzianum</i> @50g/m ² in nursery bed	24.50	25.95	51.25
T2 : T1 + <i>T. harzianum</i> @5 kg along with 2.5 tons of FYM	39.25	23.30	62.5
T3 : <i>Pseudomonas fluorescens</i> @ 10 ml/ha seed treatment	17.75	24.04	60.5
T4 : T3 + seedling treatment with <i>P. fluorescens</i> @ 150 ml/ha	23.75	21.82	54.5
T5: <i>Bacillus subtilis</i> @50g/m ² in nursery bed + <i>B. subtilis</i> 2.5 kg along with 2.5 tons of FYM/ ha	17.50	27.6	60.00
T6 : <i>B. subtilis</i> @50g/m ² in nursery bed + <i>B. subtilis</i> 5kg along with 2.5 tons of FYM/ha	21.75	26.88	69.50
T7 : Untreated Control	51.00	21.03	43.50
S. Em(±)	3.16	0.73	4.43
CD at 5%	9.38	2.17	13.16

Table 2. Effect of treatment on shoot length and root weight of tomato plant.

Treatments	Shoot length at 45 DAT(cm)	Shoot length at harvest(cm)	Fresh weight (g/plant)	Dry weight (g/plant)
T1 : <i>Trichoderma harzianum</i> @50g/m ² in nursery bed	41.9	69.9	515.0	100.00
T2 : T1 + <i>T. harzianum</i> @5 kg along with 2.5 tons of FYM	47.0	75.6	615.8	142.5
T3 : <i>Pseudomonas fluorescens</i> @ 10 ml/ha seed treatment	42.9	67.0	575.0	101.25
T4 : T3 + seedling treatment with <i>P. fluorescens</i> @ 150 ml/ha	45.9	78.1	650.0	164.00
T5: <i>Bacillus subtilis</i> @50g/m ² in nursery bed + <i>B. subtilis</i> 2.5 kg along with 2.5 tons of FYM/ ha	48.4	75.3	641.3	217.50
T6 : <i>B. subtilis</i> @50g/m ² in nursery bed + <i>B. subtilis</i> 5kg along with 2.5 tons of FYM/ ha	49.2	81.2	711.3	265.00
T7 : Untreated Control	39.6	61.0	508.8	96.25
S. Em(±)	1.5	2.4	40.1	16.88
CD at 5%	4.4	7.2	119.0	50.16

In case of initial soil nematode population study, soil sample was collected from the experimental plot. Nematode population (J_2) in 200 cm³ soil at 45 days after sowing (DAS) and at harvest collected from root zone of the plant. Nematodes were extracted from 200 cc composite samples of soil by Cobb's decanting sieving technique followed by Baermann's funnel method (Christie and Perry, 1951). The roots of the plants for examination were separated from the plant, to be mixed together and then only (2g) of roots are to be collected from the composite sample. The roots were then cleaned in tap water, cut into pieces of (2-3cm) and stained by NaOCl acid Fuchsin method (Byrd *et al.*, 1983). Stained root samples were then checked under stereoscopic microscope for taking observation on egg mass and nematode (juveniles+ female) population. The nema-suspension was counted with the help of measuring cylinder to get total nematode from 200cc of soil. (2ml) nema-suspension was taken in counting disk for getting the average juvenile population per ml of nema-suspension. Nematode count has been made under Stereoscopic microscope. No of galls, plant height and weight of seedling was taken at transplanting. Shoot length was taken

at 45 DAS and before harvest of crop from 10plants/plot. Population of root knot nematode, *M. incognita* was taken from 200cc of soil and (2g) roots both at 45 days after transplanting (DAT) and at harvest. Yield per plot and fresh and dry root weight was taken after harvest of the crop. Number of galls or knots present on the roots of tomato was counted from five randomly selected plants per plot. Root knot index was calculated using following scale (1-5) as proposed by AICRP on nematodes. 1= 0 galls, 2= 1-10 galls, 3 =11-30 galls, 4= 31-100 galls, 5= > 100 galls. The data thus recorded during the experiment were analyzed statistically.

RESULTS AND DISCUSSION

Effect of treatments on the weight, gall formation and plant height of tomato seedlings: At the time of transplanting the treatment of *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 5kg along with 2.5 tons of FYM/ha provided the greatest seedling weight (69.50) seedlings (T₆) g/25seedling, followed by (62.5) g/25seedling in T₁ + *T. harzianum* @5 kg along with 2.5 tons of FYM (T₂) and (60.5) g/25seedling in *Pseudomonas fluorescens* @ 10 ml/ha seed treatment (T₃). These

Table 3. Effect of treatment on gall index, nematode population and fruit yield of tomato.

Treatments	Gall index	Nematode population in soil (J ₂ /200cm ³) +2(g) of root		Yield (q/ha)
		45DAT	Harvest	
T1: <i>Trichoderma harzianum</i> @50g/m ² in nursery bed	2.15	272.75	307.5	248.00
T2: T1 + <i>T. harzianum</i> @5 kg along with 2.5 tons of fym	1.6	255.25	276.75	291.75
T3 : <i>Pseudomonas fluorescens</i> @ 10 ml/ha seed treatment	2.15	285.25	322.88	239.25
T4: T3 + seedling treatment with <i>P. fluorescens</i> @ 150 ml/ha	1.5	221.14	247.75	269.00
T5: <i>Bacillus subtilis</i> @50g/m ² in nursery bed + <i>B. subtilis</i> 2.5 kg along with 2.5 tons of FYM/ ha	1.35	210.62	230.63	306.00
T6: <i>B. subtilis</i> @50g/m ² in nursery bed + <i>B. subtilis</i> 5kg along with 2.5 tons of FYM/ha	1.2	177.12	216.13	335.75
T7 : Untreated Control	3.175	328	364.63	210.50
S. Em(±)	0.20	11.84	14.30	15.55
CD at 5%	0.59	35.19	42.49	46.21

two treatments were significantly higher weight over other treatment. The lowest weight was found in the untreated control (43.50) g/25 seedlings (T₇). *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 2.5 kg along with 2.5 tons of FYM/ha provided the lowest gall formation /25 seedling (17.50) (T₅) statistically at par with *P. fluorescens* @ 10 ml/ha seed treatment (17.75) (T₃). The untreated control shows the maximum no. of galls/ 25 seedlings i.e. (51) (T₇).

The height of seedling of different treatment was observed at the time of transplanting, the highest plant highest was recorded in *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 5 kg along with 2.5 tons of FYM/ ha (27.6 cm) i.e. (T₅) followed by (26.88 cm) *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 5kg along with 2.5 tons of FYM/ha (T₆), both the treatment at statistically at par with each other. The lowest height of the seedlings was observed in untreated control (21.03 cm) (T₇) (Table: 1). Cannayane *et al.* (2001) also found that seedlings treated with the *Bacillus cereus* and *B. subtilis* against J₂ of *M. incognita*/g soil improved the growth characteristics (length and weight of shoot and root) of tomato there were significant decrease of number of galls and egg masses per root system and nematode population per 200 (g) soil. *B. subtilis* strains were able to promote root elongation in seedlings of *Cicer arietinum* up to 70 74% as compare to untreated control. (Swain and Ray, 2009).

Effect of treatment on shoot length and root weight of tomato: In the above experiment it found that after 45 (DAT), all the treatments were almost effective in exhibiting the higher shoot length of shoot with regard to untreated control. Maximum shoot length (49.2 cm) was noticed in *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 5kg along with 2.5 tons of FYM/ha (T₆) followed by treatment of *Bacillus subtilis* @50g/m² in nursery bed + *B. subtilis* 5 kg along with 2.5 tons of FYM/ ha (48.4cm) (T₅).

At harvesting stage, the maximum shoot length (81.2 cm) was noticed in *B. subtilis* @50g/m² in nursery bed

+ *B. subtilis* 5kg along with 2.5 tons of FYM/ha. (T₆) with regard to all treatments and the lowest value was found in untreated control (61cm) (T₇). Fresh and dry shoot weight of tomato plants were taken at harvest. It appeared that maximum fresh shoot weight (711.3g/plant) was observed in *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 5kg along with 2.5 tons of FYM/ha (T₆). The untreated control showed lowest value i.e. (T₇) (Table: 2). Treatment with *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 5kg along with 2.5 tons of FYM/ha (T₆) showed the maximum dry shoot weight (265g/plant) and minimum and (96.25g/plant) (T₇) in untreated control (Table: 2). The treatment of *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 5kg along with 2.5 tons of FYM/ha showed a significant increase in shoot length along with simultaneous increase in fresh and dry shoot biomass production over control. These findings were agreed with those of other workers (Dawar *et al.*, 2008; Ashoub and Amara, 2010). Khalil *et al.* (2012) also reported that super strain. *Bacillus subtilis* reduced root galls and remarkably increase tomato plant growth characters significantly and egg masses on the root system, as well as, juvenile's numbers in the soil. Growth parameters in terms of shoot length, root length, shoot fresh and dry weights, root fresh and dry weights and number of nodules per plant were found significantly increased in the plants treated with the two bacteria as compared to control (Akhtar *et al.*, 2012).

Effect of treatment on gall indexing, nematode population and yield of tomato: All the treatments were reduced the gall indexing in tomato. The lowest gall indexing (1.2) was noticed in treatment *B. Subtilis* @50g/m² in nursery bed + *B. subtilis* 5kg along with 2.5 tons of FYM/ha (T₆) followed by *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 5 kg along with 2.5 tons of FYM/ ha (1.35) which was statistically at par with (T₆). The maximum gall indexing observed in untreated control (3.17), *P. Fluorescens* @ 10 ml/ha seed treatment (T₃) and *T. Harzianum* @ 50g/m² in a nursery bed

(T₁) were statistically at par.

M. incognita, (Adult female + juvenile + egg mass) were taken from 2g roots both at 45 DAT and at harvest at 45 days after transplanting, lowest infestation (177.12) was recorded in *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 2.5kg along with 2.5 tons of FYM/ha (T₆) followed by (210.62) in *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 5 kg along with 2.5 tons of FYM/ ha (T₅). The highest infestation was (328) found in untreated control (T₇). At harvest the populations of root knot nematode, *M. incognita* in roots and soil was lowest (216.13) and highest infestation was (364.63) *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 2.5kg along with 2.5 tons of FYM/ha (T₆) and untreated control, respectively. The infestation at 45 DAT and at harvest is found in the similar treatment. In a laboratory experiment on tomato, *B. subtilis* had been observed to inhibit egg-hatching of *M. arenaria* and activity of J₂. *B. subtilis* also exhibited greatest reductions (70.0-99.8%) in the number of nematode root galls, egg-masses plant⁻¹ and number of J₂ 250⁻¹ cc of soil along with simultaneous increase in the dry biomass production of shoot and root of tomato (Mokbel, 2013). This finding was also similar with the present finding. Mohamedova and Samaliev (2011) tested the efficacy of the *Bacillus subtilis* on the development of *Meloidogyne arenaria* and observed that the rate of *M. arenaria* development in potato roots treated with *B. subtilis* was lower than that untreated roots.

The highest yield of tomato (335.75q/ha) was recorded in *B. subtilis* @ 50g/m² in nursery bed + *B. subtilis* 5kg along with 2.5 tons of FYM/ha (T₆) followed by (306.00 q/ha). *B. subtilis* @50g/m² in nursery bed + *B. subtilis* 5 kg along with 2.5 tons of FYM/ ha (T₅)(Table: 3). This finding agreed with (Orhan *et al.*, 2006) who also found that *Bacillus* have a potential to increase the yield and growth of different plants.

Conclusion

From the experiment, it was found that *B. subtilis* was fruitful in reducing root galls and soil population of *M. incognita* along with simultaneous increase of fruit yield of tomato as bio-control agent for the management of *M. incognita*.

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