

Nematicidal Activity of *Bacillus subtilis* Against Root Knot Nematode *Meloidogyne incognita* (Kofoid and White) Chitwood in Tomato

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Abstract An investigation was undertaken to study the effect of *Bacillus subtilis* in different method of applications such as seed treatment, nursery treatment and in their combinations was tested against *Meloidogyne incognita* in tomato. Seed treatment with *B. subtilis* @ 10g/kg seed + nursery bed treatment with *B. subtilis* @ 50g/m² + *B. subtilis* @ 5kg with 5 tons of FYM/ha⁻¹ showed lowest gall index (1.10) as well as lowest nematode population in soil (187.63 J2/200 cm³), highest fruit yield (177 q/ha) and also provided highest shoot length and fresh dry shoot biomass yield of tomato.

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

Introduction

Tomato (*Lycopersicon esculentum* L.) is one of the significant vegetable crop popularly being consumed all over the world. It is important for health and rich in several good compounds and also the principal source of lycopene in our diet. It had been found to be a significant potent antioxidant with a quenching rate constant on single oxygen almost twice as high as that of β - carotene. Regular consumption of tomato in our diet could provide protection against a broad range of epithelial cancers and cardiovascular diseases. Nematodes have been considered as one of the important pest of crop for a long time and conceived to be presently associated with all most all commercial crop specially with the vegetable. Plant parasitic nematodes are recognized as major agricultural pathogens and are known to attack plants and cause crop losses throughout the world. 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 [1]. Root knot nematodes (*Meloidogyne* spp.) stand out as the dominant group of plant parasitic nematodes in almost all vegetable fields and cause enormous losses. *M. incognita* has 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 [2]. Keeping in view the above aspects an investigation

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was carried out for the management of root knot nematode in tomato by using biocontrol agent *Bacillus subtilis* in different combination.

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* by using *Bacillus subtilis* in different methods of application in tomato. The experiment was carried out with randomized block design with six treatments and four replications having plot size 2m × 2.5m. The cultivar Patharkuchi (Local) used for the experiment. In case of initial soil nematode population study, soil sample was collected from the experimental plot. Nematode population (J2) in 200 cm³ soil at 45 days after transplanting (DAT) and at harvest collected from root zone of the plant. Nematodes were extracted from 200 cc composite soil samples by Cobb's decanting sieving technique followed by Baermann's funnel method [3]. 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 from each plot. The roots were then cleaned in tap water, cut into pieces of 2-3 cm and stained by NaOCI acid fuchsin method [4]. Stained root samples checked under stereoscopic microscope for taking observation on nematode (juveniles+female) population. The nematode suspension was counted with the help of measuring cylinder to get total nematode from 200cc of soil. Nematode suspension 2 (ml) was taken in counting disk for getting the average juvenile population per ml of nematode suspension. Shoot length was taken at 45 DAT and before harvesting of crop from 10 plants per plot. Harvesting of tomato done regularly. Population of root knot nematode, *M. incognita* was taken from 200 cc of soil and 2g roots both at 45 DAT and at harvest. Nematode count has been made under stereoscopic microscope. 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 nursery treatments on gall formation, height and weight in tomato seedlings

The number of galls/25 seedlings was recorded at transplanting and found lowest (9) in (T₂) seed treatment with *Bacillus subtilis* @ 10g/kg seed + Nursery bed treatment with *Bacillus subtilis* 50g/m². Seed treatment with *B. subtilis* @ 10g/kg seed + *B. subtilis* @ 5kg with 5 tons of FYM ha⁻¹ (T₄), seed treatment with *B. subtilis* @ 10g/kg seed + nursery bed treatment with *B. subtilis* @ 50g/m² + *B. subtilis* @ 5 kg with 5 tons of FYM ha⁻¹ (T₅), and seed treatment with *B. subtilis* @ 10g/kg seed (T₁) were statistically at par with each other and significantly reduce the gall indexing i.e. (10), (10.25) and (10.75), respectively (Table 1).

Maximum height of seedling (19.02 cm) was found in seed treatment with *B. subtilis* @ 10g/kg seed+nursery bed treatment with *B. subtilis* @ 50g/m² (T₂) which was statistically at par with that of (18.70 cm) in seed treatment with *Bacillus subtilis* @ 10g/kg seed + nursery bed treatment with *Bacillus subtilis* @ 50g/m² + *B. subtilis* @ 5kg with 5 tons of FYM ha⁻¹ (T₅). The lowest seedling height (13.67) was observed in untreated control (T₆).

Fresh weight of 25 seedlings (30 days old) was taken before transplanting in the main field. The treatment (T₅) i.e seed treatment with *Bacillus subtilis* @ 10g/kg seed + nursery bed treatment with *Bacillus subtilis* @ 50g/m² + *B. subtilis* @ 5kg with 5 tons of FYM ha⁻¹ provided highest seedling weight (51.25 g/25 seedling) followed by (50.75g/25 seedling) in seed treatment with *Bacillus subtilis* @ 10g/kg seed + nursery bed treatment with *B. subtilis* @50g/m² (T₂) respectively. *B. subtilis* @ 5kg with 5 tons of FYM ha⁻¹ (T₃) and untreated control (T₆) were statistically at par with each other. *B. subtilis* in one type of plant growth promoting rhizobacteria which has nematocidal activity. Cannayane et al. [5] also found that seedling treated with the *Bacillus cereus* and *B. subtilis* against J2 of *M. incognita* 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

Table 1. Effect of nursey treatments on gall formation, plant height and seedling weight in tomato seedlings.

Treatments	No. of galls/25 seedlings	Plant height (cm)	Weight of 25 seedlings (g)
T ₁ = Seed treatment with <i>Bacillus subtilis</i> @10g/kg seed	10.75	15.80	40.00
T ₂ = T ₁ + Nursery bed treatment with <i>Bacillus subtilis</i> @ 50g/m ²	9.00	19.02	50.75
T ₃ = <i>B. subtilis</i> @ 5kg with 5 tons of FYM ha ⁻¹	16.00	14.27	37.5
T ₄ = T ₁ + T ₃	10.00	17.12	41.25
T ₅ = T ₂ + T ₃	10.25	18.70	51.25
T ₆ = Untreated control	15.75	13.67	38.00
SEm (±)	0.95	0.55	1.95
CD at 5%	2.85	1.64	5.87

and nematode population per 200 (g) of soil. *B. subtilis* strains were able to promote root elongation in seedlings of *Cicer arietinum* up to 74% as compare to untreated control [6].

Effect of treatment on plant shoot length and fresh and dry shoot weight of tomato

All the treatments were almost effective in exhibiting the higher shoot length of tomato seedlings after 45 DAT with regard to untreated control (Table 2). Maximum shoot length (21.19 cm) was noticed in seed treatment with *Bacillus subtilis* @ 10g/kg seed + nursery bed treatment with *B. subtilis* @ 50g/m² + *B. subtilis* @ 5kg with 5 tons of FYM ha⁻¹ i.e. (T₅). At harvesting stage, the maximum shoot length (88.65 cm) was noticed in seed treatment with *B. subtilis* @ 10g/kg seed + nursery bed treatment with *B. subtilis* @50g/m² + *B. subtilis* @ 5kg with 5 tons of FYM ha⁻¹ (T₅) when used in combination. On other hand (86.98 cm), (85.70 cm), (85.13 cm) and (82.30 cm) shoot length were recorded in *B. subtilis* @ 5kg with 5 tons of FYM ha⁻¹ (T₃), seed treatment with *B. subtilis* @ 10g/kg

seed + nursery bed treatment with *Bacillus subtilis* @ 50g/m² (T₂), and seed treatment with *B. subtilis* @ 10g/kg seed (T₁) and untreated control (T₆) respectively. Nihorimbere et al. [7] stated that treatment with *B. subtilis* significantly increases plant height in tomato.

Maximum fresh shoot weight (362.50g/plant) was recorded in the treatment of nursery bed treatment with *B. subtilis* @ 50g/m² + *B. subtilis* @ 5 kg with 5 tons of FYM ha⁻¹ (T₅) followed by (337.50 g/plant) in seed treatment with *B. subtilis* @ 10g/kg seed + *B. subtilis* @ 5 kg with 5 tons of FYM ha⁻¹ (T₄) and significantly higher than untreated control (238.75g/plant).

Similar trend in observation were also recorded in case of dry shoot weight. Maximum dry shoot weight (65.75 g/plant) was observed in nursery bed treatment with *B. subtilis* @ 50 g/m² + *B. subtilis* @ 5 kg with 5 tons of FYM ha⁻¹ (T₅).Seed treatment with *Bacillus subtilis* @ 10 g/kg seed + *B. subtilis* @ 5 kg with 5 tons of FYM ha⁻¹ (T₄) was statistically on and

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

Treatments	Shoot length at 45 DAT (cm)	Shoot length at harvest (cm)	Fresh weight (g/plant)	Dry weight (g/plant)
T ₁ = Seed treatment with <i>Bacillus subtilis</i> @ 10g/kg seed	16.52	85.13	277.50	51.25
T ₂ =T ₁ + Nursery bed treatment with <i>Bacillus subtilis</i> @ 50g/m ²	20.51	85.70	252.50	50.00
T ₃ = <i>B. subtilis</i> @ 5kg with 5 tons of FYM ha ⁻¹	17.15	86.98	297.50	57.00
T ₄ = T ₁ + T ₃	19.02	88.14	337.50	64.25
T ₅ = T ₂ + T ₃	21.19	88.65	362.50	65.75
T ₆ = Untreated control	15.30	82.30	238.75	36.25
SEm (±)	0.50	1.27	13.26	4.93
CD at 5%	1.51	3.84	39.97	14.87

Table 3. Effect of treatment on gall indexing, root knot nematode population and yield of tomato fruits.

Treatments	Nematode population in soil (J2/200 cm ²) + 2g root			Yield (q/ha)
	Gall index	45 DAT	Harvest	
T ₁ = Seed treatment with <i>Bacillus subtilis</i> @ 10g/kg seed	2.83	51.25	225.75	137.17
T ₂ = T ₁ + Nursery bed treatment with <i>Bacillus subtilis</i> @ 50g/m ²	2.68	50.00	255.75	140.04
T ₃ = <i>B. subtilis</i> @ 5kg with 5 tons of FYM ha ⁻¹	2.00	57.00	241.00	154.56
T ₄ = T ₁ + T ₃	1.30	64.25	191.38	168.75
T ₅ = T ₂ + T ₃	1.10	65.75	187.63	177.00
T ₆ = Untreated control	3.40	36.25	295.50	135.00
SEm ±	0.17	4.93	15.93	3.80
CD at 5%	0.50	14.87	48.02	11.46

at par. It appeared that *B. subtilis* showed significantly higher fresh and dry weight of the tomato plant when used in the main field treatment along with seed treatment and nursery bed treatment seedlings than untreated control. These findings are also supported earlier [8] who found *Bacillus subtilis* isolates were significantly increase fresh and dry shoot weight of potato. Khalil et al. [9] reported that super strain *Bacillus subtilis* reduced root galls and remarkably increase tomato plant growth characters. Growth parameters in terms of shoot length root length, fresh and dry shoot weight, fresh and dry root weights and number of nodules per plant was found significantly increased in the plant treated *B. subtilis* as compared to control in *Vigna mungo* [10].

Effect of treatments on gall indexing, root knot nematode population and yield of tomato

The lowest gall index (1.10) was noticed in nursery bed treatment with *B. subtilis* @ 50 g/m² + *B. subtilis* @ 5 kg with 5 tons of FYM ha⁻¹ (T₅) followed by (1.30) in seed treatment with *B. subtilis* @ 10 g/kg seed + *B. subtilis* @ 5kg with 5 tons of FYM ha⁻¹ (T₄). The highest gall index (3.40) as observed in untreated control (T₆) and significantly higher than all other treatment (Table 3).

Initial nematode population before transplanting was (232.6 J2/200 cm³ cc) of soil. At 45 DAT lowest nematode population (36.25) was observed in untreated control (T₆) followed by seed treatment with *B. subtilis* @ 10g/kg seed + nursery bed treatment with *B. subtilis* @ 50g/m² (50.00) (T₂). At the time of harvest it was found lowest (187.63 J2/200 cm³) soil in

nursery bed treatment with *B. subtilis* @ 50g/m² + *B. subtilis* @ 5 kg with 5 tons of FYM ha⁻¹ (T₅) and statistically superior than all other treatments followed by (191.38 J2/200 cm³) soil seed treatment with *B. subtilis* @ 10g/kg seed + *B. subtilis* @ 5kg with 5 tons of FYM ha⁻¹.

The highest yield of tomato (177q/ha) was recorded in seed treatment with *B. subtilis* @ 10 g/kg seed + nursery bed treatment with *B. subtilis* @ 50 g/m² + *B. subtilis* 5 kg with 5 tons of FYM ha⁻¹ (T₅) followed by (168.75q/ha) in seed treatment with *B. subtilis* 10 g/kg seed + *B. subtilis* @ 5 kg with tons of FYM ha⁻¹ (T₄). This finding agreed with earlier report [11] who also found that *Bacillus* have a potential to increase the yield and growth of raspberry plant. Roy et al. [12] also reported that *B. subtilis* was fruitful in reducing root galling and soil population of *M. incognita* along with simultaneous increase of fruit yield of tomato. This result also collaborates with earlier findings [13] who found the seedlings of bell paper treated with *B. subtilis* showed significant reductions in gall index and numbers of nematodes in roots and also helps in increasing yield of the crop and reducing mortality of the plants in the plots.

References

1. Bina L, Mohilal N, Pramodini M, Shah MM (2012) Two new species of *Boleodorus* (Nematoda : Tylenchida) from Manipur, India. The Bioscan 7 : 115—118.
2. Jain RK, Mathur KN, Singh RV (2007) Estimation of losses due to plant parasitic nematodes on different crops in India. Ind J Nematol 37 : 219—221.
3. Christie JR, Perry VG (1951) Removing nematode from soil. Proc Helminthol Soc Washington 18 : 106—108.

4. Byrd DW, Kirkpatrick T, Barker KR (1983) An improved technique for clearing and staining plant tissue for detection of nematodes. *J Nematol* 15 : 12—143.
5. Cannayane I, Rajendran G, Ramakrishnan S (2001) Biocontrol potential of *Bacillus cereus* and *Bacillus subtilis* against *Meloidogyne incognita* in tomato. *Crop Res Hisar* 21 : 164—167.
6. Swain MR, Ray RC (2009) Biocontrol and other beneficial activities of *Bacillus subtilis* isolated from cow dung microflora. *Microbiol Res* 164 : 121—130.
7. Nihorimbere V, Ongena M, Cawoy H, Brostaux Y, Kakana P, Jourdan E, Thonart P (2010) Beneficial effects of *Bacillus subtilis* on field-grown tomato in Burundi : Reduction of local *Fusarium* disease and growth promotion. *Afr J Microbiol Res* 4 : 1135—1142.
8. Algeblawi A, Adam F (2013) Biological control of *Erwinia carotovora* sub sp. *Carotovora* by *Pseudomonas fluorescens*, *Bacillus subtilis* and *Bacillus thuringiensis*. *Inter J Chem, Envir & Bio Sci* 1 : 771-774.
9. Khalil MSEH, Allam AFG, Barakar AST (2012) Nematicidal activity of some biopesticide agents and microorganisms against root-knot nematode on tomato plants under greenhouse conditions. *J Pl Prot Res* 52 : 47—52.
10. Akhtar A, Hisamuddin A, Sharf R (2012) Antagonistic effects of *Pseudomonas fluorescens* and *Bacillus subtilis* on *Meloidogyne incognita* infection *Vigna mungo* L. *Inter J Pl anim Envir Sci* 2 : 55—63
11. Orhan E, Esitken A, Ercisli S, Turan M, Sahin F (2006) Effect of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. *Sci Hort* 111 : 38—43.
12. Roy S, Rathod A, Pramanik A (2015) Bioefficacy of *Bacillus subtilis* against root knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood in tomato. *J App & Natur Sci* 7 : 1012—1015.
13. Kumar MR, Rao MS, Chaya MK, Rajnikant R, Prabhu P, Kamalnath M (2013) Effect of *Bacillus subtilis* on suppression of disease complex in bell pepper (*Capsicum annum* L.). *Pest Manag Hort Ecos* 19 : 211—215.