

## Management of root- knot nematode *Meloidogyne incognita* in tomato with liquid bioformulations

Manjunatha T Gowda\*, C Sellaperumal<sup>1</sup>, B Rajasekhar Reddy, AB Rai and B Singh

Received: August 2018 / Accepted: January 2019

### Abstract

Root-knot nematodes are one of the major plant parasitic nematodes in tomato (*Solanum lycopersicum* L.). Considering its damage potential, a field experiment was piloted for the management of root-knot nematode, *Meloidogyne incognita* in tomato. For this study, *Bacillus subtilis* (1% A.S) and *Bacillus amyloliquefaciens* (1% A.S) liquid bioformulations were evaluated. The liquid bioformulations were evaluated in two delivery mechanisms such as nursery drench (5 ml/litre of water) and soil application of bioformulations (5 l ha<sup>-1</sup>) enriched with FYM (20 t ha<sup>-1</sup>) individually and in combination and their nematicidal efficacy compared with carbofuran 3G (1 kg a.i. ha<sup>-1</sup>) and combined application of carbofuran 3G (1 kg a.i. ha<sup>-1</sup>) with FYM (20 t ha<sup>-1</sup>). Among the bioformulations, integrated application of *B. amyloliquefaciens* (1% A.S) (nursery drench and soil application of enriched FYM) consistently exhibited greater nematicidal activity by resulting maximum percent reduction of nematode population in soil 77.1, 61.5 and 74.6 during 2015-16, 2016-17 and 2017-18, respectively. Therefore, we able to harvest marketable yield of 24.0, 22.5 and 31.1 t ha<sup>-1</sup> during 2015-16, 2016-17 and 2017-18, respectively which was next to combined application of carbofuran 3G (1 kg a.i. ha<sup>-1</sup>) with FYM (20 t ha<sup>-1</sup>). Subsequently, it was followed by *B. subtilis* 1% A.S (nursery drench and soil application of enriched FYM) found promising and recorded percent reduction of nematode population in soil 52.5, 55.8 and 70.4 with marketable yield of 20.0, 22.0 and 30.4 t ha<sup>-1</sup> during 2015-16, 2016-17 and 2017-18, respectively. The present findings indicate that, liquid bioformulations of *B. amyloliquefaciens* (1% A.S) and *B. subtilis* (1% A.S) with its delivery mechanisms can be considered as a component under integrated nematode management of *M. incognita* infecting tomato under field condition.

**Key words:** Root-knot nematode, *Meloidogyne incognita*, tomato, biological control, *Bacillus amyloliquefaciens*, *Bacillus subtilis*

### Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most popular, extensively grown vegetable crops in India. It is generally considered as poor man's apple because of its appearance and nutritive richness (vitamins, minerals and antioxidants). In India, tomato cultivated in 0.809 million hectares with 19.7 million tonnes production and 24.4 t ha<sup>-1</sup> productivity (Anonymous, 2017). However, the current level of productivity and quality are constrained by the direct interference of plant parasitic nematodes on the plant root system besides several pests and diseases. Plant parasitic nematodes hinder the uptake of nutrients as well as water. Among them, root-knot nematodes (*Meloidogyne* spp.) are the frequently observed and most damaging plant parasitic nematode genera in vegetable ecosystem. In known root-knot nematode species, *Meloidogyne incognita* and *M. javanica* are widely distributed in different parts of the country causing annual yield loss to the tune of 27.2% with an estimated 2204 million rupees of monetary loss in tomato (Jain et al. 2007). Owing to their parasitic activity, the second stage infective juvenile (J<sub>2</sub>) of root-knot nematodes infect and feeds plant nutrients by developing feeding sites on root system. The primary symptom of root-knot nematode infection is formation of typical galls on root system. Affected plants express symptoms similar to mineral deficiency such as chlorosis, yellowing of leaves, wilting and stunted growth (Abad et al. 2003) because of reduced uptake and translocation of nutrients from soil to shoot (Patil et al. 2013).

The nematode management largely depends on chemical nematicides. However, their potential negative impact on environment and concerns about human health (Ferraz and Freitas 2004, Anastasiadis et al. 2008) and

ICAR- Indian Institute of Vegetable Research, Varanasi-221305

<sup>1</sup>ICAR- Indian Institute of Spices Research, Kozhikode-673012

Corresponding author\*, Email: goudru9@gmail.com

phasing out of many effective chemical nematicides necessitated research towards finding alternative strategies for the management of nematodes. In this endeavour, biological control agents (BCA) have emerged as eco-friendly and safe and cost effective alternatives to chemical nematicides (Collange et al. 2011, Rao et al. 2015). In past years, several researchers, efforts have been made to identify microbial groups which limit the nematode abundance in soil and are categorized into egg-parasitic fungi, nematode-trapping fungi, filamentous fungi, antagonistic bacteria and polyphagous predatory nematodes (Kerry and Hidalgo-Diaz 2004, Kiewnick and Sikora 2005). Among these, the egg parasitic fungi, *Purpureocillium lilacinum* (*Paecilomyces lilacinus*) and filamentous fungi, *Trichoderma viride*, *T. harzianum* and plant growth promoting rhizobacteria, *Pseudomonas fluorescens* have been extensively exploited for the suppression of root-knot nematodes *Meloidogyne* spp. (Krishnaveni and Subramanian 2004, Haseeb and Khan 2012). *Bacillus* spp. is another group of bacterial agents has recently been recognized as one of the most promising groups of nematode antagonists of which *Bacillus subtilis*, *B. licheniformis*, *B. amyloliquefaciens* and *B. cereus* are increasingly becoming important for effective management of root knot nematodes (Mohammed et al. 2008; Mohamedova 2009; Terefe et al. 2009; Xiao et al. 2013; Rao et al. 2017; Abdel-Salam et al. 2018). Considering this, efforts have been made to evaluate the liquid bioformulation of *Bacillus subtilis* (1% A.S) and *Bacillus amyloliquefaciens* (1% A.S) involving two delivery mechanisms individually and in combination for the management of root-knot nematode *M. incognita* in naturally infested tomato field.

## Materials and Methods

The experiment was conducted in tomato (cv. Kashi Aman) at Nematology experimental site (25.1821° N latitude and 82.8770° E longitude) located at ICAR-Indian Institute of Vegetable Research, Varanasi, UP for three consecutive years (2015-16, 2016-17 and 2017-18) during Rabi season. The experimental site comes under the alluvial zone of Indo-Gangetic plain, soils having silt loam soil texture with neutral to slightly alkaline in reaction (pH: 7.34) and electrical conductivity 0.31 dSm<sup>-1</sup>. The root-knot nematode (*Meloidogyne incognita*) was prevalent in nematology experimental site. Prior to experiment, initial soil population of root-knot nematode populations were assessed using Cobb's sieving and decanting method (Cobb 1918). This site had a resident nematode population of 241.3 ± 8.72, 499.9 ± 9.82, 261.9 ± 5.11 per 250 CC of soil during 2015-16, 2016-17 and 2017-18, respectively.

**Nematode identification:** Root-knot nematode species from naturally infested nematology experimental site located at ICAR-IIVR, Varanasi was identified using molecular technique. DNA was isolated from newly hatched second stage infective juvenile using standard protocol described by Adam et al. (2007). Further, identity of the root-knot nematode species *M. incognita* was confirmed through specific SCAR marker, Inc-K14-F/ Inc-K14-R (Randig et al., 2002) and also morphologically confirmed by making temporary mounts of perineal pattern of the mature females.

**Liquid bioformulations:** Liquid bioformulations of *Bacillus subtilis* (1% A.S.) and *Bacillus amyloliquefaciens* (1% A.S.) procured from, Division of entomology and nematology, ICAR-Indian Institute of Horticultural Research, Bengaluru for the present study.

**Enrichment of liquid bioformulations:** Prior to experiments, each liquid bioformulation (5 l ha<sup>-1</sup>) was thoroughly mixed with FYM (20 t ha<sup>-1</sup>) and then covered with poly ethylene sheet by maintaining optimum moisture under shade for 15 days. Further, enriched FYM was applied to respective treatments before 15 days of transplanting.

**Field efficacy:** To evaluate the nematicidal activity of liquid bioformulations of *B. subtilis* (1% A.S.) and *B. amyloliquefaciens* (1% A.S.) against root-knot nematode, *M. incognita* in field, experiment was laid out in a randomized complete block design (RCBD) with eight treatments including different delivery mechanisms and there were three replicates per treatment. The treatments were as follows; T1: Nursery drench with *Bacillus subtilis* 1% A.S. @ 5 ml/litre of water; T2: Nursery drench with *Bacillus amyloliquefaciens* - 1% A.S. @ 5 ml/litre of water; T3: T1+FYM @ 20 t ha<sup>-1</sup> enriched with 5L *Bacillus subtilis*; T4-T2+FYM @ 20 t ha<sup>-1</sup> enriched with 5L *Bacillus amyloliquefaciens*; T5: FYM at 20 t ha<sup>-1</sup> only; T6: carbofuran 3G @ 1.0 kg 1.0 kg a.i.ha<sup>-1</sup>; T7: carbofuran 3G @ 1.0 kg a.i. ha<sup>-1</sup> + FYM @ 20 t ha<sup>-1</sup>; T8: control (untreated field). In nursery, coco peat was used as substrate for raising tomato (cv. Kashi Aman) seedlings in portrays. Before sowing, one kg of substrate was treated with (5 ml per litre of water) each bioformulation separately with respective treatment. Healthy seedlings were maintained in portrays up to 21 days and transplanted to main experimental plot. Crop was raised following standard agronomic practices. At the time of harvest, observations were recorded on plant growth parameters such as plant height (cm), root weight (g) (average of 15 plants were selected randomly) and marketable yield (t ha<sup>-1</sup>). Nematode disease parameters

such as gall index (0-10) scale 0= no knots on roots; 1 = few small knots difficult to find; 2 = small knots only but clearly visible; main roots clean; 3 = some larger knots visible, but main roots clean; 4 = larger knots predominate but main roots clean; 5 = 50% of roots knotted; knotting on parts of main root system; 6 = knotting on some of main roots; 7 = majority of main roots knotted; 8 = all main roots knotted; few clean roots visible; 9 = all roots severely knotted, plant usually die; 10 = all roots severely knotted, no root were recorded (Bridge and Page, 1980). Final soil nematode population was assessed by using Cobb's sieving and decanting method (Cobb 1918). The number of egg masses per root system (average of 15 plants were selected randomly of each treatment) were counted with the help of a magnifying glass. The number of eggs per egg mass was also counted under a stereo microscope.

**Statistical Analysis:** Analysis of variance (one way ANOVA) was performed for respective year data on plant height, root weight, marketable yield, gall index, number of egg mass per root system, number of eggs per egg mass and final nematode population in soil. The significant ( $P < 0.05$ ) differences among treatments were determined by using Tukey's studentized Range (HSD) test (PROC GLM SAS version 9.2; SAS institute).

## Results

In the present study, data on nematicidal efficacy of bioformulations with carbofuran presented in Table 1, 2, 3 and Fig. 1 evidently indicates that all the treatments were considerably reduced the incidence of *M. incognita* in tomato and enhanced plant growth compared to untreated control. Among the bioformulations, the treatment (T4) involving integration of nursery drench and soil application of *B. amyloliquefaciens* (1% A.S) enriched FYM consistently provided a better protection from *M. incognita* to tomato by resulting maximum percent reduction of final nematode population in soil was of 77.1, 61.5, 74.6, number of egg mass per root system was 69.6, 70.2 and 74.2, number of eggs per egg mass were 68.0, 65.8 and 70.5, lesser root gall index (0-10 scale) of 2.0, 2.0 and 1.5 with maximum marketable yield of 24.0, 22.5 and 31.1 t ha<sup>-1</sup> during 2015-16, 2016-17 and 2017-18, respectively and which was next to carbofuran 3G @ 1.0 kg a.i. ha<sup>-1</sup> and combined application of carbofuran 3G (@ 1.0 kg a.i. ha<sup>-1</sup>) with FYM (20 t ha<sup>-1</sup>) however, it was statistically at par with carbofuran treatments (Table 1, 2 and 3).

Subsequently, it was followed by the treatment (T3) having integration of nursery drench and soil application

**Table 1:** Nematicidal efficacy of liquid bioformulation of *Bacillus amyloliquefaciens* (1% A.S) and *Bacillus subtilis* (1% A.S) on *Meloidogyne incognita* infecting tomato

Treatments	RKI (0-10)			Average (Three years)	Final soil population (250 CC) Mean±SE			Average (Three years)
	2015-16	2016-17	2017-18		2015-16	2016-17	2017-18	
T1	4.0 <sup>bc</sup>	4.0 <sup>cd</sup>	3.4 <sup>b</sup>	3.80	422.0 <sup>b</sup> ± 10.4 (-37.7)	700.0 <sup>bc</sup> ± 21.4 (-16.0)	402.0 <sup>b</sup> ± 25.6 (-24.6)	508.0 (-25.4)
T2	4.0 <sup>bc</sup>	5.0 <sup>bc</sup>	3.1 <sup>bc</sup>	4.03	410.6 <sup>b</sup> ± 5.0 (-39.4)	656.3 <sup>c</sup> ± 15.0 (-21.2)	395.5 <sup>b</sup> ± 24.0 (-25.8)	487.5 (-28.4)
T3	3.0 <sup>cd</sup>	3.0 <sup>de</sup>	1.7 <sup>cd</sup>	2.57	321.6 <sup>c</sup> ± 12.1 (-52.5)	368.0 <sup>d</sup> ± 15.0 (-55.8)	157.7 <sup>c</sup> ± 11.0 (70.4)	282.4 (-58.5)
T4	2.0 <sup>d</sup>	2.0 <sup>e</sup>	1.5 <sup>d</sup>	1.83	155.3 <sup>d</sup> ± 8.5 (-77.1)	320.6 <sup>d</sup> ± 12.3 (-61.5)	135.5 <sup>c</sup> ± 07.9 (-74.6)	203.8 (-70.0)
T5	5.0 <sup>b</sup>	6.0 <sup>ab</sup>	4.0 <sup>b</sup>	5.00	444.0 <sup>b</sup> ± 16.5 (-34.4)	805.0 <sup>ab</sup> ± 20.1 (-3.4)	453.3 <sup>a</sup> ± 11.3 (-15.0)	567.4 (-16.7)
T6	2.0 <sup>d</sup>	2.0 <sup>e</sup>	1.5 <sup>d</sup>	1.83	155.0 <sup>d</sup> ± 11.6 (-77.1)	290.3 <sup>d</sup> ± 20.1 (-65.2)	133.3 <sup>c</sup> ± 03.1 (-75.0)	192.9 (-71.6)
T7	2.0 <sup>d</sup>	2.0 <sup>e</sup>	1.3 <sup>d</sup>	1.77	155.3 <sup>d</sup> ± 11.9 (-77.1)	256.0 <sup>d</sup> ± 36.0 (-69.3)	102.2 <sup>c</sup> ± 03.6 (-80.8)	171.1 (-74.8)
T8	7.0 <sup>a</sup>	7.0 <sup>a</sup>	5.4 <sup>a</sup>	6.47	677.0 <sup>a</sup> ± 11.9 (0.0)	833.3 <sup>a</sup> ± 13.2 (0.0)	533.3 <sup>a</sup> ± 17.5 (0.0)	681.2 (0)
<b>Tukey's HSD at 0.05</b>	<b>1.23</b>	<b>1.46</b>	<b>1.20</b>		<b>68.55</b>	<b>122.54</b>	<b>91.76</b>	

Figures presented in parentheses ( ) are percent increase (+) or decrease (-) over control. RKI: Root-knot index; SE: Standard error. Different letters on each column indicate statistically significant difference between treatments at ( $P < 0.05$ ) using Tukey's HSD test. **Treatment details:** **T1:** Nursery drench with *Bacillus subtilis* 1% A.S. @ 5 ml/litre of water; **T2:** Nursery drench with *Bacillus amyloliquefaciens* - 1% A.S. @ 5 ml/litre of water; **T3:** T1+FYM @ 20 t ha<sup>-1</sup> enriched with 5L *Bacillus subtilis*; **T4:** T2+FYM @ 20 t ha<sup>-1</sup> enriched with 5L *Bacillus amyloliquefaciens*; **T5:** FYM at 20 t ha<sup>-1</sup> only; **T6:** Carbofuran 3G @ 1.0 kg 1.0 kg a.i. ha<sup>-1</sup>; **T7:** Carbofuran 3G @ 1.0 kg a.i. ha<sup>-1</sup> + FYM @ 20 t ha<sup>-1</sup>; **T8:** Control (Untreated).

of *B. subtilis* (1% A.S) enriched FYM was found promising by recording percent reduction of final nematode population in soil was 52.5, 55.8 and 70.4, number of egg mass per root system was 66.8, 65.9 and 69.9, number of eggs per egg mass were 65.4, 63.5 and 67.2, root gall index (0-10 scale) of 3.0, 3.0 and 1.7 with marketable yield of 20.0, 22.0 and 30.4 t ha<sup>-1</sup> during 2015-16, 2016-17 and 2017-18, respectively

(Table 1, 2 and 3). However, treatment (T3) differed statistically significant with the treatment (T4) *B.* and carbofuran with respect to reduction of final nematode population in soil and yield during first year field trial (Table 1 and 3). Nevertheless, in the present study the yield performance of tomato was little poor during 2015-16 and 2016-17 which may attribute to late sowing and transplanting (second fortnight of November during 2015

**Table 2:** Nematicidal efficacy of liquid bioformulation of *Bacillus amyloliquefaciens* (1% A.S) and *Bacillus subtilis* (1% A.S) on *Meloidogyne incognita* infecting tomato.

Treatments	Number of egg mass/root system Mean±SE			Average (Three years)	Number of eggs/egg mass Mean±SE			Average (Three years)
	2015-16	2016-17	2017-18		2015-16	2016-17	2017-18	
T1	65.8 <sup>b</sup> ± 1.89 (-17.7)	74.4 <sup>ab</sup> ± 3.89 (-19.7)	50.8 <sup>b</sup> ± 2.45 (-20.9)	63.7 (-18.2)	271.0 <sup>b</sup> ± 6.85 (-11.1)	253.7 <sup>b</sup> ± 9.49 (-15.1)	244.1 <sup>b</sup> ± 7.81 (-15.9)	256.3 (-14.0)
T2	64.2 <sup>b</sup> ± 3.27 (-19.8)	71.0 <sup>b</sup> ± 6.16 (-23.4)	48.4 <sup>b</sup> ± 1.61 (-24.6)	61.2 (-22.5)	250.9 <sup>b</sup> ± 4.99 (-17.7)	252.1 <sup>b</sup> ± 5.20 (-15.7)	228.7 <sup>b</sup> ± 9.54 (-21.3)	243.9 (-18.2)
T3	26.6 <sup>c</sup> ± 2.64 (-66.8)	31.6 <sup>c</sup> ± 3.98 (-65.9)	19.3 <sup>c</sup> ± 1.66 (-69.9)	25.8 (-67.3)	105.5 <sup>c</sup> ± 4.43 (-65.4)	109.1 <sup>c</sup> ± 6.42 (-63.5)	95.3 <sup>c</sup> ± 4.34 (-67.2)	100.0 (-65.4)
T4	24.3 <sup>c</sup> ± 2.37 (-69.6)	27.6 <sup>c</sup> ± 2.14 (-70.2)	16.6 <sup>c</sup> ± 1.50 (-74.2)	22.8 (-71.1)	97.6 <sup>c</sup> ± 3.34 (-68.0)	102.1 <sup>c</sup> ± 5.24 (-65.8)	85.6 <sup>c</sup> ± 5.78 (-70.5)	95.1 (-68.1)
T5	74.5 <sup>ab</sup> ± 2.59 (-6.8)	85.0 <sup>ab</sup> ± 8.16 (-8.3)	54.8 <sup>ab</sup> ± 2.55 (-14.6)	71.4 (-9.6)	274.3 <sup>ab</sup> ± 6.64 (-10.1)	269.2 <sup>ab</sup> ± 7.64 (-9.9)	262.0 <sup>ab</sup> ± 8.71 (-9.8)	268.5 (-9.9)
T6	22.3 <sup>c</sup> ± 1.91 (-69.4)	25.3 <sup>c</sup> ± 3.42 (-72.7)	14.2 <sup>c</sup> ± 1.91 (-77.9)	20.6 (-74.0)	95.8 <sup>c</sup> ± 4.91 (-68.6)	98.6 <sup>c</sup> ± 3.02 (-67.0)	80.6 <sup>c</sup> ± 7.81 (-72.2)	91.7 (-69.2)
T7	20.5 <sup>c</sup> ± 0.64 (-74.4)	22.5 <sup>c</sup> ± 2.12 (-75.8)	12.3 <sup>c</sup> ± 1.19 (-80.9)	18.4 (-76.7)	85.8 <sup>c</sup> ± 3.76 (-71.9)	87.9 <sup>c</sup> ± 4.59 (-70.6)	74.7 <sup>c</sup> ± 4.38 (-74.3)	82.8 (-72.2)
T8	80.0 <sup>a</sup> ± 2.27 (0.0)	92.7 <sup>a</sup> ± 9.0 (0.0)	64.2 <sup>a</sup> ± 2.23 (0.0)	79.0 (0.0)	305.0 <sup>a</sup> ± 4.71 (0.0)	298.9 <sup>a</sup> ± 5.53 (0.0)	290.4 <sup>a</sup> ± 7.94 (0.0)	298.1 (0.0)
<b>Tukey's HSD at 0.05</b>	<b>13.87</b>	<b>18.91</b>	<b>11.63</b>		<b>31.64</b>	<b>37.02</b>	<b>42.65</b>	

Figures presented in parentheses ( ) are percent increase (+) or decrease (-) over control. SE: Standard error. Different letters on each column indicate statistically significant difference between treatments at ( $P < 0.05$ ) using Tukey's HSD test.

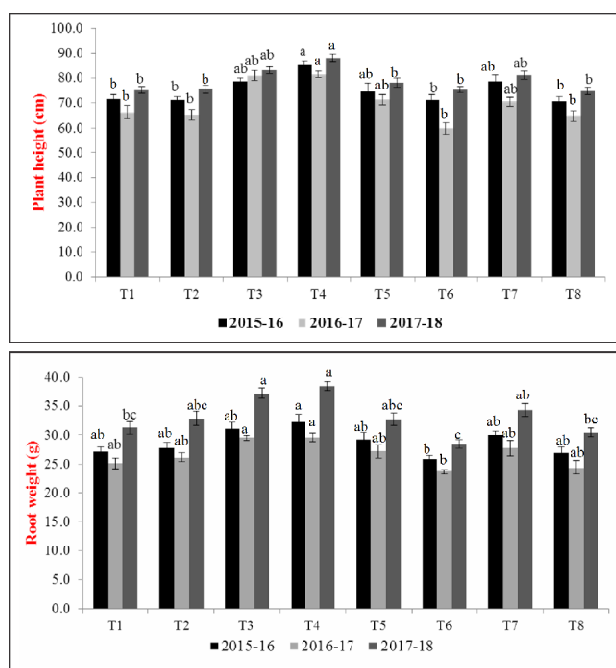
**Table 3:** Effect of liquid bioformulation, *Bacillus amyloliquefaciens* (1% A.S) and *Bacillus subtilis* (1% A.S) on yield of tomato infected by *Meloidogyne incognita*.

Treatments	Marketable yield (t/ha) Mean ±SE			Average (Three years)
	2015-16	2016-17	2017-18	
T1	18.4 <sup>cd</sup> ± 0.69 (+12.0)	20.3 <sup>bc</sup> ± 0.40 (+3.4)	28.1 <sup>cd</sup> ± 0.35 (+8.1)	22.3 (+7.6)
T2	18.7 <sup>bcd</sup> ± 0.53 (+14.2)	20.6 <sup>ab</sup> ± 0.44 (+4.8)	28.6 <sup>bcd</sup> ± 0.28 (+9.2)	22.6 (+9.1)
T3	20.0 <sup>bc</sup> ± 0.29 (+21.7)	22.0 <sup>ab</sup> ± 0.31 (+11.9)	30.4 <sup>abc</sup> ± 0.28 (+16.4)	24.1 (+16.4)
T4	24.0 <sup>a</sup> ± 0.45 (+46.1)	22.5 <sup>a</sup> ± 0.27 (+14.5)	31.1 <sup>a</sup> ± 0.34 (+18.7)	25.8 (+24.7)
T5	16.5 <sup>d</sup> ± 0.24 (+0.6)	19.7 <sup>c</sup> ± 0.26 (+0.5)	27.4 <sup>d</sup> ± 0.58 (+4.7)	21.2 (+2.2)
T6	21.4 <sup>ab</sup> ± 0.64 (+30.5)	21.8 <sup>ab</sup> ± 0.32 (+10.9)	31.0 <sup>ab</sup> ± 0.45 (+18.5)	24.7 (+19.2)
T7	24.4 <sup>a</sup> ± 0.37 (+48.6)	22.4 <sup>a</sup> ± 0.35 (+13.9)	31.8 <sup>a</sup> ± 0.53 (+21.7)	26.2 (+26.3)
T8	16.4 <sup>d</sup> ± 0.57 (0.0)	19.6 <sup>c</sup> ± 0.17 (0.0)	26.2 <sup>d</sup> ± 0.27 (0.0)	20.7 (0.0)
<b>Tukey's HSD at 0.05</b>	<b>2.97</b>	<b>1.95</b>	<b>2.42</b>	

Figures presented in parentheses ( ) are percent increase (+) or decrease (-) over control. SE: Standard error. Different letters on each column indicate statistically significant difference between treatments at ( $P < 0.05$ ) using Tukey's HSD test.

and 2016). Moreover, tomato (cv. Kashi Aman) performed better during 2017-18, since the crop was transplanted in first fortnight of October month (Table 3).

Besides nematicidal activity and improved marketable yield, bio agents were also considerably enhanced plant growth by increasing plant height and root weight reliably for three consecutive years 2015-16, 2016-17 and 2017-18. The two bioformulations as well as carbofuran 3G @ 1.0 kg a.i. ha<sup>-1</sup> with FYM (20 t ha<sup>-1</sup>) were statistically at par with their plant growth promotion activity and significantly better over carbofuran 3G @ 1.0 kg a.i. ha<sup>-1</sup> (Fig. 1).



**Fig. 1:** Effect of liquid bioformulation, *Bacillus amyloliquefaciens* (1% A.S) and *Bacillus subtilis* (1% A.S) on plant growth parameters of tomato.

Means followed by the same letter in top of error bars are not significantly different between treatments at ( $P < 0.05$ ) using Tukey's HSD test.

## Discussion

The present investigation indicated that, the liquid bioformulations of *B. amyloliquefaciens* (1% A.S) and *B. subtilis* (1% A.S) gave a good control of *M. incognita* by reducing final soil population, number of egg mass per root system, number of eggs per egg mass, gall index with considerable increase in plant height, root weight and marketable yield in tomato under field condition. The nematicidal activity of these bacterial agents might be attributed to secretion of antimicrobial compounds. Earlier reports revealed that, secretion of various types of antimicrobial metabolites and enzymes

from *Bacillus* spp. exhibit strong antagonism against root-knot nematode (Saxena *et al.* 2000, Ann 2013). Killani *et al.* (2011) revealed that, the production of five types of antimicrobial compounds such as bacitracin, bacillin, submillin, subtenolin and bacilonycin from *B. subtilis* are responsible for antimicrobial activity. Similarly, Vinodkumar *et al.* (2017) identified several antimicrobial peptide genes namely, *ituD*, *ipa14*, *bacA*, *bacD*, *bamC*, *sfp*, *spaC*, *spaS*, *alba*, and *albF*, responsible for production of the antibiotics iturin, bacilysin, bacillomycin, surfactin, subtilin, and subtilisin from *B. amyloliquefaciens*.

Furthermore, the success of bio agents with respect to their biocontrol efficacy and consistency relies upon appropriate delivery mechanisms at field condition. Earlier reports revealed that, incorporation of bio agents with organic amendments such as manures or vermicompost or oil cakes will change the soil environment in favour bioagents and provided readily available nutrients to fungal and bacterial antagonists for their survival and development (Singh and Sitramaiah, 1966; Muller and Gooch, 1982; Timper, 2014). In addition, Walker (2004) reported that the activity of bioagents was directly correlated with organic amendments. Subsequently, several researchers demonstrated that, application bio- agents enriched with organic amendments exhibited greater antagonistic activity against root-knot nematodes and many plant pathogens (Latha *et al.* 2011; Singh 2013; Singh *et al.* 2014).

Similarly, in our study, nursery drench with soil application of bioformulations enriched FYM was found to be more effective in root-knot nematode control under field condition. This study indicates nematicidal activity of bio agents have direct correlation with FYM and better control might be attributed due to enhanced multiplication and accumulation of their secondary metabolites in amended soil. In addition, our study also agree with previous studies in which they revealed that, the application of enriched organic amendments with bacterial bio agents provided successful control of root knot nematode. For example, *Bacillus cereus* enriched with organic fertilizers exhibited maximum nematicidal activity against root-knot nematodes infecting tomato and muskmelon (Xiao *et al.* 2013). Similarly, Rao *et al.* (2017) demonstrated that, soil application of vermicompost enriched with *B. subtilis* had significantly increases yield and reduces the root-knot nematode and soft rot disease complex in carrot under field condition.

In recent years, there has been greater interest in ecologically resistant, environmentally safe methods for controlling root-knot nematodes in vegetable ecosystem.

Since, the application of microbial agents creates an opportunity to cultivate vegetables without nematicides. In this endeavour, the present study indicates that, these two nematicidal liquid bioformulations *B. amyloliquefaciens* (1% A.S) and *B. subtilis* (1% A.S) with its delivery mechanism can be considered as a component under integrated nematode management of *M. incognita* infecting tomato under field condition.

### Acknowledgements

The authors are thankful to Dr. M.S. Rao, Principal Scientist (Nematology), ICAR-IIHR, Bengaluru for providing liquid bioformulations to conduct present study and AICRP (Vegetable Crops) for financial support.

### सारांश

टमाटर में जड़गॉठ सूत्रकृमि हानिकारक परजीवियों में से एक हैं। अतः नुकसान को देखते हुए जड़गॉठ सूत्रकृमि प्रबंधन के लिये *बेसिलस अमीलोलिक्वेफेसीन्स* (1 प्रतिशत ए.एस.) और *बेसिलस सबटिलिस* (1 प्रतिशत ए.एस.) द्रव्य जैवसूत्रीकरण का मूल्यांकन किया गया। द्रव्य जैवसूत्रीकरण को दो विभिन्न वितरण तंत्रों जैसे पौधशाला को भिगोना (5 मिली प्रति लीटर पानी में) और द्रव्य जैवसूत्रीकरण (5 लीटर प्रति हेक्टेयर) के साथ 20 टन खाद प्रति हेक्टेयर से समृद्ध करके मृदा उपचार किया गया। इन उपचारों को सूत्रकृमिनाशक कार्बोफुरन 3 जी (1 किलोग्राम सक्रिय तत्व/हे.) कार्बोफ्युरान 3 जी. 1 किलोग्राम सक्रिय तत्व/हे. के साथ 20 टन खाद और अनौपचारिक नियंत्रण से तुलना किया गया। द्रव्य जैवसूत्रीकरण उपचार में *बेसिलस अमीलोलिक्वेफेसीन्स* पौधशाला भिगाने एवं गोबर की खाद से मृदा उपचार लगातार तीन वर्षों तक जड़गॉठ सूत्रकृमि प्रबंधन करने में बेहतर प्रदर्शन किया और मृदा आबादी में सूत्रकृमि 77.1, 61.5 और 74.6 प्रतिशत की कमी होने के साथ ज्यादा से ज्यादा फल उपज 24.0, 22.5 और 31.1 टन प्रति हेक्टेयर 2015–16, 2016–17 और 2017–18 दौरान क्रमशः पाया गया और ये उपचार सूत्रकृमिनाशक (कार्बोफुरन 3 जी 1.0 किलोग्राम सक्रिय तत्व/हे. और कार्बोफुरन 3 जी 1.0 किलोग्राम सक्रिय तत्व/हे. के साथ 20 टन खाद) के साथ तुलनीय था। इसके बाद *बेसिलस सबटिलिस* पौधशाला भिगोने के साथ मृदा उपचार से मृदा आबादी में सूत्रकृमि 52.5, 55.8 और 70.4 प्रतिशत कमी होने के साथ 20.0, 22.0 और 30.4 टन प्रति हेक्टेयर 2015–16, 2016–17 और 2017–18 दौरान क्रमशः में फल उपज मिली और जड़गॉठ सूत्रकृमि प्रबंधन के लिये यह उपचार आशाजनक पाया गया। अंत में इस अध्ययन से स्पष्ट होता है कि *बेसिलस अमीलोलिक्वेफेसीन्स* (1 प्रतिशत ए.एस.) और *बेसिलस सबटिलिस* (1 प्रतिशत ए.एस.) द्रव्य जैवसूत्रीकरण और उनके वितरण तंत्र का टमाटर में जड़गॉठ सूत्रकृमि प्रबंधन के लिये उपयोग किया जा सकता है।

### References

Abad P, Favory B, Rosso MN and Castagnone-Sereno P (2003) Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. *Mol Plant Pathol* 4: 217-224.

- Abdel-Salam MS, Hoda HA, Gaziea MS, Elkelany US and Amira MA (2018) Improving the nematicidal potential of *Bacillus amyloliquefaciens* and *Lysinibacillus sphaericus* against the root-knot nematode *Meloidogyne incognita* using protoplast fusion technique. *Egypt J Biol Pest Co* 28: 1-6.
- Adam MAM, Phillips MS and Blok VC (2007) Molecular diagnostic key for identification of single juveniles of seven common and economically important species of root-knot nematode (*Meloidogyne* spp.) *Plant Pathol* 56: 190-197.
- Anastasiadis IA, Giannakou IO, Prophetou Athanasiadou DA and Gowen SR (2008) The combined effect of the application of a biocontrol agent *Paecilomyces lilacinus*, with various practices for the control of root-knot nematodes. *Crop Prot* 27: 352-361.
- Ann YC (2013) Screening for nematicidal activities of *Bacillus* species against root knot nematode (*Meloidogyne incognita*). *Am J Exp Agric* 3: 794-805.
- Anonymous (2017) Horticulture statistics at a glance. Department of Agriculture Cooperation and Farmers welfare, Ministry of Agriculture and Farmers Welfare, Govt. of India, pp: 151.
- Bridge J and Page SLJ (1980) Estimation of root-knot nematode infestation levels on roots using a rating chart. *Trop Pest Manage* 26: 296-298.
- Cobb NA (1918) Estimating the Nema Populations of Soil. USDA, pp. 48 (Technical Circular 1).
- Collange B, Navarrete M, Peyre G, Mateille T and Tchamitchian M (2011) Root-knot nematode (*Meloidogyne*) management in vegetable crop production: The challenge of an agronomic system analysis. *Crop Prot* 30: 1251-1262.
- Ferraz S and Freitas LG (2004) Use of antagonistic plants and natural products. In: Chen ZX, Chen, SY and Dickson, DW (ed.) *Nematology - Advances and perspectives*. Volume II: Nematode management and utilization. Beijing, China and Wallingford UK. Tsinghua University Press, CABI Publishing. pp: 931-977.
- Haseeb A and Khan MR (2012) Prospects of *Trichoderma* in nematode management. In: Status and prospects for enhancing the uptake of antagonistic organisms for nematode management in India. Nagesh M, Rajkumar BS, Bhumannavar NK, Krishna Kumar (ed) NBAII (ICAR), Bangalore, India, pp:187.
- Jain RK, Mathur KN and Singh RV (2007) Estimation of losses due to plant parasitic nematodes on different crops in India. *Indian J Nematol* 37: 219-221.
- Kerry BR and Hidalgo-Diaz L (2004) Application of *Pochonia chlamydosporia* in the integrated control of root-knot nematodes on organically grown vegetable crops in Cuba. *IOBC WPRS Bulletin* 27: 123-126.
- Kiewnick S and Sikora R (2005) Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. *Biol Control* 38: 179-187
- Killani AS, Abaidoo RC, Akintokun AK and Abiala MA (2011) Antagonistic effect of indigenous *Bacillus subtilis* on root soil borne fungal pathogens of cowpea. *Researcher* 3: 11-18.
- Krishnaveni M and Subramanian S (2004) Evaluation of biocontrol agents for the management of *Meloidogyne incognita* on cucumber (*Cucumis sativus* L.). *Curr Nematol* 15: 33-37.

- Latha P, Anand T, Prakasam V, Jonathan EI, Paramathma M and Samiyappan R (2011) Combining *Pseudomonas*, *Bacillus* and *Trichoderma* strains with organic amendments and micronutrient to enhance suppression of collar and root rot disease in physic nut. *Appl Soil Ecol* 49: 215-223.
- Mohamedova MS (2009) Efficacy of *Bacillus thuringiensis* alone and in combination with oxamyl against *Meloidogyne arenaria* infecting greenhouse tomato. *A Sci Tech*, 1: 41-44.
- Mohammed SH, Anwer M, Saedy EI, Enan MR, Ibrahim NE, Ghareeb A and Moustafa SA (2008) Biocontrol efficiency of *Bacillus thuringiensis* toxins against root-knot nematode, *Meloidogyne incognita*. *J Cell Mol Biol* 7: 57-66.
- Muller R and Gooch PS (1982) Organic amendments in nematode control. An examination of the literature. *Nematropica* 12: 319-326.
- Patil J, Miller AJ and Gaur HS (2013) Effect of nitrogen supply form on the invasion of rice roots by the root-knot nematode, *Meloidogyne graminicola*. *Nematology* 15: 483-492.
- Randig O, Leroy F, Bongiovanni M and Castagnone-Sereno P (2001) RAPD characterization of single females of the root-knot nematodes, *Meloidogyne* spp. *European J Plant Pathol* 107: 639-643.
- Rao MS, Kamalnath M, Umamaheshwari, Rajanikanth R, Prabu P, Priti K, Grace, GN, Chaya MK and Gopalakrishnan (2017) *Bacillus subtilis* IHR BS-2 enriched vermicompost controls root knot nematode and soft rot disease complex in carrot. *Sci Hort* 218: 56-62.
- Rao MS, Umamaheshwari R, Chakravarthy AK, Grace GN, Kamalnath M and Prabu P (2015) A frontier area of research on liquid biopesticides: the way forward for sustainable agriculture in India. *Curr Sci* 108: 1590-1592.
- SAS Institute: *SAS version 9.2 System options*. Cary, NC, USA, SAS Institute, USA.
- Saxena AK, Pal KK and Tilak KVBR (2000) Bacterial biocontrol agents and their role in plant disease management. In: Upadhyay RR, Mukerji KG and Chamola BP (ed) *Biocontrol potential and its exploitation in sustainable agriculture: Crop diseases, weeds, and nematodes*. Kluwer Academy Plenum, New York. pp: 25-37.
- Singh RS and Sitaramaiah IC (1966) Incidence of root-knot of okra and tomatoes on oil-cake amended soil. *Plant Disease Reporter* 50: 668-672.
- Singh S (2013) Integrated approach for the management of the root-knot nematode, *Meloidogyne incognita*, on eggplant under field conditions. *Nematology* 15: 747-757.
- Singh S, Singh B and Singh AP (2014) Integrated management of root-knot disease of okra caused by root-knot nematode, *Meloidogyne incognita*. *Indian J Nematol* 44: 172-178.
- Terefe M, Terefe T and Sakhuja PK (2009) Effect of a formulation of *Bacillus firmus* on root-knot nematode *Meloidogyne incognita* infestation and the growth of tomato plants in the greenhouse and nursery. *J Invertebr Pathol* 100: 94-99.
- Timper P (2014) Conserving and enhancing biological control of nematodes. *J Nematol* 46:75-89.
- Vinodkumar S, Nakkeeran S, Renukadevi P and Malathi VG (2017) Biocontrol potentials of antimicrobial peptide producing *Bacillus* species: multifaceted antagonists for the management of stem rot of carnation caused by *Sclerotinia sclerotiorum*. *Front Microbiol* 8: 446.
- Walker GE (2004) Effects of *Meloidogyne javanica* and organic amendments, inorganic fertilizers and nematicides on carrot growth and nematode abundance. *Nematol Mediterr* 32: 181-188.
- Xiao TJ, Chen F, Gao C, Zhao QY, Shen QR and Ran W (2013) *Bacillus cereus* X5 enhanced bio-organic fertilizers effectively control root-knot nematodes (*Meloidogyne* sp.). *Pedosphere* 23: 160-168.