

Antagonistic potential of rhizobacterial liquid formulations against *Meloidogyne incognita* infecting okra under field conditions

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Abstract

Okra (*Abelmoschus esculentus* L. Moench) is one among the commercially exploited vegetable crops. Okra fruits are low in calories and have high dietary fibre content and also rich source vitamins and minerals. However, this crop highly prone to root-knot nematodes. Largely, chemical nematicides have been opted for combating this nematode pest, nevertheless threats associated with chemical nematicides on human being and the environment led towards developing biocontrol agents as alternative approach. The biocontrol agents significantly adhere to biosafety regulations and being highly effective. In recent years, use of rhizobacteria such as *Pseudomonas* spp. and *Bacillus* spp. has shown greater biocontrol potential against root knot nematodes. In this endeavor, bio-efficacy of liquid formulation such as *Bacillus pumilus* (1% A.S) and *Pseudomonas putida* (1% A.S) were evaluated against *Meloidogyne incognita* infecting okra (cv. Kashi Pragati) under field conditions for three consecutive years (2015, 2016 and 2017). Both formulations were applied in two methods of application such as seed treatment and soil application of enriched farm yard manure. The results revealed that, seed treatment with *P. putida* (1% A.S. @ 10 ml/ kg seed) + application of 20 tonnes of FYM enriched with 5 L of *P. putida*/ ha was found most effective against *M. incognita* by consistently reducing production of egg mass per root system (73.3%, 84.6% and 76.3%), nematode final population in soil (66.6%, 61.5% and 78.3%)

with lesser root gall index (1.0, 1.0 and 1.86) during 2015, 2016 and 2017 respectively, compared to control. The results were comparable with chemical treatments. The application of *P. putida* formulation (1% A.S) in combination of seed treatment and soil application of enriched FYM significantly protect okra crop from *M. incognita* incidence and it can be considered as a component under integrated management of *M. incognita* in okra under field conditions.

Keywords: Okra, *Meloidogyne incognita*, Rhizobacterial formulation, Antagonistic.

Introduction

Okra (*Abelmoschus esculentus* L. Moench) is one of the major vegetable crops grown in tropical and sub-tropical regions of the world for its tender pods. It is grown commercially in India, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Burma, Japan, Malayasia, Brazil, Ghana, Ethiopia, Cyprus and the Southern United States. It is popularly known as “Bhindi” in India, “Lady’s finger” in England, “Gumbo” in United States. It is quite popular vegetable in India because of its easy cultivation, adaptability and economical yield. In India, okra is grown in an area of 0.53 million hectares with a production of 6.47 million tonnes (FAOSTAT 2020). Okra has several therapeutic and nutritional uses. Tender pods have antioxidant potential mostly attributed to the large amounts of polyphenols present in the seeds of immature pods. Nutritionally okra is rich in vitamins, minerals and carbohydrates. The bioactive compounds present in tender pods were polyphenols, carotene, folic acid, thiamine, riboflavin, niacin and vitamin C. The phytochemicals of okra have potential therapeutic

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activities on different chronic diseases such as type-2 diabetes, cardiovascular and digestive diseases (Elkhalifa et al. 2021).

However, the okra production was constrained by the direct interference of plant parasitic nematodes (PPNs) on the plant root system. Among them, root-knot nematodes (*Meloidogyne* spp.) are becoming major threat to okra cultivation in different parts of the country. The root-knot nematode (RKN) species i.e., *Meloidogyne incognita* and *Meloidogyne javanica* are widely distributed in India and cause annual yield loss to the tune of 19.5% with an estimated 2480.86 million rupees of monetary loss in tomato (Kumar et al. 2020). Owing to their parasitic activity, the second-stage infective juveniles (J2) infect the roots of seedlings after sowing and develop endoparasitically by feeding on vascular tissues. Females lay eggs in masses on root surface on galls and males leave the roots. The primary symptom of RKN infection is formation of typical galls or knots on root system. Affected plants express symptoms similar to nutrient deficiency such as chlorosis, yellowing of leaves, wilting and stunted growth (Abad et al. 2003). Largely nematode management depends on chemical nematicides. However, the current legal regulations/restrictions on use of chemical pesticides, to reduce their harmful effects on environment and human health, require the use of alternative approach of nematode management. Use of nematophagous microorganisms as biocontrol agents (BCA) have emerged as cost effective, durable and eco-friendly alternative approach to combat this hidden pest. Nematophagous microorganisms kill the nematodes either by trapping, capturing or producing secondary metabolites. In this view, several fungal (*Arthrobotrys*, *Pochonia*, *Purpureocillium*, *Trichoderma*, *Dactylella* and *Drechmeria*) biocontrol agents are exploited for RKNs management infecting different crops (Topalovic et al., 2020). Similarly, in recent years plant growth promoting rhizobacteria (PGPR) are being predominantly exploited as biocontrol agents against RKNs.

Among PGPR, *Bacillus* and *Pseudomonas* genera are extensively utilized for the management of RKNs infecting various crops. They exhibit nematode antagonistic activity either directly by the production of secondary metabolites and enzymes that act directly against nematodes and /or indirectly by activating plant defense mechanisms (Rani et al. 2022, Topalovic et al. 2022). Several

antimicrobial compounds i.e., 2,4-diacetylphloroglucinol, hydrogen cyanide, pyoluteorin, phenazine, pyrrolnitrin and siderophores production from *Pseudomonas* spp., and iturin, bacillomycin, bacilysin, surfactin, fengycin, mersacidin, ericin, subtilin, subtilisin, etc., production from *Bacillus* spp., are known to have nematicidal activity against *M. incognita* (Khan et al. 2016, Gowda et al. 2022). In this view, present study evaluated nematode antagonistic rhizobacterial liquid formulation i.e., *Pseudomonas putida* (1% A.S) and *Bacillus pumilus* (1% A.S) through two methods of application i.e., seed treatment and soil application of bioagent enriched farm yard manure applied singly and their combination for the management of root-knot nematode *M. incognita* infecting okra crop under naturally infested field condition for three consecutive years.

Materials and Methods

The field experiments were conducted at Nematology research field (25°11'7.23''N, 82°E52'10.92''), ICAR- Indian Institute of Vegetable Research, Varanasi (Uttar Pradesh) for three consecutive years (2015, 2016 and 2017), during Kharif 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⁻¹. The root knot nematode (*M. incognita*) was prevalent in nematology experimental site was identified based on perineal pattern of adult female using standard protocol.

Liquid formulations: Liquid formulations of two rhizobacteria such as *Bacillus pumilus* (1% A.S.) and *Pseudomonas putida* (1% A.S.) procured from, Division of Entomology and Nematology, Indian Institute of Horticultural Research, Bengaluru were used under the present investigation.

Seed treatment and enrichment of FYM with liquid formulations: For seed treatment, one kg of okra (cv. Kashi Pragati) seeds was treated with 10 mL of each formulation for 1 h and further shade dried for 12 h before sowing. Besides, prior to field experiment, each liquid formulation (5 L/ha) was thoroughly mixed with FYM (20 t/ha) and then covered with poly ethylene sheet by maintaining optimum moisture condition under shade for 15 days. Further, enriched FYM was applied to respective treatments before sowing.

T1	3.03 ^b	4.05 ^c	3.47 ^b	3.52	60.7±2.4 ^b (-20.4)	77.0±1.7 ^b (-35.4)	63.7±3.0 ^{bc} (-19.4)	67.11	-26.7
T2	3.00 ^b	4.00 ^c	3.33 ^{bc}	3.44	63.3±1.9 ^b (-17.0)	79.6±2.6 ^b (-33.2)	58.0±3.5 ^c (-26.5)	67.00	-26.8
T3	2.90 ^b	3.07 ^d	1.96 ^{bcd}	a2.64	24.3±1.6 ^c (-68.1)	44.0±1.2 ^c (-63.1)	22.3±1.5 ^d (-71.7)	30.22	-67.0
T4	1.00 ^d	1.00 ^e	1.86 ^{cd}	1.28	20.3±1.0 ^c (-73.3)	18.3±1.3 ^d (-84.6)	18.7±0.7 ^d (-76.3)	19.11	-79.1
T5	6.10 ^a	6.07 ^b	5.73 ^a	5.97	69.3±2.1 ^{ab} (-9.1)	113.6±5.5 ^a (-4.7)	74.3±1.1 ^{ab} (-5.9)	85.78	-6.3
T6	2.03 ^c	1.05 ^e	1.70 ^d	1.59	18.3±1.2 ^c (-75.9)	23.3±1.5 ^d (-80.4)	17.7±0.7 ^d (-77.63)	19.78	-78.4
T7	1.03 ^d	1.00 ^e	1.36 ^d	1.13	16.6±1.0 ^c (-78.6)	28.1±1.2 ^d (-76.4)	15.3±1.5 ^d (-80.5)	19.94	-78.2
T8	6.00 ^a	7.03 ^a	6.40 ^a	6.48	76.3±2.1 ^a (0)	119.3±2.6 ^a (0)	79.0±2.35 ^a (0)	91.56	0.0
<i>P</i> value	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001		

Data represented in Mean±SE. 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: Seed treatment with *Bacillus pumilus* 1% A.S. @ 10 mL/ kg seed, T2: Seed treatment with *Pseudomonas putida* 1% A.S. @ 10 mL/ kg seed, T3: T1+ application of 20 tonnes of FYM enriched with 5 L of *B. pumilus*/ ha, T4: T2+ application of 20 tonnes of FYM enriched with 5 L of *P. putida*/ ha, T5: Application of FYM20 t/ha, T6: Chemical treatment (Carbofuran 3G at 1 kg a.i./ha), T7: Chemical treatment (Carbofuran 3G at 1 kg a.i./ha) +FYM 20 t/ha, T8: Control.

Table 2: Bio-efficacy of liquid formulations for the management of *Meloidogyne incognita* infecting okra under field conditions

Treatments	Final nematode population in soil (200 CC)			Pooled Avg.	% decrease over control	Yield t/ha			Pooled Avg.	%decrease over control
	2015	2016	2017			2015	2016	2017		
T1	234.7±4.5 ^b (-57.3)	349.7±10.5 ^b (-37.4)	342±17.2 ^{bc} (-30.6)	308.78	-42.2	2.6±0.1 ^{bc} (+52.9)	7.4±0.1 ^{bc} (+14)	5.4±0.1 ^c (+5.2)	5.01	+13.4
T2	232.3±13.5 ^b (-57.7)	344.7±8.6 ^b (-38.3)	324.3±10.0 ^c (-34.2)	300.44	-43.7	2.6±0.1 ^{bc} (+51.0)	7.4±0.3 ^{bc} (+14)	5.4±0.1 ^{bc} (+5.8)	5.11	+15.6
T3	220.7±9.9 ^b (-59.9)	315.3±5.7 ^b (-43.5)	120.0±9.4 ^d (-75.6)	218.67	-59.1	2.5±0.1 ^{bc} (+49.0)	7.5±0.4 ^{bc} (+15.6)	6.0±0.1 ^a (+18.3)	5.34	+20.9
T4	183.7±7.1 ^b (-66.6)	215±7.1 ^c (-61.5)	106.7±14.4 ^d (-78.3)	168.44	-68.5	4.0±0.1 ^a (+135.2)	8.6±0.2 ^a (+32.6)	6.3±0.1 ^a (+22.8)	6.28	+42.0

T5	538.3±5.9 ^a (-2.2)	519.7±21.2 ^a (-7.03)	406.7±23.7 ^b (-17.5)	488.22	-8.6	2.1±0.1 ^{cd} (+21.5)	6.1±0.1 ^d (-4.5)	5.2±0.1 ^c (+2.6)	4.49	+1.6
T6	209.7±6.0 ^b (-61.8)	213.3±5.9 ^c (-61.8)	100.0±9.4 ^d (-79.7)	174.33	-67.4	2.7±0.1 ^b (+60.7)	8.4±0.1 ^{ab} (+29.5)	6.0±0.1 ^{ab} (+17.6)	5.70	+29.0
T7	181.3±3.3 ^b (-67.0)	205±3.3 ^c (-63.3)	93.3±5.4 ^d (-81.0)	159.89	-70.1	3.7±0.1 ^a (+119.6)	8.5±0.2 ^a (32.09)	6.3±0.1 ^a (+23.5)	6.19	+40.0
T8	550.0±12.5 ^a (0.0)	559.3±11.3 ^a (0.0)	493.3±14.4 ^a (0.0)	534.11	0.0	1.7±0.1 ^d (0.0)	6.5±0.1 ^{cd} (0.0)	5.1±0.1 ^c (0.0)	4.42	0.0
<i>P</i> value	<0.0001	<0.0001	<0.0001			<0.0001	<0.0001	<0.0001		

Data represented in Mean±SE. 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.

of nematodes in soil and root with lesser root-gall index than non-treated control in bottle gourd crop under field conditions (Rani et al. 2022). Similarly, nematicidal efficacy of *B. pumilus* when applied in combination of seed treatment and soil application of enriched FYM was found next effective treatment against *M. incognita* under field conditions. We agree with earlier study that, *B. pumilus* L1 inhibited egg hatch and caused juvenile mortality under in-vitro condition, while inoculation under potted soil challenged with *Meloidogyne arenaria* significantly reduced root galls and egg masses in tomato plant roots (Lee and Kim 2016). The production of secondary metabolites, enzymes and phytohormones secreted from these bioagents might have suppressed nematode incidence in okra under field conditions.

Besides, the methods of application such as seed treatment and soil application of enriched FYM were further assisted in enhancing the nematicidal efficacy of the bioagent. Since, the seed treatment protects younger plants from nematode invasion at the early stage of plant growth and reduce and degree of crop damage (Sikora et al. 2008, Gowda et al. 2022). Soil application of organic manure enriched with bioagent enhanced bacterial population in the rhizosphere region of plants and might have exhibited greater antagonistic activity by

interfering with nematode feeding, behavior and reproduction (Viaene et al. 2006, Rao et al. 2017, Engelbrecht et al. 2018). Therefore, selection of appropriate methods of application is relatively important to enhance biocontrol efficacy and consistency of bioagent and also to achieve the maximum success against any soil-borne plant pathogens under field conditions.

In addition to nematode antagonism, the application *P. putidain* combination of seed treatment and soil application of enriched FYM significantly ($P < 0.05$) enhanced okra yield compared to control. This treatment was non-significant with treatment T7 (Carbofuran 3G at 1 kg a.i./ha + FYM 20 t/ha) and significantly differed with control and other treatments except T3 treatment during 2017 field experiment (Table 1 & 2). In addition, bioagents were also considerably enhanced plant height for three consecutive years 2015, 2016 and 2017. The formulation of *P. putida* applied in combination two methods of application significantly ($P = 0.004$, $P = 0.010$) differed in plant height with control treatment. This treatment found better over other treatments in plant growth promotion though it was statistically at par i.e., T2, T3, T5, T7 during 2015 and 2016 field experiments (Figure 1).

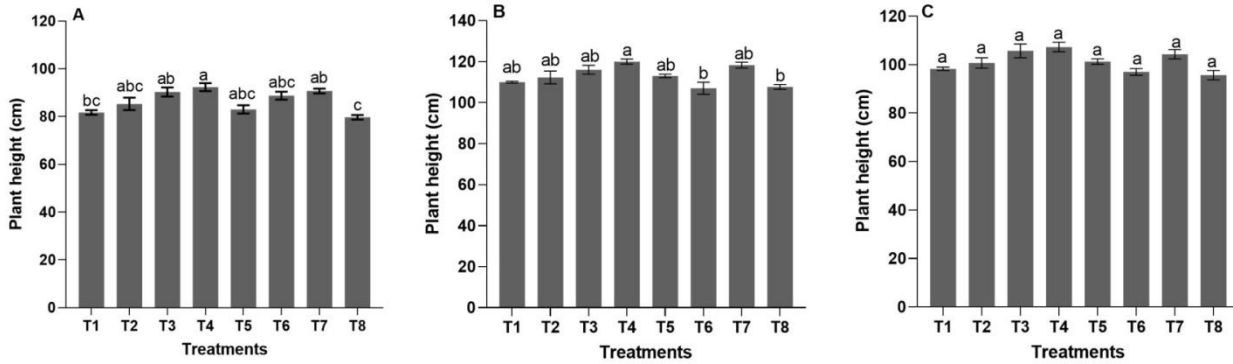


Figure 1: Effect of liquid formulation on plant height A. 2015 B. 2016 and C. 2017 field experiments. Different letters on the top of error bars indicate statistically significant difference between treatments at $P < 0.05$ using Tukey's HSD test.

The production of phytohormones like auxin derivatives and gibberellin-like substances by *Bacillus* spp. and *Pseudomonas* spp. (Arshad and Frankenberger 1991) stimulate the plant growth by modifying plant's own pool of growth regulators (Glick 1995) and also suppressing phytonematodes incidence (Khan et al. 2016). Similarly, traits associated with plant growth promotion in these bioagents might have helped in increasing plant height as well as fruit yield.

Overall, the present study demonstrates that, the application of *P. putida* formulation (1% A.S.) in combination of seed treatment and soil application of enriched FYM significantly protect okra crop from *M. incognita* incidence under field conditions. Therefore, formulation of *P. putida* with its methods of application can be considered as a component under integrated management of *M. incognita* in okra under field conditions.

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सारांश

भिण्डी (*एबलमास्कस इस्कुलेंटस* एल. मोंचे) व्यावसायिक सब्जी फसलों में से एक है। भिण्डी के फल कैलोरी में कम होते हैं जिसमें उच्च गुणवत्तायुक्त रेशा, विटामिन एवं खनिज पाये जाते हैं। हालांकि, यह फसल रूट-नॉटनेमाटोड के प्रति अत्यधिक संवेदनशील है। बड़े पैमाने पर, इसनेमाटोड कीट से लड़ने के लिए रासायनिक सूत्र कृमिनाशकों को चुना गया है,

फिर भी मानव और पर्यावरण पर रासायनिक सूत्र कृमिनाशकों से जुड़े प्रभाव ने वैकल्पिक दृष्टिकोण के रूप में जैव नियंत्रण तत्वों को विकसित करने की ओर अग्रसर है। जैविक नियंत्रण घटक जैव सुरक्षा नियमों का महत्वपूर्ण रूप से पालन करते हैं और अत्यधिक प्रभावी होते हैं। हाल के वर्षों में *स्यूडोमोनास एसपीपी* जैसे राइजो बैक्टीरिया का उपयोग और बैसिलस *एसपीपी* रूट नॉटनेमाटोड के लिए प्रभावी जैविक नियंत्रण की क्षमता है। इस प्रयास में, जैव-सूत्रीकरण की जैव-प्रभावकारिता जैसे बैसिलस-प्यूमिलस (1 प्रतिशत ए.एस.) और *स्यूडोमोनास-पुटिडा* (1 प्रतिशत ए.एस.) का लगातार तीन वर्षों तक क्षेत्र परिस्थितियों के तहत *मेलोइडो गाइनइन्कोग्निटा* संक्रमित भिण्डी (सी.वी. काशी प्रगति) के प्रतिकूल मूल्यांकन किया गया (2015–2016 और 2017)। दोनों फॉर्मूलेशन को दो तरीकों से लागू किया गया जैसे बीज उपचार और समृद्ध गोबर खाद की संस्तुति। तीन वर्षों के क्षेत्र प्रयोगों के परिणामों से पता चला है कि, उपचारों के बीच पी. पुटिडा (1 प्रतिशत ए.एस. प्रति 10 मिली. प्रतिकिग्रा. बीज) के साथ बीज उपचार + 5.0 लीटर पी. पुतिदा प्रति हेक्टेयर से समृद्ध 20 टन का अनुप्रयोग पाया गया। प्रतिजड़ तंत्र (73.3 प्रतिशत, 84.6 प्रतिशत और 76.3 प्रतिशत) में अंडे के द्रव्यमान के उत्पादन को लगातार कम करके गोबर की खाद के प्रति सबसे प्रभावी, कम रूट गॉल सूचकांक (1.0–1.0) के साथ मिट्टी में सूत्र कृमि अंतिम आबादी (66.6 प्रतिशत, 61.5 प्रतिशत और 78.3 प्रतिशत) नियंत्रण की तुलना में क्रमशः 2015, 2016 और 2017 के दौरान कम जड़ गॉल इंडेक्स (1.0, 1.0 और 1.86) के साथ। परिणाम रासायनिक उपचार के साथ तुलनीय थे। पी. पुटिडा सूत्रीकरण (1 प्रतिशत ए.एस.) तहत बीज उपचार तथा मिट्टी में गोबर की खाद प्रचुर मात्रा में देकर भिण्डी की फसल में एम. *इन्कोग्निटा* का से काफी हद तक बचाया जा सकता है और इसे खेत की परिस्थितियों में भिण्डी में एम. *इन्कोग्निटा* के एकीकृत प्रबंधन के तहत एक घटक के रूप में माना जा सकता है।

References

- Abad P, Favery 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.
- Arshad M and Frankenberger Jr. WT (1991) Microbial production of plant hormones. *Plant Soil* 133: 1–8.
- 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).
- Elkhalifa AEO, Alshammari E, Adnan M, Alcantara JC, Awadelkareem AM, Eltoum NE, Mehmood K, Panda BP and Ashraf SA (2021) Okra (*Abelmoschus esculentus*) as a potential dietary medicine with nutraceutical importance for sustainable health applications. *Molecules* 26:696.
- Engelbrecht G, IlzeHorak, Peet J, Jansen van Rensburg and Claassens S (2018) *Bacillus*-based bionematicides: development, modes of action and commercialisation. *Bio Sci and Technol* 28: 629–653.
- FAOSTAT (2020) <https://www.fao.org/faostat/en/#data/QCL>. Accessed 29 Nov 2022.
- Glick BR (1995) The enhancement of plant growth by free-living bacteria. *Can J Microbiol* 41: 109–117.
- Gowda MT, Meena BR, Nagendran K, Manjunath M, Sellaperumal C, Rai AB, Singh A, Manimurugan C, Patil J, Pandey KK and Singh J (2022) Antimicrobial peptides producing native *Bacillus* spp. for the management of root-knot nematode *Meloidogyne incognita* infecting okra (*Abelmoschus esculentus* L. Moench). *Biol Control* 171: 104951.
- Holajjer P, Dey R, Pal KK, Chakraborty K, Harish G, Nataraja MV and Deepak E (2018) Assessment of nematicidal properties of fluorescent pseudomonas using peanut root-knot nematode, *Meloidogyne arenaria*. *J Biol Control* 32:193–202.
- Khan MR, Mohidin FA, Khan U and Ahamad F (2016) Native *Pseudomonas* spp. suppressed the root-knot nematode in *in vitro* and *in vivo*, and promoted the nodulation and grain yield in the field grown mung bean. *Biol Control* 101: 159–168.
- Kumar V, Khan M R and Walia R K (2020) Crop loss estimations due to plant-parasitic nematodes in major crops in India. *Nat Acad Sci Lett* 43:409–412.
- Lee YS and Kim KY (2016) Antagonistic potential of *Bacillus pumilus* L1 against root-knot nematode, *Meloidogyne arenaria*. *J Phytopathol* 164:29–39.
- Rani P, Singh M, Prashad H and Sharma M (2022) Evaluation of bacterial formulations as potential biocontrol agents against the southern root-knot nematode, *Meloidogyne incognita*. *Egy J Biol Pest Control* 32: 29.
- 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.
- SAS Institute (2011) SAS version 9.3 System Options: Reference, 2nd ed. SAS Institute, Cary, NC.
- Sikora RA, Pocasangre L, Felde AZ, Niere B, Vu TT and Dababat AA (2008) Mutualistic endophytic fungi and in-plant suppressiveness to plant parasitic nematodes. *Biol Control* 46: 15–23.
- Topalovic O, Hussain M and Heuer H (2020) Plants and associated soil microbiota cooperatively suppress plant-parasitic nematodes. *Front Microbiol* 11:313.
- Topalovic O, Santos SS, Heuer H, Nesme J, Kanfra X, Hallmann J, Sorensen SJ and Vestergard M (2022) Deciphering bacteria associated with a pre-parasitic stage of the root-knot nematode *Meloidogyne hapla* in nemato-suppressive and nemato-conducive soils. *Appl Soil Ecol* 172:104344.
- Viaene N, Coyne DL and Kerry BR (2006) In: *Plant nematology*. CABI, Wallingford, pp. 346–369.
- Walker JT and Wilson JD (1960) The separation of nematodes from soil by modified Baermann funnel technique. *Plant Dis Reporter* 44: 94–97.