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Bacillus subtilis IIHR BS-2 enriched vermicompost controls root knot nematode and soft rot disease complex in carrot

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ABSTRACT

Root knot nematode (*Meloidogyne incognita*) and soft rot bacterium *Pectobacterium carotovorum* subsp. *carotovorum* are major pathogens of carrot (*Daucus carota*) throughout the world, reducing both quality and quantity of marketable yield. *Bacillus subtilis* IIHR BS-2, a potential biocontrol agent, was evaluated against the nematode-bacterium disease complex in carrot under *in vitro* and field conditions. Under *in vitro*, culture filtrates of *B. subtilis* not only exhibited strong nematicidal activity by inhibiting the hatching of *M. incognita* eggs (94.65%) and causing juvenile mortality (91.26%), but also inhibited the growth of *P. carotovorum* (60.6%). Liquid formulation of *B. subtilis* IIHR BS-2 (CFU -1×10^8 per ml) was tested under field conditions as seed treatment (10 ml kg^{-1} seeds) and soil application (after enrichment in vermicompost), individually and in combination, and compared with application of chemicals (carbofuran and streptocycline) and untreated control. Among all the treatments, seed treatment together with soil application of *B. subtilis* (5 l ha^{-1}) enriched vermicompost (2 tons ha $^{-1}$) recorded the maximum increase in carrot yield (28.8%) and decrease in nematode population (69.3%) and disease incidence (70.2%). This was followed by treatment with chemicals (carbofuran 1 kg a.i. ha^{-1} + streptocycline- 0.02%) which recorded 62.2% and 68.15% decrease in disease incidence and nematode population, respectively. This study proves the bioefficacy of *B. subtilis* IIHR BS-2 and demonstrates its appropriate delivery mechanism through enrichment in vermicompost for managing nematode disease complex and reaping maximum yield in carrot under field conditions.

1. Introduction

Carrot (*Daucus carota* subsp. *sativus*) is an important vegetable crop cultivated across the world for its prized tap root which are exceptionally rich in carotenes and vitamin A (Verma et al., 2013). Root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, is an important pest of carrot causing substantial damage to its roots, affecting both its quality and quantity (Sasser and Carter 1985; Gugino et al., 2006). Owing to their parasitic activity, the growing root tip is damaged resulting in root malformation exhibited as forked, distorted, or stunted tap root with excessive galling (Singh 2009; Khan et al., 2014). It is reported to cause a yield loss of 35.95% in Nilgiris of South India (Anita and Selvaraj 2011). In addition, the wounds in nematode infection sites facilitate easy entry of other soil borne pathogens especially the soft rot bacterium, *Pectobacterium carotovorum* subsp.*carotovorum* and together cause disease complex in carrot ensuing huge crop losses (Sowmya et al., 2012). *P.* carotovorum is one of the serious bacterial diseases of carrot causing more than 50% yield loss in India (Sowmya and Rao, 2011).

Globally, nematode management strategies in carrot include fallowing, crop rotation, chemical nematicides and biological control agents (Bontempo et al., 2014). However, owing to the human health and environmental safety issues, more attention is drawn towards biological control agents (BCA) and they are emerging as the way forward for cost effective, eco-friendly and sustainable agriculture (Rao et al., 2015). *Bacillus subtilis* is one such biocontrol bacterium, currently commercialized as biopesticides and numerous studies support its great potential in controlling multiple diseases and nematodes infecting a wide range of host plant species (Cawoy et al., 2011). Beside its spore forming ability, *B. subtilis* possess several characteristics that enhance its survival in the rhizosphere (Losick and Kolter, 2008). It also exhibits multiple modes of action *viz.*, antibiosis, competition, plant growth promotion and induction of systemic resistance against several pathogens and nematodes (Kloepper et al., 2004; Rao et al., 2016). Moreover,

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the USFDA (US Food and Drug Administration) has granted the "generally regarded as safe" (GRAS) status to *Bacillus subtilis* and hence recognized as non-pathogenic (Harwood and Wipat, 1996).

The success of a BCA relies on the proper delivery mechanism at field conditions. Earlier reports by Sowmya et al. (2012) and Latha et al. (2011) demonstrated that integration of BCA with organic amendments like neem cake or Farm Yard Manure had greater antagonistic activity against nematodes and fungal pathogens. As no attempt has been made so far for biological management of nematode bacterial disease complex in carrot, a study was conducted to test the *in vitro* and field efficacy of an indigeneous strain of *B. subtilis* IIHR BS-2 against *M. incognita* and *P. carotovorum* disease complex in carrot and develop an effective delivery system under field conditions through enrichment or enhancement in an organic amendment, vermicompost.

2. Materials and methods

2.1. Collection of native antagonistic microbes

Twenty soil samples were collected from five different regions of Karnataka. One gram of each soil samples was serially diluted up to 10^{-6} dilutions using sterile distilled water and 100 μl of each dilution was plated on nutrient agar (Roy et al., 2007). The plates were incubated at 28 °C for 24 h. Later, the suspected colonies were stained by gram staining method. The colonies were studied for their morphological characteristics such as shape, spore formation, gram staining and supplmentery tests such as susceptibility to penicillin, citrate hydrolysis, motility, Voges-Proskauer VP, Indole production, catalase, nitrate reduction and production of hydrogen sulphide (Amin et al., 2015).

2.2. Molecular characterization of B. subtilis

From the soil samples, one *Bacillus* strain was isolated. On nutrient agar, it appeared as luxuriant, big, white, smooth colonies with entire margin and tested positive for Gram staining and other mentioned characteristics. The identity of the bacterium was also confirmed through Polymerase chain reaction and 16S rDNA sequence results were analyzed using NCBI BLAST which showed 99% similarity with *B. subtilis*.

Bacterial strain identification was confirmed through 16S rRNA gene amplification from genomic DNA using universal primers Forward – 27F (AGAGTTTGATCMTGGCTCAG(Jiang et al., 2006) and Reverse – 1492R (GGTTACCTTGTTACGACTT-Eden et al., 1991). Final volume of 25 μl PCR mixture contained 19.7 μl of PCR grade water, 0.5 μl of each primer (10 pmol; Bio-serve Pvt. Ltd, India), 0.5 μl of dNTPs (10 mM; Fermentas), 2.5 μl (10x) of Taq buffer (Genei), 0.3 μl of Taq DNA Polymerase (Genei) and 1 μl of DNA (25 ng/μl).

Polymerase Chain Reaction (PCR) was carried out in Eppendorf Master Cycler Gradient (Vapour Protect, Eppendorf Germany). Initial denaturation was done at 95 °C for 5 min followed eby 30 cycles of denaturation at 95 °C for 45 s, primer annealing at 51 °C for 32 s and elongation at 72 °C for 40 s. An extended elongation for 5 min was done at 72 °C. Amplicons were separated in a 1.5% agarose gel and TBE buffer (0.04 M Tris-borate and 0.001 M EDTA) at 70 V for 2 h. It was visualized in Gel Documentation system (make GENE Sys) and observed for the band length of primers. Band length of 1500 bp was observed with respect to primers. The sequences results of forward and backward sequencing were compiled using the contiq0 assembly in the "Bioedit" software (Hall, 1999) and were aligned for similarity with the nucleotide database of NCBI using the blast tool. This culture was labeled as IIHR BS-2 and submitted to the repository at National Bureau of Agriculturally Important Microorganisms, Mau, U.P., India and the accession number was obtained as NBAIMCC-B-01211.

2.3. Maintenance of bio-agents

B. subtilis (IIHR BS-2), a strain of ICAR-Indian Institute Horticultural Research, Bengaluru, India) was maintained on Nutrient agar medium and used for further studies.

2.4. Maintenance of nematode inoculum

The culture of root knot nematode *M. incognita* host race 2 was maintained at Nematology lab, ICAR-IIHR in tomato cv Arka Samrat. The identity of females of *M. incognita* was confirmed through perineal cuticular pattern (Sasser and Carter 1982). Eggs of *M. incognita* were extracted from the culture plants from infected tomato plants maintained at Nematology lab, ICAR-IIHR by agitating the roots in 0.05% NaOCl for 2–3 min (Hussey and Barker 1973). The eggs were then collected and rinsed with sterile water on nested 150 and 25 μm pore sieves. The egg masses were incubated and second stage juveniles (J2) that emerged from the eggs on a sieve were collected daily for 5 days (Southey 1986). After concentration, egg masses and juveniles were used for hatching and mortality tests, respectively.

2.5. Evaluation on egg hatching

Culture filtrates were prepared by centrifugation of 24 h grown *B. subtilis* at 28 ± 2 (8000 rpm for 15 min) and the cell free culture filtrates were collected using 0.22 μm syringe filters (WHATMAN™). This was used as culture filtrates for egg hatching and mortality experiments. Approximately, hundred eggs were placed in petri dishes (3.5 cm dia) which contained cell free culture filtrates at concentrations of 100, 75, 50 and 25% and incubated at 28 ± 2 °C. Petri dishes with media as such without culture filtrates and with distilled water served as controls. All treatments were replicated four times in Completely Randomized Design and kept at room temperature (28 ± 2 °C). The egg hatching was observed under Stereo zoom microscope (Motic SMZ 168 series) every 24 h upto 120 h. Percentage inhibition of egg hatching was calculated. The whole experiment was repeated three times.

2.6. Evaluation on juvenile mortality

Three ml of cell free culture filtrates of bio-agents at concentrations of 100, 75, 50 and 25% were placed in separate petri dishes with 3.5 cm diam. Approximately, hundred second stage juveniles (J2) were transferred to petri dishes and incubated at 28 ± 2 °C. Treatments as mentioned in hatching test were evaluated for mortality with four replications maintained in Completely Randomized Design. Using Stereo zoom microscope (Olympus) the mortality of juveniles was recorded at 24 h intervals up to 120 h. Immobile juveniles were also transferred to distilled water for confirmation of death. The whole experiment was repeated three times.

2.7. Antagonism of B. subtilis *IIHR BS-2* on P. carotovorum *ssp.* carotovorum

The antagonistic activity of *B. subtilis* against *P. carotovorum* ssp. *carotovorum* was evaluated *in vitro* by disk diffusion method (Valgas et al., 2007). In a lawn culture of *P. carotovorum,* holes with a diameter of 2 mm are punched aseptically with a sterile cork borer. Into the holes, 20 μl of *B. subtilis* was added and for control, distilled water was added. Experiment was replicated three times and incu-

bated at 28° C for 24 h. After incubation, the zone of inhibition was measured and compared with control.

2.8. Field efficacy

B. subtilis IIHR Bs-2 was mass multiplied, formulated as aqueous suspension (CFU 1×10^8 ml^{-1}) and used in field trials (Rao, 2015). This suspension was added to vermicompost (Kempmann Bioorganics, Bengaluru with organic carbon-11.98%, nitrogen- 0.78%, phosphorous- 0.15%, Potassium- 0.34%) at two separate doses as 2.5 l and 5 l for 2 tons ha^{-1} of vermicompost. It was kept under shade for 15 days, with optimum moisture (25–30%) and maintained at 25–28 °C for proper enrichment of vermicompost with *B. subtilis*. Vermicompost enriched with *B. subtilis* was applied to soil in the experimental plots (4×2.5 m^2) before sowing. The experiment was conducted for two seasons in carrot (cv. Kuroda) at Block-VI, Nematology field, ICAR-Indian Institute of Horticultural Research, Bangalore, India (location 11.29°N; 75.82°E). Through serial dilurion technique, CFU of *B.subtilis* was assessed in vermicompost after enrichment (Manojkumar et al., 2013)

Using Cobb's sieving and decanting method (Cobb 1918), the initial nematode population in soil was assessed in the plots and it was 121 ± 6 per 100 CC of soil. The identity of *M. incognita* juveniles were confirmed after making temporary mounts (Sasser and Carter, 1985). The density of population of *P. carotovorum* was assesed by serial dilution in the plots and counting the colony forming units on nutrient agar plates; it was 2.2×10^3/g (Sowmya et al., 2012).

The treatments were given as per the schedule: Seed treatment with *B. subtilis* IIHR BS-2 at 10 ml kg^{-1} seed (ST); ST + soil application of 2 tons ha^{-1} of vermicompost enriched with 2.5 l of *B. subtilis* IIHR BS-2 (SA1); ST+ soil application of 2 tons ha^{-1} of vermicompost enriched with 5 l of *B. subtilis* IIHR BS-2 (SA2); Soil application of vermicompost alone at 2 tons ha^{-1} (VC); Carbofuran at 1 kg a.i. ha^{-1} + Streptocyclin (0.02%) (CHEMICAL); Untreated control (CONTROL).

The treatments were replicated five times in a Randomised Block Design (RBD). At the time of harvest at 90 days, observations were made on plant growth parameters *viz*., shoot length, root length, shoot weight and root weight. Nematode population in soil was recorded as per Cobb's sieving and decanting method (Cobb 1918) and in roots by acid fuchsin staining (Bridge et al., 1981). Root-knot index on 1–5 scale (1 = no galls, 2 = less than 25%, 3 = 26–50% 4 = 51–75%, and 5 = greater than 75%) was also recorded (Heald et al., 1989). Yield was recorded and expressed in tons ha^{-1}.

Further, data on the mortality of the plants in the field due to wilt disease were recorded by counting the number of plants completely wilted out of the total number of plants and expressed as percentage soft-rot incidence (Sowmya et al., 2012).

Root colonization by *B. subtilis* was assessed after gentle rinsing of roots with tap water. One gram root sample was grounded with mortar and pestle. The dilutions were prepared up to 10^{-7} and 1 ml aliquots of the 10^{-5}–10^{-7} dilutions were spread on Petri dishes containing Nutrient agar. Three replicates were prepared and incubated at 27 ± 1 °C. After 24 h, the colonies were observed for the morphological properties of *B. subtilis*.

Statistical analysis

In all treatments the data from repeated experiments were pooled as ANOVA indicated no difference between the experiments. The field data from two seasons were pooled and analyzed statistically. Analysis of variance (ANOVA) was executed for *in vitro* and field trials using SPSS ver.10.0 (Manojkumar et al., 2013).

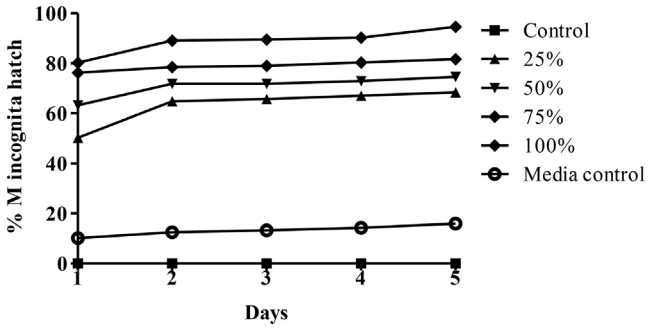

Fig. 1. Effect of cell-free culture filtrates of *B.subtilis* on egg hatching of *M.incognita*.

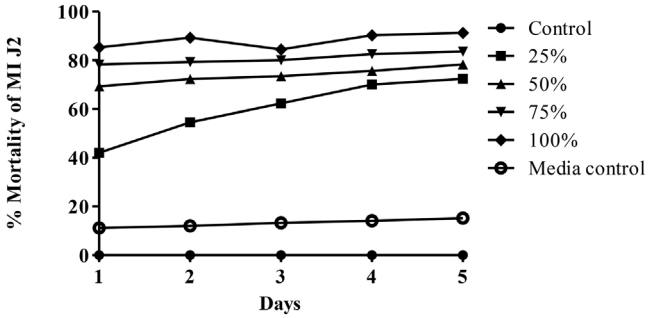

Fig. 2. Effect of cell-free culture filtrates of *B.subtilis* on mortality of *M.incognita*.

3. Results and discussion

3.1. Effect on nematode egg hatching and juvenile mortality

B. subtilis was found effective in significantly reducing the egg hatching of *M. incognita* at various concentrations compared to control. Maximum inhibition in egg hatching (94.65%) was recorded at 100% concentration after 120 h. The inhibition percentage of *M.incognita* was described by the equation in which In (1/K-Y)) = $\beta_0 + \beta_1 T$, Y = hatching percentage, K- the maximum Y reached for each Concentration, and T –Period of incubation. The Regression lines were

In (1/K-Y) = 1.648*X + 9.156, R^2 = 0.79(0%); In (1/K-Y) = 0.941*X + 48.77, R^2 = 0.89(25%); In (1/K-Y) = 0.45*X + 65.44, R^2 = 0.90(50%); In (1/K-Y) = 0.982*X + 72.76, R^2 = 0.91(75%); In (1/K-Y) = 1.232*X + 84.66, R^2 = 0.97(100%). The lethal effect of cell free culture filtrates of *B. subtilis* resulted in egg deformation and inhibited egg hatching from 24 h onwards and showed an increasing trend up to 120 h at all the concentrations tested (Fig. 1)

Similarly cell free culture filtrates of bio-agent exhibited antagonism against juveniles of the root-knot nematode as revealed by their higher mortality (59–91.26%) at various concentrations in comparison with the control(Fig. 2). With the increase in time of exposure, there was a gradual increase in J2 mortality, the highest mortality (91.26%) been recorded at 100% concentration after 120 h (Table 1). The percentage mortality of *M.incognita* was described by the regression lines In (1/K-Y) = 0.06452*X + 10.54, R^2 = 0.77(0%); In (1/K-Y) = 0.1155*X + 62.03, R^2 = 0.86(25%); In (1/K-Y) = 0.06396*X + 70.03, R^2 = 0.91(50%); In (1/K-Y) = 0.06084*X + 77.8, R^2 = 0.95(75%); In (1/K-Y) = 0.1039*X + 85.65, R^2 = 0.98(100%). All coefficients of determination were significant at P < 0.01(Tables 3 and 4)

3.2. In vitro *effect of* B. subtilis *IIHR BS-2 on* P. carotovorum

B. subtilis IIHR BS-2 showed an inhibitory effect on *P. carotovorum* in the 100% concentration and the regression lines was In

Table 1
Effect of *B. subtilis* on *M.incognita* and *P. carotovorum* infecting carrot.

Treatments	Density of *B. subtilis* (X10^6 CFU/g)	
	Soil	Root
ST	3.40^c	3.10^c
SA1	4.80^b	4.10^b
SA2	6.10^a	5.80^a
VC	0	0
Chemical	0	0
Control	0	0
C D (P = 0.05)	0.72	0.82
SEd	0.25	0.39

Values in each column followed by the same letter are not significantly different (P < 0.05). [ST – Seed treatment with *B. subtilis* IIHR BS–2 at 10 ml kg^{-1} seed; SA1 – ST + soil application of 2 tons ha^{-1} of vermicompost enriched with 2.5 l of *B. su btilis* IIHR BS–2; SA2 – ST+ soil application of 2 tons ha^{-1} of vermicompost enriched with 5 l of *B. subtilis* IIHR BS–2; VC – Soil application of vermicompost alone at 2 tons ha^{-1}; chemical – Carbofuran at 1 kg a.i. ha^{-1} + Streptocyclin (0.02%); control – Untreated control].

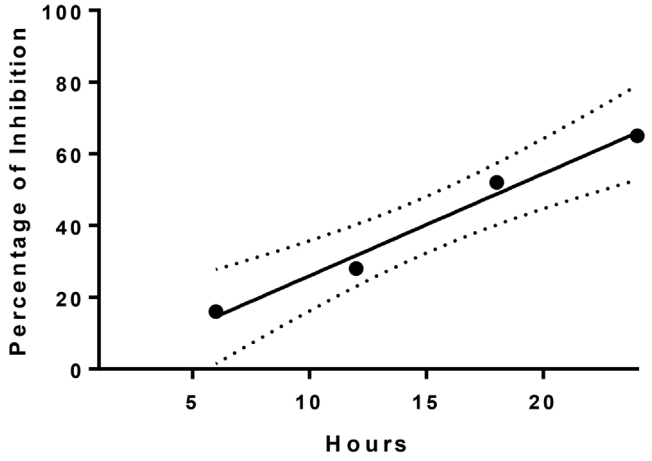

Fig. 3. Effect of *B.subtilis* on *P. carotovorum* infecting carrot.

(1/K-Y) = 0.684*X – 2.5, R^2 = 0.98 (24 h) and reduced growth of *P. carotovorum* by 60.6% compared to control within 24 h (Fig. 3).

3.3. Field bio efficacy

After enrichment in vermicompost, population of *B. subtilis* was found to be 9×10^9 CFU g^{-1}. Under field conditions, BCA application as seed treatment and soil application after enhancement in vermicompost, alone or in combination recorded significantly higher growth parameters and yield coupled with lower disease and nematode population. Seed treatment and soil application of 2 tons ha^{-1} of vermicompost enriched with 5 l of *B. subtilis* IIHR BS-2 recorded maximum plant growth parameters – root length (19.8 cm), root weight (89.6 g), shoot length (64.5 cm) and shoot weight (42.6 g) (Fig. 4 and Table 5).

Maximum reduction in *M. incognita* population (69.33%) and disease incidence (70.20%) were recorded in this treatment. It was on par with treatment with chemicals in reducing the nematode population. Maximum increase in carrot yield (28.8%) was also observed in this treatment (Table 2; Fig. 4).

It was followed by seed treatment and soil application of vermicompost (2 ton ha^{-1}) enriched with 2.5 l *B. subtilis* IIHR BS-2 which recorded significantly lower nematode and disease incidence compared to control. Seed treatment alone with *B. subtilis* IIHR BS-2 at 20 ml kg^{-1} recorded 61.75% and 33.00% reduction in nematode population in soil and root, respectively together with 32.30 reduction in disease incidence. Application of vermicompost alone also resulted in 10.1% and 24.1% decrease in nematode population and disease incidence, respectively and increase of 11.1% in yield (Table 2; Fig. 4).

3.4. Soil and root colonization of B. subtilis IIHR BS-2

B. subtilis IIHR BS-2 (5 l) enhanced in 2 tons of vermicompost recorded higher colonization (CFU of 6.1×10^6 g^{-1} soil and 5.8×10^6 g^{-1} root) followed by seed treatment and soil application of 2 tons ha^{-1} of vermicompost enhanced with 2.5 l *B. subtilis* (CFU – 4.8×10^6 g^{-1} of soil and 4.1×10^6 g^{-1} of root) (Table 1). Bacterization of carrot seeds with IIHR BS-2 at 10 ml kg^{-1} seed also recorded 3.40×10^6 g^{-1} and 3.10×10^6 g^{-1} in soil and roots, respectively.

4. Discussion

B. subtilis IIHR BS-2 showed high antagonistic activity on *M. incognita* and the effect of cell free culture filtrates on nematode hatching and juvenile mortality suggest the role of antimicrobial

Table 2
Effect of *B. subtilis* on *M. incognita* and *P. carotovora* infecting carrot.

Treatments	Nematode population		% decrease in nematode population over control		Root gall index 1–5 Scale	Disease Incidence (%)	% reduction in disease incidence by *E. carotovora* over control
	Soil/100cc	Root/5 g	Soil	Root			
ST	65.10^b	13.80^b	61.75 (51.79)	33.00 (35.06)	1.90^{cd}	24.71^c	32.30 (34.62)
SA1	63.20^b	12.30^{ab}	62.86 (52.45)	40.29 (39.35)	1.80^c	14.19^b	61.10 (51.42)
SA2	52.20^a	9.50^a	69.33 (54.78)	53.88 (47.22)	1.10^a	10.87^a	70.20 (56.98)
VC	–153.01^c	18.23^c	10.09 (18.47)	11.50 (19.75)	3.96	27.70^c	24.10 (29.37)
Chemical	54.20^a	10.23^a	68.15 (56.26)	50.33 (45.19)	1.30^b	13.80^b	62.20 (52.09)
Control	170.20^d	20.60^c	0.00 (0.52)	0.00 (0.52)	4.52^d	36.50^d	0 (0.52)
C D (P = 0.05)	3.75	3.81	3.86	4.03	0.96	4.78	4.45
SEd	1.79	1.83	1.85	1.93	0.46	2.29	2.13

Values in each column followed by the same letter are not significantly different (P < 0.05). Figures in parentheses are arc sine transformed values.
[ST – Seed treatment with *B. subtilis* IIHR BS-2 at 10 ml kg^{-1} seed; SA1 – ST + soil application of 2 tons ha^{-1} of vermicompost enriched with 2.5 l of *B. subtilis* IIHR BS-2; SA2 - ST+ soil application of 2 tons ha^{-1} of vermicompost enriched with 5 l of *B. subtilis* IIHR BS-2; VC – Soil application of vermicompost alone at 2 tons ha^{-1}; chemical – Carbofuran at 1 kg a.i. ha^{-1} + Streptocycline (0.02%); control – Untreated control]

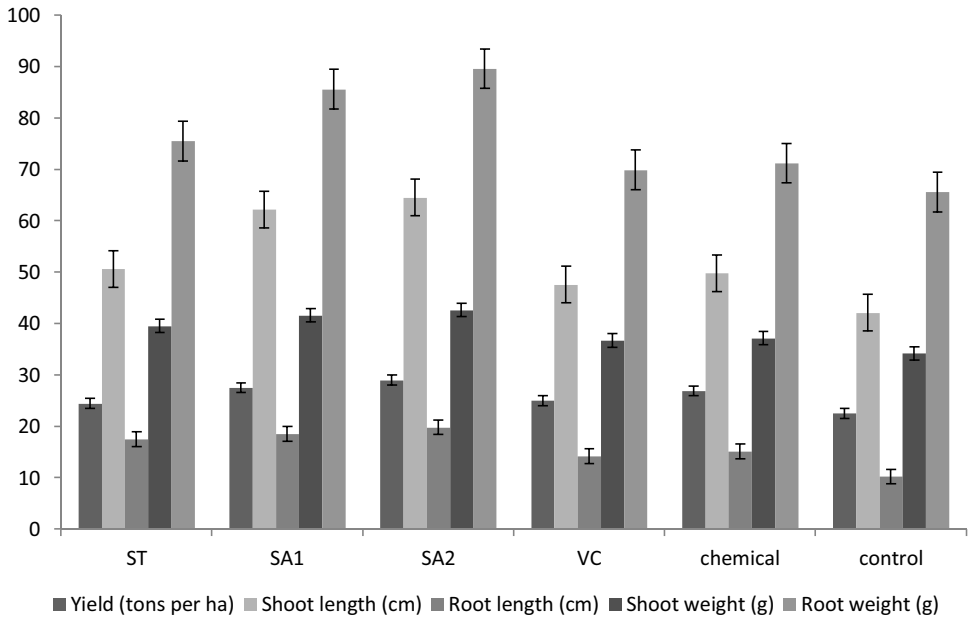

Fig. 4. Effect of *B. subtilis* IIHR BS-2 on plant growth parameters and yield of carrot.
[ST – Seed treatment with *B. subtilis* IIHR BS–2 at 10 ml kg^{-1} seed; SA1 – ST + soil application of 2 tons ha^{-1} of vermicompost enriched with 2.5 l of *B. subtilis* IIHR BS–2; SA2 – ST+ soil application of 2 tons ha^{-1} of vermicompost enriched with 5 l of *B. subtilis* IIHR BS–2; VC – Soil application of vermicompost alone at 2 tons ha^{-1}; chemical – Carbofuran at 1 kg a.i. ha^{-1} + Streptocyclin (0.02%); control – Untreated control].

Table 3
Test statistics for the effect of Egg hatching on *M.incognita* and Concentration versus Time.

Effects	Degree of freedom	S ed	CD(0.01)
Concentration	5	1.27	3.35[a]
Day	4	1.16	3.05[a]
Concentration x Day	20	2.86	7.49[a]

[a] Significant at a level of 1% probability.

Table 4
Test statistics for the effect of Mortality (J2) on *M.incognita* and Concentration versus Time.

Effects	Degree of freedom	S ed	CD(0.01)
Concentration	5	2.28	3.01[a]
Day	4	2.08	2.75[a]
Concentration x Day	20	5.10	6.74[a]

[a] Significant at a level of 1% probability.

metabolites that might be responsible for its antagonistic activity. Earlier reports by Killani et al. (2011) have shown that *B. subtilis* produces at least five types of antimicrobial compounds: subtillin, bacitracin, bacillin, subtenolin and bacilonycin. In addition, *Bacillus* spp. were also reported to produce a wide range of nematicidal volatile compounds such as benzene acetaldehyde, 2-nonanone, decanal, 2-undecanone and dimethyl disulphide, which were antagonistic towards egg hatching of *Meloidogyne* spp. and second stage juveniles (Huang et al., 2010). *B. subtilis* exhibited antinemic activity on *M. incognita* by producing several types of lipopolypeptides and antibiotics such as surfactin, fengycin and iturin (Kavitha et al., 2012). *Bacillus* spp. examined by Ann (2013) confirmed the production of proteolytic enzyme, protease which is mainly responsible for the degradation of nematode cuticle and completely destroyed *M. incognita* juveniles within 12 h

In earlier studies by El-Khair and Karima (2007), *B. subtilis* proved its bactericidal activity on soft rot pathogen in potato, *P. carotovorum* subsp. *carotovorum* by reducing the pathogen's ability to produce pectolytic enzymes: polygalacturonase and pectin methyl esterase. This bacterium is also reported to produce several hydrolytic enzymes such as glucanases or proteases and the antibiotic lipopeptides which act against several soil borne pathogenic bacteria and fungi (Cazorla et al., 2007).

This study also demonstrates that *B. subtilis* can multiply in vermicompost and soil application of such BCA enriched organic material increases the yield and decreases the nematode and associated disease complex in carrot. This confirms with earlier reports by Thilagavathi et al. (2012) which showed that bioformulations of *B. subtilis* and *P. fluorescens* enriched in vermicompost performed better in reducing root rot incidence in sugar beet. Also, Khan and Gangopadhyay (2008) reported that seed treatment of chick pea with *P. fluorescens* and soil amendment with mustard cake and vermicompost provided a better protection against *Macrophomina* root rot. Similarly, *B. cereus* enhanced in organic fertilizers

Table 5
ANOVA results for Shoot length, Root length, Shoot weight and Root weight in carrot.

	Degree of freedom	Sum of squares			
		Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)
Treatments	5	381.0373	60.54833	50.93808	445.4533
Error	24	8.318833	10.22833	26.66908	24.5875
Total	29	2104.839	548.2217	894.7484	18.11706
F-value		45.80418[a]	5.919668[a]	1.910005[a]	2817.367[a]
P-value		<0.05	<0.05	<0.05	<0.05

[a] Significative at a level of 5% probability.

showed excellent nematicidal activity against root knot nematodes in tomato and muskmelon (Xiao et al., 2013). The activity of bio-agent is reported to have direct correlation with organic amendments (Walker, 2004) and the success of the bio-agents are enhanced due to build-up of beneficial microbes and accumulation of their metabolites in amended soils (Wang et al., 2003).

Also in the present study, application of vermicompost alone in carrot fields suppressed 10–24% of nematode population and disease incidence. Soil amendment with vermicompost is reported to suppress several nematodes and soil borne phytopathogens (Stephens et al., 1993; Nakamura 1996; Szcech et al., 1993; Arancon et al., 2002, 2003). Several mechanisms are involved in nematode suppression through organic amendments such as release of nematicidal compounds like ammonia and fatty acids during degradation; enhancement of antagonistic microbes; increase in plant resistance and tolerance against pathogens; changes in soil physiology unsuitable for nematode behaviour (Oka, 2010). Hoitink and Grebus (1997) described the possible mechanisms of pathogen suppression by vermicompost through systemic plant resistance, microbial competition, antibiosis and hyper parasitism.

In Brazil, a commercial product with consortia of *Bacillus subtilis* and *Bacillus licheniformis* effectively controlled several nematode pathogens on potatoes and carrots and ultimately, replaced nematicides (Cawoy et al., 2011).

5. Conclusion

The present study proves the antagonistic potential of *Bacillus subtilis* IIHR BS-2 against the root knot nematode and soft rot complex in carrot. This study also proves the successful combination of functional microorganisms with organic amendments for effective management of soil borne pathogens. This technology of enhancing the organic material such as vermicompost with BCA has several advantages in terms of socio-economic feasibility as it can reduce the cost incurred on biopesticides and be easily multiplied on a large scale by farmers themselves in their own farm. There is a vast scope for utilizing this microbe as a biopesticide for nematode disease management in open fields, polyhouses and organic farms across several crops. Hence, these biopesticides are beyond doubt to bring about a revolution in the field of pesticide industry as a safe alternative to several chemical fungicides and nematicides.

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