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Antagonistic potential of an Egyptian entomopathogenic nematode, compost and two native endophytic bacteria isolates against the root-knot nematode (*Meloidogyne incognita*) infecting potato under field conditions

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Abstract

Background: The root-knot nematode, *Meloidogyne* spp., are one of the most dominant and dangerous group of pests. The deformations and discolorations make tubers unmarketable and/or of less quality. Therefore, management of *Meloidogyne* spp. becomes an obligatory challenge that warrants intervention. Biological control agents are the best alternative tools for controlling plant-parasitic nematodes that comply with the requirements of the development of the green agriculture and that reduce the reliance on these harmful chemicals. Therefore, this study aimed to evaluate the effectiveness of compost singly, and in combinations with the bio-agents *Heterorhabditis bacteriophora*, and two bacterial isolates Nem 212 and Nem 213 against the root-knot nematode *Meloidogyne incognita* infecting potato plants under field conditions.

Results: Among 15 bacterial isolates (Nem205-Nem219) obtained from the rhizosphere of tomato and eggplant from Giza, Egypt, the two isolates (Nem 212 and Nem 213) were molecularly characterized based on the partial 16S rDNA sequencing analysis. These two bacterial isolates were deposited in the GenBank as *Bacillus cereus* Nem 212 and *B. cereus* Nem 213 and were tested against *M.* incognita J_2s in vitro. Results showed that the cell filtrates of *B. cereus* Nem 212 and *B. cereus* Nem 213 gave the highest percentage of *M.* incognita J_2s mortality (100%), after 48 h of the in vitro application. Moreover, all the applied treatments significantly suppressed the reproductive of *M. incognita* on potato plants and enhanced the potato crop yield under the field conditions. Compost enriched with *B. cereus* Nem 212 cell suspension was the most effective treatment. The combination between the bacterial cell suspension and the compost offered an increase in the disease curing and the potato plant growth and yield production, compared to the treatment with compost alone. The entomopathogenic nematode, *Heterorhabditis bacteriophora*, was relatively less effective in controlling *M. incognita* on potato, compared to *B. cereus* Nem 212 and/or *B. cereus* Nem 213

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treatments. However, when compost was enriched with *H. bacteriophora*, it increased its capability to control the nematodes.

Conclusions: This study provides insights into the practical usage of EPNs *H. bacteriophora*, and the endophytic bacteria (*B. cereus* Nem 212 or *B. cereus* Nem 213) as biocontrol agents against *M. incognita* on potato plants. The application of compost enriched with the bacterial cell suspensions of either *B. cereus* Nem 212 or *B. cereus* Nem 213 and *H. bacteriophora* within *Galleria mellonella* cadaver proved efficient control of *M. incognita* infecting potato plants and improved the growth and yield of potato plants under field conditions.

Keywords: Potato, *Meloidogyne incognita*, Entomopathogenic nematode, Compost, Endophytic bacteria isolates, Biocontrol

Background

Potato (*Solanum tuberosum* L.) is a carbohydrate-rich vegetable crop that has a high nutritional value and is highly popular worldwide. Potato production is negatively affected by plenty of various pests and diseases, including plant-parasitic nematodes that adversely affect the quantity and quality of the tuber yields (Mugniery and Phillips 2007). The root-knot nematodes (RKNs) *Meloidogyne* spp. are considered as the most damaging plant-parasitic nematode (Abd-Elgawad 2020), especially on potato plants. Moreover, RKNs cause deformations and discolorations of potato tubers which make tubers unmarketable (Vovlas et al. 2005). Contrary to chemical nematicides, biological control agents (BCAs) are safe tools to control plant-parasitic nematodes (PPNs). They also fulfill the requirements of the development of the green agriculture and reduce the reliance on the harmful chemicals. Among these alternative control strategies, various compost treatments are gaining a lot of interest because of their low cost and positive agronomical effect on plant growth, physical, chemical and biological properties of the soil and in its effectiveness in controlling plant pathogens, which eventually leads to an increase in crop production (Al-Hendy et al. 2021). Large numbers of soil amendments could be used as plant protectants or organic fertilizers (Ntalli et al. 2020). Furthermore, these organic wastes can act in integration with the microorganisms leading to an increase in the nematode-antagonistic populations (Hernandes et al. 2020). Management of PPNs using BCAs is a promising alternative to the chemical nematicides (Viljoen et al. 2019). *Bacillus* spp. are a group of bacterial agents that have been recognized as one of the most promising groups of nematode antagonists, e.g., *B. cereus* that has been found to be effective in managing RKN and enhancing crop production (El-Wakeel et al. 2020). The action of the genus *Bacillus* on the plant-parasitic nematodes has been widely confirmed (Yin et al. 2021).

Entomopathogenic nematodes (EPNs) of the families: Steinernematidae and Heterorhabditidae, are obligate pathogens of soil insects and are employed as biocontrol agents for a number of insect pests (Kaya and Gaugler 1993) that provide environmentally safe management tools for PPNs without affecting the free living nematodes, which play an important role in nutrient cycling (Somasekhar et al. 2002). These nematodes are mutualistically associated with certain bacterial species of the genus *Xenorhabdus* that frequently associated with *Steinernema* and the genus *Photorhabdus* with *Heterorhabditis*. Third-stage infective juveniles (IJs) of EPNs of the families Steinernematidae and Heterorhabditis are the only stage of the nematode that can survive outside the host in the soil. The IJs retain cells of the bacterial symbiont in their intestines when they leave the host. When the IJs find a susceptible insect host, they enter the insect through the natural openings (anus or mouth) and penetrate into the hemocoel, where the nematodes produce toxins and release their mutualistic bacteria. The bacterial cells proliferate rapidly in the hemocoel, and the host is consequently killed by toxemia (Kusakabe et al. 2022). The endosymbionts bacteria: *Xenorhabdus* and *Photorhabdus*, produce a wide range of secondary metabolites. These metabolites are different kinds of antibiotics, proteases, adhesions, lipases and hemolysins, and they are used as biocontrol negotiators for management of virus, fungi, nematodes and insects (Lulamba et al. 2021). Only two secondary metabolites molecules, a stilbene derivative (3,5-dihydroxy-4-isopropylstilbene) and indole, had been found to have nematicidal activity. These metabolites are toxic to many species of nematodes including those of the genus *Meloidogyne* (Tomar et al. 2022).

It is well known that the efficiency of bio-agents varies upon both environmental conditions and nematode species. Bacterial strains collected from one set of environmental conditions may be less effective under other environmental conditions (Schisler et al. 1997). Therefore, one of the means to increase the potency of these biocontrol agents is to use the native ones that are well adapted to the local environmental conditions.

This study was conducted to assess the efficacy of compost singly or in combination with the Egyptian EPN,

Heterorhabditis bacteriophora, and the two bacterial isolates (*B. cereus* Nem 212 and Nem 213) against *M. incognita*, infecting potato plants under field conditions.

Methods

Source of seeds

Potato tuber seeds (*Solanum tuberosum* L. cv. Sponta) weighing 40–50 g per tuber imported from Denmark by Chance Company for Import and Export, Egypt, were used for the field experiment.

Isolation of bacterial strains

Soil samples were collected from rhizosphere region of tomato and eggplant farms from Kafr-Hakim village, Giza, Governorate, Egypt, at 10–15 cm depth. About one gram of soil was dissolved in 100 ml of sterile saline solution. Afterward, serial dilution up to 10^{-7} using sterile saline solution (0.85%, NaCl w/v) was made. After thorough mixing, 50 µl aliquot from suitable dilutions was pour plates, in triplicate, on the nutrient agar (NA) plates (Biobasic, India) and incubated for 24 h at 30 ± 0.5 °C. To obtain axenic bacterial cultures, single colonies were then suspended in sterile saline solution and distributed on NA plates as the same in soil samples. Standard microbiological methods were used to isolate bacteria from the rhizosphere (Flores-Vargas and O'Hara 2006). Fifteen pure bacterial cultures were isolated and maintained on NA slants at 4 °C for further studies and coded as Nem205 to Nem219.

Preparation of second-stage juveniles of *Meloidogyne incognita*

M. incognita population was maintained on susceptible tomato plants in the greenhouse. The nematode species was previously identified based on the morphological features of the perineal pattern (Taylor and Sasser 1978). The nematode eggs were extracted from the roots of tomato plants using a 0.5% NaOCl according to the method described by Hussey and Barker (1973). The eggs were incubated in egg hatching plastic cups at the room temperature for 72 h to obtain second-stage juveniles (J_2). The J_2s were surface sterilized by 0.01% streptomycin sulfate solution for 1 h before use.

Nematicidal activity of bacterial isolates in vitro

To determine the nematicidal activity of the bacterial isolates, axenic bacterial cultures were prepared in nutrient agar plates as described earlier. Bacterial cultures were centrifuged at 500 rpm for 15 min. The supernatant solution was passed through a 0.22 µm in diameter nitrocellulose filter, and the flow through was used as a cell-free filtrate for the bioassay test. Petri dishes (5 cm in diameter) were supplied separately with 1 ml of cell-free filtrate from each bacterial isolate plus 3 ml of nematode suspension in distilled water containing 50 ± 5 freshly hatched *M. incognita* second-stage juveniles J_2. A volume of 4 ml of distilled water containing 50 ± 5 freshly hatched *M. incognita* juveniles served as a control. All treatments and control were replicated five times. All dishes were kept in incubator at 35 °C. Dishes were partially covered to permit aeration and less evaporation. The mortality rates of juveniles were recorded after 24 and 48 h under a light microscope. After the exposure periods, the nematodes in each treatment were transferred to distilled water and left for 24 h before microscopic examination whether immobile juveniles resumed activity or not. The corrected percentages of nematode mortality were calculated according to the following equation: Mortality $\% = (m\text{-}n)/(100\text{-}n) \times 100$, where m and n indicate the percentages mortality in treatment and control, respectively (Abbott 1925).

Extraction of genomic DNA from bacterial isolates

Two bacterial isolates (*B. cereus* Nem 212 and Nem 213) were selected for amplification of the 16S rDNA. Single colonies of bacterial isolates were cultured in conical flasks containing 20 ml of nutrient broth medium for 18 h at 120 rpm and 30C°. The cultures were centrifuged at 12,000 rpm for three minutes at 4 °C. The pellets were subjected to genomic DNA extraction using the (QIAamp DNA Mini Kit, QIAGEN, Germany). The extracted DNA was used as a template for PCR to amplify the 16S rDNA gene using the universal primers: forward primer sequence (5′AGAGTTTGATCCTGGCTCAG3′) and reverse primer sequence (5′CTACGGCTACCTTGT TACGA3′), thereby producing an amplicon of ~ 1500 bp (El-Sayed et al. 2018). The amplicon obtained by PCR was purified using the QIA quick PCR purification kit (Qiagen, Germany), following resolving by electrophoresis on 1% agarose gels and compared to a 100 bp DNA ladder (Thermo scientific, USA). Purified PCR products were subjected to sequencing by sanger sequencing method using sequencer 3500 genetic analyzer, big dye X terminator kit (thermo fisher, USA) for forward and reverse directions in biomedical laboratory of colors in Clinilab, Egypt. The sequences were edited using Bioedit 7.1.10 (Hall 1999) software, and BLAST was used to detect the homology with other relatives (Altschul et al. 1997).

Assay of protease activity

A colony from each bacterial isolate (*B. cereus* Nem 212 and Nem 213) was cultured in Luria Bertani (LB) broth media pH 7 for 24 h at 30 °C and 200 rpm in an orbital shaker to develop bacterial growth. The liquid medium used for the production of alkaline protease had the following composition (% w/v): 2.0% glucose, 2.0%

hydrolysate casein, 0.04% $CaCl_2$ and 0.02% $MgCl_2$. The pH of the medium was adjusted to 8.5. The production medium was inoculated with 5% inoculum. The flasks were incubated for 72 h in a 30 °C shaking incubator (200 rpm). The contents were then centrifuged (12,000 g, 4 °C, 20 min), and the cell-free supernatant was used for determining extracellular protease activity.

Casein dissolved in pH buffer 9 was used as the substrate for the assay. The reaction mixture containing casein and the enzyme solution was incubated for 10 min at 37 °C. The amount of enzyme required to liberate 1 µg tyrosine per ml per minute under the standard conditions, defined one unit of protease activity (Asha and Palaniswamy 2018).

Source, culturing and treatments of entomopathogenic nematodes

The IJs of an Egyptian EPN *Heterorhabditis bacteriophora* routinely cultured in last-instar larvae of the greater wax moth, *Galleria mellonella* L. (Woodring and Kaya 1988), were used in two delivery forms. Five days after adding 10 *G. mellonella* larvae to a 10-cm-diameter Petri dish lined with # 1 Whatman filter paper and wetted by one ml distilled water having 200 IJs, the consequently infected insect cadavers were used for application. The cadavers were buried directly about 3 cm below the soil surface, beneath the potato seedling stem at a rate of 5 insects/seedling (cadaver application). The other delivery form used the IJs in water suspension. The IJs were collected from White traps and applied within 3 days of nematode emergence at a rate of 100 ml of IJ suspension/potato seedling (125 active juveniles/ml). A one-liter hand sprayer was used to deliver the suspension on the soil surface around the potato seedling (spray application). The two IJ treatments were applied early in the morning as soil drench; the soil was wetted by water.

Source of compost

Organic compost was purchased from Shafei Compost Company, Giza, Egypt. The chemical analysis was provided to the authors by the company (Table 1).

Nematode identification and field experiment

The field experiment was carried out during the period of January–June 2021, at Kafr-Hakim village, Giza, Governorate, Egypt. The experimental area was naturally infested with *M. incognita*. The roots of potato plants previously planted in the experimental field were collected, and the adult females were picked out from the infected roots to identify the nematode species using the morphological characteristics of their perineal pattern according to female perineal pattern (Taylor and Sasser 1978). This area was divided into 3 plots, each comprising 8 rows of 8 m. length and 50 cm. width, and the distance between plants was 30 cm. The experiment was set up in a completely randomized block design with 8 treatments; each treatment was replicated 3 times. The treatments were: 1—*M. incognita*-infested non-treated soil (control) (T1), 2—*M. incognita*-infested soil + Compost C (T2), 3—*M. incognita*-infested soil + C + *Heterorhabditis bacteriophora*-infective juveniles within infected *G. mellonella* larvae (T3) (cadaver application), 4—*M. incognita*-infested soil + C + *H. bacteriophora*-infective juveniles in water suspension (T4) (spray application), 5—*M. incognita*-infested soil + C + *Bacillus cereus* Nem 212 cell suspension (T5), 6—*M. incognita*-infested soil + C + *B. cereus* Nem 212 cell-free culture filtrate (T6), 7—*M. incognita*-infested soil + C + *B. cereus* Nem 213 cell suspension (T7), and 8—*M. incognita*-infested soil + C + *B. cereus* Nem 213 cell-free culture filtrate (T8).

In each treatment with the bacterial isolates *B. cereus* Nem 212 suspension and *B. cereus* Nem 213 suspension (T5 and T7), soil was treated with the bacterial suspension at the rate of 50 ml/hill, and concentration was (2×10^7 CFU/ml) at planting time. In treatments (T 6 and T8), the bacterial culture filtrates were added at the rate of 50 ml/hill at planting time. Compost was applied into soil, 10 days before planting for proper decompositions of the materials at the rate of 50 g/hill. Initial population densities of *M. incognita* juveniles ($J_{2)}$ were determined at planting time according to Barker (1985) from 250 g subsamples of well mixed soil from each row (5 subsamples per 15 sample per treatment). Five months later (at harvest), five potato plants were randomly selected from every row and carefully uprooted. Potato tubers were hand harvested for yield estimation and recorded in terms of their average weights. Other plant growth parameters including: root length, plant height, number and weight of tubers/plant, were recorded. For evaluation of nematode reproductive parameters (NRPs), the

Table 1 The chemical composition of the organic compost used in this study

Chemical analysis	pH	EC Ds/m	Organic matter content (OM) %	Total organic carbon (C) %	Total nitrogen (N) %	Ash %	C:N ratio %	Total phosphorus (P) %	Total potassium (K) %
Organic compost	7.0	2.65	41.75	38.95	2.15	32.8	18:1	1.09	0.31

numbers of root galls and egg masses/5 g roots as well as number of *M. incognita* juveniles/one g roots and total number of eggs/5 g roots were recorded.

Assessment of root infestation: The roots from each plant were gently washed and cut into 3.0 cm length pieces. One-gram subsample was taken from each plant. The roots were stained with acid fuchsin–lactophenol (Bybd et al 1983) and *M. incognita* (J_{2s}) were counted under light microscope and recorded.

Assessment of eggs per 5 g roots: one-gram subsample from a 5 g potato roots was taken, cut into pieces of 2-cm long. *M. incognita* eggs were extracted from the roots using a 0.5% NaOCl solution for 3 min and then obtained by rinsing the egg suspensions with sterile water in a sieve with 25-µm openings according to the method of Hussey and Barker (1973). Eggs were counted under a light microscope, and their average numbers were recorded. Final nematode soil population was extracted, and densities of *M. incognita* were determined and expressed as the number of juveniles/250 g soil (Barker 1985). Percentage nematode reduction was determined according to Henderson and Tilton formula (Puntener 1981) as follows:

$$\text{Nematode reduction \%} = \left\{1 - (\text{PTA/PTB} \times \text{PCB/PCA})\right\} \times 100$$

where PTA = population in treated plot after application, PTB = population in treated plot before application, PCB is population in check plot before application, and PCA = population in check plot after application.

Statistical analysis

Data were subjected to the analysis of variance (ANOVA) according to Snedecor and Cochran (1980), using Assistant program version 7.6 beta. The means were compared using New Multiple Range Test (DNMRT) at $P \leq 0.05$ (Duncan 1955).

Results

Screening endophytic isolates with nematicidal activity in vitro

Fifteen endophytic bacteria strains were isolated from the rhizosphere of tomato and eggplants. Based on the nematicidal activity and different appearances of dead nematodes in vitro after 24 and 48 h of treatment with the isolate's cell-free filtrates, the mortality rate of the 15 isolates was above 90%. The highly percentage mortality (100%) was recorded by two bacterial isolates (Nem 212 and Nem 213) (Table 2).

Molecular identification of *Bacillus cereus* Nem 212 and 213 isolates

The universal primers of 16 s rDNA gene amplified a fragment of approximately ~ 1550 bp (Fig. 1) in both

Table 2 Nematicidal activity of the cell-free filtrates of 15 bacterial isolates against *Meloidogyne incognita* juveniles (J_2s), in vitro

Bacterial isolates serial number	Mortality % after 24 h	Mortality % after 48 h
Control (Water only)	1.0	2.0 f
Nem 205	85.9	98.1b
Nem 206	33.6	98.2b
Nem 207	43.9	91.0d
Nem 208	31.4	98.6b
Nem 209	44.9	98.4b
Nem 210	40.8	91.1d
Nem 211	46.9	98.4 b
Nem 212	92.3	100.0a
Nem 213	89.2	100.0a
Nem 214	46.8	92.1d
Nem 215	48.4	94.5c
Nem 216	50.2	93.0c
Nem 217	46.8	90.5e
Nem 218	64.2	94.9c
Nem 219	49.4	91.5d

Each value represents mean of five replicates. Means followed by the same letter(s) are not significantly different ($P \leq 0.050$) according to Duncan's New Multiple Range Test

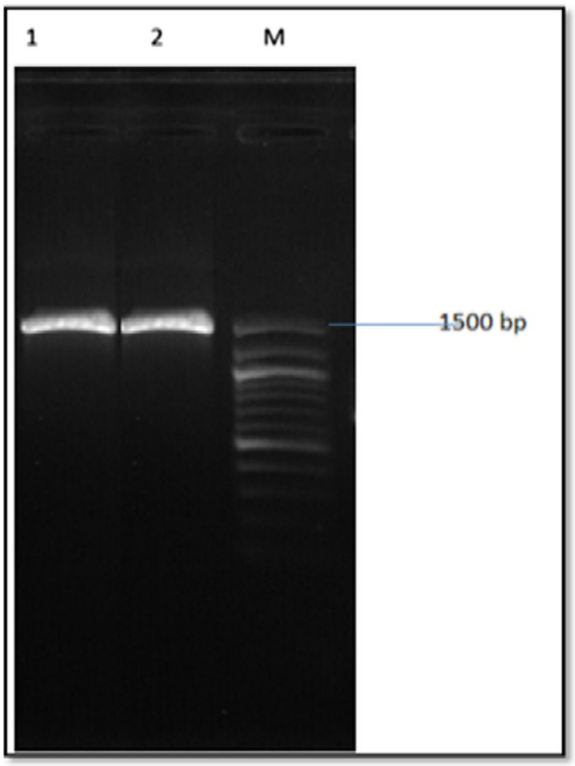

Fig. 1 Agarose gel electrophoresis for the PCR product of 16 s rDNA gene (1500 bp) in *Bacillus cereus* Nem 212 (1) and *Bacillus cereus* Nem 213 (2); M: 100 bp DNA ladder (Thermo Scientific, USA)

bacterial isolates. The amplified gene was sequenced in both directions. After trimming and sequence editing, partial DNA sequences were subjected to BLAST search on https://blast.ncbi.nlm.nih.gov/Blast against the available sequences deposited in NCBI database. Partial 16S rDNA gene sequence of bacterial isolate Nem 212 matched 100% to *Bacillus cereus* ATCC 14,579 16S ribosomal RNA accession no. NR_074540. In regard to Nem 213, partial 16S rDNA gene sequence matched 99.85% to *Bacillus cereus* strain 1H4 16S ribosomal RNA gene accession no. OK178870. Bacterial isolates Nem 212 and Nem 213 were submitted at GenBank database as *B. cereus* Nem 212 and *B. cereus* Nem 213 under accession numbers OK284601 and OK284743, respectively.

Protease activity of the bacterial strains

The results showed that *B. cereus* Nem 212 protease activity was 2.01 µg/min/ml; however, *Bacillus cereus* Nem 213 recorded 1.99 µg/min/ml.

Effects of compost enriched with endophytic bacteria and entomopathogenic nematodes on the reproduction of *Meloidogyne incognita*

All the tested treatments (Table 3) significantly suppressed the reproduction of *M. incognita* on potato plants at various time intervals, compared to the non-treated control. After two months of planting, application, T7 (C + *B. cereus* Nem 213 cell suspension) produced the highest percentage of reduction (79.4%) in the numbers of juveniles (J_2s) in the soil compared to the non-treated control, followed by T8 (*B. cereus* Nem 213 supernatant (cell-free culture filtrate) that produced 75.6%, reduction in (J_2s) in the soil. Other treatments with compost (T2, T5 and T3) exhibited 66.3%, 57.1% and 54.8% reduction in the number of J_2s in the soil, respectively, than the non-treated control. However, the lowest percent reduction in the numbers of J_2s in the soil was found in T6 (C + *B. cereus* Nem 212 supernatant (cell-free culture filtrate) that produced reduction of 21.3% in the number of J_2s in the soil compared to the non-treated control.

The treatment: T7 and T5, resulted in the greatest percentages reduction in the number of nematode stages in the roots 79.1% and 77.3%, respectively, compared to the non-treated control. However, application of T4 (C + *H. bacteriophora* juveniles in water suspension) showed the least percentage reduction in the number of juveniles in the roots (15.5%). Application of T5 and T6 exhibited the greatest percentage reduction, in number of galls (72% and 70.1%), respectively, compared to non-treated control.

At harvest, all treatments suppressed the nematode reproduction on potato under field conditions (Table 3). The treatment T5 (C + *B. cereus* Nem 212 cell suspension) provided the highest reduction in the number of galls and egg masses/plant, number of nematode stages/roots, nematode final population (Pf) and number of nematode eggs/5 g roots, followed by (T7 cell suspension (Table 3)). Application of compost alone (T2) resulted in 66.4, 38.5, 65.8, 47.1 and 46.8% decrease in the number of *M. incognita* (J_2) in soil, number of galls and egg masses/plant, number of nematode stages in the roots and total number of eggs/5 g roots, respectively, compared to the control (Table 3). Concerning the application of entomopathogenic nematode, the treatment T4 (C + *H. bacteriophora* IJs in water suspension) exhibited percentage reduction of 46.6, 17.2, 19.4, 38.8 and 29.1% in the number of *M. incognita* J_2 in soil, number of egg masses and galls/plant, number of nematode stages in the roots and the total number of eggs/5 g roots, respectively, compared to the non-treated control. Finally, the application of T3 (C + *H. bacteriophora* IJs within *G. mellonella* cadavers) resulted in a reduction of 54.9, 59.7, 64.8% in the number of *M. incognita* (J_2s) in the soil, number of egg masses/plant and total number of eggs/5 g roots, respectively.

Effects of compost enriched with endophytic bacteria and entomopathogenic nematodes on potato growth parameters

In addition to the nematicidal activity of the studied treatments, especially T5, T6, T7 and T8, the treatments generally enhanced the potato growth parameters including: root length, plant height, dry weight of plant, number and weight of potato tubers and number of leaves in the various levels of potato growth, compared to the non-treated control (Table 4). However, compost and *H. bacteriophora* IJs in water suspension treatments were the least effective in this respect (Table 4).

Treatment effects on potato yield

All treatments significantly ($P \leq 0.05$) increased potato yield production (Table 4). T7 treatment resulted in the greatest increase (221.2%) in potato yield compared to the non-treated control, followed by T8 and T5 (209% and 206.8% increase in potato yield, respectively), compared to the non-treated control. However, the application of T3 compost and *H. bacteriophora* (cadaver application) and T4 containing the compost and *H. bacteriophora* (spray application) produced an increase of 163.6% and 90.9%, respectively, in the yield of potato, compared to the non-treated control (Table 4).

Discussion

The results of the present field experiments showed that all the applied treatments significantly reduced the reproductive of *M. incognita* on potato plants and enhanced

Table 3 Effect of compost and biocontrol agents on potato plant cv. Sponta infected with root-knot nematode *Meloidogyne incognita* under field conditions after two months of planting and at harvest

Treatments	After two months									At harvest									
	Initial population /250 g soil	No. of J_2/250 g soil	% Reduction	No. of J_2/5 g roots		No. of galls/5 g roots		No. of egg masses/5 g roots		Final population /250 g soil	% Reduction	No. of J_2/5 g roots		No. of galls/5 g roots		No. of egg masses/5 g roots		Total eggs/5 g roots	
				No	% Reduction	No	% Reduction	No	% Reduction			No	% Reduction	No	% Reduction	No	% Reduction	No	% Reduction
T1	559 a	455 a	–	110 a	–	311 a	–	158 a	–	1593 a	–	221 a	–	584 a	–	325a	–	153725a	–
T2	557 a	153 f	66.3	70 cd	36.4	98 d	68.5	60 c	62.1	534 e	66.4	117 c	47.1	200 f	65.8	200 d	38.5	81,800 c	46.8
T3	555 a	204 d	54.8	57 d	48.1	101 d	67.5	54 c	65.8	714 c	54.9	133 b	39.8	250 e	57.2	131 e	59.7	54103d	64.8
T4	551 a	250 c	44.3	93 b	15.5	215 b	30.9	98 b	37.9	839 b	46.6	137 b	38.8	471 b	19.4	269 c	17.2	108945b	29.1
T5	550 a	192 e	57.1	25 e	77.3	87 d	72.0	61 c	61.3	648 d	58.7	59 f	73.3	161 g	72.4	63 g	80.6	28078f	81.7
T6	562 a	360 b	21.3	72 c	34.5	93 d	70.1	60 c	62.0	725 c	54.7	100 d	54.8	175 g	70.0	106 f	67.4	43990e	71.4
T7	560 a	94 h	79.4	23 e	79.1	125 c	50.8	55 c	65.0	349 g	77.9	49 f	77.8	286 d	51.0	110 f	66.2	47300e	69.1
T8	553 a	110 g	75.6	58 d	47.3	227 b	27.0	109 d	31.0	395 f	75.4	82 e	62.9	334 c	42.8	106 f	67.4	44838e	70.8

Each value represents mean of five replicates. Means followed by the same letter(s) within a column are not significantly ($P \leq 0.05$) different according to Duncan Multiple Range Test. T1: Control, T2: Compost (C), T3: C + *H. bacteriophora*-infective juveniles (IJs) within *Galleria mellonella* cadaver, T4: C + *H.* bacteriophora IJs in water suspension, T5: C + *B. cereus* Nem 212 cell suspension, T6: C + *B. cereus* Nem 212 supernatant (cell-free culture filtrate), T7: C + *B. cereus* Nem 213 cell suspension, T8: C + *B. cereus* Nem 213 supernatant (cell-free culture filtrate)

Table 4 Growth parameters at harvest of potato cv. Sponta infected with *Meloidogyne incognita* and treated with compost and bio-agents under field conditions

Treatments	Root length (cm)	% Increase	Plant height (cm)	% Increase	Tubers weight (g/plant)	% Increase	No. of tubers/plant	% Increase	Dry weight (g/plant)	% Increase	No. of Leaves/plant	% Increase	Yield/tons/feddan	% Increase
T1	11 e	–	21 d	–	210 f	–	3 b	–	45 c	–	11 d	–	3.3 g	–
T2	17 cb	54.6	40 c	90.5	420 e	100.0	5 a	66.7	80 c	77.8	16 c	45.4	6.6 e	100.0
T3	18 c	63.6	41 bc	95.2	600 c	185.7	6 a	100.0	119 b	164.4	22 ab	100.0	8.7d	163.6
T4	12 de	9.1	38 c	80.9	400 e	90.5	6a	100.0	120 b	166.7	18 bc	63.6	6.3 f	90.9
T5	21 bc	90.9	47 ab	123.8	640 b	204.8	5 a	66.7	61 d	35.6	25 a	127.3	10.1 b	206.8
T6	20 bc	81.8	52 a	147.6	555 d	164.3	6 a	100.0	65 d	44.4	24 a	118.2	9.5 c	187.0
T7	30 a	172.7	55 a	161.9	670 a	219.1	6 a	100.0	139 a	208.9	24 a	118.2	10.6 a	221.2
T8	25 ab	127. 0	51 ab	142.8	645 b	207.1	5 a	66.7	125 b	177.3	20 ab	81.8	10.2 b	209.0

Each value represents mean of five replicates. Means followed by the same letter(s) within a column are not significantly ($P \leq 0.05$) different according to Duncan Multiple Range Test

Feddan = 4200 m^2 T1: Control, T2: Compost (C), T3: C + *H. bacteriophora*-infective juveniles (IJs) within *Galleria mellonella* cadaver, T4: C + *H.* bacteriophora IJs in water suspension,

T5: C + *B. cereus* Nem 212 cell suspension, T6: C + *B. cereus* Nem 212 supernatant (cell-free culture filtrate), T7: C + *B. cereus* Nem 213 cell suspension, T8: C + *B. cereus* Nem 213 supernatant (cell-free culture filtrate)

the potato growth and tuber yield. The most nematode-suppressive treatments were those of the endophytic bacteria *Bacillus cereus* Nem 212 and *B. cereus* Nem 213. Antagonistic bacteria like *B. cereus* were previously found to be effective microorganisms in controlling root-knot nematodes and enhancing the plant growth (Osman et al. 2021).

The present results clearly showed that applications of *B. cereus* (Nem212 and 213 isolates) as cell suspension were more significantly potent than when used as culture-cell-free filtrate in suppressing nematode reproductive ability. Application of different delivery formulations has been reported to affect the potentiality of bacterial preparations on the host plant (Nagachandrabose 2020). Gao et al. (2016) previously found that the supernatant of *B. cereus* resulted in 90.96% mortality of *M. incognita* J_{2s} indicating that its capability to produce some extracellular substances can kill the nematodes. In a pot experiment, the control efficiency against *M. incognita* reached 81.36% for *B. cereus* S2 supernatant due to the nematicidal substance of sphingosine in the supernatant (Gao et al 2016). This can be explained in the light of *B. cereus* culture containing not only the sphingosine but also live cells or spores, which can colonize the rhizosphere of the plant to exert nematicidal activity for a long term. From another point of view, Zhou et al. (2021) stated that the endophytic bacteria *B. cereus* can produce some extracellular substances like protease that kill 100% of *M. incognita* within 72 h by degrading the cuticle and egg shell. Similarly, Yin et al. (2021) reported that *B. cereus* strain *Bc*-cm 103 caused 100% mortality of *M. incognita* J_2 but within only 12 h. They also identified volatile organic compounds caused 97.2% in *M. incognita* J_2 after 48 h. Siddiqui (2000) indicated that aqueous cell suspension and the cell-free culture filtrate considerably reduced nematode population in the root and soil and subsequently reduced *M. javanica* population in tomato plants. Aqueous cell suspension was found more effective than culture filtrate of the bacterium, indicating that the respective activity was due to cellular metabolic components. It was also speculated that compounds required for growth and suppression of nematode's reproduction might not be produced in sufficient quantity in culture media as produced by the bacterial cells in the rhizosphere.

Furthermore, the success of bio-agents with respect to their biocontrol efficacy and consistency relies upon appropriate delivery mechanisms at field conditions. Earlier reports revealed that incorporation of bio-agents with organic amendments such as compost will change the soil environment in favor of the bio-agents and provided readily available nutrients to fungal and bacterial antagonists for their survival and development (Timper 2014). Moreover, Al-Hendy et al. (2021) proposed multiple mechanisms to explain the beneficial effects of organic amendments on PPNs and plants by releasing nematode-toxic compounds from decomposing materials, stimulating the nematodes' natural enemies, improving tolerance to nematodes and altering chemicals, biological and physical properties. In addition, these amendments enhance fertility of the soils which in turn improve plant growth. Walker (2004) reported that the activity of the bio-agents was directly correlated with organic amendments. Afterward, many researchers demonstrated that using bio-agents enriched with organic amendments exhibited greater antagonistic activity against plant pathogens such as root-knot nematodes (Gowda et al. 2018). In the present study, organic compost with the endophytic bacteria was found to be more effective against *M. incognita* than the compost alone treatment.

The endophytic bacteria *B. cereus* Nem 212 and *B. cereus* Nem 213, at various delivery methods, whether cell suspension or supernatant enriched with compost were the highly effective treatments in improving plant growth and reducing nematode multiplications. These results are in harmony with findings of Park et al. (2014) who estimated the potential of *B. cereus* C1-7 against root-knot nematode *M. hapla* infecting carrot and tomato plants in pot conditions. They reported a complete inhibition of root galls and egg mass formation in treated plants, and subsequently reducing root-knot nematode damage and suppressing nematode population. From the biochemical point of view, *B. cereus* S2 could induce systemic resistance in tomato plant and enhance the activity of some defense-related enzymes for the biocontrol of *M. incognita*. These findings might be supported by several workers (Osman et al. 2021) who demonstrated that *B. cereus* is a strong producer of hydrolytic enzymes such as protease which may be partially involved in the suppression of root-knot nematodes reproduction. This enzyme can potentially cause harm to the external structures of the nematodes and their eggshells, accompanied by inhibition of egg hatching and increased juvenile's mortality (Hong et al. 2013). The suppression of *M. incognita* reproduction on potato plants as a result of the treatment with *B. cereus* strains has led to the enhancement of plant growth and tuber yield.

The efficacy of EPNs was relatively lower when compared with *B. cereus* Nem 212 or *B. cereus* Nem 213 treatments in this study. These results are in agreement with those reported by many authors under greenhouse and field conditions (Hammam et al. 2019). Our application using EPN-IJs within *G. mellonella* cadavers exhibited more inhibition in NRPs than using IJs in water suspension. Similar results were obtained under laboratory and greenhouse conditions (El Aimani et al.

2022). It has also been reported that applying the IJs within cadavers has a higher dispersal capacity and prolonged longevity compared to IJs in water suspension. Moreover, the cadavers themselves offer protection against harmful environmental conditions such as freezing and desiccation (Dolinski et al. 2015). Application of IJs in aqueous solution has some disadvantages such as decreased infectivity, survival during storage and the need for adequate irrigation equipment (Grewal 2002). Eventually, cadaver or spray application significantly increased plant growth parameters and potato yield. These results are in harmony with El Aimani et al. (2022). This increase was at least partly due to competition at the root surface, reduction in root-knot infection rate, production of allelochemicals or could be a fertilizer effect of the treatment combined with relief from *M. incognita* infection (Sayedian et al. 2020).

Conclusions

The application of compost enriched with bacterial cell suspensions of either *B. cereus* Nem 212 or *B. cereus* Nem 213 strains or *H. bacteriophora* IJs (cadaver application) gave more effective results in controlling *M. incognita* infecting potato plants and improved the growth parameters and production of potato tubers under field conditions. Further research experiments are still needed to determine the optimum dose–response as well as the time of application and economic value in each plant species.

Abbreviations

BCAs: Biological control agents; EPNs: Entomopathogenic nematodes; IJs: Infective juveniles; RKNs: Root-knot nematodes; P: Probability level; J_2: Nematode-second-stage juveniles; PPNs: Plant-parasitic nematodes; DNMRT: Duncan's New Multiple Range Test (Duncan, 1955).

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Author contributions

All authors participated in the development and implementation of the reviewing plan and subsequently written it. The first author HO discussed the different parts of the article with HA, MH, GE, UE, and MA to conduct and finalize the experimentation and write the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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