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RESEARCH

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Application of *Bacillus* species for controlling root-knot nematode *Meloidogyne incognita* in eggplant

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Abstract

Background: Eggplant (*Solanum melongena* L.) is one of the important vegetable crops infected by *Meloidogyne incognita* all over the world, including Egypt. Chemical nematicides frequently cause environmental pollution and toxic hazards to human, plants, and animals; certain biocontrol agents that are environmentally friendly and safe to humans and animals were tested against the root-knot nematode *Meloidogyne incognita* in eggplant.

Objective: This work is aimed to determine the nematicidal activity of *Bacillus* spp., viz., *B. subtilis* and *B. pumilus*, against *M. incognita* in three separated experiments to study their ability in controlling *M. incognita* and in improving the growth parameters of eggplants. *Bacillus* spp. were applied as single or in combination (experiment I), single treatment at different doses (experiment II), and different times (three times) of application (experiment III).

Results: The results of experiment I revealed that *Bacillus* sp. + *B. subtilis* significantly reduced the second-stage juvenile (J_2) in soil and galls and egg masses in roots, while *Bacillus* sp. + *B. pumilus* significantly reduced J_2 in roots. *Bacillus* spp. in pairs were more effective against *M. incognita*. In experiment II, *Bacillus* sp. (40 ml) significantly reduced the J_2 in soil and galls and egg masses in roots, while *B. pumilus* (40 ml) significantly reduced the J_2 in roots. The nematicidal activity of *Bacillus* spp. was increased by increasing the applied dose. In experiment III, *B. subtilis*, when applied three times, significantly reduced the J_2 in soil and the J_2 and galls in roots, while *B. pumilus* (applied three times) significantly reduced the egg masses in roots. All *Bacillus* spp. treatments highly increased the tested growth parameters compared to the controls.

Conclusions: The tested biocontrol agents used more than once or in combination are more effective than those used only once in controlling nematode parameters in eggplant under greenhouse conditions with a consequent increase in eggplant growth. These bacterial isolates need to be studied under different field conditions for confirmation.

Keywords: Application, *Bacillus* spp., Eggplant, *Meloidogyne incognita*, Pots experiment

Background

Eggplant (*Solanum melongena* L.) is one of the important vegetables grown over the world as well as in Egypt for producing fresh eggplant fruits (Usman and Siddiqui 2012). *Meloidogyne incognita* is a microscopic obligate soil-borne pathogen that feeds in eggplant roots and causes significant losses of yield. Application of chemicals causes ecosystem pollution. Therefore, applications of plant growth-promoting rhizobacteria (PGPR), such as *Bacillus* spp., can produce nematicidal metabolites and a promising tool for controlling nematodes and decrease the hazard due to chemical applications (Abbais et al. 2014; Ahemad and Kibret 2014; Sivasakthi et al. 2014). PGPR also can promote the plant growth parameters by secreting various regulatory compounds in the vicinity of the rhizosphere (Gu et al. 2007; Karmani et al. 2011). Application of cultural filtrates of *Bacillus subtilis*, as soil drench, reduced nematode parameters in eggplant roots in the greenhouse experiment (El-Nagdi and Abd-El-Khair 2008). *Bacillus coagulans* when applied with vermicompost or *Glomus aggregatum* decreased the root-knot nematode population and

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improved the plant growth of tomato cv. Pusa Ruby (Serfoji et al. 2010). *B. subtilis* and *Trichoderma harzianum*, alone or in combination, could suppress the *M. incognita*, viz. number of galls, immature stages, number of second-stage juveniles (J_2), and total final population of nematode, and enhance the growth parameters of eggplant cv. Patra in the greenhouse (Farfour and El-Ansary 2013). *B. subtilis*, *B. thuringiensis*, *Psudomonas fluorescens*, and *Serratia marcescens*, alone or as a mixture, inhibited egg-hatching and J_2 of *Meloidogyne javanica* and highly reduced the numbers of galls and egg masses per root system and J_2/250 soil as well as increased the dry weights of root and shoot in eggplants (Mokbel and Alharbi 2014). *Bacillus weihenstephanensis* produced the most effect against *M. incognita* in infected tomato through laboratory and glasshouse experiments. The bacterium highly reduced the J_2 population in soil and roots and gall index, and it could increase fruit yield in tomatoes (Tamalika et al., 2014). The rhizospheric bacteria, viz. *S. marcescens*, *P. fluorescens*, and *B. thuringiensis* (BT14), had antagonistic effects against *M. incognita* (Zaghloul et al. 2015). *B. subtilis* and *B. pumilus*, alone or in combination, significantly reduced the numbers of J_2 in soil and roots, females, galls, and egg-masses of *M. incognita* in pea. Treatments also significantly increased the growth parameters of pea plants, viz. shoot length, shoot fresh, and dry weight, leaf numbers, and pod fresh and dry weight (El-Nagdi et al. 2018). Application of *B. subtilis*, *B. pumilus*, and *P. fluorescens*, single or combined, controlled *M. incognita*, infecting cowpea cv. Baladi under greenhouse conditions, where most nematode reductions were obtained with *P. fluorescens*, followed by *P. fluorescens* + *B. subtilis* and *P. fluorescens* + *B. subtilis* + *B. pumilus*, respectively (El-Nagdi et al. 2019).

Therefore, this work is aimed to determine the nematicidal activity of *Bacillus* spp., viz., *B. subtilis* and *B. pumilus*, against *M. incognita* in three separated experiments to study their ability for controlling *M. incognita* and in the improvement of the growth parameters of eggplants. *Bacillus* spp. were applied as single or in combination (experiment I), single treatment at different doses (experiment II), and different times (three times) of application (experiment III).

Methods

Preparation of the root-knot nematode inocula

The roots of eggplants naturally infected with the root-knot nematode were collected from open fields. Egg masses of infected roots were extracted by using a sodium hypochlorite solution (Hussey and Barker 1973). A single egg-mass culture of the nematode was established and reared in eggplant cv. Pusa Purple Long in a greenhouse at 30 ± 5 °C. Adult females were used to identify the nematode species by the morphological characteristics according to the female perineal pattern (Taylor and Sasser 1978). Second-stage juveniles (J_2) were collected daily and stored at 15 °C, where it was applied in the experiments at less than 5 days old.

Bacillus spp. strains

Bacillus spp. strains, viz., *B. subtilis* and *B. pumilus*, were isolated from bean plant rhizospheres in Egypt, and then they were identified according to morphological, cultural, and biochemical characteristics using standard bacteriological methods in the Department of Plant Pathology, National Research Centre, and were applied in this study. The bioassay of tested *Bacillus* spp. against J_2 of *M. incognita* was examined in pervious study (Abd-El-Khair et al. 2016).

Preparation of *Bacillus* spp. inocula

For the preparation of bacterial inoculums, each *Bacillus* sp. was separately inoculated in a conical flask (250 ml) containing 200 ml of nutrient sucrose (2%) broth [NGB] medium [beef extract, 3.0 g; peptone, 5.0 g; glucose, 10.0 g; 1.0 l of distilled water, and adjusted pH at 7.2 ± 0.2]. The inoculated flasks were incubated at 28 °C for 4 days. Then, the bacterial inoculum of each bacterium was adjusted to 10^{7-9} colony-forming unit (CFU)/ml by turbidity method. *Bacillus* sp. inoculum was applied as a mixture of bacterial cells and cultural filtrates.

Pot experiment design

Three experiments were separately conducted to study the nematicidal activity of each *Bacillus* sp., *B. subtilis* and *B. pumilus*, against *M. incognita* in pots under greenhouse conditions in DPP, NRC. Four-week-old eggplant seedlings cv. Pusa Purple Long was transplanted in plastic pots (20-cm diameter) containing 2 kg of a sun-sterilized sandy and loamy soil mixture (V:V). One eggplant transplant was sown in each pot. Each pot was inoculated with 1000 newly hatched J_2 of *M. incognita* in four holes made around each plant. Four pots were used as replicates for each treatment as well as the controls. The pots in each experiment were arranged according to a completely randomized design on a bench in the glasshouse maintained at 25 ± 5 °C. The plants were irrigated regularly.

Experiment I

This experiment was conducted to study the nematicidal activity of three *Bacillus* spp. as single or in combination treatments against *M. incognita* parameters. The experiment includes ten treatments as follows: (1) *Bacillus* sp., (2) *B. subtilis*, (3) *B. pumilus*, (4) *Bacillus* sp. + *B. subtilis*, (5) *Bacillus* sp. + *B. pumilus*, (6) *B. subtilis* + *B. pumilus*, (7) *Bacillus* sp. + *B. subtilis* + *B. pumilus*, (8) Micronima®, (9) NGB medium only, and (10) *M. incognita* only. After

3 days of nematode inoculation, each pot was treated with 20 ml each of bacterial inoculum around the plant. The control pots were separately treated with 20 ml of Micronima®, 20 ml medium only and without any treatment (nematode only), respectively.

Experiment II

This experiment was designed to study the nematicidal activity of three *Bacillus* spp. against *M. incognita* when applied as a single treatment at different doses of 20, 30, and 40 ml/pot. The experiment includes 12 treatments as follows: (1) *Bacillus* sp. (20 ml/pot), (2) *Bacillus* sp. (30 ml/pot), (3) *Bacillus* sp. (40/pot), (4) *B. subtilis* (20 ml/pot), (5) *B. subtilis* (30 ml/pot), (6) *B. subtilis* (40/pot), (7) *B. pumilus* (20 ml/pot), (8) *B. pumilus* (30 ml/pot), (9) *B. pumilus* (40 ml/pot), (10) Micronima®, (11) NGB medium only, and (12) nematode only. After 3 days of nematode inoculation, the eggplant plants were treated with each bacterial inoculum at tested doses around the eggplant. For control, pots were separately treated with 20 ml of Micronima®, 20 ml NGB medium only and nematode only, respectively.

Experiment III

This experiment was conducted to study the nematicidal activity of three *Bacillus* spp. as application times (three times) of a single treatment against *M. incognita*. All *Bacillus* spp. were applied three times (one, two, and three times/pot) with a 7-day interval between each treatment and another. The experiment includes 12 treatments as follows: (1) *Bacillus* sp. (one time/pot), (2) *Bacillus* sp. (two times/pot), (3) *Bacillus* sp. (three times/pot), (4) *B. subtilis* (one time/pot), (5) *B. subtilis* (two times/pot), (6) *B. subtilis* (three times/pot), (7) *B. pumilus* (one time/pot), (8) *B. pumilus* (two times/pot), (9) *B. pumilus* (three times/pot), (10) Micronima®, (11) NGB medium only, and (12) nematode only. After 3 days of nematode inoculation, the eggplant plants were treated with each bacterial inoculum at tested doses around the eggplant. For control, the pots were separately treated with 20 ml of Micronima®, 20 ml medium only and nematode only, respectively.

Effects of *Bacillus* spp. on *M. incognita* parameters

After 6 months of nematode inoculation, the nematicidal activity of *Bacillus* spp. against *M. incognita* parameters, viz. the numbers of J_2 in soil, J_2 in roots, and both galls and egg masses in the entire root apparatus of eggplants (four plant roots per treatment), were recorded. The number of J_2 in the soil was extracted using a sieving and decanting technique (Barker, 1985). The roots of eggplants were carefully uprooted and washed thoroughly with running tap water and then a number of J_2 in roots (5 g) were recorded. All J_2 numbers of nematodes were counted under a light microscope. Numbers of galls and egg masses on the entire root system of eggplant were also recorded.

Effects of *Bacillus* spp. on growth parameters of eggplants

The role of *Bacillus* spp. in the improvement of the plant growth parameters, viz. shoot length (centimeter), fresh and dry shoot weights (grams), fresh root weight (grams), and leaf number per plant, was recorded after 6 months of nematode inoculation. Four eggplants were used as replicates.

Statistical analysis of data

Statistical analysis of the obtained data was performed through Computer Statistical Package (COSTAT) User Manual Version 3.03, Barkley Co., a computer-based program. Analyses include analysis of variance (ANOVA) procedures (Snedecor and Cochran 1999**).** Duncan's multiple range test (DMRT) was applied to compare the means for each treatment at 5% level of probability.

Results

Effects of *Bacillus* spp. on *M. incognita* parameters

Experiment I

Results show that *Bacillus* spp. reduced the J_2 numbers in soil in the ranges of 2.55 $\log_{10}$–3.10 $\log_{10}$, when applied as single or in combination, compared to 2.36 $\log_{10}$, 3.23 $\log_{10}$, and 3.34 $\log_{10}$ with Micronima®, medium only, and nematode only, respectively. The percentages of reduction were in the ranges of 42–84% (J_2 in soil), compared to 89 and 23% with Micronima® and medium only, respectively. The treatment of *Bacillus* sp. + *B. subtilis* significantly reduced the J_2, followed by treatments of *B. subtilis* + *B. pumilus*, *B. subtilis*, *Bacillus* sp. + *B. pumilus*, *Bacillus* sp. + *B. subtilis* + *B. pumilus*, *Bacillus* sp. and *B. pumilus*, respectively (Table 1). The numbers of J_2 in roots were in the ranges of 1.90 $\log_{10}$–2.48 $\log_{10}$ with *Bacillus* spp., compared to 2.19 $\log_{10}$, 3.05 $\log_{10}$ and 3.23 $\log_{10}$ with Micronima®, medium only and nematode only, respectively. *Bacillus* spp. reduced the J_2 in roots in the ranges of 82–95%, compared to 91 and 35% with Micronima® and medium only, respectively. *Bacillus* sp. + *B. pumilus* significantly reduced the J_2 in roots, followed by *B. pumilus*, *Bacillus* sp. + *B. subtilis*, *Bacillus* sp. + *B. subtilis* + *B. pumilus*, *B. subtilis* + *B. pumilus*, *B. subtilis*, and *Bacillus* sp., respectively (Table 1).

Bacillus spp. reduced the numbers of galls in eggplant roots in the ranges of 0.89 $\log_{10}$–1.53 $\log_{10}$, compared to 0.96 $\log_{10}$,1.69 $\log_{10}$, and 1.85 $\log_{10}$ with Micronima®, medium only, and nematode only, respectively. *Bacillus* spp. reduced the galls in the ranges of 52–89%, compared to 87% and 30% with Micronima® and medium only, respectively. *Bacillus* sp. + *B. subtilis* significantly reduced the galls, followed by *B. subtilis* + *B. pumilus*,

Table 1 Effects of *Bacillus* spp., as single or in combination treatments at a dose of 20 ml / pot, on nematode parameters of *Meloidogyne incognita*-infected eggplants in pots (experiment I)

Bacillus spp.	*Meloidogyne incognita* parameters							
	J_2 in soil (200 g)		J_2 in roots (5 g)		No. galls		No. egg masses	
	Count ($\log_{10}$)	Red. %	Count ($\log_{10}$)	Red. %	Count ($\log_{10}$)	Red. %	Count ($\log_{10}$)	Red. %
Nematode only	3.34^{a}*	–	3.23^{a}	–	1.85^{a}	–	1.75^{a}	–
NGB medium only	3.23^{ab}	*23*	3.05^{b}	*35*	1.69^{ab}	*30*	1.56^{b}	*36*
Micronima®	2.36^{f}	*89*	2.19^{ef}	*91*	0.96^{fg}	*87*	0.79^{ef}	*89*
Bacillus sp.	2.96^{c}	*58*	2.48^{c}	*82*	1.53^{bc}	*52*	1.41^{bc}	*55*
B. subtilis	2.67^{de}	*78*	2.43^{c}	*84*	1.53^{bc}	*52*	1.35^{c}	*61*
B. pumilus	3.10^{bc}	*42*	2.09^{f}	*93*	1.27^{de}	*73*	1.14^{d}	*75*
Bacillus sp. + *B. subtilis*	2.55^{e}	*84*	2.23^{ef}	*90*	0.89^{g}	*89*	0.72^{f}	*91*
Bacillus sp. + *B. pumilus*	2.73^{d}	*75*	1.90^{g}	*95*	1.12^{ef}	*80*	0.95^{e}	*84*
B. subtilis + *B. pumilus*	2.64^{de}	*80*	2.39^{cd}	*86*	1.06^{fg}	*83*	0.92^{e}	*85*
Bacillus sp. + *B. subtilis* + *B. pumilus*	2.78^{d}	*72*	2.27^{de}	*89*	1.42^{cd}	*63*	1.30^{cd}	*65*

Means followed by different superscript letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$
Red. reduction
*Means are averages of four replicates

Bacillus sp. + *B. pumilus*, *B. pumilus*, *Bacillus* sp. + *B. subtilis* + *B. pumilus*, *Bacillus* sp., and *B. subtilis*, respectively. The numbers of egg masses ranged from 0.72 $\log_{10}$ to 1.41 $\log_{10}$ with *Bacillus* spp., compared to 0.79 $\log_{10}$, 1.56 $\log_{10}$, and 1.75 $\log_{10}$ with Micronima®, medium only, and nematode only, respectively. *Bacillus* spp. reduced the numbers of egg masses in the ranges of 55–91%, compared to 89% and 36% with Micronima® and medium only, respectively. *Bacillus* sp. + *B. subtilis* significantly reduced the egg masses, followed by *B. subtilis* + *B. pumilus*, *Bacillus* sp. + *B. pumilus*, *B. pumilus*, *Bacillus* sp. + *B. subtilis* + *B. pumilus*, *B. subtilis*, and *Bacillus* sp., respectively (Table 1).

Experiment II

The results revealed that the numbers of J_2 in soil of eggplants ranged from 2.00 $\log_{10}$ to 3.11 $\log_{10}$ with an application of *Bacillus* spp. as a single treatment at different doses, i.e., 20, 30, and 40 ml/pot, compared to 2.36 $\log_{10}$, 3.23 $\log_{10}$, and 3.34 $\log_{10}$ with Micronima®, medium only, and nematode only, respectively. *Bacillus* spp. reduced the J_2 in the ranges of 42–95%, compared to 89% and 23% with Micronima® and medium only, respectively. *Bacillus* sp. (40 ml) significantly reduced the J_2, followed by *B. subtilis* (40 ml), *B. subtilis* (30 ml), *Bacillus* sp. (30 ml), *B. subtilis* (20 ml), *B. pumilus* (40 ml), *B. pumilus* (30 ml), *Bacillus* sp. (20 ml), and *B. pumilus* (20 ml), respectively. The numbers of J_2 in roots ranged from 1.77 $\log_{10}$ to 2.47 $\log_{10}$ with *Bacillus* spp., compared to 2.19 $\log_{10}$, 3.05 $\log_{10}$, and 3.23 $\log_{10}$ with Micronima®, medium only, and nematode only, respectively. *Bacillus* spp. reduced the J_2/roots in the ranges of 82–97%, compared to 91% and 35% with Micronima® and medium only, respectively. *B. pumilus* (40 ml) significantly reduced the J_2 in roots, followed by *B. subtilis* (40 ml), *B. pumilus* (30 ml), *Bacillus* sp. (40 ml), *B. pumilus* (20 ml), *B. subtilis* (30 ml), *Bacillus* sp. (30 ml), *B. subtilis* (20 ml), and *Bacillus* sp. (20 ml), respectively (Table 2).

The numbers of galls ranged from 0.69 $\log_{10}$ to 1.53 $\log_{10}$ with *Bacillus* sp. treatments, compared to 0.96 $\log_{10}$, 1.69 $\log_{10}$, and 1.85 $\log_{10}$ with Micronima®, medium only, and nematode only, respectively. *Bacillus* spp. reduced the galls in the ranges of 50–93%, compared to 87% and 30% with Micronima® and medium only, respectively. *Bacillus* sp. (40 ml) significantly reduced the galls, followed by *B. subtilis* (40 ml), *B. subtilis* (30 ml), *B. pumilus* (40 ml), *B. pumilus* (30 ml), *B. pumilus* (20 ml), *Bacillus* sp. (30 ml), *Bacillus* sp. (20 ml), and *B. subtilis* (20 ml), respectively. The egg masses' numbers ranged from 0.49 $\log_{10}$ to 1.44 $\log_{10}$ with *Bacillus* spp., compared to the galls of 0.82 $\log_{10}$, 1.57 $\log_{10}$, and 1.76 $\log_{10}$ with Micronima®, medium only, and nematode only, respectively. *Bacillus* spp. reduced the number of egg masses in the ranges of 52–94%, compared to 89% and 36% with Micronima® and medium only, respectively. *Bacillus* sp. (40 ml) significantly reduced the egg masses, followed by *B. subtilis* (40 ml), *B. pumilus* (40 ml), *B. subtilis* (30 ml), *B. pumilus* (30 ml), *B. pumilus* (20 ml), *B. subtilis* (20 ml), *Bacillus* sp. (30 ml), and *Bacillus* sp. (20 ml), respectively (Table 2).

Experiment III

The numbers of J_2 in soil of eggplants ranged from 2.17 $\log_{10}$ to 3.11 $\log_{10}$ with *Bacillus* spp. when applied three times with a 7-day interval between each treatment and another, compared to 2.37 $\log_{10}$, 3.23 $\log_{10}$, and 3.34 $\log_{10}$ with Micronima®, medium only, and nematode

Table 2 Effects of *Bacillus* spp., as a single treatment at doses of 20, 30, and 40 ml / pot, on nematode parameters of *Meloidogyne incognita*-infected eggplants in pots (experiment II)

Bacillus spp.		*Meloidogyne incognita* parameters							
		J_2 in soil (200 g)		J_2 in roots (5 g)		No. galls		No. egg masses	
		Count (log_{10})	Red. %	Count (log_{10})	Red. %	Count (log_{10})	Red. %	Count (log_{10})	Red. %
Nematode only		3.34^{a}	–	3.23^{a}	–	1.86^{a}	–	1.76^{a}	–
NGB medium only		3.23^{a}	*23*	3.05^{b}	*35*	1.70^{a}	*30*	1.57^{b}	*36*
Micronima®		2.36^{de}	*89*	2.19^{ef}	*91*	0.98^{d}	*87*	0.82^{fg}	*89*
Bacillus sp.	20 ml	2.96^{abc}	*59*	2.47^{c}	*82*	1.52^{b}	*54*	1.44^{bc}	*52*
	30 ml	2.61^{cd}	*81*	2.29^{de}	*88*	1.49^{b}	*57*	1.39^{bc}	*57*
	40 ml	2.00^{ef}	*95*	2.06^{f}	*93*	0.69^{e}	*93*	0.49^{h}	*94*
B. subtilis	20 ml	2.67^{cd}	*78*	2.43^{cd}	*84*	1.53^{b}	*50*	1.35^{cd}	*60*
	30 ml	2.57^{cd}	*83*	2.19^{ef}	*91*	0.96^{d}	*87*	0.86^{f}	*87*
	40 ml	2.03^{ef}	*95*	2.03^{f}	*94*	0.93^{d}	*88*	0.64^{gh}	*92*
B. pumilus	20 ml	3.11^{ab}	*42*	2.09^{f}	*93*	1.30^{c}	*72*	1.19^{de}	*73*
	30 ml	2.74^{bcd}	*75*	2.35^{cd}	*94*	1.20^{c}	*78*	1.07^{e}	*79*
	40 ml	2.72^{bcd}	*76*	1.77^{g}	*97*	1.01^{d}	*86*	0.74^{fg}	*90*

Means followed by different superscript letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$
Red. reduction
*Means are averages of four replicates

only, respectively. *Bacillus* spp. reduced J_2 in soil in the ranges of 42–93%, compared to 89% and 23% with Micronima® and medium only, respectively. *B. subtilis* (three times of application) significantly reduced the J_2, followed by *B. subtilis* (two times), *B. pumilus* (two times), *B. pumilus* (three times), *B. subtilis* (one time), *Bacillus* sp. (three times), *Bacillus* sp. (two times), *Bacillus* sp. (one time), and *B. pumilus* (one time), respectively. The numbers of J_2 in roots ranged from 1.67 log_{10} to 2.598 log_{10} with *Bacillus* spp., compared to 2.19 log_{10}, 3.05 log_{10}, and 3.24 log_{10} with Micronima®, medium only, and nematode only, respectively. *Bacillus* spp. reduced the J_2 in roots in the ranges of 77–97%, compared to 91% and 35% with Micronima® and medium only, respectively. *B. subtilis* (three times) significantly reduced the J_2 in roots, followed by *Bacillus* sp. (three times), *B. subtilis* (two times), *B. pumilus* (three times), *B. pumilus* (two times), *Bacillus* sp. (two times), *B. subtilis* (one time), *Bacillus* sp. (one time), and *B. pumilus* (one time), respectively (Table 3).

The numbers of galls ranged from 0.63 log_{10} to 1.55 log_{10} with *Bacillus* spp., compared to 0.99 log_{10}, 1.70 log_{10}, and 1.86 log_{10} with Micronima®, medium only, and nematode only, respectively. *Bacillus* spp. reduced galls in the ranges of 50–94%, compared to 86% and 31% with Micronima® and medium only. *B. subtilis* (three times) significantly reduced the galls, followed by *B. subtilis* (two times), *B. pumilus* (three times), *B. pumilus* (two times), *Bacillus* sp. (three times), *Bacillus* sp. (two times), *B. pumilus* (one time), *Bacillus* sp. (one time), and *B. subtilis* (one time), respectively. The number of egg masses ranged from 0.69 log_{10} to 1.41 log_{10} in roots with *Bacillus* spp., compared to 0.84 log_{10}, 1.56 log_{10}, and 1.76 log_{10} with Micronima®, medium only, and nematode only, respectively. *Bacillus* spp. reduced the egg masses in the ranges of 56–91%, compared to 88% and 38% with Micronima® and medium only, respectively. *B. pumilus* (three times) significantly reduced the egg masses, followed by *B. subtilis* (three times), *B. subtilis* (two times), *B. pumilus* (two times), *Bacillus* sp. (three times), *B. pumilus* (one time), *Bacillus* sp. (two times), *B. subtilis* (one time), and *Bacillus* sp. (one time), respectively (Table 3).

Effects of *Bacillus* spp. on growth parameters

Experiment I

Bacillus spp., when applied as single or in combination treatment, improved the growth parameters of eggplants under greenhouse conditions (Table 4). The averages of the shoot length (SL) of eggplants were in the ranges of 35.00–59.00 cm with *Bacillus* spp., compared to 37.75, 28.28, and 22.75 cm with Micronima®, medium only, and nematode only, respectively. *Bacillus* sp. + *B. subtilis* significantly increased the SL of eggplants, followed by *Bacillus* sp. + *B. pumilus*, *Bacillus* sp. + *B. subtilis* + *B. pumilus*, *B. subtilis* + *B. pumilus*, *B. pumilus*, *Bacillus* sp., and *B. subtilis*, respectively. The averages of the fresh shoot weight (FSW) ranged from 26.60 to 58.60 g with *Bacillus* spp., compared to 26.13, 21.90, and 15.95 g with Micronima®, medium only, and nematode only, respectively. *Bacillus* sp. + *B. subtilis* significantly increased the average of FSW, followed by *Bacillus* sp., *B. subtilis*, *Bacillus* sp. + *B. subtilis* + *B. pumilus*, *B.*

Table 3 Effects of *Bacillus* spp., as single treatment at different application times at dose 20 ml / pot, on nematode parameters of *Meloidogyne incognita*-infected eggplants in pots (experiment III)

Bacillus spp.		*Meloidogyne incognita* parameters							
		J_2 in soil (200 g)		J_2 in roots (5 g)		No. galls		No. egg masses	
		Count ($\log_{10}$)	Red. %	Count ($\log_{10}$)	Red. %	Count ($\log_{10}$)	Red. %	Count ($\log_{10}$)	Red. %
Nematode only		3.34^{a}	–	3.24^{a}	–	1.86^{a}	–	1.76^{a}	–
NGB medium only		3.23^{a}	*23*	3.05^{b}	*35*	1.70^{a}	*31*	1.56^{a}	*38*
Micronima®		2.37^{fg}	*89*	2.19^{ef}	*91*	0.99^{d}	*86*	0.84^{fg}	*88*
Bacillus sp.	one time	2.96^{c}	*58*	2.49^{cd}	*82*	1.54^{b}	*51*	1.41^{b}	*56*
	two times	2.74^{d}	*75*	1.31^{de}	*88*	1.29^{c}	*73*	1.20^{cd}	*73*
	three times	2.72^{de}	*76*	1.79^{gh}	*96*	1.18^{c}	*77*	1.04^{de}	*81*
B. subtilis	one time	2.69^{de}	*79*	2.44^{cd}	*84*	1.55^{b}	*50*	1.36^{bc}	*60*
	two times	2.45^{f}	*87*	1.87^{gh}	*96*	0.83^{d}	*90*	0.97^{ef}	*84*
	three times	2.17^{h}	*93*	1.67^{h}	*97*	0.63^{e}	*94*	0.82^{fg}	*89*
B. pumilus	one time	3.11^{b}	*42*	2.59^{c}	*77*	1.28^{c}	*73*	1.15^{d}	*75*
	two times	2.61^{e}	*82*	2.17^{ef}	*91*	1.20^{c}	*78*	0.96^{ef}	*84*
	three times	2.29^{g}	*81*	2.09^{f}	*93*	0.93^{d}	*88*	0.69^{g}	*91*

Means followed by different superscript letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$
Red. reduction
*Means are averages of four replicates

subtilis + *B. pumilus*, *Bacillus* sp. + *B. subtilis*, and *B. pumilus*, respectively. Results showed that the averages of the dry shoot weight (DSW) of eggplants ranged from 6.06 to 9.39 g with *Bacillus* spp., compared to the DSW of 3.36, 2.63, and 2.55 g with Micronima®, medium only, and nematode only, respectively. *Bacillus* sp. + *B. subtilis* also significantly increased the average of the DSW, followed by *B. subtilis* + *B. pumilus*, *Bacillus* sp. + *B. subtilis* + *B. pumilus*, *Bacillus* sp. + *B. subtilis*, *B. subtilis*, *Bacillus* sp., and *B. pumilus*, respectively (Table 4).

The averages of the eggplant leaf number ranged from 27.50 to 37.00 leaves with *Bacillus* spp., compared to 23.50, 21.25, and 16.75 leaves with Micronima®, medium only, and nematode only, respectively. *Bacillus* sp. + *B. subtilis* significantly increased the leaf number, followed by *B. subtilis*, *B. pumilus*, *Bacillus* sp., *Bacillus* sp. + *B. pumilus*, *B. subtilis* + *B. pumilus*, and *Bacillus* sp. + *B. subtilis* + *B. pumilus*, respectively The averages of the fresh root weight (FRW) of eggplants ranged from 6.10 to 13.13 g with *Bacillus* spp., compared to 5.08, 4.35, and

Table 4 Effects of *Bacillus* spp., single or in combination treatments at dose of 20 ml / pot, on vegetative parameters of eggplants in pots (Experiment, I)

Bacillus spp.	Vegetative parameters									
	Shoot length (cm)		Shoot fresh weight (g)		Shoot dry weight (g)		Leaf no.		Fresh root weight (g)	
	Length (cm)	Inc. %	Weight (g)	Inc. %	Weight (g)	Inc. %	NO.	Inc. %	Weight (g)	Inc. %
Nematode only	22.75^{d*}	–	15.95^{d}	–	2.55^{c}	–	16.75^{b}	–	4.28^{b}	–
NGB medium only	28.25^{cd}	*24*	21.90^{cd}	*37*	2.63^{f}	*3*	21.25^{b}	*27*	4.35^{b}	*2*
Micronima®	37.75^{bc}	*114*	26.13^{bc}	*64*	3.36^{e}	*32*	23.50^{ab}	*40*	5.08^{b}	*19*
Bacillus sp.	36.25^{bcd}	*59*	34.95^{b}	*119*	4.04^{d}	*58*	28.25^{ab}	*69*	7.68^{ab}	*79*
B. subtilis	35.00^{bcd}	*54*	33.45^{b}	*110*	6.06^{c}	*138*	29.75^{ab}	*78*	7.68^{ab}	*79*
B. pumilus	40.00^{bc}	*76*	26.70^{bc}	*67*	3.26^{e}	*28*	29.50^{ab}	*76*	6.30^{b}	*47*
Bacillus sp. + *B. subtilis*	59.00^{a}	*159*	58.60^{a}	*267*	9.39^{a}	*268*	37.00^{a}	*121*	13.13^{a}	*207*
Bacillus sp. + *B. pumilus*	50.00^{ab}	*120*	27.98^{bc}	*75*	6.30^{b}	*147*	27.75^{ab}	*66*	9.23^{ab}	*116*
B. subtilis + *B. pumilus*	43.25^{b}	*90*	28.28^{bc}	*77*	6.34^{b}	*149*	27.50^{ab}	*64*	6.10^{b}	*43*
Bacillus sp. + *B. subtilis* + *B. pumilus*	48.75^{ab}	*114*	29.38^{bc}	*84*	6.33^{b}	*148*	23.50^{ab}	*40*	9.10^{ab}	*113*

Means followed by different superscript letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$
Inc. increase
*Means are averages of four replicates

4.28 g with Micronima®, medium only, and nematode only, respectively. *Bacillus* sp. + *B. subtilis* significantly increased the FRW, followed by *Bacillus* sp. + *B. pumilus*, *Bacillus* sp. + *B. subtilis* + *B. pumilus*, *Bacillus* sp., *Bacillus* sp., *B. subtilis*, *B. pumilus*, and *B. subtilis* + *B. pumilus*, respectively (Table 4).

Experiment II

Bacillus sp. treatments, when applied as a single treatment at doses of 20, 30, and 40 ml/pot, improved the growth parameters of eggplants under greenhouse conditions (Table 5). The averages of the SL of eggplants ranged from 35.50 to 57.50 cm with *Bacillus* spp., compared to 38.25, 28.55, and 23.25 cm with Micronima®, medium only, and nematode only, respectively. *B. pumilus* (40 ml) significantly increased the average of the SL of plants, followed by *B. subtilis* (40 ml), *B. pumilus* (30 ml), *B. pumilus* (20 ml), *Bacillus* sp. (40 ml), *B. subtilis* (30 ml), *Bacillus* sp. (30 ml), *Bacillus* sp. (20 ml), and *B. subtilis* (20 ml), respectively. The averages of the FSW of eggplants ranged from 26.78 to 38.83 g with *Bacillus* spp., compared to 26.33, 22.10, and 16.05 g with Micronima®, medium only, and nematode only, respectively. *B. subtilis* (40 ml) significantly increased the FSW, followed by *B. pumilus* (40 ml), *B. subtilis* (30 ml), *Bacillus* sp. (40 ml), *Bacillus* sp. (30 ml), *Bacillus* sp. (20 ml), *B. subtilis* (20 ml), *B. pumilus* (30 ml), and *B. pumilus* (20 ml), respectively. The averages of the DSW of eggplants ranged from 2.37 to 8.52 g with *Bacillus* spp., compared to 3.38, 2.65, and 2.55 g with Micronima®, medium only, and nematode only, respectively. *B. pumilus* (40 ml) also significantly increased the DSW, followed by *B. subtilis* (40 ml), *B. pumilus* (30 ml), *B. subtilis* (30 ml), *Bacillus* sp. (40 ml), *Bacillus* sp. (30 ml), *B. subtilis* (20 ml), *Bacillus* sp. (20 ml), and *B. pumilus* (20 ml), respectively (Table 5).

The averages of the eggplant leaf number ranged from 26.00 to 31.50 leaves with *Bacillus* spp., compared to 23.00, 21.40, and 17.50 leaves with Micronima®, medium only, and nematode only, respectively. *B. pumilus* (40 ml) significantly increased the leaves, followed by *Bacillus* sp. (20 ml), *Bacillus* sp. (40 ml), *B. subtilis* (30 ml), *B. subtilis* (40 ml), *B. pumilus* (30 ml), *Bacillus* sp. (30 ml), *B. subtilis* (20 ml), and *B. pumilus* (20 ml), respectively. The averages of FRW ranged from 6.13 to 13.20 g with *Bacillus* spp., compared to 5.13, 4.53, and 4.45 g with Micronima®, medium only, and nematode only, respectively. *B. subtilis* (40 ml) significantly increased the FRW, followed by *B. pumilus* (40 ml), *Bacillus* sp. (40 ml), *Bacillus* sp. (30 ml), *Bacillus* sp. (20 ml), *B. subtilis* (30 ml), *B. pumilus* (30 ml), *B. subtilis* (20 ml), and *B. pumilus* (20 ml), respectively (Table 5).

Experiment III

Bacillus sp. treatments when applied three times, as a single treatment, with a 7-day interval between each treatment and another, improved the growth parameters of eggplants under greenhouse conditions (Table 6). The average of the SL of eggplants ranged from 34.75 to 50.75 cm, compared to 37.75, 28.53, and 23.55 cm with Micronima®, medium only, and nematode only, respectively. *B. subtilis* (three times) significantly increased the

Table 5 Effects of *Bacillus* spp. as a single treatment at doses of 20, 30, and 40 ml / pot, on vegetative parameters of eggplants in pots (experiment II)

Bacillus spp.		Vegetative parameters									
		Shoot length (cm)		Shoot fresh weight (g)		Shoot dry weight (g)		Leaf no.		Root fresh weight (g)	
		Length (cm)	Inc. %	Weight (g)	Inc. %	Weight (g)	Inc. %	No.	Inc. %	No.	Inc. %
Nematode only		23.25^{d}	–	16.05^{d}	–	2.55^{i}	–	17.50 ^{c}	–	*4.45^{d}*	–
NGB Medium only		28.55^{cd}	*23*	22.10^{cd}	*43*	2.65^{i}	*4*	21.40^{bc}	*20*	*4.53^{d}*	*2*
Micronima®		38.25^{bc}	*65*	26.33^{bc}	*64*	3.38^{h}	*33*	23.00^{bc}	*31*	*5.13^{d}*	*15*
Bacillus sp.	20 ml	35.75^{bc}	*58*	34.98^{ab}	*118*	4.04^{g}	*58*	28.75^{ab}	*64*	8.95^{abcd}	*101*
	30 ml	39.00^{b}	*68*	36.50^{a}	*127*	4.83^{e}	*89*	26.50^{ab}	*51*	9.70^{abcd}	*119*
	40 ml	39.50^{b}	*70*	36.90^{a}	*130*	5.75^{d}	*126*	27.50^{ab}	*57*	11.30^{abc}	*154*
B. subtilis	20 ml	35.50^{bc}	*53*	33.55^{ab}	*109*	4.43^{f}	*74*	26.00^{ab}	*49*	6.30^{bcd}	*42*
	30 ml	39.25^{b}	*69*	37.13^{a}	*131*	6.08^{c}	*138*	27.50^{ab}	*57*	7.73^{abcd}	*74*
	40 ml	43.25^{b}	*86*	38.83^{a}	*142*	8.52^{b}	*234*	27.00^{ab}	*54*	13.20^{a}	*197*
B. pumilus	20 ml	40.50^{b}	*74*	26.78^{bc}	*67*	3.27^{h}	*28*	26.00^{ab}	*49*	6.13^{cd}	*38*
	30 ml	41.25^{b}	*77*	26.80^{bc}	*67*	8.44^{b}	*231*	26.75^{ab}	*53*	6.38^{bcd}	*43*
	40 ml	57.50^{a}	*147*	38.65^{a}	*141*	9.62^{a}	*277*	31.50^{a}	*80*	11.75^{ab}	*164*

Means followed by different superscript letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$
Inc. increase
*Means are averages of four replicates

Table 6 Effects of *Bacillus* spp. as single treatment at different application times at dose 20 ml / pot on vegetative parameters of eggplants in pots (experiment III)

Bacillus spp.		Vegetative parameters									
		Shoot length (cm)		Shoot fresh weight (g)		Shoot dry weight (g)		No. leaves		Root fresh weight (g)	
		Length (cm)	Inc. %	Weight (g)	No.	Weight (g)	Weight (g)	Weight (g)	Inc. %	No.	Inc. %
Nematode only		23.55^{f}	–	16.03^{d}	17.25^{b}	4.50c	4.50^{c}	4.50^{c}	–	17.25^{b}	–
NGB medium only		28.53^{ef}	*21*	21.98^{cd}	21.35^{ab}	4.53c	4.53^{c}	4.53^{c}	*1*	21.35^{ab}	*24*
Nematode + Micronima®		37.75^{bcde}	*60*	26.30^{bc}	23.50^{ab}	5.15^{c}	5.15^{c}	5.15^{c}	*14*	23.50^{ab}	*36*
Bacillus sp.	one time	34.75^{de}	*48*	28.75^{a}	4.68^{c}	4.68^{c}	*59*	4.68^{c}	*4*	28.75^{a}	*67*
	two times	42.50^{abcd}	*81*	28.00^{a}	4.90^{c}	4.90^{c}	*80*	4.90^{c}	*9*	28.00^{a}	*62*
	three times	44.75^{abc}	*90*	28.75^{a}	9.75^{a}	9.75^{a}	*107*	9.75^{a}	*117*	28.75^{a}	*67*
B. subtilis	one time	35.50^{cde}	*51*	22.25^{ab}	4.50^{c}	4.50^{c}	*73*	4.50^{c}	*1*	22.25^{ab}	*29*
	two times	49.00^{a}	*108*	25.25^{ab}	6.10^{bc}	6.10^{bc}	*120*	6.10^{bc}	*36*	25.25^{ab}	*50*
	three times	50.75^{a}	*116*	28.75^{a}	7.73^{ab}	7.73^{ab}	*138*	7.73^{ab}	*72*	28.75^{a}	*67*
B. pumilus	one time	40.75^{abcd}	*73*	25.50^{ab}	4.68^{c}	4.68^{c}	*29*	4.68^{c}	*4*	25.50^{ab}	*45*
	two times	46.25^{ab}	*96*	29.75^{a}	6.35^{bc}	6.35^{bc}	*179*	6.35^{bc}	*41*	29.75^{a}	*73*
	three times	50.25^{a}	*113*	29.75^{a}	8.40^{a}	8.40^{a}	*192*	8.40^{a}	*87*	29.75^{a}	*73*

Means followed by different superscript letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$
Inc. increase
*Means are averages of four replicates

average of the SL of eggplants, followed by *B. pumilus* (three times), *B. subtilis* (two times), *B. pumilus* (two times), *Bacillus* sp. (three times), *Bacillus* sp. (two times), *B. pumilus* (one time), *B. subtilis* (one time), and *Bacillus* sp. (one time), respectively. The averages of the FSW of eggplants ranged from 26.75 to 39.40 g with *Bacillus* spp., compared to 26.30, 21.98, and 16.03 g with treatments of Micronima®, medium only, and nematode only, respectively. *Bacillus* sp. (three times) significantly increased the averages of FSW, followed by *B. subtilis* (three times), *Bacillus* sp. (two times), *B. subtilis* (two times), *Bacillus* sp. (one time), *B. subtilis* (one time), *B. pumilus* (three times), *B. pumilus* (two times), and *B. pumilus* (one time), respectively. The averages of the DSW of eggplants ranged from 3.28 to 7.44 g with *Bacillus* spp., compared to 5.18, 4.53, 4.53, and 4.50 g with treatments of Micronima®, medium only, and nematode only, respectively. *B. pumilus* (three times) significantly increased the average of the DSW, followed by *B. pumilus* (two times), *B. subtilis* (three times), *B. subtilis* (two times), *Bacillus* sp. (three times), *Bacillus* sp. (two times), *B. subtilis* (one time), *Bacillus* sp. (one time), and *B. pumilus* (one time), respectively (Table 6).

The averages of the eggplant leaf number ranged from 22.75 to 29.75 leaves with *Bacillus* spp., compared to 23.50, 21.35, and 17.25 leaves with treatments of Micronima®, medium only, and nematode only, respectively. *B. pumilus* (three times) significantly increased the leaf number, followed by *B. pumilus* (two times), *B. subtilis* (three times), *Bacillus* sp. (three times), *Bacillus* sp. (one time), *Bacillus* sp. (two times), *B. subtilis* (two times), *B. pumilus* (one time), and *B. subtilis* (one time), respectively. The averages of the FRW of eggplants ranged from 4.50 to 9.75 g with *Bacillus* spp., compared to 5.15, 4.53, and 4.50 g with Micronima®, medium only, and nematode only (control), respectively. *Bacillus* sp. (three times) significantly increased the average of the FRW, followed by *B. pumilus* (three times), *B. pumilus* (two times), *B. subtilis* (three times), *B. subtilis* (two times), *Bacillus* sp. (two times), *Bacillus* sp. (one time), *B. pumilus* (one time), and *B. subtilis* (one time), respectively (Table 6).

Discussion

The root-knot nematode, *M. incognita*, is one of the most soil-borne pathogens which attack a wide range of crops (Kavitha et al. 2012). The application of chemicals continues to be the major tactic to mitigate crop diseases that cause hazards to human and the environment. Therefore, the use of biocontrol agents to suppress soil-borne disease-causing activity of plant pathogens is very important, where biological agents could colonize the plant rhizosphere, suppress the disease through several mechanisms, viz. antibiosis, competition, myco-parasitism, and cell wall degradation, and induce resistance, as well as produce the plant growth promotion (Junaid et al. 2013). *Pseudomonas* or *Bacillus* could colonize the rhizosphere of plants in, on, or around plant tissues, stimulate plant growth, and reduce nematode populations by antagonistic behavior. The application of some of these bacteria recorded promising results. Their beneficial events could be a biological control of a nematode, plant growth promotion, and increase in crop yields and quality (Akhtar et al. 2012).

In this work, the combination treatment between *Bacillus* spp., especially in two pairs, highly reduced more the tested nematode parameters than in a single treatment or three pairs. The two pair combination differed in suppressive activity against nematode, where *Bacillus* sp. mixed with *B. subtilis* highly reduced the J_2 in soil, galls, and the number of egg masses, while *Bacillus* sp. mixed with *B. pumilus* highly reduced the J_2 in roots. It may be due to the antagonistic effect of a single treatment. *Bacillus* spp., when applied as single at different doses, were more effective against nematode parameters, where their nematicidal activity was increased by increasing the applied dose. It was clear with the application of a dose (40 ml/pot). The same trend was noticed when *Bacillus* spp. applied at different times, where the nematicidal effect of the tested bacteria was increased with increased time of application. Our results suggest that the application of *Bacillus* spp. in two pairs was more effective in reducing tested nematode parameters. The nematicidal activity of *Bacillus* spp. increased with the increase of the used dose as well as the increase of the time of application.

These results are in agreement with the colonization of plant roots by non-pathogenic bacteria that can induce systemic resistance to pathogen infections in plants (Siddiqui and Shauka 2003; Rahanandeh and Moshaiedy 2014). *Bacillus* spp. also have a wide distribution of cuticle-degrading proteases that play an important role as a nematicidal factor in balancing nematode populations in the soil (Lian et al. 2007). *B. amyloliquefaciens*, *B. subtilis*, *B. pasteurii*, *B. cereus*, *B. pumilus*, *B. mycoides*, and *B. sphaericus* elicit significant reductions of root-knot nematodes (Choudhary and Johri 2009), where colonization of plant roots is an essential step for both soil-borne pathogenic and beneficial rhizobacteria. Our results are in agreement with microbes that could be a biological control indirectly by reducing the level of disease which includes antibiosis, induction of systemic resistance, and competition for nutrients and niches (Lugtenberg and Kamilova 2009). *Bacillus lipopeptides*, producing surfactins, iturins, and fengycins, have antagonistic activity against a wide range of phytopathogens which include nematodes. *B. subtilis* also highly produced surfactin and iturin activity that could suppress egg hatching and kill the J_2 of *M. incognita* under in vitro condition (Kavitha et al. 2012). The strain of *Bacillus alvei* showed potentiality against the nematode's eggs and larvae, where the strain used hydrolytic enzymes to directly hydrolyze the nematode's eggs and larvae. The action of the strain or its metabolites against eggs and larvae is also associated with the release of high levels of reducing sugars and lytic enzymes, viz. chitinase, chitosanase, and proteases (Abdel-Aziz et al. 2013).

In this work, the application of *Bacillus* spp. as single or in combination resulted in a variable increase in tested growth parameters, where treatments in two pairs or three pairs showed that the *Bacillus* spp. increased the shoot length or shoot dry weight, compared with the single treatment. *Bacillus* sp. mixed with *B. subtilis* was the most effective in increasing the tested growth parameters. Results showed that *Bacillus* spp. at a high dose (40 ml) were more enhanced in the tested growth parameters than the other doses. *B. pumilus* (40 ml) highly increased all tested growth parameters except the shoot fresh weight, while *B. subtilis* (40 ml) highly increased the shoot fresh weight. Results showed that the frequency of application of *Bacillus* spp. highly increased the growth parameters of eggplants except for the root fresh weight compared with the medium only and nematode only.

These results are in agreement with the rhizobacteria that are capable of stimulating plant growth through a variety of mechanisms that include the improvement of plant nutrition, production, and regulation of phytohormones and suppression of disease-causing organisms (Martínez-Viveros et al. 2010). The inoculation of crop plants with PGPR strains at an early stage of development improves biomass production through direct effects on root and shoot growth as well as enhance the seedling germination, stand health, plant vigor, plant height, shoot weight, nutrient content of shoot tissues, early bloom, chlorophyll content, and increased nodulation in legumes (Saharan and Nehra 2011). PGPR are beneficial bacteria that could colonize the plant rhizosphere which resulted in an enhancement of root length, shoot fresh, and dry weight/plant on plants infected with *M. incognita* (El-Sayed and Edrees 2014). The microbes are directly beneficial to plant growth by means of biofertilization, stimulation of root growth, rhizoremediation, and plant stress control (Lugtenberg and Kamilova 2009).

Conclusions

The biocontrol agents used more than once or in combination are more effective than those used only once in controlling nematode parameters and thus increased the growth parameters compared to the controls. However, it is necessary to further affirm these results and investigate various mechanisms involved that are caused by the studied bacterial isolates under field conditions.

Abbreviations

ANOVA: Analysis of variance; *B. amyloliquefaciens*: *Bacillus amyloliquefaciens*; *B. cereus*: *Bacillus cereus*; *B. mycoides*: *Bacillus mycoides*; *B. pasteurii*: *Bacillus pasteurii*; *B. pumilus*: *Bacillus pumilus*; *B. sphaericus*: *Bacillus sphaericus*; *B. subtilis*: *Bacillus subtilis*; *B. thuringiensis*: *Bacillus thuringiensis*; *B. weihenstephanensis*: *Bacillus weihenstephanensis*; CFU: Colony-forming unit; DMRT: Duncan's multiple range test; DSW: The averages of dry shoot weight; FRW: The averages of fresh root weight; FSW: The averages of fresh shoot weight; Inc.: Increase; J_2: Second-stage juveniles; *M. incognita*: *Meloidogyne* *incognita*; NGB: Nutrient Glucose Beef Extract; *P. fluorescens*: Psudomonas fluorescens; PGPR: Plant growth-promoting rhizobacteria; Red.: Reduction; *S. marcescens*: *Serratia marcescens*; SL: The averages of shoot length

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