International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.10, pp 097-107, 2016

Antagonistic effects of rhizobacteria isolates against *Meloidogyne incognita* infecting tomato plants under greenhouse conditions

Hassan Abd-El-Khair[1]*, Wafaa, M.A. El-Nagdi[2] and Hoda, H. Ameen[2]

[1]Plant Pathology Department, National Research Centre, Giza, Egypt.
[2]Nematology Laboratory, National Research Centre, Giza, Egypt.

Abstract : Thirty rhizobacteria (RB) isolates isolated from rhizospheres of healthy plants - free from nematode infection *viz*. 13 isolates (RBba1-RBba13) from banana; 6 isolates (Rbbe1-RBbe6) from bean ; and 11 isolates (RBcu1 - RBcu11) from cucumber. All the thirty RB isolates were primarily identified according to cultural characters using standard bacteriological method and their nematicidal activity were evaluated against *Meloidogyne incognita* at second stage juveniles (J_2) *in vitro*. Results of primary bioassay test of the thirty RB isolates against *Meloidogyne incognita* J_2 showed that the percentages of mortality ranged from 81 – 97%. RB isolates of banana, bean and cucumber reduced the mortality of *M. incognita* J_2 in the ranges of 81-97%, 85-96% and 84-95%, respectively. Isolates of RBba9, RBba10, RBba12, RBba13, RBbe5, RBbe6 RBcu1and RBcu6 showing the highest net mortality of nematode about ≥ 95% were selected and identified as *Bacillus* sp.ba9, *Bacillus* sp.ba10, *Bacillus* sp.ba12, *Bacillus* sp.ba13, *Bacillus* sp.be5, *Bacillus* sp.be6, *Bacillus* sp.cu1 and *Bacillus* sp.cu6 according to morphological, cultural and biochemical characters. Under greenhouse conditions, the eight select *Bacillus* spp. significantly reduced the root-knot nematode parameters, i.e. numbers of J_2 in soil (82.7 – 97.6%); J_2 in roots (91.7 – 95.8%) ; Galls (61.1- 85.3%) and egg-masses (63.8 - 87.0%), compared to untreated controls. The treatments also improved tomato plant growth parameters such as shoot length, shoot and root dry weight, compared to untreated controls.
Key words: *Bacillus* spp., Nematicidal activity, *Meloidogyne incognita*, Rhizo-bacteria, Tomato.

Introduction

Plant growth promoting rhizo-bacteria (PGPR) are the soil bacteria inhabiting around/on the root surface, they affect plant growth directly through synthesized compounds i.e. phytohormones, auxin, facilitating the uptake of certain nutrients from the environment or indirectly when they prevent the deleterious pathogens to came in contact with the plant [1]. Rhizo-bacteria affect nematode by more than one mechanisms: direct effects on egg hatch and nematode mobility through the production of toxins; interference with plant-nematode recognition ; competition for nutrients; plant growth promotion; synthesize enzymes; production of metabolites such as protease, chitinase, glucanase, antibiotics, alteration of root exudates which make roots less attractive to nematodes and induced systemic resistance [2,3,4] . Out of several PGPR genera; *Bacillus* spp. have considerable potential effect in the biocontrol of plant parasitic nematodes by their effective root colonization, multiple modes of action and promising ability to sporulate under stressed conditions [5]. Moussa and Zawam[6] reported that *Bacillus amyloliquefaciens*, *Brevibacterium otitidis* and *Sanguibacter inulinus* inhibited the egg-masses hatching of *M. incognita in vitro* and exhibited strong nematicidal activity by killing the second stage juveniles

(J_2) of nematode in tomato plants under greenhouse conditions. El-Hadad *et al*.[7]found that application of *Bacillus polymyxa* NFB7, *B. megaterium* PSB2 and *B. circulans* KSB2 gave the highest reduction in root-knot nematode population on tomato plants under greenhouse conditions, comparing with the un-inoculated nematode-infested control**.**

Xiao *et al*.**[8]** noted that *Bacillus cereus* X5-gfp can kill J_2 of root -knot nematodes and reduce egg hatching rates in pot experiment by colonized the rhizosphere soil and the root surfaces of tomato plants. Khalil *et al*.**[9]** reported that *Bacillus subtilis*, *Pseudomonas fluorescens* and *Paecilomyces lilacinus* reduced root galls of *M. incognita* and egg masses on root system as well as J_2 numbers in the soil on the tomato plants under greenhouse conditions. Their data obvious that, *B. subtilis* recorded the highest increase in fresh root weight, followed by *P. fluorescens* with values of 125.75 and 86.57%, consecutively, while the great increase in root dry weight 68.14% resulted from *P. fluorescens* followed by *B. subtilis* which recorded 35.40%. Khalil *et al*.**[9]** found that application of *B. subtilis* and *B. thuringiensis* suppressed populations of *M. incognita* infesting tomato plants in the soil with 82.6 and 80.5%, respectively as compared to control. They mentioned that *B. thuringiensis* also increased shoot weight and root length than *B. subtilis*. Almaghrabi *et al***.[10]** noted that *B. amyloliquefaciens, B. subtilis* and *B. cereus* were effective in reducing numbers of J_2 in soil, galls and egg masses/root of root-knot nematode in tomato plant after 45 days from nematode infection. They mentioned that treatments increased shoot dry weight, plant height, fruit/plant and weight of plant yield, compared to infected untreated plants. Amin *et al*.**[11]** reported that *Bacillus brevis*, *B. cereus* and *Bacillus firmus* showed significant reduction in *M. incognita* development and reproduction in tomato under greenhouse condition. El-Nagdi and Abd-El-Khair**[12]** mentioned that *B. subtilis* (as commercial product of Rhizo-N®) highly reduced the number of rook knot nematode J_2 in soil.

This work is aimed to 1) Isolate of common rhizo-bacteria from the rhizospheres of banana , bean and cucumber healthy plants-free from root knot nematode infection ; 2) Study the nematicidal activity of the isolated rhizo-bacteria against *M. incognita* second stage juveniles (J_2) *in vitro* ;and 3) Determine their antagonistic activity on nematodes parameters *viz*. numbers of J_2 in soil and roots and number of galls and egg-masses on tomato roots in pot experiment.

Materials and methods

1. Sampling

Thirty rhizosphere soil samples were collected from healthy plants -free from root-knot nematode infection- from banana (13 samples), bean (6 samples) and cucumber (11 samples) from Gazeret El-Dahab, Giza Governorate, Egypt. About 200 g soil as collective sample were collected around each plant root at a depth of 20–30 cm. The samples were kept in polyethylene bags and immediately transferred to the Plant Pathology Department, National Research Centre for isolation of common rhizo-bacteria.

2. Isolation and identification of common rhizo-bacteria (RB)

The isolation of RB was made according to total plate count technique and dilution method**[13]**. Ten grams of each collective sample were transferred into 250 ml conical flask containing 90 ml of sterile distilled water to give dilution of 10^{-1}. Serial dilutions from 10^{-2} to 10^{-7} were prepared. One ml from each dilution was pipette into sterile Petri-dish (9 cm diam.) containing Nutrient Glucose 1% Agar (NGA) medium [Beef extract ,3.0g ; Peptone ,5.0g ; Glucose , 10.0g ; Agar 15.0g ; in 1.0 liter of distilled water and pH ,7.4 ± 0.2]**[14]**. Each dilution was replicated three times and incubated at 28°C for 48h. The thirty RB isolates were firstly identified according to the growth cultural characters on NGA medium using standard bacteriological methods**[15].** Then, the eight RB isolates named RBba9, RBba10, RBba12, RBba13, RBbe5, RBbe6, RBcu1, and RBcu6 which showed the net mortality of *M. incognita* J_2 about ≥ 95% were selected to identify to genera level using cultural, morphological and biochemical characters as *Bacillus* sp.ba9, *Bacillus* sp.ba10, *Bacillus* sp. ba12, *Bacillus* sp. ba13, *Bacillus* sp. be5, *Bacillus* sp.be6, *Bacillus* sp.cu1 and *Bacillus* sp.cu6. **[15, 16]**

3. *In vitro* experiments

3.1. Preparation of RB suspension

For the preparation of RB suspension, each bacterial isolate was separately grown in Nutrient Glucose 1% Broth (NGB) medium for four days at 28°C and then adjusted to 10^7- 10^9 colony forming unit (CFU)/ml using turbidity method for *in vitro* studies[17] and considering as S concentration.

3.2. Root knot nematode inoculums

The second stage juveniles (J_2) of root-knot nematode used throughout the study were extracted according to Hussey and Barker[18] from a pure culture maintained in eggplant roots in the greenhouse of the National Research Centre and previously identified as *Meloidogyne incognita* according to Taylor and Sasser [19].

3.2.1. Primary bioassay test

Twenty five ml plastic capsule supplied with nine ml from each of the thirty RB isolates suspension plus one ml of nematode suspension containing 300 *M. incognita* J_2. Sterile distilled water and NGB medium without bacteria were served as control. The numbers of viable and dead nematodes were counted under a light microscope after 24, 48 and 72 h of exposure at 25°C. Nematodes were considered alive if they moved or assumed a winding shape and dead if they were straight and immobile. After the exposure periods the nematodes in each treatment were transferred to distilled water and left for 24 h to see whether immobile nematodes resumed activity or not. The corrected percentages of nematode mortality were calculated according to Abbott's[20] formula:

$$\textbf{Mortality (\%)} = (m - n)/(100 - n) \times 100$$

Where: *m* and *n* indicate the percentages of mortality in treatments and control, respectively.

3.2.2. Secondary bioassay test

The nematicidal activity of the eight RB isolates *viz. Bacillus* sp.ba9, *Bacillus* sp.ba10, *Bacillus* sp.ba12, *Bacillus* sp.ba13, *Bacillus* sp.be5, *Bacillus* sp.be6, *Bacillus* sp.cu1and *Bacillus* sp.cu6, which gave ≥ 95%, mortality in the primary bioassay test , were re-evaluated for their nematicidal activity against *M. incognita* J_2 mortality *in vitro* as previously described.

4. Greenhouse study

A pot experiment was conducted to assess the nematicidal effects of the eight selected *Bacillus* spp. isolates *viz. Bacillus* sp.ba9, *Bacillus* sp.ba10, *Bacillus* sp. ba12, *Bacillus* sp.ba13, *Bacillus* sp.be5, *Bacillus* sp.be6, *Bacillus* sp.cu1 and *Bacillus* sp.cu6 against *M. incognita* reproduction infecting tomato plants as well as plant growth parameters in comparing with Micronema® as commercial bio-product (containing10^9 CFU/ml of *Serratia* sp., *Pseudomonas* sp., *Azotobacter* sp., *Bacillus circulans* and *Bacillus thuringiensis*) and NGB medium and untreated plant as control under greenhouse conditions at Plant Pathology Department, National Research Centre (NRC) . Plastic pots (20 cm in diam.) containing 2 kg autoclaved sandy loamy soil (1:1 v/v) were arranged in randomized completely design on a bench in the greenhouse at 25 ± 5 °C. Soil of each pot was separately mixed with 20 ml from each of the previous RB suspension and NGB medium without bacteria then transplanted with two -one month old- tomato seedlings. One week later, each pot received 1,000 newly hatched *M. incognita* J_2 (in four holes around plants). Pots received only 1,000 newly hatched *M. incognita* J_2 served as control. The plants were watered after inoculation and thereafter, whenever required. Each treatment replicated four times as well as the control. After 6 months from nematode infestation, the following data were collected on nematode reproduction [No. of J_2 in soil and roots, No. of root galls, and egg-masses / root system (eight plant roots per treatment)] as well as dry shoot length and weight. All collected data were subjected to analysis of variance ANOVA procedures which reported by Snedecor and Cochran[21] and means of treatments were compared by the least significant difference test "LSD" at 5% level of probability using Computer Statistical Package (CO-STATE) User Manual Version 3.03, Barkley Co.

Results

1. Rhizo-bacteria (RB) isolates

The thirty RB isolated from healthy plant were classified into thirteen isolates from banana rhizosphere (RBba1 to RBba13); six isolates from bean rhizosphere (RBbe1 to RBbe6) and eleven isolates from cucumber rhizosphere (RBcu1 to RBcu11), respectively. Their cultural characters details according to standard bacteriological methods are listed in Table (1).

Table 1. Cultural characters of rhizo-bacteria isolates (RB) isolated from the rhizosphere of some plant species on nutrient glucose 1% agar medium.

No.	Rhizo-Bacteria isolates	Plant species	Cultural characters*
1	RBba1	Banana	Irregular , Raised , Undulate , Smooth , Transluscent , Butyrous , Creamy white
2	RBba2		Irregular , Flat , Erose , Smooth , Transluscent , Butyrous , Creamy white
3	RBba3		Circular , Flat , Entire , Smooth , Translucent , Botyrous , Creamy white
4	RBba4		Circular , Convex , Entire , Smooth , Translucent , Botyrous , Creamy white
5	RBba5		Circular , Raised , Entire , Smooth , Opaque , Butyrous , White
6	RBba6		Circular , Raised , Entre , Smooth , Opaque , Butyrous , White
7	RBba7		Circular , Convex, Entire , Smooth , Opaque , Butyrous , creamy white
8	RBba8		Circular , Convex , Entire , Smooth , Opaque , Viscoud , White
9	RBba9		Rhizoid , Flat , Filamentous , Rough , Translucent , Membranous ,Creamy white
10	RBba10		Irregular , Raised , Undulate , Rough , Opaque , Brittle, White
11	RBba11		Irregular , Flat , Lobate , Rough , Opaque , Brittle , White
12	RBba12		Irregular , Flat , Lobate , Rough , Opaque , Brtittle , White
13	RBba13		Filamentous , Flat , Filamentous , Rough , Translucent , Brtittle , white
14	RBbe1	Bean	Circular , Convex , Entre , Smooth , Opaque , Viscoud , Creamy white
15	RBbe2		Rhizoid , Flat , Filamentous , Rough , Translucent , Membranous, White
16	RBbe3		Circular , Convex , Entire , Smooth , Opaque, butyrous , Creamy white
17	RBbe4		Irregular , Flat , Lobate , Rough , Translucent , Brittle , Creamy white
18	RBbe5		Irregular , Flat , Undulate , Rough , Translucent , Membranous , Creamy white
19	RBbe6		Circular , Convex, Entire , Smooth , Opaque , Butyrous , creamy white
20	RBcu1	Cucumber	Irregular, Raised, Undulate, Rough, Opaque, Butyrous, Creamy white.
21	RBcu2		Irregular , Raised , Undulate , Smooth , Translucent , Butyrous , Creamy while
22	RBcu3		Circular , Raised , Entire , Smooth , Opaque , Butyrous , White
23	RBcu4		Circular , Raised , Entire , smooth, Opaque , Butyrous , Creamy white
24	RBcu5		Rhizoid , Flat , Filamentous , Rough , Translucent , Membranous, White
25	RBcu6		Circular , Convex , Entire , Smooth , Opaque, Viscoud , Creamy white
26	RBcu7		Circular , Convex , Entire , Smooth , Opaque, butyrous , Creamy white
27	RBcu8		Irregular , Flat , Lobate , Rough , Opaque , Membranous , Creamy white
28	RBcu9		Circular , Convex , Entire , Smooth , Opaque , Butyrous , Creamy white
29	RBcu10		Circular , Raised , Entire ,Smooth , Opaque, Butyrous , Creamy white
30	RBcu11		Circular , Convex , Entire , Smooth , Opaque , Butyrous , White

*Cultural of signal bacteria colony as Form, Elevation, Margin of edge, Surface, Optical features, and Consistency

2. *In vitro* bioassay

2.1. Primary bioassay test

As shown in (Table 2), all the 30 RB isolates at concentration (S) could kill *M. incognita* J_2 and the percentage mortality increased by increasing the exposure time. The percentages of mortality after 24 h of exposure were in the ranges of 81-92%, while it's were in the range of 86-94% and 90- 97% after 48 and 72 h, respectively. The net mortality was in the ranges of 90 - 97%. No recovery % was occurred. The RB isolates of banana; bean and cucumber reduced the J_2 of nematode in the ranges of 81-97%; 85-96% and 84-95% after the different exposure periods, respectively. The net mortality of J_2 was in the ranges of 90-97%, 92-96% and 92-95% with RB isolates of banana, bean and cucumber rhizospheres, respectively. Results obvious that the eight of RB isolates *viz*. RBba9, RBba10, RBba12, RBba13, RBbe5, RBbe6, RBcu1 and RBcu6 which showing mortality of *M. incognita* J_2 by $\geq$ 95 % were identified as *Bacillus* spp.

Table 2. *In vitro* nematicidal activities of 30 rhizosphere bacteria from banana bean and cucumber plants against *Meloidogyne incognita* based on juvenile mortality.

No.	Rhizo-bacteria isolates	Mortality %			Net Mortality %
		24h	48h	72h	
1	RBba1	81	86	90	90
2	RBba2	83	88	91	91
3	RBba3	89	91	94	94
4	RBba4	86	90	93	93
5	RBba5	87	89	91	91
6	RBba6	90	92	94	94
7	RBba7	89	91	94	94
8	RBba8	87	89	92	92
9	RBba9	**88**	**90**	**95**	**95**
10	RBba10	**90**	**93**	**97**	**97**
11	RBba11	89	91	94	94
12	RBba12	**91**	**94**	**96**	**96**
13	RBba13	**92**	**93**	**95**	**95**
14	RBbe1	85	90	93	93
15	RBbe2	86	90	93	93
16	RBbe3	87	90	92	92
17	RBbe4	85	89	93	93
18	RBbe5	**90**	**93**	**96**	**96**
19	RBbe6	**88**	**92**	**95**	**95**
20	RBcu1	**89**	**93**	**95**	**95**
21	RBcu2	88	92	93	93
22	RBcu3	84	88	92	92
23	RBcu4	86	89	92	92
24	RBcu5	89	92	93	93
25	RBcu6	**90**	**94**	**95**	**95**
26	RBcu7	88	91	94	94
27	RBcu8	87	90	92	92
28	RBcu9	88	90	93	93
29	RBcu10	87	92	93	93
30	RBcu11	89	90	94	94
Medium		6	8	10	10
Water only		0	0	0	0
L.S.D. 0.05		Bacterial isolates (B)		Period (P)	B x P
		0.7		0.2	1.1

2.2. Second bioassay test

Data in Table (3) showed that the eight *Bacillus* spp. isolates *viz*. *Bacillus* sp.ba9, *Bacillus* sp. ba10, *Bacillus* sp.ba12, *Bacillus* sp.ba13, *Bacillus* sp.be5, *Bacillus* sp.be6, *Bacillus* sp.cu1 and *Bacillus* sp.cu6 at concentration (S) could kill *M. incognita* J_2. The percentages of mortality after 24 h of exposure were in the ranges of 88-95%, while it's were in the ranges of 90 – 96% after 48 and 72 h of exposure period, respectively. The net mortality was in the ranges of 90 – 96%. *Bacillus* sp.ba10 gave the highly percentage of mortality in *M .incognita* J_2 , followed by *Bacillus* sp.ba12, *Bacillus* sp.be6 , *Bacillus* sp.cu6 , *Bacillus* sp.be5 , *Bacillus* sp.ba9 and *Bacillus* sp.cu1, where the net mortalities were 96 , 95 , 95 , 95 , 95 ,93 , 90 and 90% , respectively. While Micronema® resulted in 93, 95 and 95% mortality for 24, 48 and 72 h of exposure. The NGB medium induced only 6, 8 and 8 % mortality during the previous exposure time, respectively. No recovery % was occurred. As observed in Table (3), the nematicidal effect of the selected bacterial isolates was increased by increasing the exposure periods.

Table 3. Effects of *Bacillus* spp. isolates on the mortality of second-stage juveniles of *Meloidogyne incognita in vitro* test (Second bioassay).

Rhizo-bacteria treatments	Mortality %			Net Mortality %
	24h	48h	72h	
M. incognita + water only	0	0	0	0
M. incognita + NGB Medium only	6	8	8	8
M. incognita + Micronema®	93	95	95	95
M. incognita + *Bacillus* sp.ba9	88	90	90	90
M. incognita + *Bacillus* sp.ba10	96	96	96	96
M. incognita + *Bacillus* sp.ba12	93	95	95	95
M. incognita + *Bacillus* sp.ba13	94	95	95	95
M. incognita + *Bacillus* sp.be5	91	93	93	93
M. incognita + *Bacillus* sp.be6	95	95	95	95
M. incognita + *Bacillus* sp.cu1	89	90	90	90
M. incognita + *Bacillus* sp.cu6	92	94	95	95

3. Greenhouse experiment

3.1. Effect on root-knot nematode parameters

Data in Table (4) showed that the entire eight selected *Bacillus* spp. isolates as well as the commercial product Micronema® significantly reduced numbers of *M. incognita* J_2 in soil and roots as well as root galls and egg-masses counts in roots, comparing with untreated control after 6 months from nematode infestation. The percentages of reduction in *M. incognita* J_2/200 g soil due to the application of the *Bacillus* spp. isolates ranged between 82.7 - 97.6%, while Micronema® and the NGB medium resulted in 93.1 and 79.5% reduction ,respectively, as compared to untreated control. Data showed that *Bacillus* sp. be5 gave the highest reduction in *M. incognita* J_2 in soil , followed by *Bacillus* sp. ba12, *Bacillus* sp.cu6 , *Bacillus* sp.be6, *Bacillus* sp.ba9 , *Bacillus* sp.ba10 , *Bacillus* sp.cu1 and *Bacillus* sp.cu13 , where the percentages of reduction recorded were 97.6 , 95.2 , 95.2 , 93.7 , 92.2 , 91.3 , 89.4 and 82.7% , respectively (Table,4). While the percentages of reduction recorded in *M. incognita* J_2 in roots ranged from 91.7 to 95.8%, while those resulted from the application of Micronema® and NGB medium were 94.8 and 75.5 %, respectively, compared to untreated control. *Bacillus* sp. be5 also resulted in the highly reduction in *M. incognita* J_2 in roots , followed by *Bacillus* sp.ba13 , *Bacillus* sp.ba9 , *Bacillus* sp.ba10 , *Bacillus* sp.cu6 , *Bacillus* sp.ba12 , *Bacillus* sp.cu1and *Bacillus* sp.be6 , where the percentages of reduction were 95.8 , 95.8 , 95.7 , 95.2 , 94.3 , 93.4 , 92.9 and 91.7% , respectively (Table, 4).

Table 4. Nematicidal effects of *Bacillus* spp. isolates on *Meloidogyne incognita* reproduction infecting tomato plants.

Rhizo-bacteria treatments	*M. incognita* parameters							
	J_2 in /200g soil		J_2 in /5g roots		Root galls /5g roots		egg-masses/5g roots	
	Count	Red. %	Count	Red. %	Count	Red. %	Count	Red. %
Nematode only	2600	-	2100	-	95	-	69	-
M.incognita+NGB Medium only	533	79.5	515	75.5	66	30.5	45	34.8
M. incognita + Micronema®	180	93.1	110	94.8	18	81.1	14	79.7
M. incognita + *Bacillus* sp.ba9	203	92.2	90	95.7	27	71.6	20	71.0
M. incognita + *Bacillus* sp.ba10	225	91.3	100	95.2	37	61.1	32	53.6
M. incognita + *Bacillus* sp.ba12	125	95.2	138	93.4	22	76.8	18	73.9
M. incognita + *Bacillus* sp.ba13	450	82.7	88	95.8	15	4.2	11	84.1
M. incognita + *Bacillus* sp.be5	63	97.6	88	95.8	18	81.1	13	81.2
M. incognita + *Bacillus* sp.be6	165	93.7	175	91.7	35	63.2	25	63.8
M. incognita + *Bacillus* sp.cu1	275	89.4	150	92.9	14	85.3	9	87.0
M. incognita + *Bacillus* sp.cu6	125	95.2	120	94.3	17	82.1	12	82.6
L.S.D. $_{0.05}$	144.4	-	76.4	-	7.6	-	6.0	-

Also the numbers of root galls were affected by the application of the eight of *Bacillus* spp. isolates, the detected reduction percentages ranged between 61.1-85.3%, while the application of Micronema® and NGB medium resulted in 81.1 and 30.5% reduction, respectively, as compared to untreated control. As shown in (Table, 4) , *Bacillus* sp.cu1 showed the highly reduction in root galls followed by *Bacillus* sp.ba13 , *Bacillus* sp.cu6, *Bacillus* sp.be5, *Bacillus* sp.ba12, *Bacillus* sp.ba9, *Bacillus* sp.be6 and *Bacillus* sp.ba10, where the percentages of reduction were 85.3, 84.2, 82.1, 81.1, 76.8, 71.6, 63.2 and 61.1% , respectively. All *Bacillus* spp. isolates suppressed number of egg-masses in tomato roots, as compared to control. The percentages of reduction ranged between 53.6 – 87.0% and for Micronema® and NGB medium were 79.7 and 34.8% reduction respectively as compared to untreated control. *Bacillus* sp.cu1 induced the highly reduction in egg-masses numbers, followed by *Bacillus* sp.ba13, *Bacillus* sp.cu6, *Bacillus* sp.be5, *Bacillus* sp.ba12, *Bacillus* sp.ba9, *Bacillus* sp.be6 and *Bacillus* sp.ba10, where the percentages of reduction were 87.0 , 84.1 , 82.6 , 81.2 , 73.9 , 71.0 , 63.8 and 53.6% , respectively (Table, 4).

3.2. Effect on Growth parameters of tomato plants

All the eight of *Bacillus* spp. isolates increased the growth parameters of tomato plants *viz.* plant length (cm), shoot and root dry weight (g) , compared to untreated control. Results showed that the percentage increase in plant length due to *Bacillus* spp. isolates ranged between 12.5-31.0%, while Micronema® and NGB medium showed 5.2 and 20.3% increase, respectively as compared to control. *Bacillus* sp.ba12 gave the highly increase in plant length, followed by *Bacillus* sp.cu1, *Bacillus* sp.ba13 , *Bacillus* sp.cu6 , *Bacillus* sp.ba9 , *Bacillus* sp.be6 , *Bacillus* sp.be5 and *Bacillus* sp.ba10 , where the percentages increase were 31.0 , 29.0 , 27.0 , 21.7 , 16.8 , 15.9 , 15.4 and 12.5% , respectively , as compared to untreated control .Also, all *Bacillus* spp. isolates increased shoot dry weight as compared to control. The increase was in the ranges of 9.2-107.7%, while it was 7.7 and 35.4 % with Micronema® and NGB medium, respectively (Table, 5).

Table 5. Effects of *Bacillus* spp. isolates on growth parameters of tomato plants infected with *Meloidogyne incognita.*

Rhizo-bacteria treatments	Growth parameters					
	Shoot length		Shoot dry weight		Root dry weight	
	Length (cm)	% Inc.	Weight (g)	% Inc.	Weight (g)	% Inc.
Nematode only	34.5	-	6.5	-	2.5	-
M. incognita +NGB medium only	41.5	20.3	8.8	35.4	3.3	32.0
M. incognita + Micronema®	36.3	5.2	7.0	7.7	3.1	24.0
M. incognita + *Bacillus* sp.ba9	40.3	16.8	9.2	41.5	3.2	28.0
M. incognita + *Bacillus* sp.ba10	38.8	12.5	7.1	9.2	3.7	48.0
M. incognita + *Bacillus* sp.ba12	45.3	31.0	13.5	107.7	4.6	84.0
M. incognita + *Bacillus* sp.ba13	43.8	27.o	8.8	35.4	5.6	124.0
M. incognita + *Bacillus* sp.be5	39.8	15.4	9.8	50.8	4.4	76.0
M. incognita + *Bacillus* sp.be6	40.0	15.9	8.4	29.3	3.2	28.0
M. incognita + *Bacillus* sp. cu1	44.5	29.0	10.4	60.0	5.1	104.0
M. incognita + *Bacillus* sp. cu6	42.0	21.7	8.8	35.4	3.0	20.0
L.S.D. $_{0.05}$	9.5	-	3.9	-	2.0	-

Bacillus sp.ba12 induced the highly increased in shoot dry weight of treated plants, followed by *Bacillus* sp.cu1**,** *Bacillus* sp.be5 **,** *Bacillus* sp.ba9**,** *Bacillus* sp.ba13 , *Bacillus* sp.be6 , *Bacillus* sp.be6 and *Bacillus* sp.ba10 , where the percentages of increase were 107.7 , 60.0 , 50.8 , 41.5 , 35.4 , 35.4 , 29.3 and 9.2% , respectively (Table,5). As well as the root dry weight increased in all treated plants as compared to control. The percentages of increase ranged between 20.0-124.0%, while Micronema® and NGB medium increased root dry weight by 24.0 and 32.0%, respectively. *Bacillus* sp.ba13 resulted in the highly increase in root dry weight, followed by *Bacillus* sp.cu1, *Bacillus* sp.ba12, *Bacillus* sp.be5, *Bacillus* sp.ba10, *Bacillus* sp.ba9, *Bacillus* sp.be6 and *Bacillus* sp.cu6, where the percentages of increase were 124.0 , 104.0 , 84.0 , 76.0 ; 48.0 , 28.0 , 28.0 and 20.0% ,respectively (Table, 5).

Discussion

The use of pesticides to control plant diseases became limited because of the environmental concerns, health conscious attitude of human beings and other hazards associated with the use of chemicals. It is clear that using PGPR to suppress the plant pathogens is gaining importance. They exhibit diverse modes of action against plant parasitic nematodes include antibiosis, competition, myco-parasitism, cell wall degradation, induced resistance, plant growth promotion and rhizosphere colonization capability[22-28]**.** This work is aimed to isolate the common rhizo-bacteria from rhizosphere of some plants to control root-knot nematode, *M. incognita in vitro* and in pots. Our results clearly illustrated the potential of the thirty isolated rhizo-bacteria from the rhizosphere of healthy banana, bean and cucumber plants to kill and immobilized *M. incognita* second stage juveniles under laboratory conditions with variation in their potentialities to kill. These results are in agreement with that cited by Becker *et al*.[29] who reported that bacteria isolated from rhizosphere of different plants affected the vitality of second-stage juveniles of *M. incognita in vitro* test, also ElSayed and Edrees[30]found that microorganisms that can grow in the rhizosphere are ideal for use as bio-control agents where the rhizosphere provides the front line defense for roots against attack by pathogens. Present results showed that the most potent rhizo-bacteria for killing *M. incognita* J_2 are identified as *Bacillus spp*. these are in accordance with Daward *et al***.**[31] who found that application of *Bacillus* spp., significantly reduced hatching of *M. javanica* eggs, whereas mortality of larvae was significantly increased with the increase in time. El-Nagdi and Abd-El-Khair[32]**,** Ruiz *et al*.[33] and Wei *et al***.**[34]found that cell-free culture filtrate of *B. subtilis* increased *M. incognita* J_2 mortality and inhibited egg hatch.

The greenhouse evaluation confirm the results observed *in vitro* bioassay where the selected *Bacillus spp*. significantly reduced the numbers of J_2 in soil and roots as well as galls and egg-masses/root system infected with *M. incognita* this documented the findings of Mannanov and Sattarova[35] who reported that *Bacillus* species are effective in the management of plant pathogens owing to their ability to produce

antimicrobial compounds and the wide distribution of the cuticle-degrading proteases with nematicidal activity which play an important role in bacteria–nematode interactions and serve as important nematicidal factors in balancing nematode populations in the soil. Ahmad *et al*.[36]noted that the nematicidal activity of *Bacillus* spp. may due to their capabilities to produce indole acetic acid and hydrogen cyanide. While Oliveira *et al*.[37] refer the nematicidal of *B. megaterium* to the production of threonine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine and histidine that cause significantly higher J_2 mortalities of *M. exigua* than control (water). Kavitha *et al*.[5] mentioned that *B. subtilis* with high surfactin and iturin activity suppressed hatching of eggs and killed second stage juveniles of the nematode under *in vitro* condition. Abdel –Aziz *et al*.[38]noted that the potentiality of *B. alvei* against nematode's eggs and larvae due to the hydrolytic enzymes which directly hydrolyze nematode's eggs and larvae .The strain also produced lytic enzymes viz. chitinase, chitosanase, proteases, as well as other potential bioactive metabolite.

Obtained results demonstrated the effect of the selected *Bacillus* spp. on tomato growth parameters by enhancing plant length and shoot and root dry weight over control. This is in agreement with Siddiqui and Mahmood[39] who reported that the rhizosphere supports large and active microbial populations capable of exerting beneficial, neutral or detrimental effects on plant growth and confirm the previous findings of Antoun and Prevot[3] who found that PGPR may induce plant growth promotion directly by invade plant roots and produce different metabolites like phyto-hormones , auxin derivatives and gibberellin-like substances which have been reported to elongate stem, enlarge cells and leaves, expand the root system, induce cell-division and cause early flowering and fruiting, as well as, the metabolites of *Bacillus spp*. increase the mineral availability to plants by solubilization of inorganic phosphate and mineralization of organic phosphate or indirect action by stimulation of disease-resistance mechanisms[40].

It is clear that PGPR are the most abundant in plant rhizosphere and exhibit diverse modes of action against nematodes includes antibiotics , enzymes and toxins production; parasitizing; competing for nutrients; inducing systemic resistance of plants and improve of plant health[23,24] **.** Rhizo-bacteria are capable of stimulating plant growth through a variety of mechanisms that include improvement of plant nutrition, production and regulation of phyto-hormones, and suppression of disease causing organisms[40]. PGPR are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion. These results demonstrated that inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production through direct effects on root and shoots growth and controlling of nematode infection.

Acknowledgement

This research work was supported in part by the In-house Project No. 10120603 entitled "Improving Activity of some Microorganisms through Biotechnological Approaches to Control plant Parasitic Nematodes" funded by National Research Centre, Egypt.

References

1. Khalil , M. S. ; Kenawy, A. ; Gohrab, M. A. and Mohammed , E. E. (2012). Impact of microbial agents on *Meloidogyne incognita* management and morphogenesis of tomato. J. Biopest. 5(1):28-35.
2. Kerry, B.R. (2000). Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant-parasitic nematodes Annual Review of Phytopathology, 38: 423-441.
3. Antoun, H. and Prevost, D. (2005). Ecology of plant growth promoting Rhizo-bacteria. PGPR: Biocontrol and Biofertilization, 1–38. *Z. A. Siddiqui (ed.)*, 2005 Springer. Printed in the Netherlands.
4. Sivasakthi, S.; Usharani, G. and Saranraj, P. (2014). Biocontrol potentiality of plant growth promoting bacteria (PGPR) - *Pseudomonas fluorescens* and *Bacillus subtilis*: A review. African Journal of Agricultural, 9 (16): 1265-1277.
5. Kavitha, P.G.; Jonathan, E.I. and Nakkeeran, S. (2012). Effects of crude antibiotic of *Bacillus subtilis* on hatching of eggs and mortality of juveniles of *Meloidogyne incognita*. Nematol. medit. , 40: 203-206.
6. Moussa, Lobna and Zawam, Hanaa (2010). Efficacy of some bio-control agents on reproduction and development of *Meloidogyne incognita* infecting tomato. Journal of American Science, 6(11):495-509.

7. El-Hadad , M. E.; Mustafa , M.I.; Selim , Sh. M.; El-Tayeb T.S.; Mahgoob, A.E.A.; Abdel Aziz , H. Norhan (2011). The nematicidal effect of some bacterial bio-fertilizers on *Meloidogyne incognita* in sandy soil. Braz. J. Microbiol., 42 (1) São Paulo Jan. /Mar.
8. Xiao, T. J.; Tan, S. Y.; Shen, Q. R. and Ran, W. (2012). *Bacillus cereus* X5 suppresses root-knot nematode of tomato by colonizing in roots and soil. African Journal of Microbiology Research, 6 (10):2321-2327.
9. Khalil, M. S. H.; Allam, A. F. G. and Barakat, A. S. T. (2012). Nematicidal activity of some bio-pesticide agents and microorganisms against root-knot nematode on tomato plants under greenhouse conditions. Journal of Plant Protection Research, 52 (1):47- 52.
10. Almaghrabi, O. A.; Massoud, S. I. and Abdelmoneim, T. S. (2013). Influence of inoculation with plant growth promoting rhizo-bacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions. Saudi Journal of Biological Sciences , 20(1): 57–61.
11. Amin, A.W.; Anter, A.A.; Ashoub, A.H. and El-Nuby, A.S. (2014). Evaluation of rhizo-bacteria as resistance inducers or bio-control agents for the control of *Meloidogyne incognita* in tomato. Pakistan Journal of Nematology, 32 (2): 211-211.
12. El-Nagdi, Wafaa, M.A. and Abd-El-Khair, H. (2014). Biological control of *Meloidogyne incognita* and *Fusarium solani* in dry common bean in the field. Archives of Phytopathology and Plant Protection, 47(4): 388–397.
13. Ghini, R.F.; Patrico, R.A.; Bettiol, W.; de Almeida, M.G. M.G. and Maia, N. H. A. (2007). Effect of sewage sludge on suppressiveness to soil-borne plant pathogens. Soil Biol. Biochem. , 39:2797–2805.
14. Bridson, E. Y. (1995). The Oxide Manual 7th Ed., Published by Unipath Limited , Wade Koad, Basingstoke Hampshire , RG 248 PW, England.
15. Schaad, N. W. (1980). Laboratory Guide for Identification of Plant Pathogenic Bacteria . Bacteriology Committee of American Phyt. Society , St. Paul. Minnesota , 72 pp.
16. Goszczynska, T.; Serfontein, J. J. and Serfontein, S. (2000). Introduction to Practical Phytobacteriology. Sponsored by SDC, Switzerland, 82 pp.
17. Abd-El-Khair, H. and El-Mougy, Nehal, S. (2003). Field biological approach under organic cultivations conditions for controlling black mould disease infection during storage. Egyptian J. Appl. Sci., 18(6):50–69.
18. Hussey, R.S. and Barker,K.R.(1973). A comparison of methods of collecting inoculation of *Meloidogyne* species, including a new technique. Plant Dis., 57:1025–1028.
19. Taylor, A.L. and Sasser, J.N. (1978). Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Raleigh (NC): IMP, North Carolina State University Graphics.
20. Abbott, M.S. (1925). A method for computing the effectiveness of an insecticide . Journal of Economic Entomology, 18: 265-267.
21. Snedecor, G.W. and Cochran, W.G. (1980). Statistical methods, 5th ed. Ames (IA): Iowa State Univ. Press; p. 593.
22. Lian, L.H. ; Tian, B.Y.; Xiong, R.; Zhu, M.Z. ; Xu, J. and Zhang, K.Q. (2007). Proteases from *Bacillus*: a new insight into the mechanism of action for rhizo-bacteria suppression of nematode populations. Letters in Applied Microbiology, doi:10.1111/j.1472-765X.2007.02184.x
23. Tian, B.; Yang, J. and Zhang, K.Q. (2007). Bacteria used in the biological control of plant-parasitic nematodes: populations, mechanisms of action, and future prospects. FEMS Microbiol. Ecol. , 61(2):197-213.
24. Lugtenberg, B. and Kamilova, F. (2009). Plant-Growth-Promoting Rhizobacteria , Annual Review of Microbiology, 63: 541-556.
25. Junaid, J. M.; Dar, N. A.; Bhat, T. A.; Bhat, A. H. and Bhat, M. A. (2013). Commercial bio-control agents and their mechanism of action in the management of plant pathogens . International Journal of Modern Plant & Animal Sciences, 1(2): 39 – 57.
26. El-Nagdi, Wafaa, M. A. and Yossef, M. M. A. (2016). Plant growth and yield of cowpea as affected by population density of root-knot nematode, *Meloidgyne incognita*, International Journal of ChemTech Research, 9 (4):126-130.
27. El-Nagdi, Wafaa, M. A.; Yossef, M. M. A. and El-Rokik , Kowthar ,G.(2016). Allelopathic effect of dry leaves of lantana and guava for controlling root-knot nematode, *Meloidgyne incognita* on cowpea and some associated weed., International Journal of ChemTech Research, 9 (6):55-62.

28. Abd-El-Khair, H.; Haggag, Karima, H. E. and ElShahawy, I. E. (2016). Soil application of *Bacillus pumilus* and *Bacillus subtilis* for suppression of *Macrophomina phaseolina* and *Rhizoctinia solani* and yield enhancement in peanut., International Journal of ChemTech Research, 9 (6):142-152.
29. Becker, J. O.; Zavaleta-Mejia, E. ; Colbert, S. F.; Schroth, M. N.; Weinhold, A. R.; Hancock, J. G. and Van Gundy, S. D. (1988) . Effects of rhizo-bacteria on root-knot nematodes and gall formation. Phytopathology, 78:1466-1469.
30. ElSayed, I.A. and Nada, Edrees, O. (2014).Using of plant growth promoting rhizo-bacteria as biocontrol agents for root-knot nematode under greenhouse. Nature and Science, 12 (12) 41-49.
31. Daward, S., M. Tariq and M. J. Zaki. 2008. Application of *Bacillus* species in control of *Meloidogyne javanica* (Treub) Chitwood on cowpea and mash bean. Pak. J. Bota., 40: 439-444.
32. El-Nagdi, Wafaa, M.A. and Abd-El-Khair, H. (2008).Biological control of *Meloidogyne incognita* and *Rhizoctonia solani* in eggplant. Nematol. Medit., 36:85–92.
33. Ruiz, S.E.; Cristobal, A.J.; Reyes, R.A.; Tun, S.J.; Garcia, R.A. and Pacheco, A.J. (2014). *In vitro* antagonistic activity of *Bacillus subtilis* strains isolated from soils of the Yucatan Peninsula against *Macrophomina phaseolina* and *Meloidogyne incognita* . Phyton (B. Aires), 83: 45-47.
34. Wei, L. ; Shao , Y. ; Wan , J.; Feng , H. ; Zhu , H.; Huang , H. and Zhou , Y. (2014). Isolation and characterization of a rhizo-bacterial antagonist of root-knot nematodes . Plos One, 9(1): e85988. doi:10.1371/journal.pone.0085988.
35. Mannanov, R.N. and Sattarova ,R.K. (2001) Antibiotics produced by Bacillus bacteria. Chem. Nat. Comp., 37:117–123.
36. Ahmad, F.; Ahmad , I. and Khan , M.S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities . Microbiological Research , 163 (2): 173–181.
37. Oliveira, D.F.; Carvalho, H.W.P.; Nunes, A.S.; Campos, V.P.; Silva, G.H. and Campos, V.A.C. (2009). Active substances against *Meloidogyne exigua* produced in a liquid medium by *Bacillus megaterium*. Nematologia Brasileira Piracicaba (SP) Brasil, 33(4):271 – 277.
38. Abdel -Aziz , Shadia , M. ; El –Nagdi , Wafaa, M. A. and Moharam , Maysa , E. (2013). Efficiency of the novel strain *Bacillus alvei* NRC -14 for bio-control of parasitic Nematode. J. Agric. Food. Tech., 3 (12)31-40.
39. Siddiqui, Z. A. and Mahmood , H. (1999). Role of bacteria in the management of plant parasitic nematodes: A review. Bioresource Technology , 69 (2):167–179.
40. Martínez-Viveros, O.; Jorquera, M. A.; Crowley, D.E.; Guajardo, G. and Mora, M.L. (2010).Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J. Soil Sci. Plant Nutr., 10 (3): 293 – 319.

*****