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THE NEMATICIDAL EFFECT OF SOME BACTERIAL BIOFERTILIZERS ON *MELOIDOGYNE INCOGNITA* IN SANDY SOIL

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ABSTRACT

In a greenhouse experiment, the nematicidal effect of some bacterial biofertilizers including the nitrogen fixing bacteria (NFB) *Paenibacillus polymyxa* (four strains), the phosphate solubilizing bacteria (PSB) *Bacillus megaterium* (three strains) and the potassium solubilizing bacteria (KSB) *B. circulans* (three strains) were evaluated individually on tomato plants infested with the root-knot nematode *Meloidogyne incognita* in potted sandy soil. Comparing with the uninoculated nematode-infested control, the inoculation with *P. polymyxa* NFB7, *B. megaterium* PSB2 and *B. circulans* KSB2, increased the counts of total bacteria and total bacterial spores in plants potted soil from 1.2 to 2.6 folds estimated 60 days post-inoculation. Consequently, the inoculation with *P. polymyxa* NFB7 increased significantly the shoot length (cm), number of leaves / plant, shoot dry weight (g) / plant and root dry weight (g) / plant by 32.6 %, 30.8 %, 70.3 % and 14.2 %, respectively. Generally, the majority treatments significantly reduced the nematode multiplication which was more obvious after 60 days of inoculation. Among the applied strains, *P. polymyxa* NFB7, *B. megaterium* PSB2 and *B. circulans* KSB2 inoculations resulted in the highest reduction in nematode population comparing with the uninoculated nematode-infested control. They recorded the highest reduction in numbers of hatched juveniles/root by 95.8 %, females/root by 63.75 % and juveniles/1kg soil by 57.8 %. These results indicated that these bacterial biofertilizers are promising double purpose microorganisms for mobilizing of soil nutrients (nitrogen, phosphate and potassium) and for the biological control of *M. incognita*.

Key words: *Paenibacillus polymyxa*, *Bacillus megaterium*, *B. circulans*, biofertilizers, biocontrol, root-knot nematodes, *Meloidogyne incognita*.

INTRODUCTION

Root-knot nematodes *Meloidogyne* spp., are obligate endoparasites of great economic importance, being among the major limiting factors in the production of field and plantation crops, predominantly in the tropics but also in Europe and North America (17).

A number of methods for the management of root-knot nematode such as chemical control, organic amendments, and biological control have been tried with different levels of successes for the protection of tomato plants (22, 24). Chemical management is effective, but expensive and may lead

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to soil pollution problems (32). Over the last decades, researchers all over the world are engaged in standardizing the nematode management strategies by following non-chemical and ecofriendly approaches (3, 21, 31). A great diversity of rhizospheric microorganisms has been described, characterized, and tested for activity as biocontrol agents against soil nematodes. These microorganisms possess some mechanisms to promote plant growth and control pathogens. Explanation among ecological interactions that occur between nematodes and bacteria, amensalism and parasitism happens in soil largely as the result of the stepwise bacterial degradation of plant and animal residues. The widely recognized mechanisms of biocontrol mediated by plant growth promoting bacteria (PGPB) are competition for an ecological niche or a substrate, production of inhibitory substances, and induction of systemic resistance in host plants to a broad spectrum of pathogens and/or abiotic stresses (7, 13). *Paenibacillus polymyxa*, *B. megaterium* and *B. circulans* are common soil bacterial biofertilizers belonging to plant growth promoting bacteria (PGPB). Activities associated with these bacteria include nitrogen fixation (6), soil phosphorus solubilization (9) and solubilizing insoluble potassium (10, 26). Research into the impact of PGPB has provided a greater understanding of the multiple facets of disease suppression by these biocontrol agents. Much remains to be learned from these bacteria that have unique associations and a more pronounced growth-enhancing effect on host plants. Moreover, additional environmentally safe and economically feasible root-knot nematode control practices needs to be available (7, 14, 27, 32).

The main focus of this work was to evaluate the dual effect of some bacterial strains as biofertilizers and biocontrol agents against the root-knot nematode *M. incognita* infestation in sandy soil cultivated with tomato.

MATERIALS

Bacterial biofertilizers and nematode used

Strains of bacterial biofertilizers including nitrogen fixing bacteria (*Paenibacillus polymyxa* NFB2, NFB5, NFB6, NFB7), phosphate solubilizing bacteria (*Bacillus megaterium* PSB2, PSB4, PSB5), potassium solubilizing bacteria (*B. circulans* KSB2, KSB4, KSB7) and the root-knot nematode *Meloidogyne incognita* were isolated and identified by the authors in a previous work (8). Bacterial strains were maintained on nutrient agar slants at 4ºC, while pure culture of *M. incognita* was maintained on roots of tomato plants (*Lycopersicon esculantium* Mill cv. Castel Rock) growing in 20-cm diameter pots in sterile sandy-loam soil in greenhouse.

Microbiological Media used

a- *Nutrient agar medium* (12) was used to determine the total bacterial count. b- *Soil extract agar medium* (5) was used to determine the densities of spore forming bacteria. c- *Modified Buntt and Rovira medium* (16) was used for counting of phosphate solubilizing bacteria. d- *Modified Aleksandrov's medium* (34) was used for counting of potassium solubilizing bacteria. e- *MBS medium* (18) was used for counting of nitrogen fixing bacteria. On all media, counting was done by using the plate count method (12).

Plant and soil used

Thirty days old seedlings of tomato *(Lycopersicon esculantium* Mill cv. Castel Rock) were used as the host plant for *M. incognita*. They were kindly provided from Central Laboratory of Agricultural Climate, Agricultural Research Center, Dokki, Giza, Egypt. Seedlings were transplanted into 25-cm diameter plastic pots (one seedling/pot) filled with 5 kg of sterilized sandy soil. They were watered daily with tap water and with a nutrient solution once a week.

Chemical fertilizers

Ammonium sulfate (20.5% N), Potassium sulfate (48% K_2O) and phosphoric acid (80% P_2O_5) were used for nitrogen, potassium and phosphorus fertilization, respectively. Rock phosphate (16.7% P_2O_5) and Feldspar (Potassium ore 11% K_2O) were used as cheap sources for phosphorus and potassium fertilizers. All chemical fertilizers were provided from Al-Ahram Company for Mining and Natural Fertilizers, Giza, Egypt.

METHODS

Preparation of bacterial inocula

For each bacterial strain, a conical flask (250 ml) containing 100 ml of nutrient broth medium consisted of 5.0 g peptone, 3.0 g beef extract, 1000 ml tap water, (pH was adjusted to 7.0) was inoculated and incubated at 28-30°C with shaking at 150 rpm for two days prior to application.

Preparation of *M. incognita* inoculum

When nematode inoculum as the second stage juveniles (J_{2s}) was needed, galled tomato roots were washed thoroughly with tap water, cut into pieces then placed in mist chamber for egg hatching (23). The first catch was discarded, and the following emerged J_{2s} were collected daily and refrigerated at 6ºC for the experimental use. J_{2s} were placed in 0.5% sodium hypochlorite, agitated and rinsed with sterile water immediately before infestation (15).

Greenhouse pot experiment

Four days after seedlings transplantation, seedlings were infested with 1000 fresh J_{2s}/plant in 10 ml suspension. Two days later, each pot was inoculated with a single bacterial culture at the rate of 10 ml/pot (containing $2x10^7$ cfu/ml). There were also two uninoculated controls: In uninoculated control 1, the nematode was applied but the bacteria were not, whilst in uninoculated control 2, neither the nematode nor the bacteria were applied. The inoculated plants were maintained in greenhouse for 60 days at 28 ± 2°C. Throughout 60 days after bacterial inoculation, the plants were carefully removed from pots and the rhizosphere and soil of each pot were thoroughly mixed to compose representative samples then collected in labeled plastic bags for the determination of bacterial populations, CO_2 evolution and second stage juveniles (J_{2s}). Roots of plants taken 30 and 60 days post-bacterial inoculation were gently washed to remove soil particles for the determination of nematode population in roots. Plant growth was determined 60 days post-bacterial inoculation. The shoot length was measured from the soil line to the tip of the stem and expressed in cm. The dry weight of shoots and roots was recorded after drying in oven at 70°C. Nitrogen, phosphorus and potassium contents of tomato plants were determined according to Jackson (11). The rate of CO_2 evolution was determined according to the method described by Shehata (25) and calculated according to Alef and Nannipieri (1). Ten replicates of each treatment were done and average response was taken. The obtained data were subjected to an analysis by using Statistical Analysis System (SAS) (version 6.12, SAS, Institute Inc., Cary, NC, USA). Duncan`s Multiple Range Test was used to test significance of means according to Snedecor and Cochran (29). Application treatments of this experiment are illustrated in Table (1).

Table 1. Application treatments of tomato plants infested with *M. incognita* and inoculated with different *P. polymyxa*, *B. megaterium* and *B. circulans* strains in potted sandy soil.

Treatments	Chemical fertilizations			Bacterial* inoculation	Infestation with *M. incognita*
	N	P**	K**		
1 (control)	Full dose of $(NH_4)_2SO_4$	Full dose of H_3PO_4	Full dose of K_2SO_4	-	+
2 (control)	Full dose of $(NH_4)_2SO_4$	Full dose of H_3PO_4	Full dose of K_2SO_4	-	-
3	75% of full dose of $(NH_4)_2SO_4$	Full dose of H_3PO_4	Full dose of K_2SO_4	*Paenibacillus polymyxa* (4 strains)	+
4	Full dose of $(NH_4)_2SO_4$	Rock phosphate	Full dose of K_2SO_4	*Bacillus megaterium* (3 strains)	+
5	Full dose of $(NH_4)_2SO_4$	Full dose of H_3PO_4	Feldspar	*B. circulans* (3 strains)	+

* Each strain was tested alone.
**Rock phosphate and feldspar were added in equivalent to (P%, K %) in full recommended dose of H_3PO_4 and K_2SO_4.

***M. incognita* measurements**

For counting of J_{2s}, the soil of each pot was mixed thoroughly and about 250 g of soil was used for nematode extraction by using the Baermann funnel technique (30). Extracted J_{2s} were counted in 1 ml suspension using counting slides. Number of J_{2s} was attributed to 1 kg soil. Washed root was placed in a mist chamber for egg hatching (23). The hatched J_{2s} were collected daily and refrigerated at 6°C for 5 days after which were counted. In one ml of extracted nematode suspension, J_{2s} were counted using nematodes counting slides. Roots were removed from the mist chamber and stained with acid fuchsin in cold lactophenol for more than 24 h. The stained roots were rinsed with tap water and cut into pieces to facilitate counting of galls, females and egg masses using a stereomicroscope. The reduction of nematode population due to different bacterial inoculations was calculated compared with the uninoculated infested tomato control.

RESULTS AND DISCUSSION

The benefits of different bacterial inoculations on the growth of infested tomato plants with the root-knot nematode *M. incognita*, and their impact as biocontrol agents were studied. Within 60 days after tomato seedlings inoculation, the counts of bacterial strains were increasing (Table 2). The inoculation with *P. polymyxa* NFB7 exhibited the highest total nitrogen fixing bacterial (NFB) counts comparing with other *P. polymyxa* strains (131 x 10^4 and 172 x 10^4 cells / g dry soil after 30 and 60 days of inoculation, respectively). Also, this treatment achieved the highest total bacterial count and total bacterial spores count after 60 days of inoculation (221 x 10^6 and 195 x 10^5 / g dry soil, respectively). Comparing with control 1, this treatment enhanced the growth of total bacteria and total bacterial spores populations after 60 days of inoculation by 2.6 and 1.89 folds, respectively. The CO_2 evolution induced under the effect of *P. polymyxa* NFB7 inoculation were 13 and 19.6 mg / g dry soil / h, after 30 and 60 days, respectively.

Among *B. megaterium* and *B. circulans* strains inoculations, *B. megaterium* PSB2 and *B. circulans* KSB2 recorded the highest PSB and KSB counts (130 x 10^4, 190 x 10^4 and 146 x 10^4, 190 x 10^4 / g dry soil after 30 and 60 days of inoculation, respectively (Table 2). The equivalent CO_2 evolutions due to the inoculation with the two strains were 15.01, 18.3 and 15.41, 19.4 mg / g dry soil / h, respectively. *B. megaterium* PSB2 and *B. circulans* KSB2 inoculations recorded the highest total bacterial count and total bacterial spores count after 60 days of inoculation (104 x 10^6, 193 x 10^5 and 198 x 10^6, 189 x 10^5 / g dry soil, respectively), which represented 1.2, 1.87 and 1.5, 1.83 folds comparing with control 1. These results indicated that these bacterial biofertilizers persisted in soil up to 60 days after inoculation despite of the lack of organic matter and depending on the inorganic compounds used for plants fertilization.

By comparing the plant growth of uninoculated infested plants (control 1) with those of uninoculated uninfested plants (control 2), a great reduction was achieved (Table 3). The reduction in shoot length (cm), number of leaves / plant, shoot dry weight / plant and root dry weight / plant were 21.2 %, 13.3 %, 23.7 % and 17.4 %, respectively. The majority of bacterial strains inoculations resulted in prevention or even reduction of the consequences of nematode infection which reflected on increasing the plants growth comparing with control 1 (Table 3). The inoculation with *P. polymyxa* NFB7 achieved the most promising results. This treatment increased the shoot length (cm), number of leaves / plant, shoot dry weight (g) / plant and root dry weight (g) / plant by 32.6 %, 30.8 %, 70.3 % and 14.2 %, respectively. Lower percentages could be obtained if results were compared with those of uninfested plants (control 2) (4.4 %, 13.3 %, 29.9 % and – 5.6 %, respectively) (Table 3). *B. megaterium* PSB2 and *B. circulans* KSB2 inoculations were the most efficient among the other PSB and KSB strains. They increased the shoot length (cm), number of leaves / plant, shoot dry weight (g) / plant, root dry weight (g) / plant comparing with uninoculated infested plants (control 1) by 25.6 %, 15.4 %, 29.2 %, 3.8 % and 2.3 %, 7.7 %, 66.8 %, 9.7 %, respectively. The root weight was not relevant to the weight of

the shoot. This could be due to root galling on infested plants and induced systemic resistance or multiple potential defense mechanisms due to the interaction between the host, the bacteria, and the nematode (33).

Table 2. Bacterial activity and CO_2 evolution per gram of dry potted soil of tomato plants infested with *M. incognita* and inoculated with different *P. polymyxa*, *B. megaterium* and *B. circulans* strains in sandy soil.

Treatments	Time after bacterial inoculation (days)	Total bacterial count / g dry soil ($x10^6$)	Total spores count / g dry soil ($x10^5$)	Nitrogen fixing bacterial count / g dry soil ($x10^4$)	Phosphate solubilizing bacterial count / g dry soil ($x10^4$)	Potassium solubilizing bacterial count / g dry soil ($x10^4$)	CO_2 evolution (mg/g dry soil/h)
	0	55^{d}	55^{d}	28^{e}	35^{d}	45^{d}	4.13^{d}
Control(1)*	30	73^{f}	71^{i}	54^{e}	54^{d}	92^{d}	8.2^{d}
	60	85^{hi}	103^{f}	133^{c}	73^{c}	132^{d}	9.2^{e}
	0	28^{g}	73^{b}	42^{d}	-	-	2.8^{e}
P. polymyxa	30	82^{e}	104^{fg}	82^{d}	-	-	8.2^{d}
NFB2	60	123^{d}	132^{e}	122^{d}	-	-	12.6^{d}
	0	63^{c}	63^{c}	56^{c}	-	-	6.3^{c}
NFB5	30	102^{c}	98^{g}	110^{c}	-	-	10^{c}
	60	173^{c}	153^{d}	146^{b}	-	-	17.7^{ab}
	0	73^{b}	82^{a}	76^{b}	-	-	7.3^{bc}
NFB6	30	114^{b}	106^{ef}	115^{b}	-	-	11.5^{c}
	60	198^{b}	165^{c}	169^{a}	-	-	15.5^{c}
	0	85^{a}	85^{a}	84^{a}	-	-	8.5^{b}
NFB7	30	129^{a}	116^{d}	131^{a}	-	-	13^{b}
	60	221^{a}	195^{a}	172^{a}	-	-	19.6^{a}
	0	54^{d}	87^{a}	-	61^{b}	-	10.48^{a}
B. megaterium	30	81^{e}	162^{a}	-	130^{a}	-	15.01^{a}
PSB2	60	104^{g}	193^{a}	-	190^{a}	-	18.3^{a}
	0	36^{f}	73^{b}	-	51^{c}	-	8.17^{b}
PSB4	30	63^{g}	142^{b}	-	115^{b}	-	13.48^{b}
	60	91^{h}	174^{b}	-	163^{b}	-	16.1^{b}
	0	46^{e}	61^{c}	-	72^{a}	-	10.48^{a}
PSB5	30	75^{f}	90^{h}	-	94^{c}	-	8.23^{d}
	60	83^{i}	131^{e}	-	166^{b}	-	11.9^{d}
	0	89^{a}	82^{a}	-	-	100^{a}	8.14^{b}
B. circulans	30	132^{a}	134^{c}	-	-	146^{a}	15.41^{a}
KSB2	60	198^{b}	189^{a}	-	-	190^{a}	19.4^{a}
	0	65^{c}	63^{c}	-	-	76^{b}	5.62^{cd}
KSB4	30	74^{f}	112^{e}	-	-	105^{c}	12.45^{bc}
	60	124^{e}	153^{d}	-	-	165^{b}	17.74^{ab}
	0	44^{e}	73^{b}	-	-	63^{c}	7.01^{bc}
KSB7	30	88^{d}	123^{d}	-	-	124^{b}	13.21^{b}
	60	116^{f}	174^{b}	-	-	153^{c}	15.96^{bc}

* Uninoculated tomato plants infested with *M. incognita* were supplemented with full-dose of chemical NPK fertilizers before transplantation.
Means within the same column not followed by the same letter are significantly different ($P < 0.05$).

Table 3. Effect of various bacterial biofertilizers treatments on growth of tomato plants infested with *M. incognita* 60 days post-inoculation.

Treatments	Growth parameters									
	Shoot length (cm)	No. of leaves / plant	Shoot dry weight (g/ plant)	Root dry weight (g/ plant)	Shoot content (%)			Root content (%)		
					N	P	K	N	P	K
P. polymyxa										
NFB2	44.1^{e}	11^{e}	3.12^{h}	2.21^{c}	1.23^{e}	0.62^{ab}	1.46^{c}	0.26^{e}	0.43^{b}	0.30^{c}
NFB5	46.6^{d}	15^{bc}	6.11^{f}	3.18^{b}	2.2^{a}	0.61^{b}	1.45^{cd}	0.52^{b}	0.42^{b}	0.18^{f}
NFB6	54^{b}	15^{b}	10.25^{b}	4.01^{a}	1.43^{d}	0.53^{c}	1.03^{e}	0.26^{e}	0.38^{d}	0.37^{b}
NFB7	57^{a}	17^{a}	11.63^{a}	4.01^{a}	2.32^{a}	0.63^{a}	1.53^{b}	0.58^{a}	0.47^{a}	0.40^{a}
B. megaterium										
PSB2	54^{bc}	15^{b}	8.82^{c}	3.64^{b}	2.2^{a}	0.61^{b}	1.46^{c}	0.48^{c}	0.43^{b}	0.36^{b}
PSB4	52^{c}	13^{cd}	5.14^{g}	2.04^{c}	1.82^{c}	0.51^{a}	1.64^{a}	0.46^{c}	0.41^{bc}	0.23^{e}
PSB5	53^{c}	12^{d}	6.02^{f}	2.5^{c}	2.20^{a}	0.55^{c}	1.43^{cd}	0.32^{d}	0.41^{bc}	0.23^{e}
B. circulans										
KSB2	44^{e}	14^{bc}	11.39^{a}	3.85^{a}	2.38^{a}	0.65^{a}	1.44^{cd}	0.54^{b}	0.44^{b}	0.39^{a}
KSB4	38^{g}	13^{cd}	7.02^{ed}	1.06^{d}	2.22^{a}	0.52^{c}	1. 39^{d}	0.51^{b}	0.43^{b}	0.28^{d}
KSB7	42^{f}	13^{cd}	6.14^{fe}	2.21^{c}	1.89^{c}	0.56^{c}	1.09^{e}	0.46^{c}	0.42^{b}	0.18^{f}
Control(1)	43^{e}	13^{cd}	6.83^{d}	3.51^{b}	2.17^{b}	0.53^{c}	1.46^{c}	0.52^{b}	0.42^{b}	0.35^{b}
Control(2)	54.6^{b}	15^{b}	8.95^{c}	4.25^{d}	2.24^{a}	0.61^{b}	1.48^{c}	0.54^{b}	0.43^{b}	0.39^{a}

Control (1): Uninoculated tomato plants infested with *M. incognita* were supplemented with full-dose of chemical NPK fertilizers before transplantation.
Control (2): Uninoculated uninfested tomato plants were supplemented with full-dose of chemical NPK fertilizers before transplantation.
Means within the same column not followed by the same letter are significantly different ($P < 0.05$).

N P K contents of inoculated plants by any bacterial strain gave close or in some cases slightly higher determinations comparing with control 1 (Table 3). *P. polymyxa* NFB7, *B. megaterium* PSB2 and *B. circulans* KSB2 inoculations achieved the highest N P K contents. *P. polymyxa* NFB7 increased the N P K contents of shoots by 6.91 %, 65.86 %, 41.20 % and roots by 11.5 %, 11.9 %, 14.2 %, respectively. Bacterial inoculations induced tomato plants to endure the nematode infection, N P K contents of endured plants were in general close to those of uninfested plants (control 2).

Regarding to the effectiveness of the strains of bacterial biofertilizers in suppressing *M. incognita* reproduction, it could be observed that, most of bacterial inoculations had significantly reduced nematode population by comparing with the uninoculated infested plants (control 1), especially for the counts of hatched J_{2s}/root, females/root and J_{2s}/1kg soil (Table 4). The reduction in nematode population was more obvious after 60 days of inoculation by most inoculated treatments. However, inoculating *P. polymyxa* NFB7, *B. megaterium* PSB2 and *B. circulans* KSB2 resulted in the highest control effect comparing with control 1. After 30 days of inoculation, they recorded the highest reduction in numbers of females/root by 14.3%, hatched J_{2s}/root by 87.2% and in J_{2s}/1kg soil by 79.3%, respectively (Table 5). While, after sixty days of inoculation, the highest reduction by the three biofertilizers were obtained on numbers of hatched J_{2s}/root (95.8 %), females/root (63.75%) and J_{2s}/1kg soil (57.8%), respectively (Table 5).

The present pot experiment indicated that bacterial biofertilizers reduced significantly the ability of *M. incognita* to reproduce in soil which was a strain dependant. Several reports were focused on the benefits of rhizosphere colonizing bacteria especially nitrogen fixing bacteria (NFB), phosphate solubilizing bacteria (PSB) and potassium solubilizing bacteria (KSB) as biocontrol agents, some of important genera include *Agrobacterium*, *Alcaligenes*, *Bacillus*, *Clostridium*, *Desulfovibrio*, *Pseudomonas*, *Serratia*, *Paenibacillus* and *Streptomyces*. Application of these bacteria has given very promising results (28, 13, 14). Whole cultures and supernatants (exotoxins) of *Bacillus thuringiensis* subsp. *Brasiliensis* and *B. laterosporus* caused high mortality of *Meloidogyne javanica* in *in-vitro* bioassays and greenhouse tests on tomato (4). Neipp and Becker (19) reported that several strains of *B. megaterium*

were found to be effective against *Heterodera schachtii*, they reduced J_{2s} penetration of sugar beet by 38%-59%. Similarly, *B. megaterium* was reported to reduce by 50% the penetration of both *M. chitwoodi* and *Pratylenchus penetrans* in potato (2). Padgham and Sikora (20) found that the inoculation with *B. megaterium* reduced more than 40% of nematode penetration and gall formation compared with non–treated rice root. In a separate study, colonization of rice roots with *B. megaterium* decreased migration of *M. graminicola* to the root zone by nearly 60%, compared with that of nontreated roots. Exposure of *M. graminicola* eggs to secondary metabolites of *B. megaterium* reduced hatching eggs by over 60% compared with eggs not exposed to the bacteria.

Table 4. Effect of different biofertilizers strains inoculations on *M. incognita* infestation throughout 60 days post-inoculation in sandy soil.

Treatments	Time after bacterial inoculation (days)	*M. incognita* measurements				
		No. of galls / root	No. of egg masses / root	No. of hatched J_{2s} / root	No. of females / root	No. of J_{2s} /1kg soil
P. polymyxa						
NFB2	30	135^{f}	79^{e}	8790^{h}	64^{d}	16200^{i}
	60	56^{b}	31^{a}	990^{c}	18^{a}	4800^{b}
NFB5	30	76^{bc}	49^{bc}	6060^{g}	35^{b}	11880^{g}
	60	60^{b}	36^{a}	2580^{d}	22^{a}	9240^{d}
NFB6	30	89^{d}	62^{d}	6960^{g}	49^{c}	12240^{h}
	60	66^{bc}	35^{a}	3300^{e}	25^{ab}	5760^{c}
NFB7	30	63^{b}	40^{b}	4050^{f}	30^{b}	10560^{f}
	60	41^{a}	28^{a}	390^{b}	12^{a}	1452^{a}
B. megaterium						
PSB2	30	50^{a}	26^{a}	360^{a}	22^{a}	1560^{a}
	60	70^{c}	44^{b}	4980^{f}	29^{b}	11000^{e}
PSB4	30	67^{b}	41^{b}	690^{b}	50^{c}	4670^{d}
	60	89^{d}	59^{bc}	7980^{i}	52^{c}	17450^{h}
PSB5	30	61^{b}	35^{b}	660^{b}	$40b^{c}$	3480^{c}
	60	82^{d}	56^{b}	5970^{h}	33^{b}	14660^{g}
B. circulans						
KSB2	30	68^{b}	44^{b}	1320^{c}	15^{a}	2520^{b}
	60	88^{d}	49^{b}	5490^{g}	47^{c}	12650^{f}
KSB4	30	92^{d}	60^{d}	2280^{d}	22^{a}	8760^{e}
	60	112^{e}	80^{d}	296^{a}	55^{cd}	19230^{i}
KSB7	30	102^{e}	65^{d}	2970^{e}	26^{ab}	22600^{j}
	60	123^{f}	83^{d}	9900^{k}	70^{e}	26400^{j}
Control(1)*	30	71^{b}	46^{b}	4050^{f}	35^{b}	12180^{h}
	60	133^{g}	86^{d}	9360^{j}	80^{f}	30000^{k}

* Uninoculated tomato plants infested with *Meloidogyne incognita* were supplemented with full-dose of chemical NPK fertilizers before transplantation.
Means within the same column not followed by the same letter are significantly different ($P < 0.05$).

Table 5. Effect of the most efficient biofertilizer strains treatments on nematode population after 30 and 60 days of inoculation comparing with control 1*.

Treatments	Time after bacterial inoculation (days)	Reduction of nematode population				
		No. of galls / root	No. of egg masses / root	No. of hatched J_{2s} / root	No. of females / root	No. of J_{2s} /1kg soil
P. polymyxa NFB7	30	11.3	13	0	14.3	13.3
	60	69.2	67.4	95.8	85	95.16
B. megaterium PSB2	30	1.4	4.3	23	17.1	87.2
	60	47.4	48.8	46.8	63.75	63.3
B. circulans KSB2	30	4.2	4.3	67.4	57.1	79.3
	60	33.8	43	41.3	41.25	57.8

*Control 1: Uninoculated tomato plants infested with *M. incognita* were supplemented with full-dose of chemical NPK fertilizers before transplantation.

CONCLUSIONS

The present greenhouse pot experiment indicated that the individual inoculations of the bacterial biofertilizers *P. polymyxa* NFB7, *B. megaterium* PSB2 and *B. circulans* KSB2, enhanced significantly the growth of tomato plants, and suppressed the nematode population in infested plants roots and soil. Such effect was prolonged and increased through the 60 days after plant transplantation. Depending on these results, the combined addition of these bacterial cultures may be more effective to increase the nematode suppression in infested plants. The reliance on bacterial biofertilizers that have the ability to fix nitrogen or solubilize either insoluble phosphate or potassium, in the biological control of root-knot nematode helps in the same way to minimize the quantities of chemical nitrogen fertilizers used and may open the door for a wide application of cheap and more available crude sources of insoluble phosphate and potassium in agriculture.

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