Biocontrol Activity of Some Bacterial Genera Against *Root-Knot nematode, Meloidogyne incognita.*

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Abstract: Biological control is considered as new efficient method that becomes widely used for controlling plant parasitic nematodes, as aim to decrease the extent of environment degradation and the effect of the excessive toxic nematicides. So, this study was done to investigate the role of some bacterial genera as biocontrol agent against *Meloidogyne incognita*. The results of *in vitro* and *in vivo* experiments indicated that, all tested bacteria have a greatly significant effectiveness for suppressing *M. incognita*. *In vitro* results showed that all biovars of *Bacillus thuringiensis* and *Pseudomonas fluorescens* besides, *Rhizobium leguminosarum* can achieve *M. incognita* juveniles mortality to 100% at 72 hrs. Mixtures of bacteria genera varied in their effects, with regard to the presence of genus *Rhizobium* in the bacterial mixture give it an obvious lethal efficacy against *M. incognita* juveniles. *In vivo* study exhibited that, the most effective bacterium which can restrict and cease *M. incognita* reproduction was *Pseudomonas fluorescens* RR, followed by *Rhizobium leguminosarum*. Also, an enhancement in plant growth occurs. [Journal of American Science. 2010;6(10):321-328]. (ISSN: 1545-1003).

Key words: Biocontrol agent**,** bacteria, *Meloidogyne incognita, root-knot* nematodes, *Bacillus thuringiensis, Pseudomonas* spp., *Rhizobium leguminosarum*.

1. Introduction

The continuing world population pressure ensures the need to maximize the average yields of most major crops. So, increment utilization for the nematicides was occurred. Consequently, the environmental problems become aggravated which induced an importance of urgency to search for alternative methods for managing plant parasitic nematodes. One of these successful methods is the biological control, where bacteria represent an important useful group for microbial control that is capable for limiting nematode multiplication.

Chahal and Chahal (1993) and Zukerman et al. (1993) reported that *B. thuringiensis* suppressed population of *M. javanica* and *M. incognita.* In 1999 Ismail and Fadel evaluated three Egyptian-isolates of *B. thuringiensis*, they found positive significant correlations between reduction percentage in the nematodes populations and bacteria doses. Dhawan *et al.* (2004) evaluated four strains of *Bacillus thuringiensis*, they found that the mobility of *M. incognita* juveniles completely ceased after 24-hr. exposure in S and S/10 dilutions. However, all dilutions above S/25 were ineffective. Naghesh et al. in 2005 confirmed the importance of genus *Bacillus.* Their results indicated that, cell-free culture filtrates of *B. cereus* reduced egg hatching (90%) and caused 100% mortality of juveniles. These results were in harmony with that of Mekete *et al*. (2009) which indicate *in vivo* experiment the role of *Bacillus pumilis* and *Bacillus mycoides* in reducing the number of galls and eggmasses by 33 and 39%, respectively. Also, we can not ignore the importance of genus *Pseudomonas* against phytoparasitic nematodes as well as genus *Bacillus*. Hanna et al. (1999) evaluated *Pseudomonas fluorescens* for the control of *Meloidogyne incognita* on tomato plants. They found that the percentage of gall formation and root gall index were decreased when the bacteria were introduced prior to nematodes. Siddiqui and Shaukat, (2002) noted that *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* reduced *M. javanica* juvenile penetration into tomato plants. In 2004 El-Hamshary *et al*. found that *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* affected *M. incognita* juveniles survival *in vitro* study, and the mortality percentages of the nematode were dependent on the bacterial concentration and exposure time. Moreover, Siddiqui and Shaukat in 2004 concluded that fluorescent pseudomonads induce systemic resistance against *Meloidogyne javanica* via a signal transduction pathways which is independent of salicylic acid accumulation in roots. About *Corynebacterium* spp. El -Sherif *et al.* (1994) proved

that culture of bacteria. inhibited hatching of *Meloidogyne incognita* and was highly toxic to juveniles. Concerning *Rhizobium*, Sidiqqui *et al.* in 2001 noted that, this genus can significantly reduced egg hatching and caused mortality of *M. javanica in vitro*. Martina *et al.* (2002) concluded that the oligosaccharides of the core-region of *Rhizobium* are the main trigger of systemic resistance in potato roots towards *Globodera pallida*. In 2006 Siddiqui *et al*. demonstrated that, the using of *Rhizobium* plus *Pseudomonas putida* caused maximum reduction in *Meloidogyne javanica* galling and multiplication on lentil plants.

Accordingly, this current study aims to detect the role of some bacterial genera which used individually and their antagonistic or synergistic effect when used as mixtures against *Meloidogyne incognita* juveniles *in vitro*. In addition to, *in vivo* experiment was done to confirm the suppressive lethal effect of certain bacterial genera that were chosen from *in vitro* study to evaluate them as biocontrol agents to control *M. incognita.*

2. Material and Methods

To assert the nematostatic and nematocide effect of some bacterial genera on the pathogenicty of *Meloidogyne incognita* these experiments were carried out.

In vitro study

Bacterial isolates

Bacterial isolates used in this study are listed in table (1). Bacteria were routinely cultivated in Tryptic soy broth.

Table (1): Code and source of some bacterial isolates.

Isolates	Source of isolates	Code of isolates
Bacillus thuringiensis	Larvae of Lepidoptra	Biovar $_1$
Bacillus thurengiensis	Larvae of Lepidoptra	Biovar $_2$
Bacillus thurengiensis	Larvae of Lepidoptra	Biovar $_3$
Pseudomonas fluorescens	Wheat rhizosphere	WR
Pseudomonas fluorescens	Onion rhizosphere	OR
Pseudomonas fluorescens	Radish rhizosphere	RR
Pseudomonas fluorescens	Waste water	WW
Pseudomonas putida	Wheat rhizosphere	*P. putida*
Pseudomonas aeruginosa	Wheat rhizosphere	*P. aeruginosa*
Rhizobium leguminosarum	Broad bean roots	*R..leguminosarum*
Pseudomonas fluorescens	Wheat rhizosphere	Biovar I
Pseudomonas fluorescens	Wheat rhizosphere	Biovar II
Pseudomonas fluorescens	Wheat rhizosphere	Biovar III
Ochrobactrum anthropi	Polluted soil with oil	*O.anthropi*
Cellulomonas cellulans	Polluted soil with oil	*C.cellulans*
Mycobacterium marinum	Red sea water	*M.marinum*
Proteus mirabilis	Red sea water	*P.mirabilis*
Corynebacterium ulcerans	Red sea water	*C.ulcerans*

Identification of isolates

Different isolates were completely identified by using the Biology technique of epidemiology and etiology which concerns to the U.S. Naval Medical Research Unit (NAMRU) No.3 Cairo Egypt.

Preparation of culture

Heavy cell suspension of all isolates was prepared at rate 1.8 x 10^8 cells on tryptic soy broth and was kept at 4ºC until used *in vitro* as bioagent against *M. incognita.*

Effect of bacterial cells and filtrates on *M. incognita* juveniles (J2) mortality

Two separated experiments were done consequently to evaluate the effectiveness of some bacterial isolates on *M. incognita* juveniles (J_2) mortality under different exposure times (24, 48 and 72 hours). In the first experiment twenty bacteria isolates were tested through three sets; *Bacillus* set, *Pseudomonas* set and diversity set *besides, control set (media and distilled water).* Petri dishes were filled with 2 cm^3 of different tested bacterial cultures. Hundred freshly second stage juveniles (J_2) of *M. incognita,* reared in the greenhouse on eggplant roots as pure culture, were added to each Petri dish by a pipette. Each treatment was replicated tripled. All dishes were kept in the laboratory at room temperature in completely randomized design. Number of dead nematodes was account daily for periods; 24, 48 and 72 hours. Percentage mortality was calculated as: [mean of dead number of juveniles in treatment / total number of juveniles in treatment] x 100. At the end of the exposure times (72 h), the juveniles were transferred to distilled water for 24 hrs, to be ensure that no recovery will be occur. The second experiment was done by preparing mixtures from the previous bacterial isolates to evaluate their antagonism or synergism effect on *M. incognita* juveniles. Two cm^3 from mixed bacterial cultures were poured into Petri dishes in addition to control treatments as well as the previous experiment. Using a pipette, hundred freshly second stage juveniles (J_2) of *M. incognita* were added to each dish. Each treatment was replicated three times and arranged in completely randomized design under laboratory conditions. The results were recorded after 24, 48 and 72 hours as the previous manner.

In vivo experiment

From *in vitro* study, eight bacteria isoletes of highly suppressive effect against *M. incognita* were chosen for testing under greenhouse conditions besides control treatment (untreated infected plants). Plastic pots were filled with sterilized mixed soil from sand and clay as 2:1 (v/v). Each treatment was replicated thrice. Eggplant, *Solanum melongena* cultivar Baladi, seedlings (one month old) was added to the center of pots as one seedling for each pot.

After ten days from transplanting, 10 cm3 of bacterial cultures (cells and filtrates) were added to the soil. Forty eight hours later (until bacterial cells reach its maximum growth peak) plants were inoculated with 1000 freshly hatched second stage juveniles (J_2) of *M. incognita.* Pots were fertilized with recommended dose and kept at 20ºC ± 2ºC in complete randomized design. After two months plants were uprooted then galls and egg-masses were counted and their indices were recorded according to Sharma *et al*. 1994. Also, fresh weight of shoots and roots were registered. Data were statistically analyzed according to the procedure "Anova" reported by Senedecor and Cochran (1980). Treatments means were compared by the Duncan s Multiple Range Test at 5% level of probability.

3. Results and Discussion

The *in vitro* study

The effectiveness of certain bacteria isolates against *M. incognita* juveniles

Data presented in table (2) shows that, the suppressive activity of all bacterial isolates in the different three sets increase gradually with the increment of the exposure periods. All biovars of *Bacillus thuringiensis* exhibited high ability to kill second stage juveniles (J_2) as 100% after 72 hours. However, after 24 hours biovar$_3$ exhibited distinct effect, it recorded 90.33% as juveniles morality. During the two beginning exposure periods the weakly biovar was bv.$_1$ where it registered 70.33 and 88.67 as mortality percentage. It was clear that the nematostatic and nematocide effect of *Bacillus thuringiensis* mixture was highest after 24 and 48 hours which listed 92% and 94.33% respectively, then still constant until 72 hours. The remaining biovars (bv.$_2$ and bv.$_3$) their effect was nearly the same after 24 and 48 hrs. and after 72 hrs. mortality percentage reached 100% for all of them.

As concerning as *Pseudomonas* set, a great variation in the nematostatic and namadicidal effect was recorded. Five biovars of *Pseudomonas fluorescens* registered 100% mortality at 72 hrs. The most effective biovar was RR at all exposure times. A descending arrangement for these lethal biovars can be done as follows: RR> WW>WR>OR>bv.II. *Pseudomonas aeruginosa* exhibited some success to kill *M. incognita* juveniles after 72 hrs. (94.33%) comparing with *Pseudomonas putida* that recorded (87.33%). It can be notice *that Peudomonas fluorescens* bv.I was less effective (82.00%). It must be mentioned that *Pseudomonas* isolates mixture had best nematostatic and mematicidal effect during 24 and 48 hrs. However, it can not reach 100% juveniles mortality after 72 hrs.

Regarding to the diversity set, the topped genus was *Rhizobium leguminosarum*, where it gave distinctive results at all exposure times until reached 100% mortality after 72 hrs. On the other side, both of *Corynebacterium ulcerans* and *Mycobacterium marinum* were close to each other in their results after 72 hrs. According to the results occurred within *Cellulosmicrobium cellulans* and *Proteus mirabilis* they were considered as the less suppressive genera. It is important to notice that *M. incognita* juveniles still alive in the two treatments of control sets (media and distilled water).

According to Lindberg 1981, our *Bacillus* results, may be due to the nematicidal volatile products produced by this bacterium and characterized to include mainly the benzeneacetaldehyde, 2-nonanone, decanal, 2-undecanone and dimethyl disulphide, which were active against *M. incognita* juveniles. Also, fluorescent pseudomonads can produce a large number of toxic secondary metabolites such as; phenazines, indoles, compounds, phenylpyrroles and pterines.

Reitz *et al*. (2000) showed that lipopolysaccharides, LPS (lipid.A) which is defined as an integral part of the outer membrane of the cell, which can extracted from bacterial cultures has an antagonistisc agent against nematodes. So, this can explain the results obtained in studying the *Rhizobium* effect on *M. incognita.*

Moreover, it is known that LPS (lipid A) is an endotoxin that release from bacterial cell membrane after death (Tortora *et al.* 2009). Also, a recent confirmation was done by Bin *et al.* 2005, they mentioned that culture filtrates of rhizobacterium are heat stable and resistant to extreme pH values, which suggested that the antibiotic rather than protein might be responsible for the nematicidal activity**.**

The effectiveness of certain bacteria genera mixtures against *M. incognita* juveniles

Data as shown in table (3) exhibited how our tested bacteria can synergy or antagonize each other through different mixtures. It is noticeable to focusing genus *Rhizobium leguminosarum* where its presence in the mixture makes mortality percentage stable as well as the mixture of *Rhizobium* and *Corynebacterium* or *Rhizobium* and *Mycobacterium* (table3). Although the mixture of *Corynebacterium* and *Mycobacterium* give an antagonistic impact on juveniles mortality that registered 87% facing to 93% & 94.33% for their separately effect (table1).

While when *Rhizobium* added to the mixture of the previous two genera, a synergistic effect appears to record 94% juveniles mortality (*Mycobacterium*+*Rhizobium*) comparing to 87% (*Mycobacterium*+*Corynebacterium*).

Regarding to *Bacillus* mixture with other genera, its capability to cause juveniles death records nearly

(94%) after 72 hours but can reach to 100% as in single culture (table2) except when *Rhizobium* with *Mycobacterium* were added to the mixture its lethal effect can reach to 100% mortality at 72 hrs. as well as *Pseudomonas* mixture with the same two previous

Table (2): Mortality percentages of *Meloidogyne incognita juveniles* affected some bacterial isolates at different exposure periods.

Bacterial treatments	Juveniles (J_2) mortality percentages after different exposure periods		
	24 h	48 h	72 h
Bacillus Set			
Bacillus thuringiensis bv.$_1$	70.33	88.67	100
Bacillus thuringiensis bv. $_2$	85.33	90.67	100
Bacillus thuringiensis bv.$_3$	90.33	93.67	100
Bacillus thuringiensis bvs. mixture	92.00	94.33	94.33
Pseudomonas Set			
Pseudomonas fluorescens bv. WR	81.33	94.67	100
Pseudomonas fluorescens bv. OR	74.33	90.67	100
Pseudomonas fluorescens bv. WW	85.33	90.00	100
Pseudomonas aeruginosa	47.67	82.67	94.33
Pseudomonas fluorescens bv.I	44.00	72.00	82.00
Pseudomonas fluorescens bv.II	72.67	87.33	100
Pseudomonas putida	72.33	84.33	87.33
Pseudomonas bv.III	42.33	64.00	92.67
Pseudomones bv.RR	90.67	96.00	100
Pseudomonas spp.. mixture	81.67	88.00	94.33
Diversity Set			
Rhizobium leguminosarum	90.67	94.67	100
Ochrobactrum anthropi	47.33	73.33	90.00
Cellulosimicrobium cellulans	42.67	63.00	84.67
Proteus mirabilis	45.67	52.33	88.33
Mycobacterium marinum	52.33	73.00	94.33
Corynebacterium ulcerans	54.67	83.33	93.00
Control Set			
Media	0	0	0
Distilled water	0	0	0

genera. It is clear to note that an antagonism was occurred with *Rhizobium* genus in the mixture cultures which its effectiveness decreased to 94% than its effect in the single culture. However, when *Bacillus* mixture contains *Rhizobium* with *Corynebacterium* the mortality percentage of this mixture decreases to 96.33% after 72 h. respectively. While the mixture of *Bacillus*, *Corynebacterium* and *Mycobacterium* registered 95% juveniles mortality.

As the same trend as *Bacillus*, the addition of *Corynebacterium* and *Mycobacterium* to *Pseudomonas* mixture synergized juveniles mortality to 96.67% (table3) compared to 94.33% (table2) after 72 hrs. On the other hand, the using of *Rhizobium* in spite of *Corynebacterium* with *Mycobacterium*, the mortality percentage for this previous mixture reaches to 100%. When a displacement of *Mycobacterium* by *Corynebacterium* was done the mortality percentage diminished to 94.33%. Also, the mixture of *Corynebacterium* and *Pseudomonas* exhibited an antagonistic effect since the percentage death of mortality decreases to 92%. Control treatments (either media or distilled water) there was no any lethal effect on *M. incognita* juveniles.

From these previous results we can detect that *Rhizobium* has antagonistic effect against *M. incognita* juveniles when added to the growth-

promoting rhizobacteria (as mixture preparation) these results are in harmony with those of Siddiqui *et al*. (2006).

The *in vivo* study

To confirm *in vitro* study, an experiment under greenhouse conditions was done. Eight bacterial isolates were chosen from the laboratory results, which their efficacy to control *M. incognita* juveniles reach to 100% after 72 hrs. In general, data indicated that all chosen bacterial isolates had suppressive nematicidal effect on *M. incognita*, where galls formation and eggmasses production were significantly greatly affected.

The overtopped results gained was with genus *Pseudomonas fluorescens* (RR) which recorded 4 galls without any eggmasses per plant. The second rank was obtained by genus *Rhizobium leguminosarum* where it formed 4 galls per plant and produced 2 eggmasses only. A similarity in gall formation was noted between *Bacillus thuringiensis* biovar$_3$ and *Pseudomonas fluorescens* (WW), 6&7 galls/plant, respectively.
Also, another similarity was recorded between *Bacillus thuringiensis* biovar$_2$ and *Pseudomonas fluorescens* OR (8 galls/plant) and their product of eggmasses were approximately the same; 4 and 3, respectively. The two rested genera that exhibited less relatively nematicidal effect were *Bacillus thurigiensis* biovar$_1$ and *Pseudomonas fluorescens* WR where they gave 20 and 33 galls besides 8 and 4 eggmasses per plant, respectively. On the other hand, control treatment (untreated infected plants) registered 110 galls and 65 eggmasses per plant.

Table (3): Mortality pecentages of *Meloidogyne incognita juveniles* affected by some bacterial genera mixtures under different exposure periods.

Bacterial mixtures	Juveniles(J_2) mortality percentages after different exposure periods		
	24 h.	48 h.	72 h.
Mix Ps + Mix B + Co	44.67	81.33	92.67
Mix B + Co	65.00	85.00	94.33
Mix B + Rh	67.00	90.00	94.00
Mix Ps + Co	67.00	90.00	92.00
Mix Ps + Mix B + Rh	65.00	90.00	92.00
Mix B + My	59.00	85.67	94.00
Myco + Rh	72.00	94.00	94.00
Myco + Co	68.67	80.00	87.67
Co + Rh	70.00	83.33	94.00
My + Co + Rh	60.00	85.67	94.00
Mix B + Rh + Co	82.33	92.33	94.00
Mix Ps + Rh + Co	88.00	90.00	94.33
Mix Ps + Rh + My	89.00	95.67	100
Mix Ps + My + Co	89.00	94.67	96.67
Mix Ps + Rh + Co	87.67	94.67	94.67
Mix B + Rh + My	90.00	93.33	100
Mix B + My + Co	89.67	89.00	95
Mix B + Co + Rh	90.00	86.67	96.33
Rh + Co	86.67	88.67	95.00
Control (media)	0	0	0
Control (distilled water)	0	0	0

Ps = *Pseudomonas*, B = *Bacillus*, Co = *orynobacterium*, Rh = *Rhizobium*, My = *Mycobacterium*

The high suppressive effect of these chosen bacteria against *Meloidogyne incognita* may be attributed to the distinctive properties for these genera. As concerning as *B. thuringiensis* it is known that this bacteria produce chitinolytic enzymes, i.e., chitinases which is responsible for degrading chitin in cell walls of the nematode eggs also in nematode eggmasses, so

this nematode name as chitinolytic bacteria (Muzzarelli 1977). Chitin is a structural element (poly-B (1-4)-N-acetyl-D-Glucosamine) in the middle layer of nematode egg shell and in the exoskeletons of invertebrates. It is conceivable that the chitinases degraded eggshell chitin to be extended that juveniles were released prematurely. So, many of these juveniles were dead, probably because its development had been interrupted and they could not survive in the environment outside the egg (Wharton, 1980). Thus, there must be enough spores present to prevent a large proportion of *Meloidogyne* juveniles from invading roots, and to ensure that most of the nematodes which penetrate roots become infected, and therefore do not reproduce. Also, it is important to remember the mentioned effect of the volatile nematicidal products of genus *Bacillus* against juveniles and eggs, Huang *et al*. (2009).

Table (4): Effect of certain bacterial isolates on *Meloidogyne incognita* indices associated with eggplant roots, *Solanum melongena L*

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Bacterial treatments	Galls / plant	Reduction %	Eggmasses/ Plant	Reduction %	G / E .I.
Bacillus bv.$_1$	20 b	81.82	8 a	87.69	4 / 3
Bacillus bv.$_2$	8 c	92.73	4 b	93.85	3 / 2
Bacillus bv.$_3$	6 cd	94.55	4 b	93.85	3 / 2
Pseudomonas fluorescens (OR)	8 c	92.73	3 b	95.38	3 / 2
Pseudomonas fluorescens(WW)	7 cd	93.64	2 bc	96.92	3 / 2
Pseudomonas fluorescens(WR)	33 a	70.00	4 b	93.85	6 / 2
Pseudomonas fluorescens(RR)	2 e	98.18	0 c	100.00	2 / 1
Rhizobium leguminosarum	4 de	96.36	2 bc	96.92	2 / 2
LSD $_{05}$	3.568		2.235		
Untreated infected plant (control)	110	-	65	-	9 / 7

Gall index, Eggmass index: 1 = no galls or eggmasses, 2 = 1-5, 3 = 6-10, 4 = 11-20, 5 = 21-30, 6 = 31-50, 7 = 51-70, 8 = 71-100 and 9 >100 galls or eggmasses / plant. In each column, values followed by the same letter (s) are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Related to the greatly impact of genus *Rhizobium* against *M. incognita*, Reitz et al. in 2002 demonstrated that the oligosaccharide of the core-region of this genus are the main resistance roots towards in potato roots *Globodera*. Also, Siddiqui *et al*. (2006) reported the effect of *Rhizobium* to the greater colonization and siderophore production. Besides, this genus shows high potential in suppressing the root-knot nematodes with interfere with the host finding processes of the nematodes or produces metabolite that are toxic to the nematodes. In addition to, induce systemic resistance takes place according to this genus.

Regarding, fluorescent pseudomonads, Wescott and Kluepfel (1990) showed that all these previous bacterial species inhibited egg hatch whereas it can produce exotoxic compounds as a result of cellular metabolism, and also can affect nematode juveniles. This antagonistic effect against *M. incognita* is due to the permeability changes of juvenile cuticle which is characterized by its selective permeability and this effect is more pronounced with molting inside eggs.

It is important to not neglect the indirect role for the almost of these bacteria genera as inducers for plant systemic resistance toward soil pathogens i.e. phytonematodes, in addition to their direct function that exhibited as competition and antibiosis, Buchenauer 1998.

Concerning plant growth, a significant augmentation of both shoot and root fresh weights were illustrated in figure1.Three bacterial treatments encouraged significantly shoot fresh weight than the

un-infected plants, healthy plants, these treatments can be ranked in descending order as follows: *Pseudomonas fluorescens* WR> *Rhizobium leguminosarum*> *Pseudomonas fluorescens* RR. On the other hand, *Pseudomonas fluorescens* WW decreased significantly shoot weight than the healthy plants, while the shoot weight values of the other treatments were insignificant.

As well as shoot weight, a significant variation in root fresh weight occurred. The best value was exhibited by *Bacillus thuringiensis* bv.$_3$, while *Rhizobium leguminosarum* recorded the minimum fresh root weight value.

We can conclude that microbial residents of the rhizosphere represent a potential reservoir of biological control agents that can capable of challenger nematode multiplication. So, these previous tested bacterial genera can be considered as potential source for suppressing plant parasitic nematodes by drawing them through integrated pest magement programs. It is noticeable to note that rhizosphere of antagonistic plants may be useful sources of potential biological control agents for pathogenic nematodes, (Kloepper *et al*.,1992). This note was confirmed through the suppressive results of *Pseudomonas fluorescens* RR on *M. incognita*, whereas this biovar was isolated from radish rhizosphere and it is known that radish is not preferable host for *M.* spp.

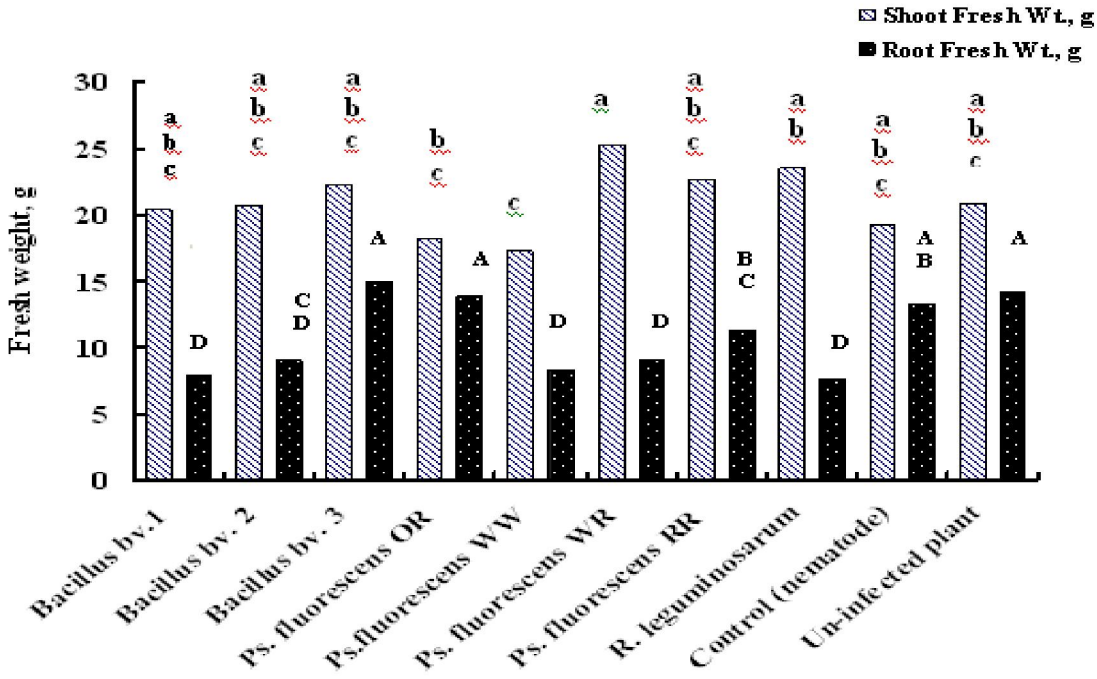

Figure (1): Effect of some bacterial isolates on shoot and root fresh weights of eggplant, *Solanum melongena* L., affected by *Meloidogyne incognita.*

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