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IMPACT OF RHIZOSPHERE ANTAGONISTIC BACTERIA AND UREA FERTILIZER ON ROOT KNOT NEMATODE (*MELOIDOGYNEINCOGNITA*) UNDER GREEN HOUSE CONDITION

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ABSTRACT

The effect of rhizosphere bacteria on activity of root-knot nematode (*Meloidogyne incognita*) was investigated under laboratory and greenhouse conditions. Rhizosphere bacteria were isolated from roots of plants infected with root knot nematode. The effect of the rhizosphere bacteria on second stage juvenile mortality of *M. incognita* under laboratory conditions was studied after 24, 48 and 72 hs. Three bacterial isolates, *Serratia* sp., *Pseudomonas fluorescens*CHA0, and *Pseudomonas putida*, that caused the higher mortality of second stage juvenile, were used in greenhouse experiment. They were evaluated with and without application of 50 mg urea fertilizer per kg of soil, on tomato (*Solanumly copersicum*) infected with *M. incognita*. All treatments had positive effect on plant growth parameters, and decreased the nematode-related parameters, such as number of gall, egg and egg mass, as well as the reproduction factor. Moreover, application of *Serratia* sp. in combination with urea fertilizer had the greatest effect, as compared to other treatments. The experiments were repeated and similar results were obtained.

Key words: Nitrogen, *Pseudomonas fluorescens* CHA0, *Pseudomonas putida*, Tomato, *Serratia* sp.

INTRODUCTION

Tomato is one of the most important vegetable crops in the world. Root knot nematodes are known as the most damaging plant parasitic nematodes worldwide. They are one of the main limitation of producing theadequate food with a reduction of approximately 5% of agricultural products. Nematodes of the genus *Meloidogyne*, cause more than 50% crop losses to tomato (Mukhtar *et al.* 2014). More than 2,000 plant species have been reported as hosts of root knot nematodes (Hussey and Janssen, 2002). In Iran, *M. javanica* and *M.incognita*, are two most dominant species of root knot nematodes among four main species, respectively (Akhiani *et al.* 1984).

Traditional methods of nematode management include sanitation measures, fallow, crop rotation, cultivation of resistant varieties, application of nematicides and etc. Chemicals are used as a common strategy to control pathogens and reduce damage; however, due to environmental hazards, their application should be reduced. Application of biological agents is an important way to control the plant parasitic nematodes. Due to the presence of some microorganisms such as the plant growth promoting rhizobacteria (PGPR), the rhizosphere has the potential capability to protect itself against diseases caused by nematodes. It has been described as external plant defense against root pathogens attack (Siddiqui and Mahmood, 2001). The process of exudation from active root and releasing the organic compounds are key factors in rhizosphere activity. Rhizosphere is affected by hostplant characteristics, soil factors, environmental conditions, planting techniques and soil microbial interactions. The various microorganisms can help in absorption of phosphorus, nitrogen, microelements and water and improve the plant growth and also production efficiency (Compant *et al.* 2005). Rhizosphere fungi and bacteria can assist to provide a proper defense against soil-borne plant pathogens. The mechanisms of action of these fungi and bacteria include inhibition of penetration, reduction of reproduction, and also delay in egg hatching and movement of nematodes (Sikora and Fernandez, 2005). Macro and micro fertilizers are frequently used to provide the essential elements for optimal plant growth. Charegani *et al.* (2010) showed that the use of ideal fertilizer levels improved the plant growth and reduced the damage caused by root knot nematodes.

Bacteria which are the most active microorganisms of the soil, are the most common rhizosphere microorganisms. They play a fundamental role in all biological reactions of the soil. Enormous experiments have been carried out in association with the use of bacteria as biocontrol agents against *Meloidogyne* spp. For instance, the individual and combined application of the bacterium *Pseudomonas aeruginosa* and the fungus *Memnoniellae chinata* reduced activity of root pathogenic fungi such as *Macrophominaphaseolina*, *Fusariumsolani* and *Rhizoctoniasolani* as well as *M.javanica* on mung bean (*Vignaradiata*) (Siddiqui *et al.* 2000). Moreover, these results showed that the use of

urea and potash fertilizers increased the antagonistic effect of *P. aeruginosa* and *M. echinata* in the soil.

Application of Nemaless, a commercial biofertilizer contains a strain of the bacterium *Serratiamarcescens*, as soil treatment, led to decrease thenematode population (El-Nagdi and Youssef, 2004; Noweer and Hasabo, 2005).

The results of a study on chemical fertilizers showed that application of NPK fertilizer or urea fertilizer increased plant growth factors and also decreased the gall formation of *M. javanica* (Irshad *et al.* 2006).

In an experiment, effects of two biocontrol agents, *Pseudomonas aeruginosa* and *Bradyrhizobium japonicum*, as well as two inorganic fertilizer, urea and potash fertilizer, individually and in combination, were investigated on tomato infected with *M. javanica*. The maximum shoot growth was observed in treatments with *P. aeruginosa* and both fertilizers. Besides, the maximum fresh weight of shoot was obtained in using both bacteria in combination with both fertilizers (Parveen *et al.* 2008).

The objective of the present study was to investigate the effect of application of plant growth-promoting rhizobacteria and urea fertilizer, individually and in combination with each other, on tomato growth parameters and the reproduction rate of root knot nematode (*M. incognita*).

MATERIALS AND METHODS

Preparation of nematode: The single egg mass of root knot nematode, *M. incognita* was propagated on root of tomato, the variety "Early Urbana". Nematode eggs were extracted by the technique of Hussey and Baker (1973), then they were incubated at 27°C for 72 hrs, to prepare second-stage juveniles (J2).

Preparation of bacteria: Bacteria were isolated from rhizosphere of tomatoes and cucumbers in farms and greenhouses. The roots were cut into 2-3 cm pieces, then, one gram of the root pieces were transferred to test tubes containing 10 ml distilled water and were shaken for 1 hr. The tubes were kept aside for 30 min, at room temperature. Serial dilutions of the supernatant were cultured on Plate Count Agar (PCA) and Petri plates were incubated at 25°C for 48 hrs. Based on the color and the appearance, colonies were purified and single colonies were cultured on Nutrient agar (NA)(Holt, 2002).Two bacterial isolates, 26 (*P. fluorescens* CHA0) and 27 (*P. putida*), were provided by Department of Plant Protection, Shiraz University.

Effect of antagonistic bacteria on mortality of second-stage juveniles of *Meloidogyneincognita*: One ml of suspension of each bacterial isolate at a concentration of 1 x 10^8 cells/ml, was added to one ml of 70 newly hatched second-stage juveniles of nematode. Petri dishes were incubated at 27°C and distilled water was used in control petri dishes. After 24, 48 and 72 hrs, the number of dead larvae were counted under a stereomicroscope and percentages of mortality were calculated(Meyer *et al*. 2000).

Identification of Bacteria: Three isolates of bacteria which caused the maximum mortality of nematode J_2,were identified by the method of Holt (2002). The used diagnostic tests were gram stain, oxidase, catalase, levan formation, fluorescent pigmentation on KB agar, metallic green sheen on EMBagar, yellow colonies on YDC agar medium, growth at 40°C, anaerobic growth, acid production from sugars, gelatin hydrolysis, starch hydrolysis and tween 80 hydrolysis.

Greenhouse experiments: Four leaf seedlings of Early Urbana tomato variety were transplanted to plastic pots containing 1.5 kg mixture of peat moss and vermiculite (1:1 ratio),and 50 mg urea fertilizer per kg of soil were added to pots(U). After 24 hrs, 15 ml of 1 x 10^8 cells/ml of suspension of each one of three selected bacterial isolates were added to the rhizosphere of root tomato in pots(B). After seven days(Siddiqui and Shaukat, 2003), 2000 eggs and J2s of nematode per kg of soil were added to pots(N).Therefore, sixteen treatments were included N, U, B1, B2, B3, U+N, B1+N, B2+N, B3+N, B1+U, B2+U, B3+U, B1+N+U, B2+N+U andB3+N+U. Control treatment was without any bacteria, nematode and urea (C).Plants were maintained in greenhouse with 30/25°C day/night temperatures and16 hours of daylight, and fertilized weekly with a 20-20-20 (N-P-K) fertilizer solution. Plants were irrigated with distilled water. After 60 days, plant growth parameters (plant height, shoot fresh weight, shoot dry weight and root fresh weight)and the nematode parameters (number of galls, egg masses and eggs and reproduction factor) were evaluated. To calculate the reproduction factor (RF), final population of nematode (number of female + number of eggs + number of J_2 in soil) divided to the initial population (2000). The experiments were repeated and similar results were obtained.

Statistical analysis: The laboratory and greenhouse experiments were carried out in completely randomized designs with three and six replicates, respectively. In greenhouse experiment, a factorial arrangement of4 x 2 x 2 (bacterial isolate, urea, nematode inoculation) was used. Data were analyzed with the help of SAS software (SAS, 2004) and treatments were compared with Duncan's multiple range test ($P \leq 0.05$) (Little and Hills, 1978).

RESULTS

Effect of antagonistic bacteria on mortality of the second-stage juveniles of *Meloidogyneincognita*: Results of *In vitro* study showed that the isolates number 20 (isolated from rhizosphere), 26 and 27 (provided by Department of Plant Protection, Shiraz University) caused maximum mortality of second-stage juveniles of nematode, respectively (Table 1).These isolates were used in greenhouse experiments.

Identification of Bacteria: The results showed that the isolates number 2 and 3 belonged to the genera *Pantoea* and *Bacillus*, respectively. The isolate number 20 belongs to *Serratia*, a genus of plant pathogenic bacteria

Greenhouse experiments

Plant growth parameters: Application of bacteria andurea fertilizer, aloneorin combination with each other, significantly increased the height and dry weight of shoot in all of tomato plants, inoculated with nematode, as compared to control treatment. Combined application of urea fertilizer and bacterial isolates significantly increased the shoot fresh weight in inoculated plants, as compared to application of only the bacterial isolates. The maximum root fresh weight were obtained in nematode inoculated plants (Table 2and 3).

Nematode parameters: The number of galls, egg masses, eggs as well as the reproduction factor significantly decreased by using bacterial isolates alone and also in combination with urea fertilizer, but the rate of nematode reproduction had more reduction in treatments with combination of bacteria and urea fertilizer .The best results belonged to the treatment with combination of *Serratia* sp. and urea fertilizer (Table 4and 5).

Table 1. Comparison of the effect of the antagonistic bacterial isolates on percentage mortality of second-stage juveniles of *Meloidogyne incognita* after 24, 48 and 72 hours in three replications

Number of bacterial isolates	24 h	48 h	72 h
1	19.3 (1.2)^{de}	45 (2.1)^{de}	59.7 (1.2)^{fgh}
2	30 (0.9)^{a}	60.3 (0.3)^{a}	75.7 (0.7)^{b}
3	29.7 (0.7)^{a}	61.7 (1.5)^{a}	76 (1.5)^{b}
4	16 (1.5)^{f}	49.7 (0.3)^{c}	63.3 (1.2)^{ef}
5	15.3 (0.7)^{fg}	46.3 (0.7)^{cd}	70.7 (0.3)^{c}
6	12.7 (0.3)^{ij}	41.7 (1.2)^{efg}	57.7 (0.7)^{ghi}
7	18.3 (0.3)^{e}	37 (1.2)^{hij}	51.3 (1.5)^{jkl}
8	22.3 (1)^{c}	46.3 (1.2)^{cd}	61.7 (1.5)^{fg}
9	25 (1.2)^{b}	39.7 (0.7)^{fgh}	64.7 (0.9)^{ef}
10	13.7 (0.7)^{hij}	34.3 (0.7)^{ijk}	49 (1)^{kl}
11	16.3 (1)^{f}	44.7 (0.3)^{de}	64.7 (1.5)^{ef}
12	20.3 (0.3)^{d}	50.7 (0.9)^{c}	67.3 (1.2)^{cde}
13	16 (0.6)^{f}	33.7 (1.2)^{ijk}	49.3 (1.5)^{kl}
14	14 (0.9)^{hig}	31.3 (1.5)^{kl}	47.7 (0.3)^{l}
15	15.7 (0.9)^{f}	38 (0.6)^{ghi}	55.3 (0.3)^{hij}
16	20.3 (1.2)^{d}	42.7 (1.2)^{def}	64.3 (1.9)^{ef}
17	18 (0.6)^{e}	46.3 (0.7)^{cd}	70 (1.2)^{cd}
18	16 (0)^{f}	41 (0.6)^{efgh}	56 (1.5)^{hij}
19	19.3 (1.5)^{de}	33.7 (1.2)^{ijk}	52.3 (1.9)^{jkl}
20	24.3 (1.5)^{b}	55.7 (0.9)^{b}	84.7 (1.9)^{a}
21	15 (0.6)^{fgh}	38 (0.6)^{ghi}	61.3 (1.2)^{fg}
22	12.3 (1)^{j}	27.3 (0.9)^{l}	47.3 (0.3)^{l}
23	18.7 (0.7)^{e}	31.7 (0.9)^{k}	53.7 (1.5)^{ijk}
24	15.7 (0.7)^{f}	34.7 (1.3)^{ijk}	65 (0.6)^{def}
25	16 (0.9)^{f}	33 (1.5)^{jk}	62.3 (0.9)^{efg}
26	30.7 (0.9)^{a}	55.3 (1.8)^{b}	80.3 (0.9)^{ab}
27	24.7 (0.7)^{b}	59.3 (1.2)^{ab}	81.7 (2.7)^{a}
Control	3.3 (0.6)^{k}	11 (0.6)^{m}	23.3 (1.2)^{m}

*Means and standard errors (in parentheses).Different letters represent values within columns that are significantly different at P<0.05 based on the Duncan's multiple range test.

Table 2. Effect of the antagonistic bacterial isolates and urea fertilizer on growth of Early Urbana cultivar of tomato infected with *Meloidogyne incognita* in six replications in the first experiment.

Treatments*			Root fresh weight (g)	Shoot dry weight (g)	Shoot fresh weight (g)	Plant length (cm)
Bacterium	**Urea**	**Nematode**				
No bacteria	No urea	No inoculum	4.38 (0.17)^{j}	3.88 (0.16)^{bc}	33.13 (1.19)^{bc}	23.27 (0.42)^{d}
No bacteria	No urea	Inoculated	5.83 (0.13)^{a}	1.86 (0.1)^{e}	21.46 (0.8)^{e}	17.81 (0.32)^{g}
No bacteria	Urea	No inoculum	4.56 (0.1)^{hi}	4.26 (0.11)^{ab}	36.9 (1.99)^{ab}	26.21 (0.16)^{ab}
No bacteria	Urea	Inoculated	5.48 (0.18)^{b}	2.51 (0.12)^{d}	25.7 (0.65)^{d}	19.5 (0.32)^{f}
P. fluorescens CHA0	No urea	No inoculum	4.83 (0.23)^{ef}	3.61 (0.12)^{c}	32.97 (1.84)^{bc}	23.23 (0.61)^{d}
P. fluorescens CHA0	No urea	Inoculated	5.24 (0.1)^{cd}	2.75 (0.15)^{d}	25.1 (1.34)^{d}	19.2 (0.56)^{f}
P. fluorescens CHA0	Urea	No inoculum	4.63 (0.13)^{gh}	4.46 (0.18)^{ab}	37.2 (1.17)^{ab}	26.53 (0.27)^{a}
P. fluorescens CHA0	Urea	Inoculated	4.98 (0.13)^{e}	3.50 (0.15)^{c}	29 (1.14)^{c}	21.46 (0.63)^{e}
P. putida	No urea	No inoculum	4.84 (0.12)^{ef}	3.99 (0.13)^{b}	33.37 (1.17)^{bc}	23.57 (0.55)^{cd}
P. putida	No urea	Inoculated	5.22 (0.07)^{cd}	3.1 (0.28)^{cd}	25.13 (0.37)^{d}	18 (0.32)^{g}
P. putida	Urea	No inoculum	4.98 (0.1)^{e}	4.69 (0.45)^{a}	37.6 (1.23)^{ab}	25.93 (0.42)^{b}
P. putida	Urea	Inoculated	5.35 (0.07)^{bc}	3.82 (0.31)^{c}	30.1 (1.34)^{c}	22.9 (0.8)^{d}
Serratia sp.	No urea	No inoculum	4.45 (0.23)^{ij}	4.09 (0.25)^{b}	34.93 (2.01)^{b}	24.27 (0.67)^{c}
Serratia sp.	No urea	Inoculated	5.16 (0.06)^{d}	3.11 (0.14)^{cd}	25.47 (0.54)^{d}	19.6 (0.38)^{f}
Serratia sp.	Urea	No inoculum	4.77 (0.25)^{fg}	4.83 (0.29)^{a}	38.27 (1.3)^{a}	26.47 (1.18)^{a}
Serratia sp.	Urea	Inoculated	5.31 (0.09)^{bcd}	4.16 (0.42)^{b}	34.86 (1.39)^{b}	22.6 (0.28)^{d}

*Means and standard errors (in parentheses). Different letters represent values within columns that are significantly different at $p<0.05$ based on the Duncan's multiple range test.

Table 3. Effect of the antagonistic bacterial isolates and urea fertilizer on growth of Early Urbana cultivar of tomato infected with *Meloidogyne incognita* in six replications in the second experiment.

Treatments*			Root fresh weight (g)	Shoot dry weight (g)	Shoot fresh weight (g)	Plant length (cm)
Bacterium	**Urea**	**Nematode**				
No bacteria	No urea	No inoculum	4.78 (0.14)^{i}	4.02 (0.2)^{bc}	33.79 (0.99)^{bc}	23.18 (0.22)^{d}
No bacteria	No urea	Inoculated	6.64 (0.13)^{a}	2.12 (0.1)^{e}	22.07 (0.92)^{e}	18.15 (0.36)^{g}
No bacteria	Urea	No inoculum	5.08 (0.15)^{h}	4.33 (0.13)^{ab}	37.54 (1.68)^{a}	26.36 (0.43)^{ab}
No bacteria	Urea	Inoculated	5.9 (0.16)^{b}	2.72 (0.07)^{d}	26.19 (0.95)^{d}	20.06 (0.12)^{f}
P. fluorescens CHA0	No urea	No inoculum	5.32 (0.19)^{ef}	3.85 (0.18)^{c}	33.24 (1.73)^{bc}	23.74 (0.52)^{cd}
P. fluorescens CHA0	No urea	Inoculated	5.7 (0.11)^{cd}	2.83 (0.1)^{d}	25.52 (1.55)^{d}	19.69 (0.81)^{f}
P. fluorescens CHA0	Urea	No inoculum	5.15 (0.13)^{gh}	4.46 (0.12)^{ab}	37.66 (1.33)^{a}	26.75 (0.33)^{a}
P. fluorescens CHA0	Urea	Inoculated	5.45 (0.16)^{e}	3.73 (0.19)^{c}	29.61 (1.12)^{c}	21.92 (0.68)^{e}
P. putida	No urea	No inoculum	5.31 (0.09)^{ef}	4.16 (0.1)^{b}	33.6 (1.54)^{bc}	23.97 (0.71)^{cd}
P. putida	No urea	Inoculated	5.74 (0.12)^{cd}	3.31 (0.2)^{cd}	25.73 (0.78)^{d}	18.43 (0.29)^{g}
P. putida	Urea	No inoculum	5.5 (0.14)^{e}	4.67 (0.39)^{a}	38.12 (0.93)^{a}	26.13 (0.37)^{b}
P. putida	Urea	Inoculated	5.83 (0.06)^{bc}	3.88 (0.19)^{c}	30.57 (1.08)^{c}	23.26 (0.74)^{d}
Serratia sp.	No urea	No inoculum	4.9 (0.2)^{i}	4.26 (0.21)^{b}	35.47 (1.31)^{b}	24.39 (0.47)^{c}
Serratia sp.	No urea	Inoculated	5.64 (0.1)^{d}	3.35 (0.11)^{cd}	26.04(0.77)^{d}	20.23 (0.74)^{f}
Serratia sp.	Urea	No inoculum	5.24 (0.19)^{fg}	4.86 (0.24)^{a}	38.24 (1.28)^{a}	27.11 (0.43)^{a}
Serratia sp.	Urea	Inoculated	5.8 (0.05)^{bc}	4.68 (0.32)^{a}	36.5 (1.84)^{ab}	23.55 (0.41)^{d}

Table 4.Effect of the antagonistic bacterial isolates and urea fertilizer on the reproduction of *Meloidogyne incognita*on Early Urbana cultivar of tomato in six replications in the first experiment

Treatments*		Number of Gall/root	Number of Egg masses/root	Number of Eggs/root	RF
Bacterium	**Urea**				
No bacteria	No urea	158 (4.85) ^{a}	150 (7.68)^{a}	21143 (884)^{a}	7.15 (0.34)^{a}
No bacteria	Urea	138 (5.67)^{a}	119 (4.16)^{b}	14510 (430)^{b}	4.92 (0.23)^{b}
P. fluorescens CHA0	No urea	95 (7.61)^{b}	75 (2.28)^{c}	10763 (499)^{c}	3.64 (0.16)^{c}
P. fluorescens CHA0	Urea	75.7 (3.2)^{c}	65.3 (5.05)^{d}	9856 (621)^{c}	3.33 (0.15)^{c}

P. putida	No urea	79.7 (5.55)^{c}	63 (4.71)^{d}	9125 (350)^{d}	3.09 (0.21)^{cd}
P. putida	Urea	68 (4.13)^{cd}	54 (3.33)^{e}	8443 (241)^{e}	2.85 (0.12)^{d}
Serratia sp.	No urea	78.7 (7.19)^{c}	64.3 (1.9)^{d}	8926 (330)^{de}	3.02 (0.2)^{cd}
Serratia sp.	Urea	58 (2.5)^{d}	47.7 (2.24)^{f}	7506 (426)^{f}	2.54 (0.08)^{e}

*Means and standard errors (in parentheses). Different letters represent values within columns that are significantly different at $p<0.05$ based on the Duncan's multiple range test.

Table 5.Effect of the antagonistic bacterial isolates and urea fertilizer on the reproduction of *Meloidogyne incognita*on Early Urbana cultivar of tomato in six replications in the second experiment

Treatments*		Number of Gall/root	Number of Egg masses/root	Number of Eggs/root	RF
Bacterium	**Urea**				
No bacteria	No urea	176 (12.6) ^{a}	152 (14.52)^{a}	20436 (690)^{a}	6.92 (0.16)^{a}
No bacteria	Urea	142 (14.8)^{a}	113 (12.8)^{b}	13988 (410)^{b}	4.74 (0.08)^{b}
P. fluorescens CHA0	No urea	90.6 (3.48)^{b}	73.3 (1.4)^{c}	11020 (396)^{c}	3.73 (0.26)^{c}
P. fluorescens CHA0	Urea	81.2 (6.6)^{c}	67 (7.64)^{d}	10250 (728)^{c}	3.46 (0.2)^{c}
P. putida	No urea	77 (9.1)^{c}	66 (6.18)^{d}	8980 (212)^{d}	3.04 (0.28)^{cd}
P. putida	Urea	64.4 (6.66)^{cd}	53 (3.33)^{e}	8320 (126)^{e}	2.81 (0.19)^{d}
Serratia sp.	No urea	72.6 (4.8)^{c}	60.2 (3.4)^{d}	8700 (406)^{de}	2.94 (0.11)^{cd}
Serratia sp.	Urea	56 (6.2)^{d}	44 (6.9)^{f}	7150 (566)^{f}	2.42 (0.1)^{e}

DISCUSSION

The findings reported here showed that all of three bacterial isolates (*Serratia* sp., *P.putida* and *P. fluorescens* CHA0) with or without using 50 mgurea per kg of soil enhanced the plant growth and decreased the nematode reproduction. It seems that these bacteria accumulate in the plant rhizosphere and improve the plant growth. Moreover, the bacteria prevent the root from nematode attack. These rhizobacteria can promote the plant growth, directly (with nitrogen fixation, hormone production and increase of nutrient uptake) or indirectly (with production of siderophore and antibiotics, competition for food and space, induction of systemic resistance and decreasing the environmental stress) (Compant *et al.* 2005). It should be noted that, depending on the bacterial isolate and other environmental conditions, the growth rate of plant is different (Glick *et al.* 2001).

Based on the earlier studies, use of appropriate levels of NPK fertilizers have good effects on plant growth factors (Irshad *et al.* 2006). The use of inorganic fertilizers can improve the plant defense and reduce the development of plant pathogens. The findings of the present study were consistent with previous studies which showed the effects of urea fertilizer (Babatola and Oyedunmade, 1992; Noweer and Hasabo, 2005; Irshad *et al.* 2006), bacteria (Siddiqui and Shaukat, 2003; El-Nagdi and Youssef, 2004; Siddiqui and Shakeel, 2007; Mohammed *et al.* 2008), or combined application of the both (Siddiqui *et al.* 2000; Parveen *et al.* 2008) on plant growth factors.

Different mechanisms of nematode damage reduction are reported. For instance, fluorescent *Pseudomonas* bacteria which are one of the most dominant, active and effective rhizobacteria can decrease the damage of pathogen directly and/or indirectly. It is noteworthy that different isolates of these bacteria have different effects on nematode (Siddiqui and Mahmood, 2001). The inhibitory effect of *P. florescence* CHA0 on root knot nematode have been shown (Jahanbazian *et al.*, 2015a and 2015b; Moradi *et al.*, 2015). However, other bacteria may reduce damage caused by nematode by production of siderophore and secondary metabolites and also increasing of the host tolerance or induction of systemic resistance (Kokalis-Burelle and Samac, 2003). The results of the present study were also consistent with the findings of Becker *et al.* (1988), Khan and Kounsar (2000), Meyer *et al*. (2000), Siddiqui and Mahmood (2001), Siddiqui*et al*. (2001), Kokalis-Burelle and Samas (2003), Kaskavalci *et al*. (2006) and Majzoob*et al*. (2012) who reported that application of bacteria led to reduce the number of egg masses and galls of nematode. Besides, using appropriate levels of fertilizers reduced reproduction rate and damage caused by root knot nematodes. Moreover, improving the agronomic conditions for plant growth is an important factor for increasing the plant tolerance to nematodes (Charegani *et al.*, 2010). The results of present study were confirmed by results of Rodriguez-Kabana (1986), Noweer and Hasabo (2005) and Irshad *et al*. (2006) who reported that application of chemical fertilizers reduce the damage of root knot nematodes. Our finding were consistent with previous studies which showed that using combination of antagonistic bacteria and urea fertilizer decreases the nematode population (Siddiqui *et al.* 2000; Parveen *et al.* 2008).

In spite of the positive results which observed in this study, it should be emphasized that biological control may reduce pathogen populations or their virulence but

rarely can causes complete elimination of pathogens in a region.

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