Rhizobacteria cooperative effect against *Meloidogyne javanica* in rhizosphere of legume seedlings

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Summary Root-knot nematodes are major pests of legume fields in Iran. This research evaluated the effect of *Rhizobium leguminosarum* bv. *phaseoli* and *Pseudomonas fluorescens* CHA0 (stand alone and combination treatment) on galling and reproduction of root-knot nematode, *Meloidogyne javanica*, in legum (chickpea, bean, lentil, pea) seedling rhizosphere, and the growth properties of the host plants. The legumes seeds were sown in 1kg sterilized sandy loam soil. Inocula were 5 J₂/g of soil, in the case of the nematode, while considering the bacteria 1×10⁷ cfu/kg soil. A treatment of nematicide (cadusafos) was performed, as a commonly used nematicide in Iran, at 2g/kg soil. Two months after inoculation, the following parameters were recorded: the number of knots, egg masses and reproduction factor of the nematode, bacterial nodules per root, and growth properties of seedlings in the treatments (control, nematode, nematode+nematicide, and nematode+rhizobacteria). The greatest bacterial effect on the control of the nematode was observed in the rhizosphere of the bean treatments. Inoculation with *Rhizobium* in the soil decreased the galling on the legumes' roots, and the combined inoculation with *Pseudomonas* and *Rhizobium* resulted in a higher decrease of the galling.

Additional keywords: antagonism, biocontrol, management, nodule, *Pseudomonas*, root-knot nematode

Introduction

Legumes are well recognized for their seeds, which are rich of proteins, minerals and vitamins (Bojnanska *et al.*, 2012), and their significant role in maintaining productivity in agricultural systems (Dashadi *et al.*, 2011). After cereals, leguminous crops are the most important crops in Iran (Alizadeh *et al.*, 2013).

Root-knot nematodes (*Meloidogyne* spp.) are known as a major constraint to leguminous plants, causing yield losses in heavily infested fields (Onyeke and Akueshi, 2012). They are very important plant parasitic nematodes due to their worldwide distribution, wide host range, many species and interactions with root rot and wilt fungi and bacteria (Nicol, 2002; Perry and Moens, 2006). *Meloidogyne javanica* (Treub) Chitwood has a broad host range and great dispersal, making plant roots more vulnerable to root rot pathogens

in Iran (Hosseini Nejad and Khan, 2001).

A number of biocontrol agents have demonstrated high potency in reducing damage by nematodes in different crops (Gupta *et al.*, 2015). Natural enemies of nematodes are successful in reducing the activity of plant parasitic nematodes through parasitism, secretions (toxin, antibiotic and enzyme), competition, inducing systemic resistance and stimulation of host resistance (Tian *et al.*, 2007). There are many examples of research in the field of biological control of plant parasitic nematodes, especially root-knot nematodes, due to the application of various microorganisms, including bacteria (Ashoub and Amara, 2010; Jang *et al.*, 2016; Ntalli and Caboni, 2012; Saeedizadeh, 2016; Sokhandani *et al.*, 2016).

In a rhizosphere, significant and intensive interactions take place between the plant, soil and microorganisms (Panizzon *et al.*, 2016). Plant growth-promoting rhizobacteria (PGPR) are free-living bacteria that may impart beneficial effects on plants (Nihorimbere *et al.*, 2011). PGPR enhance emergence, colonize roots and stimulate overall plant development. The presence of rhizobia in a

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rhizosphere may protect the host root from damage induced by pathogens (Lipiec and Glinski, 2011). Therefore, the handling of rhizosphere by inoculation of PGPR has shown considerable promise for biocontrol of plant pathogens (Ahemad and Kibret, 2014).

Rhizobacteria-nematodes interactions have been extensively considered to manage plant-parasitic nematodes (Shaikh *et al.*, 2016; Siddiqui and Akhtar, 2009). Rhizobacteria genera such as *Pasteuria*, *Pseudomonas* and *Bacillus* have a respectable potential as biological control agents against plant parasitic nematodes (Beneduzi *et al.*, 2012). Most frequently studied antagonistic rhizobacteria to affect the nematodes are *Bacillus subtilis*, *Bacillus sphaericus* and *Pseudomonas fluorescens* (Ashoub and Amara, 2010; Muthulakshmi *et al.*, 2010). On the other hand, infestation by nematodes is known to suppress bacterial nodulation and consequently decrease nitrogen fixation in leguminous plants (Siddiqui *et al.*, 2013).

Rhizobium forms an endosymbiotic nitrogen-fixing association with roots of legumes and it can also act as a biocontrol agent on root-knot nematodes exhibiting the capacity to colonize plant roots and the nematode galls (Siddiqui and Akhtar, 2009). *P. fluorescens* CHA0 has been introduced as biocontrol agent of root-knot nematodes. The bacterium has been able to suppress the nematodes populations through the production of secondary metabolites (Siddiqui *et al.*, 2006).

Taking into account the importance of root-knot nematodes and the need for biological control methods, the scope of the research was to evaluate the effect of *R. leguminosarum* bv. *phaseoli* and *P. fluorescens* CHA0 as rhizobacteria against *M. javanica* in legumes seedling rhizosphere, and growth properties of the plants.

Materials and Methods

Preparation of nematode inoculum

Infested roots with root-knot nematodes were sampled from a population maintained

in the fields of tomato plants (cv. Walter) in the southern Tehran city, Iran. Extraction and preparation of *M. javanica* inoculum were applied according to the Hussey and Barker (1973) using the single egg mass method. According to the morphological and morphometrical characteristics of body and perineal pattern, the nematodes were initially identified (Aydinli and Mennan, 2016). The egg mass of the species was added to the rhizosphere of tomato seedling cv. Local; the nematode was multiplied and second stage juveniles (J_2) were finally obtained in a glasshouse (10 hours lighting and $25\pm 2^\circ\text{C}$ temperature). Infected tomato roots bearing large egg masses were incubated in water for three days at $28\pm 2^\circ\text{C}$ and hatched J_2 were collected and counted. The nematode inoculum level of the treatments was determined as $5 J_2/\text{g}$ of soil (Gharabadiyan *et al.*, 2013).

Preparation of bacterial inoculum

P. fluorescens CHA0 and *R. leguminosarum* bv. *phaseoli* were obtained from the culture collection of the Department of Plant Pathology, Shahed University, Tehran, Iran.

P. fluorescens CHA0

The bacterium was prepared as a bacterial inoculum suspension according to Weller and Cook (1983). A full loop of 48-hour culture of the bacterium on King's medium B (King B) was transferred to a flask containing 100ml King B liquid medium and incubated for 48 hours on a shaker (120 RPM) at 27°C . Bacterial suspension was centrifuged for 10 min @ 6000g, and washed for 2-3 times with a natural salt solution (NaCl 0.14M) to remove residual nutrient medium. Bacterial cells were extracted by recentrifuging and suspending to a solution of $1\times 10^7\text{cfu/ml}$, which was prepared using the standard curve spectrophotometrically in a carboxymethyl cellulose solution. To develop the optimal amount of inoculants, this solution was diluted by Tween80 (0.02%) (Weller and Cook, 1983).

R. leguminosarum bv. *phaseoli*

To activate the bacterium, 1ml of liquid

medium of YMA (Yeast Mannitol Agar) under sterile conditions was added to bacteria. For bacterial proliferation, one inoculation loop of the bacterium dissolved in 100ml of liquid YMA and incubated on an orbital shaker @ 200 RPM for 24 h. For concentration determination of the bacterial suspension, the Rhizobium was cultured in a liquid medium of YMA and incubated on an orbital shaker @ 200 RPM for 24 h at 25°C (Sadovinkova *et al.*, 2003). The culture was centrifuged @ 1000g for 10 min and was re-suspended with phosphate buffer. If the optical density (OD₆₂₀) of this solution was 0.1, it means 108 cfu/ml. To develop the optimal amount of inoculants (107cfu/ml), this solution was diluted by phosphate buffer (Bai *et al.*, 2003).

Plant material and inoculation

The seeds of four legumes, including chickpea (*Cicer arietinum* L., cv. Sefid), bean (*Phaseolus vulgaris* L., cv. Bahman), lentil (*Lens culinaris* Medikus, cv. Gachsaran), and pea (*Pisum sativum* L., cv. Shamshiri) were obtained from Department of Plant Protection, Shahed University, Tehran, Iran. They were surface-sterilized by 70% ethanol for 2 min and 1% sodium hypochlorite for 5 min, and rinsed 5 times with distilled water (Wang and Oyaizu, 2009). Four seeds were sown in 1kg steam-sterilized sandy loam soil in the greenhouse; and one week after germination thinning was done to save a single seedling per pot. The plants were inoculated at the trifoliate emergence stage. The seedlings were kept in a greenhouse (natural light and 25±2°C).

The nematode inoculum was set at 5 J₂/g soil, the bacteria at 1×10⁷ cfu/kg soil, and the nematicide RUGBY® 10 G (Cadusafos), as a reference product, at 2g/kg soil (Safdar *et al.*, 2012). The inocula were individually applied to the pots in a volume of 5ml suspension in a ring from around the plant root to a depth of 3cm. Control comprised of healthy untreated seedlings (free of the nematode and bacteria inocula). Each pot plant represented one replication.

Measurement of growth properties of the plants

Plants were uprooted 60 days after inoculation and the root systems were gently washed. The plants were cut with a knife above the base of the root emergence zone. Excess water was removed by blotting before weighing shoots and roots separately.

Evaluation of *M. javanica* activity in the plants

According to the proposed method of Hussey and Janssen (2002), activity of *M. javanica* was evaluated in the control and treated plants as the number of egg masses and knots per root, and final population and reproduction factor per pot. At first, the roots of the plants were washed with tap water and drained on blotting paper. To specify the number of egg masses, roots were divided into 3-4cm parts and egg masses were stained with Floxin solution B (0.15g/l of water), bleached with lactophenol and counted under a dissecting microscope (Hussey and Janssen, 2002). For determination of the nematode population in the pot soil, a 250g subsample of well mixed soil was processed according to Jenkins (1964), known as centrifuge or sugar flotation method. The number of nematodes in prepared suspension was used to calculate the population of nematodes per pot (1kg soil). To count the number of juveniles, eggs and females inside the root, 1g sub-sample of the root was macerated in a Waring blender and counts were done on the suspension thus obtained. The numbers of nematodes present in the root were calculated by multiplying the numbers of nematodes present in 1g of the root with the total weight of the root. The reproduction factor was calculated utilizing the equation $RF = Pf/Pi$, which is RF: reproduction factor, Pf: final population of the nematode, and Pi: primary population of the nematode (Osei *et al.*, 2010).

Statistical analysis

This experiment was based on a Complete Randomized design with four legumes species and 12 treatments for the

management of *M. javanica*, including: control (with no the inoculum of the nematode and the bacteria), rhizobium (R), pseudomonas (P), nematode (N), rhizobium + pseudomonas (RP), rhizobium + nematode (RN), pseudomonas + nematode (PN), rhizobium + pseudomonas + nematode (RPN), rhizobium + nematode (a week later) (Rn), pseudomonas + nematode (a week later) (Pn), rhizobium + pseudomonas + nematode (a week later) (RPn), and nematode + nematicide (NN). The treatments were replicated four times. The data were subjected to one way analysis of variance (ANOVA). Mean treatments were compared using a Duncan multiple range test. All analyses were performed by using SAS software version 9.1.

Results

The experiment revealed significant differences between the various treatment and crop combinations (ANOVA, $p \leq 0.05$). The greatest bacterial effect on the control of the nematode was observed in bean treatments (Table 1). The plant roots in the *Pseudomonas* treatment produced less galls than those in the nematode treatment (Table 1). However, higher galling occurred on the roots treated with *Rhizobium*. Combined application of *Rhizobium* and *Pseudomonas* caused a lower reduction in galling and multiplication of the nematode than the nematicide (Table 1).

The level of the nematode activity (gall, egg mass and reproduction factor) was higher on the treatments of lentil plants followed by bean, pea and chickpea (Table 1). The lowest nematode activity was noted in the nematicide treatment (Table 1). Inoculation of *Pseudomonas* and *Rhizobium* reduced nematode activity, especially in the treatments Pn, Rn and RPn, where the inoculation with the nematode was conducted one week after the bacteria inoculation (Table 1). The treatments of *Pseudomonas* have been more effective than *Rhizobium* on reducing the nematode activity (Table 1).

The bacterial nodulation of *Rhizobium* on

the legume roots infested by the nematode was lower than on those at the *Rhizobium* treatment (R) (Table 2). Also the infested roots by the nematode, in the treatments which received the co-inoculation of *Pseudomonas* and *Rhizobium*, produced fewer nodules than those in the stand alone treatment of *Rhizobium* (Table 2). The descending order of the number of bacterial nodules obtained was: chickpea>pea>bean>lentil (Table 2). The nematode was more effective in reducing bacterial nodulation by *Rhizobium* in comparison with *Pseudomonas*. The number of bacterial nodules obtained in the treatments was in descending order: R>RP>RPn>Rn>RPN>RN (Table 2).

Data analysis of plant growth properties indicated a difference between the fresh weight of root and shoot in all treatments, compared with the control (Figures 1 and 2); this difference was positive in all treatments, except the treatment with the nematode (N). The greatest increase (positive difference) of root and shoot fresh weight were obtained in the treatments with the bacteria (*Pseudomonas* and *Rhizobium*) inoculation, either with or without the nematode (*Meloidogyne*) inoculations (P, R, RP) (Figures 1, 2). The highest increase in fresh weight of root and shoot was observed in the treatments RP, R and P (descending order). The inoculation with the nematode resulted in the least weight of roots and shoots. The highest reduction in root and shoot weight was obtained in the nematode treatment of lentil and bean (Figures 1 and 2).

Discussion

Our results indicate that inoculation with *Rhizobium* and *Pseudomonas* in stand-alone application can reduce *Meloidogyne* activity in the rhizosphere of the legumes seedlings. *Rhizobium* decreases the nematode galling while the nematode reduces the bacterial nodules on the roots of the plants and consequently decreases nitrogen fixation in leguminous plants (Siddiqui *et al.*, 2013). Other studies have also indicated an increased

Table 1. Activity of *Meloidogyne javanica* (galls, egg mass, reproduction factor) (mean $\pm$ StE) on root of legume seedlings (chickpea, bean, lentil, pea) after inoculation with *Rhizobium leguminosarum* bv. *phaseoli* and *Pseudomonas fluorescens* CHA0.

	Host	Treatment*									
		N	PN	Pn	RN	Rn	RPN	Rpn	NN		
Gall†	Bean	29.5 $\pm$ 1.258 d	24.5 $\pm$ 0.957 gh	19.75 $\pm$ 1.315 ijkl	27.5 $\pm$ 1.19 def	22.5 $\pm$ 0.866 hi	21.25 $\pm$ 1.493 ij	15.75 $\pm$ 0.854 nop	0.75 $\pm$ 0.479 q		
	Chickpea	26.5 $\pm$ 0.645 efg	18.25 $\pm$ 1.109 klmn	15.5 $\pm$ 0.645 op	20.75 $\pm$ 1.25 ijk	17.5 $\pm$ 0.645 lmno	17 $\pm$ 0.816 mno	13.75 $\pm$ 0.854 p	1.5 $\pm$ 0.645 q		
	Lentil	40.25 $\pm$ 0.479 a	36.25 $\pm$ 0.629 b	27.5 $\pm$ 0.866 def	37.5 $\pm$ 0.645 b	32.75 $\pm$ 0.946 c	33.25 $\pm$ 0.629 c	21.5 $\pm$ 1.19 ij	1.5 $\pm$ 0.645 q		
Egg mass	Pea	29 $\pm$ 1.08 de	22.5 $\pm$ 1.323 hi	19.25 $\pm$ 1.315 jklm	26 $\pm$ 0.408 fg	25.5 $\pm$ 0.645 fg	20.5 $\pm$ 0.645 ijk	16 $\pm$ 0.707 nop	0.5 $\pm$ 0.289 q		
	Bean	19.5 $\pm$ 1.555 e	16 $\pm$ 1.472 ghi	10.25 $\pm$ 0.854 jk	18 $\pm$ 0.707 efg	15.5 $\pm$ 0.645 hi	15 $\pm$ 0.408 i	7.75 $\pm$ 0.479 lm	0.25 $\pm$ 0.25 o		
	Chickpea	18.25 $\pm$ 0.854 ef	8.5 $\pm$ 0.645 klm	5 $\pm$ 0.707 n	10.25 $\pm$ 0.629 jk	7.75 $\pm$ 0.946 lm	7 $\pm$ 0.707 m	4.75 $\pm$ 0.479 n	0.5 $\pm$ 0.289 o		
RF	Lentil	30.5 $\pm$ 0.645 a	25.75 $\pm$ 0.75 c	17.5 $\pm$ 0.645 efgh	27.75 $\pm$ 0.479 b	23 $\pm$ 1.08 d	23.5 $\pm$ 0.645 d	14.75 $\pm$ 0.946 i	0.75 $\pm$ 0.479 o		
	Pea	19 $\pm$ 1.08 ef	15.5 $\pm$ 1.323 hi	9.75 $\pm$ 0.629 jkl	17.75 $\pm$ 0.854 efg	17.25 $\pm$ 0.479 fgh	11.25 $\pm$ 0.629 j	9 $\pm$ 0.408 klm	0.25 $\pm$ 0.25 o		
	Bean	1.25 $\pm$ 0.016 de	0.9125 $\pm$ 0.042 gh	0.5975 $\pm$ 0.015 lm	1.215 $\pm$ 0.013 e	0.81 $\pm$ 0.009 j	0.79 $\pm$ 0.009 j	0.525 $\pm$ 0.013 n	0.00375 $\pm$ 0.004 p		
	Chickpea	1.1575 $\pm$ 0.039 f	0.5875 $\pm$ 0.005 lm	0.22 $\pm$ 0.011 o	0.5775 $\pm$ 0.006 lmn	0.575 $\pm$ 0.017 lmn	0.545 $\pm$ 0.006 mn	0.2275 $\pm$ 0.009 o	0.0075 $\pm$ 0.005 p		
	Lentil	1.9025 $\pm$ 0.054 a	1.3475 $\pm$ 0.023 c	0.9375 $\pm$ 0.009 g	1.405 $\pm$ 0.01 b	1.285 $\pm$ 0.013 d	1.265 $\pm$ 0.023 de	0.7225 $\pm$ 0.049 k	0.00875 $\pm$ 0.006 p		
	Pea	1.2825 $\pm$ 0.009 d	0.83 $\pm$ 0.025 ij	0.6 $\pm$ 0.011 lm	0.955 $\pm$ 0.014 g	0.875 $\pm$ 0.006 hi	0.6075 $\pm$ 0.018 l	0.545 $\pm$ 0.023 mn	0.0025 $\pm$ 0.003 p		

* Treatment abbreviations are as follows:

N (nematode), PN (pseudomonas + nematode), Pn (pseudomonas + nematode (a week later)), RN (rhizobium + nematode), Rn (rhizobium + nematode (a week later)), RPN (rhizobium + pseudomonas + nematode), RPN (rhizobium + pseudomonas + nematode (a week later)) and NN (nematode + nematode).

† Activity of *Meloidogyne javanica* was evaluated as the number of galls (knots) and egg masses per root; and RF (reproduction factor) per pot.• Values shown are the mean of four replications (n = 4) $\pm$ standard error. Means of each trait (Gall, Egg mass and RF) that do not share the same letter are significantly different ($P \leq 0.05$).

Table 2. Number of bacterial nodules of *Rhizobium leguminosarum* bv. *phaseoli* (Mean±StE) on root of legume seedlings.

Plant†	Treatment*					
	R	RN	Rn	RP	RPN	RPn
Bean	17±0.41 abc	9.25±0.63 k	13±0.91 fghi	14±0.41 defg	12.25±0.85 ghi	14.25±0.85 defg
Chickpea	19±0.91 a	11.75±0.85 hij	17.5±0.65 ab	17.5±0.87 ab	15.5±0.65 bcd	17±1.08 abc
Lentil	15±0.41 cdef	6.5±0.65 l	11±0.71 ijk	12.25±0.85 ghi	11.75±0.85 hij	12.25±0.85 ghi
Pea	17.5±0.65 ab	10±0.41 jk	12.75±1.11 ghi	15.5±0.65 bcde	13.75±0.63 efgh	16±0.41 bcd

* Treatment abbreviations are as follows:

R (rhizobium), RN (rhizobium + nematode), Rn (rhizobium + nematode (a week later)), RP (rhizobium + pseudomonas), RPN (rhizobium + pseudomonas + nematode) and RPn (rhizobium + pseudomonas + nematode (a week later)).

† Activity of *Rhizobium leguminosarum* bv. *phaseoli* was evaluated as the number of nodules per root.

• Values shown are the mean of four replications (n = 4) ± standard error. Means that do not share the same letter are significantly different ($p \leq 0.05$).

Figure 1. Effect of inoculation with *Rhizobium leguminosarum* bv. *phaseoli* and *Pseudomonas fluorescens* CHA0 on root fresh weight of legume seedlings infested with root-knot nematode *Meloidogyne javanica*, as compared with control♦

† Each value has been calculated using the equation: $\frac{\text{Treatment mean} - \text{control mean}}{\text{control mean}} \times 100$

* Completely randomized design with four replications. Treatment abbreviations are as follows:

N (nematode), P (pseudomonas), PN (pseudomonas + nematode), Pn (pseudomonas + nematode (a week later)), R (rhizobium), RN (rhizobium + nematode), Rn (rhizobium + nematode (a week later)), RP (rhizobium + pseudomonas), RPN (rhizobium + pseudomonas + nematode), RPn (rhizobium + pseudomonas + nematode (a week later)) and NN (nematode + nematicide).

♦ Control comprised of healthy untreated seedlings (free of the nematode and bacteria inocula).

Comparisons were carried out using Duncan test ($\alpha = 0.05$); means that do not share the same letter are significantly different ($p \leq 0.05$).

Figure 2. Effect of inoculation with *Rhizobium leguminosarum* bv. *phaseoli* and *Pseudomonas fluorescens* CHA0 on shoot fresh weight of legume seedlings infested with root-knot nematode *Meloidogyne javanica*, as compared with control*

† Each value has been calculated using the equation: $\frac{\text{Treatment mean} - \text{control mean}}{\text{control mean}} \times 100$

* Completely randomized design with four replications. Treatment abbreviations are as follows: N (nematode), P (pseudomonas), PN (pseudomonas + nematode), Pn (pseudomonas + nematode (a week later)), R (rhizobium), RN (rhizobium + nematode), Rn (rhizobium + nematode (a week later)), RP (rhizobium + pseudomonas), RPN (rhizobium + pseudomonas + nematode), RPN (rhizobium + pseudomonas + nematode (a week later)) and NN (nematode + nematicide).

† Control comprised of healthy untreated seedlings (free of the nematode and bacteria inocula). Comparisons were carried out using Duncan test ($\alpha=0.05$); means that do not share the same letter are significantly different ($p \leq 0.05$).

effect of nematodes on the bacterial nodulation on host roots (Ugwuoke and Eze, 2010). Rhizobia are reported to produce toxic metabolites inhibitory to many plant pathogens (Hmissi *et al.*, 2011). However, in our case, it seems that competition for the host root tissue is the most important concern between the antagonizing organisms (*Rhizobium* and *Meloidogyne*).

Pseudomonas can inhibit galling and reproduction of the nematode. These findings coincide with other studies suggesting that *P. fluorescens* CHA0 can be considered as a common biological agent to root-knot nematodes (Saeedizadeh, 2016; Siddiqui *et al.*, 2005).

The combined use of *Rhizobium* and *Pseudomonas* was more beneficial in increasing plant growth and reducing nem-

atode multiplication, probably due to positive interaction. This positive interaction is evident by the increase in nodulation and the reduction in nematode multiplication. Some strains of *R. leguminosarum* and *P. fluorescens* have been used in combination to control root-knot nematodes affecting legumes (Ashoub and Amara, 2010). In our trial, positive cooperative effect of *P. fluorescens* CHA0 and *R. leguminosarum* bv. *phaseoli* on the plants has been demonstrated by the suppression of activity of *M. javanica* as well as by the host growth enhancement of the legume seedlings. Bayat *et al.* (2014) showed that the root fresh weight of legumes increased significantly upon rhizobial inoculation. Similar results have been reported in *Vicia faba* (Dashadi *et al.*, 2011). In *faba* bean, individual inoculation and co-in-

oculation of *Rhizobium* and *Azotobacter* increased most of growth indicators such as root dry weight. Co-inoculation of *Rhizobium* and *Azotobacter* could improve some of the faba bean growth properties under water stress conditions (Dashadi *et al.*, 2011).

Several management strategies have been developed for *Meloidogyne* spp., nevertheless their usage in subsistence agriculture is restricted. Chemical compounds used to control nematodes are too expensive, and non-chemical practices, such as crop rotation, are limited because of the wide host range of the root-knot nematodes. Biological control agents, such as *Pseudomonas* and *Rhizobium*, can allow significant a reduction in the use of nematicides and chemical fertilizers through their numerous direct or indirect mechanisms of action.

In conclusion, according to our results, using a co-inoculum of *P. fluorescens* CHAO and *R. leguminosarum* bv. *phaseoli* can be more effective against *M. javanica*, compared with stand-alone application of either bacterium, and will reduce the level of its pathogenic activity. Biological control of the nematode using the bacteria in a joint application cannot be as effective as the commercial synthetic pesticides in the greenhouse. However, the prolonged use of biocontrol agents will reduce the nematode population in long-term, minimizing the risk for development of nematicide resistance, adverse effects to the environment and humans, which are associated with the chemical control. Moreover, bacterial nodulation induced on the legumes root inoculated with *R. leguminosarum* can improve plant growth besides the contribution to biological control of the nematode on the legume roots.

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Συνδυαστική δράση ριζοβακτηρίων κατά του κομβονηματώδη *Meloidogyne javanica* στη ριζόσφαιρα ψυχανθών

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Περίληψη Οι κομβονηματώδεις είναι σημαντικοί εχθροί στις καλλιέργειες ψυχανθών στο Ιράν. Η παρούσα εργασία αξιολόγησε την επίδραση των βακτηρίων *Rhizobium leguminosarum* bv. *phaseoli* και *Pseudomonas fluorescens* CHA0 (απλές και συνδυασμένες επεμβάσεις) στο σχηματισμό κόμβων και την αναπαραγωγή του κομβονηματώδη, *Meloidogyne javanica*, στη ριζόσφαιρα ψυχανθών (ρεβίθι, φασόλι, φακή, μπιζέλι), καθώς και στην ανάπτυξη των φυτών-ξενιστών. Οι σπόροι των ψυχανθών σπάρθηκαν σε 1kg αποστειρωμένου αμμοπηλώδους εδάφους. Έγινε εμβολιασμός με 5 προνύμφες δευτέρου σταδίου (J2) του νηματώδη/g εδάφους, και 1×10^7 CFU/kg εδάφους για τα βακτήρια. Πραγματοποιήθηκε μία εφαρμογή με νηματωδοκτόνο που περιέχει δ.ο. cadusafos, ως ένα συνήθως χρησιμοποιούμενο νηματωδοκτόνο στο Ιράν, σε 2g/kg εδάφους. Δύο μήνες μετά τον εμβολιασμό, καταγράφονταν οι ακόλουθες παράμετροι: ο αριθμός των κόμβων στις ρίζες και των ωόσακκων, ο ρυθμός αναπαραγωγής του νηματώδους, ο αριθμός φυματίων στη ρίζα, και διάφορες παράμετροι ανάπτυξης των φυτών στις επεμβάσεις (μάρτυρας, νηματώδης, νηματώδης + νηματωδοκτόνο, νηματώδης + ριζοβακτήριο). Η μεγαλύτερη βιολογική δράση των βακτηρίων κατά του νηματώδους παρατηρήθηκε στη ριζόσφαιρα των φυτών φασολιού. Ο εμβολιασμός με *Rhizobium* στο έδαφος μείωσε το σχηματισμό κόμβων στις ρίζες των ψυχανθών, και ο συνδυασμένος εμβολιασμός με *Pseudomonas* και *Rhizobium* οδήγησε σε μεγαλύτερη μείωση σχηματισμού κόμβων.

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