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Research Article

THE EFFECTS OF HELPING BACTERIA (*PSEUDOMONAS SPP.*) IN NITROGEN GREEN BEANS FIXATION AND NODULATION WITH *RHIZOBIUM LEGUMINOSARUM BY PHASEOLI*

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ABSTRACT

Some- bacteria settle in the rhizosphere of legume plants and enhance the performance of ribosome bacteria to nitrogen fixation and nodulation. In this paper, we used four isolated from two species of Pseudomonas containing *P.putida*, *P.fluorescens* Chao, *P.Flouresence* Tabriz, *P.flouresence* B119 and *Rhizobium leguminosarumbv.phaseoli*. In a factorial experiment with complete randomized blocks were used 5 levels of helping bacteria(*Pseudomonas* spp.) and two rhizobium levels, four replicates were employed. Jamaran418 green bean was utilized as host plant. At the end, nodulation, growth and plant's nitrogen indexes were measured. The results showed that all above mentioned helping bacteria enhance the growth and nodulation performance of green bean. It should be said that *P.putida* had the highest effect on the green bean nodulation increase along with rhizobium (130%) followed by *P.fluorescens* Tabriz, *P. fluorescens* Chao and *P.fluorescens* B119, (83, 63 and 17%, respectively). Also, we observed 45, 33, 22 and 8% performance increase under the effect of *P.putida*, *P.* fluorescens Chao, P. fluorescens Tabriz and P. fluorescens B119, respectively.

Keywords: helping bacteria, Rhizobium, green bean, nodulation, Pseudomonas

INTRODUCTION

The widespread use of chemical fertilizers and pesticides is discussed worldwide because of their adverse effects on the environment and human health. The natural process of rhizobacteria is a suitable way for plant protection (DelipKoomar, 2001).

Private and public investment has increased to mass production of biological fertilizers and especially microbiological ones after more attention on these matters. Rhizobium inoculants are one of the most important and widespread of these fertilizers which are used for different legume plants.

Along with the extensive researches in Iran and worldwide to introduce the most efficient ways of these bacteria performance, further research is underway to improve the quality of these fertilizers. Some evidences show that the bacteria as*Pseudomonas, Streptomyces* and *Bacillus* as a helping one to enhance nodulation and fixation performance of *Rhizobium* bacteria (Ming and Alexander, 1988). The results are patent and the *Rhizobium* inoculants producers companies access them.

Some PGPR(*Plant growth-promoting rhizobacteria*) bacteria with synergistic effect on Rhizobium can enhance the nodulation and fixation of nitrogen in legumes. According to conducted researches, most of the bacteria which are effective in the field belong to phosphate solubilizing microorganisms (Pisney and Azcon, 1987; Ming and Alexander, 1988). Rasi poor and Ali Asgarzade (2002) investigated the role of four phosphates solubilizing bacteria, *P.flourescens*, *P.putida* and *Aeromonashidrophyla* with *Bradyrhizobiumjaponicum* on soya nodulation and performance and concluded that these bacteria enhance phosphorus-absorption, compared with control group. The results show that *P.putida* treatment had the most effect on soya nodulation, nitrogen fixation and phosphorus absorption, comparing with the other treatments only with *Bradyrhizobiumjaponicum*.

Ali khani and SalehRastin (2006) announced that the phosphate solving bacteria enhance the performance of different rhizobia in Iran soils.

Rozras et al. (2002) in a farm experiment with soya investigated the mutual effects between soya bacteria and **Pseudomonas piotida**phosphate solving bacteria. They reported a significant increase in stem nodulation; nitrogen fixation and soya shoot weight. Also, in a farm experiment in India, the effect of phosphate solving bacteria, *Pseudomonas staritaand Bradyrhizobiumjaponicum*on nodules and plant dry weight were investigated. The combined inoculation with *Pseudomonas strait* resulted in a significant increase of nodule dry weight and number (Vasole et al., 2002).

Rhizosphere bacteria do not always enhance the nitrogen fixation and nodulation by legumes and sometimes can have negative or neutral effects on these indexes. Pan et al. (2002) investigated the effects of two rhizosphere bacteria (Serratiaproteamawlans and Serratiali*q*uefaciens along with

Bradyrhyzobiumjaponicum Strain 5326 on two soyabean species. It was showed a significant difference on plant performance treated with Rhizosphere bacteria, but these bacteria had no significant effect on the nitrogen fixation and nodulation. AnjelaBergern et al. (2001) had conducted an experiment in Sweden and investigated the effects of *P.putida* strain A313 in symbiosis with pea Rhizobium bacteria and the results showed reduction of nitrogen fixation.

We should consider these items to increase Rhizobium inoculants production in the country as a good replacement of nitrogen fertilizers. Also, It is necessary an extensive researches due to the lack of their efficiency in farm. This study introduces the most effective helping bacteria.

MATERIALS AND METHODS

Proliferation of used bacteria:

In this experiment, we used *P.flourescens* B119 (H2), *P.flourescens*Chao (H1) (Tehran Plant Pests and Diseases Research Institute), *P. flourescens*Tabriz (H3) (Tabriz university Soil Biology Laboratory) and *P.putida* (H4) (Tabriz isolated) as the helping bacteria and a control ones (H0) only with *R. leguminosarum*bv.phaseoli. King B broth was used for *Pseudomonas* proliferation and YEM broth (Yeast Extract-Mannitol B***roth***) for Rhizobium proliferation.

Pseudomonas spp. flasks were groth for 2 days in a shaker incubator at 28 ˚C and 120 rpm and *Rhizobium* growth was obtained in this condition for 3 days.

We used turbidimetry and Mac Farland to identify the number of bacteria in the suspension.

Microbial carrier preparation:

Microbial carrier was obtained from milled vermiculite which was sieved through 100 micrometer sieve. Each flask was filled with 50 g of processed vermiculite and then sterilized.

Microbial suspension inoculation on the carrier:

Twelve ml from each microbial suspension was poured in the flasks containing processed vermiculite and then stirred. Another flask containing 12ml sterile culture media was considered as control treatment.

Selection and preparetion of soil for greenhouse experiment:

We used soil from Agriculture faculty at Karkaj, which was placed in plastic pots after passing thought 4mm sieve , after that they were sterilized in autoclave for 2 hours.

Preparation of treatments and planting pots:

Fertilizer was applied based on soil test and recommendation for green beans.

Green- bean seeds were soaked in distilled water for 2 hours and then superficially sterilized by placement in 95% ethanol for 30 seconds and thereafter in 0.1% acid Mercuric chloride for 15 minutes. Finally, they were soaked in a solution of 1% sodium hypochlorite for 30 minutes; Seeds were washed 5 times with sterile distilled water and then germinated in Petri dishes containing water-agar for 3 days. To create the same condition for all of the pots, they were moved every 2 days. Plants were grown until pods and seeds (75 days).

Experimental design and statistical analysis:

In a factorial experiment with complete randomized blocks base and 5 levels of helping bacteria and two Rhizobium levels were inoculated with four repetition of Jamaran418 green bean. At the end, nodulation indexes, growth and plant's nitrogen indexes were measured. We used variance analysis and mean comparisons via MSTATC. Dunkan multi-range test (5% significance level) was used to compare treatment means. The graphs were drawn by Excel.

Vitro biochemical tests:

Sperber culture media was prepared to quantitatively measure the phosphate dissolution ability of bacteria. The culture media was sterilized in autoclave at 1/5 pressure atmosphere for 20 minutes and 121 ˚C. The bacteria were grown on solid medium (48 hours of incubation) in Colony and clear zone diameters around colonies were used to measure phosphate dissolution.

Measurement potential of siderophore production by bacteria:

Ten ml of Chrome azurol-s culture medium and 90 ml of King's B were sterilized in autoclave and then poured in Petri dishes. After 48 hours of bacterial growth, colony diameter and clear zone diameter were measured and the ratio between them calculated (Bernhard et al, 1987).

Germination and seedling growth of helpful bacteria in the presence of green beans and *Rhizobium:*

Water-agar(0.8 gram per 100 ml water) was transferred to Petri dishes after sterilization(1/5 pressure atmosphere for 20 minutes and 121 ˚C in autoclave). The bacteria were moved on the solid medium using a sterile wooden toothpick.

The time required for maximum germination was calculated as % Maximum number of germinated seeds= Germination rate (% per day)

Results and Discussion:

Physical and chemical analysis of soil used in pot culture experiment is described in Table 1.

Number, fresh weight and dry weight of nodules:

Mean comparisons show that all helping bacteria except *P. fluorescens* B119 increased the bean nodulation. *P. putida* induced the higher nodule number and fresh and dry weight.

P. putida inoculated plants had the lower nitrogen percent compare to control treatment (without inoculation).

Shoot weight:

The treatments inoculated with *Rhizobium* and helping bacteria had the highest dry weight. *P. putida* with *R.leguminosarum*bv. phaseoli caused the highest dry and fresh weight of shoot. Figures 2 and 3 show of 5%, the significant differences between bacterial and control treatments.

pH	**ECe(s /cm)**	**Organic carbon %**	**Neutral matter %**	**Moisture of Field capacity %**	**Nitrogen %**	**Available Phosphor**	**Available Potassium**	**Clay %**	**Silt %**	**Sand %**	**tissue**
7/5 6	948	0/6	9/1	15	0/12	5/4	232	24	21	55	Loamy sand

Table1: Analysis of physical and chemical soil test

Phosphorus: Method of Olsen ($Na_2 CO_3$,0/5 Molar and PH=8/5) [10]

Potassium: Ammonium acetate 1N and PH=7) [10]

Organic carbon: method of Valkely Black [10].

Nodule Dry weight per plant(g)	Nodule Fresh weight per plant (g)	Number of Nodule per plant	Helping bacteria specie
b0/024	c0/325	d*19/75	control
ab0/079	c0/405	cd23	*P.flourescensB119*
ab0/086	ab0/942	ab36/5	*P.flourescensTABRIZ*
a0/122	a1/273	a45/5	*P.putida*
ab0/084	bc0/782	bc31/5	*P.flourescensCHAO*

Table2: Helping bacteria effects on green bean nodulation.

Different letters in each column indicate a significant difference of**5%** .

Siderophore production	Phosphate solving	Bacteria species
Diameter of halo /the colony	Diameter of halo /the colony	
b2/015	bc2/713	*P.flourescensB119*
b2/110	b3/585	*P.flourescensTABRIZ*
b1/990	a4/475	*P.putida*
a2/378	c1/974	*P.flourescensCHAO*

Table3: Biochemical tests in helping bacteria species.

Different letters in each column indicate a significant difference of **5%** .

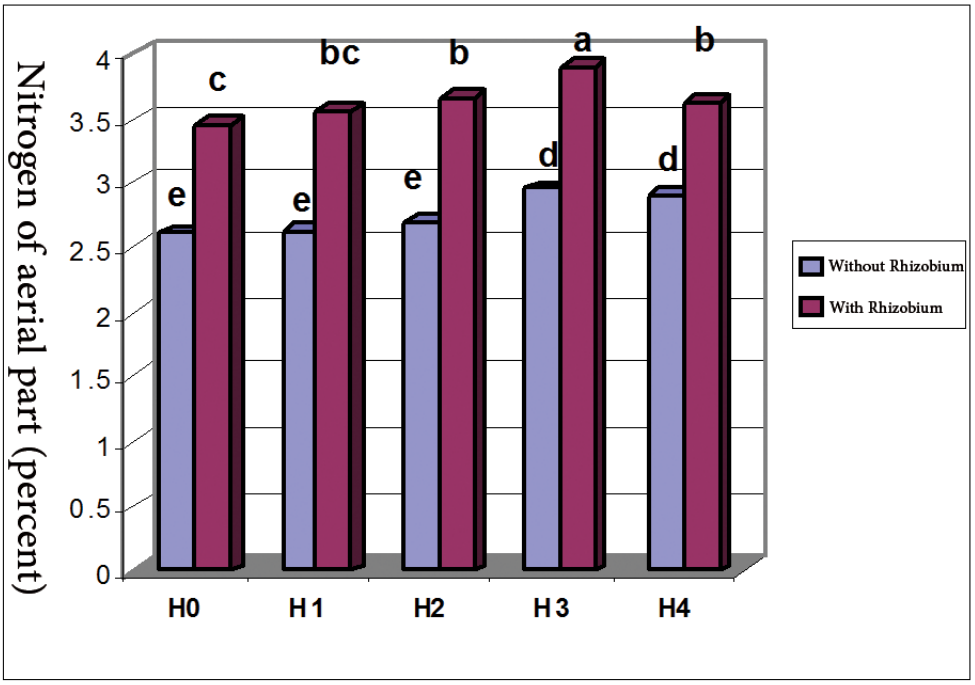

Figure1: Rhizobium leguminosarum*bv. phaseoli* and *Pseudomonas spp*. combination effects on shoot nitrogen concentration.

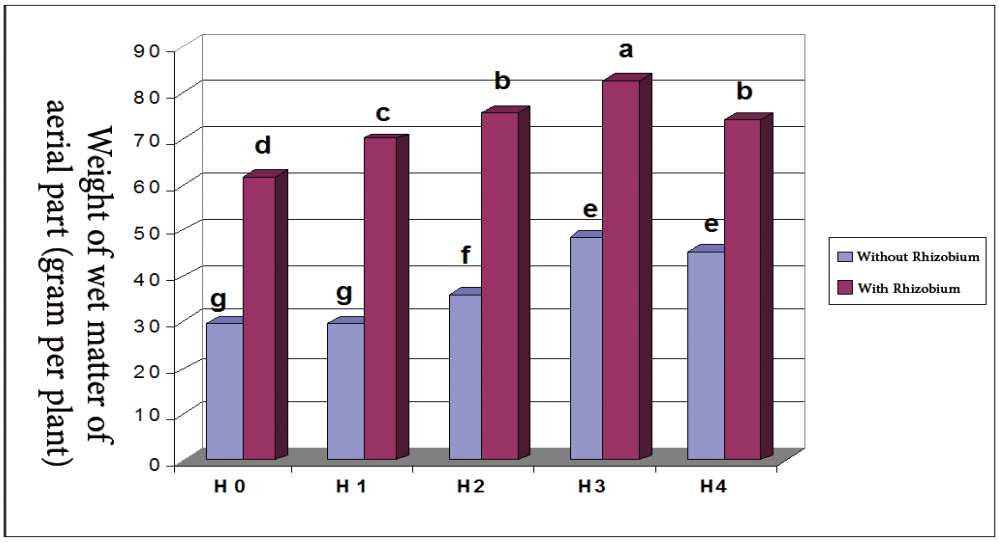

Figure 2:*Rhizobium leguminosarumbv.phaseoli* and *Pseudomonas spp.* combination effects on green bean shoot weight.

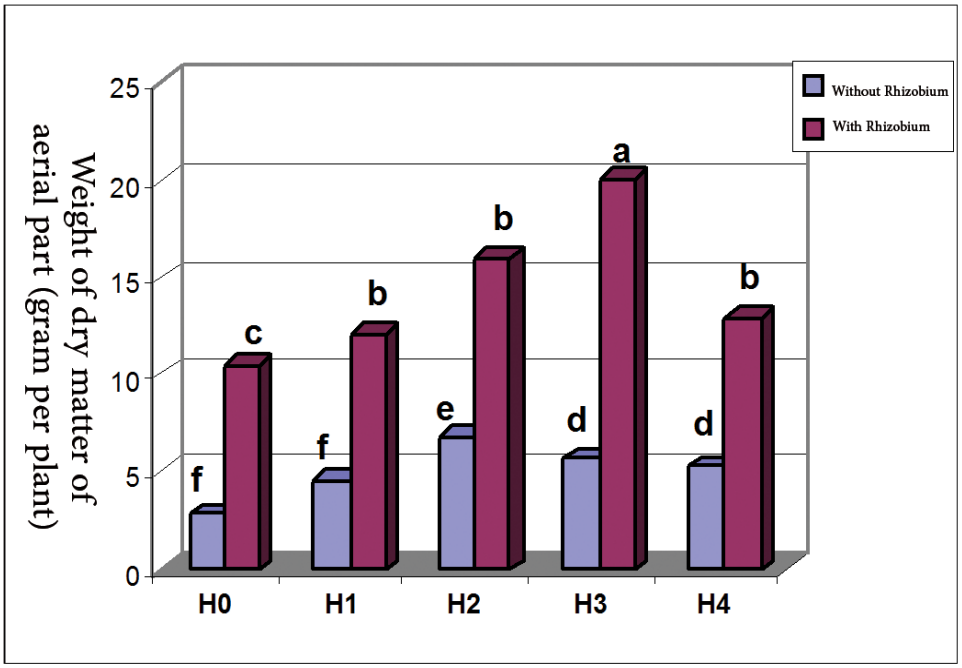

Figure 3:*Rhizobium leguminosarumbv.phaseoli* and *Pseudomonas spp.* combination effects on green bean dry weight.

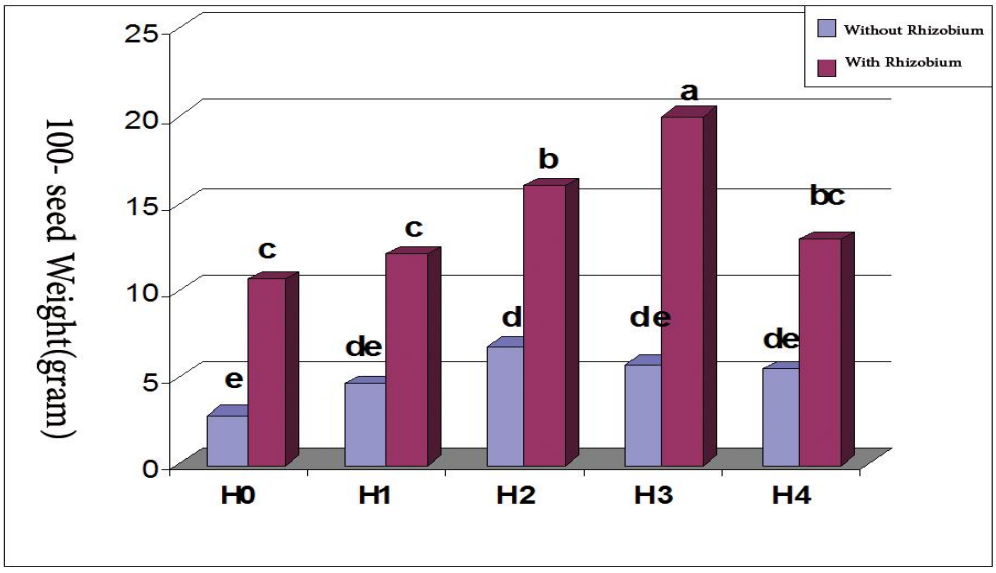

Figure 4:*Rhizobium leguminosarumbv.phaseoli* and *Pseudomonas spp.* combination effects on green bean seed weight.

Weight of 100 seeds per plant:

The treatments with *Rhizobium* and helping *Pseudomonas* spp. had the highest dry weight.

P. putida with R. leguminosarumbv. phaseoli treatment plants had the better performance in this field, but had a significant difference with the other bacterial treatments.

The results of biochemical tests on helping bacteria:

Colony and clear zone diameters were measured and the ratio of the diameter to the colony diameter. There was a significant difference between P.flourescens CHAO and the other ones.

Mean comparison shows that all helping bacteria except P.flourescens B119 increased the bean nodulation. P.putida had the more number of nodules, fresh weight and dry weight in green bean.

DISCUSSION

The results show that the helping *Pseudomonas* spp. with *R.legminosarum*bv. phaseoli had a significant effects on green bean behavior and enhanced its performances. *P. putida* and *R.leguminosarum*bv. phaseoli were the most effective on nodulation and nitrogen and phosphorus percentage, and general in plant performance.

Also, we can say that the phosphate solving power of *P putida* and *P.fluorescens* TABRIZ enhance the nodulation, nitrogen fixation and green bean performance in combination with *R.leguminosarum*bv.phaseoli; but as the results show *P.fluorescens*B119 has the highest phosphate solving power .AnjelaBergern et al (2001) had conducted an experiment in Sweden and investigated the effects on pea of *P. putida* strain A313 in mixture with *Rhizobium* ; the results showed the reduction of nitrogen proportion with *P. putida* strain A313. It should be mentioned that the positive effects of the helping bacteria on growth and legumes nodulation is not limited to the above mentioned mechanisms. Because, Pseudomonas has many positive effects such as: HCN production, ACC-D aminase activity, anti biotic production, etc.

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