# بررسی تأثیر سویه های باکتریایی گرم منفی و مثبت بر جوانه زنی و رشد گیاهچه های برخی غلات و گیاهان دانه روغنی

**گلـــــکار، پــــوراندخت*:** پژوهشکده زیست فناوری و مهندسی زیستی، دانشگاه صنعتی اصفهان، pooran60@yahoo.com
**طباطبـــــائی، ســـــمیرا:** پژوهشکده زیست فناوری و مهندسی زیستی، دانشگاه صنعتی اصفهان، ss_tabatabai@yahoo.com
**مســــــاوات، نیمــــــا:** پژوهشکده زیست فناوری و مهندسی زیستی، دانشگاه صنعتی اصفهان، nima_mosavat@yahoo.com

## چکیده

**مقدمه:** در سال های بسیاری از تحقیقات به سمت استفاده از عوامل بیولوژیکی به عنوان جایگزینی برای کودهای شیمیایی و همینطور به منظور افزایش تولید و کشاورزی پایدار سوق پیدا کرده است. برخی میکروارگانیزم می توانند به عنوان محرک رشد و عملکرد در گیاه عمل کنند. به همین منظور در این پژوهش تأثیر چند سویه باکتریایی محرک رشد بر جوانه زنی و رشد گیاهچه چهار گونه مختلف گیاهی شامل کنجد، کلزا، گندم و جو را مورد ارزیابی قرار گرفت.

**مواد و روش ها:** بذرهای چهار گونه گیاهی ذکر شده توسط پنج سویه باکتریایی شامل Bacillus subtilis، Bacillus pumilus، Azotobacter chroococcum، Rhizobium meliloti و Stenotrophomonas در شرایط آزمایشگاهی تلقیح شده و جوانه زنی و رشد آنها مورد ارزیابی قرار گرفت.

**نتایج:** نتایج نشان داد که در میان باکتری های گرم منفی گونه Stenotrophomonas، به طور معنی داری جوانه زنی بذر، تعداد ریشه، طول ریشه (سانتی متر)، وزن تر ریشه و ساقه (گرم) را افزایش داد. همچنین در میان باکتری های گرم مثبت Bacillus subtilis باعث افزایش میزان رشد و جوانه زنی گردید.

**بحث و نتیجه گیری:** در این تحقیق، برای اولین بار اثر باکتری B. Pumilus بر جوانه زنی و رشد گیاهچه در غلات گزارش شده است. نتایج کاربرد بالقوه گونه Stenotrophomonas را در افزایش جوانه زنی بذر نشان دادند که این اثر مثبت بر محصولات دانه روغنی در مقایسه با بذر غلات کمتر بود. در مقابل باکتری B. Pumilus بیشترین اثر منفی را بر جوانه زنی گیاهان کلزا و جو ایجاد کرد.

**واژه‌های کلیدی:** جوانه زنی، گیاهچه، ریزوباکترهای محرک رشد گیاهی، محصولات دانه روغنی.

* نویسنده مسؤول مکاتبات

تاریخ دریافت: ۱۳۹۷/۱۰/۱۱- تاریخ پذیرش: ۱۳۹۸/۰۲/۳۱

Biological Journal of Microorganism
Document Type: Research Paper
8th Year, Vol. 8, No. 32, Winter 2020
**Received:** January 1, 2019/ **Accepted:** May 21, 2019. **Page:** 139-151
Doi: 10.22108/BJM.2019.114798.1176

# A Comparative Study on the Effect of Gram Negative and Positive Bacterial Strains on Germination and Seedling Growth of Cereals and Oil Seed Crops

**Pooran Golkar***
Research Institute for Biotechnology and Bioengineering, Isfahan University of Technology, 8415683111, Isfahan, Iran, golkar@cc.iut.ac.ir
**Samira Tabatabaei**
Research Institute for Biotechnology and Bioengineering, Isfahan University of Technology, 8415683111, Isfahan, Iran, ss_tabatabai@yahoo.com
**Nima Mosavat**
Research Institute for Biotechnology and Bioengineering, Isfahan University of Technology, 8415683111, Isfahan, Iran, nima_mosavat@yahoo.com

## Abstract

**Introduction:** In recent years, numerous studies have been done on using biological approaches instead of chemical fertilizers and increasing sustainable production in agriculture systems. Some microorganisms can promote growth and yield in the plant. In this study, the effect of several plant-growth-promoting bacterial strains were evaluated on the germination and seedling growth of four different plant species including sesame (*Sasamum indicum*), canola (*Brassica napus*), wheat (*Triticum turgidum* L.*)* and barley (*Hordeum vulgar* L.).

**Materials and methods:** The seeds of four plant species were inoculated by five bacterial strains including *Bacillus subtilis*, *Bacillus pumilus*, *Azotobacter chroococcum*, *Rhizobium meliloti* and *Stenotrophomonas*. Their effects were evaluated on seed germination and growth parameters of seedlings under *in vitro* condition.

**Results:** The results of this experiment showed that among gram-negative bacteria, the *Stenotrophomonas sp.* enhanced seed germination, root number, root length (cm), shoot length (cm), root fresh weight (g) and shoot fresh weight (g) significantly. Among the gram-positive strains, *Basillus subtilis* mostly promoted germination and the seedling growth.

**Discussion and conclusion:** The effects of *B. Pumilus* on germination and seedling growth of cereals was evaluated first. The results indicated the potential application of *Stenotrophomonas sp.* On the enhancement of plant seed germination, but its positive effect on oil seed crops was less than cereals seed. *B. pumilus* showed the most negative effect on germination of barely and canola.

**Key words:** Germination, Seedling, Plant-growth-promoting Rhizobacteria, Oil Seed Crops.

*Corresponding Author

## Introduction

A wide range of microorganisms ranging from pathogenic to beneficial, interact continuously with higher plants in the soil ecosystem (1). These microorganisms influence the growth, development and functions of plants (1, 2). *Rhizobacteria* are used as inoculants to enhance crop yield and control fungal pathogens biologically (12).

Bacteria that colonize plant roots and promote plant growth are called plant growth-promoting rhizobacteria (PGPR) (3, 4). PGPRs isolated as free-living soil bacteria from plant rhizosphere, which can decrease the use of chemical fertilizer-N (4). The corresponding PGPR associated to plant roots and/or other plant parts could increase the growth and seed yield of plants (4).

PGPR could affect plant growth either indirectly or directly (8). The indirect promotion of plant growth occurs when PGPR lessens or prevents the deleterious effects of one or more phytopathogenic organisms (4). The direct promotion of plant growth by PGPR involves either providing the plants with certain bacterial-synthesized compounds or facilitating the uptake of certain nutrients from soil (4, 8). In general, microbial inoculation is considered as an important component for agricultural practice, due to loss of topsoil, soil infertility, decrease in plant growth and plant yield, and insufficient diversity of indigenous microbes (9).

PGRP could be classified into four groups: 1. Bio fertilizers (solubilisation of mineral phosphates, asymbiotic nitrogen fixation) 2. Phytostimulators (ability to produce phytohormones) 3. Rhizoremediators (degrading organic pollutants) and 4. Biopesticides (siderophores production and synthesis of antibiotics, enzymes and/or fungicidal compounds) (10).

*Rhizobium meliloti*, as a free-living microorganism in the soil, is grouped as *Rhizobiaceae* (1). It is considered as nitrogen-fixing bacteria, which have symbiotic relationships with legumes, especially alfalfa.

Some species of Pseudomonas bacteria like Azotobacter are useful due to their ability to make the unavailable nutrient elements accessible (13). For instance, inoculation with pseudomonas caused a significant increase in yield of wheat, sorghum and corn by 10 to 30% (5-12). Azadikhah et al. (14) showed that the *Pseudomonas fluorescens* strain had produced siderophore with promoting iron absorption by the plant and increasing the availability of iron in the surrounding soil of the root. Hamaoui et al. (15) reported that *Azospirillum brasilense* improved root and shoot development significantly, when compared with non-inoculated controls in *Faba bea*ns and chickpea. The *Azotobacter* strains had increased the disease tolerance in plants.

*Bacillus sp.* (as PGPR) could increase plant growth and yield when contacting the plant roots (4, 19). *Bacillus* species is inhibitory to several plant pathogens and improves the growth of many plants in streamed and natural soils (18). For example, inoculation of tea plants by *Bacillus sp.* reduced the disease incidence of blister blight for two seasons, which was almost comparable with the performance of chemical fungicide (16). *Bacillus* species could be considered as facultative anaerobes (17).

*Bacillus subtilis* is a rhizobacteria, which is attributed to synthesis of phytohormones, such as indole acetic acid, abscisic acid, gibberellins and cytokinins (20). This function of *B. subtilis* promotes root growth and increases the number of root hairs (20). The positive effect of *B. subtilis* has been documented in previous studies (5, 18). The cellular structure of *B. pumilus* is similar to other *Bacillus* species such as *B. subtilis, B. megaterium*, which generally shows high resistance to environmental stresses (21, 22). Kuan et al. (5) reported the significant effect of *B. pumilus* s1R1 on nitrogen fixation in maize.

*Stenotrophomonas sp*. is a gram-negative obligate aerobe (23). The rod isolate is an environmental bacterium found in aqueous habitats, including plant rhizospheres, animals, foods, and water sources (23).

Several bacteria such as *Klebsiella*, *Azospirillum*, *Pseudomonas*, *Bacillus* and *Burkholderia* are identified as PGPR through biological nitrogen fixation (BNF), phytohormone production (e.g., auxin, gibberellin and cytokinin) and biological control of soil pathogens in different plant species as maize (5,7). Kuan et al. (5) reported the positive effect of four bacterial strains including *Klebsiella* sp. Br1, *Klebsiella* pneumoniae Fr1, *Bacillus pumilus* S1r1 ‹*Acinetobacter sp. S3r2* on growth characteristics of maize. Safdarpour and Khodakaramian (31) reported the promoting effects of bacterial strains of *Pseudomonas mosselli*, *Pseudomonas fuorescence* and *Stenotrophomonas maltophilia* on germination and disease tolerance in tomato under *in vitro* conditions.

In PGPR bacteria group, the deleterious rhizosphere bacteria (DRB) inhibits the growth of plants without causing symptoms of disease infection. One of the main mechanisms for growth inhibition by this undesirable group of rhizobacteria have been proposed as the production of phytotoxins such as cyanide and other volatile and non-volatile compounds (24). A certain concentration of indole-3-acetic acid (IAA), which is produced by DRB, has an inhibitory effect on root growth in sugar beet and blackcurrant (24). Tabatabaei et al. (25) reported the inhibitory effect of *Pseudomonas,* as a DRB strain, in seedling growth of durum wheat. Plant inoculation with PGPRs have different benefits in increasing different indices such as germination rate, higher mass production and better control of disease and microbial activities (26).

Identifying the native strains of PGPR as promoters of plant growth and/or inducers of plant defense response, may offer a practical way to achieve plant growth promotion and better management for plant diseases (11). In recent years, several studies have carried out to evaluate the effects of PGPRs on various plants, but there are few reports on the effects of some bacterial species as *Stenotrophomonas sp*. *Rhizobium meliloti* and *B. pumilus* on seedling growth of different plant species. In addition, the PGPR function mechanisms are not fully understood in different plant species. This experiment was conducted to study the effect of seed inoculation with five different bacterial strains (*Azotobacter chroococcum, Rhizobium meliloti, Bacillus subtilis, Bacillus pumilus* and *Stenotrophomonas sp*.) on the germination and primary growth of mentioned plants under *in vitro* condition. This study reports the effect of *B. Pumilus* on germination and seedling growth of the studied plant species.

This experiment was conducted to study the effect of seed inoculation with five different bacterial strains (*Azotobacter chroococcum, Rhizobium meliloti, Bacillus subtilis, Bacillus pumilus* and *Stenotrophomonas sp*.) on germination and primary growth of mentioned plants under *in vitro* condition. This study reports, for the first time, the effect *B. Pumilus* on germination and seedling growth of the plants.

## Materials and Methods

**Bacterial Strains and Growth Conditions:** Two *Bacillus* strains including *B. subtilis* (PTCC No:1204) and *B. pumilus* (PTCC No: 1733), *Azotobacter chroococcum* (PTCC No: 1658), *Rhizobium meliloti* (PTCC No:1684) and *Stenotrophomonas sp*. (Access No.: AB1616885.1) were obtained from the Department of Biology, University of Isfahan, Iran. The isolates were grown on nutrient agar (NA) (2 g yeast extract, 1 g meat extract, 5 g peptone, 5 g sodium chloride, 20 g agar-agar, 1000 mL distilled water) for routine use. The strains were maintained in NB with 20% glycerol at –80 °C for long-term storage. For preparing the bacterial cultures, single colony of each bacterial isolate was grown in 250 mL flasks containing 100 mL NB medium and incubated for 24 h at 28 ± 2 °C on a rotary shaker at 120 rpm. After incubation, the cell suspension was centrifuged at 5,000 (rpm) for 5 min at 4 °C and washed twice with sterile distilled water. The final pellet was re-suspended in sterilized distilled water and the bacterial cultures were standardized to $1\times10^8$ colony-forming units (CFU)/mL and used, immediately, for seed germination experiments.

**Effects of Bacterial Species on Seed Germination and Seedling Growth Traits:** The effect of five different bacteria strains were evaluated in four different plant species. The examined species were both monocotyledon (wheat and barley) and dicotyledon (canola and sesame). The germination and seedling growth traits were evaluated at seeds of durum wheat (*Triticum turgidum* L. var durum), barley seeds (*Hordeum vulgar* L. cv. Valfajr), rape seeds (*Brassica napus* cv. Hayola) and sesame seeds (*Sasamum indicum* cv. Oltan). The experiment was conducted as a completely randomized design (CRD) with four replicates. Six treatments were made as follows: 1) seeds treated with *Stenotrophomonas sp.*; 2) seeds treated with *B. subtilis*; 3) seed treated with *B. pumilus*; 4) seeds treated with *A. chroococcum*; 5) seeds treated with *R. meliloti*; and 6) un-inoculated as control. Seeds were surface disinfected with 2% (v/v) solution of sodium hypochlorite for 15 min and were rinsed four times with sterile distilled water, and air-dried before being used in the germination experiments. All further manipulations were carried out under sterile conditions. The surface-sterilized seeds were immersed into individual bacterial suspensions for 30 min, shaking at 120 rpm. Twenty seeds were placed into sterile petri dishes (12 cm diameter), containing 1.5% (w/v) of distilled water agar. The Petri dishes were transferred into a growth chamber at 25 ± 2 °C for 7 days. Final percentage for germination and its rate were measured after 7 days from incubation. At the end of vegetative growth (30 days), different growth parameters viz., seedling fresh weight and seedling dry weight was measured from the base to the tip of the plant. Fresh weight was determined by uprooting the plant carefully, washing them thoroughly to remove remnants of soil particles. Dry weight was determined by drying the plants in an oven at 65 ºC until the weight remained constant.

**Statistical Analysis:** Analysis of variances (ANOVA) was conducted on the data when $F$ values were significant ($P \leq 0.05$). Mean comparisons were conducted using least significant differences (LSD, 0.05) procedure. Orthogonal independent comparisons were conducted for differences within and between the two crop groups, i.e. dicotyledonous versus monocotyledon and for their interaction with bacteria strain. The mean comparisons were conducted using Fisher’s least significant difference (LSD) at $P \leq 0.05$.

## Results

The bacterial strains, plant species and bacterial strains × plant species interaction showed significant effect on all the studied traits (data not shown). This result implied different responses of plant species to inoculation with bacterial strains. The highest germination percentage was related to sesame (85%) and wheat (86%) but the least one was associated to barley (36.4%) (Table1). All of the bacteria strains showed a significant increase in germination (%) of plant species, except *B. pumilus* (Table 1). The comparison effect of two *Bacillus sp*. (*B. subtilis* and B. *pumilus*) on wheat germination is presented in Figure 1.

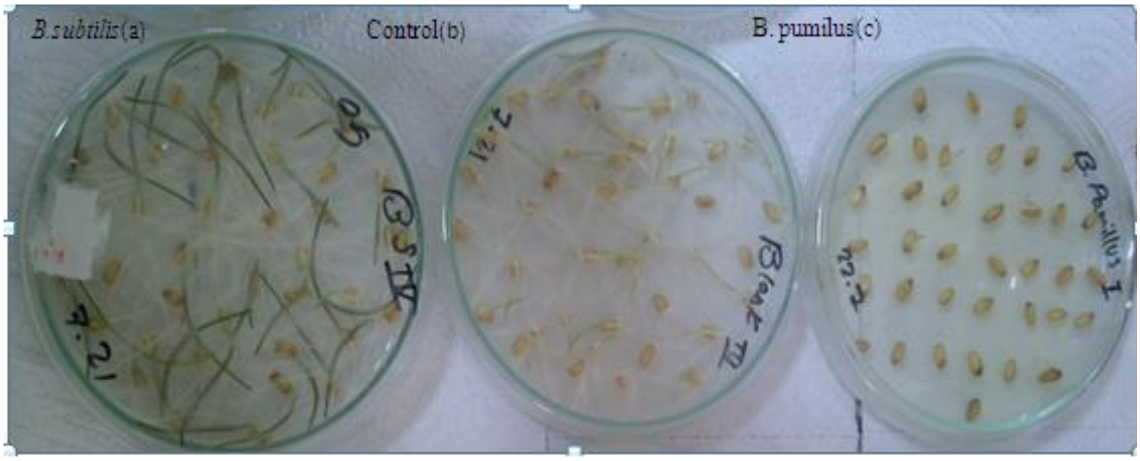



Fig. 1- The Effects of *B. Pumilus* (a), and *B. Subtilis* (c) Wheat Blank (b) on Germination of Wheats

Table 1- Mean Comparisons of Plant Species and Bacteria Strains for Seed Germination (%), Root Number, Root Length, Shoot Length, Root Fresh Weight, Root Dry Weight, Shoot Fresh Weight and Shoot Dry Weight

| Plant Species | G (%) | RN | RL (cm) | SL (cm) | RFW (g) | RDW (g) | SFW (g) | SDW (g) ¥ |
|---|---|---|---|---|---|---|---|---|
| Sesame | 85.0 $^{a}$ | 1.00 $^{c}$ | 1.78 $^{c}$ | 1.99 $^{b}$ | 0.011 $^{c}$ | 0.001 $^{c}$ | 0.025 $^{c}$ | 0.002 $^{b}$ |
| Canola | 60.4 $^{b}$ | 1.00 c | 1.41 $^{c}$ | 1.85 $^{b}$ | 0.008 $^{c}$ | 0.005 $^{a}$ | 0.017 $^{c}$ | 0.002 $^{b}$ |
| Wheat | 85.0 $^{a}$ | 4.82 $^{a}$ | 9.40 $^{a}$ | 9.38 $^{a}$ | 0.034 $^{a}$ | 0.005 $^{a}$ | 0.072 $^{b}$ | 0.007 $^{ab}$ |
| Barley | 36.4 $^{c}$ | 4.43 $^{b}$ | 5.54 $^{b}$ | 9.61 $^{a}$ | 0.025 $^{b}$ | 0.003 $^{b}$ | 0.086 $^{a}$ | 0.012 $^{a}$ |
| Bacteria strains | | | | | | | | |
| Blank | 61.13 $^{b}$ | 2.86 $^{b}$ | 3.18 $^{c}$ | 4.17 $^{c}$ | 0.013 $^{c}$ | 0.003 $^{c}$ | 0.03 $^{c}$ | 0.003 $^{b}$ |
| *B. subtilis* | 75.13 $^{a}$ | 3.17 $^{ab}$ | 4.99 $^{b}$ | 5.88 $^{b}$ | 0.023 $^{b}$ | 0.003 $^{bc}$ | 0.05 $^{b}$ | 0.005 $^{ab}$ |
| *B. pumiluspumilus* | 18.12 $^{c}$ | 1.12 $^{c}$ | 0.17 $^{d}$ | 0.40 $^{d}$ | 0.002 $^{d}$ | 0.0006 $^{d}$ | 0.003 $^{d}$ | 0.0004 $^{b}$ |
| *A chroococcum* | 82.50 $^{a}$ | 3.17$^{ab}$ | 6.45 $^{a}$ | 7.57 $^{a}$ | 0.023 $^{b}$ | 0.010 $^{a}$ | 0.07 $^{a}$ | 0.006 $^{ab}$ |
| *R. meliloti* | 82.50 $^{a}$ | 3.46 $^{a}$ | 5.33 $^{b}$ | 7.71 $^{a}$ | 0.023$^{b}$ | 0.003 $^{c}$ | 0.07 $^{a}$ | 0.012 $^{a}$ |
| *Stenotrophomonas* sp | 80.83$^{a}$ | 3.08$^{b}$ | 7.07 $^{a}$ | 8.53 $^{a}$ | 0.035 $^{a}$ | 0.004 $^{b}$ | 0.08 $^{a}$ | 0.007 $^{ab}$ |

Values within a column bearing different superscript are significantly different at P≤ 0.05. ¥: Abbreviations: germination (G); root number (RN), root length (RL), shoot length (SL), root fresh weight (RFW), root dry weight (RDW), shoot fresh weight (SFW), shoot dry weight (SDW).

*B. subtilis* caused the highest germination (56.25%) in sesame (Fig. 2). *B. pumilus* had the most negative effect on the germination of barley and canola (0%) (Fig. 2). In canola, inoculation with all bacterial strains, except *B. pumilus*, showed significant increase on germination rather than the control (Fig. 2). Inoculation with bacterial strains had no significant effect on germination of wheat, barley and sesame (Fig. 2).

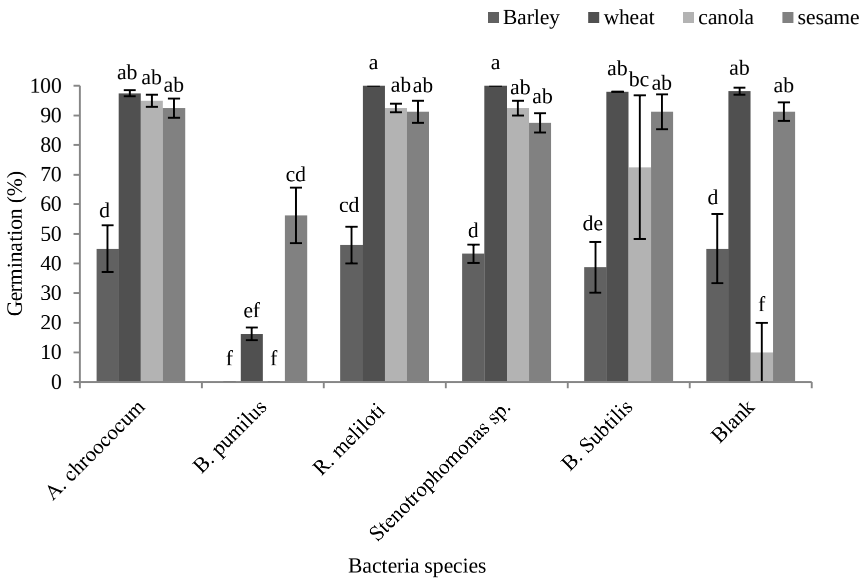
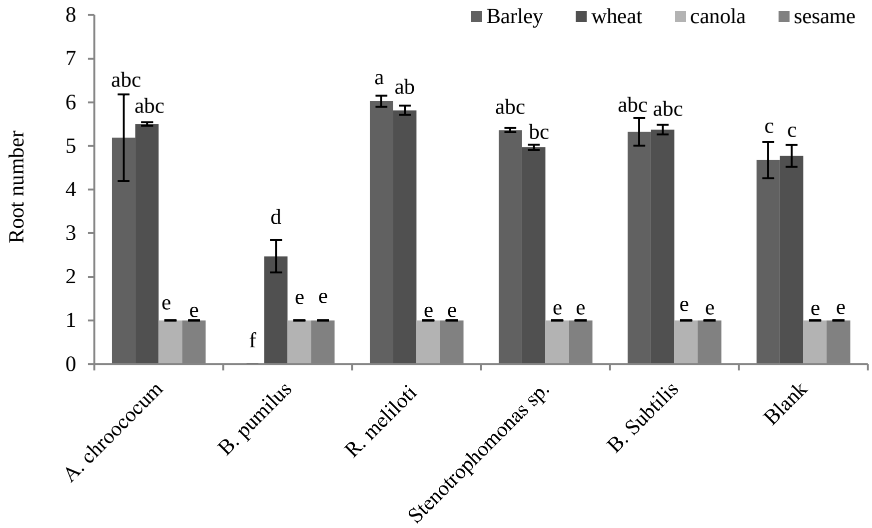



Fig. 2- The Plant Species ×Bacteria Species Interaction on Germination (%)

**Root Number and Length:** The highest number of root was observed under inoculation with *R. meliloti* that increased the number of roots by 2(%) in comparison with control (Table 1). The strains of *B. subtilis*, *A. chroococcum* and *Stenotrophomonas sp*. showed no significant effect on root number of the studied plant species, while the *B. pumilus* reduced the root number (83%) in comparison with control (Table 1). The non-significant effect of *B. subtilis* and *A. chroococcum* on all of the plant species could be explained by plant species × bacterial strains interaction. The negative effect of *B. pumilus* on root number, in comparison with control, was more obvious in barley (100%) rather than wheat (48.21%) (Fig. 3a).

The highest increase in root length was associated with *Stenotrophomonas* sp (7.07 cm) and *A. chroococcum* (6.45 cm), but the least one was related to *B. pumilus* (0.17 cm) (Table 1). Inoculation with all strains, except *B. pumilus*, showed an increase in the root length (Fig. 3b). *B. pumilus* reduced the root length by 95(%) in comparison with control (Fig. 3b). In comparison between different plant species, the highest root length was associated with wheat (9.4 cm), but the least ones were observed in the sesame (1.78 cm) and canola (1.41 cm) (Table 1). In barley, the root length increased significantly by 296 (%), 208 (%) and 210 (%) under inoculation with *Stenotrophomonas* sp, *A. chroococcum* and *Rhizobium meliloti*, respectively (Fig. 3b), but *B. subtilis* decreased the root length (100%) in comparison with control (Fig. 3b).

In wheat, *Stenotrophomonas* sp (183%), *B. subtilis* (193%) and *A. chroococcum* (159 %) were considered as the effective strains for the enhancement of root length, in spite of *B. pumilus*, which reduced the root length (86%) significantly (Fig. 3). In sesame, root length was reduced under inoculation with *B. pumilus* (51%) and *B. subtilis* (51%) (Fig. 3b), but *A. chroococcum* (109%), *R. meliloti* (59%) and *Stenotrophomonas* sp (47%) increased the root length in comparison with control (Fig. 3).



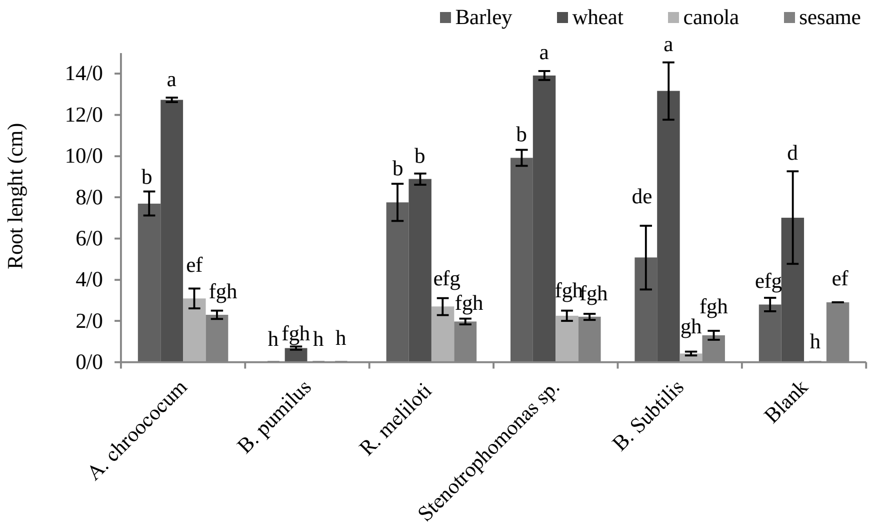




Fig. 3- The Plant Species × Bacteria Species Interaction on Root Number (a) and Root Length (b)

**Shoot Length:** Inoculation with all bacteria strains (except *B. pumilus*) showed a significant increase in shoot length (Table 1). *Stenotrophomonas* sp (105%), *A. chroococcum* (81%) and *R. meliloti* (73%) were considered as the most effective strains (Table 1). *B. pumilus* decreased the shoot length (90%), significantly. Inoculation with all strains, except *B. pumilus*, increased the shoot length in barley, wheat and canola, significantly (Fig. 4). In sesame *B. subtilis* (41%) and *B. pumilus* (100%) the shoot length was reduced (Fig. 4). *B. pumilus* showed the most increasing effect on shoot length (104%) of wheat in comparison with control (Fig.4). In barley (213%) and canola (180%), the highest increase in shoot length was caused by *Stenotrophomonas* sp., whereas in sesame the most one (14%) was observed under *A. chroococcum* inoculation (Fig.4).

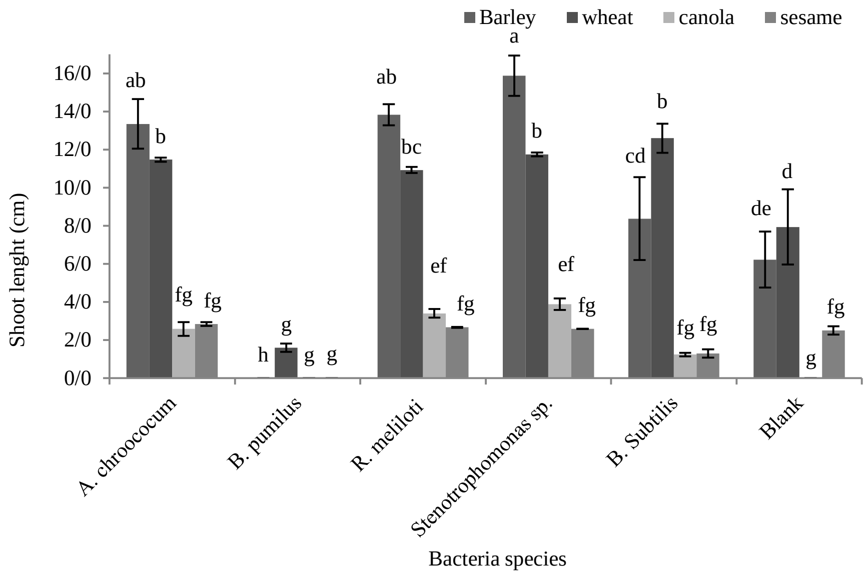


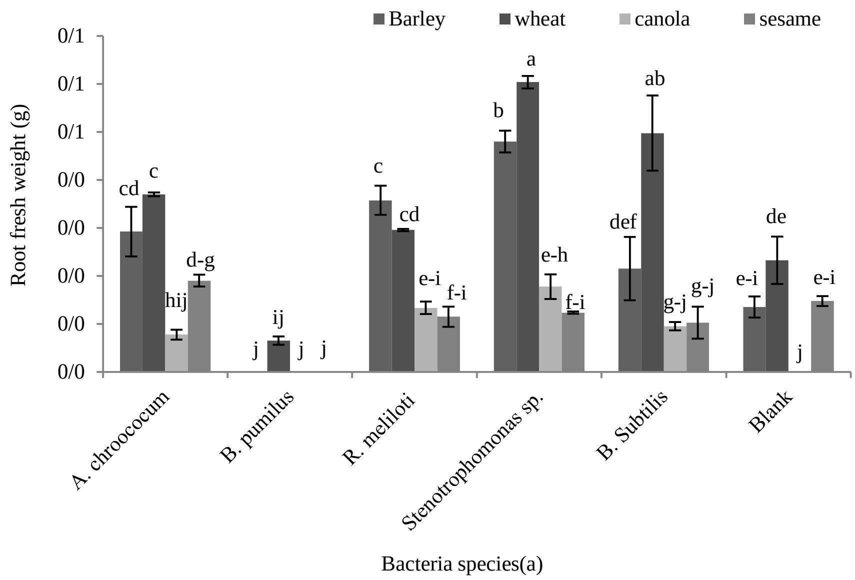

Fig. 4- The Plant Species × Bacteria Species Interaction on Shoot Length

**Root Fresh and Dry Weight:** The highest fresh weight (0.034 g) and dry weight (0.005 g.) was associated with wheat, whereas the least was related to canola (0.008 g) and sesame (0.001 g), respectively (Table 1). The most effective strains for the increase of root fresh weight and root dry weight was *Stenotrophomonas* sp (166%) and *A. chroococcum* (267%), respectively (Table 1). Inoculation with *B. pumilus* reduced root fresh weight in all plant species, compared to control (Fig. 5a). The highest increase in root fresh weight of wheat (202%), barley (336%) and canola (180%) were related to *Stenotrophomonas* sp. (Fig. 6a). The highest increase in root fresh weight of sesame (27%) was associated with *A. chroococcum* (Fig. 6a). Al the bacterial strains, except *A. chroococcum*, reduced the root fresh weight in sesame (Fig. 6a). This inhibitory effect could be compromised by the significant effect for plant species × bacteria strain interaction.

The bacteria strains showed different root dry weight in the studied plants (Fig. 6b). The highest increase of dry root weight in wheat (142%) was achieved under inoculation with *B. subtilis*, whereas, in barley (262%) and canola (20%) the most increase was obtained under inoculation with *Stenotrophomonas* sp. (Fig. 6b). All the bacteria species reduced the dry root weight in sesame, in comparison with control (Fig. 6b). The differences in water absorption capacity between species could be a reason for this result.

**Shoot fresh and dry weight:** Bacterial inoculation increased the shoot fresh weight, significantly (Table 1), but the highest shoot fresh weight (0.086 g) was obtained in barley (Table 1). Among different bacterial strains, the highest increase in shoot fresh weight (150%) was related to *Stenotrophomonas* sp, whereas the least one (90%) was associated with *B. subtilis* (Fig. 7a). The effect of inoculation on increased shoot fresh and dry weight was more in wheat and barley rather than sesame and canola (Fig. 6a). In wheat and barley, all strains, except *B. pumilus*, showed an increasing effect on the shoot fresh weigh (Table 1). The highest shoot dry weight was observed in barley (0.012 g) and wheat (0.007 g) (Table 1). Inoculation with all strains of bacteria showed the beneficial role of these rhizobacteria on increasing shoot dry weight.

The highest increase in shoot dry weight (312%) was observed under inoculation with *R. meliloti* (Fig 6a). The highest shoot dry weight in sesame (56%) was observed under inoculation with *R. meliloti* (Fig. 6a). Shoot dry weight showed a significant increase in all of the plant species under inoculation with *B. pumilus* (Fig. 6b). The highest increase in shoot dry weight of wheat (163%), barley (608%), canola (523%) and sesame (8%) was observed under elicitation with *B. subtilis*, *R. meliloti*, *Stenotrophomonas s*p and *A. chroococcum*, respectively (Fig. 6a). The bacterial strains had the least positive effect on shoot dry weight of sesame.



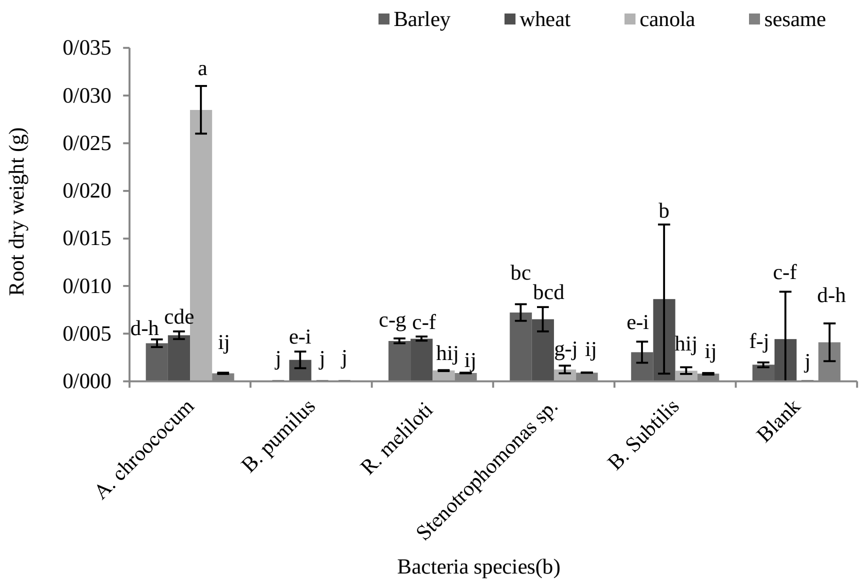
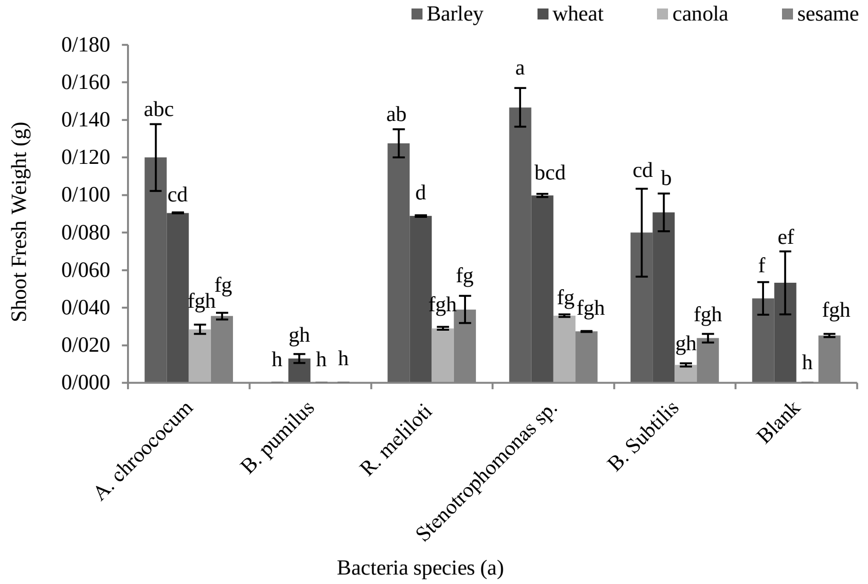




Fig. 5 (a, b)- The plant species × bacteria species interaction on root fresh (a) and dry (b) weight



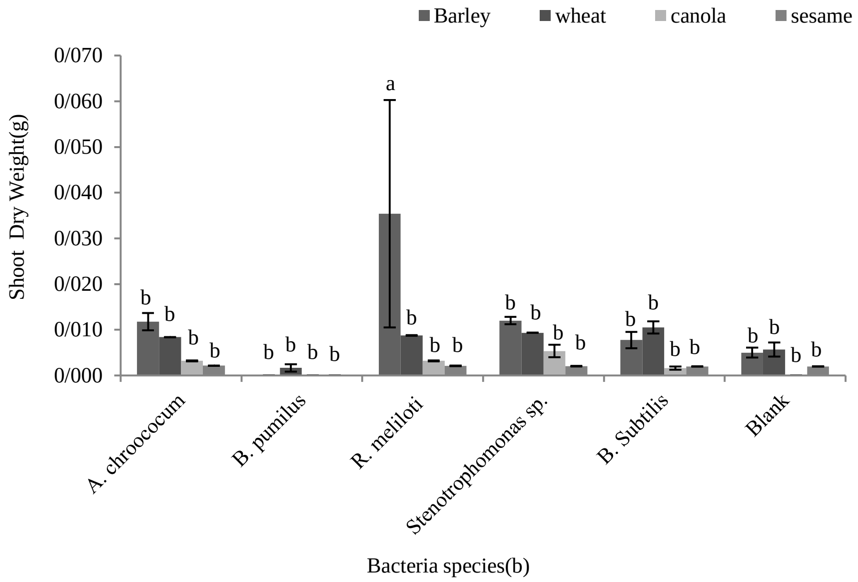




Fig. 6 (a, b)- The Plant Species × Bacteria Species Interaction on Shoot Fresh Weight (a) and Shoot Dry Weight (b)

The stimulation effects of plant growth-promoting rhizobacteria are considered as an important issue in biological studies. In the present study, all the bacterial strains, except of *B. pumilus* had positive effect on increasing seed germination and seedling vigor (fresh weight and dry weight) of studied plant species (canola, sesame wheat and barley). This finding was consistent with previous reports on positive effects of *Azotobacter* strains on increasing seed yield in canola (27, 28) and seedling growth in rice (29). Kloepper and Beauchamp (30) reported that seed yield of wheat increased up to 30% and 43% with *Azotobacter* and *Bacillus* inoculation, respectively. Inoculation with *Azotobacter* strains was reported as producers of gibberellic acid, IAA and cytokinins with promoting effects on seed germination and plant growth (29).

It seems that *R. meliloti* and *B. subtilis* and *Stenotrophomonas sp.* could improve dry matter production, which is due to increase in photosynthesis and development in cell growth. According to our findings, *R. meliloti* could produce internal IAA that has positive effect on the length of shoot seedlings. Jahanian *et al.* (35) showed that either sole or integrated application of promoting rhizobacteria led to significant increase in germination, shoot weight and shoot length in *Cynara scolymus*. Previous reports demonstrated the positive effect of *B. subtilis* on increasing seed germination in pearl millet (11), *Surgum bicolor* (18) and maize (20). In addition, the promoting effect of *R. meliloti* on root number confirmed these bacteria as promoting strains. The positive effect of *A. chroococcum* on nitrogen uptake and seedling growth of wheat was reported (32). Mrkovacki and Milic (33) reported the significant effect of *A. chroococcum* on seedling vigor and nutrient uptake in plants. Inoculation of seeds with *B. subtilis* caused a significant increase in fresh and dry weights of roots and leaves, photosynthetic pigments, proline, total free amino acids and crude protein contents of inoculants under salinity in maize seeds (20). Araujo and Marchesi (34) observed higher growth and weight in roots of the tomato treated with *B. subtilis.*

*Stenotrophomonas* sp. was considered as the most effective strain to increase root length in the studied species. This increase could be caused by the higher water absorption and subsequent increase in fresh weight of root. Therefore, *Stenotrophomonas* could be effective for inoculation with the seeds that are cultivated in arid and semi-arid regions. The present study showed that all strains except *B. pumilus* were most effective on increasing shoot fresh and dry weight and length. In this study, the least reduction in fresh weigh of shoot (90%) was associated with *B. subtilis*. Contrary to this finding, inoculation with *B. subtilis* showed a significant increase on early germination, vigor index, plant height, leaf area and fresh weight and dry weight in *pearl millet* (11). The negative effect of this strain has not been reported previously. This study demonstrated that the negative effect of *B. Pumilus* on germination could be due to GAF (Germination Arrest Factors) or inhibitory components at germination. The GAFs are defined as microbial-derived compounds that lead to irreversible arrest of seed germination in a wide range of graminaceous species (36). According McPhail et al. (37), the *Pseudomonas flurescens* bacteria have capacity in producing GAF, which inhibited the germination when inoculated with germinated seeds. The GAF effect in seedling growth of *Graminea* was also reported by *Pumilus sp* (36). Similar to the findings of the present study, Asghari *et al.* (38) reported that the variation in stimulatory effects of rhizobacteria greatly depends on species and/or strains of the same species.

The inhibitory effects of *B. pumilus* on germination and seedling growth of the studied plant species may be explained by the internal auxin in these species. Accordingly, it is more advisable to inoculate the seeds with *B. pumilus* after the termination of the germination process. Interestingly, in spite of involving the GAF factors in *B. pumilus*, the growth of seedling was reduced. The inconsistency of the results with the reports of Junges *et al.* (20) could be due to the internal level of IAA that has been produced by this strain. Contrary to the results of the present study, inoculations of maize seeds with *B. subtilis* has significantly increased the total nitrogen content and dry biomass of maize (1, 2). This result suggests that the promoting or inhibitory effect of these bacteria could be dependent on plant species, IAA internal level and bacteria strain × plant species interaction.

Finally, the obtained results suggest the application of the mentioned bacterial strains, except *B. pumilus* on improving seed germination and seedling vigor of the studied plant species under poor environmental conditions.

## Acknowledgments

The authors would like to thank Research Institute for Biotechnology and Bioengineering, Isfahan University of Technology, Isfahan, Iran. The authors would like to thank Dr. N. Habibi for preparation of Bacterial strains.

## References

(1) O'Callaghan M. Microbial inoculation of seed for improved crop performance: issues and opportunities. *Applied Microbiology Biotechnology* 2016; 100(13): 5729.

(2) Taghavi S, Garafola C, Monchy S, Newman L, Hoffman A, Weyens N, Barac T, Vangronsveld J, van der Lelie D. Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. *Apply Microbiology Biotechnology* 2009; 75(3): 748.

(3) Beneduzi A, Ambrosini A, Passaglia LM. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genetic and Molecular Biology* 2012; 35(4): 1044.

(4) Lugtenberg B, Kamilova F. Plant-growth-promoting rhizobacteria. *Annual Review Microbiology* 2009; 63(1): 541.

(5) Kuan K.B, Othman R, Rahim K.A, Shamsuddin Z.H. Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilization of maize under greenhouse conditions. *PLoS ONE 2016; 11(3): e0152478.*

(6) Okon Y, Labandera-Gonzalez CA. Agronomic applications of Azospirillum: an evaluation of 20 years worldwide field inoculation. *Soil Biology and Biochemistry* 1994; 26 (12): 1591.

(7) Raj SN, Shetty NP, Shetty HS. Seed bio-priming with *Pseudomonas fluorescens* isolates enhances growth of pearl millet plants and induces resistance against downy mildew. *International Journal of Pest Management* 2004; 50(1): 41.

(8) Glick BR. The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* 1995; 41(2): 109.

(9) Patten CL, Glick BR. Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. *Applied and Environmental Microbiology* 2002; 68(8): 3795.

(10) Ahmad F, Ahmad I, Khan M. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiology Research* 2008; 163(2): 173.

(11) Saxena A, Raj N, Sarosh B, Kini R, Shetty H. Rhizobacteria mediated growth enhancement in Pearl millet. *Indian Journal of Scientific Research* 2013; 4(2): 41.

(12) Kapulnik Y. Plant-Growth-Promoting Rhizobacteria, In: *Plant Roots. The Hidden Half*, Waisel, Y., Eshel, A. and Kafkafi, U., Eds., pp. 717-729. New York: Marcel Dekker, U.S.A. (1991).

(13) Ladha J, Reddy P. Nitrogen fixation in rice systems: State of knowledge and future prospects. *Plant and Soil* 2003; 252(1): 151.

(14) Azadikhah M., Jamali F., Nooryazdan HR. and Bayat F. Screening *Pseudomonas fluorescens* strains for plant growth promoting properties and salinity tolerance. *Biological Journal of Microorganism* 2017; 6(23): 95.

(15) Hamaoui B, Abbadi J, Burdman S, Rashid A, Sarig S, Okon Y. Effects of inoculation with *Azospirillum brasilense* on chickpeas (*Cicer arietinum*) and faba beans (*Vicia faba*) under different growth conditions. *Agronomie* 2001; 21(6-7): 553.

(16) Saravanakumar D, Vijayakumar C, Kumar N, Samiyappan R. PGPR-induced defense responses in the tea plant against blister blight disease. *Crop Protection* 2007; 26(4): 556.

(17) Graumann P. *Bacillus: Cellular and Molecular Biology* (2nd ed.). Caister Academic Press. (2012).

(18) Prathibha K, Siddalingeshwara K. Original research article effect of plant growth promoting *Bacillus subtilis* and *Pseudomonas fluorescence* as Rhizobacteria on seed quality of sorghum. *International Journal Current Microbiology and Applied Science* 2013; 2(3): 11.

(19) Kilian M, Steiner U, Krebs B, Junge H, Schmiedeknecht G, Hain R. FZB24 *Bacillus*

*subtilis*–mode of action of a microbial agent enhancing plant vitality. *Pflanzenschutz-Nachrichten Bayer* 2000; 1(00): 1.

(20) Junges E, Toebe M, Santos RFD, Finger G, Muniz MFB. Effect of priming and seed-coating when associated with Bacillus subtilis in maize seeds. *Revista Ciência Agronômica* 2013; 44(3): 520.

(21) Parvathi A, Krishna K, Jose J, Joseph N, Nair S. Biochemical and molecular characterization of *Bacillus pumilus* isolated from coastal environment in Cochin, India. *Brazilian Journal of Microbiology* 2009; 40(2): 269.

(22) Vivas A, Marulanda A, Ruiz-Lozano JM, Barea JM, Azcón R. Influence of a *Bacillus* sp. on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEG-induced drought stress. *Mycorrhiza* 2003; 13(5): 249.

(23) Brooke JS. *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. *Clinical Microbiology Reviews* 2012; 25(1): 2.

(24) Brimecombe M, De Leij F, Lynch J. Rhizodeposition and microbial populations. In: Pinton R., Veranini Z. & Nannipieri P., eds. *The rhizosphere biochemistry and organic substances at the soil-plant interface*. New York, USA: Taylor & Francis Group. (2007).

(25) Tabatabaei S, Ehsanzadeh P, Etesami H, Alikhani HA, Glick BR. Indole-3-acetic acid (IAA) producing Pseudomonas isolates inhibit seed germination and α-amylase activity in durum wheat (*Triticum turgidum* L.). *Spanish Journal of Agricultural Research* 2016; 14(1): 0802.

(26) Lucy M, Reed E, Glick BR. Applications of free living plant growth-promoting rhizobacteria. *Antonie van leeuwenhoek* 2004; 86(1): 1.

(27) Yasari E, Patwardhan A. Effects of (*Azotobacter* and *Azospirillum*) inoculants and chemical fertilizers on growth and productivity of canola (*Brassica nap*us L.). *Asian Journal of Plant Science* 2007; 6(1): 77.

(28) Naderifar M, Daneshian J. Effect of seed inoculation with *Azotobacter* and *Azospirillum* and different nitrogen levels on yield and yield components of canola (*Brassica na*pus L.). *Iranian Journal of Plant Physiology* 2012; 3(1): 619.

(29) Keyeo F, Aishah O.N, Amir H. Diazotroph in promoting growth of rice seedlings. *Biotechnology Advances* 2011; 10, 267.

(30) Kloepper JW, Beauchamp CJ. A review of issues related to measuring colonization of plant roots by bacteria. *Canadian Journal of Microbiology* 1992; 38(12): 1219.

(31) Safdarpour F. and Khodakaramian G. Assessment of antagonistic and plant growth promoting activities of tomato endophytic bacteria in challenging with *Verticillium dahliae* under in-vitro and in- vivo conditions. *Biological Journal of Microorganism* 2019; 7(28): 77 - 90.

(32) Narula N, Kumar V, Behl RK, Deubel A, Gransee A, Merbach W. Effect of P-solubilizing Azotobacter chroococcum on N, P, K uptake in P-responsive wheat genotypes grown under greenhouse conditions. *Journal of Plant Nutrition and Soil Science* 2000; 163, 393.

(33) Mrkovacki N, Milic V. Use of *Azotobacter chroococcum* as potentially useful in agricultural application. *Annals of Microbiology* 2001; 51(2): 145.

(34) Araújo FFD, Marchesi GVP, Use of *Bacillus subtilis* in the control of root-knot nematode and the growth promotion in tomato. *Ciência Rural* 2009; 39(5): 1558.

(35) Jahanian A, Chaichi MR, Rezaei K, Rezayazdi K, Khavazi K. The effect of plant growth promoting rhizobacteria (PGPR) on germination and primary growth of artichoke (*Cynara scolymus*). *International Journal of Agricultural and Crop Science* 2012; 4(14): 923.

(36) Banowetz GM, Azevedo MD, Armstrong DJ, Halgren AB, Mills DI. Germination-Arrest Factor (GAF): Biological properties of a novel, naturally-occurring herbicide produced by selected isolates of rhizosphere bacteria. *Biological Conservation* 2008; 46(3): 380.

(37) McPhail KL, Armstrong DJ, Azevedo MD, Banowetz GM, Mills DI. 4-Formylaminooxyvinylglycine, an herbicidal germination-arrest factor from Pseudomonas rhizosphere bacteria. *Journal of Natural Product* 2010; 73(11): 1853.

(38) Asghari H, Zahir Z, Arshad M, Khaliq A. Relationship between *in vitro* production of auxins by rhizobacteria and their growth-promoting activities in *Brassica juncea* L. *Biology and Fertility of Soils* 2002; 35(4): 231.