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Research Article

Effect of three commercial bio-fertilizers prepared with *Pseudomonas* on yield and morphophysiological traits of lettuce (*Lactuca sativa* L.)

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ABSTRACT- Bio-fertilizers are microbial inoculants or a combination of carriers of effective microbial strains with high efficiency to provide one or more nutrients needed by the plant, which are important materials as reducing agents to reduce the environmental damage of chemical fertilizers, to reduce diseases, to improve the soil structure, to stimulate plant growth and to increase the quantity and quality of the crops. This study was conducted to investigate the effect of three commercial bio-fertilizers, including *Pseudomonas aeruginosa, P. putida*, and *P. fluorescens*, on some morphological and physiological traits of lettuce under greenhouse conditions. The experiments were held on Nuclear Agriculture Research School in a completely randomized design (CRD) with four replications. The results indicated that inoculation of lettuce at growth stage with these biological fertilizers caused a significant increase in plant growth components such as plant height, leaf number, plant diameter, dry and fresh weights. In the case of physiological indicators, the use of bio-fertilizers increased expression of total protein, and also improved the production of oxidative enzymes (peroxidase and polyphenol oxidase) in the leaf compared to the control. According to the results of this study, it can be concluded that the use of bio-fertilizers contributes to the development of organic agriculture which in turn improves the quantity and quality of the product.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a leafy herbaceous in the family Asteraceae. Lettuce is a salad vegetable and has cultivated since 4500 BC in the Mediterranean area. This plant is a source of vitamins and nutrients which are highly required for human health, and because of high cellulose content, it facilitates digestion.

Although chemical fertilizers are vital to agriculture, concerns have been raised due to their adverse effects on public health, the environment and government spending (Rodriguez and Fraga,1999). Scientists have considered the use of bio-fertilizers as promising alternatives, particularly in developing countries. The application of biological fertilizers, especially plant growth-promoting rhizobacteria such as *Pseudomonas*, has been promoted as a nutritional strategy in the sustainable management of agricultural systems instead of chemical fertilizers. Iran's large-scale development policies emphasize on increasing the production through use of biological fertilizers and biological control of pests, disease and weeds. Several projects on production of bio-fertilizers and biocides are on the agendas of agricultural researchers. Among the soil microorganisms, rhizospheric bacteria have a positive effect on the nutrition and growth of plants and can guarantee plant health and soil fertility; thus, they are called plant-growth-promoting rhizobacteria (PGPR) (Wu et al.,2005).

PGPRs were first defined by Kloepper and Schroth (1978) to describe soil bacteria that colonize the roots of plants and, in a mutualistic manner, enhance plant growth. Researches have been shown that their broad application in the agricultural system is gaining confidence among growers (Reddy et al.,2014). It has been reported that these microbes directly assist fundamental processes required for plant growth, such as fixation of atmospheric nitrogen (N) (Hirel et al., 2011), solubilization of inorganic phosphates (Sharma et al.,2013), sequestration of iron (Sayyed et al., 2013) and synthesis of phytohormones (Maheshwari et al.,2015). Most growth-promoting bacteria used in the researches in recent years are related to the genus *Pseudomonads* (Esmaeil, 2007). *Pseudomonads* spp. are aerobic, gram negative, rod shaped, non-spore forming and fast growing bacteria. *Pseudomonads* is the largest plant growth promoting bacterial group and includes both fluorescent and non-fluorescent species (Weller, 2007). The most important fluorescent species are *Pseudomonas aeruginosa*, *P. putida*, and *P. fluorescens* (Scarpellini et al., 2004). These three species are well-known non- pathogenic species. The non-pathogenicity of these species has already been confirmed by various scientists and the identification of the bacterial species which in this study were used, were confirmed by Soil Biology Department of the Soil and Water Research Institute in Karaj.

Today, the production of organic greenhouse lettuce are many fans among consumers. Bio-fertilizers are the most useful compounds for producing these products. Thus, this study aimed to evaluate the effects of *Pseudomonas aeruginosa*, *P. putida*, and *P. fluorescens* applications on morphological and physiological traits in lettuce plants under greenhouse conditions.

MATERIALS AND METHODS

Experimental Design and Treatments

Three commercial bio-fertilizers including *P. aeruginosa*, *P. putida (*P168*)* and *P. fluorescens* (P169) were obtained from the Soil and Water Research Institute in Karaj, Iran to use in greenhouse for evaluation their effects on morphological and physiological traits in lettuce plants under greenhouse conditions using a completely randomized design with four replications.

Greenhouse Preparations

To conduct the experiments of this study, the temperature in the greenhouse was maintained at 28 ± 2° with a relative humidity of 65% and a 16 h photoperiod which created by using supplemental lighting from high-pressure sodium lamps. Half of the 4-kg plastic pots used were filled with farm soil (sandy-loamy, it contained 45%–85% sand, but had enough silt and up to 20% clay, %0.86 organic carbon, %3 Organic matter with CEC equal to 6.55 meq.100g^{-1}) and half with peat moss. The soil pH was adjusted to 6.6 (if the pH was higher than desired, it was adjusted using a hydrochloric acid solution. If the pH was lower than desired, it was adjusted using a sodium hydroxide solution.). Then the pots were sterilized in an autoclave at a temperature of 120$^{\circ C}$ and a pressure of 1.5 atmospheres and a humidity of 30-40% for 20 minutes. Bio-fertilizer (10^4 bacteria cell per milliliter) was added to each pot three times during the growth stage from the leafy stage of the seedlings at three-week intervals before flowering of the lettuce according to manufacturer recommendations. The test period was 3 months.

Morphological Traits

The number of leaves, plant height (shoot and root), plant diameter, fresh weight (yield), plant dry weight were measured.

Evaluation of Protein, Peroxidase, Polyphenol Oxidase Activity, Proline and Malondialdehyde

The Bradford method was used to determine the Bradford Total proteins. At 72 h after each treatment, 0.5 g of leaf sample was collected and powdered in liquid nitrogen. Fifty ml of buffer was added to each frozen sample for extraction of protein and antioxidant enzymes. The extraction was centrifuged for 15 min at 13000 rpm by using a Hettich™ Universal 320 R Benchtop Centre centrifuge. After that the upper phase was separated and the protein level and enzyme activity were read (Bradford, 1976).

The amount of protein was calculated using bovine serum albumin (BSA) as a standard. A standard curve was prepared using 0, 2, 8, 12, 20 and 25 µg BSA per ml of water. The standards and tests were replicated three times. The absorbance was read at 595 nm using a spectrophotometer (Jenway, USA). Protein samples (50 ml) from plant leaves were deposited with acetone (50 ml) and kept at -70˚C to freeze until they were used. The molecular weight of the extra cellular enzymes was identified by sodium dodecyl sulfate-poly-acrylamide gel electrophoresis (SDS-PAGE) using a 5% stacking and 12% separating polyacrylamide gel by the method of Laemmli (1970). Before electrophoresis, equal volume of sample buffer (100 µl) that comprised 65 mM Tris-Hcl, PH 6.8, 10% (v.v) glycerol, 2% (v.v) SDS, 5% (v.v) 2-mercaptoethanol, and 0.2% (w.v) bromophenol blue was added to the protein sample (100 µl) and boiled for 5 min and applied to loading on the gels. The proteins were separated at constant voltage of 250 V using the running buffer containing 25 Mm Tris, 192 mM glycine, and 0.1% (w.v) SDS, pH 8.3.The gels were stained with Coomassie Brilliant Blue R-250 in methanol-acetic acid-water (5:1:4, v/v), and decolorized in methanol-acetic acid-water (1:1:8,v/v) (Laemmli 1970).

Measurement of the guaiacol peroxidase enzyme activity was based on the amount of oxidation of guaiacol by this enzyme at 470 nm wave readings according to the Chance and Maehly method (Chance and Maehly,1955). The activity of the polyphenol oxidase enzyme was calculated using phosphate buffer and pyrogallol by the Kar and Mishra method (Kar and Mishra, 1976). The absorbance of the purpurogallin formed was taken at 420 nm. The amount of proline was measured by the method of Bates et al. (1973). In order to measure the malondialdehyde concentration, a value of 0.5 g of fresh leaf samples was used and the malondialdehyde concentration was measured at 532 nm according to Stewart and Bewley (1980).

Evaluation of Chlorophyll a and b

To measure the chlorophyll a and b content in accordance with the Arnon (1949) method, one g of leaf was covered with foil, placed in liquid nitrogen and crushed with 85% acetone. The resulting extract was centrifuged at 8000 rpm for 10 min by a Hettich™

Universal 320 R Benchtop Centre centrifuge. The upper phase was separated and the volume of that was brought to 25 ml in a volumetric balloon using 80% acetone. The absorbance of solution was read at 663 and 646 nm, respectively, using a spectrophotometer and the chlorophyll a and b were calculated using the following relations.

Chlorophyll a (U/g FW) = (12.25 Abs 663 nm – 2.79Abs 646 nm)

Chlorophyll b (U/g FW) = (21.21 Abs 646 nm – 5.1 Abs 663 nm)

Statistical Analysis

Statistical analysis was performed using SPSS Version 13 and means comparison were performed using Duncan's multi-range test at $P < 0.05$.

RESULTS AND DISCUSSION

Evaluation of Morphological Traits

The growth components such as plant height, dry and fresh weight, leaf number, plant diameter, leaf area (unexposed data) increased under the influence of the bio-fertilizers.

The effect of bio-fertilizer treatments on plant height showed that there was no significant difference between the results of the strain of bacteria in the bio-fertilizers, but all treatments were significantly different from the control at the 5% level (Fig. 1). The evaluation of treatments on plant height indicated that bio-fertilizers increased this index significantly in comparison with the control (11.02 cm), however, *P. aeroginosa* with 13.09 cm average height led to the highest average value in treated plants and *P. fluorescens* with 12.69 cm average height and *P. putida* with 12.64 cm average height were approximately the same.

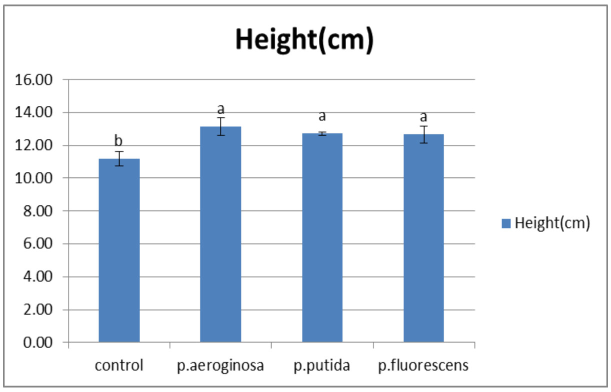

Fig. 1. The effect of bio-fertilizer treatment on plant height Different small characters (a and b) on the top of columns for each plant responses denote significant differences (p<0.05, Duncan's multiple range test). Bookmarks in each column represent Error Bar.

The effect of bio-fertilizer treatments on dry and fresh weight showed that the highest plant dry and fresh weight was related to *Pseudomonas fluorescens* and the lowest plant dry and fresh weight was related to the control. The fresh weight index showed better differences between treatments on plants. By comparing the mean fresh weight of plants treated with biological fertilizers and the control (68.47 gr), it was found that the reaction of lettuce plants to each of species of *Pseudomonas* was different, so that the treatment of *P. fluorescens* (87.7gr) had the best effect on increasing the fresh weight of the plants. Although the use of *P. aeroginosa* (78.72gr) produced the high longitudinal growth in the plant, in comparison with *P. fluorescens* species, it was the weakest species according to the fresh weight of the plant, which indicates the use of this species as a biological fertilizer is not a good option for increasing the yield of lettuce (Fig. 2).

The effect of bio-fertilizer treatments on number of leaves showed that the highest leaf number was related to *p. fluorescens* (55) and *p. putida* (50.80), respectively, and the lowest number of leaves was related to the control (26.60). The number of leaves in lettuce plants which treated with *P. aeroginosa* was 44.80. So, the greatest effect of bio-fertilizer application was observed on the number of leaves in lettuce plants which treated with *P. fluorescens* and *P.putida* where, the number of leaves was twice as high as the control plants. (Fig. 3).

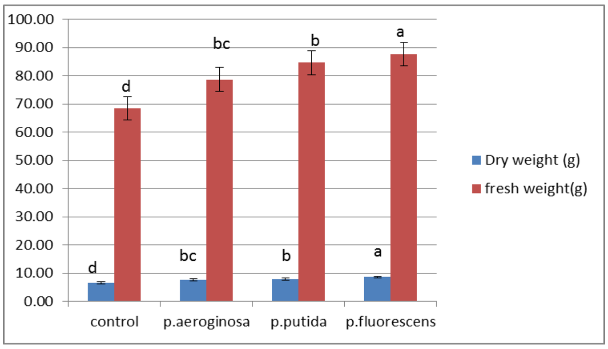

Fig. 2. The effect of bio-fertilizer treatment on plant dry and fresh weight

Different small characters (a-d) on the top of columns for each plant responses denote significant differences (p<0.05, Duncan's multiple range test). Bookmarks in each column represent Error Bar.

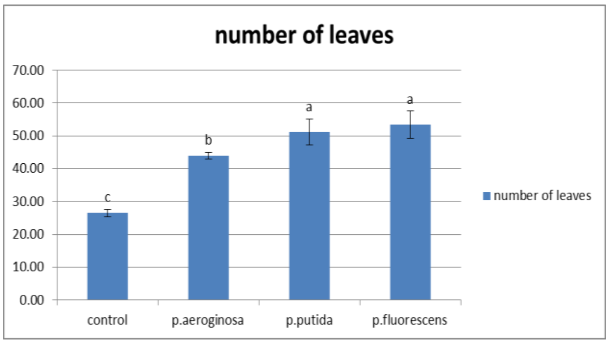

Fig. 3. The effect of bio-fertilizers treatment on the number of leaves Different small characters (a-c) on the top of columns for each plant responses denote significant differences (p<0.05, Duncan's multiple range test). Bookmarks in each column represent Error Bar.

Results indicated that different bio-fertilizers increased the plant diameter compared to the control

(Fig. 4). The effect of bio-fertilizer treatments on the plant diameter showed that the highest plant diameter was related to *p. fluorescens* (20.25 cm) and the lowest plant diameter was related to the control (7.72 cm). *p. putida* (17.13cm) and *p. aeroginosa* (16.12 cm) were in the same group for their effect on the plant diameter. The best treatment on the plant diameter was *p. fluorescens*.

Glick et al. (1997), Frommel et al. (1993) and De Freitas and Germia. (1992) showed that application of *P. putida* and *P. fluorescens* could increase root and shoot length in canola, lettuce, tomatoes, wheat and potatoes. It has been found that inoculating *Nigella sativa* L. seeds with *Azospirillum*, *Azotobacter* and *Pseudomonads* improved growth factors such as plant height (Shaalan, 2005; Khoramdel et al., 2008). Researchers have argued that bacterial synthesis of auxin and regulation of ethylene production in young seedlings are the most important mechanisms of rhizospheric bacteria for stimulating plant growth (Glick et al., 1998).

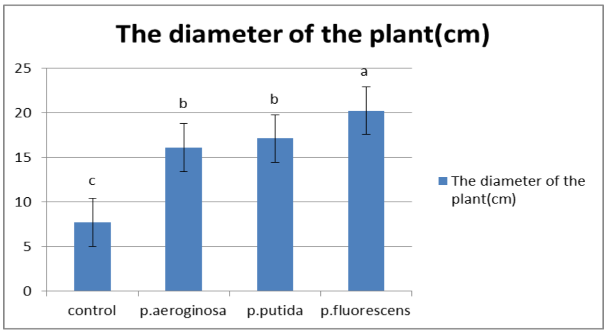

Fig. 4. The effect of bio-fertilizers treatment on the diameter of the plant Different small characters (a-c) on the top of columns for each plant responses denote significant differences (p<0.05, Duncan's multiple range test). Bookmarks in each column represent Error Bar.

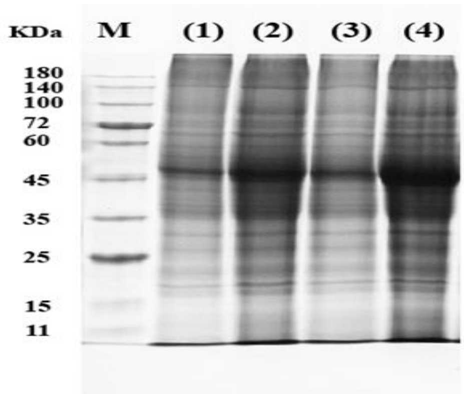

Fig. 5. SDS-PAGE of extractive proteins from lettuce leaf samples: (1) Control plant, (2) A plant treated with *P. fluorescens*, (3) A plant treated with *P .aeroginosa*, (4) A plant treated with *P. putida*. "M" indicates a molecular weight marker (SinaClon BioScience, Prestained protein ladder, PR901641).

Asghar et al. (2002), Bashan et al. (2004) and Biswas et al. (2000) reported significant increases in the growth and yield of crops including *Brassica juncea* L. and rice in response to inoculation with PGPR were seen and Chamangasht et al. (2012) found that bio-fertilizers could improve lettuce yield. Rodriguez and Fraga (1999) and Anzuay et al. (2015) showed that organic and inorganic phosphate solubilizing bacteria increased the fresh and dry weight of the plant, which was similar to the results of this study. Kohler et al.(2007) observed that inoculation of lettuce with growth promoting bacterium *Bacillus subtilis* caused to increase the dry weight of the plant. Gholami et al. (2009) indicated that increasing of leaf dry weight of maize and increasing in total fresh weight of this plant with application of *Azospirilum berazilens* and *Pseudomonas putida* were due to the ability of these plant growth promoting rhizobacteria to produce hormonal substances and biological stabilization of nitrogen. Results of Khosravi et al. (2017) showed that inoculation of phosphate solubilizing bacterium to seed of plants caused increasing fresh and dry weight of lettuce. It seems that increasing the absorption of nutrients by the plant can lead to increase accumulation of dry matter and minerals in the stems and leaves of the plant. Another reason for the increasing of aerial parts weight was mentioned to be hydrolyze of the ACC (1-aminocyclopropane-1-carboxylic acid) by bacteria to reduce the level of ethylene around the roots of the plant and cause to increase the weight of the roots and shoots (Shaharoona et al., 2007).

Banchio et al. (2008) reported that inoculation of marjoram (*Origanum majorana* L.) with *Pseudomonas fluorescens* increased the number of leaves by 80%, per each plant than the control which were similar to the results of this experiment. Fasciglione et al.(2012) reported that inoculation of lettuce with a species of *Azospirillum* increased the number of lettuce leaves. Results of Noumavo et al. (2013) study showed that inoculation of corn with growth promoting bacteria could increase plant growth. Kouchaki et al. (2008) stated that the use of *Pseudomonas fluorescent* bacteria leads to an increase in the diameter of the stem. This could be due to the production and release of plant growth stimulating compounds or some growth regulating hormones that have been implanted by the bacterium through the leaf which in turn they have affected plant production and growth. It was also reported that plant growth promoting rhizobacteria have increased the diameter of corn stalks (Ehteshami et al., 2009). The reason for the increase in diameter can be discussed by increasing accumulation of protein fractions and higher biomass of plant. Also, the production of higher biomass of inoculated plants with phosphate soluble microorganisms can be attributed to the involvement of these microorganisms in increasing host plant nutrition as a result of increased water and food intake. This increase in nutrient uptake by plants might be explained through organic acid production by the plants and PGPRs, and decreasing the soil pH in rhizosphere. Growth promoting substances are likely to be produced in large quantities by these rhizosphere microorganisms that influence indirectly on the overall morphology of the plants and the growth of plant basically depends on a number of factors like plant genotype, nature of PGPR inoculants as well as environmental conditions.

Evaluation of Protein, Peroxidase Activity, Polyphenol Oxidase, Chlorophyll a and b, Proline, Malondialdehyde

The results of all morphological and physiological treatments performed in this study showed not only the non-pathogenicity of the bacterial isolates (*P. aeruginosa*, *P. putida* (P168) and *P. fluorescens* (P169)), but also their effect as bio-fertilizers in increasing plant growth as previously reported by other researchers (Dashawn et al 2013 and Nakkeeran et al 2005). By considering the positive effects of plant growth-promoting rhizobacteria on plant growth by mechanisms such as hydrogen cyanide production, iron supply through Siderophores production and dissolution of insoluble phosphate, and production of growth hormones and regulators, it can be concluded that physiological traits such as antioxidant enzymes can change under the influence of plant growth-promoting bacteria. Decreasing in plant growth seems to be the result of altering many physiological activities in plants such as photosynthesis, mineral uptake, and antioxidant activity.

The best treatment of this study that causing the maximum production of chlorophyll, protein, proline and antioxidant enzymes in tested plants was *Pseudomonas putida*. This treatment kept the plants in better and healthier conditions and in a better quality. Among the physiological indicators, the use of bio-fertilizers increased expression of total proteins, proline, and antioxidant enzymes, chlorophyll content compared to the control (Tables 1, 2).

Inoculation of tested plants with bacteria caused significant changes in the expression of proteins involved in the metabolism of amino acids and carboxylic acid cycles. The results obtained by other researchers showed that rapeseed plants inoculated with *Pseudomonas florescence FY32*, a plant growth promoter, had better growth characteristics than control plants to osmotic and salinity stresses, and the expression levels of protein in the inoculated plants were increased. In addition, proteins involved in energy metabolism, photosynthesis, and membrane proteins had the most significant expression changes in bacteria inoculated plants (Banaei et al., 2016; Shokri Gharelo et al., 2016).

Enzymatic antioxidant systems protect cells from free radical scavenging oxygen (ROS). The metabolism of free oxygen radicals is dependent on several interacting structural oxidants such as superoxide dismutase, catalase, and peroxidase. Rhizospheric bacteria induce resistance through different pathways. Enzymes such as polyphenol oxidase, peroxidase and chitinas and the phenolic content are related to systemic induction resistance (Chen et al.,2000; Jung et al.,2007). Therefore, plant growth-promoting bacteria increase plant antioxidant levels by protecting the plant (Pourebtehaj et al.,2012). The positive role of plant growth metabolites in the production of secondary metabolites in plants has been well defined (Sangwan et al.,2001) and the results of the current study indicated the increasing of physiological traits such as the amount of chlorophyll a and b and antioxidant enzymes in plants inoculated with the bacteria. Increasing the antioxidant enzymes in plants increases the quality of the product. The bacteria that increased plant growth in this study increased the antioxidant enzymes compared to the control.

Table 1. The average data showing effect of bio-fertilizers prepared from *Pseudomonas* species on physiological traits of lettuce compare to control

Treatment	Antioxidant enzyme(U/gfw)		Protein (mg/gfw)
	Peroxidase	Polyphenol oxidase	
control	4.41^{b}±0.09	26.65^{b} ± 0.66	2.30^{b}±0.05
P.fluorescens	5.78^{b}±0.33	30.36^{b} ± 0.61	2.24^{b}±0.05
P.putida	6.14^{a}±0.67	37.58^{a} ± 0.63	2.75^{a}±0.02
P.aeroginosa	6.80^{a}±0.05	38.13^{a} ± 0.75	2.24^{b}±0.04

Different superscript small letters (a-d) within a column denote significant differences (p<0.05, Duncan's multiple range test)

In a study conducted by Islam et al. (2014), it was shown that *Pseudomonads* bacteria increased the activity level of antioxidant enzymes of superoxide dismutase, catalase and peroxidase, and also reduced the hydrogen peroxide and malondialdehyde. It was concluded that there is a negative correlation between increase of leaf malondialdehyde concentration and plant growth.

Saghafi et al. (2013) reported that *Pseudomonads* strains increased the physiological traits regarding the chlorophyll content of wheat in comparison with control treatment, so that the highest chlorophyll index, chlorophyll a content were found in the treatments that *Pseudomonads* bacteria were used. Similarly, Abbas et al. (2013) reported that phosphate solubilizing bacteria increased the amount of chlorophyll content in corn. Plantlets co-cultured with *Pseudomonas* spp. produced more phenolic and chlorophyll than the non-bacterized control (Nowak 1998). The chlorophyll content also increased significantly in all the PGPR treatments. Other researchers have confirmed that inoculation of lettuce with plant growth promoting bacteria effected the growth and total chlorophyll content under salinity stress (Han and Lee,2005). Marius et al. (2005) showed that the effect of bacterial inoculation on sunflowers increased chlorophyll a and b contents and carotene pigments before and after flowering and that sunflower plant growth improved with the bio-fertilizer treatments compared to the control. In fact, it can be said that the bacterium provides more water and nutrients to the plant, thereby increasing the amount of pigment production and facilitating the transfer of water and photosynthetic materials in the plant. Therefore, according to the obtained results in this study, it can be admitted that the higher chlorophyll content of the leaves leads to an increase in the photosynthesis of the plant.

Table 2. The average data showing effect of bio-fertilizers prepared from *Pseudomonas* species on physiological traits of lettuce compare to control

Treatments	Chlorophyll (μg/gfw)		proline (Ug/gfw)	Malondialdehyd (n mol/gfw)
	Chlorophyll b	chlorophyll a		
control	$14.56^{d}\pm0.69$	$17.94^{b}\pm0.01$	$56.98^{d}\pm 0.96$	$4.71^{a}\pm0.09$
P.fluorescens	$16.26^{c}\pm0.61$	$29.69^{b}\pm0.61$	$68.38^{b}\pm0.60$	$3.16^{c}\pm 0.26$
P.putida	$19.15^{b}\pm0.67$	$39.60^{a}\pm0.53$	$75.07^{a}\pm0.62$	$2.19^{d}\pm0.19$
P. aeroginosa	$24.68^{a}\pm0.88$	$94.12^{a}\pm0.67$	$65.93^{c}\pm 1.20$	$3.73^{b}\pm0.09$

Different superscript small letters (a-d) within a column denote significant differences (p<0.05, Duncan's multiple range test)

In other words, increasing the production of photosynthetic materials in this way increased %10-20 of plant yield (Fig. 2).

The results of this study also showed that these microorganisms caused higher plant yields due to their high efficiency in phosphorus absorption from the soil, which indicates the role of phosphorus in the synthesis of chlorophyll and consequently the amount of plant photosynthesis.

It has been shown that soil microorganisms have a significant effect on the amount of proline. In a study conducted on wheat by saghafi et al. (2013), it was shown that the highest amount of proline was found in *Pseudomonas* bacteria treatments which was similar to the results of this study. Jiang and Huang (2000) reported that amino acids such as proline may have a protective role for chloroplast thylakoids and other membrane systems. Proline prevents the change of nature by affecting the solubility of proteins and enzymes.

The protein profiles of all treatment including plants treated with *p. fluorescens*, *p. aeruginosa* and *p.putida* and also control plant were compared by SDS-PAGE (Fig. 5). Reducing the expression of proteins has been done in both of inoculated plants and non-inoculated plant, but the amount of protein degradation in non-inoculated plants has occurred less.

The banding patterns of proteins of plant treated with *P. fluorescens* and plant treated with *P. putida* were very similar and identical. The control plant and plant treated with *P. aeruginosa* contained much fewer protein bands than the other two treatments. *P. fluorescens* is a robust expression host for high yield expression of secreted proteins. These results can indicate the positive effect of bacteria on lettuce and show the ability of this bacterium to modify the effects of stress on the plant. Plants inoculated with bacteria showed better growth characteristics than non-inoculated plants. Proteins in bacteria inoculated and non-stressed plants were also more expressed than control plants. The electrophoretic pattern of the proteins associated with the leaves of the lettuce treated with *Pseudomonas* strain and its comparison with the control showed that significant quantitative and qualitative changes were observed in protein bands whose molecular weights were above 25 and below 72 kDa. With the use of *Pseudomonas fluorescens* and *Pseudomonas putida*, the quantity and quality of the protein bands of the lettuce have increased. Also, the presence of unchanged bands in the control and control group can indicate the resistance of some proteins to *Pseudomonas*.

CONCLUSIONS

The results of this study showed a positive effect of *Pseudomonads* rhizobacteria on the morphological traits of lettuce including height, yield, number of leaves and dry weight, plant diameter compared to the control plant. In general, it can be stated that the use of biological treatments improves the efficiency of growth factors and physiological activities of the plant in comparison to the time of non-use of biological treatments. However, the strategy of using PGPR to enrich amounts of minerals and vitamins in major food crops has also gained consideration of workers. Increased demands for organic products indicate consumer preference for reduced chemical use.

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REFERENCES

Abbas, Z., Zia, M. A., Ali, S., Abbas, Z., Waheed, A., Bahadur, A., Hameed, T., Iqbal, A., Muhammad, I., Roomi, S., Ahmad, M. Z., Sultan, T. (2013). Integrated effect of plant growth promoting rhizobacteria, phosphate solubilizing bacteria and chemical fertilizers on growth of maize original research article. *International Journal of Agriculture and Crop Sciences*, 6(13), 913-921.

Anzuay, M. S., Ludueña, L. M., Angelini, J. G., Fabra, A., & Taurian, T. (2015). Beneficial effects of native phosphate solubilizing bacteria on peanut (*Arachis hypogaea* L) growth and phosphorus acquisition. *Symbiosis*, 66(2), 89-97.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant Physiology*, 24(1), 1-15.

Asghar, H., Zahir, Z., Arshad, M., & Khaliq, A. (2002). Relationship between in vitro production of auxins by rhizobacteria and their growth-promoting activities in *Brassica juncea* L. *Biology and Fertility of Soils*, 35(4), 231-237.

Banaei-Asl, F., Farajzadeh, D., Bandehagh, A., & Komatsu, S. (2016). Comprehensive proteomic analysis of canola leaf inoculated with a plant growth-promoting bacterium, *Pseudomonas fluorescens*, under salt stress. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomic,* 1864(9), 1222-1236.

Banchio, E., Bogino, P. C., Zygadlo, J., & Giordano, W. (2008). Plant growth promoting rhizobacteria improve growth and essential oil yield in *Origanum majorana* L. *Biochemical Systematics and Ecology*, 36(10),766-771.

Bashan, Y., Holguin, G., & De-Bashan, L. E. (2004). *Azospirillum*-plant relationships: Physiological, molecular, agricultural, and environmental advances (1997-2003). *Canadian Journal of Microbiology*, 50(8), 521-577.

Bates, L. S., Waldren, R. P., & Teare, I. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39(1), 205-207.

Biswas, J., Ladha, J., & Dazzo, F. (2000). Rhizobia inoculation improves nutrient uptake and growth of lowland rice. *Soil Science Society of America Journal*, 64, 1644-1650.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248-254.

Chamangasht, S., Ardakani, M. R., Khavazi, K., Abbaszadeh, B., & Mafakheri, S. (2012). Improving lettuce (*Lactuca sativa* L.) growth and yield by the application of biofertilizers. *Annals of Biological Research*, 3(4), 1876-1879.

Chance, B., & Maehly, A. C. (1955). *Assay of catalases and peroxidases. Methods in Enzymology*, 2, 764-775.

Chen, C., Belanger, R. R, Benhamou, N., & Paulitz, T. C. (2000). Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. *Physiological and Molecular Plant Pathology*, 56(1), 13-23.

Dashawn, V. K., & Kumar, P. (2013). Production of plant growth promoting substance by pseudomonads. *Journal of Academia and Industrial Research (JAIR)*, 2(4), 221-225.

De Freitas, J., & Germida, J. (1992). Growth promotion of winter wheat by *fluorescent pseudomonads* under field conditions. *Soil Biology and Biochemistry*, 24(11), 1137-1146.

Ehteshami, S. M. R., Aghaalikhani, M., Chaichi, M. R., & Khavazi, K. (2009). Effect of phosphate biofertilizers on yield and yield components of grain corn (*Zea mays* L. S.C.704) under water deficit stress conditions. *Iranian Journal of Field Crop Science*, 40(1), unpaginated. Retrieved from: https://www.researchgate.net/publication/291831532

Esmaeil, Y. A. M. P. (2007). Effects of (*Azotobacter* and *Azosprillium*) inoculants and chemical fertilizers on growth and productivity of canola (*Brassica napus* L). *Asian Journal of Plant Sciences*, 6(1),77-82.

Fasciglione, G., Casanovas, E. M., Yommi, A., Sueldo, R. J., & Barassi, C. A. (2012). *Azospirillum* improves lettuce growth and transplant under saline conditions. *Journal of the Science of Food and Agriculture*, 92(12), 2518-2523.

Frommel, M., Nowak, J., & Lazarovits, G. (1993). Treatment of potato tubers with a growth promoting *Pseudomonas* sp.: Plant growth responses and bacterium distribution in the rhizosphere. *Plant and Soil*, 150(1), 51-60.

Gholami, A., Shahsavani, S., & Nezarat, S. (2009). The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *International Journal of Biological and Life Science*, 1(1), 35-40.

Glick, B. R., Penrose, D. M., & Li, J. (1998). A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *Journal of Theoretical Biology*, 190(1), 63-68.

Glick, B. R., Liu, C., Ghosh, S., & Dumbroff, E. B. (1997). Early development of canola seedlings in the presence of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2. *Soil Biology and Biochemistry*, 29(8), 1233-1239.

Han, H., & Lee, K. (2005). Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. *Research Journal of Agriculture and Biological Sciences*, 1(3), 210-215.

Hirel, B., Tétu, T., Lea, P. J., & Dubois, F. (2011). Improving nitrogen use efficiency in crops for sustainable agriculture. *Sustainability*, 3(9), 1452-1485.

Islam, F., Yasmeen, T., Ali, Q., Ali, S., Arif, M. S., Hussain, S., Rizvi, H. (2014). Influence of *Pseudomonas aeruginosa* as PGPR on oxidative stress tolerance in wheat under Zn stress. *Ecotoxicology and Environmental Safety*, 104, 285-293.

Jiang, Y., & Huang, B. (2000). Effects of drought or heat stress alone and in combination on Kentucky bluegrass. *Crop Science*, 40(5), 1358-1362.

Jung, W. J., Mabood, F., Kim, T. H., & Smith, D. .L. (2007). Induction of pathogenesis-related proteins during biocontrol of *Rhizoctonia solani* with *Pseudomonas aureofaciens* in soybean (*Glycine max* L. Merr.) plants. *BioControl*, 52(6), 895-904.

Kar, M., & Mishra, D. (1976). Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. *Plant Physiology*, 57(2), 315-319.

Khoramdel, S., Kouchaki, A. R., Nasiri, M. M., & Ghorbani, R. (2008). Application effects of biofertilizers on the growth indices of black cumin (*Nigella sativa* L.). *Iranian Journal of Field Crop Research,* 6(2), 285-294.

Khosravi, A., Zarei, M., & Ronaghi, A. (2017). Effect of phosphate solubilizing bacterium, vermicompost and phosphate sources on growth of lettuce in a calcareous soil. *Journal of Soil Biology*, 5(1), 81-93.

Kloepper, J., & Schroth, M. (1978). Association of in vitro antibiosis with inducibility of increased plant growth by *Pseudomonas* spp.(Abstr). *Phytopathol. News*, 12, 136.

Kohler, J., Caravaca, F., Carrasco, L., & Roldán, A. (2007). Interactions between a plant growth-promoting rhizobacterium, an AM fungus and a phosphate-solubilising fungus in the rhizosphere of *Lactuca sativa*. *Applied Soil Ecology*, 35(3), 480-487.

Kouchaki, A. R., Tabrizi, L., & Ghorbani, R. (2008). Effect of biofertilizers on agronomic and quality criteria of Hyssop (*Hyssopus officinalis*). *Iranian Journal Of Field Crops Research*, 6(1), 127-137.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227(5259), 680-685.

Maheshwari, D. K., Dheeman, S., & Agarwal, M. (2015). Phytohormone-producing PGPR for sustainable agriculture. In Maheshwari D. K, (Ed.), *Bacterial metabolites in sustainable agroecosystem* (pp 159-182). Cham: Springer International Publishing.

Marius, S., Octavita, A., Eugen, U., & Vlad, A. (2005). Study of a microbial inoculation on several biochemical indices in sunflower (*Helianthus anuus* L.). *Genetics and Molecular Biology*, 12(2), 11-14.

Nakkeeran, S., Fernando, W. D., & Siddiqui, Z. A. (2005). Plant growth promoting rhizobacteria formulations and its scope in commercialization for the management of pests and diseases. In Siddiqui, Z. A. (Ed.) PGPR: *Biocontrol and biofertilization* (pp. 257-296). Dordrecht: Springer.

Noumavo, P. A., Kochoni, E., Didagbé, Y.O., Adjanohoun, A., Allagbé, M., Sikirou, R., Gachomo, E. W., Kotchoni, and S. O., Baba-Moussa, L. (2013). Effect of different plant growth promoting rhizobacteria on maize seed germination and seedling development. *American Journal of Plant Sciences*, 4(05), 1013-1021.

Nowak, J. (1998). Benefits of *in vitro* “biotization” of plant tissue cultures with microbial inoculants. *In Vitro Cellular & Developmental Biology-Plant*, 34(2), 122-130.

Pourebtehaj, M., Habibi, D., Paknejad, F., Fazeli, F., & Davoodi, F. M. (2012). Effect of plant growth promoting rhizobacteria and foliar application of silicic acid and amino acids on destruction antioxidant enzyme activity of barley under drought stress *(Hordeum Vulgar* L). *Iranian Journal of Agronomy and Plant Breeding*, 8(2), 147-160.

Reddy, M., Ilao, R. I., & Faylon, P. S. (2014). *Recent advances in biofertilizers and biofungicides (PGPR) for sustainable agriculture*. England: Cambridge Scholars Publishing.

Rodriguez, H., & Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances*, 17, 319-339.

Saghafi, K., Ahmadi, J., Asgharzadeh, A., & Bakhtiari, S. (2013). The effect of microbial inoculants on physiological responses of two wheat cultivars under salt stress. *International Journal of Advanced Biological and Biomedical Research*, 1(4), 421-431.

Sangwan, N., Farooqi, A., Shabih, F., & Sangwan, R. (2001). Regulation of essential oil production in plants. *Plant Growth Regulation*, 34(1), 3-21.

Shaalan, M. (2005). Influence of biofertilizers and chicken manure on growth, yield and seeds quality of (Nigella Sativa L.) plants. *Egyptian Journal Agricultural Research*, 83, 811-828.

Shaharoona, B., Arshad, M., Zahir, Z. A., & Khalid, A. (2007). "Performance of *Pseudomonas* spp. containing ACC deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer." *Soil Biology and Biochemistry*, 38(9), 2971-2975.

Sayyed, R., Chincholkar, S., Reddy, M., Gangurde, N., & Patel, P. (2013). Siderophore producing PGPR for crop nutrition and phytopathogen suppression. In Maheshwari, D. K, (Ed.), *Bacteria in Agrobiology*: *Disease Management* (pp. 449-471). London: Springer.

Scarpellini, M., Franzetti, L., & Galli, A. (2004). Development of PCR assay to identify *Pseudomonas fluorescens* and its biotype. *Federation of European Microbiological Societies Microbiology Letters*, 236(2), 257-260.

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., & Gobi, T. A. (2013). Phosphate solubilizing microbes: Sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus*, 2(1), 1-14.

Shokri Gharelo, R., Bandehagh, A., Toorchi, M., & Farajzadeh, D. (2016). Canola 2-dimensional proteom profile under osmotic stress and inoculation with *Pseudomonas fluorescens FY32*. *Plant Cell Biotechnology and Molecular Biology*, 17, 257-266.

Stewart, R. R., & Bewley, J. D. (1980). Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiology*, 65(2), 245-248.

Weller, D. M. (2007). Pseudomonas biocontrol agents of soilborne pathogens: Looking back over 30 years. *Phytopathology*, 97(2), 250-256.

Wu, S. C., Cao, Z. H., Li, Z. G., Cheung, K. C., & Wong, M. H. (2005). Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: A greenhouse trial. *Geoderma*, 125(1), 155-166.

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تاثیرسه نوع کودزیستی تجاری تهیه شده ازباکتری سودوموناس برعملکرد و برخی صفات مورفوفیزیولوژیک گیاه کاهو

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عملکرد

چکیده- کودهای زیستی، مایه میکروبی یا یک ترکیب حامل سوش های میکروبی موثر با راندمان بالا برای تامین یک یا چند عنصر غذایی مورد نیاز گیاه می باشد که دارای اهمیتی چون کاهش زیان های زیست محیطی کودهای شیمیایی، کاهش بیماری ها، بهبود ساختمان خاک و تحریک رشد گیاه و افزایش کمی و کیفی محصول می گردند. این مطالعه به منظوربررسی تأثیر سه نوع کود زیستی تجاری شامل *سودوموناس آئروجینوزا، سودوموناس پوتیدا، سودوموناس فلورسنس* بر برخی صفات مورفوفیزیولوژیک گیاه کاهو در شرایط گلخانه انجام شد .آزمایشات در پژوهشکده کشاورزی هسته ای ودر قالب یک طرح کاملا تصادفی درچهار تکرار انجام شد. نتایج نشان داد که مایه زنی نشاهای کاهو با کودهای زیستی مذکور، باعث افزایش معنی دار فاکتورهای رشدی مانند ارتفاع گیاه، تعداد برگ، قطر گیاه و وزن خشک و وزن تر گیاه گردید. در مورد شاخص های فیزیولوژیک، مصرف کودهای زیستی باعث بهبود تولید آنزیم های اکسیدانی و تجمع بالاتر پروتئین در برگ درمقایسه باشاهد شد. با توجه به نتایج این مطالعه ، استفاده از کودهای زیستی به توسعه کشاورزی ارگانیک کمک می کند که باعث بهبود کمی و کیفی محصول می شود.