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Research Article

Investigation of the Effects of Some Plant Growth Promoting Bacteria on Curly Lettuce (*Lactuca sativa L.* var. *crispa*) Seedling Development[1]

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Abstract

Lettuce is an annual vegetable crop with an edible leaf and widely grown and consumed around the world. It is consumed in all seasons of the year because it can be grown in the open fields and greenhouses. However, to meet the fresh lettuce demand of the increasing world population, it has brought along the use of excessive synthetic fertilizers, which cause environmental pollution and are harmful to human health. Nowadays, by developing environmentally friendly agricultural techniques, alternative applications have gained momentum in order to minimize the use of chemical fertilizers. In our study, 13 strains of PGPR (RC631, RC67 and RC11 strains of *Bacillus subtilis* RC34 and RC33 strains of *B. megaterium* RC356 strain of *B. pumilus,*, RC77e, RC86 and RC9 strains of *Pseudomonas fluoresence*, biotype B RC14 strain of *P. putida*, RC36 and RC16 strains of *Pantoea agglomerans* and RC49 strain of *Rodococcus erythropolis*) were used. Bacterial strains in 5 different combination groups (B1, B2, B3, B4, B5) were applied to lettuce seeds and their effects on seedling growth were investigated. The study was carried out in Eskişehir Osmangazi University, Faculty of Agriculture, Department of Horticulture experimental fields and the "Sürpriz" curly leaf salad variety was used as plant material.
PGPR applications significantly increased the seedling's leaf fresh weight, root length and fresh weight and root collar diameter compared to control applications and had no effect on leaf number and dry weight and root dry weight. As a result, it was found that the use of a mixture of different PGPR strains as a biofertilizer had significant effects on the development of lettuce seedlings and concluded that the research should be done more comprehensively with different plant species, different application forms and doses.

Keywords: Curly lettuce (*Lactuca sativa L.* var. *crispa*), PGPR, Plant Growth, Seedling

Bitki Gelişimini Teşvik Eden Bazı Bakterilerin Kıvırcık Salata (*Lactuca sativa L.* var. *crispa*) Fide Gelişimi Üzerine Etkilerinin Araştırılması

Öz

Marul, tek yıllık, yaprakları yenilen, dünya genelinde yaygın olarak yetiştirilen ve çok tüketilen bir sebze türüdür. Marul örtüaltında ve açık alanda yetiştiriciliği yapılabildiği için yılın her mevsiminde tüketilmektedir. Artan dünya nüfusun taze marul talebini karşılamak için üretimde, çevre kirliliğine neden olan ve insan sağlığına zararlı olan aşırı kimyasal gübre kullanımı giderek artmaktadır. Günümüzde çevre dostu tarım tekniklerinin geliştirilerek kimyasal gübre kullanımın en aza indirmek için alternatif uygulamalar hız kazanmıştır. Çalışmamızda 13 adet PGPR straini (*Bacillus subtilis* RC631, RC67, RC11, *B. megaterium* RC34, RC33, *B. pumılus* RC356,

[1] This study was produced from Abdukadır Yusuf HUSSEIN's master's thesis titled "Investigation of the Effect of Some Plant Growth Promoting Bacteria on Plant Growth in Curly lettuce (Lactuca sativa L. var. crispa)".
Root bacteria combination solutions used in practice, were obtained from Çanakkale 18 Mart University at Prof. Dr. Ramazan Çakmakçı's private collection.
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Pseudomonas fluoresence RC77e, RC86, RC9, *P. putida* biyotip B RC14, *Pantoea agglomerans* RC 36, RC 16 ve *Rodococcus erythropolis* RC49) kullanılmıştır. Bakteri strainleri 5'erli grup halinde 5 (B1, B2, B3, B4, B5) farklı kombinasyon olarak marul tohumlarına uygulanarak fide gelişimi üzerindeki etkileri araştırılmıştır. Çalışma ESOGÜ Ziraat Fakültesi Bahçe Bitkileri Bölümünde yürütülmüş olup bitkisel materyal olarak "Sürpriz" kıvırcık yapraklı salata çeşidi kullanılmıştır.
Fidelerin yaprak yaş ağırlığı, kök uzunluğu ve yaş ağırlığı ile kök boğaz çapı üzerine PGPR uygulamalarının kontrol uygulamalarına göre önemli oranda arttırdığı, yaprak sayısı ve kuru ağırlığı ile kök kuru ağırlığı üzerine etkisinin olmadığı istatistiksel olarak tespit edilmiştir. Sonuç olarak farklı PGPR strainleri karışımının biyogübre olarak kullanımının marul fidelerinin gelişmesi üzerine önemli etkileri olduğu belirlenmiş olup, araştırmanın farklı bitki türleri, farklı uygulama şekil ve dozları ile daha kapsamlı olarak yapılması sonucuna varılmıştır.

Anahtar kelimer: Kıvırcık Salata (*Lactuca sativa* L. var. *crispa*), PGPR, Bitki Gelişimi, Fide

1. Introduction

After the importance of vegetables in human nutrition was understood, researches on their properties increased even more (Vural et al., 2000). Vegetables are a natural source of fiber, minerals, antioxidants, vitamins, carbohydrates, and proteins (George, 2009).

Lettuce (*Lactuca sativa* L.) group of vegetables are in the *Asteraceae* (*Compositae*) family and widely grown all over the world, especially in temperate and subtropical climates (Mou, 2008). Lettuce is an annual, highly self-pollinated, and important commercial vegetable crop, mostly used in prepared foods such as salads and sandwiches. In some countries, such as China and Egypt, the stem of lettuce is consumed instead of its leaves (Hasan et al., 2021; Mou, 2008).

The curly lettuce (*Lactuca sativa* L. var. *crispa*) variety are gaining popularity among consumers daily. Curly salad contains more fiber, minerals, and vitamins than head lettuce because it forms a large structure with leaves of various shapes and colors without forming a solid head (Koudela and Petříková, 2008).

Since lettuce group of vegetables are grown open fields and in greenhouses, it is possible to consume them in all four seasons of the year (De Vries, 1997). Achieving high yield and quality in agricultural production to meet the demand of consumer markets has brought about the excessive use of chemical fertilizers (Ekinci et al., 2015; dos Santos et al., 2020). Excessive use of chemical fertilizers not only causes an environmental pollution, but also damages human health and soil structure, and increases plant production inputs (Altaf et al., 2019). To reduce the harmful effects of chemical fertilizers on the environment and human health, scientists are searching for applications that are cost-effective, ecologically friendly, and simple to use. Due to their current relative affordability, accessibility, and ease of use, plant growth promoting root bacteria (also known as PGPRs) have gained popularity. In addition to their plant growth-promoting effects, PGPRs also have species that are effective in biological war against soil-borne pathogens (Altın and Bora, 2005). It is known that when rhizobacteria are used alone or in a mixture, they have a positive effect on the cultivation of horticulture crops. Plant growth stimulating bacteria (PGPR) are advantageous for plant growth, yield, and quality, according to recent studies (Eşitken et al., 2010).

Plant growth promoting root bacteria are soil bacteria that live in the rhizosphere or root surfaces of plants and play a direct or indirect role in promoting plant growth and development (Ahemad and Kibret, 2014). For plant growth stimulating root bacteria "Plant Growth Promoting Rhizobacteria" - the term PGPR was first used in 1978 (Kloepper and Schroth, 1978). It was discovered that certain bacterial strains, including *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus, Rhizobium,* and *Serratia,* promote plant development (Saharan and Nehra, 2011).

Plant growth-promoting root bacteria (PGPR) have the impact of allowing the plant to use a compound produced by the bacteria or of making it easier for the plant to absorb specific nutrients from the environment. While PGPR has a direct impact on plant growth through the fixation of atmospheric nitrogen, synthesis of siderophores, production of specific plant hormones, solubilization of phosphorus, and synthesis of plant-useful enzymes, it also has an indirect impact by reducing or preventing the negative effects of one or more plant pathogens. The mechanism of indirect effect of root bacteria on plant growth can be achieved by synthesizing siderophores and antibiotics and inhibiting the proliferation of phytopathogens (Glick, 1995). Biofertilizers are root bacteria that improve nutrient availability and support plant development through nitrogen fixation, phosphorus solubility, or iron uptake. Root bacteria that produce plant hormones like Indole-3-acetic acid (IAA), ethylene, gibberellic acid, and others are referred to as phyto-stimulators, while those that contribute to the degradation of organic pollutants and reduce metal toxicity in polluted soils are referred to as bio-remediators. Root bacteria that suppress phytopathogens through antagonism and competition are known as biocontrol agents (Aloo et al., 2019). The most crucial nutrient for plant development and productivity is nitrogen (N). In crops, free-living N-fixing bacteria are crucial. Non-symbiotic N-fixing bacterial species include Cyanobacteria (Anabaena, nostoc), Azospirillum, Azotobacter, Gluconoacetobacter diazotrophicus, and Azokarus (Ahemad and Kibret, 2014; Mehmood et al., 2018). Phosphorus (P), which occurs abundantly in soil in both organic and inorganic forms, is the second most significant mineral element that influences plant development after nitrogen (N). Although there is a lot of phosphorous, there is typically little of it that is in forms that plants can use. Because they can supply plants with phosphorus from scarce sources through a variety of processes, phosphorus solubilizing root bacteria (PSB) are regarded as biofertilizers with a promising future. (Ahemad and Kibret, 2014). Inoculation of phosphate solubilizing bacteria not only increases plant growth, but also significantly increases the overall yield of plants (Mehmood et al., 2018). It has been expressed those genera such as *Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium* and *Serratia* are the most important phosphate-solving bacteria (Ahemad and Kibret, 2014)., PGPRs are crucial for enhancing plant development parameters by creating phytohormones. Such as ethylene, abscisic acid, auxins, cytokinins and gibberellins. The first group of bacteria capable of producing indole-3-acetic acid (IAA) was named Rhizobia. *Pseudomonas putida* and *P. fluorescens* are the two most important strains of bacteria that show significant results in auxin production and in promoting plant growth. It is known that the bacterial strain of *Pseudomonas fluorescens* secretes phytohormones such as cytokinin, which significantly affect cell division and increase cell growth in the plant (Mehmood et al., 2018). Additionally, it is understood that *Azospirillum* rhizobacteria can produce phytohormones (including gibberellins, cytokinins, and auxins) (Siddiqui, 2006).

Although it has been clearly shown in earlier studies that PGPR improves plant growth and yield by ensuring that plants receive better nutrients, boosting phytohormonal activity, and preventing harmful microflora in plant roots, the goal of our study was to ascertain the effects of specific plant growth-promoting rhizobacteria on the growth of lettuce seedlings and to explore potential applications.

2. Material and Method

2.1. Material

In the study, "Sürpriz" curly leaf salad (*Lactuca sativa* L. *crispa*) (Dikmen Tarım Ürünleri) cultivar was used as plant material. “Sürpriz” curly lettuce is a late stem growing variety suitable for greenhouse cultivation in winter and open field cultivation in summer. This variety has a large number of leaves with the leaf color of medium green (Anonymous, 2022). Combinations of bacterial groups used in the study were given in table 1.

Table 1. PGPR strain combinations used in seed application

Group No.	PGPR strain combinations
B1	*Pseudomonas fluorescens* RC77e + *Bacillus subtilis* RC631 + *Bacillus megaterium* RC33 + *Pantoea agglomerans* RC36 + *Pseudomonas putida* biyotip B RC14
B2	*Pseudomonas fluorescens* RC86 + *Bacillus subtilis* RC67 + *Bacillus subtilis* RC631 + *Pseudomonas putida* biyotip B RC14+ *Bacillus megaterium* RC34
B3	*Pseudomonas fluorescens* RC77e + *Bacillus subtilis* RC631 + *Bacillus megaterium* RC34 + *Pantoea agglomerans* RC16 + *Rhodococcus erythropolis* RC49
B4	*Pseudomonas fluorescens* RC86 + *Bacillus subtilis RC*11 + *Bacillus pumilus* RC356 + *Pantoea agglomerans* RC36 + *Bacillus megaterium* RC33
B5	*Pseudomonas fluorescens* RC9 + *Bacillus subtilis* RC631 + *Bacillus megaterium* RC34 + *Bacillus pumilus* RC356 + *Pantoea agglomerans* RC16

2.2. Method

Before the seeds were sown in seedling trays, 10 mL of PGPR was applied with a density of 10^9 CFU/ml, and 10 mL of water was applied to the control application. Seed treatments with PGPR were made in petri dishes and shaken in a shaker (Gerhardt RO500) at 100 rpm for two hours (Çakmakcı et al., 2009) and after 2 hours the seeds were sown in seedling trays having 630 holes filled with peat/vermiculite mixture (Figure 1).

Figure 1. Application of bacterial suspensions and sowing seeds. A. Application of PGPR suspensions to lettuce seeds in petri dishes. B. Sowing PGPR-treated seeds into seedling trays.

3. Results and Discussion

The effects of different rhizobacteria seed treatments on leaf and root fresh and dry weight, leaf number, root collar diameter and root length of lettuce seedlings are given in table 2.

Table 2. The effects of PGPR seed treatments on , e seedling seaf fresh weight, number of leaves, dry leaf weight, root fresh weight, root collar diameter, root length and root dry weight.

Treatments	Leaf fresh weight (g)	Leaf number	Leaf dry weight (g)	Root fresh weight (g)	Root collar diameter (mm)	Root length (cm)	Root dry weight (g)
B1	**0.594±0.167a**	4.400±0.503	0.045±0.016	**0.386±0.107 a**	2.825±0.365ab	4.360±0.256bc	0.046±0.012
B2	0.762±0.218ab	4.550±0.826	0.058±0.018	0.450±0.136 ab	2.860±0.372ab	4.305±0.475bc	0.056±0.016
B3	**0.879±0.273b**	4.800±0.696	0.061±0.019	0.482±0.139bc	**3.219±0.467c**	4.150±0.227a-c	0.055±0.016
B4	0.848±0.343b	4.500±1.000	0.058±0.025	0.529±0.205bc	3.0680.562bc	4.105±0.411ab	0.056±0.023
B5	0.740±0.224ab	4.700±0.923	0.078±0.093	**0.551±0.132 c**	3.039±0.434bc	**4.393±0.272c**	0.061±0.015
Control	0.706±0.229ab	4.300±0.979	0.053±0.015	**0.379±0.126 a**	**2.686±0.572a**	**4.000±0.525a**	0.068±0.092
Anova P value	**	**Ns**	**Ns**	**	**	**	**Ns**

**P≤0.01: the difference between the mean levels is significant.

Ns: the difference between means is not significant.

Seedling leaf fresh weight was statistically significant at p≤0.01 confidence interval. The lowest leaf fresh weight was obtained from the B1 application, the highest leaf fresh weight was obtained from the B3 application (Figure 2). Samancioğlu et al., 2016, reported that different PGPR applications increased leaf fresh weight of cabbage seedlings and Ekinci et al., 2014and Özaktan et al., 2016 determined that shoot fresh weight increments in cauliflower and tomato seedlings .

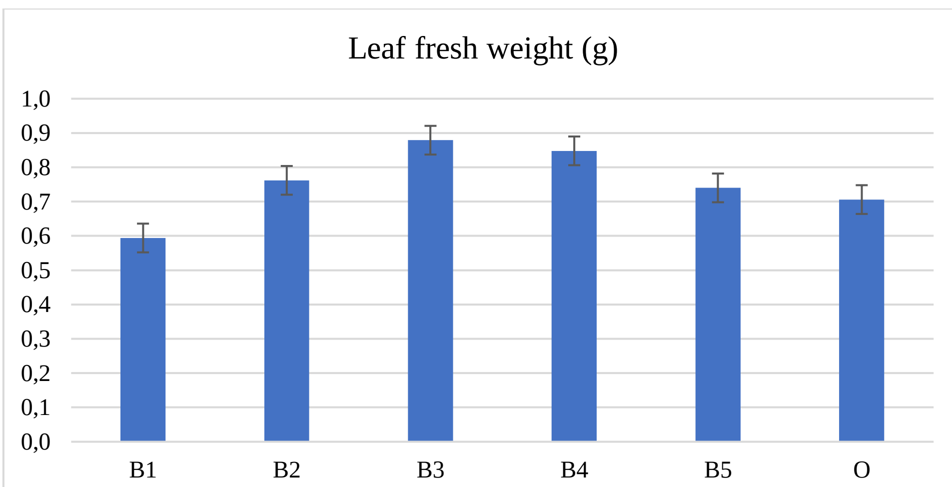

Figure 2. The effects of seed treatments with different PGPR strains on lettuce seedling's leaf fresh weight.

The seed treatments of various PGPR strains had no statistically significant impact on the leaf number in curly-leaved lettuce seedlings (Table 2). All applications were in the same group, but B3 treatment had the highest leaf number with 4.8, and the control group, which did not receive PGPR treatment, had the lowest leaf number with 4.3 (Figure 3). Turan et al., 2014 determined that PGPRs had no effect on the true leaf number of cabbage seedlings.

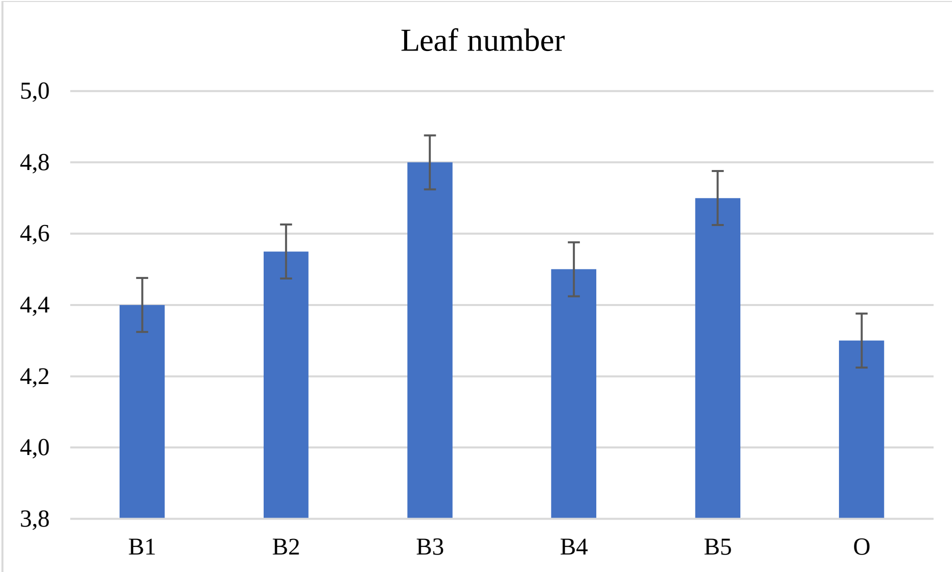

Figure 3. The effect of seed treatments with PGPR on leaf number of lettuce seedlings

It was determined that the effect of seed treatments with PGPR on seedling leaf dry weight was not statistically significant (Table 2). The highest leaf dry weight was obtained from B5 seed treatment, and the lowest value was obtained from the B1 seed treatment (Figure 4). A similar study was done by Ekinci et al. 2014 reported that root bacterial strain did not have a positive effect on the shoot dry weight of cauliflower seedlings.

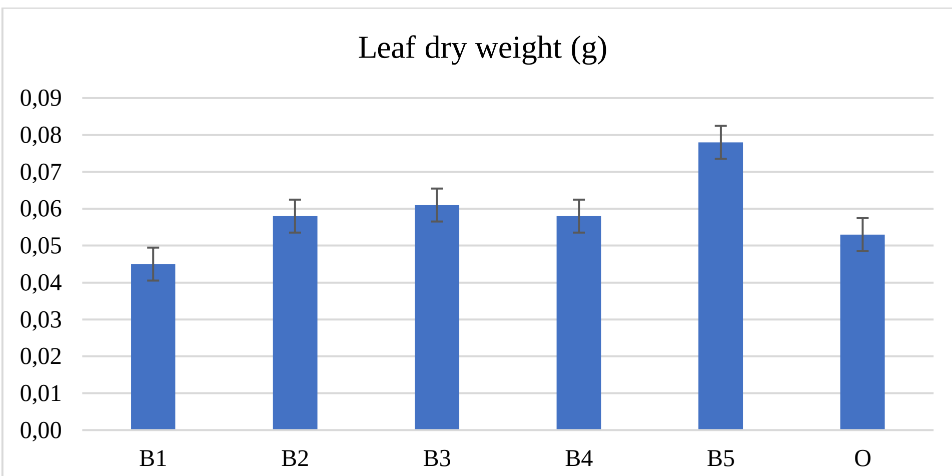

Figure 4. The effects of seed treatments with different PGPR strains on leaf dry weight of lettuce seedlings.

Seed treatments of PGPR were found to have a statistically significant impact on the root fresh weight at the P≤0.01 level (Table 2). It was found that the B5 seed treatmets provided the highest root fresh weight, whereas the control treatment gave the lowest (Figure 5). Özaktan et al. (2016) found that root bacteria had a substantial impact on tomato seedlings' root fresh weight.

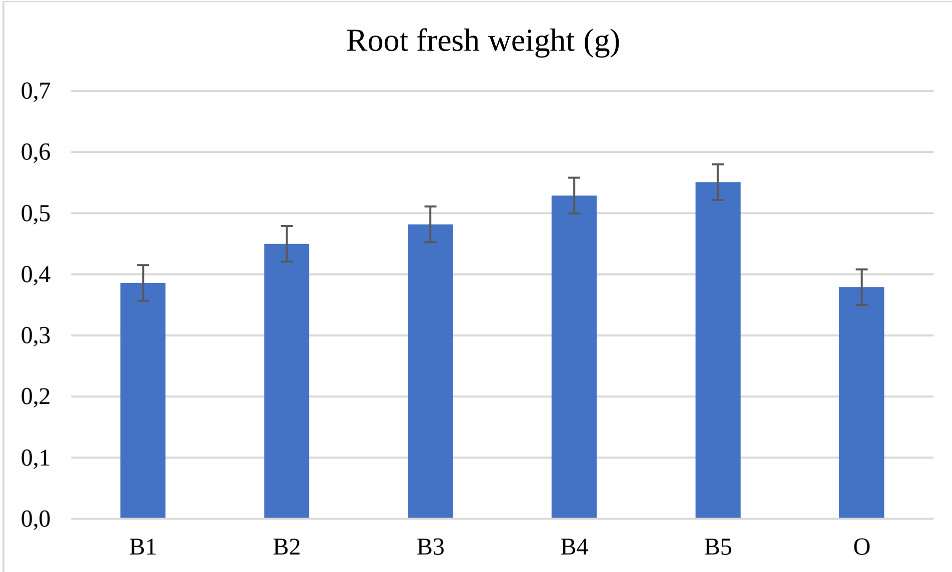

Figure 5. The effects of different seed treatments with PGPR strains on root fresh weight of lettuce seedlings.

It was determined that the effect of the seed treatments with PGPR on the root collar diameter of the seedlings was statistically significant and different in terms of applications (Table 2). The highest root collar diameter was obtained from the B3 seed treatment and the lowest from the control treatment. It was found that there was no difference between B1 and B2 , and B4 and B5 treatments were also in the same group (Figure 6). Ekinci et al. (2014) reported that bacterial application increased the root collar diameter of cauliflower seedlings.

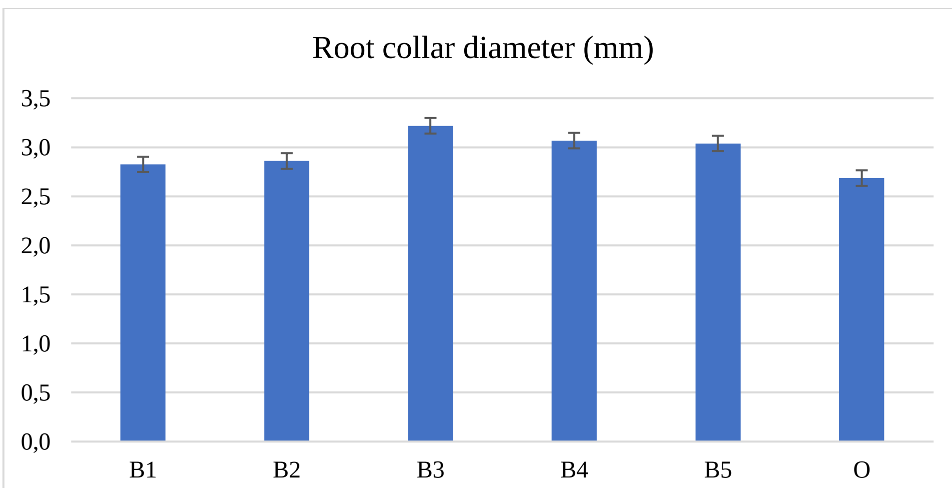

Figure 6. The effects of seed treatments with PGPR strains on root collar diameter of lettuce seedlings.

At a rate of P≤0.01, The effect of seed treatments with different PGPR strains on seedling root length was determined statistically significant. (Table 2). The B5 seed treatment gave the highest root length, and the lowest root length was deterimined in control treatment. (Figure 7). In contrast to our findings Ekinci et al. 2015 reported that the impact of PGPR application on the root length of broccoli seedlings was not statistically significant. Similar results with our findings reported by Roychowdhury et al. 2016, PGPR strains significantly affected the root length of spinach seedlings compared to the control.

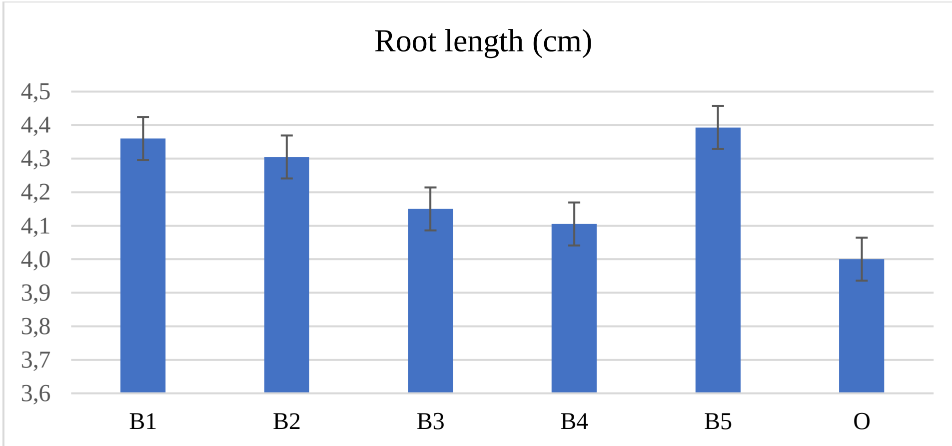

Figure 7. The effects of seed treatments with PGPR strains on seedling root length of lettuce seedlings.

When the seedling root dry weight results were evaluated, there were no statistically significant differences between seed treatments (Table 2). However, it was determined that the seedling root dry weight of the control was the highest compared to the PGPR treatments (Figure 8). Ekinci et al., 2015 stated that the effect of bacteria on seedling root dry weight was statistically insignificant in their study on broccoli.

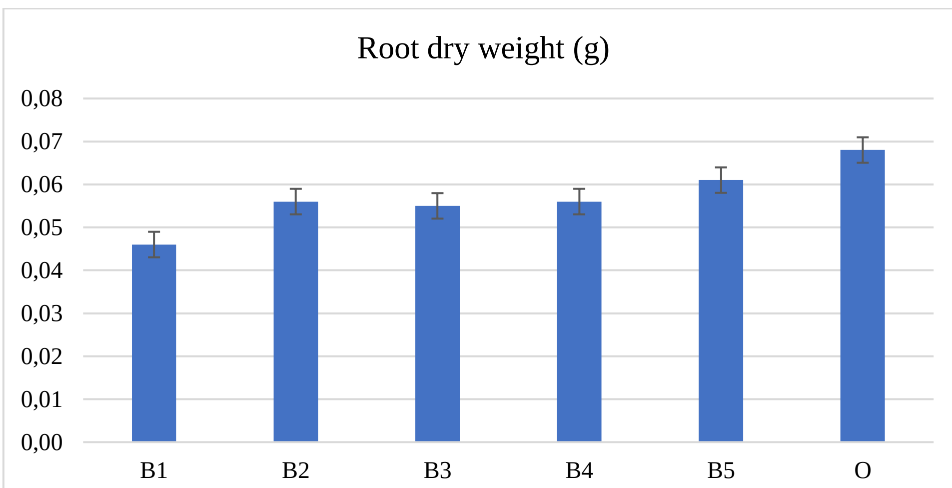

Figure 8. The effect of seed treatment with PGPR strains on seedling root dry weight of lettuce seedlings.

4.Conclusions and Recommendations

When the effects of seed treatments with different PGPR strains on the growth parameters of lettuce seedlings were evaluated , it was found that PGPR strains increased the growth of curly leaf lettuce seedlings compared to control applications. As a result, it was found that the effect of seed treatments of PGPR strains on leaf and root fresh weight, root collar diameter and length of seedlings was positive. According to the results we obtained, when the efficiency of PGPRs were examined, it was determined that the bacterial suspension no. B2 was more effective, followed by the PGPR suspensions no. B3, B4, B5, and lower values were obtained from the bacterial suspension no. B1 compared to the control application.

Lettuce is a group of vegetables are grown at increasing rates in the world and in Turkey. For more yield in lettuce, chemical fertilizers with high nitrogen content are used. The use of environmentally friendly PGPRs instead of chemical fertilizers can help ensure sustainable soil flora while ensuring plant growth. However, factors such as bacterial strain, soil type, PGPR application timing and shape, and plant species are thought to be important in determining the degree of effect of PGPR on plant growth and yield increase. As a result of our study, it was concluded that the use of root bacterial strains as biofertilizers can help improve the growth and quality of lettuce seedlings.

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