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Determination Of The Efficiency Of Some Plant Growth- Promoting Bacteria In Strawberry Growing Under Cover And Field Conditions Organically Single And Dual Applications On Plant Development And Good Production Parameters

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Abstract

With this study, in 2010 and 2011; bacteria species belonging to *Pseudomonas fluorescens* RC77, *Bacillus pumilus* RC23, *Bacillus megaterium* RC07, *Bacillus subtilis* RC521, *Pantoea agglomerans*, RCYE58, *Burkholderia pyrrocinia* RCYE64, *Paenibacillus polymyxa* RCYE283, *Stenotrophomonas acidaminiphila* RCYE47, which promotes some plant growth in greenhouse and field organic conditions, isolates were used. Frigo strawberry seedlings (Fern cv.) were used for 60 minutes before planting, for 60 minutes *B. megaterium* RC07, *B. pumilus* RC23, *B. subtilis* RC521, *B. pyrrocinia* RCYE64, *P. polymyxa* RCYE283, *P. fluorescens* RC77, *S. acidaminiphila* RCYE47 (single solution-7 pieces), (*B. pumilus* RC23 + *P. agglomerans* RCYE58, *B. subtilis* RC521 + *S . acidaminiphila* RCYE47, *P. polymyxa* RCYE283 + *B. pyrrocinia* RCYE64 ve *P. fluorescens* RC77 + *B. megaterium* RC07 (dual solution-4 pieces). At the end of the study, *B. subtilis* RC521 + *S . acidaminiphila* RCYE47 was obtained from application, while the highest fruit yield per plant was obtained from *B. pumilus RC23* + *P. agglomerans* RCYE58 application with 99.68g. The best average fruit weight was obtained from *S. acidaminiphila* RCYE47 application with 6.70 g, while the highest total fruit yield was obtained from *B. pyrrocinia* RCYE64 application with 1155 g. *P. polymyxa* RCYE283 application had the highest chemical content in the fruit (23.07%) and at the end of the 1st year (12.43%), while in terms of ascorbic acid content (84mg/100g) and at the end of the 1st year (1000ppm) it was found to be the highest. Obtained from *Bacillus pumilus* RC23 application.
In the study; It was determined that the bacterial applications provided an increase in the macro and micro nutrient content of the strawberry leaves under the cover and in the open field, and these increases were statistically significant.

Key Words: *Strawberry, PGPR, root and leaf application, yield.*

Örtü Altı Ve Açık Alandaki Organik Çilek Yetiştiriciliğinde Bitki Gelişimini Teşvik Edici Bazı Bakterilerin Tekli Ve İkili Kombinasyon Uygulamalarının Bitki Gelişimi İle Verim Parametrelerine Etkinliklerinin Belirlenmesi

Özet

Bu çalışma ile, 2010 ve 2011 yıllarında; örtü altı ve açıkta organik koşullarda bazı bitki gelişimini teşvik eden *Pseudomonas fluorescens* RC77, *Bacillus pumilus* RC23, *Bacillus megaterium* RC07, *Bacillus subtilis* RC521, *Pantoea agglomerans*, RCYE58, *Burkholderia pyrrocinia* RCYE64, *Paenibacillus polymyxa* RCYE283, *Stenotrophomonas acidaminiphila* RCYE47 türlerine ait bakteri izolatları kullanılmıştır. Fern çeşidine ait frigo çilek fideleri dikimden önce 60 dakika süreyle *B. megaterium* RC07, *B. pumilus* RC23, *B. subtilis* RC521, *B. pyrrocinia* RCYE64, *P. polymyxa* RCYE283, *P. fluorescens* RC77, *S. acidaminiphila* RCYE47 (7 adet tekli), (*B. pumilus* RC23 + *P. agglomerans* RCYE58, *B. subtilis* RC521 + *S . acidaminiphila* RCYE47, *P. polymyxa* RCYE283 + *B. pyrrocinia* RCYE64 ve *P. fluorescens* RC77 + *B. megaterium* RC07 (4 adet ikili) izolat içeren solusyonlarda 60 dk bekletilmiş ve dikilmiştir. Yapılan çalışma sonunda yılların genel ortalamasına göre en fazla yaprak alanı 25,28cm^2 ile *B. subtilis* RC521 + *S . acidaminiphila* RCYE47 uygulamasından elde edilirken, bitki başına en yüksek meyve verim 99,68g ile *B. pumilus* RC23 + *P. agglomerans* RCYE58 uygulamasından elde edilmiştir. Ortalama meyve ağırlığının en iyi olduğu uygulama 6,70g ile *S. acidaminiphila* RCYE47 uygulaması iken en fazla toplam meyve verimi 1155g ile *B. pyrrocinia* RCYE64 uygulamasından elde edilmiştir. Meyvedeki kimyasal içerik açısından en yüksek SÇKM (%23,07) ve 1. yıl sonunda (%12,43) *P. polymyxa* RCYE283 uygulamasından elde edilirken, askorbik asit içeriği bakımından (84mg/100g) ve 1. yıl sonunda (1000ppm) ile *Bacillus pumilus* RC23 uygulamasından elde edilmiştir. Çalışmada; bakteri uygulamalarının örtü altında ve açık alanda çilek yapraklarının makro ve mikro besin elementi içeriklerinin büyük bir kısmında artış sağladığı, bu artışların da istatistiksel olarak önemli olduğu belirlenmiştir.

Anahtar Kelimeler: *Çilek, PGPR, kök ve yaprak uygulaması, verim unsurları*

Introduction

Turkey's climate and soil conditions have provided the opportunity to grow a large number of fruit genus and fruit cultivars, so that our country has been among the important fruit producer countries of the world in terms of both fruit species and varieties and production amount. One of these fruit species is strawberry. *Fragaria vesca*, known as forest strawberries in the early days, has been cultivated with the frustration and since the 1970s, it has started to be grown economically (Yılmaz 2009).

The strawberry belongs to the *Rosaceae* Family of the *Rosa* genus and the north (*F. virginiana*) and the south (*F. chiloensis*) hemispheres at the end of the 18th century emerged as a result of coincidental hybridization in France. For this reason, strawberry (*Fragaria* × *Pineapple* Duch.) Is one of the youngest fruit species in the world (Hancock, et al., 2008, Demirsoy and Serçe 2016).

Strawberry, which is a species that can be grown in a wide range of ecological conditions and in a wide area in the world, is generally spread in the northern hemisphere, but also in all areas of agriculture from the tropics to the Arctic regions and in the areas of 2000 m from sea level to 2000 m It is a species that can be produced (Aslantaş and Karakurt, 2007). Since the investments in production return in a short time, the earnings from the unit area are higher than many other products. All of this has led to a continuous increase in interest in strawberry cultivation and its production in the world has increased every year. The high adaptation capability and variety richness plays an important role in the fact that strawberries can be grown in such a wide range (Demirsoy and Serçe, 2016).

In the control of diseases and pests in plant production, the use of unconscious chemical fertilizer and pesticide ultimately causes deterioration of soil health and environmental pollution (Glick 1995; Keles and Ertürk 2021; Ertürk 2022). In particular, the increase in environmental pollution and the increasingly contamination due to the synthetic inputs used in the production activities of agricultural areas have revealed the recipe of organic cultivation. Organic agriculture is an important tool for reducing the negative impact of intensive synthetic chemicals on soil quality and ensuring sustainability (Çakmakçı and Erdoğan 2005).

In recent years, studies on alternative applications and inputs have increased to reduce chemical fertilizer requirements. The most remarkable application in these studies, which focuses on the use of organic materials, stands out as the use of microorganisms (Ertürk 2022). There are many common microorganism groups in the soil, including bacteria, fungus, actinomyst, protozoa and algae. Microorganisms, which are used as free-living, promoting plant growth, used as biologic agents or biological fertilizer, are called Plant Growth-Promoting Rhizobacteria = PGPR), which promotes plant growth and development. These bacteria are mostly more *Acetobacter, Acinetobacter, Achromobacter, Aereobacter, Agrobacterium, Alcaligenes, Artrobacter, Azospirillum, Nitrotobacter, Bacillus, Burkholderia, Clostridium, Enterobacter, Erwinia, Flavobacterium, Klebsiellalla, Pseudomonas, Rhizobium, Serratia* and *Xanthomonas*. This group of microorganisms make significant contributions to cultivation by using different mechanisms in the development process of the plant.

The first group of bacteria that increase plant growth; It manufactures direct growth substances (hormones, vitamins, enzymes and siderofor, etc.), prevents ethylene synthesis, fixes atmospheric nitrogen, increases iron uptake, organic and inorganic phosphate solubilisation, increases the durability of plants against drought, salty, metal toxide and pesticide harm; The second group reduces the harmful effects of phytopatogenic microorganisms (Glick 2012, Bashan et.al 2014, Ertürk 2022).

Increased inorganic and organic phosphate solubilisation and mineralisation with microbial metabolites encourages plant development. In particular, the production of bacterial organic acid and acid phosphatase increases the uptake of nutrients (Çakmakçı et al. 2006; 2007, Ertürk 2022). Bacterial treatments that encourage plant development are carried out in laboratory, greenhouse and field conditions, but some conditions that cannot be predicted in field trials sometimes make it difficult to obtain appropriate results (Miransari 2013; Ahemad and Cabret 2014, Keles and Ertürk 2021). Infections in the soil, pH changes, high temperature, low rainfall and humidity deficiency in growth period, nutritional deficiencies, such as non-appropriate conditions reduce bacterial colonization. As a matter of fact, the effectiveness of these bacteria is affected by the soil and environmental factors (Çakmakçı 2005, Miransari 2013; Ertürk 2022).

Some bacteria, which may be effective in controlled conditions, may be insufficient in field conditions. PGPR (Plant Growth-Promoting Rhizobacteria) activity emerged during the early development of the plant and contributed especially on vegetative development (Çakmakçı 2005, Ertürk 2022). In recent years, some fruit (hazelnuts, bananas, apricots, rosehip, cherry, citrus fruits, blueberry, strawberry, kiwi, apple, etc.) species have increased in numerically.

Especially in garden plants where perennial and woody plant species are high compared to field plants, the activities of these applications are more variable and long-term due to the fact that they are tried in different ecologies in terms of all species (Çakmakçı et al. 2010a, Ertürk 2022). Bacterial applications, especially in different ecologies, especially in different ecologies, affected many parameters such as vegetative development of some bacterial breeds, leaf area, yield, nutritional intake, fruit quality and content, some bacterial breeds were neutralized, some bacterial races were found ineffective. It has come to the forefront with their binary or triple

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usage rather than single use (Vestberg et al.. Equalgen 2018; Ünal 2019, Ertürk et al. 2021; Ertürk and Çakmakçı 2022)
With this study; In recent years, it is aimed to determine bacterial applications that can be used in increasing organic strawberry cultivation in Erzincan and its region and their effects on yield -related elements. It has been found that the size of the bacterial isolates used can contribute positively under organic conditions. Additionally, this study; For the first time under field conditions and under the cover in Erzincan, it is the first in terms of the application of some bacterial isolates in terms of single and dual application.

Materyal ve Yöntem

Bacterial Isolates
This study was conducted on 11 different biological fertilizer formulations (*Pseudomonas fluorescens* RC77, *Paenibacillus polymyxa* RCYE283, *Stenotrophomonas acidaminiphila* RCYE47, *Bacillus pumilus* RC23, *Bacillus megaterium* RC07, *Bacillus subtilis* RC521, *Burkholderia pyrrocinia* RCYE64, *Pseudomonas fluorescens* RC77 + *Bacillus megaterium* RC07, *Paenibacillus polymyxa* RCYE283,+ *Burkholderia pyrrocinia* RCYE64, *Bacillus subtilis* RC521+ *Stenotrophomonas acidaminiphila* RCYE47 ve *Bacillus pumilus* RC23+ *Pantoea agglomerans* R RCYE58) plant in the Fern strawberry variety grown under greenhouse and open field under organic conditions. This study was carried out to determine the development, yield and yield elements and its effect on leaf macro and micro nutrient content. *P. fluorescens* RC77 (Çakmakçı et al. 2008; Çakmakçı et al. 2013a) and *B. pumilus* RC23 (Ertürk et al. 2010), used as biological fertilizer, were obtained from wild raspberry rhizosphere soil; *B. megaterium* RC07 from wheat rhizosphere (Çakmakçı et al. 2006, 2007), *B. subtilis* RC521 isolate from saline-calcareous wild grapevine rhizosphere soil (Çakmakçı et al. 2013a, Çakmakçı et al. 2013b); *P. agglomerans*, RCYE58, *B. pyrrocinia* RCYE64 and *P. polymyxa* RCYE283 from acidic tea rhizosphere soils of the Eastern Black Sea Region (Çakmakçı et al. 2011, 2012, 2013a,bc,d); *S. acidaminiphila* RCYE47 was isolated from the acidic wild strawberry rhizosphere soil of Rize Hemşin region (Çakmakçı et al. 2010; Çakmakçı et al. 2013 d) and was characterized by some biochemical properties. Diagnosis, characterization and some test results of bacteria tested on different plants in previous studies are given in Table 1.

Table 1. Some laboratory test results of the bacterial isolates used in the study.

MIS Identification	**Oxydase test**	**Catalase test**	**Growth in N-free media**	**Sucrose Test**	**Growth in NBRIP-BPB media**	**Amylase Test**	**ACC-Deaminase Test**
Bacillus megaterium RC07	-	+	SP+	-	SP+	WP+	+
Bacillus pumilus RC23	-	SP+	SP+	-	+	-	+
Bacillus subtilis RC521	-	SP+	SP+	+	-	+	+
Burkholderia pyrrocinia RCYE64	+	WP+	SP+	WP+	SP+	-	+
Paenibacillus polymyxa RCYE283	-	+	SP+	+	WP+	WP+	+
Pantoea agglomerans R RCYE58	-	+	SP+	+	SP+	-	ND
Pseudomonas fluorescens RC77	SP+	SP+	SP+	WP+	+	-	+
Stenotrophomonas acidaminiphila RCYE47	+	SP+	WP+	-	-	-	ND
Bacterial Formulations/Combinations							
Paenibacillus polymyxa RCYE283+ *Burkholderia pyrrocinia* RCYE64							
Bacillus subtilis RC521+ *Stenotrophomonas acidaminiphila* RCYE47							
Bacillus pumilus RC23+ *Pantoea agglomerans* R RCYE58							

**SP+: strong positive, WP+: weak positive, -: negative, +: positive, ND: Not Determined*

The strawberry seedlings used in this study belong to the Fern cultivar with day-neutral characteristics, bred by the University of California, and this variety, which does not have a strong vegetative structure, is recommended for cultivation in our country, especially in high-altitude plateau areas. The field studies of this research were carried out in the open and in a high tunnel (roof height 3m) on the producer land in Çayırlı district of Erzincan province in 2010-2011. Laboratory studies were carried out in the laboratories of Atatürk University, Faculty of Agriculture, Department of Horticulture and Soil-plant Nutrition .

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In the experiment, black plastic mulch with UV additive was used as standard in the tubes both in the field and in the high tunnels, and the mulching process was carried out before planting the seedlings. Seedlings were planted and cared for on tubes equipped with a drip irrigation system in which an automatic watering unit was mounted.
The study was carried out with a total of 864 frigo-seedlings of the Fern cv. 432 under cover and 432 in the field, with 4 parcel for greenhouse and field, 3 separate applications in each tube, 3 repetitions in each application and 12 plants in each repetition. .
Animal manure and 200 kg of elemental sulfur were applied to the rollers at the rate of 3 tons per decare to regulate the soil structure.

PGPR suspension preparation and Application

48-hour cultures were used in the preparation of the bacterial solution, and a loopful of each growing bacteria was taken and transferred to a conical flask containing 250 ml of nutrient broth. Liquid media inoculated with bacteria were incubated in a shaker at 91 rpm for 24 hours. The prepared bacterial suspensions were diluted with sterile pure water and the final concentration was adjusted to 108 CFU ml-1 by spectrophotometric measurement. Before planting, frigo strawberry seedlings of Fern variety were treated with *B. megaterium* RC07, *B. pumilus* RC23, *B. subtilis* RC521, *B. pyrrocinia* RCYE64, *P. polymyxa* RCYE283, *P. fluorescens* RC77, *S. acidaminiphila* RCYE47, *B. pumilus* RC23 + *P. agglomerans* RCYE58, *B. subtilis* RC521 + *S . acidaminiphila* RCYE47. They were kept in solutions containing, *P. polymyxa* RCYE283 + *B. pyrrocinia* RCYE64 and *P. fluorescens* RC77 + *B. megaterium* RC07 for 60 minutes and planted.
After planting, solutions containing bacterial isolates were evenly injected into the root rhizosphere. No application was made to the parcel designated as control. In the study, under the title of evaluations regarding efficiency; yield per plant (g/plant), average fruit weight (g) and total fruit yield (g); regarding the chemical composition of the fruit, SSC (%) and ascorbic acid (vitamin C) values were determined.
Also in the research; regarding the physical and chemical properties of the leaf, leaf area (cm^2) and macro and micronutrient contents in the leaf were determined. In the research; All studies were conducted according to the randomized parcel trial design. Values in all parameters were analyzed using SPSS package program with analysis of variance in 1 (variety) x 2 (growing medium) x 11 (application) factorial order.
The difference between the means was subjected to Duncan's multiple comparison test. In characters where interactions are important, interactions are explained with a simple main effect. Statistical analyzes were performed using the Duncan multiple comparison test to see the differences between the average values.

Results and Discussion

In the yield parameters examined, it was determined that bacterial applications had a positive effect on fruit yield in strawberries both in the open and under cover. The applied *B. pumilus* RC23 + *P. agglomerans* RCYE58 combination increased the yield per plant (99.16 g) according to the general average of the years, average fruit weight (6.70 g) with the *S. acidaminiphila* RCYE47 isolate, and total fruit yield (6.70 g) with the *B. pyrrocinia* RCYE64 isolate. 1155 g) were the isolates that provided the highest yield increase (table 2). The values obtained at the end of the study are similar to other studies.
As a matter of fact, Ertürk et al. (2012) reported fruit yield per plant in strawberry as 1.98-20.85%, average fruit yield as 3.05-19.26%; Equalken et al (2010), Koç et al (2016) reported that fruit yield in strawberry was significantly increased with bacterial applications. While the differences between applications in yield values are significant, the reason for the very low yield obtained in different regions can be shown to be that the applications are carried out under organic conditions. It is possible that the mentioned isolates have very different functions, such as nitrogen fixation, phosphate solubilization, and ACC deaminase activity (table 1), resulting in more balanced nutrition of the treated plants and thus increases in the values of yield and yield parameters.
The effect of the applications on the TSS rate and the general average in the 1st and 2nd years was found to be statistically very significant. At the end of the trial, the general average of the years (23.07%) and the highest TSS rate at the end of the 1st year (12.43%) were obtained from the *P. polymyxa* RCYE283 application, and in the 2nd year, the highest rate was obtained from the *P. fluorescens* RC77 + *B. megaterium* RC07 application. The TSS rate (11.37%) was reached.
In the determination of ascorbic acid (vitamin C), which we used to determine the chemical content of the fruits, it was determined that they contained more Ascorbic acid (vitamin C) than the control group, according to the general average of the applications (table 3), and this was statistically significant ($p < 0.01$). Among the treatments, especially *B. pumilis* RC23 and *P. fluorescens* RC77 isolates increased their vitamin C content (table 3). In the study, bacterial isolates applied to strawberries, especially *B. subtilis* RC521 and *B. subtilis* RC521+*S. acidaminiphila* RCYE47 It was determined that increased leaf area both under cover and in the field condition (table 2). These bacteria are reported to have nitrogen-fixing and phosphate-solubilizing properties in laboratory tests (table 1). It is highly likely that there will be increases in leaf chlorophyll content and leaf area in plants that

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are well nourished in terms of nitrogen (Marschner 2012). Similar studies have also revealed that bacteria increase the leaf area. Sudhakar et al. (2000) observed that the leaf area of the mulberry plant increased with *Azotobacter* application, and in another study, De Silva et al (2000) stated that the leaf area of blueberries increased with *P. fluorescens* Pf5. Also; Ertürk et al. (2012), in their studies conducted with the same variety under Çoruh Valley conditions, reported that the leaf area increased with almost all bacterial applications, and accordingly the yield increased. It can be said that the bacteria we applied directly or indirectly increased the leaf area by increasing the plant's nutrient uptake from the soil. It was determined that bacteria-treated strawberry leaves contained more macro and micro (P, K, Ca, B, Cu, Fe, Mg, Mn, Na, S, Zn) nutrients than the control, and these values were found to be statistically significant (table 3). In similar studies, researchers detected similar effects in different plant or strawberry varieties to which they applied bacterial isolates.

As a matter of fact, Equalken et al. (2003) reported that with bacterial applications in apricot, N, P, K, Ca and Mg contents in the leaves of trees increased compared to the control; Jaizme-Vega et al. (2003) *Bacillus spp*. into two banana varieties grown under in vitro conditions. As a result of the application, they found that the nutritional content of both varieties increased compared to the control.

Çakmakçı et al. (2009) reported that 236 of 281 original bacterial isolates could fix free nitrogen, 177 isolates could solubilize phosphate, and 152 could both fix nitrogen and dissolve phosphate. In a parallel study they conducted, they observed that bacterial application in tea clones made significant contributions to the development parameters as a result (Çakmakçı et al. 2010). Similarly, Ertürk et al. (2011) reported that the bacteria applied to Tombul hazelnut cultivar had an effect on the plant nutrient content (N, P, K, Ca, Mg, Mn, Zn, Fe, Na), and Eşitken et al. (2010) reported that they reported that the N, P, K, Fe, Mn and Zn contents of the leaves of Fern cv. increased significantly as a result of bacterial application to the strawberry variety.

Since the applied bacteria have different functions such as dissolving phosphate in the rhizosphere, fixing atmospheric nitrogen, and producing hormones such as IAA that increase plant growth (table 1), they significantly contributed to the nutrient content of leaf plants.

In conclusion; the effects of bacterial isolates on yield per plant, average fruit weight, total fruit yield, plant leaf area and chemical content values varied according to years and growing environments. Especially considering the physiology of perennial plants, it is reported that it takes time to observe the effects of bacterial applications completely and homogeneously and may vary depending on soil ecology and climate (Çakmakçı 2005; Turan et. al. 2009, Miransari 2013, Bashan et.al 2014, İpek et. al 2014, Pehluvan and Güleryüz 2014, Kumar et.al 2015, Kurokura et.al. 2017, Geçer et al. 2018, Ertürk 2022). However, applications include *B. subtilis* RC521, *B. pyrrocinia* RCYE64, *B. pumilis* RC23+*P. agglomerans* RCYE58, it has been observed that isolates are promising, make a positive contribution to data on yield and yield elements, and cause significant increases in leaf nutrient element contents. In the light of these evaluations, it has been concluded that the mentioned isolates can be used as biological fertilizer in strawberry cultivation in the Erzincan region under organic conditions, since organic strawberry cultivation is becoming increasingly widespread and therefore biological fertilizer alternatives are needed.

***** This study includes a part of the Master's thesis of H. İbrahim Tuzlacı

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