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Determination of the Effects of Combined use of *Paenibacillus* sp. S1S22 Strain and *Ulva lactuca* Extract on Seed Germination and Growth of Tomato Plant

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ABSTRACT
In recent years, plant growth promoting bacteria (PGPB) have been used as biofertilizers that increase agricultural productivity and plant resistance to changing environmental conditions such as drought, salinity and heat stress. One of the proposed new approaches to increase plant productivity, especially under stress conditions, is using algae extracts, which usually consist of naturally occurring bioactive compounds. *Paenibacillus* sp. S1S22 isolated from sediment was tested for its performance in indole acetic acid production (IAA), nitrogen (N) fixation, phosphate solubilization, proteolytic activity, and cellulose activity. Its antimicrobial activity was also determined against different pathogens of *Paenibacillus* sp. S1S22. Tomato seeds were incubated with the S1S22 strain, and the S1S22 strain supplemented with *U. lactuca*. The seeds incubated with deionized water were used as control. As a result, we demonstrated that the addition of *U. lactuca* extract to tomato seedlings incubated with *Paenibacillus* sp. S1S22 strain increased the root and stem length compared to the control. The results indicate that macroalgae in combination with PGPR may be a much more effective way of promoting plant growth.

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Keywords
Paenibacillus sp.
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Paenibacillus sp. S1S22 suşu ile *Ulva lactuca* Ekstresinin Kombine Kullanımının Domates Bitkisinin Tohum Çimlenmesi ve Büyüme Üzerine Etkilerinin Belirlenmesi

ÖZET
Son yıllarda, bitki büyümesini teşvik eden bakteriler (PGPB), tarımsal üretkenliği ve kuraklık, tuzluluk ve sıcaklık stresi gibi değişen çevresel koşullara karşı bitki direncini artıran biyogübreler olarak kullanılmaktadır. Özellikle stres koşulları altında bitki verimliliğini artırmak için önerilen yeni yaklaşımlardan biri, doğal olarak oluşan biyoaktif bileşikler içeren alg ekstraktlarının kullanılmasıdır. Sedimentten izole edilen *Paenibacillus* sp. S1S22 suşunun bitki büyümesini teşvik etme performansı indol asetik asit üretimi (IAA), azot (N) fiksasyonu, fosfat çözünürlüğü, proteolitik aktivite ve selüloz aktivitesi açısından test edilmiştir. Ayrıca bu suşun farklı patojenlere karşı antimikrobiyal aktiviteleri de belirlenmiştir. Domates tohumları, S1S22 suşu ve *U. lactuca* ekstraktı eklenmiş S1S22 suşu ile inkübe edilmiştir. Deiyonize su ile inkübe edilen tohumlar kontrol grubu olarak kullanılmıştır. Elde edilen sonuçlar, *Paenibacillus* sp. S1S22 suşu ile inkübe edilen domates fidelerine *U. lactuca* ekstraktı eklenmesinin kontrole göre kök ve gövde uzunluğunu arttırdığını göstermiştir. Sonuçlar, makroalglerin PGPR ile kombinasyon halinde, bitki büyümesini teşvik etmede tek başına kullanılmalarından çok daha etkili bir araç olabileceğini göstermektedir.

Bitki Fizyolojisi

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi :
Kabul Tarihi :

Anahtar Kelimeler
Paenibacillus sp.
Ulva lactuca
Bitki büyümesini teşvik eden bakteriler (PPGPB)

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INTRODUCTION

Agricultural practices such as synthetic chemical fertilizer applications and excessive use of pesticides to increase crop yields have led to negative consequences such as groundwater pollution, deterioration of soil quality and reduction in biological diversity, especially in recent years (Chandini et al., 2019). The human population is expected to reach 9 billion in the next 30 years and it has become imperative to increase product yield with environmentally friendly and sustainable agricultural practices to meet the needs of humanity (Wise, 2013). Microorganisms, one of the most important components of the soil ecosystem, are very important in increasing soil fertility, nutrient cycling and supporting plant diversity (Hardoim et al., 2009). Plant growth promoting bacteria (PGPB) have been used as biofertilizer and biocontrol agents because of their beneficial effects on soil and agricultural products (Pathma et al., 2021).

PGPB microorganisms, which play a major role in plant growth, can promote plant growth through mechanisms such as changing plant hormone levels, facilitating the uptake of substances from the soil, or inhibiting the inhibitory effects of pathogenic agents on plant growth and development (Glick, 2012). It is necessary to clarify the interactions between the plant and the PGPB in order to be used effectively in the solution of the above-mentioned agricultural problems. Developing plant-PGPB interactions will allow the development of microbial inoculants that can be used to increase crop yields. PGPBs are shown as a natural technology to improve soil quality and reduce chemical use, in this context.

One of the most important features of *Paenibacillus* species is their ability to promote plant growth. For example, it has been reported that they can trigger plant defense mechanisms and contribute to tolerance against biotic stresses by neutralizing various phytopathogens and herbivorous insects. A study by Weselowski et al. (2016) showed *Paenibacillus* species with plant growth promoting properties could directly affect plant growth by producing phytohormones such as indole-3-acetic acid (IAA). PGPR use is currently finite due to the incomplete elucidation of the mechanisms underlying the interactions between plant and bacteria, the diversity and species specificity of plant and bacterial species. Algae are exposed to permanent biotic and abiotic stress factors throughout their life cycle, and this has caused the algae to develop mechanisms to protect themselves from drought, salinity, different light intensity, cold, bacterial or fungal colonization. As a result, algal cells have become rich sources of compounds that play an important role in plant growth and defense, such as growth hormones such as cytokinins and auxins, minerals, carbohydrates, sterols and antioxidants (Michalak et al., 2016). Because of these compounds, which play a role in increasing yield by regulating plant cell metabolism, algae have been identified as phytohormone-like plant growth regulators (PGRs) (Patel et al., 2019).

Studies on the combined use of PGPR and algae in order to increase plant yield have recently attracted attention in the literature. For example, the combination of *Macrocystis pyrifera* algae extracts used as biofertilizer with PGPB *Azospirillum brasilense* showed that the germination ratio of lettuce seedlings (*Lactuca sativa*) increased (Julia et al., 2020). In addition, *M. pyrifera* extract treatment increased root mass and promoted adaptation to water deficit stress (Julia et al., 2020). Another study showed that maize plants treated with both algal biochar and *Serratia odorifera* had significantly greater height and more leaves per pot than control. However, it has also been shown that co-treatment of algae and PGPB increases photosynthetic yield in maize (Ullah et al., 2020). Similarly, leaf area of high bush blueberry was increased by PGPR application (de Silva et al., 2000).

Ulva lactuca is a widely distributed green algae mainly in the mid to low intertidal zones. Generally found in nutrient rich areas and blooms, especially nearby sewage outfalls. Besides its usage in the cosmetics industry, it is also edible and consumed in many countries. Growth promoting effects of *U. lactuca* extracts on seed germination and root development of tomato and mung beans (Hernandez-Herrera et al., 2016), on seed germination and growth promotion of tomato (Mzibra et al., 2021). Also plant growth regulators such as abscisic acid, auxins, cytokinins, gibberellins, jasmonates and salicylates were determined in the aqueous extract of *U. lactuca* (Garcia et al., 2020). *U. lactuca* appears as an important source for plant promoting studies by containing diverse and remarkable active compounds. Due to the diversity of PGRs found in algae, their physiological effects on plants differ. In addition, the mechanisms of action on different plants are still questionable in many respects. In this study, the effects of a combination of *Paenibacillus* sp. S1S22 isolated from the Sakarya River sediment and green algea *U. lactuca* extract collected from Tirilye, on plant growth were determined.

MATERIAL and METHOD

Paenibacillus sp. Isolation from Sediment

Isolation was carried out by dilution plate method from

the sediment sample taken from the source of the Sakarya River in the Cifteler district of Eskisehir province (39°21'08.3"N 31°03'29.0"E), at a depth of 1 m in the sun-drenched region (Sembiring, 2000; Sivakumar, 2008). SM3 agar supplemented with nalidixic acid (10 mg mL^{-1}), rifampicin (0.5 µg mL^{-1}) and cycloheximide (50 µg mL^{-1}), was used for isolation.

Genomic DNA Isolation and Analysis of 16S rDNA Gene Region

The amplification of the 16S rDNA gene region of the S1S22 isolate, whose DNA was obtained using the DNA Isolation Kit (Invitrogen,USA), was carried out with 27F and 1525R primers (Write original paper) . 16S rDNA sequences were determined by the ABI 3730XL automatic base array device by MacroGen. Sequences obtained from 5 different primers were assembled using the Mega 7 (Kumar et al., 2016) package program. Sequences obtained using Ez Taxon Server (https://www.ezbiocloud.net/) and NCBI (https://www.ncbi.nlm.nih.gov/) were analyzed. Multiple alignments were performed in MEGA 7 program with the most closely related organisms from which 16S rDNA gene sequences were obtained in fasta format. After multiple alignment, phylogenetic tree was built using the neighbor-joining algorithm (Saitou & Nei, 1987). Jukes-Cantor metod was used as the distance matrix (Jukes & Cantor, 1969).

Plant Growth Activities of *Paenibacillus* sp. S1S22 Strain

The strain was investigated for the presence of indole acetic acid (IAA) and its ability to fix free nitrogen (N) (Ozdemir Kocak et al., 2020). For the assignment of indole acetic acid, *Paenibacillus* sp. S1S22 was grown in Luria-Bertani (LB) broth supplemented with 100 mg L^{-1} L-tryptophan and used tryptophan-free LB broth as a control group. The strain was incubated at 30 °C in the dark for 7 days. After the incubation, the cultures were centrifuged at 10,000 rpm for 15 minutes, and 2 mL of Salkowski solution (2 mL FeCl3 (1.35 %), 49 mL water, and 49 mL 60 % (v v) perchloric acid) were added on to 1 mL of supernatant, and left 30 minutes in the dark (Gordon & Weber, 1951). The pink color formation was considered positive for IAA production.

Strain N fixation ability was determined according to its ability to grow in the prepared nitrogen-free environment. The strain was inoculated into test tubes containing 10 mL of semi-solid agar, prepared with 1 % Yeast Carbon Base and 1 % Noble Agar and incubated in the dark at 28 °C for three weeks. Semi-solid agar supplemented with (NH4)2SO4 (2 g L^{-1}) was used as a positive control (Trujillo et al., 2010).

S1S22 strain was evaluated to assign its proteolytic activity. According to a modified procedure, nutrient agar (NA) and skim milk determined proteolytic activity. The strain inoculated into these media was incubated for three days at 37 °C. The formation of a clear halo region around the strain was evaluated as positive for proteolytic activity (Kazanas, 1968).

Cellulase activity was determined according to the method used by Li et al. (2018). The test isolate was inoculated on CMC agar consisting of tryptone, yeast extract, NaCI, CMC, agar and incubated at 37 °C overnight. Cellulase (glucanase) activity was considered positive according to the formation of the yellow hydrolysis zone. The phosphate solubilization ability of the strain was determined using the National Botanical Research Institutes Phosphate-Bromophenol blue (NBRIP- BPB) media. The strain inoculated on the NBRIP-BPB medium was left to incubate at 30 °C for seven days. The formation of the clear halo region around the colony was evaluated as positive (Nautiyal, 1999).

Antimicrobial Activity Properties of S1S22 Strain

In the study, 2 Gram-negative bacteria (*Escherichia coli* (*E.coli*) W3110, *Pseudomonas vulgaris* (*P. vulgaris*) NRRL B-123), 2 Gram-positive bacteria (*Bacillus subtilis* (*B. subtilis*) IMG 22, *Staphylococcus aureus* (*S. aureus*) ATCC 25923), two yeast (*Candida albicans* (*C. albicans*) ATCC 1326, *Saccharomyces cerevisiae* (*S. cerevisiae*) ATCC 9763) and two fungi (*Aspergillus parasiticus* (*A. parasiticus*) NRLL 465, *Fusarium* sp.) were used. Inoculation of *Paenibacillus* strain was done by spot seeding on modified Bennett's Agar the using agar spot method (Jones, 1949) without antibiotics (Williams & Cavanaugh, 1983). After inoculation, it was incubated for 24 hours at 28 °C. 3-5 ml of chloroform was poured into the developing colony, allowing it to die. The density of pathogen test organisms growing for 1-2 days was set at 0.1 OD. The smear plate method inoculated pathogenic microorganisms prepared on the dead colony. After a 1-day incubation at 28 °C, the zones of inhibition against the test pathogens around the colony were recorded by measuring with a cumpas.

Collection and Extraction of *Ulva lactuca* Samples

Ulva lactuca samples were collected from Tirilye region of Mudanya district of Bursa (40°23'29.3"N 28°48'17.0"E). Fresh samples were washed with dH_2O and then dried under laboratory conditions. Dry algae samples were thoroughly crushed in a mortar before extraction and mechanical fragmentation was performed. The extraction process was carried out with distilled water at a concentration of 10% at 100 °C for 1 hour by boiling method (Unal et al., 2022). When the obtained extract came to room temperature, it was filtered first with sterile filter paper and then using sterile syringe filters with a diameter of 0.45 µm.

Plant Material and Cultivation

The tomato plant (*Lycopersicon esculentum* L.) was used as the study material. After the seeds of the plant were grown in 10% hypochlorite solution for surface sterilization, the seeds were washed 5 times with sterile deionized water. Approximately half of the seeds were incubated with dH_2O and the other half with S1S22 bacterial culture at 0.1 OD for 2 hours at 30 °C. Petri dishes with sterilized filter paper were used for planting. While the filter papers in the petri dish were wetted with 5 mL of dH_2O for the control and groups to be treated only with S1S22, they were wetted with 5 mL of *Ulva lactuca* extract for the groups to which *Ulva lactuca* extract would be applied. Seeds incubated with S1S22 were planted in petri dishes belonging to the groups to which bacteria were to be applied, and seeds incubated with water only were planted in the other groups. The trial design of the study is shown in Figure 1.

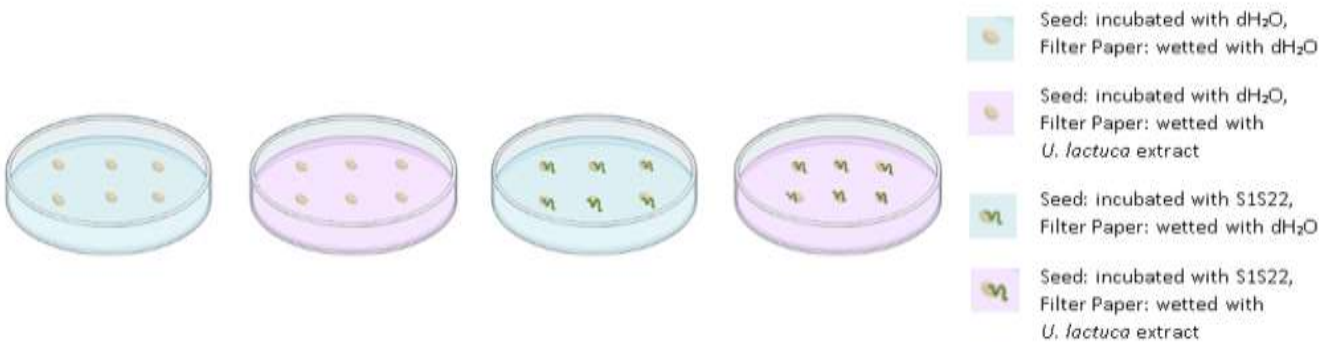

Figure 1. Schematic representation of the experimental design
Şekil 1. *Deney tasarımının şematik gösterimi*

Controlled conditions applied during cultivation; a photoperiod of 16 hours light/8 hours dark was carried out at a density of 650 µ mol m^{-2} s^{-1}, a temperature of 24-25 °C and a humidity of 42 %. Trials were carried out in 3 repetitions with at least 50 individuals per trial group in each repetition. The samples were harvested after 15 days. During the experimental period, the germination of *S. lycopersicum* seeds was recorded for five days. After harvesting, shoot and root lengths were measured. Each experiment was set up independently three time.

Statistical Analysis

SPSS 15.0 software was used for statistical analysis. Continuous variables with normal distribution were presented as mean ± SD. The median value was used where normal distribution was absent. Qualitative variables were given as a percent. Statistical analysis for the parametric variables was performed using the Student's t-test between two groups. A p-value of 0.05 was considered statistically significant.

RESULTS and DISCUSSION

Paenibacillus sp. Isolation from Sediment

S1S22 isolate was isolated by the dilution plate method using sample which collected from river sediment. The isolate was coded as S1S22 and given based on location (S: Sakarya River; 1: 1 m deep; S: sediment; 22: sample number).

16S rDNA Gene Region Analysis of S1S22 Isolate

16S rDNA gene region analysis of S1S22 isolate was conducted using five oligonucleotide sequences were obtained by purchasing service from Macrogen company. Sequences were assembled in the MEGA 7 program and analyzed in the ez-taxon program. Sequences of close relatives obtained in fasta format were analyzed in the MEGA 7 program, and a phylogenetic tree was created in this program. The phylogenetic tree is given in Figure 2.

The base sequence of *Paenibacillus* sp. S1S22 was aligned with the type species, resulting in a total aligned sequence of 1433 nucleotides. It was determined that the S1S22 isolate was related to *P. dendritiformis* and showed 99.6 % similarity. It was determined that this type differs by 4 nucleotides (Figure 2).

Plant Growth Activities of *Paenibacillus* sp. S1S22 Strain

Paenibacillus sp. S1S22 strain isolated from the sediment was examined in terms of IAA production, proteolytic activity and N fixation ability. It was determined that it gave positive results in the tests. Cellulose activity and phosphate solubilization ability of the S1S22 strain were negative (Table 1).

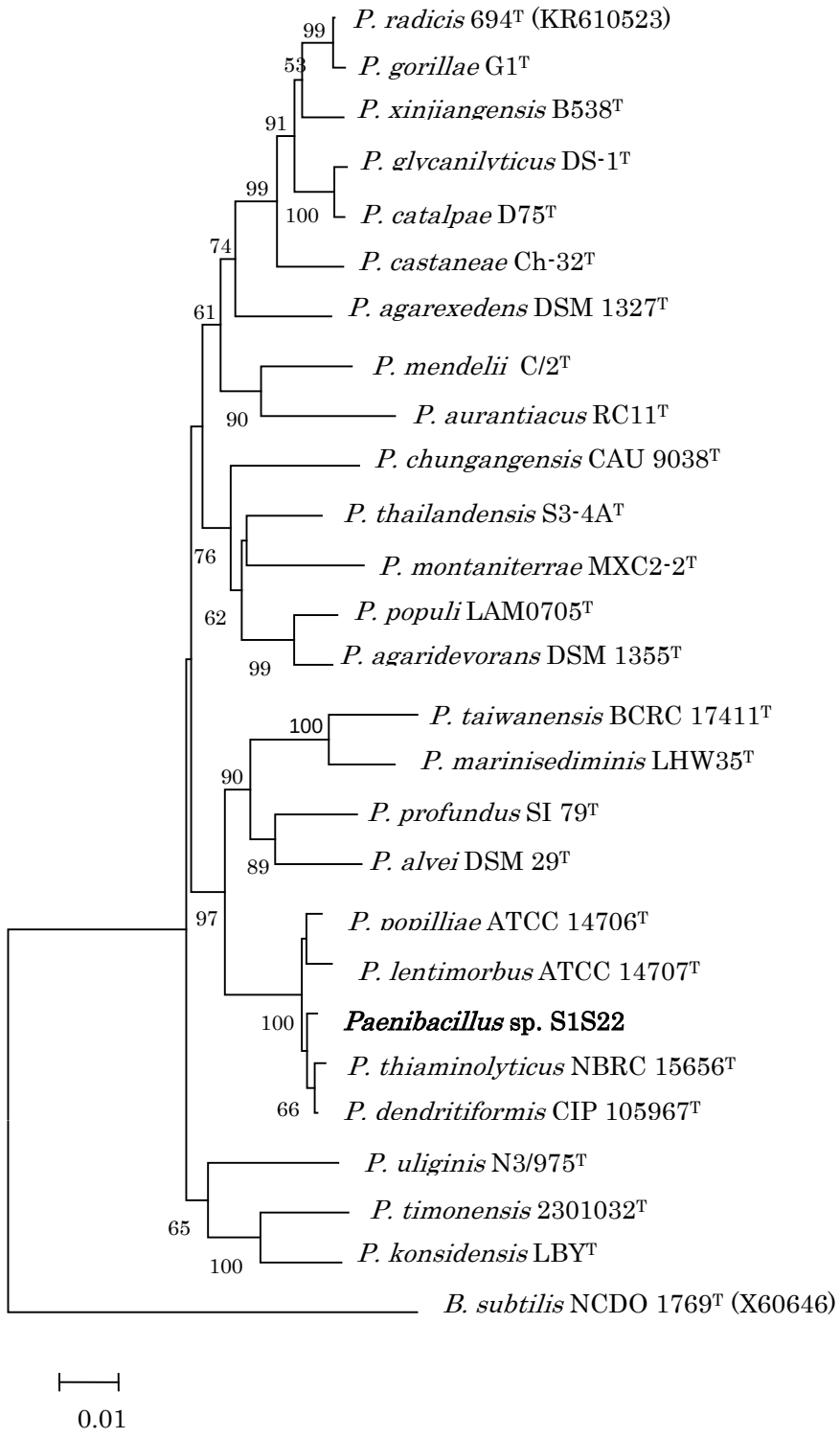

Figure 2. Neighbor-joining (Saitou & Nei, 1987) phylogenetic tree based on 16S rDNA base sequence analysis of test organism and type species belonging to the genus *Paenibacillus* and *B.subtilis* NCDO 1769T as outgroup was used.

Şekil 2. *Paenibacillus cinsine ait türlerin test organizasının 16S rDNA baz dizi analizine dayalı komşu birleştirme (Saitou & Nei, 1987) filogenetik ağacı ve ve B.subtilis NCDO 1769T dış grup olarak kullanılmıştır.*

Table 1. Plant growth-promoting properties of the S1S22 strain.

Çizelge 1. *S1S22 suşunun bitki büyümesini teşvik edici özellikleri.*

Isolate Code	IAA production	N fixation	Caseinase	Cellulose	Phosphate solubilization
S1S22	+	+	+	-	-

Plant growth-promoting bacteria (Bashan & Holguin, 1998) provide the plant with increased yield, pathogen neutralization, and stress defense (Welbaum et al., 2004, Lugtenberg & Kamilova, 2009). These microorganisms, which have been used as biofertilizers, natural pesticides, and phytoremediation agents due to their benefits to plants, have attracted attention in agricultural applications (Berg, 2009; Weyens et al., 2009). The effect of *Paenibacillus* sp. S1S22 strain on tomato plant growth was investigated due to its IAA production, proteolytic activity, and N fixation ability.

Antimicrobial Activity of S1S22 Strain

The ability of the test strain isolated from the sediment to inhibit the growth of 8 pathogenic test organisms (Gram-positive and negative, yeast and fungus) was determined. The zone diameters were measured with cumpas and given in the table below (Table 2).

It was determined that the *Paenibacillus* sp. S1S22 strain showed high activity against Gram-positive, Gram-negative and yeast but had no activity against fungi.

Table 2. Antibacterial activity results of *Paenibacillus* sp. S1S22 strain
Çizelge 2. *Paenibacillus sp. S1S22 suşunun antibakteriyel aktivite sonuçları*

	E. coli	*P. vulgaris*	*S. aureus*	*B. subtilis*	*C. albicans*	*S.cerevisiae*	*A. parasiticus*	*Fusarium* sp.
S1S22	60 mm	30 mm	45 mm	35 mm	50 mm	30 mm	-	-

Effects of *Paenibacillus* sp. S1S22 Strain and *U. lactuca* Application on Plant Growth

As a result of the applications, the experimental groups' root (Figure 3.) and stem (Figure 4.) lengths compared to the control plants are given below. According to the results, plant roots treated with only S1S22 strain and only *U. lactuca* extract were 5.26 % and 9.28 % longer than the control, respectively. The greatest difference in root length is that individuals treated with the S1S22 strain and *U. lactuca* extract have 18 % longer roots. Similarly, the most striking difference in stem length was observed with the combination treatment of *U. lactuca* and S1S22 strain, which had an 18 % longer stem than the control. However, stem length was 2.47 % shorter in plants inoculated with only S1S22 and 4.29 % in plants treated with only *U. lactuca* extract.

In this study, the growth rates of plants treated with *Paenibacillus* sp. S1S22 strain differs in root and stem compared to control groups. While root growth increased compared to control, a decrease in stem length was observed. At this point, it is important to emphasize that there may be a change in the hormonal balance of the plant. In parallel with this point of view, a study has shown that *Burkholderia phytofirmans* promote plant shoot and root growth, related to the modulation of plant growth hormones (Kurepin et al., 2015). However, not every bacterium affects every plant in the same way. For example, *Pseudomonas fluorescens* inhibited root growth in cherries but increased it in blackcurrant (Olanrewaju et al., 2017). These findings are important for further studies to elucidate the mechanism underlying these physiological changes caused by S1S22 bacteria in the tomato plant.

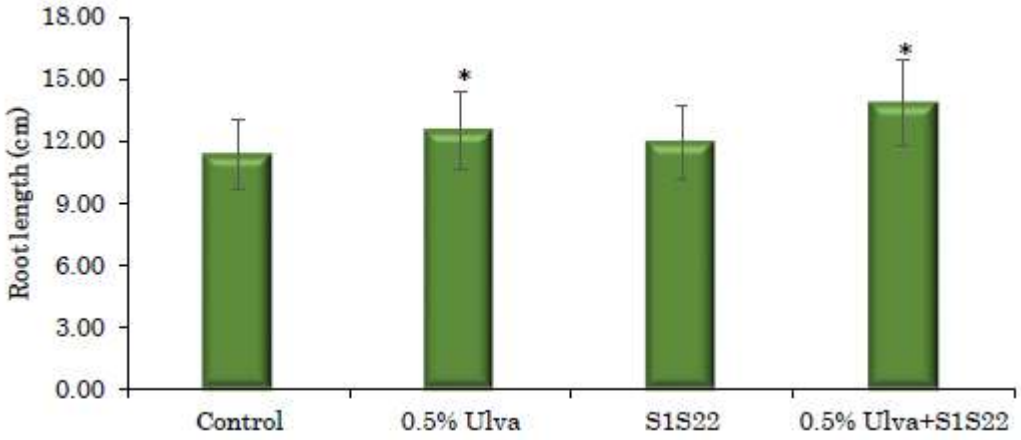

Figure 3. Root length differences of plants applied with *Paenibacillus* sp. S1S22, *U. lactuca* extract, and both *U. lactuca* extract and S1S22 compared to control
Şekil 3. *Kontrole kıyasla Paenibacillus sp. S1S22, U. lactuca ekstresi ve hem U. lactuca ekstresi hem de S1S22 uygulanan bitkilerin kök uzunluğu farklılıkları*

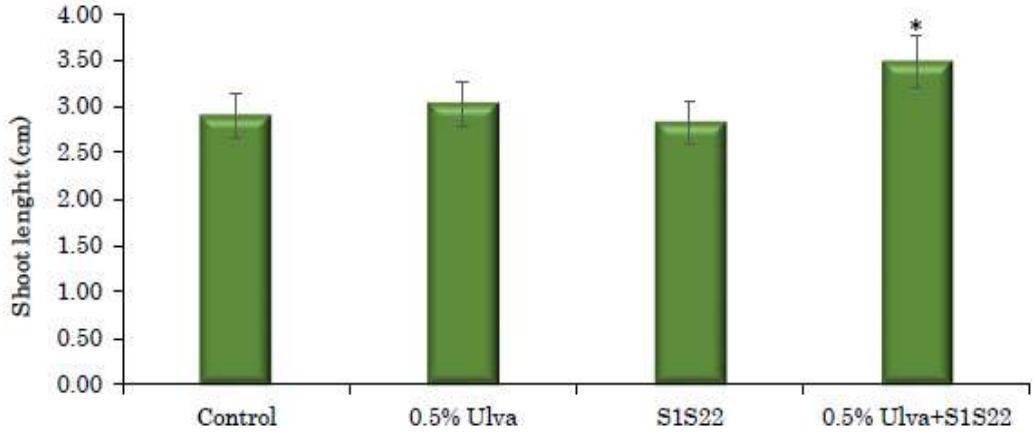

Figure 4. Stem length differences of plants applied with *Paenibacillus* sp. S1S22, *U. lactuca* extract, and both *U. lactuca* extract and S1S22 compared to control

Şekil 4. *Kontrole kıyasla Paenibacillus sp. S1S22, U. lactuca özütü ve hem U. lactuca özü hem de S1S22 ile uygulanan bitkilerin gövde uzunluğu farklılıkları*

It has been revealed that there are significant amounts of photosynthetic pigments (chlorophyll and b, carotenoids and phycobiliproteins), lipids, and phenolic substances in different species of the genus *Ulva*, especially *U. lactuca* (Chojnacka et al., 2012). In parallel with these results, the treatment of the *Brassica napus* plant with *U. lactuca* extracts caused significant morphological, biochemical, and physiological changes. In addition, it is recommended to be used as a biofertilizer due to this stimulating effect in terms of growth and yield. The extracts of *U. lactuca* increased IAA, cytokinin, chlorophyll a, b, and carbohydrate levels in *B. napus*. In another study, its extract from the brown macroalgae *Ascophyllum nodosome* affected the regulation of phytohormone biosynthesis and accumulation in *Arabidopsis* (Jithesh et al., 2012). It was reported that *A. nodosum* extracts induced cytokinin-like activity in *A. thaliana* (Khan et al., 2011). Obtained results, compared to the control plant; the growth increase in both roots and stems of the plants treated with *U. lactuca* extract supports the approaches to increasing the agricultural productivity of algae extracts. However, the growth induction observed in plants treated with *U. lactuca* and S1S22 strain was greater than in plants treated only with bacteria or algae. When bacteria and algae are applied to the plant together, they show different effects when applied alone because they both synthesis their polysaccharides and growth hormones such as auxin and cytokines and stimulate the plant defense systems (Kang et al., 2021). This study, a preliminary experiment, draws attention to support the idea that the positive effects of PGPBs on growth can be increased with algae extracts.

The plant's combined application of PGPB and algae with the PGP effect can lead to a synergistic effect and increase yield. For example, co-treatment of *P. fluorescens* and *Pantoea ananatis* promoted the shoot and root length of *Pisum sativum* plants due to IAA production and phosphate solubility (Anwar et al., 2019). In another co-administration of *C. vulgaris* and PGPBs, *Azospirillum* sp., *B. licheniformis, Azotobacter* sp., *Bacillus megatherium*, and *Herbaspirillum* sp. caused an increase in leaf weight of 22.7 % in lettuce (Kopta et al., 2018). Moreover, triple treatment of *Anabaena cylindrica, Rhizobium tropici* and *Azospirillum* sp. to the common bean increased plant growth and grain production by 84 % (Horacio et al., 2020). However, co-cultivation is much more successful than the monoculture system in promoting plant growth because with the co-culture system, algae and bacteria both act on each other and affect the plant individually, increasing plant growth more (Spadaro & Gullino, 2005).

CONCLUSION

It is necessary to investigate the basic reasons for the morphological changes caused by the application of S1S22 strain in combination with the *U. lactuca* extracts to the plant, expands this interaction range with different algae extracts and with different bacterial species and reveals the basic mechanisms of plant-bacteria-algae interactions in this way. Thus, it will be possible to increase the effectiveness of natural tools such as PGPB away from chemical and environmentally harmful stimulants.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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