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Effect of Different Plant Growth-Promoting Rhizobacteria on Biological Soil Properties, Growth, Yield and Quality of Oregano (*Origanum onites* L.)

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Abstract: Intensive agriculture uses continuous chemical fertilizers to increase crop yields, but excessive use of fertilizers leads to environmental pollution, permanent changes in physicochemical conditions in soil ecology, deterioration of soil biological health, leaching of nutrients, surface and groundwater pollution and eutrophication. Plant growth-promoting rhizobacteria (PGPR) are becoming increasingly important for ensuring crop safety, increasing nutrient uptake and output, lowering fertilizer costs, preventing environmental contamination and promoting sustainable agriculture and agricultural resources. Therefore, the purpose of this study was to identify and evaluate the effects of fifteen bacteria strains that were isolated from various acidic rhizospheric soils as biofertilizers on soil biological properties. Growth, yield and quality traits were analyzed, and various PGPR were identified using 16S ribosomal RNA of Turkish oregano. Fifteen bacterial inoculations with 1-aminocyclopropane-1-carboxylate (ACC) deaminase, N_2-fixing, P-solubilizing and/or IAA-producing genes were used in the experiment, which was carried out in a randomized block design with five replicates (each with three pots) and a control without inoculation. Increased biological activity in soil inoculated with bacteria with multiple traits was confirmed by high C and N content in microbial biomass, urease, dehydrogenase and acid and alkaline phosphatase activities. Essential oil content, oil yield, thymol and carvacrol contents increased by 0.5–40.1%, 5.9–71.9%, 0.07–16.7% and 0.3–9.2%, respectively, as a result of bacterial inoculation. Oil content ranged from 2.02% to 2.83%; carvacrol (66.1–72.2%) was the main constituent, followed by thymol (14.5–16.9%) and linalool (1.38–3.68%). Two large PGPR groups were formed based on genetic distance analysis. Responses were variable and depended on the inoculant strain and the parameters being evaluated. The results indicate PGPR has clear potential for improving the yield of cultivated aromatic and essential oil plants, such as oregano.

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1. Introduction

The most prominent medicinal plant exported from Turkey is oregano (*Origanum onites* L.), which is grown in Western and Southern Anatolia and is used as a spice and in herbal teas. Both cultivated plants and wild populations found in nature are used to make dried oregano. Its vegetative elements and biochemical extracts are widely employed as a seasoning herb to flavor fish, soups, salads, olives, chicken, pork, vegetables, salad dressing and wine in the food and spice sectors [1]. They are becoming more and more well-liked as organic antioxidants and antibacterial agents that can be applied to food preservation [2]. The main constituents of *O. onites* essential oil are carvacrol, thymol, p-cymene, gamma-terpinene, borneol, linalool and alpha-terpinene [3]. Carvacrol is an oxidized monoterpene that has a variety of medicinal effects [4] and is a key component influencing oregano's scent [5]. Additionally, it has been found to have antispasmodic, lipid peroxidase inhibitory, radical scavenging, insecticidal, antiangiogenic, anticarcinogenic and cardiac depressive properties [6].

Cultivation of oregano has been established and developed rapidly in recent years in order to protect natural resources and provide products of high standards and quality. Since many medicinal and aromatic plants such as oregano are consumed without further processing, production without the use of chemical fertilizers and the absence of synthetic compounds in the harvested product are of great importance in the food and pharmaceutical industries. The most ideal strategy is to develop growth-promoting strategies with plant growth-promoting bacteria [7]. Plant growth-promoting rhizobacteria (PGPR) can be used in agricultural production that is more sustainable, given the harm that excessive fertilizer use has done to the environment and the high production costs that follow from it [8]. Through a variety of methods, including nitrogen fixation and the solubilization and mineralization of organic/inorganic P and/or other nutrients, PGPR promotes plant development both directly and indirectly. The production of siderophores, ammonia, salicylic acid and phytohormones such as indole acetic acid (IAA), cytokinins, gibberellins, etc. play an important role, recycling of essential elements and the uptake of essential nutrients from the soil and contributing to the mineralization of organic matter containing ACC deaminase, the reduction of ethylene levels in plants, the degradation of organic pollutants and the improvement of the symbiosis of plants and microorganisms [9–11]. By reducing production costs and environmental pollutants with PGPR, a new idea and alternative approach to increasing plant growth and yield can help improve agronomic efficiency. Rhizobacteria might be able to provide a long-lasting, environmentally benign substitute for the hazardous synthetic fertilizers now utilized in nutrient management and sustainable production of aromatic and medicinal plants. Understanding the factors that regulate or affect the biosynthesis of secondary metabolites and the accumulation of monoterpenes will enable strategies that boost the cultivation and productivity of aromatic plants and other agricultural crops without the use of chemical fertilizers or pesticides [12]. An important crop factor influencing the quantity and quality of essential oils is appropriate and balanced mineral fertilization of aromatic plants, adapted to their nutritional needs and growth environment. This process is analogous to bio-fertilization [13].

Studies on the bacterial–plant relationship has focused on cereals and grass plants, and only a few studies have been conducted on medicinal plants. For medicinal and aromatic plants, experiments with PGPR have shown increases in yield and essential oil in Italian oregano and *Origanum majorana* L. [14], rosemary [15], *Pelargonium graveolens* [16], sweet fennel [17], dill plant [18], common basil [19], coriander [20], Mexican marigold [21], *Thymus daenensis* [22], summer savory [23] and peppermint [24]. Although several studies have focused on the impact of PGPR inoculation in aromatic plants [21], it is unclear how PGPR affects the formation of secondary metabolites [25]. Overall, the morphological and

physiological characteristics of rhizobacteria-associated aromatic plants remain limited and fragmented [12].

Enzyme activities in soil are considered a major contributor to overall soil microbial activity and soil quality [26] and are good indicators for predicting changes in soil quality and productivity [27] and ecosystem health and sustainability [28]. Ureases, dehydrogenase (DHA) and acid and alkaline phosphatase (ACP and ALP) are the most important soil enzymes involved in carbon, nitrogen and phosphorus cycling and the transformation of various plant nutrients [29]. The microbial biomass is a potential source of enzymes in soil and an important labile fraction of soil organic matter, functioning as a factor in both the transformation and cycling of organic matter and plant nutrients. PGPR, enzymatic activity and microbial biomass are closely linked, as the transformation of important organic elements occur via microorganisms [30,31].

The 16S rRNA gene has been a staple of decades of historical analysis of bacteria [32]. Since the advent of high-throughput sequencing, 16S sequences amplified by PCR are typically clustered based on similarity to generate operational taxonomic units and representative sequences of operational taxonomic units that are compared to reference databases to understand taxonomy [33]. One of the most attractive potential applications of 16S rRNA gene sequence informatics is genus identification for isolates that do not fit any known biochemical profile, for strains that produce only "low research" or "acceptable" definitions according to commercial systems. Limited cumulative production results show that 16S rRNA gene sequencing alerts genus identification in most cases (>90%), but less so for species (65% to 83%) and 1% to 14% for genus identification of the remaining unidentified isolates after testing [34].

Our literature survey showed that there are limited resources on agronomic factors such as the use of organic fertilizers in addition to rhizobacteria that stimulate plant growth on soil biological properties, yield and essential oils, as well as the identification of different bacterial strains using 16S ribosomal RNA of Turkish oregano [35]. The purpose of this study was to investigate the effects of multi-trait rhizobacteria on soil biological quality indicators (microbial C and N biomass and dehydrogenase, urease, acid and alkaline phosphatase activities), growth, yield and essential oil content, essential oil components, chlorophyll value and macro- and micronutrient concentrations in the leaves of *O. onites*, an important medicinal and aromatic plant and a natural source of carvacrol.

2. Materials and Methods

2.1. Bacterial Strains

We selected 15 different isolates from a pool of 476 rizobacterial isolates based on their 1-aminocyclopropane-1-carboxylate (ACC) deaminase-containing, auxin (IAA)-producing, N_2-fixing, P-solubilizing abilities and their ability to utilize different carbon sources. Of these, six were isolated from grapevine (RC39, RC236, TE142, TE565, TE28 and RC32), five from wild red raspberries (RC125, RC521, RC43, RC66 and RC96), and four strains from tea (RC613, RC636, RC213 and RC42) rhizosphere. Isolation procedures were carried out according to the procedure already described [36]. Using the MIDI system, BIOLOG tests, 16S rRNA amplification and sequencing and fatty acid methyl ester (FAMEs) analysis, rhizobacteria isolates were randomly chosen from soy broth with tryptase solidified on agar and identified. In addition, the capacity of bacterial strains to consume 95 various carbon sources was determined using bacterial species that were further described using BIOLOG GP2 MicroPlates (Biolog, Inc., Hayward, CA, USA) [36]. Identification of the tested bacteria was confirmed using the microbial identification system (MIS). Preparation and analysis of FAMEs from whole-cell fatty acids of bacterial strains were performed according to the manufacturer's manual (Sherlock Microbial Identification System version 4.0, MIDI, Inc., Newark, DE, USA). FAMEs were separated using gas chromatography (HP-6890, Hewlett Packard, Palo Alto, CA, USA) with a fused silica capillary column (25 m × 0.2 mm × 0.25 µm with cross-linked 5% phenyl methyl silicone). FAME profiles of each bacterial strain were identified by comparing commercial databases (TSBA 40 aerobe

library) with the MIS software package version 4.5 [37]. Two days prior, utilizing the Biolog microplate system BIOLOG GP2 and GN2 Plates (Biolog, Inc., Hayward, CA, USA), bacterial strains were inoculated into TSA or BUG agar plates for bacteria identification. The MicroPlates are 96-well plates with 95 various carbon sources and a negative control in the water well. A suspension of Gram-positive or Gram-negative bacteria containing 125 µL was added to each well of Biolog GP2 or GN2 microtiter plates, adjusted to the proper density (108 cfu/mL) and then incubated at 27 ± 1 °C for 24 and 48 h, respectively. Using a microplate reader with a 590 nm filter, the color evolution was automatically captured. Using the automated threshold option in the BIOLOG420/Databases/GN601 and GP601 KID program, identification (Biolog Microlog 34.20 database) and recording of results in an ASCII file were carried out [38].

2.2. Identification of Bacterial Isolates by 16S rRNA Gene Amplification

Utilizing the Pitcher et al.-provided procedure, genomic DNA was extracted [39]. With the use of the universal primers Bac8f (5′-AGAGTTTGATCCTGGCTCAG-3′) and Univ529 (5′-ACCGCGGCKGCTGGC-3′), a small portion of the 16S rRNA gene bordering the V1–V3 region was amplified (around ~500 bp). The PCR program included 30 cycles of denaturation at 95 °C for 60 s, annealing at 54 °C for 30 s, extension at 72 °C for 90 s, and a final extension at 72 °C for 10 min. The PCR was performed using a 100 pM primer and 50 ng of DNA [40].

2.3. Sequencing and Phylogenetic Analyses

The 16S rDNA amplicons were custom sequenced at MedSanTek, Istanbul. The obtained nucleotide sequences were assigned a bacterial taxonomic affiliation based on the closest match to sequences available in the NCBI database (http://www.ncbi.nlm.nih.gov/) accessed on 12 July 2023. Sequences of the 16S rRNA gene were aligned using the ClustalX 2.1 multiple sequence alignment tool. Phylogenetic and molecular evolutionary analysis was carried out using CLC Sequence Viewer 7.6 software. The phylogenetic tree was constructed with the neighbor-joining method, using a distance matrix from alignment.

2.4. Acetylene Reduction Assay and Phosphate Solubilization

An acetylene reduction test was used to measure the nitrogen-fixing capacity of isolates in nitrogen-free media [41]. Using a Hewlett Packard 6890 N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA), ethylene production was monitored. All pure isolates were evaluated in triplicate for their ability to solubilize phosphate in sucrose–tricalcium agar media [42] by inoculating 1 mL of 6-day-old culture (density 4×10^9 CFU mL^{-1}) in 250 mL Erlenmeyer flasks containing 500 µg mL^{-1} of P as rock phosphate at 30 ± 1 °C [36]. Acetylene reduction activity was expressed as nmol C_2H_4 $(10^7$ cfu$)^{-1}$ h^{-1}.

2.5. Quantification of IAA Production and Activity of ACC Deaminase

The ability of PGPR to produce auxins similar to IAA has been studied. Erlenmeyer flasks (125 mL) containing 40 mL of semi-concentrated tryptophan broth (TSB) were incubated for 18 h at 27 °C on a rotary shaker at 100 rpm with 25 µg tryptophan mL^{-1}, and then each flask was inoculated with 1 mL of each PGPR (10^8 CFU mL^{-1}) [43]. The concentration of IAA in the culture medium was measured using Salkowski reagent [50 mL 35% (*v*/*v*) $HClO_4$ containing 1 mL 0.5 M $FeCl_3$]. Using a Shimadzu UV-1208 spectrophotometer, absorbance was measured at 530 nm. Centrifugation was used to separate the bacterial cells from the supernatant for 30 min at 4 °C at 10,000 rpm. The concentration of IAA (0–100 µg mL^{-1}) in each culture medium was determined by comparison with a standard curve. After incubation, the density of each culture growth was measured spectrophotometrically at 600 nm; IAA production was estimated from the optical density at 600 nm (OD_{600}) by comparison with the prepared standard curve and expressed as µg of IAA secreted per unit of optical density. Isolates were tested for. By evaluating their capacity to grow in minimal DF salt medium [44] supplemented with 3 mmol ACC as the only nitrogen source,

ACC deaminase activity can be determined [45]. According to the Honma and Shimomura technique, the amount of α-ketobutyrate generated (nmoles mg protein^{-1} h^{-1}) was used to measure the amount of cell extracts and ACC-deaminase activity [46]. The isolates' ACC deaminase activity was assessed using a spectrophotometer that measures absorbance at 540 nm [47].

2.6. Greenhouse Experiment and Growth Conditions

This study was conducted in greenhouse at Atatürk University, Faculty of Agriculture, during the 2011–2012 growing season as part of two trial sets. The pots were sterilized with 20% sodium hypochlorite solution and filled with sandy loam, which is a primary field soil with 3.2% organic matter and 0.18% nitrogen, available Olsen-P and exchangeable contents of K, Ca and Mg of 16.2, 448, 3420 and 472 mg kg^{-1}, respectively. The available Fe, Mn, Zn and Cu contents were 5.9, 9.6, 1.6 and 1.8 ppm, respectively. At the beginning of the study, the initial microbial biomass C (MBC) and microbial biomass N (MBN) of the soil used were 380 and 42 mg kg^{-1}.

Two sets of experiments were conducted. The experiment was organized as a completely randomized block design with 15 PGPR applications and a non-inoculated control with five replications (each with three pots). Pure cultures were grown in 50% tryptic–soy broth (Merck, Darmstadt, Germany) on a rotary shaker (120 rpm, 25 °C) for 3 days. Bacteria were then harvested by centrifugation (about 3000 g for 10 min), washed and resuspended in 10 mM sterile phosphate buffer (SPB), pH 7, to a density of 10^9 colony forming units (CFU) mL^{-1} for bacterial strains. Bacterial inoculation consisted of immersing the root systems of the seedlings in a suspension of each PGPR strain 60 min before planting. Control plants received 5 mL of diluted SPB without bacteria. Seeds of oregano (*Origanum onites* L.) were germinated in a seed tray containing garden soil/peat/sand (2:1:1 [*v*/*v*]) at 24 ± 5 °C with 60% relative humidity, under 16 h day and 8 h night conditions in the greenhouse. The seedlings were removed from the seed trays after one month. Later, three uniform 30-day-old inoculated seedlings were transferred to each pot containing a virgin garden, and, after 2 weeks, the seedlings were thinned to one.

All plants were grown in the greenhouse on a day/night cycle of 16/8 h natural light (intensity of 900 to 1.300 µmol m^{-2} s^{-1}), temperature of 25/17 °C and relative humidity of 60/70%. Pots were watered to 70% of water capacity and this humidity was maintained by watering every 4 days. If necessary, weeds were controlled by hand. In the second year of bacterial inoculation, 5 mL of bacterial suspension (10^8 CFU mL^{-1}) was injected into the rhizosphere of each oregano plant. About three months after planting, oregano plants were harvested twice, in mid-July and September each year. Experimental and greenhouse studies on the plants, including collection of plant material, are in accordance with relevant institutional, national and international guidelines and regulations.

2.7. Microbial Biomass and Enzyme Activities Analysis

Samples from the rhizosphere, the soil strongly attached to the root, were taken at the end of the second year of the study. From all treatments and replications, 10–15 cm cores of soil in the rhizosphere zone were taken at random and, after mixing, were combined into one composite sample. The soil samples were thoroughly homogenized, sieved through a 2 mm sieve and stored at 4 °C until analysis. Soil microbial biomass C (MBC) and N (MBN) were determined by chloroform extraction method [48]. Soil MBC and MBN were calculated based on the difference between total C and N in fermented K_2SO_4 extracts from fumigated and non-fumigated 10 g of soil samples, and 0.45 and 0.54 for the extract correction factor, respectively. Urease enzyme activity was determined by measuring the rate of NH_4^+ release when soil was incubated with urea for 90 min at 30 °C [49]. The activity of dehydrogenase in 1 g of soil was determined according to the method of Thalmann [50], and the activity was expressed as µmol iodonitrotetrazolium formazan (INTF) g^{-1} h^{-1}. The activities of acid and alkaline phosphatase (ACP and ALP) enzymes were determined according to the method described by Tabatabai and Bremner [51], using *p*-nitrophenyl

phosphate (*p*-NP) as substrate. Phosphatase activity was expressed as µmol *p*-NP per gram of dry soil and incubation time (µmol *p*-NP g^{-1} h^{-1}). All estimated soil biological properties were calculated on the basis of soil dry weight.

2.8. Essential Oil Extraction and GC-MS Analysis

To calculate the yield of fresh herb, plants were picked when fully flowering, trimmed 5–6 cm above ground, and weighed. After being picked, the oregano herb was dried naturally in a dry, open area with some shade. Using Clevenger equipment, the plants' essential oil content was ascertained. Hydrodistillation was used to separate the essential oil from the dried herb for two hours on average. According to the methodology outlined by Kordali et al., the essential oil was analyzed using a Thermofinnigan Trace GC/Trace DSQ/A1300 apparatus (E.I. Quadrapole) outfitted with an SGE-BPX5 MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm) [52]. Identification of individual compounds was based on comparison of their relative retention times with those of authentic samples on the SGE-BPX5 capillary column and on matching the mass spectra of their peaks with those obtained from authentic samples and/or spectra of Wiley 7N and TRLIB libraries and published data [53].

2.9. Plant Analysis

Leaf samples of 2 g were dried in an oven at 68 °C for 48 h and ground to pass through a 1 mm sieve. The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine the total N content of oregano leaves. The P, K, Ca, Mg, Fe, Mn, Zn and Cu contents of the tissues were determined using an inductively coupled plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Perkin-Elmer, Waltham, MA, USA). A chlorophyll meter (SPAD-502, Minolta, Tokyo, Japan), which gauges how green a plant's leaves are, was used to measure the amount of chlorophyll in the upper fourth and fifth leaves. Four spots on each leaf, two on either side of the nerve, and an average were recorded for every plant.

2.10. Statistical Analysis

The studies were performed in a completely randomized block design with five replicates (each with three pots), and they were repeated twice. Three samples from each replication of the enzyme were used to measure its activity. Means were separated based on Duncan's multiple range test (Genstat v. 23), and data were subjected to analysis of variance using SPSS 13.0 (SPSS Inc., Waltham, MA, USA).

3. Results

3.1. PGPR and Their Characteristics

Of the strains used in this study, 13 of the 15 selected bacterial strains produce both 1-aminocyclopropane-1-carboxylate (ACC) deaminase and indole acetic acid (IAA), and all of them have the ability to fix N_2 and solubilize inorganic P. All of these isolates showed nitrogenase and P solubilization activities ranging from 0.24 to 0.86 nmol C_2H_4 10^7 CFU h^{-1} and from 14.3 to 124.7 µg P mL^{-1} d^{-1}, respectively. The levels of IAA produced by the various PGPR evaluated, in the presence of 25 µg mL^{-1} tryptophan, ranged from 6.3 µg mL^{-1} for *Bacillus pumilus* RC39, to 71.6 µg mL^{-1} for *Bacillus velezensis* RC521 (Table 1). There were large differences in C utilizations between the strains tested. Gram-positive strains *B. megaterium* RC213 and *A. ramosus* RC32 used the highest number of substrates (65 and 63), followed by *B. simplex* TE142, *B. licheniformis* RC636, *B. simplex* RC236 and *B. megaterium* RC42 (transforming 57, 55, 51 and 48 substrates, respectively), while *P. castaneae* RC66 and *B. amyloliquefaciens* RC613 used the lowest number of substrates (27 and 35) (Table 1).

Table 1. Biochemical characteristics of the bacterial strains tested.

Closest NCBI Match/Closest Type Strain	SIM Index	BSI	Nitrogenase Activity (nmol C_2H_4, 10^7 CFU h^{-1})	P-Solubilization (μg P $mL^{-1}d^{-1}$)	ACC-Deaminase Activity (nmol α-Ketobutyrate mg^{-1} Protein h^{-1})	IAA-Production (μg mL^{-1} OD_{600} $unit^{-1}$)	Number of Carbon Sources Used
Bacillus amyloliquefaciens RC613	0.600	0.50	0.74 ± 0.17	34.6 ± 0.08	972.0 ± 28.3	41.4 ± 2.5	35
Bacillus licheniformis RC636	0.397	0.28	0.48 ± 0.12	39.3 ± 1.5	138.1 ± 6.5	29.7 ± 1.9	55
Bacillus megaterium RC213	0.797	0.44	0.65 ± 0.13	48.3 ± 1.7	688.3 ± 27.4	26.6 ± 2.5	65
Bacillus megaterium RC42	0.857	0.59	0.52 ± 0.15	24 ± 0.09	62.3 ± 9.4	26 ± 1.4	48
Bacillus pumilus RC125	0.726	0.47	0.58 ± 0.12	26.5 ± 1.6	32.5 ± 8.6	35.6 ± 6.1	42
Bacillus pumilus RC39	0.543	0.43	0.28 ± 0.5	14.3 ± 1.1	443.4 ± 22.7	6.3 ± 1.2	37
Bacillus simplex RC236	0.676	0.57	0.33 ± 0.12	15.6 ± 0.43	ND	41.3 ± 3.6	51
Bacillus simplex TE142	0.507	0.41	0.81 ± 0.21	37.8 ± 1.2	321.3 ± 18.5	63.8 ±1.1	57
Bacillus subtilis TE565	0.494	0.27	0.51 ± 0.11	42.2 ± 2.3	236.5 ± 14.4	21.6 ± 1.3	39
Bacillus subtilis TE28	0.608	0.44	0.24 ± 0.06	23.4 ± 0.07	94.8 ± 4.9	23.1 ± 0.7	33
Bacillus velezensis RC521	0.682	0.42	0.86 ± 0.14	124.7 ± 6.18	317.8 ± 13.6	71.6 ± 7.4	41
Paenibacillus barcinonensis RC43	0.426	0.23	0.61 ± 0.13	28.8 ± 1.2	132.1 ± 16.5	ND	45
Paenibacillus castaneae RC66	0.728	0.50	0.78 ± 0.17	25.8 ± 0.9	132.1 ± 16.5	36 ± 2.2	27
Arthrobacter ramosus RC32	0.595	0.42	0.24 ± 0.06	33.8 ± 1.1	ND	ND	63
Stenotrophomonas maltophilia RC96	0.808	0.58	0.39 ± 0.11	29.4 ± 1.6	77.8 ± 9.7	52.6 ± 0.9	36

The percentage identity with the 16S rRNA gene sequence of the closest phylogenetic relative. SIM: similarity index from FAME analysis; BSI: BIOLOG similarity index; ND: not determined; data were the mean ± standard error of three independent cultures of each strain (biochemical characteristics by each strain were measured in triplicate); IAA production in average 72 h pure cultures.

3.2. Plant Growth Parameters

Different rhizobacteria varied in their impact on the soil's biological characteristics, plant growth indicators, essential oil content and the yield of *Origanum onites* (both positive and negative) (Table 2). On average, in pot experiments in both years, the maximum height of the oregano plants was found after inoculation with *B. simplex* TE142, followed by *B. megaterium* RC213, *S. maltophilia* RC96, *P. castaneae* RC66 and *B. subtilis* TE565. Among bacterial inoculants, the maximum oregano canopy diameter was measured for *B. velezensis* RC521, followed by *B. amyloliquefaciens* RC613, *B. licheniformis* RC636 and TE142 inoculations. Inoculation of Turkish oregano with strains TE142, RC521, RC66, RC213, RC613, RC636 and RC565 showed a significant increase in dry herb and leaf yield and leaf chlorophyll content compared to control plants. Considering all values of fresh oregano herb, dry herb and leaf yield, plant height, canopy diameter and chlorophyll concentration (SPAD value), the most effective inoculations of N_2-fixing and P-solubilizing bacteria were TE142, RC521, RC66, RC213, RC613, TE565 and RC636 in terms of yield and yield components in Turkish oregano. Comparing inoculated responses to those of control plants that were not inoculated, the results were as follows: −0.5% to +19.0% for chlorophyll concentration, −11.6% to +24.6% for fresh herbage yield, −2.0% to 32.2% for the dry herbage yield and −4.7% to 36.5% for dry leaf yield. As an average across years and treatments, values ranged from 37.9 to 48.5 cm plant height, 38.3–45.9 cm canopy diameter, 39.7 to 47.5 chlorophyll concentration (SPAD value), 48.1–67.8 g fresh herbage, 14.6 to 19.7 g dry herbage and 8.1 to 11.6 g dry leaf yield per plant.

Table 2. Effect of *Origanum onites* L. inoculation with different PGPR strains on plant growth parameters and essential oil content and yield.

Treat-ments [a]	Plant Height (cm)	Canopy Diameter (cm)	Chlorophyll Content (SPAD Unit)	Fresh Herbage Yield (g Plant^{-1})	Dry Herbage Yield (g Plant^{-1})	Dry Leaf Yield (g Plant^{-1})	Essential Oil Content (%)	Oil Yield (mL Dry Herb^{-1})
Control	39.1 ^{cd}	38.4 ^{d}	39.9 ^{de}	54.4 $^{b–d}$	14.9 ^{de}	8.5 ^{c}	2.02 ^{d}	0.32 ^{e}
RC613	44.7 $^{a–c}$	45.7 ^{a}	45.6 $^{a–c}$	63.2 ^{ab}	18.1 $^{a–c}$	11.3 ^{ab}	2.83 ^{a}	0.55 ^{a}
RC636	43.5 $^{a–d}$	45.0 ^{ab}	45.6 $^{a–c}$	62.6 ^{ab}	17.9 $^{a–c}$	10.9 ^{ab}	2.62 ^{ab}	0.50 ^{ab}
RC213	47.2 ^{a}	44.5 $^{a–c}$	46.7 ^{ab}	66.2 ^{a}	18.8 ^{ab}	10.9 ^{ab}	2.39 ^{bc}	0.48 ^{ab}
RC42	42.7 $^{a–d}$	41.5 $^{a–d}$	45.6 $^{a–c}$	61.9 $^{a–c}$	17.4 $^{a–c}$	9.7 ^{bc}	2.03 ^{d}	0.37 $^{c–e}$
RC125	37.9 ^{d}	38.4 ^{d}	39.7 ^{e}	48.1 ^{d}	14.6 ^{e}	8.1 ^{c}	2.26 ^{cd}	0.35 ^{de}
RC39	40.6 $^{b–d}$	40.5 $^{b–d}$	42.7 $^{b–e}$	59.7 $^{a–c}$	17.1 $^{b–d}$	9.7 ^{bc}	2.30 ^{cd}	0.42 $^{b–d}$
RC236	38.0 ^{d}	38.3 ^{d}	42.2 $^{b–e}$	51.0 ^{cd}	14.7 ^{e}	8.3 ^{c}	2.04 ^{d}	0.34 ^{e}
TE142	48.5 ^{a}	44.8 ^{ab}	47.4 ^{a}	67.8 ^{a}	19.7 ^{a}	11.5 ^{a}	2.36 ^{bc}	0.50 ^{ab}
TE565	45.0 ^{ab}	43.9 $^{a–c}$	45.8 $^{a–c}$	65.2 ^{ab}	17.9 $^{a–c}$	10.9 ^{ab}	2.36 ^{bc}	0.45 $^{b–c}$
TE28	42.9 $^{a–d}$	40.0 ^{cd}	40.3 ^{de}	54.5 $^{b–d}$	14.6 ^{e}	8.1 ^{c}	2.07 ^{d}	0.34 ^{e}
RC521	44.1 $^{a–c}$	45.9 ^{a}	47.5 ^{a}	66.2 ^{a}	19.1 ^{ab}	11.6 ^{a}	2.72 ^{a}	0.55 ^{a}
RC43	44.1 $^{a–c}$	42.6 $^{a–d}$	43.2 $^{a–e}$	58.7 $^{a–d}$	16.3 $^{c–e}$	9.7 ^{bc}	2.15 ^{cd}	0.37 $^{c–e}$
RC66	46.4 ^{ab}	44.1 $^{a–c}$	46.8 ^{ab}	66.1 ^{a}	19.1 ^{ab}	11.4 ^{a}	2.34 ^{bc}	0.48 ^{ab}
RC32	43.5 $^{a–d}$	43.5 $^{a–c}$	42.0 $^{b–e}$	58.9 $^{a–d}$	16.1 $^{c–e}$	9.1 ^{c}	2.03 ^{d}	0.35 ^{de}
RC96	47.0 ^{a}	38.8 ^{d}	44.1 $^{a–d}$	62.9 ^{ab}	16.4 $^{c–e}$	9.6 ^{bc}	2.25 ^{cd}	0.39 $^{c–e}$
Average	43.5	42.2	44.1	60.5	17.0	10.0	2.29	0.42

Control: without the use of mineral fertilizers or bacterial inoculation. Using Duncan's multiple range test, values in columns followed by various letters were found to be substantially different ($p < 0.05$).

3.3. Oil Yield, Content and Chemoarray

Based on two-year findings, the maximum essential oil content and oil yield per oregano plant were observed after bacterial inoculation with RC613, RC521 and RC636, followed by TE142, RC213 and RC66. With the exception of RC42, RC125, RC39, RC234, TE28, RC43, RC32 and RC96, other bacterial inoculation significantly increased the essential oil ratio and oil yield in *O. onites*. Values ranged from 2.01 to 2.83% essential oil content and 0.31–0.55 mL oil yield per plant depending on the treatments. Carvacrol made up the majority of the oil's composition (66.13–71.79%), with thymol (14.47–16.89%), linalool (1.38–3.68%), borneol (1.43–2.36%), γ-terpinene (0.63–3.92%) and p-cymene (1.92–3.91%) following closely behind. While the highest concentration of *p*-cymene, γ-terpinene and linalool in the oil were determined in the control application, other components varied according to the bacterial inoculations. While all bacterial inoculations significantly increased borneol and β-bisabolene content compared to the control, terpinen-4-ol content increased with inoculation of strains RC125, TE28, RC42, RC213, RC236 and RC636. Of the strains used in this study, four PGPR (RC636, RC213, RC42 and TE142) significantly increased the β-caryophyllene content of oregano, and nine of them stimulated caryophyllene oxide content, except for RC42, RC213, RC39, RC236, RC43 and RC66. Thymol content increased with inoculation, except for strains RC613, RC636, RC32 and RC96, in a similar manner; carvacrol content increased with inoculation, except for strains RC42, RC236, TE565 and RC66. With the exception of strains RC613, RC636, RC32 and RC96, bacterial inoculation significantly increased thymol content in a similar manner and eleven of them significantly increased carvacrol content compared to the control, with the exception of RC42, RC236, RC565 and RC43. The highest thymol content was determined in TE565 inoculations, followed by RC236, RC42, RC43, RC66 and RC521. The highest carvacrol content was obtained from inoculations of RC96, followed by RC521, RC636, RC32, RC613 and RC66 (Table 3).

Table 3. Effect of PGPR on *main essential oil* compounds (%) of Turkish oregano.

Treatments	*p*-Cymene	γ-Terpinene	Linalool	Borneol	Terpinen-4-ol	Thymol	Carvacrol	β-Caryop-Hyllene	β-Bisabolene	Caryophyllene Oxide
Control	3.91 ^{a}	3.92 ^{a}	3.67 ^{a}	1.43 ^{e}	0.73 ^{d}	14.47 ^{c}	66.13 ^{e}	1.46 ^{cd}	0.54 ^{d}	0.20 ^{cd}
RC613	2.19 ^{cd}	0.69 ^{de}	2.03 ^{cd}	1.89 ^{d}	0.83 $^{b–d}$	15.40 ^{bc}	70.19 $^{a–c}$	1.51 $^{b–d}$	1.13 ^{c}	0.36 ^{ab}
RC636	2.42 $^{b–d}$	1.43 ^{b}	1.38 ^{d}	2.32 ^{ab}	1.01 $^{a–c}$	14.58 ^{c}	70.42 ^{ab}	1.70 ^{a}	1.37 ^{ab}	0.35 ^{ab}
RC213	1.92 ^{d}	1.04 $^{b–e}$	2.25 ^{cd}	2.34 ^{ab}	1.04 ^{ab}	15.75 ^{ab}	68.60 $^{b–d}$	1.69 ^{a}	1.42 ^{a}	0.21 ^{cd}
RC42	2.52 ^{bc}	1.04 $^{b–e}$	2.89 $^{a–c}$	2.34 ^{ab}	1.04 ^{ab}	16.74 ^{a}	67.33 ^{de}	1.69 ^{a}	1.42 ^{a}	0.22 ^{cd}
RC125	1.99 ^{d}	1.03 $^{b–e}$	2.45 $^{b–d}$	2.30 ^{ab}	1.09 ^{a}	16.13 ^{ab}	69.99 $^{a–c}$	1.39 ^{d}	1.32 $^{a–c}$	0.39 ^{a}
RC39	1.98 ^{d}	0.63 ^{e}	3.28 ^{ab}	1.95 ^{cd}	0.74 ^{d}	15.98 ^{ab}	68.64 $^{b–d}$	1.39 ^{d}	1.15 ^{c}	0.28 ^{bc}
RC236	2.76 ^{b}	0.96 $^{c–e}$	3.68 ^{a}	2.25 $^{a–c}$	0.99 $^{a–c}$	16.79 ^{a}	66.43 ^{e}	1.57 $^{a–c}$	1.33 $^{a–c}$	0.19 ^{d}
TE142	1.94 ^{d}	1.36 ^{bc}	2.05 ^{cd}	2.14 $^{a–d}$	0.88 $^{a–d}$	15.87 ^{ab}	68.77 $^{b–d}$	1.62 ^{ab}	1.21 ^{bc}	0.36 ^{ab}
TE565	2.04 ^{cd}	0.76 ^{de}	2.02 ^{cd}	2.31 ^{ab}	0.85 $^{b–d}$	16.89 ^{a}	68.05 $^{c–e}$	1.46 ^{cd}	1.19 ^{bc}	0.32 ^{ab}
TE28	2.12 ^{cd}	1.11 $^{b–d}$	2.18 ^{cd}	2.20 $^{a–c}$	1.09 ^{a}	15.80 ^{ab}	69.49 ^{bc}	1.40 ^{d}	1.26 $^{a–c}$	0.36 ^{ab}
RC521	2.12 ^{cd}	0.73 ^{de}	1.44 ^{d}	1.94 ^{cd}	0.84 $^{b–d}$	16.34 ^{ab}	70.47 ^{ab}	1.49 ^{cd}	1.17 ^{c}	0.35 ^{ab}
RC43	2.12 ^{cd}	0.74 ^{de}	3.67 ^{a}	1.95 ^{cd}	0.73 ^{d}	16.71 ^{a}	66.30 ^{e}	1.46 ^{cd}	1.21 ^{bc}	0.21 ^{cd}
RC66	1.97 ^{d}	0.75 ^{de}	2.18 ^{cd}	2.36 ^{a}	0.91 $^{a–d}$	16.56 ^{a}	70.16 $^{a–c}$	1.49 $^{b–d}$	1.14 ^{c}	0.19 ^{d}
RC32	2.39 $^{b–d}$	0.93 ^{de}	2.04 ^{cd}	2.04 $^{b–d}$	0.80 ^{cd}	15.30 ^{bc}	70.24 ^{ab}	1.52 $^{b–d}$	1.18 ^{c}	0.33 ^{ab}
RC96	2.13 ^{cd}	0.83 ^{de}	1.86 ^{cd}	2.03 $^{b–d}$	0.80 ^{cd}	14.48 ^{c}	72.19 ^{a}	1.52 $^{b–d}$	1.16 ^{c}	0.34 ^{ab}

Using Duncan's multiple range test, values in columns followed by various letters were found to be substantially different ($p < 0.05$).

3.4. Nutrient Uptake

Inoculation of RC521, RC66, RC613 and RC636 among the bacteria tested increased the macro- and micronutrient content of Turkish oregano leaves compared to the control. With the exception of PGPR strains RC125, RC39, RC236, TE28 and RC32, other bacterial inoculations significantly increased the N and P content in oregano. Inoculation with strains RC613, RC521, RC43, RC66, RC636 and TE565 increased the uptake of Ca and Mg by oregano, while RC613, RC521, RC213, RC636, TE142, TE565, RC43 and RC66 increased the content of K. Among the bacterial inoculants, the maximum S content in oregano was measured for strains RC636 and RC96. Inoculation with strains RC613, RC636, RC213, TE142, TE565, RC521, RC66 and RC96 increased Fe uptake by oregano plants, while only isolates RC613, RC636, RC521, RC43 and RC66 increased Cu and Mn content (Table 4). Five PGPR strains (RC42, RC125, RC39, RC236 and TE28) did not change the Zn content of oregano plants. Based on B and Mo content, the efficient strains were RC613, RC521, TE565, RC66, RC43 and RC636.

Table 4. Effect of plant growth-promoting rhizobacteria (PGPR) on macro- and micronutrient concentrations in *Origanum onites* leaves.

Treatments	(%) N	Macronutrient (g kg^{-1} DW)							Micronutrient (mg kg^{-1} DW)					
		P	K	Ca	Mg	S	Al	Na	Fe	Cu	Mn	Zn	B	Mo
Control	1.93 ^{d}	2.17 $_{e}$	31.1 $_{e}$	9.5 ^{ef}	2.5 ^{ef}	9.2 ^{de}	0.47 ^{ab}	1.08 ^{d}	601 ^{e}	16.5 ^{ef}	41.8 ^{f}	101 ^{f}	58.3 ^{e}	3.1 ^{e}
RC613	3.49 ^{ab}	2.94 $_{a}$	43.1 $_{a}$	12.3 ^{a}	3.4 ^{a}	22.4 ^{ab}	0.50 ^{ab}	1.72 ^{a}	712 $^{a–d}$	19.3 ^{a}	50.6 ^{a}	138 ^{b}	82.0 ^{a}	6.8 ^{a}
RC636	3.18 ^{ab}	2.66 $_{a–c}$	38.1 $_{a–d}$	11.6 $^{a–d}$	3.1 $^{a–c}$	27.1 ^{a}	0.56 ^{a}	1.48 $^{a–c}$	786 ^{a}	18.9 $^{a–d}$	47.8 $^{a–e}$	161 ^{a}	72.2 $^{a–d}$	5.2 $^{a–d}$
RC213	3.33 ^{ab}	2.91 $_{a}$	39.7 $_{a–c}$	10.3 $^{b–f}$	2.7 $^{c–f}$	11.3 $^{c–e}$	0.58 ^{a}	1.27 $^{b–d}$	726 $^{a–c}$	17.3 $^{a–f}$	43.7 $^{b–f}$	126 $^{b–d}$	62.9 ^{de}	5.0 $^{a–e}$
RC42	2.91 ^{bc}	2.67 $_{a–c}$	35.7 $_{b–e}$	10.6 $^{a–f}$	2.8 $^{b–f}$	18.4 $^{a–c}$	0.52 ^{ab}	1.32 $^{b–d}$	610 ^{e}	18.2 $^{a–f}$	43.8 $^{b–f}$	110 ^{ef}	66.3 $^{b–e}$	5.6 $^{a–d}$
RC125	2.12 ^{d}	2.20 $_{e}$	31.9 $_{de}$	9.4 ^{ef}	2.5 ^{ef}	11.0 $^{c–e}$	0.49 ^{ab}	1.23 ^{cd}	654 $^{c–e}$	16.9 $^{c–f}$	42.8 $^{c–f}$	106 ^{f}	63.8 ^{de}	3.9 ^{de}
RC39	2.06 ^{d}	2.19 $_{e}$	30.9 $_{e}$	9.0 ^{f}	2.4 ^{f}	10.8 $^{c–e}$	0.46 ^{ab}	1.24 ^{cd}	608 ^{e}	16.1 ^{f}	40.1 ^{f}	101 ^{f}	65.6 $^{c–e}$	4.0 $^{c–e}$
RC236	1.96 ^{d}	2.42 $_{c–e}$	31.4 $_{de}$	9.0 ^{f}	2.4 ^{f}	8.0 ^{e}	0.46 ^{ab}	1.27 $^{b–d}$	654 $^{c–e}$	16.1 ^{f}	42.4 $^{d–f}$	101 ^{f}	64.6 ^{de}	4.2 $^{c–e}$
TE142	3.04 ^{ab}	2.72 $_{ab}$	39.6 $_{a–c}$	9.7 $^{d–f}$	2.6 $^{d–f}$	8.1 ^{e}	0.53 ^{ab}	1.33 $^{b–d}$	784 ^{a}	17.4 $^{a–f}$	48.4 $^{a–c}$	132 $^{b–d}$	66.7 $^{b–e}$	5.2 $^{a–d}$
TE565	3.10 ^{ab}	2.66 $_{a–c}$	38.0 $_{a–d}$	11.5 $^{a–d}$	3.0 $^{a–d}$	17.6 $^{b–d}$	0.44 ^{ab}	1.52 $^{a–c}$	770 ^{ab}	17.1 $^{b–f}$	44.2 $^{b–f}$	124 ^{cd}	77.6 $^{a–c}$	5.9 $^{a–c}$
TE28	2.24 ^{cd}	2.42 $_{c–e}$	35.3 $_{b–e}$	10.0 $^{c–f}$	2.7 $^{c–f}$	9.4 ^{de}	0.45 ^{ab}	1.44 $^{a–c}$	601 ^{e}	16.9 $^{c–f}$	43.4 $^{b–f}$	108 ^{ef}	71.9 $^{a–d}$	5.0 $^{a–e}$
RC521	3.75 ^{a}	2.94 $_{a}$	41.6 $_{ab}$	12.0 ^{ab}	3.3 ^{ab}	21.1 ^{ab}	0.52 ^{ab}	1.61 ^{ab}	760 ^{ab}	19.1 ^{ab}	50.6 ^{a}	129 $^{b–d}$	78.2 ^{ab}	6.2 ^{ab}
RC43	2.91 ^{bc}	2.57 $_{b–d}$	38.1 $_{a–d}$	12.1 ^{ab}	3.2 $^{a–c}$	26.3 ^{ab}	0.52 ^{ab}	1.53 $^{a–c}$	679 $^{b–e}$	19.0 $^{a–c}$	49.3 ^{ab}	124 ^{cd}	73.8 $^{a–d}$	5.4 $^{a–d}$
RC66	3.39 ^{ab}	2.94 $_{a}$	39.3 $_{a–c}$	11.8 $^{a–c}$	3.1 $^{a–c}$	23.3 ^{ab}	0.51 ^{ab}	1.49 $^{a–c}$	768 ^{ab}	19.3 ^{a}	48.2 $^{a–d}$	120 ^{de}	75.1 $^{a–d}$	5.8 $^{a–d}$
RC32	2.23 ^{cd}	2.35 $_{de}$	34.0 $_{c–e}$	9.6 ^{ef}	2.6 $^{d–f}$	21.5 ^{ab}	0.42 ^{b}	1.41 $^{a–d}$	619 ^{de}	16.8 $^{d–f}$	42.2 ^{ef}	131 $^{b–d}$	67.7 $^{b–e}$	4.7 $^{b–e}$
RC96	3.32 ^{ab}	2.54 $_{b–d}$	35.9 $_{b–e}$	10.8 $^{a–f}$	2.9 $^{a–e}$	26.8 ^{a}	0.56 ^{ab}	1.35 $^{b–d}$	728 $^{a–c}$	18.5 $^{a–e}$	43.6 $^{b–f}$	136 ^{bc}	67.9 $^{b–e}$	4.4 $^{b–e}$

Using Duncan's multiple range test, values in a column that were followed by various letters were found to be substantially different ($p < 0.05$).

3.5. Biological Soil Properties

In terms of soil biological activity, inoculated PGPR increased dehydrogenase (DHA), urease, acid and alkaline phosphatase (ACP and ALP), microbial biomass carbon (MBC)

and nitrogen (MBN) in the rhizosphere soil compared to the uninoculated control. As with the efficiency of macro- and microelement uptake, mainly N, P and K, the highest IAA-producing, N_2-fixing and P-solubilizing isolates were determined as isolates 613 and 52, followed by isolates 565, 66 and 142 with respect to soil biological properties (microbial biomass, activities of urease, dehydrogenase, acid and alkaline phosphatase). These isolates, which were particularly effective based on urease, DH, ACP, ALP, MBC and MBN, showed significant effects on nutrient uptake, growth and yield of herbage and essential oil (Figure 1).

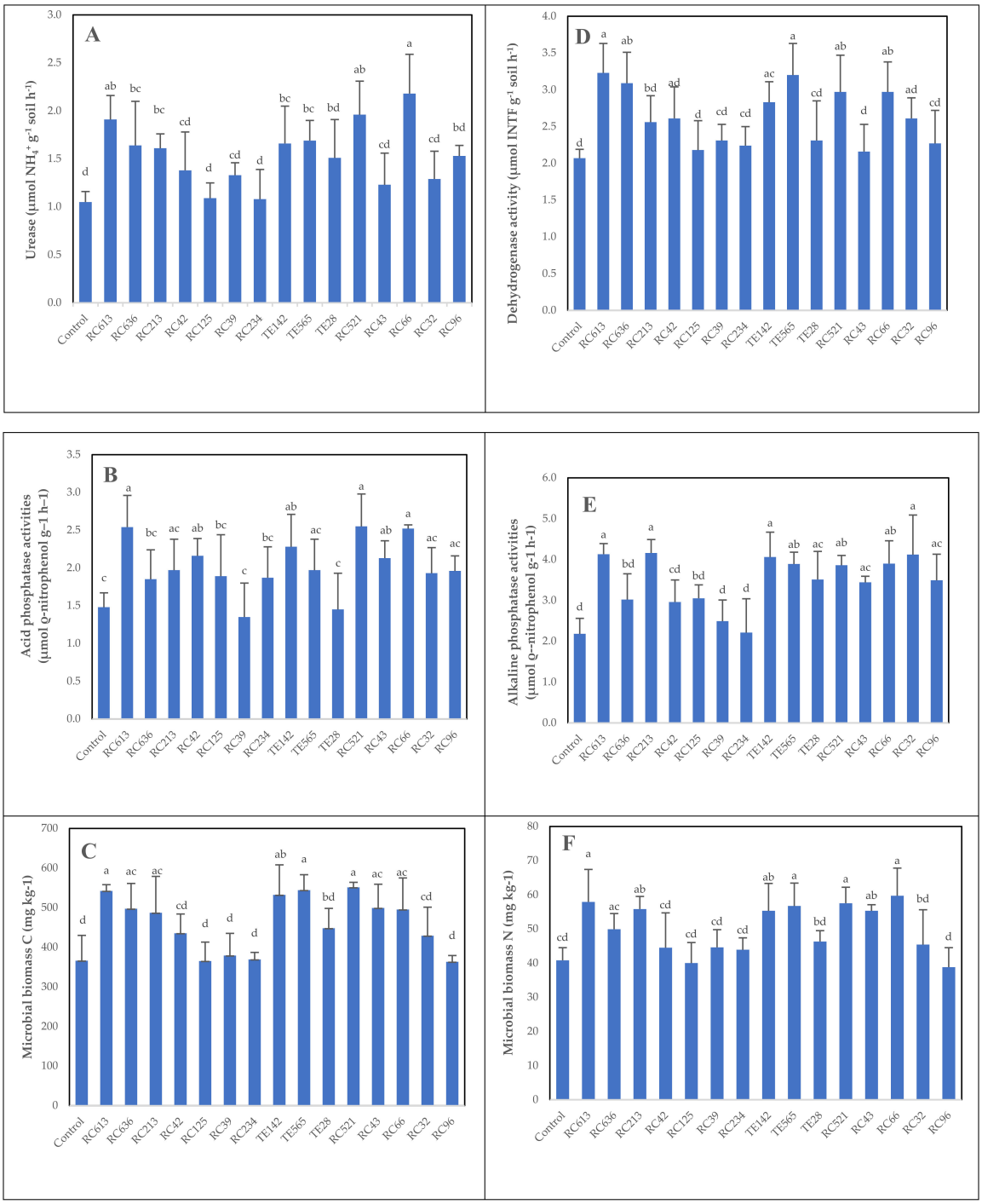

Figure 1. Urease (**A**), dehydrogenase (**B**), acid phosphatase (**C**), alkaline phosphatase (**D**) activity, microbial biomass C (**E**) and microbial biomass N (**F**). Different letters indicate significant difference ($p < 0.05$), bars denote mean + SE (n = 5).

3.6. Identification of Bacterial Isolates by 16S rRNA Gene Amplification and Sequencing and Phylogenetic Analyses

Custom sequencing of the 16S rDNA amplicons was performed at MedSanTek in Istanbul. The obtained nucleotide sequences are displayed in Table 5 and were given a bacterial taxonomic affiliation based on the sequences that most closely matched those in the NCBI database (http://www.ncbi.nlm.nih.gov/) accessed on 12 July 2023. One isolate was 100% similar to related standard-type strains: *Bacillus subtilis* (TE28). Isolates with 99% similarity were *Bacillus licheniformis* (RC636), *Bacillus megaterium* (RC42), *Bacillus pumilus* (RC125), *Bacillus pumilus* (RC39), *Bacillus subtilis* (TE565), *Bacillus velezensis* (RC521) and *Arthrobacter ramosus* (RC32). Isolates with 98% similarity were *Bacillus amyloliquefaciens* (RC613), *Bacillus simplex* (TE142) and *Paenibacillus barcinonensis* (RC43).

Table 5. Identification of bacterial isolates by 16S rRNA gene amplification.

Closest NCBI Match/Closest Type Strain	Max Score	Query Cover (%)	E-Value	Percent Identity (%)	Accession Lenght	Accession
Bacillus amyloliquefaciens RC613	878	97	0.0	98.60	4,034,955	CP122460.1
Bacillus licheniformis RC636	902	98	0.0	99.40	1513	KF242348.1
Bacillus megaterium RC213	857	96	0.0	97.98	1517	KC443085.1
Bacillus megaterium RC42	887	95	0.0	99.39	1475	FJ796434.2
Bacillus pumilus RC125	889	96	0.0	99.19	1505	OQ473589.1
Bacillus pumilus RC39	876	96	0.0	99.18	1513	EU855197.1
Bacillus simplex RC236	601	88	6×10^{-167}	91.11	1485	OL851775.1
Bacillus simplex TE142	835	93	0.0	98.13	870	MK484264.1
Bacillus subtilis TE565	1871	98	0.0	99.51	1488	KR061403.1
Bacillus subtilis TE28	881	46	0.0	100	1041	LN885092.1
Bacillus velezensis RC521	896	96	0.0	99.59	3,929,662	CP055160.1
Paenibacillus barcinonensis RC43	869	96	0.0	98.19	6,393,895	CP054614.1
Paenibacillus castaneae RC66	758	97	0.0	94.37	1483	KJ589459.1
Arthrobacter ramosus RC32	798	44	0.0	99.54	1663	LN890160.1
Stenotrophomonas maltophilia RC96	782	95	0.0	96.24	1500	EF690418.1

According to the cluster analysis that followed 16S rDNA-PCR, the fifteen different PGPR isolates were classified into two main clusters, and both had two subclusters (Figure 2). The dendrogram showed a clear separation. Eleven isolates formed the first cluster, which were *Bacillus simplex* (RC236), *Bacillus simplex* (TE142), *Bacillus megaterium* (RC213), *Bacillus megaterium* (RC42), *Bacillus licheniformis* (RC636), *Bacillus amyloliquefaciens* (RC613), *Bacillus velezensis* (RC521), *Bacillus pumilus* (RC125), *Bacillus pumilus* (RC39), *Paenibacillus barcinonensis* (RC43) and *Paenibacillus castaneae* (RC66). The second main cluster had two subclusters: the first subcluster consisted of *Stenotrophomonoas maltophilia* (RC96) and the second subcluster contained *Arthrobacter ramosus* (RC32), *Bacillus subtilis* (TE28) and *Bacillus subtilis* (TE565).

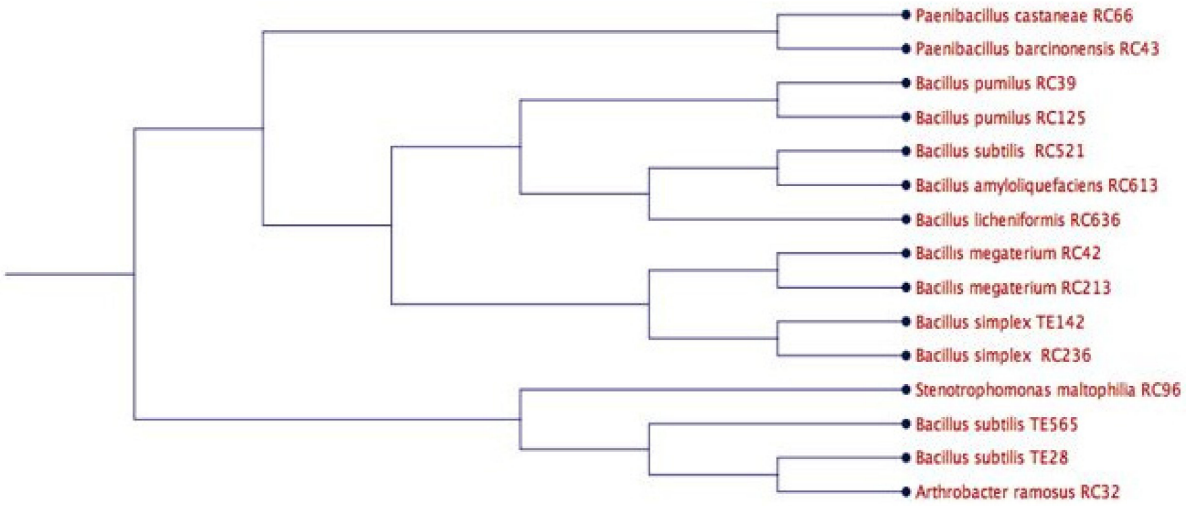

Figure 2. Phylogenetic tree of bacterial isolates from the acidic rhizospheric soil of native tea, grapevine and wild red raspberries.

4. Discussion

The two-year study showed that inoculations with multi-trait bacteria increased the dry herbage yield of *O. onites* by 8.1–32.2%, essential oil content by 0.5–40.1% and oil yield per plant by 5.9–71.9% compared to the control. The oil content ranged from 2.02 to 2.83%; the content of the main components thymol, carvacrol and borneol increased by 0.07–16.7%, 0.3–9.2% and 0.32.2–65.0%, respectively, as a result of bacterial inoculation compared to the control. This study shows that the application of PGPR not only improves growth parameters, green yield and essential oil yield in *O. onites* but also plays a significant role in increasing its bioactive compounds and plant nutrition. The effectiveness of the bacteria was variable and depended on the inoculant strain and the parameters evaluated. A number of studies conducted on various aromatic plant species have concluded that the application of PGPR is strategic for increasing growth, yield and production of secondary metabolites [54,55]. Essential oil, dry herb and leaf yield, chlorophyll content and biosynthesis of major essential oil components such as carvacrol, thymol, linalool and borneol of *O. onites* were significantly affected by inoculation with effective PGPR species such as *P. fluorescens*, *P. putida*, *B. subtilis* and *P. polymyxa* [35].

In previous studies, PGPR inoculations also had positive effects on essential oil yields in *Origanum syriacum* [56], *Mentha* × *piperita* [57], *Thymus kotschyanus* [58], *Pelargonium graveolens* [59], *Origanum vulgare* [60], *Melissa officinalis* [61], *Ocimum basilicum* and *Satureja hortensis* [62], *Lippia palmeri* [63], *Salvia sclarea* [64], *Salvia officinalis* [65], *Trachyspermum ammi* [66], *Mentha pulegium* [55] and *Cupressus arizonica* [54]. Essential oil content and composition are the most important quality criteria for oregano in all purposes [67]. In the context of our data, multicellular PGPR inoculation of oregano plants appear to be an important factor in modifying their aromatic profile and both the content and composition of the essential oil. Moreover, the increase in the total essential oil yield in response to PGPR inoculation was due to increased plant dry weight and oil content and/or terpene biosynthesis. Previous research has demonstrated that PGPR inoculation boosted shoot and root biomass, leaf area and stomatal density and caused noticeable qualitative and quantitative alterations in the number of monoterpenes [12,21]. The PGPR inoculation has many properties, such as nitrogen fixation, phosphate solubilization, indole-3-acetic acid (IAA) and siderophore production, and has significantly affected the total essential oil yield, chemical composition and biosynthesis of the main essential oil components of medicinal and aromatic plants [68]. However, there has not been much investigation into how PGPR inoculation affects plant development or the creation of secondary metabolites in significant aromatic plants.

Effective PGPR species with multiple traits, such as RC613, RC521, RC636, TE142, RC213, RC66 and TE565, improved oregano nutrition in N, P, K, Ca, Mg, S, Fe, Cu, Mn, Zn, B and Mo, and thus stimulated plant growth and the quantity and quality of the essential oils. The use of particularly effective strains makes it possible to grow Turkish oregano without the use of chemical fertilizers, without loss of yield and quality. The beneficial role of these PGPRs, which can be attributed to IAA production, N_2 fixation, P solubilization, ACC deaminase activity and even other valuable PGPR traits that stimulate the plant, is indicated by the positive effects of these strains on the growth, yield, oil content and composition of oregano plants. Utilizing the variety and high rate of carbon sources and using efficient strains to metabolize root secretions might give a competitive advantage and be crucial for plant and soil adaptability. It was previously claimed that the strains investigated used various carbon sources differently, with the PGPR strain, one of the best fertilizer strains, favoring a variety of carbon sources [7,36,47]. Similar findings from earlier studies have demonstrated that nutrients including N, P, K, S, Ca, Mg and microelements can also change the yield and composition of essential oils [13]. In addition, phosphorus is an important source of essential oil synthesis by plants, so increased P uptake by PGPR can stimulate essential oil synthesis in medicinal plants [69]. Oregano's essential oil yield dramatically increased after Ca and Mg were applied topically [70], and minerals can also improve the quantity and quality of oil produced by medicinal plants [71]. Moreover, some researchers have shown that the increase in growth characteristics of medicinal plants may be due to the fact that plants inoculated with PGPR were able to absorb nutrients from the solution at a faster rate than uninoculated plants, resulting in the accumulation of more N, P and K in the leaves [72]. The study showed that PGPR screening with multiple traits can be very effective in improving the growth and nutrient uptake of aromatic oregano. Increased uptake of nutrients necessary for chlorophyll production may potentially contribute to the favorable effect of PGPR on boosting total chlorophyll concentration. Çakmakçı et al. [47] discovered that the use of PGPR improved the amount of chlorophyll in the leaves and the amount of minerals that tea plants absorbed. The findings of Vafadar et al. [73] and Cappellari et al. [12] that the chlorophyll content of inoculated plants can be represented in increased rates of carbon absorption and photosynthetic activity are supported by comparable data. A higher rate of photosynthesis was achieved by infected plants with more chlorophyll, which enhanced biomass and plant nutrition.

Increased soil microbial biomass and enzyme activity following PGPR inoculation demonstrated higher soil biological quality, which is associated with greater plant growth. Soil microbial biomass and soil enzymes were activated and increased to varying degrees by PGPR inoculation. Increased microbial activity is a certain sign of better soil quality and will have an impact on crucial soil functions for crop growth, such as carbon and nitrogen cycling. In addition, increases in soil enzyme activity can result from physical and chemical changes in the soil, so there is a direct relationship between microbial biomass expression and soil enzyme activity [74]. Enzymatic activity and soil microbial biomass are regarded as reliable indices of soil quality and microbial activity [75]. An increase in dehydrogenase activity in the rhizosphere of inoculated plants is an indicator of increased microbial activity [31]. In soil ecosystems, phosphatase activity is often predicted as an indicator of soil potential for organic phosphorus mineralization and biological activity [76]. The increase in phosphatase activity observed after PGPR application in the present experiment may improve soil nutrient P status and correlate with plant growth. Bowles et al. [77] showed that the activity of C-cycling enzymes increased with the availability of inorganic N, while the activity of N-cycling enzymes increased with the availability of C. The observed increase in the activities of urease, DHA, ACP and ALP may be related to the increase in the microbial population in the rhizosphere as a result of inoculation. The rhizosphere microbial community influences soil quality through its involvement in the biogeochemical and nutrient cycle and long-term soil equilibrium and has a major impact on crop production [78]. The secretion of phosphatases by phosphate-solubilizing bacteria (PSB) plays a key role in P mineralization and is a common way to facilitate the conversion of

insoluble forms of P to plant-available forms, thereby increasing P uptake by plants. The importance of microbial properties in soil implies significant changes in soil quality related to the balance of nutrient cycling and plant development, and phosphatases can be stimulated when soil phosphate levels are low [79]. Taktek et al. [80] suggest that the studied PSBs selected for their high solubilization of inorganic phosphate, through the production of organic acids, may also play an important role in the mineralization of organic phosphate in the soil.

5. Conclusions

For herbaceous plants, microbial methods present an alluring alternative to chemical fertilizers, but little is known about their potential benefits and the capacity of PGPR to boost secondary plant metabolites. There is currently little information on how PGPR inoculation affects aromatic and medicinal plants [25]. Egamberdieva et al. [71] indicated that further research is needed to explore possible mechanisms by which bacteria increase phytochemical in medicinal plants at the tissue, cellular or molecular level. Specifically, IAA-producing, N_2-fixing, P-solubilizing and ACC deaminase-containing strains of the bacteria studied—*B. simplex* TE142, *B. velezensis* RC521, *P. castaneae* RC66, *B. megaterium* RC213, *B. amyloliquefaciens* RC613, *B. subtilis* TE565 and *B. licheniformis* RC636—increased macro- and micronutrient absorption, chlorophyll and essential oil content and oregano oil yield; they also encouraged overall plant development, including plant height, canopy diameter, fresh and dry herbage and leaf yield. The increase in enzyme activity and microbial biomass suggests that the proliferation and activity of inoculated beneficial and known bacteria in the rhizosphere may also have contributed to improved plant growth and soil biological quality. In addition, they had potential as biofertilizers in conventional, improved and sustainable organic cultivation of Turkish oregano. In this study, the 16S rRNA gene of bacterial isolates from acidic rhizospheric soil of native tea, grapevine and wild red raspberry was sequenced for the first time.

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