THE USE OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)'S EFFECT ON ESSENTIAL OIL RATE, ESSENTIAL OIL CONTENT, SOME MORPHOLOGICAL PARAMETERS AND NUTRIENT UPTAKE OF TURKISH OREGANO

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Abstract. The objective of this study was to evaluate the effects of mineral fertilizer (NP) and thirty-five N_2-fixing and/or P-solubilizing and different carbon sources utilizing-bacterial strains (7 isolates each of *Bacillıs megaterium, Bacillus subtilis*, *Paenibacillus polymyxa*, *Pseudomonas putida* and *Pseudomonas fluorescens*) isolated from the acidic rhizospheric soil of native tea, grapevine and wild red raspberries, as bio-fertilizers on growth, yield and quality characteristics of the plant, on chlorophyll content, on macro- and micronutrient concentrations, on essential oil content and on major component of the essential of Turkish oregano (*Origanum onites* L.). The isolates were identified based on whole-cell fatty acid methyl ester (FAMEs) analysis using the MIDI system and BIOLOG assays. The study contains both applications NP-fertilizer and a control application without inoculation of bacteria and fertilizer application. This study was carried out in Greenhouse at Atatürk University, Faculty of Agriculture and Department of Agronomy in 2011-2012 growing season. As an average of years the treatments showed that the plant has 35.3-48.5 cm of length, 34.29-48.30 cm of canopy diameter, 36.56-47.19 SPAD units of chlorophyll concentration, 41.88-74.64 g/plant of green herb yield, 11.52-15.52 g/plant of drug herb yield, 6.53-12.18 g/plant of drug leaf yield and 1.85-2.78% of essential oil content rate. Increasing carbon source utilization rates significantly increased plant height, canopy diameter, green herb, drug herb and drug leaf yield. The main components of the oil were carvacrol (58.19-70.08%), followed by thymol (17.85-26.27%), linalool (1.64-8.13%), borneol (1.36-2.39%) and p-cymene (0.37-3.21%) which were the most abundant components. In addition, with PGPR inoculation, macronutrient concentrations (N, P and K,) of oregano leaves increased, main component of the essential oil also changed. In terms of growth, yield and quality criteria of Turkish oregano, in particular, the most effective N_2-fixing and P-solubilizing strains *P. fluorescens* (8/2, 58/3, 9/7, 53/6 and 51/2), *B. subtilis* (52/1, 6/3 and R 3/3), *B. megaterium* (21/3, K5E and 35/6), *P. polymyxa* (R2/2), and *P. putida* (55/2, 3/10 and 53/5) may be used instead of mineral fertilizer as bio-fertilizers in sustainable organic oregano cultivation.
Keywords: *medicinal and aromatic plants, Origanum onites, carvacrol, main component, nutrient uptake*

Introduction

Turkish oregano (*Origanum onites* L.), distributed in Western and Southern Anatolia is widely used as a spice and herbal tea in Turkey. In Turkey, *O. onites* is on the top of the list in case of its commercial *origanum* species which has a dominant position in the worldwide trade (Başer, 2002). Dried oregano is obtained both from wild populations in nature and from cultivated plants, its vegetative parts and biochemical essence are quite used in the food and spice industrial area, and also as a condiment herb for flavouring

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fish, soups, salads, olives, chicken, meat, vegetables, salad dressing, and wine (Tonk et al., 2010). As food preservation, natural antimicrobial and antioxidant, *O. onites* becoming increasingly popular (Stefanakis et al., 2013). Carvacrol, thymol, p-cymene, gamma-terpinene, borneol, linalool, and alpha-terpinene is main component of essential oil of *O. onites* (Bokov et al., 2015). Carvacrol is main component of essential oils of this species (Avcı and Bayram, 2013), which is an oxygenated monoterpene with multiple pharmacological actions (Baser, 2008), and an important impact compound of oregano aroma (Bansleben et al., 2009). It is also known for its antibacterial, antifungal, antioxidant, insecticidal, anti-angiogenic and anticarcinogenic activities, antispasmodic effects, lipid peroxidase inhibition, radical scavenging effect and cardiac depressant activity (Kotan et al., 2014).

In recent years, oregano cultivation has expanded rapidly because of its conservation of natural resources and production with high standard and quality. Consumed without further processing in medicinal and aromatic plant species is important because its components have not synthetic compounds in the harvested crop. Undoubtedly, excessive use of chemical fertilizer has adverse effects on plant and soil health. In view of environmental pollution and high costs of the production due to excessive use of fertilizers; plant growth- promoting rhizobacteria (PGPR) may be used in sustainable agricultural production (Cakmakçi et al., 2006). Plant growth with excrete of vitamins and phytohormones, decline of plant ethylene level, resistance to stress, contribution to food intake, solubilisation of inorganic phosphate, fixing N_2, and mineralization of organic phosphate can provide with applications of PGPR. As a new concept and alternative strategy emerging in plant growth and yield increase, PGPR can provide increase in agricultural productivity, decline in product cost and environmental protection against pollution. It is evident that rhizobacteria could possibly serve as eco-friendly, safe and sustainable alternative to the harmful synthetic fertilizers used for the nutrient management and sustainable production in medicinal and aromatic crops.

Knowledge of about the monoterpene accumulation and biosynthesis of secondary metabolites can provide new procedure for medical plant cultivation and other agricultural application without chemical input (Cappellari et al., 2015). Similarly to bio-fertilization, optimal and balanced mineral fertilization of aromatic plants, adjusted to their nutritional requirements and growing conditions, is an important cultivation factor determining the quantity and quality of essential oil (Nurzynska-Wierdak, 2013). Bacteria and plant related studies are usually focused on cereals and grassy plants and studies on medical plants are very few. In medicinal and aromatic plants, experiments with PGPR indicated yield and essential oil increases in Italian oregano and *Origanum majorana* L. (Banchio et al., 2008, 2010), rosemary (Leithy et al., 2006), *Pelargonium graveolens* (Mishra et al., 2010), sweet fennel (Rezvani Moghaddam et al., 2011), dill plant (Hellal et al., 2011), common basil (Ordookhani et al., 2011), coriander (Hassan et al., 2012), Mexican marigold (Cappellari et al., 2013), *Thymus daenensis* (Bahadori et al., 2013), summer savoury (Farahani et al., 2015). Although some experiments have addressed the role of PGPR added in medicinal plants (Santoro et al., 2011), the impact of PGPR on production of secondary metabolites is poorly known (Banchio et al., 2008). In general, physiological and morphological property of medicinal plants connected with rhizobacteria leftovers restricted and piecemeal (Cappellari et al., 2015).

In particular, the possibilities of medicinal and aromatic plants development, yield, volatile oil content and components to be increased or replaced have not been extensively investigated using multi-traits bacteria. However, there is no study on

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agronomic factors such as application of bio-fertilizers as well as plant growth promoting rhizobacteria on biological soil properties, yield and essential oils of Oregano. In addition, there is a trend towards biological fertilizer applications and sustainable agricultural systems in the production of medical plants due to environmental impacts, which are caused by over application of chemical fertilizers, energies and expenses. Therefore, a study was conducted in order to investigate the effect of plant-associated beneficial multi-traits 35 bacteria on growth, yield and content and composition of essential oils of Turkish oregano.

Materials and methods

Bacterial strains

In this study, thirty-five different isolates of PGPR were selected from stock of 460 rizobacterial isolates obtained from the native grapevine, wild red raspberries and tea rhizosphere Selection criteria of these bacteria was made according to whether 1-aminocyclopropane-1-carboxylate (ACC) deaminase-containing, IAA-producing, N_2-fixing, P-solubilizing, and different carbon sources using ability. In the study, gram-positive *Bacillus megaterium*, *Bacillus subtilis* and *Paenibacillus polymyxa*, gram-negative *P. fluorescens* and *P. putida* species were used for at least seven different isolates from each species to be used in *Origanum onites*. The isolates were identified based on whole-cell fatty acid methyl ester (FAMEs) analysis using the Sherlock Microbial Identification System (Version 4.5) and Biolog microplate assays (Biolog Inc., Hayward, CA, USA). Also, characterized by using BIOLOG GN2 and GP2 MicroPlates, were used to determine the ability of bacterial strains to utilize 95 different carbon sources (Çakmakçı et al., 2010).

Acetylene reduction assay and phosphate solubilisation

Nitrogen fixation of the isolates was determined in a nitrogen free medium by the acetylene reduction assay (Hardy et al., 1968). Using a Hewlett Packard gas chromatograph, Ethylene production was measured (Model 6890, USA). Entire of the bacteria were tried in threefold for their phosphate solvent talent in sucrose-tricalcium phosphate agar media (Pikovskaya, 1948) by added 1 ml of 6-day-old culture (density 4×10^9) in 250-ml Erlenmeyer flasks containing 500-μg ml^{-1} of P as rock phosphate at 29-31°C (Çakmakçı et al., 2010).

Greenhouse experiment and growth conditions

This study was carried out in the Department of Agronomy at Atatürk University under greenhouse conditions in Erzurum, Turkey, in 2011-2012 growing season under two trial sets. Flowerpot were sterilized by 20% NaClO and replete with a sandy clay-loam which is virgin field soil with an organic matter content of 3.2 and 0.17% nitrogen, an available Olsen-P and changeable potassium calcium and magnesium content of 16.2, 448, 3420, and 472 mg kg^{-1}, respectively. Iron, manganese, zinc and copper contents were 5.9, 9.6, 1.2, and 1.8 ppm. Two set of experiment was conducted with the same treatments. The experiment was conducted in a completely randomized design with five replicates (each having five pots), having 37 treatments as 35 high N_2-fixing and/or P-solubilizing and different carbon sources utilizing-bacteria, NP fertilizer (200 mg N $plant^{-1}$ + 200 mg P $plant^{-1}$) applications as well as a control

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treatment without inoculation and any fertilizer application. In our study, bacteria were developed in fifty percent tryptic soy broth. Bacteria in Rotary shaker was stayed three days (120 rpm; 25°C). Control applications were stayed 5 ml of diluted SPB without bacteria. Oregano seeds were germinated in a seed trays containing garden soil/peat/sandy (2:1:1 [v/v]). Our plants were grown conditions which 16 h day, and 8 h night conditions, at 19-29°C with about sixty percent humidity in greenhouse. Seedlings were removed from seed trays after one month. Uniform oregano seedlings were dipped and kept in bacteria solution for an hour, later three uniform 30-day-old inoculated seedlings were transferred into each pots containing virgin garden soil, and seedlings were thinned to one after two weeks. In all plants, irrigation was done every 4 days, keeping 70% water holding capacity. Weed control was done by hand when required. In the second year of bacterial inoculation, 5 ml bacterial suspension was injected into rhizosphere of each oregano plant. About three months after planting, the oregano plants were harvested twice approximately in mid-July and September in each year.

Essential oil extraction and GC–MS analysis

Plants were harvested at full flowering stage, were cut 5-6 cm above ground level and weighed to determine fresh herbage yield. Then, harvested the oregano herb was dried under natural conditions, in a dry, airy and shaded place. Essential oil of plants was obtained with using Clevenger apparatus. Dry herb was made hidrodistilation for 3 h v/w and essential oil of these plant was obtained. The essential oil' analyses was performed (Kordali et al., 2008). Oil components and RI (relative retention times) values detected according to Adams (1997).

Plant analysis

Before 2 g of leaf samples was grinded to be 1 mm, oven-dried at 68°C for 48 h. For determine total N the oregano leaves were used Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany). After determined P and K with an inductively Coupled Plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Perkin-Elmer, Waltham MA, USA), extraction was made. For measuring chlorophyll contents of leaves which top fourth and fifth was used chlorophyll meter (SPAD-502, Minolta, Japan) which is used to measure leaf greenness of the plants. Firstly, it was measured at four locations on each leaf for each plant. After, this numbers were received for average.

Statistical analysis

The experiment was performed in a completely randomized design with five replicates. Each replicate consisted of five plants. The experiment was repeated twice. Our data were analyzed by SPSS 20 and the means were separated according to Duncan's Multiple Range Test.

Results

Plant growth parameters

Different rhizobacteria had variable effects (both negative and positive) on the measured plant growth parameters, the essential oil content and yield of *Origanum onites* (*Table 1*). In terms of fresh herbage yield in oregano, all application except *B.*

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megaterium 44, *B. megaterium* R2C, *B. subtilis* 36/10, *P. putida* 48/2, *P. fluorescens* 22B and *P. fluorescens* 8/6 were detected to be higher according to control. The maximum fresh herbage yield in oregano was obtained with the mineral NP fertilizer application, followed by *P. fluorescens* 8/2 inoculation. The maximum dry herbage yield in oregano was found after *P. polymyxa* R2/2 and *P. fluorescens* 58/3 inoculation, followed by *B. megaterium* 21/3, *B. subtilis* 52/1, and *P. fluorescens* 8/2, whereas the highest levels of dry leaf yield per plants were determined in treatments with *P. fluorescens* 58/3, followed by *B. subtilis* 52/1, *B. subtilis* 6/3, and *P. polymyxa* R2/2 (*Table 1*).

Table 1. *The effect of mineral fertilizer application and bacterial inoculations on plant growth parameter in Turkish oregano*

Treatments	Fresh herb yield (g/plant)*	Dry herb yield (g/plant)	Dry leaf yield (g/plant)	Plant height (cm)	Canopy diameter (cm)	Chlorophyll content (SPAD)	Essential oil yield (%)
Control	48.3 n-f	13.8 n-p	7.54 h-j	36.85 lm	34.92 op	37.38 kl	1.94 l-o
Mineral fertilizer (NP)	70.3 a	17.7 b-d	10.81 a-c	43.40 c-h	46.01 a	44.24 a-f	2.64 a-b
Bacillus megaterium K5E	59.4 g-k	16.9 c-g	9.78 e-h	39.90 h-l	38.62 ı-n	40.77 c-j	2.58 a-c
Bacillus megaterium 22D	52.1 m-o	16.0 f-ı	9.20 h-j	36.90 lm	38.38 ı-o	40.31 e-l	2.55 b-d
Bacillus megaterium 66/5	42.7 s	12.5 p-s	7.10 p-s	36.80 m	34.83 n-p	37.16 l	2.33e-h
Bacillus megaterium 21/3	65.7 b-d	18.5 ab	10.63 b-d	48.03 a	38.13 j-p	43.44 a-g	2.30 f-ı
Bacillus megaterium 44	46.0 p-s	14.5 j-n	8.27 l-n	38.20 ı-m	34.78 op	42.66 a-ı	1.86 o
Bacillus megaterium 35/6	66.8 b-c	17.1 c-f	9.81 e-h	40.57 g-l	44.00 a-f	44.42 a-e	2.10 ı-n
Bacillus megaterium R2C	43.0 s	12.0 rs	6.53 s	36.71 m	34.88 p	39.09 h-l	1.85 o
Bacillus subtilis R 3/3	62.6 d-g	17.7 a-d	10.07 d-g	40.38 g-l	39.89 g-k	45.02 a-c	1.98 l-o
Bacillus subtilis 2/8	48.9 n-p	12.9 o-r	7.07 p-s	41.05 f-k	36.96 k-p	37.18 kl	1.86 o
Bacillus subtilis 39/3	52.5 k-m	17.1 c-f	10.14 d-f	41.95 d-j	44.70 a-d	43.54 a-g	2.27 g-j
Bacillus subtilis 52/1	64.3 c-f	18.3 ab	11.23 ab	42.08 d-ı	45.95 ab	45.41 ab	2.71 a-b
Bacillus subtilis 6/3	61.9 d-h	17.8 a-d	11.22 ab	41.97 d-j	44.54 a-e	43.84 a-g	2.78 a
Bacillus subtilis 36/10	42.5 s	12.5 p-s	6.93 rs	36.85lm	34.79 op	38.83 ı-l	2.36 d-h
Bacillus subtilis 20D	57.9 h-l	15.9 f-ı	9.38 g-ı	43.28 c-h	38.46 ı-o	42.74 a-ı	2.53 b-e
Paenibacillus polymyxa R2/2	64.7 c-f	18.6 a	11.18 ab	45.28a-e	42.77 a-h	44.95 a-c	2.27 g-j
Paenibacillus polymyxa 2/.5	55.0 k-m	17.1 c-f	9.78 e-h	41.22 f-k	41.90 c-j	42.83 a-ı	2.01 l-o
Paenibacillus polymyxa 11/4	53.8 l-n	15.7 g-j	8.99 ı-k	41.80 d-j	40.01 g-k	44.65 a-d	1.93 m-o
Paenibacillus polymyxa 43/5	56.5 j-m	15.9 g-ı	9.44 g-ı	43.25 c-h	41.37 d-j	40.26 e-l	2.17g-l
Paenibacillus polymyxa 24/3	56.0 k-m	13.6 n-p	7.69 n-p	37.87 j-m	40.75 e-k	43.88 a-g	2.06 j-o
Paenibacillus polymyxa 31/5	56.1 k-m	16.5 e-h	9.54 f-ı	41.10 f-k	42.25 b-ı	41.77 b-ı	2.14 h-m
Paenibacillus polymyxa 56/4	57.6 ı-l	15.0 ı-l	8.48 k-m	40.31 g-l	40.50 f-k	40.92 c-j	2.09 ı-n
Pseudomonas putida 20c	55.0 k-m	15.3 h-k	8.50 k-m	41.46 e-k	40.41 f-k	40.02 f-l	1.92 n-o
Pseudomonas putida 3/10	61.2 e-ı	15.7 g-j	9.38 g-ı	44.77 a-f	46.01 a	42.68 a-ı	2.26 g-j
Pseudomonas putida 27/3	54.7 l-m	15.0 ı-l	8.26 l-n	45.90 a-d	39.54 g-l	42.56 a-ı	2.04 k-o
Pseudomonas putida 48/2	44.8 r-s	14.1 l-n	7.84 m-o	38.39 ı-m	36.00 l-p	40.23 e-l	2.24 g-k
Pseudomonas putida 53/5	52.4 m-o	14.2 k-n	8.52 j-l	41.30 e-k	42.74 a-h	43.42 a-g	2.58 a-c
Pseudomonas putida 55/2	60.7 f-j	17.4 b-e	10.54 c-e	44.70 b-g	43.13 a-g	43.93 a-g	2.59a-c
Pseudomonas putida 62/5	52.5 mn	14.9 ı-m	8.36 k-m	37.65k-m	38.45 ı-o	43.13 a-h	2.25 g-k
Pseudomonas fluorescens 22B	46.1 pr	11.9 s	6.57 s	39.06ı-m	35.14 m-o	39.76 g-l	2.17 g-l
Pseudomonas fluorescens 8/2	69.8 ab	18.0 a-c	10.85 a-c	47.61 ab	45.94 a-c	46.42 a	2.38c-g
Pseudomonas fluorescens 8/6	48.1 o-r	14.3 k-n	8.59 j-l	36.74 m	35.86 l-p	42.65 a-ı	2.29 f-ı
Pseudomonas fluorescens 9/7	57.0 ı-l	15.7 g-j	9.58 f-ı	39.87 h-l	40.41 f-k	43.44 a-g	2.52 b-e
Pseudomonas fluorescens 53/6	59.7 g-k	16.6 d-g	10.30 c-e	46.87 a-c	41.26 d-j	43.12 a-h	2.62 ab
Pseudomonas fluorescens 51/2	56.1 k-m	15.8 g-ı	9.43 g-ı	45.83 a-d	38.97 h-m	40.65 d-k	2.50 b-f
Pseudomonas fluorescens 58/3	65. 0 c-f	18.6 a	11.25 a	43.62 c-h	40.92 d-k	44.36 a-e	2.59 a-c
Mean	**55.9**	**15.7**	**9.15**	**41.34**	**40.11**	**42.20**	**2.27**

*Values followed by different lower-case letters in a column were significantly different ($P \leq 0.05$) using Duncan's multiple range test; Values are the averages from the two experiments with five replications

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In terms of dry herbage yield, 31 applications were found to be effective according to control. In terms of plant height, 5 applications were detected as taller according to mineral fertilizer. The maximum plant height in oregano was found after *B. megaterium* 21/3 (48.3 cm) inoculation, followed by *P. fluorescens* 8/2, whereas the highest levels of canopy diameter per plants were measured in treatments with mineral NP fertilizer and *P. putida* 3/10 (46.01 cm), followed by *B. subtilis* 52/1 and *P. fluorescens* 8/2. In terms of chlorophyll content (SPAD value), applications except *B. megaterium* 66/5, *B. megaterium* R2C and *B. subtilis* 36/10 were found as effective according to control. The maximum chlorophyll content measured as *P. fluorescens* 8/2 (*Table 1*). Control plants gave the lowest water content of air drying (71.4%) while NP application gave the highest water content (74.8%), bacterial inoculations having values generally higher than control but lower than NP applied plants.

Oil yield, content and chemoarray

According to two years results, in terms of essential oil yield, only 4 applications were found as less than control. All of *P. fluorescens* and *P. putida* applications were increased yield of essential oil when compared to control. The maximum essential oil yield in oregano was found as *B. subtilis* 6/3 inoculation, followed by *B. subtilis* 52/1, *P. fluorescens* 53/6, and mineral fertilizer (NP). Essential oil yields ranged from 1.85 to 2.78%. The main components of the oil were carvacrol (58.19-70.08%), followed by thymol (17.85-26.27%), linalool (1.64-8.13%), borneol (1.38-2.39%), and p-cymene (0.37-3.21%). Highest p-cymene (3.21%) in oil was determined in control application. Other components were changed with bacteria inoculations. The maximum linalool content of oregano oil was obtained with the inoculation of *P. polymyxa* 2/5 (8.13%), followed *P. polymyxa* 11/4 and *P. polymyxa* R2/2 (*Table 2*). Of the 37 treatments, the maximum borneol and thymol components of oregano oil were seen in *P. fluorescens* 51/2, *P. fluorescens* 9/7, *P. fluorescens* 53/6 and *P. fluorescens* 58/3 inoculations. Borneol and thymol components in all four applications were detected respectively as % 2.39 and 26.27. The maximum carvacrol content essential oil in oregano was obtained with the *P. putida* 20C and *P. putida* 3/10 inoculations, followed by *P. putida* 27/3 and *B. subtilis* 52/1 (*Table 2*).

Nutrient uptake

All applications were found to be higher according to control in leaf nitrogen content. Twenty-four, ten and eleven of the 35 PGPR strains test selectively increased nitrogen, phosphorus and potassium content leaf, and most of them improved the plant growth parameters and yield in oregano plants. In terms of leaf phosphorus and potassium content, all applications except *B. magaterium* 22D inoculation was detected more effective than control. While the highest leaf nitrogen contents were determined on *P. putida* 3/10 inoculated oregano leaves, followed by *P. putida* 53/5, 55/2 and 48/2; *P. polymyxa* 43/5 and *P. polymyxa* R2/2 inoculations (*Table 3*). The leaf nitrogen content of inoculated *P. putida* 3/10 was higher (16.3%) than mineral fertilization. In terms of leaf phosphorus content, *P. polymyxa* 43/5, *P. polymyxa* 31/5, *P. polymyxa* R2/2, *B. subtilis* 52/1 and *P. polymyxa* 24/3 applications were found effective. As in terms of leaf phosphorus content, *P. polymyxa* 43/5, *P. polymyxa* 31/5, *P. polymyxa* R2/2, *B. subtilis* 52/1 and *P. polymyxa* 24/3 applications were found effective, in terms of leaf potassium content, *B. subtilis* 6/3, *B. subtilis* 52/1 and *P. polymyxa* R2/2 applications were detected as effective (*Table 3*).

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Table 2. *The effect of mineral fertilizer application and bacterial inoculations on basic essential oil components in Turkish oregano*

Treatments	p-cymene* (%)	Linalool (%)	Borneol (%)	Thymol (%)	Carvacrol (%)
RI[a]	1020	1095	1165	1289	1298
RT	14:66	18:32	22:05	27:75	29:39
Control	3.21 a	6.63 b	1.42 f	18.63 f	61.01h-j
Mineral fertilizer (NP)	1.21 bc	2.43 h-j	1.38 f	20.98 b-f	66.96 a-f
Bacillus megaterium K5E	0.54 ef	4.55 d-e	2.29 a-d	25.56 a	61.27 h-j
Bacillus megaterium 22D	0.67 ef	3.11 f-i	2.14 a-e	24.10 ab	63.86 e-i
Bacillus megaterium 66/5	0.39 f	3.26 f-h	2.34 ab	24.91 ab	63.37 f-i
Bacillus megaterium 21/3	0.73 c-f	5.12 cd	2.33 a-c	24.84 ab	60.68 ij
Bacillus megaterium 44	0.82 b-f	4.70 c-e	2.28 a-d	23.17 a-c	62.47 g-j
Bacillus megaterium 35/6	0.75 c-f	5.12 cd	2.33 a-c	24.68 ab	60.68 ij
Bacillus megaterium R2C	0.73 c-f	5.79 bc	2.22 a-e	24.43 ab	60.32 ij
Bacillus subtilis R 3/3	0.75 c-f	2.30 h-j	2.30 a-d	21.26 b-f	66.57 a-g
Bacillus subtilis 2/8	0.75 c-f	2.10 h-j	2.30 a-d	21.26 b-f	66.97 a-f
Bacillus subtilis 39/3	1.03 b-e	2.41 h-j	1.97 de	20.92 b-f	66.71 a-g
Bacillus subtilis 52/1	0.39 f	1.64 j	2.01 b-e	21.39 b-f	68.87 a-c
Bacillus subtilis 6/3	0.37 f	2.10 hj	1.88 e	21.09 b-f	68.47 a-d
Bacillus subtilis 36/10	0.49 f	1.92 i-j	2.01 b-e	21.13 b-f	68.63 a-d
Bacillus subtilis 20D	0.67 ef	2.10 hj	1.88 e	21.03 b-f	68.22 a-e
Paenibacillus polymyxa R2/2	0.47 f	7.69 a	2.00 b-e	23.33 ab	61.38 h-j
Paenibacillus polymyxa 2/5	0.43 f	8.13 a	1.98 c-e	23.93 ab	60.50 ij
Paenibacillus polymyxa 11/4	0.47 f	8.12 a	1.98 c-e	23.33 ab	60.98 h-j
Paenibacillus polymyxa 43/5	0.64 ef	4.28 d-f	1.98 c-e	23.38 ab	64.22 d-i
Paenibacillus polymyxa 24/3	0.83 b-f	3.21 f-h	1.91 e	22.67 a-e	65.29 b-h
Paenibacillus polymyxa 31/5	0.66 ef	4.63 de	2.02 b-e	24.00 ab	62.98 f-i
Paenibacillus polymyxa 56/4	0.64 ef	4.26 d-f	1.98 c-e	23.14 a-d	64.42 c-i
Pseudomonas putida 20c	0.60ef	2.61 g-j	2.05 a-e	18.59 f	70.09 a
Pseudomonas putida 3/10	0.60ef	2.41 h-j	2.05 a-e	18.89 ef	70.08 a
Pseudomonas putida 27/3	0.69 d-f	2.65 g-j	1.92 e	19.35 c-f	69.24 ab
Pseudomonas putida 48/2	1.19 b-d	2.38 h-j	2.29 a-d	18.41 f	68.40 a-e
Pseudomonas putida 53/5	1.19 b-d	2.38 h-j	2.29 a-d	18.35 f	68.50 a-d
Pseudomonas putida 55/2	1.19 b-d	2.18 h-j	2.29 a-d	18.35 f	68.70 a-d
Pseudomonas putida 62/5	1.03 b-e	2.16 h-j	2.21 a-e	19.23 d-f	68.39 a-e
Pseudomonas fluorescens 22B	1.26 b	3.63 e-h	2.15 a-e	17.85 f	68.05 a-e
Pseudomonas fluorescens 8/2	1.26 b	3.63 e-h	2.15 a-e	17.85 f	68.05 a-e
Pseudomonas fluorescens 8/6	1.26 b	3.63 e-h	2.15 a-e	17.85 f	68.05 a-e
Pseudomonas fluorescens 9/7	1.03 b-e	4.52 de	2.39 a	26.27 a	58.19 j
Pseudomonas fluorescens 53/6	1.03 b-e	4.53 de	2.39 a	26.26 a	58.20 j
Pseudomonas fluorescens 51/2	1.02 b-e	4.51 de	2.40 a	26.28 a	58.19 j
Pseudomonas fluorescens 58/3	1.03 b-e	4.52 de	2.39 a	26.27 a	58.19 j
Mean	**0.86**	**3.80**	**2.10**	**21.85**	**64.92**

*Values followed by different lower-case letters in a column were significantly different ($P \leq 0.01$) using Duncan's multiple range test. [a]Retention index relative to *n*-alkanes on SGE-BPX5 capillary column; GC: identification based on retention times of authentic compounds on SGE-BPX5 capillary column; MS, RI: tentatively identified based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data, and comparison of retention index of the compounds compared with published data (Adams, 2007)

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Table 3. *Effect of chemical fertilizer application and bacterial inoculations for nitrogen, phosphorus and potassium on contents leaf of Turkish oregano*

Treatments	N (%)*	P (g/kg)	K (g/kg)
Control	1.63 g	2.10 g	30.3 f
Mineral fertilizer (NP)	3.47 a-c	2.73 a-f	37.5 a-e
Bacillus megaterium K5E	1.85 e-g	2.08 g	30.3 f
Bacillus megaterium 22D	3.21 a-e	2.54 b-g	35.1 c-f
Bacillus megaterium 66/5	1.77 fg	2.08 g	37.8 e-f
Bacillus megaterium 21/3	2.46 b-g	2.41 d-g	33.8 c-f
Bacillus megaterium 44	2.10 d-g	2.27 fg	32.9 c-f
Bacillus megaterium 35/6	3.29 a-d	2.61 b-g	36.5 a-f
Bacillus megaterium R2C	1.77 fg	2.08 g	30.8 e-f
Bacillus subtilis R 3/3	3.29 a-d	2.78 a-f	39.2 a-d
Bacillus subtilis 2/8	2.10 d-g	2.36 e-g	35.1 c-f
Bacillus subtilis 39/3	3.01 a-f	2.65 b-g	36.1 a-f
Bacillus subtilis 52/1	3.41 a-d	2.98 a-d	42.3 ab
Bacillus subtilis 6/3	3.49 a-c	2.96 a-e	42.8 a
Bacillus subtilis 36/10	2.30 c-g	2.53 b-g	38.5 a-d
Bacillus subtilis 20D	2.80 c-g	2.61 b-g	36.9 a-f
Paenibacillus polymyxa R2/2	3.59 a-c	3.02 a-c	39.9 a-c
Paenibacillus polymyxa 2/.5	3.33 a-d	2.83 a-f	37.5 a-e
Paenibacillus polymyxa 11/4	3.33 a-d	2.83 a-f	37.5 a-e
Paenibacillus polymyxa 43/5	3.73 ab	3.27 a	38.5 a-d
Paenibacillus polymyxa 24/3	3.55 a-c	2.97 a-d	35.9 b-f
Paenibacillus polymyxa 31/5	3.48 a-c	3.11 a-b	37.5 a-e
Paenibacillus polymyxa 56/4	2.97 a-f	2.66 b-g	35.6 c-f
Pseudomonas putida 20c	3.55 a-c	2.34 fg	34.2 c-f
Pseudomonas putida 3/10	4.07 a	2.58 b-g	36.5 a-f
Pseudomonas putida 27/3	3.45 a-d	2.27 fg	32.5 d-f
Pseudomonas putida 48/2	3.75 ab	2.50 c-g	37.1 a-e
Pseudomonas putida 53/5	3.93 a	2.63 b-g	36.9 a-f
Pseudomonas putida 55/2	3.93 a	2.74 a-f	38.03 a-d
Pseudomonas putida 62/5	3.31 a-d	2.30 fg	33.9 c-f
Pseudomonas fluorescens 22B	1.92 e-g	2.27 fg	33.7 c-f
Pseudomonas fluorescens 8/2	1.92 e-g	2.27 fg	33.7 c-f
Pseudomonas fluorescens 8/6	2.77 a-g	2.5 c-g	34.4 c-f
Pseudomonas fluorescens 9/7	3.43 a-d	2.52 c-g	35.4 c-f
Pseudomonas fluorescens 53/6	3.43 a-d	2.52 c-g	35.4 c-f
Pseudomonas fluorescens 51/2	3.43 a-d	2.52 c-g	35.4 c-f
Pseudomonas fluorescens 58/3	3.54 a-c	2.60 b-g	36.5 a-f
Mean	**3.04**	**2.57**	**36.00**

*Values followed by different lower-case letters in a column were significantly different ($P \leq 0.05$) using Duncan's multiple range test

Discussion

Inoculation with multi-traits bacteria increased fresh and dry herbage yield, dry leaf yield, plant height, canopy diameter, chlorophyll content (SPAD) and essential oil yield in oregano compared with the control. The responses to inoculation, compared to uninoculated control plants, were: -13.7% to +34.8% for dry herbage yield per plant, -13.4% to +49.2% for dry leaf yield, -0.4% to +30.3% for plant height, -0.6% to +24.2% for chlorophyll content, and -4.1% to +43.3% for essential oil yield. Plant growth responses were variable and dependent on the inoculant strain used, as well as on the growth parameter being evaluated. Increased plant dry weight and the oil content and biosynthesis of terpenes provided the increases in total essential oil yield by PGPRs inoculation. Using PGPR in the study was promoted the growth, yields and essential oil

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content of oregano and chemical fertilizer application was equal or lower according to bacterial inoculations. Several authors have indicated that PGPR inoculation increased in shoot and root biomass, leaf area, and stomatal density, and marked qualitative and quantitative changes in monoterpene content in different medicinal and aromatic crops (Banchio et al., 2009; Banchio et al., 2010; Cappellari et al., 2013; Çakmakçı, 2016).

According to our data, aromatic structure, content and essential composition of oregano oil changed positively with multi-traits PGPR inoculation. In our study, major components of oregano oil have been carvacrol, thymol and borneol. Turkish oregano' essential oil was described as high content of phenolic compounds enclosed thymol and carvacrol (Bokov et al., 2015) and they have various biologic and pharmacological role (Dundar et al., 2008), antioxidant activity and they are important effect for component of oregano aroma (Bansleben et al., 2009). In addition, essential oil content and its composition are the most significant of quality criterion for oregano in all purposes (Baydar et al., 2004; Yaldiz et al., 2005; Baser, 2008; Bansleben et al., 2009; Ekren et al., 2013). Total essential oil yield, chemical composition and biosynthesis of major essential oil components of medicinal and aromatic plants were significantly affected by inoculation with PGPR (Banchio et al., 2008, 2009, 2010; Santoro et al., 2011, 2015; Prasad et al., 2012; Bahadori et al., 2013; Cappellari et al., 2013). However, there are not enough studies on the effect of inoculation with PGPR on plant growth or on production of secondary metabolites in important aromatic plants.

Effective multi-traits PGPR species, such as *P. polymyxa* R2/2, *P. putida* 3/10, *P. fluorescens* 8/2 and *P. fluorescens* 58/3 improved the N, P and K, nutrition in oregano, and therefore encouraged plant growth, essential oil quantity and quality. When particularly effective strains are used, it is possible that without using of chemical fertilizers to grow Turkish oregano organically without any loss in yield and quality. Beneficial role of these PGPR in growth, yield, oil content and compositions of oregano plants can have related to IAA-production, N_2-fixation, P-solubilisation, ACC deaminase activity utilization of variety and high rate of carbon sources and metabolize root exudates by the effective strains may be possible to afford a competitive advantage and play an important role in adapting to plants and soil. Previous studies suggested that carbon sources which were differentially utilized by the strains tested and many carbon sources were preferred by PGPR strain, which was one of the best bio fertilizers strains (Çakmakçı et al., 2010). Nutrients such as N, P, K, S, Ca, Mg and microelements can change of essential oil yield and composition according to reported similar studies (Nurzynska-Wierdak, 2013). In addition, for essential oils synthesized by plants, phosphorus is an important source. Therefore, PGPR can stimulate essential oil synthesis in medicinal plants when increased P uptake (Lichtenthaler, 2009). Moreover, according to researchers, leaves of PGPR inoculated plants are more contain to N, P and K than un-inoculated plants. Therefore, PGPR can provide increase in growth characters of medicinal plants (Saharan and Nehra, 2011). According to result of this study, versatile PGPR can be effective for improving growth and nutrient uptake of aromatic oregano crop. Also, more uptakes of nutrients that involved in chlorophyll formation can provide increasing for total chlorophyll due to beneficial effect of PGPR uptake of mineral elements in tea plants by application of PGPR was provide increased the chlorophyll content in leaves (Çakmakçı, 2016).

The better nutrient status were positively correlated with growth rates and yield of essential oils (Trivino and Johnson, 2000) and resulted in increased assimilation and translocation of photosynthates (Singh et al., 2016). While, N strongly affected not only

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herb yield, but also its essential oil content and major oil constituents (Ozguven et al., 2006), an increase in the amount of phosphorous of the plants resulted in the enhanced accumulation of essential oil (Khalid, 2014) and affects the primary and secondary metabolites (Pal et al., 2016). While an adequate nutrient supply results in larger biomass production and consequently higher oil yield, changes in the essential oil composition may be related to better nutrition. On the other hand, mineral element composition of medicinal and aromatic plants has gained an interest and essential macronutrients may have nutritive, preventive and curative role in human.

Rate of carbon assimilation and photosynthetic activity can have provided by higher chlorophyll content in inoculated plants and for evaluation of plant photosynthetic efficiency, chlorophyll is an important parameter (Cappellari et al., 2015). For replace the use of chemical fertilizers, Microbial strategy is an attractive way for herbal plants, but little is known about their potential effect and ability of PGPR to increase plant secondary metabolites. At this point, limited knowledge is available in respect to effects of inoculation with PGPR in aromatic and medicinal plants (Banchio et al., 2008). For investigate the possible mechanisms by which bacteria increase phytochemical constituents in medicinal plants at the tissue, cell, or molecular level are need with more studies (Egamberdieva and da Silva, 2015).

Conclusion

PGPR have clear potential for improving the productivity of aromatic plants, and may significantly increase plant growth and reduce the amount of fertilizers required for economically sustainable crop production. Bacterial inoculants may be an efficient biotechnological tool for stimulating secondary metabolism in oregano plants, and studies of their activities will increase our understanding of processes that affect the accumulation of monoterpenes and phenolic compounds for a variety of applications in food and cosmetic industries, and poorly understood at present. In particular, *P. fluorescens* (8/2, 58/3, 9/7, 53/6 and 51/2), *B. subtilis* (52/1, 6/3 and R3/3), *B. megaterium* (21/3, K5E and 35/6), *P. polymyxa* (R2/2), and *P. putida* (55/2, 3/10 and 53/5) stimulated overall plant growth, including plant height, canopy diameter, fresh and dry herbage and leaf yield; improving macro- and micronutrient uptake, chlorophyll and essential oil content, and oil yield of oregano. Responses were variable and dependent on the inoculants strain and parameter evaluated. In addition, these bacteria can be used instead of mineral fertilizer as bio-fertilizer in sustainable organic oregano cultivation. Beneficial plant-associated bacteria exhibiting plant growth promoting traits can play a key role in supporting and/or enhancing plant growth, dry herb yield, and essential oil content, yield and its components. The action of PGPR on the essential oil and their components in medicinal aromatic plants remains a focus area for future research. Additional field trials are required to confirm the effects of PGPR strains on the growth characters, yield, essential oil and its constituents and nutrient contents, in this and so on plant species under different conditions. Future researches will also need to focus on elucidating the exact mechanisms by which PGPR have their growth-promoting effect and biosynthesis of secondary metabolites and monoterpene accumulation in medicinal and aromatic plants.

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