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Growth and Active Substances of Summer Savory as Affected by PGPR

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ABSTRACT: Summer savory, member of Labiatae family, is an annual plant. The plant growth promoting rhizobacteria (PGPR) can influence plant growth and metabolism. *Azotobacter* and *Pseudomonas* are two genera of the PGPR. In this study we applied two strains of *Azotobacter* (O4 and O6) and *Pseudomonas* strain MC1 in order to determine their effects on growth and active substances of summer savory. The experiment was carried out using a completely randomized design (CRD) with six replications. The bacteria altered growth characteristics and active substances of this plant. Essential oil percent was the maximum (1.87%) by application of *Azotobacter* strain O6 which was not significantly different when compared to other bacterial treatments. The highest value of oil yield (27.32 mg/plant) was achieved on *Pseudomonas* treatment which increased oil yield by 66% when compared to control. Thirty seven compounds were identified in the essential oils. γ-terpinene (45.3-49.4%) and carvacrol (37.0-41.3%) were the major components.

***Keywords*:** *Satureja hortensis*, PGPR, biofertilizer, medicinal plants, carvacrol.

INTRODUCTION

Summer savory (*Satureja hortensis* L.) is an annual plant from family Labiatae (Lamiaceae). It is native to southern Europe and central and south-west of Asia countries such as Iran (Boskabady et al., 2007; Gontaru et al., 2008). Shoot parts of this plant are frequently used as additives for many foods. This plant is also used in the traditional medicine to treat muscle pains, indigestion, diarrhoea, and infection diseases (Gontaru et al., 2008). Major oil constituents of this plant are thymol, carvacrol, γ-terpinene and borneol (Boskabady et al., 2007).

Some biofertilizers are microbial inoculants contain living cells of micro-organism such as bacteria, algae and fungi which may help plant growth. Biological activities are markedly enhanced by microbial interactions in the rhizosphere of plants (Tilak and Reddy, 2006). The plant growth promoting rhizobacteria (PGPR) can influence plant growth directly through the production of phytohormones and indirectly through nitrogen fixation and production of bio-control agents against soil-borne phytopathogens (Glick, 2003). Agronomical factors can alter composition and quantity of active substances in medicinal plants (Naghdi badi et al., 2004; Jordan et al., 2006).

Application of biofertilizers as substitute for inorganic fertilizers should not be considered as a simple objective and short term benefits, but as a mean to improve environmental conditions and human health.

Azotobacter and Pseudomonas are two genera of the PGPR. The aim of this experiment was to study the effects of PGPR on growth and essential oils of summer savory.

MATERIALS AND METHODS

Plant materials and experimental conditions:

The experiment was carried out in the glasshouse of Islamic Azad University, Firoozabad Branch, Iran (28°35' N, 52°40' E; 1327 m above sea level). The seeds of summer savory were sown in the pots containing 2/5 soil, 2/5 sand and 1/5 leaf mold (v/v) and thinned at 4-6 leaves stage to two plants per each pot. The pot mixture were tested before applying treatments and the texture was sandy with PH=7.56, organic C=1.26%, total N=0.13%, available P=89.4 mg/kg, available K=252 mg/kg and EC=5.01 dS/m. Plants kept at 22±3/14±3°C day/night temperatures. The

treatments were strains O4 and O6 of *Azotobacter*, *Pseudomonas* strain MC1 and comparing them to control (without using bacteria). The strains were prepared from Soil and Water Research Institute, Karaj, Iran. Bacteria were mixed with surface of the pot soil. Experiment was carried out using a completely randomized design (CRD) with six replications. Each replicate contained 3 pots. Plants were harvested at full bloom. Shoot height and fresh weights of shoot and root were measured then shoots were dried at room temperature and roots were dried at 60°C for 72 hours.

Isolation of essential oils:

Isolation of essential oils was performed using hydrodistillation of dried sample of shoots using a Clevenger-type apparatus over 3 hours. The oils were dried over sodium sulphate. Isolation of oil was done with four replications.

Qualitative and quantitative analysis of essential oils:

Gas Chromatography (GC) analysis was performed on an Agilent technologies model (7890A) equipped with flame ionization detector and capillary column HP-5(30m × 0.32 mm, 0.25 µm film thicknesses). The chromatographic conditions were as follows: The oven temperature increased from 60 to 210ºC at a rate of 3ºC/min. The injector and detector temperatures were 280 and 290ºC, respectively. N2 used as the carrier gas (1 ml/min). Essential oil was also analysed by GC-MS (Agilent Technologies-5975C-MS, 7890A-GC) operating at 70e V ionization energy, equipped with a HP-5 capillary column (phenyl methyl siloxane (30 m × 0.25 mm, 0.25 µm film thickness) with He as the carrier gas and a split ratio of 1:50. The retention indices for all the components were determined according to the Van Den Doll method using n-alkanes as standard. The compounds were identified by comparison of retention indices (RRI- AP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley and mass finder 3 libraries or with the published mass spectra.

Statistical analysis:

The data were subjected to analysis of variance (ANOVA) using SAS computer software and means compared with DNMRT at 5% level of probability.

RESULTS AND DISCUSSION

The bacteria altered growth characteristics and active substances of summer savory (Table 1). The highest value of shoot height (43.38 cm) was obtained at control which was not significantly different when compared to *Pseudomonas* treatment. The maximum shoot fresh and dry weights (7.22 g/plant and 1.80 g/plant, respectively) were achieved on control which was not significantly different when compared to *Pseudomonas* treatment. Root fresh and dry weights were the maximum at control. Essential oil percent was maximum (1.87%) by application of *Azotobacter* strain O6 which was not significantly different when compared to other bacterial treatments. The highest value of oil yield (27.32 mg/plant) was achieved on *Pseudomonas* treatment which increased oil yield by 66% when compared to control.

Qualitative and quantitative analysis of essential oils have been shown in Table 2. Thirty seven compounds were identified. γ-terpinene (45.3-49.4%) and carvacrol (37.0-41.3%) were the major components. Carvacrol was the maximum (41.3%) at *Pseudomonas* treatment. Carvacrol has been reported to act as antioxidant (Kulisic et al., 2005), antimicrobial agent (Deans and Ritchie, 1987; Prabuseenivasan et al., 2006), antifungal agent (Klaric et al., 2007) treatment for respiratory tract diseases (Inouye et al., 2001), wound healing, a stomachic carminative, diuretic and urinary disinfectant (Boskabady et al., 2006).

Production of the secondary metabolites like antibiotics, cyanide, and hormonelike substances is one role of PGPR. Another mechanism is antagonism to soilborne root pathogens and phosphate solubilization. These bacteria may improve plant P acquisition by solubilizing organic and inorganic phosphate sources through phosphatase synthesis or by lowering the pH of the soil (Dart, 1986; Goldstein, 1986; Sharafzadeh, 2012). According Sharafzadeh (2011), nutrients are able to change growth and total phenolic content of garden thyme.

Brown, (1974), revealed that *Azotobacter paspali* can release IAA in the medium and Reda et al. (2005), reported that growth regulators increase total phenolic content in thyme.

PGPR can biologically fix nitrogen (Glick, 2003). Nutrient availability affects the production of polyphenolic compounds in three cultivars (Dark Opal, Genovese, and Sweet Thai) of basil (Nguyen and Niemeyer, 2008). Nitrogen and phosphorus play important role in essential oil biosynthesis. Besides production of pyruvate, these elements are present in ATP, NADP and CoA structure which terpenoid biosynthesis depend on such coenzymes (Sell, 2003; Kapoor et al., 2004).

In conclusion, in our experimental conditions, *Psedomonas* strain MC1 resulted in the highest values of oil yield and carvacrol.

Table 1. Effects of PGPR treatments on growth, oil percent and oil yield of summer savory.

Treatment	Shoot height (cm)	Shoot fw (g/plant)	Shoot dw (g/plant)	Root fw (g/plant)	Root dw (g/plant)	Oil percent(%)	Oil yield (mg/plant)
Co	43.38a	7.22a	1.80a	0.90a	0.24a	0.92b	16.42a
O4	35.68b	5.95b	1.40bc	0.56b	0.17b	1.72a	26.83a
O6	31.80b	5.30b	1.25c	0.43b	0.15b	1.87a	23.62a
P	37.94ab	6.31ab	1.61ab	0.62b	0.20ab	1.62a	27.32a

In each column, means with the same letters are not significantly different at 5% level of Duncan's new multiple range test

Table 2. The chemical components of summer savory oil in different treatments of PGPR

No	RI	Compound name	control	O4	O6	P
1	928	α-Thujene	0.8	0.6	0.4	0.7
2	936	α-Pinene	0.6	0.4	0.3	0.5
3	951	Camphene	0.1	t	t	0.1
4	976	Sabinene	0.1	0.1	0.1	0.1
5	980	β-Pinene	0.1	0.4	0.3	0.4
6	993	Myrcene	1.8	1.5	1.3	1.7
7	1000	α-Phellandrene	-	t	t	t
8	1008	δ-3-Carene	0.3	0.3	0.2	0.3
9	1013	α-Terpinene	0.1	0.1	t	0.1
10	1022	*p*-Cymene	4.2	3.6	3.2	3.8
11	1028	Limonene	1.2	1.1	1.1	1.3
12	1029	β-Phellandrene	0.8	1.3	1.4	0.9
13	1032	1,8-Cineole	0.5	0.4	0.4	0.5
14	1038	(Z)-β-Ocimene	t	t	t	t
15	1049	(E)-β-Ocimene	-	0.1	0.1	0.1
16	1062	γ-Terpinene	49.4	46.1	46.1	45.3
17	1075	cis-Sabinene hydrate	0.1	0.1	0.1	0.1
18	1092	Methyl benzoate	t	t	t	t
19	1101	trans-Sabinene hydrate	t	t	t	t
20	1104	n-Nonanal	t	0.1	t	t
21	1169	Borneol	t	0.1	t	t
22	1180	Terpinene-4-ol	t	0.1	0.1	0.1
23	1208	trans-dihydrocarvone	-	-	t	-
24	1244	Carvacrol methyl ether	0.1	0.1	0.2	0.1
25	1287	Thymol	t	-	-	-
26	1303	Carvacrol	37.2	37.0	40.6	41.3
27	1341	δ-Elemene	0.3	0.1	0.1	0.1
28	1354	Eugenol	-	t	-	-
29	1377	Carvacrol acetate	0.2	0.5	0.5	0.2
30	1422	(E)-Caryophyllene	0.3	0.4	0.6	0.4
31	1436	trans-α-Bergamotene	t	t	t	t
32	1441	Aromadendrene	t	-	t	t
33	1456	α-Humulene	0.1	0.1	0.1	0.1
34	1499	Bicyclogermacrene	0.3	0.4	0.5	0.5
35	1511	β-Bisabolene	0.8	1.2	0.5	1.0
36	1530	(E)-γ-Bisabolene	-	0.2	-	-
37	1595	Spathulenol	t	0.1	0.1	t
		Total (%)	99.89	96.36	98.45	99.71

All data show as percent (%), RI: retention index, t: trace (< 0.05)

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