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Effects of Inoculation Bacteria on Chemical Content, Yield and Growth in Rocket (*Eruca vesicaria* subsp. sativa)

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This study was conducted to determine the effects of inoculation bacteria on mineral content, yield and growth in rocket. Strains of bacteria, *Burkholderia gladii* BA-7, *Pseudomonas putidae* BA-8, *Bacillus subtilis* OSU-142 and MFD-5, *Bacillus megatorium* M3, *Agrobacterium rubi* A-1, A-16 and A-18, were used. The effects of the bacterial treatments on the plant nutrient elements of leaves were evaluated. The parameters of yield per m^2, average leaf number, leaf weight, leaf length, leaf stem diameter, leaf area, leaf dry matter and average root length, root weight and root dry matter were also determined. The effects of bacterial application on plant mineral contents were significant. Bacterial applications increased mineral contents of rocket leaves as compared to control treatment. All bacterial applications particularly affect on increasing in N, K, P, Zn, Fe, Mn, Na, Ca and Mg contents of plant. The effects of bacterial application on the parameters were also significant. The highest yield (4586.54 g/m^2), average leaf weight (1.63 g), leaf length (27.48 cm), leaf stem diameter (2.06 mm), leaf area (93.57 cm^2) and root weight (0.60 g) were obtained from *Pseudomonas* BA-7 applications as comparing to that of the other applications. The highest leaf number (8.23), leaf dry matter (6.70 %) and root dry matter (11.85 %) were determined in A-18, BA-142 and MFD-5 applications, respectively. The results of this study showed that especially *Burkholderia gladii* BA-7, *Pseudomonas* BA-8 and *Bacillus* OSU-142 have a great potential to increase the parameters of plant growth of rocket.

Key Words: Bacteria, Plant growth, Yield, Mineral content, Rocket.

INTRODUCTION

Vegetable production is getting increase in all around of the world. Turkey has favourable ecological conditions for vegetable growth and is

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one of the most important vegetable producers in the world. Turkey is fourth important producer (25.3 million tones) country regarding of vegetable production in the world[1].

Vegetables commonly grown in Turkey today consist of annual crops including Solanaceous, Crucifers, Cucurbits, Bulb crops, Leguminous and other indigenous vegetable species. In terms of economic value, nutrition, consumers preference, general adaptability and extent of cultivation, the most commonly grown vegetable crops are tomato, watermelon, cucumber, pepper (hot and sweet), eggplant, squash, onion, snap bean, melon, salad vegetables, *etc*.

Intensive farming practices require extensive use of chemical fertilizers, which are costly and create environmental problems, for warranting high yield and quality. Hence, there has recently been a resurgence of interest in environmentally friendly, sustainable and organic agricultural practices[2,3]. Because of the reason, uses of bio-fertilizers containing beneficial micro-organisms instead of inorganic chemicals are positively known to affect on plant growth in terms of supplying of plant nutrients and may help to sustain environmental health and soil productivity[3,4].

A number of inoculated bacterial species mostly associated with the plant rhizosphere have been tested and determined to be beneficial for plant growth, yield and crop quality so far. They have been called plant growth promoting rhizobacteria (PGPR)' including the strains in the genera *Acinetobacter Alcaligenes, Arthrobacter, Azospirillium, Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Rhizo-bium* and *Serratia*[3,5-7]. These bacteria were previously reported as plant growth promoting bacteria and had potential bio-control agents against a wide range of bacterial and fungal pathogens causing economically important problems in agriculture[3,8-12]. They affect on fixation of nitrogen[13-15] and are one of the most plausible mechanisms of action affecting plant growth[3]. The reason is that nitrogen-fixing bacteria may be important for plant nutrition by increasing nitrogen uptake by the plants and playing as significant role as plant growth PGPR in the biofertilization of the crops[16,17]. Many researchers determined that PGPR can stimulate growth and increase yield in pepper and tomato[12,18], in sugar beet[19,20], in spring barley[21], in apricot[11,22], in raspberry[23] and in apple[16]. However, not much is known about promoting effects on yield, growth and nutrient contents of rocket vegetable species.

The objective of this study was to determine the effects of inoculation bacteria (*Burkholderia gladii* BA-7, *Pseudomonas putidae* BA-8, *Bacillus subtilis* OSU-142 and MFD-5, *Bacillus megatorium* M3, *Agrobacterium rubi* A-1, A-16 and A-18) on chemical content, yield and growth in rocket in unheated greenhouse conditions.

EXPERIMENTAL

Strains of bacteria, *Burkholderia gladii* BA-7, *Pseudomonas putidae* BA-8, *Bacillus subtilis* OSU-142, MFD-5, *Bacillus megatorium* M3, *Agrobacterium rubi* A-1, A-16 and A-18, were obtained from Department of Plant Protection at Ataturk University. Bacteria were grown on nutrient agar (NA) for routine use and maintained in nutrient broth (NB) with 30 % glycerol at -80 ºC for long-term storage. For this experiment, the bacterial strains were grown on nutrient agar. A single colony was transferred to 250 mL flasks containing nutrient broth and grown aerobically in flasks on a rotating shaker (95 rpm) for 48 h at 27 ºC. The bacterial suspension was then diluted in sterile distilled water to a final concentration of 10^8 CFU mL^{-1} and the resulting suspensions were used to treat rocket plants. The plants were sprayed with bacterial suspension (10^8 CFU mL^{-1}) at one week interval for three times after first true leaf development.

Unheated greenhouse experiment: The experiment was carried out on rocket (*Eruca vesicaria* subsp. sativa) in the Department of Horticulture at Ataturk University under unheated greenhouse condition in Erzurum, Turkey, in 2006. It was made based on a completely randomized design with four replicates.

The effect of the bacterial treatments on the plant nutrient elements of leaves was evaluated. Growth promoting effects of bacterial treatments were also evaluated by determining yield/m^2, average leaf number, leaf weight, leaf length, leaf stem diameter, leaf area, dry matter and average root length, root weight and dry matter.

Leaf analysis: In order to determine the mineral contents of plant shoot and root, plants samples were oven-dried at 68 ºC for 48 h and then ground. The micro-Kjeldahl procedure was applied for determination of N. K^+, Ca^{2+} and Mg^{2+} were determined after wet digestion of dried and ground sub-samples in a H_2SO_4-Se-salisilic acid mixture. In the diluted digests, phosphorus was measured spectrophotometrically by the indophenol-blue method and after reaction with ascorbic acid. K^+ and Ca^{2+} were determined by flame photometry, Mg^{2+}, Mn, Zn and Cu by atomic absorption spectrometry using the method of AOAC[24].

Data analysis: All data were subjected to a one-way analysis of variance (ANOVA) and separated by Duncan's multiple range tests using SAS statistical software[25].

RESULTS AND DISCUSSION

The effects of bacterial application on plant mineral (N, K, P, Zn, Fe, Mn, Na, Ca and Mg) contents were significant at $p < 0.05$ and 0.01 (Table-1). In present study, we determined that bacterial applications increased mineral contents of rocket leaves as compared with control treatment. All bacterial applications particularly affect on increasing in N, K, P, Zn, Fe, Mn, Na, Ca and Mg contents of plant. The highest N, K, P, Zn, Fe, Mn, Na, Ca and Mg contents were obtained from A-16 (4.62 %), BA-7 (4571.25 ppm), MFD-5 (287.65 ppm), M3 (47.03 ppm), BA-8 (919.30 ppm), BA-8 (4.75 ppm), MFD-5 (1241.50 ppm), BA-8 (9601.00 ppm) and A-18 (1309.00), respectively. Bacterial strains effecting on Cu were not significant (Table-1). The higher mineral contents in the bacteria treated plant may have resulted from the producing plant hormone such as auxins, cytokinins, gibberellins and ethylene[16] ability of these bacteria, as reported that many kinds of bacteria had given same results on different plant species in previous studies[8,11,18,23]. Marschner[17] and Aslantas *et. al.*[16] stated that increasing mineral contents in plants results in greater uptake of nutrient elements from soil. This evidence confirms the data showing that the quantity of N, P, K, Zn, Fe, Mn, Na, Ca and Mg was significantly or relatively increased in the bacteria-treated plants, which may be explained by higher concentration of nitrogen and phosphorus stimulated by bacterial application and resulted from the producing plant hormone.

Plant growth: Growth promoting effects of bacterial application on yield, average leaf number, leaf weight, leaf length, leaf stem size, leaf area, leaf dry matter and average root length, root weight and root dry matter of roka were significant at $p < 0.01$ (Table-2). The highest yield (4586.54 g/m^2) was obtained from *Pseudomonas* BA-7 applications as comparing to that of the other applications and average leaf weight (1.63 g), leaf length (27.48 cm), leaf stem size (2.06 mm), leaf area (93.57 cm^2) and root weight (0.60 g) were also obtained from *Pseudomonas* BA-7 applications when comparing to the other treatments. The highest leaf number (8.23), leaf dry matter(6.70 %) and root dry matter (11.85 %) were determined in A-18, BA-142 and MFD-5 applications, respectively. This is the first report on growth promoting effect of bacterial application on plant growth parameters of rocket. However, similar reports were determined in different plant species. Researchers stated that bacterial applications including *Pseudomonas* and *Bacillus* strains can stimulate growth and increase yield in pepper and tomato[12,18], in sugar beet[19,20], in spring barley[21], in apricot[11,22], in raspberry[23] and in apple[16]. The reason of growth promoting effect of bacterial applications on plant growth is that they affect on fixation capacity of nitrogen[13-15] and are one of the most plausible mechanisms of action affecting plant growth[3]. The used bacterial strains showed the same results in our findings. Thus, the present finding is good agreement to previous studies mentioned above.

Conclusion

The effects of bacterial applications depend on the crop species. Bacterial application is safe, effective and easily adopted by farmers. In terms of first report on rocket, the results of this study showed that

TABLE-1
EFFECTS OF BACTERIAL APPLICATIONS ON PLANT CHEMICAL CONTENTS OF LEAVES IN ROCKET

Applications	N (%)	K (ppm)	P (ppm)	Zn (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Na (ppm)	Ca (ppm)	Mg (ppm)
Control	4.03c‡	3466.00b‡	213.59e‡	32.02d‡	418.84c‡	0.64	3.57b†	912.30b†	8918.00cd‡	1292.50ab†
M3	3.98c	4547.75a	257.92cd	47.03a	542.95bc	0.64	3.80ab	1198.63a	8683.00d	1298.00ab
BA-142	4.41ab	4327.25a	259.91bcd	37.11c	560.03bc	0.64	4.05ab	1237.00a	9034.25bcd	1298.50ab
BA-7	4.39b	4571.25a	250.56d	36.49c	464.65bc	0.64	3.33b	1117.20ab	9142.75abcd	1292.50ab
BA-8	4.45ab	4443.25a	250.99d	39.62bc	919.30a	0.64	4.75a	1165.18a	9601.00a	1287.00b
A-18	4.47ab	4195.25a	254.01d	47.56a	1139.38a	0.64	3.80ab	1220.93a	9435.50abc	1309.00a
A-16	4.62a	4451.25a	281.70abc	39.20bc	547.58bc	0.64	3.80ab	1209.78a	9352.00abc	1298.00ab
A-1	4.50ab	4059.25a	285.99ab	39.31bc	584.32bc	0.64	4.12ab	1192.40a	9518.50ab	1292.50ab
MFD5	4.03c	4142.00a	287.65a	41.48b	680.75b	0.64	3.80ab	1241.50a	9350.00abc	1298.00ab
LSD (0.05)	0.07	149.60	8.46	1.18	75.45	NS	0.29	78.57	156.80	5.33

TABLE-2
EFFECTS OF BACTERIAL APPLICATIONS ON YIELD, AVERAGE LEAF NUMBER, LEAF WEIGHT, LEAF LENGTH, LEAF STEM SIZE, LEAF AREA, DRY MATTER AND AVERAGE ROOT LENGTH, ROOT WEIGHT AND DRY MATTER

Applications	Yield/m^2 (g)	Average leaf number	Average leaf weight (g)	Average leaf length (cm)	Average leaf stem size (mm)	Average leaf area (cm^2)	Leaf dry matter (%)	Average root length (cm)	Average root weight (g)	Root dry matter (%)
Control	2660.58d‡	6.80de‡	1.05d‡	22.05c‡	1.46d‡	64.96b‡	5.88cd‡	11.67d‡	0.31d‡	9.56c‡
M3	3314.42bcd	6.70de	1.56ab	25.91ab	1.81c	68.75b	6.56ab	12.33c	0.31d	10.96ab
BA-142	3027.88bcd	7.05cd	1.39abcd	25.97ab	1.85bc	77.15b	6.70a	13.60a	0.44bc	11.50a
BA-7	4586.54a	8.10ab	1.63a	27.48a	2.06a	93.57a	6.61a	13.27ab	0.60a	10.15bc
BA-8	3574.04bc	7.73ab	1.55ab	26.60ab	2.04ab	89.76a	6.15bc	11.63d	0.51b	9.90bc
A-18	3069.23bcd	8.23a	1.10cd	24.00bc	1.93abc	74.98b	5.65d	12.93b	0.35cd	7.94d
A-16	3823.08b	7.08cd	1.41abc	26.53ab	1.74c	94.66a	5.85cd	11.98cd	0.30d	9.86bc
A-1	2720.77cd	7.58bc	1.55ab	23.83bc	1.82c	75.70b	6.29abc	13.30ab	0.41c	11.65a
MFD5	3000.00bcd	6.33e	1.28bcd	24.60abc	1.84c	75.09b	6.37ab	13.08ab	0.32d	11.85a
LSD (0.05)	251.10	0.19	0.11	0.89	0.06	3.83	0.14	0.20	0.03	0.34

†Significantly important at $p < 0.05$; ‡Significantly important at $p < 0.01$; NS = Not Significant.

Burkholderia gladii BA-7, *Pseudomonas putidae* BA-8, *Bacillus subtilis* OSU-142 and MFD-5, *Bacillus megatorium* M3, *Agrobacterium rubi* A-1, A-16 and A-18 have a great potential to increase the yield, growth and mineral contents of rocket plant. They also have the potential to benefit such farmers in many ways and hence, its importance has been recognized by farmers as well as researchers. Therefore, they may be put to good use as biofertilizer for fruit and vegetable production in sustainable and ecological agricultural systems.

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