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N_2-fixing Plant Growth-Promoting Rhizobacteria: Potential to Increase Yield, Growth and Element Contents of *Mentha piperita* L. Leaves

Haluk Çağlar Kaymak[1*] • Mesude Figen Dönmez[2] • Ramazan Çakmakçı[3]

[1] Department of Horticulture, Faculty of Agriculture, Atatürk University, 25240 Erzurum, Turkey
[2] Department of Plant Protection, Faculty of Agriculture, Atatürk University, 25240 Erzurum, Turkey
[3] Department of Agronomy, Faculty of Agriculture, Atatürk University, 25240 Erzurum, Turkey

Corresponding author: * **hckaymak@atauni.edu.tr; hckaymak@yahoo.com**

ABSTRACT

During 2009 and 2010, plant growth promoting effects of N_2-fixing rhizobacteria *Pseudomonas putida* biotype B C3/101 and *Paenibacillus polymyxa* RC105, and urea (150 kg ha^{-1}) were tested on yield, growth and element composition of leaves of mint (*Mentha piperita* L.) grown in the Erzurum province of Turkey. Fresh and dry yield, shoot length and diameter, dry matter content and element composition of leaves were determined in both the 1st and 2nd harvest in both experiment years. *P. putida* biotype B C3/101, *P. polymyxa* RC105 and urea treatments stimulated plant growth and resulted in significantly higher total fresh and dry yields than the control. Inoculation of the mint rhizosphere with *P. polymyxa* RC105 showed better performance than *P. putida* biotype B C3/101 on total fresh and dry yields and shoot length; moreover, yield obtained from bacteria inoculation was lower than urea treatment but more than control. Thus, the highest total fresh (3181.0 kg $decare^{-1}$) and dry (622.7 kg $decare^{-1}$) herbage yield, shoot length (58.9 cm) and diameter (3.2 mm) were obtained in urea application. However, the highest total fresh (1926.7 kg $decare^{-1}$; in 2010) and dry (402 kg $decare^{-1}$; in 2009) yield for bacteria strains were observed from *P. polymyxa* RC105 treatment. Additionally, dry and fresh yield increase in bacteria inoculations compared to control treatment ranged from 15% to 57% in both experiment years. Bacterial applications also increased in element contents of mint leaves as compared to control treatment. Inoculation with *P. putida* biotype B C3/101 and *P. polymyxa* RC105 particularly affect on increasing in B, Ca, Cu, Fe, K, Mg, Mn, Na, P, Pb, S, Zn and N contents of leaves. Although the highest values for evaluated parameters were obtained from urea application, the results of this study suggest that *P. putida* biotype B C3/101 and *P. polymyxa* RC105 have the potential to increase the yield, growth and element composition of leaves of mint for environmental friendly mint production.

Keywords: mint, *Mentha piperita* L., N_2-fixing rhizobacteria, dry and fresh yield, element content
Abbreviations: **N**, nitrogen; **NBRIB-BPB**: National Botanical Research Institute's Phosphate Growth Medium; **IAA**, indole-3-acetic acid; **ACC**, 1-aminocyclopropane-1-carboxylate; **P**, phosphate; **S**, strongly; ***Pap* RC105**, *Paenibacillus polymyxa* RC105; ***Psp* C3/101**, *Pseudomonas putida* biotype B C3/101

INTRODUCTION

Mint is belonging to family Labiatae and the genus *Mentha*. It is a green plant with a particular smell aromatic, perennial, with height of up to 100 cm and its leaves is picked and dried in the shade, crushed, sifted and used until needed (Abbas 2009). Besides its dry usage, its fresh leaves are utilized as a leafy vegetable in Turkey. Although commercial production areas are Southeast Anatolia and Mediterranean, wild and cultivated mint plants have been spreading throughout Turkey with 11772 tons annual production (TUIK 2010). Additionally, Turkey is an important producer country like Morocco, Argentina and Spain in the world (FAO 2009; TUIK 2010).

Although mint is a perennial plant and productive up to 15 years, irrigation and fertilizer application are the principal limiting factors in the growing of mint (Jeliazkova *et al.* 1999; Alsafar and Al-Hassan 2009). A number of papers have been reported about fertilization of mint (Zheljazkov and Margina 1996; Jeliazkova *et al.* 1999; Shormin *et al.* 2009; Abbas 2009; Alsafar and Al-Hassan 2009). Recently, excessive amounts of fertilizers have been used on many soils for commercial vegetable production in Turkey (Güvenç 2002). Nitrogen is usually more important and key element for increasing plant growth and development, especially leafy vegetables, than any other nutrients. However, the global nitrogen cycle pollutes groundwater and increases risk of chemical spills. High-input farming practices achieving high yields have created environmental problems and degradation in natural resources (Şahin *et al.* 2004).

Since Kloepper and Schroth (1978) reported that microbial communities that exert beneficial for plant growth and have been called 'plant growth promoting rhizobacteria' (PGPR), there has been an increasing effort in advancing bacterial inoculants such as *Azotobacter*, *Azoarcus*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Gluconacetobacter*, *Klebsiella*, *Pseudomonas*, *Paenibacillus*, *Serratia*, etc. for plant growth promotion in agriculture. Therefore, Figueiredo *et al.* (2008) reported that during the past couple of decades, the use of PGPR for sustainable and environment friendly agriculture have been increased tremendously in various parts of the world. Increasing and extending the role of bio-fertilizing with PGPR would reduce the need for chemical fertilizers and decrease adverse environmental effects. Microorganisms are gaining importance in agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers (Şahin *et al.* 2004; Orhan *et al.* 2006).

Several investigations have reported on the effect of plant growth promoting rhizobacteria on different vegetable

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species. For instance, N_2-fixing bacterial strains *Pseudomonas putida* RC06, *Paenibacillus polymyxa* RC05 and RC14, and *Bacillus* OSU-142 have great potential as biofertilizers for better yield and quality of spinach growth (Cakmakci *et al.* 2007). Similarly, significant positive effects on growth and yield of soybean were obtained after inoculation with *Bradyrhizobium* spp. strains S62 and S63 (Egamberdiyeva *et al.* 2004). Additionally, similar studies showed that PGPR stimulated growth and increased yield in several vegetable species including tomato, sweet fennel, corn, bean, soybean, pea and lettuce (Chanway *et al.* 1989; Gagné *et al.* 1993; Noel *et al.* 1996; Burdman *et al.* 1996; Cattelan *et al.* 1999; Mehnaz and Lazarovits 2006; Siddiqui and Akhtar 2009; Moradi *et al.* 2011).

In our literature search, although, there are a lot of studies about the positive effects of PGPR on growth and yield in vegetable species, we have not found any detailed report on the effect of PGPR on growth and yield of mint, except for root formation of mint cuttings (Kaymak *et al.* 2008). Therefore, the objective of this study is to investigate the growth promoting effects of soil application of N_2-fixing rhizobacteria *Pseudomonas putida* biotype B C3/101 and *Paenibacillus polymyxa* RC105 on yield, growth and element content of mint (*Mentha piperita* L.) for environmental friendly mint production.

MATERIALS AND METHODS

This study was conducted in field conditions at Atatürk University, Agriculture Faculty, Erzurum, Turkey, during 2009 and 2010. The mother plant material of mint (*Mentha piperita* L.) was collected from Coruh Valley and they were cultivated in the field from 2005 in Erzurum (located at 40° 57′ and 39° 10′ N latitude, 40° 15′ and 42° 35′ E longitude, 1850 m altitude). The soil of the cultivated area was loamy texture (clay 22.17%, silt 31.82%, sand 46.01%) Ustorthent great soil group with neutral pH (7.13) and EC 415 µmhos cm^{-1}. It had 1.88% organic matter, 10.07 cmol kg^{-1} Ca, 2.34 cmol kg^{-1} Mg, 1.89 cmol kg^{-1} K, 0.52 cmol kg^{-1} Na, 16.71 mg kg^{-1} P and 16.9 cmol kg^{-1} Cation Exchange Capacity. The cultivated area was not fertilized, only irrigated in 2007 and 2008 to see the effect of N_2-fixing rhizobacteria better and clearly.

N_2-fixing rhizobacteria *P. putida* biotype B C3/101 and *P. polymyxa* RC105 were obtained from the culture collection of Department of Agronomy, Faculty of Agriculture, University of Atatürk, Erzurum, Turkey. They were stored in LB (Lauryl broth) amended with 30% glycerol at -80°C prior to use. Bacterial cell suspensions were prepared by first streaking the isolates onto Nutrient Agar and incubating at 27°C for 24 h to check for purity, then transferring single colonies to Nutrient Agar plates. After 24 h, the bacterial cells were harvested from the plates in nutrient broth. The optical density of the suspension was adjusted using a UV-visible spectrophotometer (Shimadzu, Japan, UV 1201, SN A1080) following the method of Mortensen (1992) to obtain a final density of 10^8 cfu/ml. The resulting suspensions were used to treat mint plots. Plots not exposed to bacterial suspensions and 150 kg ha^{-1} urea treatment (Singh *et al.* 1989; Ram *et al.* 1995) served as controls. Additionally, some biochemical characteristics of the bacterial strains C3/101 and RC105 were given in **Table 1**.

Mint plots were harvested before full blossoming 1st (July) and 2nd harvest (September) in both experiment years owing to the fact that when peppermint is grown for dry production plants are harvested at the stage of the bud formation (Jeliazkova *et al.* 1999). Afterwards, growth promoting effects of bacterial treatments were evaluated by determining total fresh and dry herbage yields (kg $decare^{-1}$), shoot length (cm) and diameter (mm), and dry matter content of leaves (%). In addition, the effect of the bacterial treatments on the element contents of leaves was evaluated.

In order to determine the mineral contents of leaves, plants samples were oven-dried at 68°C for 48 h and then grounded to pass 1 mm sieve. Macro- (P, K, Ca, Mg, S and Na) and micro-elements (B, Cd, Ni, Pb, Fe, Mn, Zn, and Cu) were determined after wet digestion of dried and ground subsamples using a HNO_3–H_2O_2 acid mixture (2:3, v/v) with three steps (first step: 145°C, 75% RF, 5 min; second step: 180°C, 90% RF, 10 min; third step: 100°C, 40% RF, 10 min) in a microwave oven (Bergof Speedwave Microwave Digestion Equipment MWS-2) (Mertens 2005a). Tissue P, K, Ca, Mg, S, Na B, Cd, Ni, Pb, Fe, Mn, Zn and Cu were determined by using an Inductively Couple Plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT, USA) (Mertens 2005b). The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N (Bremner 1996).

The experimental design was a completely randomized block design with 3 replications. ANOVA was applied on the data obtained in this study and the differences between means were compared by using Duncan's multiple range test ($P < 0.05$). There were no statistical differences between years in both 1st and 2nd harvest for elemental analyses; therefore, the data were pooled for only element contents. Data were analysed by SPSS 18.0.

RESULTS AND DISCUSSION

Data for the different treatments illustrating shoot length and diameter and, dry matter contents of leaves of mint are presented in **Table 2**. The mean value of shoot length and diameter of mint varied depending on treatments. As seen in **Table 2**, there was a numerical increase in shoot length in *P. putida* C3/101 and *P. polymyxa* RC105 according to control. And also, the lowest values of shoot diameter were determined in control and the highest values obtained in urea treatment in both first and second harvest during experiment years. When bacteria inoculations are taken into consideration, while the range of results obtained in first harvest for shoot length were between 28.6 cm (*P. putida* C3/101) and 35.4 cm (*P. polymyxa* RC105), the results for second harvest ranged from 15.7 cm (*P. putida* C3/101) to 18.5 cm (*P. polymyxa* RC105). Similarly, shoot diameter was ranged from 2.2 to 2.6 mm in application of *P. putida*

Table 1 Some biochemical characteristics of the bacterial strains C3/101 and RC105.

	***Pseudomonas putida* biotype B C3/101**	***Paenibacillus polymyxa* RC105**
Biolog test	61	27
Oxidase activity	S+	+
Catalase activity	+	+
N-free medium growth	S+	+
Sucrose test	S+	+
Growth in NBRIB-BPB	+	+
IAA production (µg ml^{-1})	8.2	6.4
ACC Deaminase activity	+	+
P solubilization	+	+

N: nitrogen, NBRIB-BPB: National Botanical Research Institute's Phosphate Growth Medium, IAA: indole-3-acetic acid, ACC: 1-aminocyclopropane-1-carboxylate, P: phosphate, S: strongly

Table 2 The effects of treatments on shoot length and diameter and, dry matter contents of leaves

	2009		**2010**	
	1st harvest	**2nd harvest**	**1st harvest**	**2nd harvest**
		Shoot length (cm)		
Control	23.8 b	13.0 b	28.3 b	11.4 c
Pap RC105	30.6 b	18.5 b	35.4 b	17.0 b
Psp C3/101	28.6 b	17.2 b	34.1 b	15.7 b
Urea	58.9 a	24.7 a	55.6 a	23.9 a
		Shoot diameter (mm)		
Control	1.9 c	1.4 b	2.1 c	1.6 b
Pap RC105	2.4 b	1.6 ab	2.3 c	1.9 a
Psp C3/101	2.2 bc	1.7 a	2.6 b	2.0 a
Urea	3.2 a	1.7 a	3.1 a	2.1 a
		Dry matter contents of leaves (%)		
Control	20.1 a	26.0 ^{NS}	21.9 ab	24.2 ^{NS}
Pap RC105	20.7 a	26.9	22.8 a	25.9
Psp C3/101	20.6 a	26.4	20.3 b	25.5
Urea	17.6 b	26.7	21.2 ab	27.9

Pap RC105: *Paenibacillus polymyxa* RC105, *Psp* C3/101: *Pseudomonas putida* biotype B C3/101

* Different letters within a column indicate significant differences among applications ($P < 0.05$).

** NS: Non significant at $P < 0.05$.

Table 3 The effects of treatments on element contents of leaves of mint.

	Mean of 2009 and 2010			
Elements	**Control**	***Pap* RC105**	***Psp* C3/101**	**Urea**
1st Harvest				
B (mg kg^{-1})	30.5 b	32.2 b	31.2 b	39.1 a
Ca (mg kg^{-1})	20702 c	23412 b	23564 b	28295 a
Cd (mg kg^{-1})	0.44 ab	0.54 a	0.17 b	0.21 b
Cu (mg kg^{-1})	29.8 b	26.9 b	29.8 b	34.6 a
Fe (mg kg^{-1})	499.7 b	545.0 b	630.3 a	518.8 b
K (mg kg^{-1})	42386 b	50222 a	50365 a	48370 a
Mg (mg kg^{-1})	6237.7 b	6482.5 ab	6967.5 ab	7865.2 a
Mn (mg kg^{-1})	57.3 b	61.7 b	62.1 b	73.0 a
Na (mg kg^{-1})	293.7 a	158.8 b	176.7 b	145.7 b
Ni (mg kg^{-1})	2.7 ^{NS}	2.9	2.9	2.7
P (mg kg^{-1})	3139 a	2863 ab	3101 ab	2713 b
Pb (mg kg^{-1})	57.5 b	70.4 a	41.2 bc	48.5 c
S (mg kg^{-1})	195.0 b	249.3 a	245.3 a	242.8 a
Zn (mg kg^{-1})	64.6 a	59.5 a	49.6 b	62.2 a
N (%)	2.1 b	2.4 a	2.4 a	2.4 a
2nd Harvest				
B (mg kg^{-1})	25.9 ^{NS}	25.2	25.4	33.3
Ca (mg kg^{-1})	19610 b	20543 b	26652 ab	29863 a
Cd (mg kg^{-1})	0.24 ^{NS}	0.16	0.25	0.14
Cu (mg kg^{-1})	22.7 a	18.4 b	23.2 a	21.0 ab
Fe (mg kg^{-1})	341.3 ab	275.2 b	443.2 a	369.2 ab
K (mg kg^{-1})	28733 b	29847 ab	32850 ab	35122 a
Mg (mg kg^{-1})	4498.7 b	5840.2 a	4311.2 b	5747.5 a
Mn (mg kg^{-1})	51.6 ab	42.7 b	62.4 a	58.5 a
Na (mg kg^{-1})	159.2 b	147.5 b	206.2 ab	245.5 a
Ni (mg kg^{-1})	1.7 ^{NS}	1.5	1. 7	1.9
P (mg kg^{-1})	3104 ^{NS}	3291	3371	2484
Pb (mg kg^{-1})	57.2 ^{NS}	55.9	64.2	65.5
S (mg kg^{-1})	228.7 b	273.7 b	232.2 ab	253.2 a
Zn (mg kg^{-1})	36.3 ^{NS}	33.3	42.1	39.6
N (%)	1.6 c	1.7 b	1.9 a	1.9 a

Pap RC105: *Paenibacillus polymyxa* RC105, *Psp* C3/101: *Pseudomonas putida* biotype B C3/101

* Different letters within a column indicate significant differences among applications ($P < 0.05$).

** NS: Non significant at $P < 0.05$.

C3/101 in the first harvest, and 1.6 mm (*P. polymyxa* RC105) to 2.0 mm (*P. putida* C3/101) in the second harvest. Although, there were significant differences between treatments concerning the average leaf dry matter in first harvest, there were no significant differences in second harvest. The urea treatment provided the highest leaf dry matter (27.9%) but there was no statistically significance between the control and bacterial applications in second harvest in 2010. On the other hand, when bacteria applications compared to the control, the highest leaf dry matter (22.8%) was obtained from *P. polymyxa* RC105 application in first harvest in 2010.

The response of agriculturally important crops to inoculation with PGPR was investigated in numerous experiments carried out in various countries. Based on given data, PGPR can affect plant growth in a number of ways and enhancement of vegetative and reproductive growth is documented in a range of crops such as vegetables, fruits or other crops. For instance, including production of plant hormones like auxins, cytokinins, gibberellins, and lowering of plant ethylene levels, N_2 fixing, phosphate solubilising can be provided positive effects on plant growth by PGPR (Glick 1995; Costacurta and Vanderleyden 1995; Lucy *et al.* 2004; Şahin *et al.* 2004). The plant growth enhancement effects of bacteria used in this work on mint could be explained by the similar reasons in mentioned studies.

The element composition of plant leaves treated by PGPR strains may provide important information about the effect of bacterial inoculation in plant nutrient element uptake (Orhan *et al.* 2006). Therefore, the effect of *P. polymyxa* RC105, *P. putida* C3/101 and urea on plant nutrient element uptake of mint were given in **Table 3**. In this study it was found that bacterial treatments and urea significantly increased element contents of mint leaves compared with the control except for Na, P and Zn. In particular, when bacterial treatments was taken into consideration, inoculation with both *P. polymyxa* RC105 and *P. putida* C3/101 promoted N, Ca, Cd, Fe, K, Mg, Mn, Pb and S uptake according to the control in both harvests. In addition, the highest Fe (630.3 mg kg^{-1}), K (50365 mg kg^{-1}) and S (249.3 mg kg^{-1}) contents in first harvest and the highest Fe (443.2 mg kg^{-1}), Mn (62.4 mg kg^{-1}) and S (273.7 mg kg^{-1}) contents in second harvest were obtained from bacterial inoculations when compared with both control and urea. The percentage of element uptake increase from bacterial inoculations for N in first and second harvests were 14% and 18% according to the control, respectively. According to Orhan *et al.* (2006) report, this increase may also explained by organic acids production by plants and bacteria in the rhizosphere, which decrease soil pH and stimulate the availability of Cd, Fe, Mn and Pb. It has also been recommended that PGPR may have increase uptake of elements such as N, Ca, K, and Mg near Cd, Fe, Mn and Pb via stimulation of the proton pump ATPase (Mantelin and Touraine 2004). These findings in the present study were supported by a number of previous studies (Eşitken *et al.* 2003; Mantelin and Touraine 2004; Eşitken *et al.* 2005; Orhan *et al.* 2006; Naveed *et al.* 2008; Yang *et al.* 2009).

The experiments in this study showed that treatments with of N_2-fixing plant growth promoting rhizobacteria *P. putida* C3/101 and *P. polymyxa* RC105 and urea application affected fresh and dry yield (**Table 4**). Although, the highest yield values were obtained with urea, significant fresh and dry yield increase was determined with *P. putida* C3/101 and *P. polymyxa* RC105 treatments as compared with control in both 1st and 2nd harvest. The highest values for fresh and dry yield were obtained at the 1st harvest of 2009 (2284.0 kg decare^{-1}) and, 2009 and 2010 (321.0 and 359.3 kg decare^{-1}) in urea application, respectively. Besides, the fresh and dry yields for the 1st harvests were higher than those of the 2nd harvests in both experiments years for all treatments. When the bacterial treatments were compared with control, the highest fresh yield (1130.7 kg decare^{-1}) in 2009 and the highest dry yield (231.3 kg decare^{-1}) in 2010 in first harvest were obtained with *P. polymyxa* RC105.

Table 4 The effects of treatments on fresh and dry yield of mint.

	2009			2010		
	1st harvest	**2nd harvest**	**Total**	**1st harvest**	**2nd harvest**	**Total**
Fresh Yield (kg decare^{-1})						
Control	897.3 c	451.3 c	1348.7 c	724.7 b	498.7 c	1223.3 c
Pap RC105	1130.7 b	622.7 b	1753.3 b	1294.7 ab	632.0 b	1926.7 b
Psp C3/101	986.0 bc	624.7 b	1610.7 b	952.0 b	653.3 b	1622.0 bc
Urea	2284.0 a	897.3 a	3181.3 a	1792.0 a	856.0 a	2648.0 a
Dry Yield (kg decare^{-1})						
Control	185.0 b	130.7 c	315.7 c	189.3 c	129.0 c	318.3 c
Pap RC105	230.0 b	172.7 b	402.7 b	231.3 b	158.0 b	389.3 b
Psp C3/101	186.3 b	175.3 b	361.7 bc	229.7 b	168.3 b	398.0 b
Urea	321.0 a	258.7 a	579.7 a	359.3 a	263.3 a	622.7 a

Pap RC105: *Paenibacillus polymyxa* RC105, *Psp* C3/101: *Pseudomonas putida* biotype B C3/101

* Different letters within a column indicate significant differences among applications ($P < 0.05$).

Table 5 Examples of promoting effect of PGPR in different vegetable species.

Promoting effect	Species	PGPR	References
marketable fruit yield	tomato (*Lycopersicum esculentum* L.)	*Pseudomonas fluorescens* strain 63-28	Gagné *et al.* 1993
root hair formation, total and upper nodule numbers	common bean (*Phaseolus vulgaris*)	*Azospirillum brasilense* Cd (10^7 CFU ml^{-1})	Burdman *et al.* 1996
root and shoot weight	sweet corn varieties (*Zea mays* L. var. *saccharata*)	*Pseudomonas putida*, *Gluconacetobacter azotocaptans*, *Azospirillum lipoferum* (10^8 cells mL^{-1})	Mehnaz and Lazarovits 2006
fruit yield	bell pepper (*Capsicum annuum* L.)	*Bacillus subtilis* GB03, *Bacillus amyloliquefaciens* IN937a, PGPR-containing product BioYield ™ (Bayer CropScience LP, Research Triangle Park, NC, USA)	Herman *et al*. 2008
root and shoot growth, and nodulation	pea (*Pisum sativum*)	*Pseudomonas*, *Bacillus*, *Kocuria*, *Microbacterium*, *Cellulomonas* species	Egamberdieva 2008
seed germination	radish (*Raphanus sativus* L.)	*Agrobacterium rubi* strain A16, *Burkholderia gladii* strain BA7, *Pseudomonas putida* strain BA8, *Bacillus subtilis* strain BA142, *Bacillus megaterium* strain M3 (10^8 cfu ml^{-1})	Kaymak *et al.* 2009
shoot dry mass of nematode-inoculated plants	tomato (*Solanum lycopersicum* L.)	*Burkholderia cepacia* 4684, *Bacillus subtilis* 7612 (1.5×10^7 cells ml^{-1})	Siddiqui and Akhtar 2009
fruit yield and essential oil	sweet fennel (*Foeniculum vulgare* var. dulce)	*Pseudomonas putida*, *Azotobacter chroococcum* (10^7 CFU mL^{-1})	Moradi *et al*. 2011

Otherwise, *P. polymyxa* RC105 was generally more effective than *P. putida* C3/101 for fresh and dry yield. When the total yield (1st + 2nd harvest) was considered for fresh and dry yield, the percentage of yield increase for *P. polymyxa* RC105 was 29% (2009) and 57% (2010) for fresh yield according to the control. Similarly, the percentage of yield increase for dry yield was 28% (2009) and 22% (2010) when *P. putida* C3/101 was applied.

Bashan *et al.* (2004) reported that *Azospirillum* was the first microorganism suggested to promote the growth of plants by N_2-fixation. Similarly, recent studies have confirmed that a number of bacterial species (PGPR) such as *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Serratia*, *Rhizobium*, etc. mostly associated with the plant rhizosphere, have been found to be beneficial for plant growth, yield and crop quality (Eşitken *et al.* 2010). On the other hand, some greenhouse and field experiments have shown repeatedly that the transfer of nitrogen fixed by PGPR to the plant is not enough and cannot fulfil all of the nitrogen requirements of the plants, nevertheless contribute significant amounts of nitrogen (Bashan and Holguin 1997; Bashan *et al.* 2004). These findings clearly explained that yield obtained from bacteria inoculation was lower than urea treatment but more than control. Moreover, previous studies with PGPR were tested on different vegetable species such as tomato or sweet fennel have been reported similar findings presented in **Table 5** confirming results of this work.

CONCLUSIONS

The results of the present work suggested that N_2-fixing and also phosphate solubilising rhizobacteria *P. putida* C3/101 and *P. polymyxa* RC105 have potential to increase the yield, growth and N, Ca, Fe, K, Mg, Mn and S content of leaves of mint (*Mentha piperita* L.). The high-input agriculture practices achieving high yields have created seriously environmental problems and degradation in natural resources such as global nitrogen cycle and increases risk of chemical spills with large amount usage of synthetic fertilizers and their high production costs are taken into consideration, the bacteria tested in this study is a promising alternative as a bio-fertilizer for environmental friendly mint and vegetables production.

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