



Influence of Bacteria Isolated from Different Ecological Zone of Turkey on Maize Growth and Nutrient Uptake[#]

Amer Abdulhadi Jawad^{1,a}, Ali Coşkan^{1,b,*}

¹Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Isparta University of Applied Sciences, 32000 Isparta, Turkey

*Corresponding author

ARTICLE INFO	ABSTRACT
<p>[#]This study derived from PhD thesis of Amer Abdulhadi JAWAD and presented as an oral presentation at the 13th National, 1st International Field Crops Conference (Antalya, TABKON 2019)</p> <p><i>Research Article</i></p> <p>Received : 24/11/2019 Accepted : 05/12/2019</p> <p>Keywords: PGPR Bacteria isolates Maize Biofertilizer Maize nutrition</p>	<p>The aim of this study was to find potential PGPR from sub-forest soil located different region soils of Turkey. Previous research indicated that the existing bacteria in arable soil are not capable to represent their individual performance most probably due to the competition. To overcome this phenomenon, soils are collected from sub-forest soil of Adana (Ad), Antalya (An), Hatay (Ha), Isparta (Is), Ordu (Or) and Sivas (Si) provinces. Experiment was carried out on the soil existing in Isparta in a greenhouse condition. Four fast growing bacteria colonies in tryptic soy (CASO) agar medium from each province were isolated and then, each isolate cultivated at liquid CASO broth until they reach 10⁶ cfu ml⁻¹. Experiments were carried out with a total of 24 bacteria including 6 province and 4 bacteria cultures from each region. The effects of those bacteria on biomass development and nutrient uptake of maize (<i>Zea mays</i>) were investigated. Sterile broth was applied treatment defined as control. The results revealed that 23 isolates out of 24 stimulated plants shoot dry weight. The highest value observed in the Or1 and Is4 isolates as 12.8 and 12.7 g plant⁻¹ which around 77% higher than control whereas the lowest was in Or2 as 6.45 g plant⁻¹. Plant nutrient concentrations were also influenced from inoculates where An1, Ad1, Or1, Is1 and Is3 significantly increased macro nutrients uptake where total N, available P, K, Ca and Mg were higher by 19%, 14%, 14%, 59% and 41% over the control, respectively. The Fe concentration was found 48% higher in Ad3 isolate. The Cu, Mn and Zn were the highest in Si3 as 43%, 30% and 31%, respectively. In general 4 out of 24 isolates were selected as promising PGPR for both plant development and nutrient uptake of maize.</p>

^a aa70ir@yahoo.com

^{id} <https://orcid.org/0000-0003-4295-5078>

^b alicoskan@isparta.edu.tr

^{id} <https://orcid.org/0000-0001-5473-3515>



This work is licensed under Creative Commons Attribution 4.0 International License

Introduction

“Plant Growth Promoting Rhizobacteria” which is shortened as PGPR term was first used in 1978 (Kloepper and Schroth, 1978). Since 1978, many researchers have interested in this topic in many countries around the world. The PGPR refers to bacteria that a beneficial effect on plant growth and nutrient uptake. Rhizosphere is a region where the biological activity is maximum, is a closed food pool containing all the macro and micro nutrients necessary for the plants (Vejan et al., 2016). PGPR have a series of mechanisms to stimulate plant growth or nutrient uptake. These mechanisms are referred as direct and indirect effects in the literature. Direct mechanisms are symbiotic and non-symbiotic nitrogen fixation (Hubbell and Kidder, 2003; Pereg et al., 2016; Ghaly and Alanos, 2016; Youseif, 2018; Richard et al., 2018; Fukami et al., 2018;) production of phytohormones such as auxin, gibberellin and cytokine, preventing ethylene production via 1-aminosiklopropan-1-karboksilat (ACC) deaminase activity, reducing environmental stress factors (Dar et al., 2018; Glick 2014;

Ali and Kim, 2018) increasing the solubility of inorganic phosphorus and mineralization of organic phosphorus compounds (Richardson et al., 2009; Zaidi et al., 2009; Zhang et al., 2014; Khosravi et al., 2018; Singh and Gera, 2018), increasing K uptake (Meena and Verma, 2014) and producing siderophore to improve Fe carriage to inner root zone (Patel et al., 2018; Dimkpa et al., 2009; Sandy and Butler, 2009). Indirect mechanisms include the reduction of the harmful effects of plant pathogens via enzyme production such as chitinase, cellulase, 1,3 glucanase, protease, lipase (Kundan et al., 2015), antibiotic secretion (Labuschagne et al., 2010) and inhibiting the establishment of phytopathogens by sequestration iron from the environment (Glick, 2014). Several studies have shown the potential of PGPR to increase plant growth (plant height, stem diameter, dry biomass of shoot, root length and root dry weight) of maize also increase grain yield (Gholami et al., 2009; Morais et al., 2016; Agbodjato et al., 2016; Ghaly and Alanos, 2016; Mosimann et al., 2017).

Egamberdiyeva (2007) reported that the beneficial effects of PGPR better stimulating plant growth and nutrient uptake of maize in nutrient deficient calcisol soil than rich nutrient loamy sand soil. Hence this study was conducted to isolate possible PGPRs that capable to promote growth and nutrient uptake on maize.

Materials and Methods

Bacteria Isolation

Bacteria were isolated from the soils collected from soils under forest located 6 different province as Ordu (Or) in the north; Sivas (Si) in the middle; Hatay (Ha) and Adana (Ad) in the south; Antalya (An) and Isparta (Is) in the south west of Turkey. Ten grams of soil placed to erlenmayer flask containing 90 ml of sterile saline solution (0.85% NaCl). After shaking 30 min at 200 rpm, this solution was diluted repeatedly to reach 10^6 dilution level. From each dilution level a 1 ml of samples inoculated to tryptic soy broth agar (TSB) culture media (Ottow, 1984). Petri dishes were placed in the incubator for 24 h at 28°C, all plates were observed and the fastest growing 4 colonies representing each region were purified by streaking into new petri dishes to get the pure colony. Each isolates was transferred onto agar slant (TSB) and kept in the refrigerator at 4°C for maintain a stock of pure culture for the next subsequent experiment. When needed 24 pure cultures in slant were transferred to 250 ml flask containing 100 ml nutrient broth (TSB) and cultivated aerobically on a rotating shaker at 140 rpm for 24 h at 28°C (Merck KGaA, Germany). From these suspensions which containing at least 10^6 cfu ml⁻¹, 1 ml applied to the soil, 2 cm around the stem when plant height reach approximately 20 cm. Sterile nutrient broth liquid media was applied to the untreated control (ctrl).

Pot Experiment

The 75 pots that have 5.5 liter capacity were filled by the soil collected from Agricultural Research Station of Suleyman Demirel University. Experiment was started at 19/01/2018 and harvested after two months of sowing. Completely randomized design with three replicate was used in the experiment. All treatments including control was fertilized by 150 mg kg⁻¹ N, 100 mg kg⁻¹ P₂O₅ and 100 mg kg⁻¹ K₂O as 0.82 g MAP, 2 g NH₄NO₃, 1 g K₂SO₄ before sowing the seed. To each pot 5 maize (*Zea mays*) seeds were sown and thinned to one plant after 3 leaves development. All pots were irrigated considering water holding capacity. The properties of soil used for trial was clay loam with 31.2% sand, 34.6% silt and 34.1% clay contents. The pH (1:2.5 soil/water), E.C, organic matter, total N, available P and K were 7.8, 1.01 ds m⁻¹, 1.9%, 0.17%, 78.3 and 458 mg kg⁻¹ respectively.

Plant Measurement and Analysis

After 60 days of the seed sowing, plant height was measured from the base to top of leaf with measuring tape. Stem diameter was measured followed a manual caliper (both sides of stem measured and average reading were taken). Plant samples were collected by cutting plants from soil surface, harvested and roots were cleaned by washing with tap water then washing by purified water. Plant samples and roots were dried at 80°C until constant weight obtained, and dry weight was recorded. Shoot samples were dried and

grinded, afterwards macro (P, K, Mg and Ca) and micro element (Fe, Cu, Mn and Zn) contents were analyzed by digestion of 0.5 g of samples at microwave oven (CEM-MARS Reaction System) with acid mixture (nitric and perchloric acid). Out of N and P the element contents were determined via atomic absorption spectrophotometer (Kacar and Inal 2010). Phosphorus content was measured using spectrophotometer at 420 nm, according to Barton (1948). Total nitrogen (N%) was determined according to Kjeldahl method (Kacar and Inal 2010) which 0.5 g from fine grinded sample with 10 ml of concentrated sulfuric acid, digested at 400°C until the mixture become clear.

Statistical Analysis

Analysis of variance was performed using MSTAT-C software (Crop and Soil Science Department, Michigan State University, Version 1.2) according to randomized block design. Duncan test was applied to determine the differences among the mean of three replication at P<0.05.

Results and Discussion

Effects of PGPR on Growth Parameters

In this study plant growth has been investigated as vegetative growth parameters such as plant height (cm), plant stem diameter (mm), shoot dry weight (g) and root dry weight (g). Obtained results presented in Table 1.

All the isolates except Isparta were increased plant height statistically. There was no difference between bacteria isolated from Isparta and the control application by mean of plant height. The highest plant height was observed at Si3 as 127.3 cm which was 24.8% higher than control. The effects of the bacteria on plant height was found to be statistically significant (P<0.05). Molina et al. (2017) reported 22% improvement on plant height in case of bacteria inoculation at their 45 days experiment at greenhouse conditions. Additionally, a number of researchers are reported longer plants as a result of PGPR inoculation (Agbodjato et al., 2016; Ghaly et al., 2016; Jarak et al., 2012).

No significant differences were observed between mean stem diameter values at different region isolates. The highest statistically significant stem diameter value was observed at Or1 isolates as 16.1 mm whereas the lowest value was at Ha4 isolates as 12.4 mm. The stem diameter value at Or1 was 22% higher than control treatment. Similar results repeatedly reported by the researchers (Lin et al., 2018; Picazevicz et al., 2017, Chattha et al., 2017 and Gholami et al., 2012) where Molina et al. (2017) measured 12% thicker stem at PGPR inoculated conditions.

Significant shoot dry weight found in Or1, Is4 and An4 isolates. The increases were around 77%, 77% and 75%, respectively over the control treatment. Root dry weights were also influenced by bacteria inoculation where Is2 and Or1 provided 59.7% and 52.9% higher root development than control treatment. In this context, Gholami et al. (2012) reported *Azotobacter* s-5 + *Azospirillum* s-21 inoculated maize seeds significantly increased the stem height by 17% and stem diameter by 28% and increased ear dry weight up to 115% under field condition. Similarly following the inoculation of three rhizobacteria combinations had induced growth of maize plants with an increase for about 17%, Agbodjato et al. (2016).

Table 1 Plant height, stem diameter, shoot and root dry weight values

	Ad	An	Ha	Is	Or	Si	Ctrl
Plant height (cm)							
1	111 ^{abc}	112 ^{abc}	122 ^{abc}	98 ^{bc}	125 ^{abc}	116 ^{abc}	102 ^{bc}
2	119 ^{abc}	112 ^{abc}	123 ^{abc}	108 ^{abc}	105 ^{abc}	120 ^{abc}	102 ^{bc}
3	115 ^{abc}	118 ^{abc}	113 ^{abc}	93 ^c	118 ^{abc}	127 ^a	102 ^{bc}
4	113 ^{abc}	120 ^{abc}	111 ^{abc}	114 ^{abc}	111 ^{abc}	121 ^{ab}	102 ^{bc}
\bar{x}	115 ^A	116 ^A	117 ^A	103 ^B	116 ^A	121 ^A	102 ^B
Stem diameter (mm)							
1	13.7 ^{ab}	13.9 ^{ab}	12.7 ^{ab}	13.5 ^{ab}	16.1 ^a	13.0 ^{ab}	13.2 ^{ab}
2	13.8 ^{ab}	12.9 ^{ab}	14.0 ^{ab}	14.9 ^{ab}	12.6 ^{ab}	13.5 ^{ab}	13.2 ^{ab}
3	13.9 ^{ab}	13.8 ^{ab}	14.5 ^{ab}	13.1 ^{ab}	13.9 ^{ab}	12.5 ^{ab}	13.2 ^{ab}
4	14.7 ^{ab}	14.8 ^{ab}	12.4 ^b	14.4 ^{ab}	14.1 ^{ab}	13.2 ^{ab}	13.2 ^{ab}
\bar{x}	14.0 ^A	13.9 ^A	13.4 ^A	14.0 ^A	14.2 ^A	13.1 ^A	13.2 ^A
Shoot dry weight (g)							
1	8.6 ^{ab}	8.8 ^{ab}	9.3 ^{ab}	8.3 ^{ab}	12.8 ^a	7.5 ^{ab}	7.2 ^{ab}
2	10.8 ^{ab}	8.6 ^{ab}	11.7 ^{ab}	11.6 ^{ab}	6.5 ^b	8.7 ^{ab}	7.2 ^{ab}
3	10.2 ^{ab}	9.8 ^{ab}	10.0 ^{ab}	7.4 ^{ab}	10.7 ^{ab}	8.4 ^{ab}	7.2 ^{ab}
4	9.6 ^{ab}	12.6 ^a	8.9 ^{ab}	12.7 ^a	8.5 ^{ab}	8.4 ^{ab}	7.2 ^{ab}
\bar{x}	9.8 ^A	9.9 ^A	10.0 ^A	10.0 ^A	9.6 ^A	8.2 ^B	7.2 ^C
Root dry weight (g)							
1	2.39 ^{abc}	1.71 ^{abc}	2.29 ^{abc}	2.07 ^{abc}	3.15 ^{ab}	1.72 ^{abc}	2.06 ^{abc}
2	2.69 ^{abc}	1.42 ^c	2.53 ^{abc}	3.29 ^a	1.62 ^{bc}	1.99 ^{abc}	2.06 ^{abc}
3	2.40 ^{abc}	1.87 ^{abc}	2.09 ^{abc}	1.84 ^{abc}	2.03 ^{abc}	2.42 ^{abc}	2.06 ^{abc}
4	2.04 ^{abc}	3.02 ^{abc}	2.40 ^{abc}	2.98 ^{abc}	2.43 ^{abc}	2.42 ^{abc}	2.06 ^{abc}
\bar{x}	2.38 ^A	2.01 ^A	2.33 ^A	2.54 ^A	2.31 ^A	2.14 ^A	2.06 ^A

Table 2 The N, P, K, Mg and Ca concentration of maize shoot

	Adana	Antalya	Hatay	Isparta	Ordu	Sivas	Control
Nitrogen (%)							
1	2.92 ^{ab}	2.97 ^a	2.92 ^{ab}	2.60 ^{b-e}	2.74 ^{a-e}	2.94 ^{ab}	2.50 ^{cde}
2	2.91 ^{ab}	2.69 ^{a-e}	2.88 ^{ab}	2.46 ^e	2.79 ^{a-e}	2.78 ^{a-e}	2.50 ^{cde}
3	2.93 ^{ab}	2.85 ^{ab}	2.88 ^{ab}	2.50 ^{cde}	2.77 ^{a-e}	2.80 ^{a-d}	2.50 ^{cde}
4	2.71 ^{a-e}	2.81 ^{a-d}	2.90 ^{ab}	2.50 ^{cde}	2.94 ^{ab}	2.84 ^{abc}	2.50 ^{cde}
\bar{x}	2.87 ^A	2.83 ^A	2.89 ^A	2.52 ^B	2.81 ^A	2.84 ^A	2.50 ^B
Phosphorus (%)							
1	0.193 ^a	0.176 ^{a-d}	0.188 ^{abc}	0.141 ^{de}	0.191 ^{ab}	0.177 ^{a-d}	0.169 ^{a-e}
2	0.159 ^{abc}	0.150 ^{cde}	0.163 ^{a-e}	0.173 ^{a-e}	0.156 ^{a-e}	0.155 ^{a-e}	0.169 ^{a-e}
3	0.155 ^{a-e}	0.161 ^{a-e}	0.162 ^{a-e}	0.153 ^{a-e}	0.169 ^{a-e}	0.182 ^{abc}	0.169 ^{a-e}
4	0.135 ^e	0.189 ^{abc}	0.168 ^{a-e}	0.159 ^{a-e}	0.171 ^{a-e}	0.172 ^{a-e}	0.169 ^{a-e}
\bar{x}	0.160 ^A	0.169 ^A	0.170 ^A	0.156 ^B	0.172 ^A	0.171 ^A	0.169 ^A
Potassium (%)							
1	3.86 ^{c-f}	4.97 ^{ab}	3.03 ^{fg}	3.16 ^{efg}	5.46 ^a	4.21 ^{bcd}	4.81 ^{abc}
2	3.80 ^{def}	4.98 ^{ab}	4.98 ^{ab}	2.82 ^g	4.95 ^{ab}	4.72 ^{abc}	4.81 ^{abc}
3	3.52 ^{d-g}	3.97 ^{cde}	4.95 ^{ab}	3.01 ^{fg}	5.03 ^{ab}	4.19 ^{bcd}	4.81 ^{abc}
4	4.25 ^{bcd}	4.00 ^{cde}	4.97 ^{ab}	3.06 ^{fg}	5.01 ^{ab}	4.15 ^{bcd}	4.81 ^{abc}
\bar{x}	3.86 ^C	4.48 ^B	4.48 ^B	3.01 ^D	5.11 ^A	4.32 ^B	4.81 ^{AB}
Calcium (%)							
1	1.63 ^c	1.88 ^{bc}	1.93 ^{bc}	3.14 ^a	1.67 ^c	1.80 ^c	1.98 ^{bc}
2	1.77 ^c	2.11 ^{bc}	1.75 ^c	1.87 ^{bc}	1.93 ^{bc}	1.52 ^c	1.98 ^{bc}
3	1.80 ^c	1.76 ^c	2.13 ^{bc}	2.83 ^{ab}	1.69 ^c	1.83 ^c	1.98 ^{bc}
4	2.03 ^{bc}	1.56 ^c	2.12 ^{bc}	1.70 ^c	2.04 ^{bc}	1.78 ^c	1.98 ^{bc}
\bar{x}	1.81 ^B	1.83 ^B	1.98 ^B	2.39 ^A	1.83 ^B	1.73 ^B	1.98 ^B
Magnesium (%)							
1	0.285 ^{a-g}	0.244 ^{c-h}	0.268 ^{q-b-h}	0.338 ^a	0.297 ^{a-d}	0.235 ^{d-h}	0.240 ^{c-h}
2	0.263 ^{b-h}	0.241 ^{c-h}	0.230 ^{fgh}	0.293 ^{a-f}	0.282 ^{a-g}	0.218 ^h	0.240 ^{c-h}
3	0.303 ^{abc}	0.235 ^{d-h}	0.262 ^{b-h}	0.333 ^a	0.294 ^{a-e}	0.252 ^{b-h}	0.240 ^{c-h}
4	0.295 ^{a-d}	0.232 ^{e-h}	0.244 ^{c-h}	0.310 ^{ab}	0.296 ^{a-d}	0.229 ^{gh}	0.240 ^{c-h}
\bar{x}	0.286 ^B	0.238 ^C	0.251 ^C	0.318 ^A	0.292 ^{AB}	0.234 ^C	0.240 ^C

A greenhouse pot study conducted by Lin et al. (2018) showed that maize plant inoculation with PGPR at tassel stage increases in shoot biomass of 36.4% and root biomass of 56.4%, also increased plant height 9.5% compared to non-PGPR application. Under greenhouse condition Calvo et al. (2017) found that, by microbial-based treatments provide a significant increase in plant growth parameters such as plant height by 26%, stem width by 34%, shoot dry weight by 57% and root dry weight up to 9% compared to the control. These results are in line with previous findings of Chattha et al. (2017) that they reported sorghum plant inoculated with PGPR increased stem diameter up to 20%, plant height up to 7% and ear dry weight up to 9%, under field conditions.

Macro Nutrient Concentrations

The effect of PGPR inoculation on macro nutrient uptake by maize plant was assessed after 60 days of sowing and the results are presented in Table 2. The nitrogen content of the plants was positively affected by bacterial inoculation. The highest N concentration was in An1 isolates as 2.97% which was higher than the control up to 18.8% ($P < 0.05$). Ad1 was the most stimulant isolate in term of shoot P concentration. Determined P was 0.193% which was 14% higher than the control application. There was no accordance over the isolates in terms of nutrient concentration. For each element one of the isolates showed the higher values than the others. Considering this conclusion, for each limited nutrient condition, one of the specific isolate should be used to prevent nutritional disorders. The K concentration was also statistically influenced by bacteria inoculation where the highest value was in Or1 with 5.46% which was 15% higher than the

control. According to Table 2, Is1 and Is3 isolates provides higher Mg concentration with 0.34% and 0.33% respectively. Those isolates improved Mg concentration up to 42%. Calcium content in shoot biomass of maize plant was positively affected by bacterial inoculated pots significantly ($P < 0.05$). The highest Ca concentration was observed in the applications with isolates Is1 by 3.14% which is higher than the control up to 59% (Table 2).

In accordance with our results, Calvo et al. (2017) found that, when maize plants were evaluated at 72 days after planting, plant N concentration by microbial-based treatments up to 54%, increasing P concentration by 138% and average increasing K concentration was 71% more than the control treatment. Hussain et al. (2016) reported that, due to rhizobial inoculation of maize plant, under well-watered conditions, have induced uptake of the NPK contents of shoot by 34%, 31% and 27% respectively compared to control. In consistent with the result presented here, Rojas et al. (2012) reported increases on accumulation of nutrients (K, Ca and Mg) in biomass due to the inoculation with *Azotobacter* sp. up to 38%, 18% and 78% respectively under salt stress. Increment on macronutrient uptake is also reported by Gulnaz et al. (2017) that maize plant inoculation with PGPR (*P. fluorescens* + *B. megaterium* + *A. brasilense*) increased nitrogen, phosphorus and potassium uptake by 13%, 20% and 54% respectively over the control. Moreover, Agbodjato et al (2016) stated that maize plants treated with *A. lipoferum* and their combination with chitosan *Pseudomonas* bacteria was increased nitrogen by 42%, phosphorus 7% and potassium 6% content in the aerial part after 30 days over the control.

Table 3 The Fe, Cu, Mn ve Zn concentration of maize shoot

	Adana	Antalya	Hatay	Isparta	Ordu	Sivas	Control
Iron ($\mu\text{g g}^{-1}$)							
1	43.2 ^{a-d}	41.9 ^{a-d}	59.9 ^{ab}	51.3 ^{a-d}	43.1 ^{a-d}	47.0 ^{a-d}	41.7 ^{a-d}
2	46.7 ^{a-d}	34.6 ^{cd}	42.6 ^{a-d}	40.9 ^{a-d}	47.2 ^{a-d}	40.9 ^{a-d}	41.7 ^{a-d}
3	61.6 ^a	31.7 ^d	39.9 ^{bcd}	55.9 ^{abc}	51.0 ^{a-d}	47.1 ^{a-d}	41.7 ^{a-d}
4	49.9 ^{a-d}	44.9 ^{a-d}	46.6 ^{a-d}	45.6 ^{a-d}	51.0 ^{a-d}	56.2 ^{ab}	41.7 ^{a-d}
\bar{x}	50.3 ^A	38.3 ^B	47.3 ^A	48.4 ^A	48.1 ^A	47.8 ^A	41.7 ^B
Zinc ($\mu\text{g g}^{-1}$)							
1	55.3 ^{b-f}	44.1 ^f	61.3 ^{a-d}	50.1 ^{def}	46.6 ^{ef}	57.2 ^{b-f}	53.1 ^{c-f}
2	43.8 ^f	45.3 ^{ef}	49.4 ^{def}	42.8 ^f	44.9 ^{ef}	47.0 ^{ef}	53.1 ^{c-f}
3	45.5 ^{ef}	47.1 ^{ef}	46.2 ^{ef}	64.5 ^{abc}	48.0 ^{ef}	69.5 ^a	53.1 ^{c-f}
4	53.8 ^{c-f}	53.2 ^{c-f}	53.0 ^{c-f}	54.2 ^{c-f}	49.0 ^{def}	66.5 ^{ab}	53.1 ^{c-f}
\bar{x}	49.6 ^B	47.4 ^B	52.5 ^B	52.9 ^B	47.1 ^B	60.0 ^A	53.1 ^B
Copper ($\mu\text{g g}^{-1}$)							
1	5.58 ^{efg}	4.65 ^{fgh}	9.90 ^{bc}	3.02 ^{ghi}	10.10 ^{bc}	8.92 ^c	9.48 ^{bc}
2	1.60 ⁱ	3.43 ^{f-i}	7.98 ^{cde}	2.98 ^{ghi}	9.03 ^c	8.20 ^{de}	9.48 ^{bc}
3	2.05 ^{hi}	9.80 ^{bc}	8.43 ^{cd}	2.37 ^{hi}	8.47 ^{cd}	13.60 ^a	9.48 ^{bc}
4	5.80 ^{def}	12.10 ^{ab}	7.95 ^{cde}	2.25 ^{hi}	9.05 ^c	11.90 ^{ab}	9.48 ^{bc}
\bar{x}	3.76 ^D	7.48 ^C	8.57 ^B	2.65 ^E	9.17 ^B	10.70 ^A	9.48 ^B
Manganase ($\mu\text{g g}^{-1}$)							
1	80 ^{a-d}	85 ^{a-d}	89 ^{a-d}	66 ^{bcd}	80 ^{a-d}	95 ^{ab}	80 ^{a-d}
2	71 ^{bcd}	83 ^{a-d}	85 ^{a-d}	62 ^{cd}	80 ^{a-d}	92 ^{abc}	80 ^{a-d}
3	78 ^{a-d}	82 ^{a-d}	78 ^{a-d}	67 ^{bcd}	90 ^{a-d}	104 ^a	80 ^{a-d}
4	61 ^d	93 ^{ab}	88 ^{a-d}	63 ^{cd}	88 ^{a-d}	92 ^{abc}	80 ^{a-d}
\bar{x}	73 ^{BC}	86 ^{AB}	85 ^{AB}	64 ^C	84 ^{AB}	96 ^A	80 ^B

Micro Nutrient Concentrations

The micro nutrient concentration values of the maize plant are presented in Table 3. Results revealed that the bacterial inoculations were effective on enhancing and stimulating plant micro nutrient uptake. Fe concentration was $61.6 \mu\text{g g}^{-1}$ at the highest in Ad3, which was higher up to 48% compared to the control. Cu, Mn and Zn concentration in shoot were significantly ($P < 0.05$) increased by the application of isolate Si3. The values were 13.6, 104 and $69.5 \mu\text{g g}^{-1}$ for Cu, Mn and Zn respectively. That values were higher than the control up to 44%, 30% and 31%. In consistent with our results, Biari et al. (2008) reported that nutrient uptake of the plants increased due to the bacteria inoculation. The rate of differences were +130% for nitrogen, +113% for phosphorus, +100% for potassium, +153% for iron, +107% for zinc, +147% for manganese and +127% for copper. None of the inoculant was reduced plant nutrient uptake. Moreover, Yolcu et al. (2012) reported higher macro and micro nutrient uptake in case of 12 different plant growth-promoting rhizobacteria inoculations. Ekinci, et al. (2014) reported that Fe, Cu, Mn and Zn were higher PGPR applied cauliflower plants by 31%, 15%, 20% and 10% compared to control. Turan and Sahin (2013) found that, inoculation of barley with OSU-142 + M3 + *Azospirillum* sp.245 were tightly increased uptake of macro-nutrients (N, P, K, Ca, Mg and S) and micro-nutrients (Fe, Mn, Zn, and Cu) of grain, leaf, and straw part of plant compared to the control.

Conclusion

From 24 bacterial isolated from of sub-forest soil tested to capable to induce growth and nutrient uptake of maize. The results indicated that some of PGPR isolates had a positive effect on the growth, and some of isolates increased uptake of macro and micro-element of maize plant, recommended that these isolates could be used as biofertilizer and sustainable agriculture and could be use an alternative fertilizer, in future these isolates should be teste in the field applications and with the different crops.

Results revealed that forest soil is highly promising origin to find PGPR that effective on plant growth and nutrient uptake. PGPRs are either not existing in arable soil or they are not capable to present their effects as Mutlu and Coskan (2018) reported. In both cases, inoculation with the bacteria isolated from forest soil seems to be good idea to improve soil biological productivity.

References

- Agbodjato NA, Noumavo PA, Adjanohoun A, Agbessi L, Baba-Moussa L. 2016. Synergistic effects of plant growth promoting rhizobacteria and chitosan on in vitro seeds germination, greenhouse growth, and nutrient uptake of maize (*Zea mays* L.). *Biotechnology research international*, Article ID 7830182, 11:2016.
- Ali S, Kim WC. 2018. Plant growth promotion under water: Decrease of water logging-induced ACC and ethylene levels by ACC deaminase-producing bacteria. *Frontiers in microbiology*, 9.
- Barton CJ. 1948. Photometric analysis on phosphate rock. *Ind. And. Eng. Chem. Anal. Ed.* 20:1068-1073.
- Biari A, Gholami A, Rahmani HÁ. 2008. Growth promotion and enhanced nutrient uptake of maize (*Zea mays* L.) by application of plant growth promoting rhizobacteria in arid region of Iran. *J. Biol. Sci.* 8(6):1015-1020.
- Calvo P, Watts DB, Klopper JW, Torbert HA. 2017. Effect of microbial-based inoculants on nutrient concentrations and early root morphology of corn (*Zea mays*). *Journal of Plant Nutrition and Soil Science*, 180(1):56-70.
- Chattha MB, Iqbal A, Chattha MU, Hassan MU, Khan I, Ashraf I, Faisal M, Usman M. 2017. PGPR Inoculated-Seed Increases the Productivity of Forage Sorghum under Fertilized Conditions. *Journal of Basic and Applied Sciences*, 13:150-153.
- Dar ZM, Masood A, Mughal AH, Asif M, Malik MA. 2018. Review on drought tolerance in plants induced by plant growth promoting rhizobacteria. *International Journal of Current Microbiology and Applied Sciences*, 7:(5), ISSN: 2319-7706.
- Dimkpa C, Weinand T, Asch F. 2009. Plant-rhizobacteria interactions alleviate abiotic stress conditions. *Plant Cell Environ.* 32, 1682–1694.
- Egamberdiyeva D. 2007. The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. *Applied Soil Ecology*, 36(2-3): 184-189.
- Ekinci M, Turan M, Yildirim E, Güneş A, Kotan R, Dursun A. 2014. Effect of plant growth promoting rhizobacteria on growth, nutrient, organic acid, amino acid and hormone content of cauliflower (*Brassica oleracea* l. var. botrytis) transplants. *Acta Scientiarum Polonorum Hortorum Cultus*, 13(6): 71-85.
- Fukami J, Cerezini P, Hungria M. 2018. *Azospirillum*: benefits that go far beyond biological nitrogen fixation. *AMB Express*, 8(1):73.
- Ghaly FM, Mosaad ISM, Alanos MATE. 2016. Effect of Bio Fertilizer, Nitrogen and Sulfur Levels on Maize Production in Saline Soil of North Delta of Egypt. *J. Soil Sci. and Agric. Eng., Mansoura Univ.*, 7(8): 541-546.
- Gholami A, Biyari A, Gholipoor M, Asadi Rahmani H. 2012. Growth promotion of maize (*Zea mays* L.) by plant-growth-promoting rhizobacteria under field conditions. *Communications in soil science and plant analysis*, 43(9): 1263-1272.
- Gholami A, Shahsavani S, Nezarat S. 2009. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *Int J Biol Life Sci*, 5(1): 35-40.
- Glick BR. 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiological research*, 169(1): 30-39.
- Gulnaz Y, Fathima PS, Denesh GR, Kulmitra AK, Shivraj Kumar HS. 2017. Effect of plant growth promoting rhizobacteria (PGPR) and PSB on root parameters, nutrient uptake and nutrient use efficiency of irrigated maize under varying levels of phosphorus. *Journal of Entomology and Zoology Studies*; 5(6): 166-169.
- Hubbell DH, Kidder G. 2003. Biological Nitrogen Fixation. Fact sheet document SL-16, of the Soil and Water Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.
- Hussain MB, Mehmood S, Zahir ZA, Naveed M, Imran M, Ahmad I, Ahmed N, Nawaz H. 2016. Modulating nutrition, physiology and production of maize through *Rhizobium phaseoli* and *Mesorhizobium ciceri* inoculation under drought stress conditions. 7 th International Conference on Water Resources and Arid Environments (ICWRAE 7): 549-559.
- Jarak M, Mrkovački N, Bjelić D, Joscaron D, Hajnal-Jafari T, Stamenov D. 2012. Effects of plant growth promoting rhizobacteria on maize in greenhouse and field trial. *African Journal of Microbiology Research*, 6(27): 5683-5690.

- Kacar B, Inal A. 2010. Plant Analyses. Nobel Academic Publish: LTD.ŞTİ. 892s, Ankara.
- Khosravi A Zarei M and Ronaghi A 2018. Effect of PGPR, Phosphate sources and vermicompost on growth and nutrients uptake by lettuce in a calcareous soil. Journal of Plant Nutrition, 41(1): 80-89.
- Kloepper JW. 1978. Plant growth-promoting rhizobacteria on radishes. In Proc. of the 4th Internat. Conf. on Plant Pathogenic Bacter, Station de Pathologie Vegetale et Phytobacteriologie, INRA, Angers, France, 1978 (2): 879-882.
- Kundan R, Pant G, Jadon N, Agrawal PK. 2015. Plant growth promoting rhizobacteria: mechanism and current prospective. J Fertil Pestic, 6(2), p.9.
- Labuschagne N, Pretorius T, Idris AH. 2010. Plant growth promoting rhizobacteria as biocontrol agents against soil-borne plant diseases. In: Maheshwari, D.K. (Ed.), Plant Growth and Health Promoting Bacteria. Springer Verlag, Berlin, Heidelberg, pp. 211–230.
- Lin Y, Watts DB, Kloepper JW, Torbert HA. 2018. Influence of Plant Growth-Promoting Rhizobacteria on Corn Growth Under Different Fertility. Sources Communications in Soil Science and Plant Analysis, 49(10): 1239-1255.
- Meena VS, Maurya BR, and Verma JP. 2014. Does a rhizospheric microorganism enhance K⁺ availability in agricultural soils?. Microbiological research, 169(5-6): 337-347.
- Molina-Romero D, Baez A, Quintero-Hernández V, Castañeda-Lucio M, Fuentes Ramírez LE, del Rocio Bustillos-Cristales M, Rodríguez-Andrade O, Morales García YE, Munive A, Muñoz-Rojas J, 2017. Compatible bacterial mixture, tolerant to desiccation, improves maize plant growth. PloS one, 12(11): 0187913.
- Morais TPD, Brito CHD, Brandão AM, Rezende WS. 2016. Inoculation of maize with *Azospirillum brasilense* in the seed furrow. Revista Ciência Agronômica, 47(2): 290-298.
- Mosimann C, Oberhänsli T, Ziegler D, Nassal D, Kandeler E, Boller T, Mäder P, Thonar C. 2017. Tracing of two *Pseudomonas* strains in the root and rhizosphere of maize, as related to their plant growth-promoting effect in contrasting soils. Frontiers in microbiology, 7: 2150.
- Mutlu H, Coskan A. 2018. The restriction of individual performance of PGPR on maize nutrient uptake by antagonistic relations. In "Agriculture for Life, Life for Agriculture" Conference Proceedings (Vol. 1, No. 1, pp. 93-100). Sciendo.
- Ottow JCG. 1984. Bodenmikrobiologisch-biochemisches-Praktikum. S. 1-2.
- Patel P, Trivedi G, Saraf M, 2018. Iron biofortification in mungbean using siderophore producing plant growth promoting bacteria. Environmental Sustainability, 1(4): 357-365.
- Pereg L, de-Bashan LE, Bashan Y. 2016. Assessment of affinity and specificity of *Azospirillum* for plants. Plant and soil, 399(1-2): 389-414.
- Picazevicz AA, Kusdra JF, Moreno ADL. 2017. Maize growth in response to *Azospirillum brasilense*, *Rhizobium tropici*, molybdenum and nitrogen. Revista Brasileira de Engenharia Agrícola e Ambiental, 21(9): 623-627.
- Richard PO, Adekanmbi AO, Ogunjobi AA. 2018. Screening of bacteria isolated from the rhizosphere of maize plant (*Zea mays* L.) for ammonia production and nitrogen fixation. African Journal of Microbiology Research, Vol. 12(34): 829-834.
- Richardson AE, Barea JM, McNeill AM, Prigent Combaret C. 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant and soil, 321(1-2): 305-339.
- Rojas-Tapias D, Moreno-Galván A, Pardo-Díaz S, Obando M, Rivera D, Bonilla R. 2012. Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (*Zea mays*). Applied Soil Ecology, 61: 264-272.
- Sandy M, Butler A. 2009. Microbial iron acquisition: marine and terrestrial siderophores. Chemical reviews, 109(10): 4580-4595.
- Singh K, Gera R. 2018. Assessing phosphate solubilization ability of *Sesbania grandiflora* rhizobia isolated from root nodules using diverse agroecological zones of Indian soils for biofertilizer production. International Journal of Chemical Studies, 6(4): 398-402.
- Turan M, Sahin F. 2013. Role of plant growth promoting rhizobacter strain reduce application rates mineral fertilizer in barley. ProEnvironment Promediun, 6(14).
- Vejan P, Abdullah R, Khadiran T, Ismail S, Nasrullah Boyce A. 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability -a review. Molecules, 21(5): 573.
- Yolcu H, Gunes A, Gullap MK, Cakmakci R. 2012. Effects of plant growth-promoting rhizobacteria on some morphologic characteristics, yield and quality contents of Hungarian vetch. Turkish Journal of Field Crops, 17(2): 208-214.
- Youseif SH. 2018. Genetic diversity of plant growth promoting rhizobacteria and their effects on the growth of maize plants under greenhouse conditions. Annals of Agricultural Sciences, 63(1): 25-35.
- Zaidi A, Khan M, Ahemad M, Oves M. 2009. Plant growth promotion by phosphate solubilizing bacteria. Acta microbiologica et immunologica Hungarica, 56(3): 263-284.
- Zhang L, Fan J, Ding X, He X, Zhang F, Feng G. 2014. Hyphosphere interactions between an arbuscular mycorrhizal fungus and a phosphate solubilizing bacterium promote phytate mineralization in soil. Soil Biology and Biochemistry, 74: 177-183.