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Improvement of Corn Growth Parameters by Biofertilization

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ABSTRACT

Laboratory experiments were conducted to study seed germination and growth parameters of corn (Zea mays L.), after treated by 7 treatments using indigenous soil isolates of *Bacillus* and *Pseudomonas* in addition to well-known *Azospirillum*. Results indicated that, seed germination was increased by about 90 % when using the three bacterial isolates in combination, and the ratio was lower when the isolates were used individually. The growth parameters of corn were examined in greenhouse experiment, the results indicated that, the plant height and dry weight were enhanced by using the three isolates or when using Pseudomonas + Azospirillum in combination. .

INTRODUCTION

Maize (Zea mays L.) is a very important crop in Saudi Arabia and is cultivated in wide area of the country. Nitrogen is important in large quantities for plant growth and it is provided in the form of chemical synthetic fertilizers such protects refer a health risk and microbial problem in soil, in addition to making the production cost higher (Kouchebagh *et al*.,2012). Therefore, there is a need to amend crop productivity in a safe manner to enhance the fertility and organic matter (Laditi *et al*., 2012). Plant growth promoting rhizobacteria (PGPR) are very important for nutrition of plant by increasing nitrogen and phosphorous absorption by plants, and playing important role in biofertilization of crops (Cakmakci *et al.,* 2005). Maize activate different nitrogen-fixers in its rhizosphere, most of them belong to Enterobacteraceae and Azotobacteraceae (Yazdani *et al*.,, 2009). Soil microflora play important role in organic matter decomposition and release plant nutrient such as nitrogen, phosphorous and potassium.

Therefore, this study was conducted to:

1- Isolation of indigenous soil microbial isolates, and testing their ability to solubilizing phosphate, siderophores production, hydrocyanic acid production and indole acetic acid production.
2- Study the effect of these isolates on maize growth parameters in greenhouse experiment.

MATERIALS AND METHODS

1- Indigenous bacterial isolates.

PGPR are isolated from corn rhizosphere which growing in soil from Jazan area. Isolates' selection was done according to the PGPR traits. Identification of the isolates was done using morphological and biochemical characters. Yeast mannitol agar amended by 6 % NaCl for salt tolerance and nutrient agar medium at different pH (from 5-9) for pH tolerance.

2- Examination of plant growth-promoting properties.

a-phosphate solubilization:

Inoculation of Pikovskaya agar (0.5 % tricalcium phosphate), by bacterial isolates, were done for solubilization experiment (Pikovskaya, 1948). Appearance of clear zone was in positive correlation with phosphate solubilization ability. Clear zones' diameters around colonies after 5 days inoculation at 28°C were measured.

b- Production of siderophores:

Chrome azural S (CAS) test was applied for estimating of siderophore production, Schwyn and Neilands (1987). Plates were inoculated with 10µl of ~ $5x10^6$ CFU/ ml on CAS medium. The degree of siderophores production was determined color change zone (green-blue to orange) after 5 days incubation at 28°C.

c- Production of hydrocyanic acid (HCN):

HCN production was examined on king B medium amended by glycine (Ayyadurani *et al.,* 2007). Filter papers were impregnated with 0.5 % picric acid & 2 % sodium carbonate put on the lid of inverted plates and closed with paraffin. Inverted plates were incubated during 4 days at 28°C and production of cyanide was detected by discoloration of yellow filter paper to orange or brown.

d- Indole acetic acid (IAA) production:

Quantitative analysis of IAA production was done. Ten µl of bacterial suspension,were inoculated in liquid nutrient agar medium with and without 5 mM of tryptophan (Glickman and Dessaux, 1995) then incubated 36 h at selected temperature. Salkowski reagent (2 % 0.5 M ferric chloride in 30 % per-chloric acid) was migled with the supernatant in (2-1 v/v) and degree of the color was measured at 530 nm for IAA production.

3- Bacterial treatment

Three bacterial strains were selected for their plant growth-promoting properties. Bacterial cell suspensions were diluted with sterile distilled water and cell concentrations were adjusted at 10^8 cfu mL^{-1}. Seed treatments took place in plastic bags, maize seeds were put in a plastic bag then 30 ml of bacterial cell suspensions were added. The bags were shaken for 5 min. till the seeds were coated. Then the seeds were spread on paper towels and let to air dried overnight. Madhin *et al.,* 2016.

Seven treatments were conducted as the following:

1- Bacillus
2- Pseudomonus
3- Azospirillum
4- Bacillus + Pseudomonus
5- Bacillus + Azospirillum
6- Pseudomonus + Azospirillum
7- Bacillus + Pseudomonus + Azospirillum

4- Germination bioassay

Germination experiments were carried out using a paper towel technique following the procedures described by Gholami *et al.,* 2009. Seeds treated were compared with untreated control. Each treatment was carried out in three replicated. Seeds were germinated in a growth chamber at 28°C. After five days, the number of germinated seeds was determined.

5- Greenhouse experiment

Greenhouse experiment was applied in pots at temperatures 28± 2°C. The experiment was designed in seven treatments and three replicates, ten seeds were planted at 5 cm depth in soil on each pot. After 8 weeks, maize plants were harvested carefully separated from soil.

6- Growth parameters.

After 8 weeks, plant height (cm plant-1), dry weight (g plant -1) and (shoot & root length) were determined and the mean per plant was determined. Dry weight was listed after drying into an oven at 50°C to constant weight.

7- Data analysis

One way ANOVA was used to analyze data from greenhouse; LSD and Duncan test were used to separate treatment means when there was a significant difference at $P < 0.05$ level.

RESULTS

Bacterial strains

PGPR were isolated from corn rhizosphere and identified according to their morphological and physiological characteristics as well as their ability for producing plant growth hormones and solubilizing phosphate, Table (1) and Figs.(1&2). Five strains were isolated from maize rhizosphere, belonging to Bacillus and Pseudomonas were tested for their ability for producing plant growth promoting material. All isolates were able to produce indole acetic acid ranging from 0.73-7.9 µg ml^{-1}. Isolate Ps.2 produced the highest amount of indole acetic acid being 7.9 µg ml^{-1}, followed by Ps1 (7.6 9 µg ml^{-1}) then B.2 isolate (0.89 µg ml^{-1}). All isolate produce HCN in a moderate amount. Phosphate solubilization function was noticed by all isolates. The highest was the isolate Ps.2 found to be (10.6 µg ml^{-1}). shown in Table (1).

Table 1. Plant growth promoting properties of the isolates

	Ps.1	Ps.2	B.1	B.2	B.3
Phosphate solubilization µg ml^{-1}	9.1 ± 1.3	10.6 ± 1.3	4.6 ± 1.3	5.1 ± 1.3	4.6 ± 1.3
Siderophores production µg ml^{-1}	6.9 ± 0.3	7.6 ± 0.3	4.1 ± 0.3	4.8 ± 0.3	3.9 ± 0.3
HCN production µg ml^{-1}	+	+	+	+	+
IAA production (µg ml^{-1})	7.6 ± 0.3	7.9 ± 0.3	0.73 ± 0.3	0.89 ± 0.3	0.69 ± 0.3

[a], (+) bacterial growth; (±) poor bacterial growth; (-) no growth; [b], means and standard deviations values.

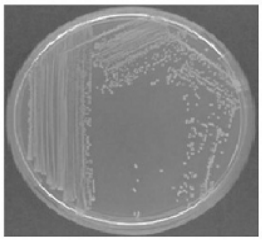

Fig. (1): Pseudomonus 2 isolates

Fig. (2): Bacillus 2 isolates

Germination bioassay

Germination rate was calculated after 5 days of treatment, compared to untreated control (Table2). Results of germination rate experiment assured that treatment with the three bacterial isolates (Bacillus + Pseudomonus + Azospirillum) significantly increased germination ratio with 82.2%. Followed by treatment with (Bacillus + Pseudomonus), (Bacillus + Azospirillum) and (Pseudomonus + Azospirillum) which reported % germination of 79.8, 77.4 and 76.9 respectively. No significant difference was reported between the treatments of using the isolates individually and control.

Greenhouse experiment & Growth parameters

Application of seven of PGPB as seed as seed treatments, resulted in increases in corn growth parameters compared to untreated control. (Table2) & (Fig.3). It was insignificantly when use the isolates individually. The highest plant height was when *Bacillus+ Pseudomonus* were used in combined (29.9 cm), followed by the treatment with the three isolates (28.6 cm). The highest dry weight was obtained also when *Bacillus+ Pseudomonus* were used (0.45g plant $^{-1}$). And the highest shoot and root length were recorded when the treatment with the three isolates, 16.2 and 18.5 cm for shoot and root length, respectively.

Fig. 3. Efficacy of inoculation by isolates on corn growth using the three bacterial strains.

Table 2. Efficacy of inoculation by isolates on corn growth parameters.

	Tested growth parameters					
	Germination %	Plant height (cm plant-1)	Dry weight (g plant -1)	Shoot length (cm)	Root length (cm)	Shoot/ Root ratio
1- Bacillus	61.6^{c}	25.9	0.42*	7.88^{d}	2.88^{e}	2.74
2- Pseudomonus	63.4^{c}	27.6	0.41*	10.31^{c}	12.76^{c}	0.8
3- Azospirillum	59.4^{c}	26.4	0.37*	9.80^{c}	6.66^{e}	1.47
1 + 2	79.8^{ab}	29.9*	0.45*	15.7^{ab}	14.3	1.1
1 + 3	77.4^{ab}	28.3*	0.41*	12.9^{b}	11.4^{c}	0.89
2 + 3	76.9^{ab}	28.9*	0.43*	16.1^{a}	16.3^{b}	0.99
1 + 2 +3	82.2^{a}	28.6*	0.50*	16.2^{a}	18.5$^{d\,a}$	0.88
Non-inoculated control	41.8^{d}	23.7	0.33	6.9^{d}	2.97	2.3
LSD 5%	22.1	3.72	0.02	6.8	6.2	

*p ≤ 0.05 (significant)

DISCUSSION

In the year of 2015, corn production from non-commercial agriculture was 442,200 and 231,600 for yellow and white species. About sixty % of the corn production in the non-commercial part was planted in the Eastern Cape meanwhile poor soil fertility is a challenge. (Dredge, 2016). According to Kolawole, 2014, two week prior application Also, according to the results of Siqueira *et al*., 1993 and Gholami *et al*., 2009, authors positive effect of biofertilizer treatments was observed on germination. A favorable effect of direct seed and filter paper treatment on germination, which concurs with these authors results. As was published by Kloepper and Beauchamp (1992), the Azotobacter and Bacillus inoculation increase wheat yield up to 43% and 30 %, respectively.

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تعزيز مؤشرات النمو لنبات الذرة بإستخدام التسميد الحيوى

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تم اجراء تجربة معملية لدراسة الانبات و مؤشرات النمو لنبات الذرة بعد المعاملة بسبعة معاملات مختلفة باستخدام عزلات بكتيرية محلية للبكتيريا *Bacillus* و *Pseudomonas* وعزلة معرفة من *Azospirillum* وقد اثبتت النتائج ان النسبة المئوية للانبات زادت بمعدل 90 % عند استخدام المعاملة بالثلاث عزلات البكتيرية مقارنة بنسب اقل عند استخدام العزلات البكتيرية منفردة. كذلك تم تعزيز مؤشرات النمو للنباتات عند اختبارها في تجربة داخل الصوبة الزراعية، حيث زادت اطوال النباتات و الوزن الجاف عند المعاملة بالثلاث عزلات و كذلك عند استخدام Pseudomonas + Azospirillum معا. حيث زادت اطوال النباتات بنسبة 21 و 20 % على التوالي. بينما زاد الوزن الجاف بنسبة 66 % عند المعاملة بالثلاث عزلات البكتيرية.