



Effect of Super Absorbent Polymer and Bio fertilization on Maize Productivity and Soil Fertility under Drought Stress Conditions



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THIS STUDY aimed to investigate the response of maize to super absorbent polymer (SAP) and exopolysaccharides (EPS) producing bacteria under drought stress conditions at Panjar El -Soukkar region, Alexandria government, Egypt. The functional groups and heavy metal contents of SAP were determined. The swelling capacity of the polymer increased with increasing the hydrolysis time up to 30 min and the de-swelling water ratios of the polymer varied from 0.25 to 8 days. EPS-producing bacteria, *Klebsiella oxytoca*, *Serratia marcescens*, and *Pseudomonas aeruginosa* were selected and classified based on their phenotypic, biochemical, and also their molecular criteria. The EPS production by the selected isolates ranged from 0.85 to 1.24 g/100ml and the total activity of antioxidant of the extracted EPS ranged from 1.464 to 1.827%. The bacterial isolates could tolerate up to -2.24 MPa of water stress and their production of EPS increased with increasing drought stress levels. Different plant growth promoting parameters of selected isolates were evaluated. Two field experiments were conducted during 2018 and 2019 summer growing seasons. The obtained data revealed that drought stress had significant adverse effects on growth and yield parameters of maize plant, NPK uptake by grains, nutrient availability and microbial activities in soil. Seed bacterization of maize with EPS-producing bacterial isolates in combination with SAP improved all measured characters compared to PGPR inocula or SAP used alone. The findings suggested that applying superabsorbent polymer in combination with EPS-producing bacteria could improve tolerance of maize plant toward drought.

Keyword: Corn (*Zea mays*), Antioxidant exopolysaccharide, PGPR, Superabsorbent polymer, FTIR, Drought stress.

Introduction

In Egypt, *Zea mays* is among the greatest essential cereal crops in terms of cultivated area or nutritional value. It is the third-largest cereal crop behind wheat and rice. Maize is a food source not only for human but also for animals, poultry, and used as a healthy forage crop (El-Rasoul et al., 2020). *Zea mays* as a summer crop is highly affected by the climate change thus, the applied irrigation amount is expected to increase by 10-19% in all Egypt governorates in 2040 (Ouda et al., 2016). Drought is among the most major abiotic stresses, which induces the most extreme financial damage

in livestock and decreases agricultural output in arid and semi-arid areas (Marulanda et al. 2007). High competition for water supplies would need new energy-saving irrigation techniques and these techniques may sustain productive output rates in arid and semi-arid regions (Abu-hashim and Negm, 2018). Superabsorbent polymer (SAP), as a soil conditioner, enhances soil quality by reducing water leaching, thereby saving soil water for agronomic crops. Ekebafé et al. (2011) pointed out that hydrogels may hold enough amounts of water and elements and release them as needed by the plant, thereby enhancing

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plant growth with limited supply of water. They claimed that the usage of SAP plays a significant role in improving water absorption and alleviating largely the detrimental consequences of drought stress. Parvathy and Jyothi (2014) indicated that hydrogels could improve soil properties, mainly under conditions of reduced availability of moisture. They concluded that these polymers have excellent water retaining capability and slow release properties, suggesting possible usage in agriculture as they increase fertilizers and water use efficiency.

Plant inoculation with beneficial native microorganisms could also increase plant tolerance to drought. Soil microbiota have developed numerous methods to survive desiccation in soil. For instance, bacteria have been identified to alter their membrane structure to increase their presence during periods of low external water potential. Polysaccharides are hygroscopic and thus may sustain higher water content in the micro-environment of the colony than in the bulk soil as water capacity decreased (Vurukonda *et al.*, 2016). Polysaccharides producing bacteria were able to sustain higher soil moisture content and plant production even in sandy soils (Marchus *et al.*, 2018). Indeed, the increased release of soluble carbohydrates into the rhizosphere soil of plants inoculated with plant growth promoting rhizobacteria (PGPR) may have improved microorganisms' survival efficiency under water deficit condition. In addition, Costa *et al.* (2018) stated that the exo-polysaccharides formed by PGPR in the rhizosphere with the corresponding mineral particles can form rhizosheath around the plant roots and thereby shield them from desiccation over a longer period of time, which can also participate in increasing macroaggregate production as an additional indirect impact.

The present investigation was conducted to evaluate the effect of superabsorbent polymer alone and/or in combination with EPS-producing bacteria on maize grown under drought stress conditions.

Material and Methods

Cultivar

Maize seeds tripartite hybrid cultivar -310 (*Zea mays*) were obtained from Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.

Characterization of superabsorbent polymer (SAP)

Sodium Polyacrylate-based superabsorbent polymer manufactured by SUMITOMO SEIKA

Chemicals Company, Japan. The polymer appearance is white granular, its water retention is not less than 95% and moisture content is not more than 6%.

Fourier transform infrared spectroscopy

The functional groups in the SAP, used in this study, were characterized by Fourier transform infrared (FT-IR) spectroscopy (Nicolet Avatar 230 spectrometer).

Heavy metals content

The heavy metals content in polymer was determined by the Ionic Coupled Plasma according to (Ure, 1995) where 1.00 g polymer was finely grounded, moistened with distilled water, heated in 100 ml Teflon beaker with 10 ml Conc. HNO₃ and evaporated to small volume. Then 5 ml Conc. HNO₃, 5 ml 70% HClO₄ and 10 ml Conc. HF are added and the whole heated to perchlorate fumes. After 30 min. fuming, 10 ml of HCl (1/1, v/v) is added and mixture boiled for 10 min. and then ventilated and diluted to 100 ml with distilled water.

Swelling and de-swelling measurements

The absorption capacity and rate of the SAP used in the study was investigated by using the tea bag method (Sadeghi and Hosseinzadeh, 2008). A tea bag (nylon screen) containing an accurately weighed powdered polymer (0.05 ± 0.001 g), was fully submerged in 200 mL distilled water and permitted to soak at room temperature for 180 min. The tea bag was held up for 15 min in order to eliminate any excess water. Then, the value of equilibrated swelling (ES) was calculated two times using the given formula:

$$ES_{(g/g)} = \frac{W_2 - W_1}{W_1}$$

where W₁ and W₂ are dry and swollen polymer weights, respectively.

The de-swelling water ratio of each sample was evaluated from the following equation:

$$\text{Deswelling water ratio} = \frac{W_t}{W_{t_0}} \times 100$$

where W_{t₀} and W_t are the initial weight of the fully swollen polymer, and the weight of polymer at de-swelling time (t), respectively.

Isolation of exopolysaccharide (EPS) producing bacteria

The soil EPS producing bacteria, were isolated from agricultural soil (28° '37 "0 N and 30° '20 "0 E) of North West West of Minya, Cairo, Egypt. The isolation of bacterial culture was done by

means of serial dilution followed by spread plate method using sucrose medium (Amellal et al., 1998). Inoculated plates were incubated at 28 °C for 72 hr. Individual bacterial colonies were picked and further purified by sub-culturing on nutrient agar (NA) medium. Stock culture was maintained by sub-culturing at a regular time interval, the slants were preserved at -20 °C. Exopolysaccharid production (EPS) has been tested using the method described by Emtiazi et al. (2004). Production of EPS was assessed by cultivating the isolates on Congo Red Agar (CRA) using the method described by Kaiser et al. (2013). Selected isolates were cultivated on NA medium amended with Congo red (0.08%) and sucrose (5%). The plates were incubated at 35 °C for 48 hr.

Characterization of bacterial Isolates

Thirty-eight bacterial isolates were isolated from soil and screened for EPS production. Three bacterial isolates that produced the highest amount of EPS were selected and identified on the basis of morphological and microscopic observations, followed by molecular level identification. The technique of Higgins et al. (2007) was used to isolate the genomic DNA and partial sequencing of 16S rRNA was performed using universal primers, F (5' AGA GTT TGA TCC TGG CTC AG-3') and R (5' -GGT TAC CTT GTT ACG ACTT-3'), (Weisburg et al., 1991). The neighbor joining method of Saitou and Nei, (1987) was used to build the phylogenetic tree of identified isolates.

Isolation and purification of exopolysaccharide

Selected isolates were grown in sucrose medium (4% sucrose, 0.5% yeast extract and 0.5% peptone) on a rotary shaker at 28°C and 150 rpm. After incubation for 10 d, the culture broth was centrifuged at 6000 rpm in 1 mM EDTA for 20 min at 4°C. The protein content in cell free supernatant was denaturated using 10% trichloroacetic acid. The pH of supernatant was adjusted to 7.2 with NaOH, centrifuged, and the precipitate protein was discarded. The supernatant was evaporated and concentrated at 40°C under reduced pressure. The EPS were extracted from the supernatant by the addition of two fold ice cold ethanol (95%), the solution was left overnight in the refrigerator for complete precipitation. The polysaccharide precipitate was collected by centrifugation and washed with ethanol and then dried at 45°C and weighted (Kumar et al. 2011).

Determination of total antioxidant activity of EPS product

The potential of antioxidant of produced EPS was evaluated according to Prieto et al. (1999). Briefly, 0.1 g of extracted EPS was blended with 3 ml of reagent solution (36 ml of 0.6 M sulphuric acid, 5g ammonium molybdate and 4g sodium phosphate/up to 1L). The mixture was incubated at 95 °C for 90 min and cooled to room temperature. Then, the solution absorbance was calculated against the blank at 695 nm. A calibration curve was prepared with ascorbic acid, and on a dry weight basis the overall antioxidant intensity was measured as ascorbic acid equal milligrams per gram of sample.

Effect of drought stress on EPS produced bacteria

The ability of isolates to grow and produce EPS in the presence of different osmotic potentials was examined; Polyethylene glycol 6000 (PEG) compounds have been used to stimulate drought stress effects. The concentration of PEG-6000 for each water stress was determined using the equation of Villeda et al. (1991).

$$\text{Water potential (MPa)} = (-1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7}) C^2T$$

where C is the concentration of PEG-6000 in g/l H₂O and T is the temperature in Celsius degree. Bacterial isolates were cultured in test tube containing 9 ml sucrose broth (SB) medium supplemented with different concentrations of PEG 6000 (60, 80, 100, 120 and 140 g/l) to generate osmotic potential of (-0.52, -0.84, -1.23, -1.7 and -2.24 MPa) respectively. Tubes were incubated in an orbital shaker (100 rpm) at 30°C for 48 h and optical density was measured at 600 nm by a spectrophotometer (Jenway 6105 U.V/V.S spectrophotometer). EPS was extracted and quantified as mentioned above.

Plant growth promoting properties of selected bacteria

The bacterial isolates were tested in vitro for plant growth promoting properties. Nitrogen fixation was determined according to Döbereiner and Day (1976) by growing the bacterial isolates in nitrogen-free malate medium. For studying phosphate solubilization, the method of Liu et al. (2014) was followed. Hydrogen cyanide (HCN) production, was determined according to the method described by Bakker and Schipper (1987). The method of Apine, and Jadhav, (2011) was followed for the estimation of indole acetic acid (IAA). For the estimation of gibberellic acid, 2 ml of zinc acetate were added

followed by 2 ml of potassium ferrocyanide to 15 ml of the culture supernatant raised in minimal media. After centrifugation at low speed for 15 min, 5 ml of the supernatant was taken in a test tube, and 5 ml of 30% HCL were added followed by incubation at 20°C for 75 min; then the absorbance was read at 254 nm (Holbrook *et al.* 1961). The bacterial organic acid production test was conducted using the method described by Trivedi *et al.*, (2013). Ammonia production was tested according to Dey *et al.* (2004).

Field experiment

The study was conducted during 2018 and 2019 summer growing seasons to investigate the response of maize tripartite hybrid cultivar -310 to super absorbent polymer and bio fertilizer under drought stress conditions at Panjar El -soukkar region, Alexandria Governorate, Egypt. The physical and chemical analysis of experimental field soil was determined according to Page *et al.* (1982) and presented in Table 1. Each experiment included 12 treatments, which were the combinations of three treatments of water requirements and four treatments of polymer and bio-fertilizer. The statistical design of the experiment was split plot design with three replicates, whereas water application treatments occupied the main plots and super absorbent polymer and bio fertilizer were arranged in sub main plots. Water irrigation treatments were as follows; 50, 75 and 100% of water requirements (7680 m³ ha⁻¹), while the treatments of sub main were as follows; control (no application), superabsorbent polymer (SAP) at 48 kg ha⁻¹, exopolysaccharides producing bacteria (10⁸ cfu) and the polymer with EPS producing bacteria. The plot area was 21 m² (5.0 m × 4.2 m) *i.e.* 1/200 feddan, contained of 7 rows, 5 m in length and 60 cm a part. Planting was done on 1th June 2018 and

27th May 2019. Standard cultural practices were adopted during the crop-growing season.

During soil preparation, maize plots were fertilized with phosphorous at 75kg P₂O₅ ha⁻¹ in the form of calcium super phosphate (15.5% P₂O₅), nitrogen at 120 kg N ha⁻¹ in the form of ammonium nitrate (33.5% N) in two equal doses where the 1st dose was applied after 30 days of planting and the second one was applied 2 weeks later and potassium at 115 kg K₂O ha⁻¹ as potassium sulfate (48% K₂O) after two weeks of the application of the 2nd nitrogen dose. Upon planting, herbicide was sprayed at 2.4 L ha⁻¹ for weed control in growing plots at the producer. Before planting, super absorbent polymer applied (SAP) at 48 kg ha⁻¹ in soil at a depth of 15cm. For bacterial treatments, maize grains were immersed for 30 min in liquid bacterial culture (mixture of three bacterial isolates) before sowing. Untreated control seeds were maintained. Maize plants were harvested manually on 9th December 2018 and 13th December 2019. Ten plants were selected randomly from third and fifth rows of each plot to determine plant height, ear diameter, ear length, number of rows/ ear, number of grains /row ear weight, ear grain weight, 100 grain weight, grain and that calculated by following formula according to (Giriappa, 1983).

$$100\text{- grain weight} = \frac{100 \times \text{ear grain weight (g)}}{\text{No of rows/ear} \times \text{No of grain/rows}}$$

$$\text{Water use efficiency (kg/m}^3\text{)} = \frac{\text{Grain yield (kr/ha)}}{\text{Total applied water (m}^3\text{/ha)}}$$

$$\text{Nutrients uptake (kg/ha)} = \text{nutrients uptake kg/fad} \times 2.4$$

TABLE 1. Analysis of experimental soil

Depth (cm)	Physical analysis %			Soil texture								
	Sand	Silt	Clay									
0-30	58.42	12.23	29.35	Sandy clay loam								
Chemical analysis												
pH	EC dSm ⁻¹	Available nutrients (mg kg ⁻¹)			Soluble cations and anions in saturated soil extract							
		N	P	K	Cation (cmol _c kg ⁻¹)				Anion (cmol _c kg ⁻¹)			
					Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻
7.74	1.5	19.6	5.9	123.8	5.9	4.5	3.6	0.94	0.0	2.1	2.3	10.6

Determination of microbial activities

Total microbial counts were estimated according to Allen, (1959). The method described by Amellal et al. (1988) was used to estimate the counts of EPS producing bacteria. Soil dehydrogenase activity was estimated according to Casida et al. (1964).

Determination of N, P and P in soil and in maize grains

Nitrogen, phosphorous and potassium uptake by maize grains were determined in acid digested solution according to Cottenie et al. (1982). Available nitrogen in soil samples was extracted by 2M potassium chloride solution and determined according to Dhank and Johnson (1990). Available potassium and phosphorous were measured according to the method described by Soltanpour (1985).

Statistical analysis

The results from the present study were evaluated statistically and the variations between the treatment approaches were rendered significant when they were more than the least significant differences (LSD) at the 5% stage using Statistix version 9 computer software (Analytical software, 2008).

Results and discussion

Characterization of Super Absorbent Polymer

The functional groups in the superabsorbent polymer were evaluated using FTIR and the spectra were illustrated in Fig. 1. The strong bands

appeared between 1048 and 1319 cm^{-1} were attributed to C—O—C stretching that indicated the presence of ethyl group. The carboxylate groups of the intact SAP display a strong band at 1540 cm^{-1} and a medium wide band at 3360 and 2919 cm^{-1} (attributed to carboxylic acid). The spectra for C—H groups, which are presented in the entire cross linked samples, were attributed to the methyl group of the crosslinker. The two peaks appeared at 2362 and 2341 are due to O=C=O of CO_2 present in the air. In the spectra, absorption peaks characteristic of the expected functional groups are in agreement with previous studies of Ghasri et al. (2019) and Pourjavadi et al. (2012).

Heavy metal content

Data shown in Table 2 revealed that the polymer is free of any harmful elements such as (As, Cd and Hg) according to Dragun (1988); Eissa et al. (2017) and Khalifa and Gad (2018).

Time effect of swelling and deswelling of polymer

The swelling capacity of the polymer was greatly influenced by the hydrolysis time during irrigation time, as shown in Fig. 2. The reaction period of the hydrolysis time varied from 5 to 90 min., generally the water absorbency increased as the hydrolysis time increased, and then the time required to reach the equilibrium swelling was achieved at 30 min. These results were in accordance with those obtained by Rosa and Casquilho (2012).

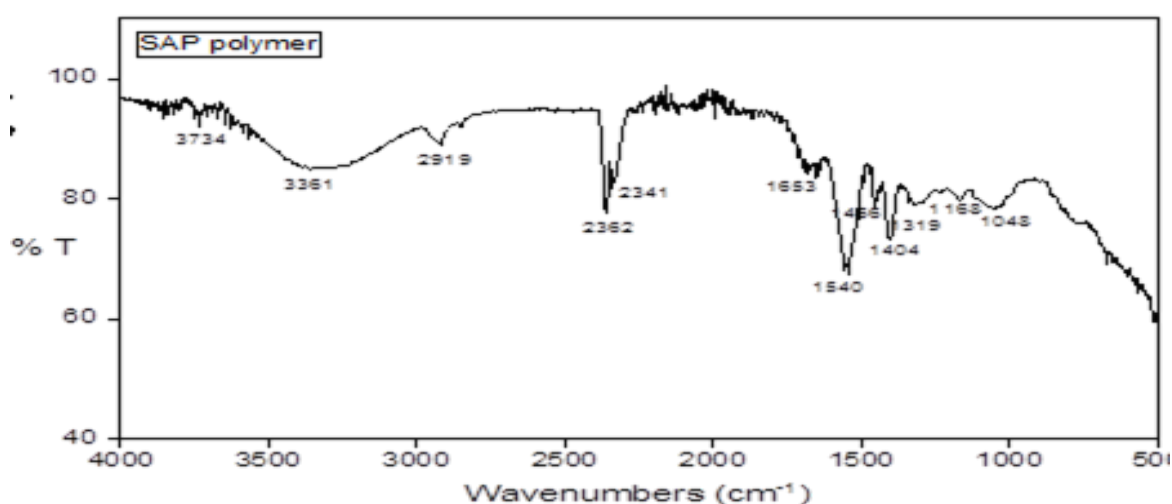
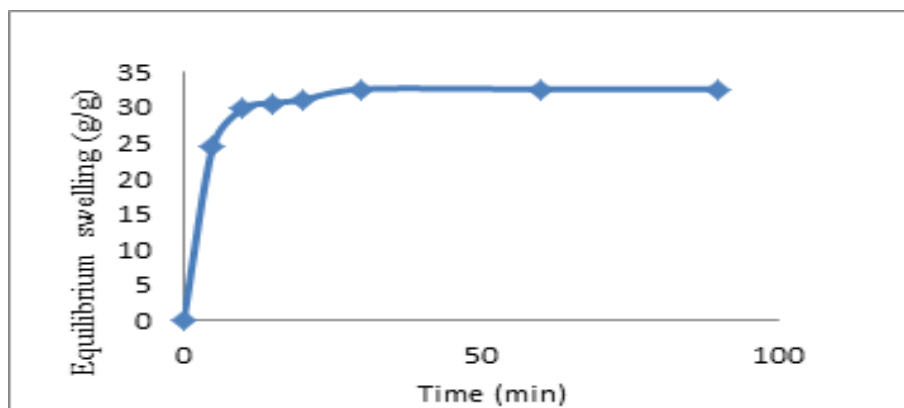


Fig. 1. FTIR spectra of superabsorbent polymer

TABLE 2. Some elements contents in superabsorbent polymer

Elements	Al	As	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	Hg
Conc. (mg kg ⁻¹)	1069.3	-	17.6	11.2	3190	-	3.3	19.9	60.8	1901	-
Elements	Li	Mg	Mn	Mo	Ni	Pb	Se	Si	Sr	Tl	V
Conc. (mg kg ⁻¹)	94.6	643.8	178	182.5	18.1	99.7	-	436.3	24.5	0.145	1.7

**Fig. 2. Effect of hydrolysis time on the swelling capacity of the polymer**

The de-swelling water ratios of the polymer after irrigation were measured. As shown in Fig. 3, the reaction period of the hydrolysis time varied from 0.25 to 8 days (This time represents the different irrigation periods). The de-swelling water ratios of the polymer after irrigation indicated a weight reduction of about 23.5 at 20°C (Average soil temperature in winter) and about 3.11 from its original weight at 40 °C (Average soil temperature in summer) after 4 days. SAP is hydrogel that can absorb a considerable amount of water. These polymers besides having high speed and capacity of water absorption also give water easily, if required. Some of the advantages of using SAP are maximizing the use of water, fertilizer, and pesticide, and reducing the pressures of moisture variations in the soil of the arid region (Montazar, 2008).

Isolation and identification of EPS-producing bacteria

Sixteen of thirty-eight bacterial isolates, isolated from agricultural soil of North West West of Minya, were able to produce EPS. The production of EPS was confirmed by cultivation of the purified isolates on Congo red agar (CRA) according to Kaiser *et al.* (2013). The CRA enables exopolysaccharide development to be observed through differences in colonial colour. The colors of the colonies ranged from brown to nearly black with clear zone around colonies considered ideal for EPS development. Whereas colonies with red color are considered negative for EPS development (Fig. 4). The highly production of EPS were obtained by three isolates, so that, they were chosen for further research analysis. Identification of the three selected bacteria was done based on their morphological and biochemical parameters (Table 3). The three bacterial isolates were

gram negative and bacilli. Isolate numbered I1 was non-motile while I2 and I3 were motile. The isolates I1 and I2 were positive for catalase and negative for oxidase, whereas I3 was positive for both oxidase and catalase tests. Molecular characterization of bacterial isolate was performed by partial sequencing of 16S rRNA and isolates were belonging to *Klebsiella oxytoca*, *Serratia marcescens* and *Pseudomonas aeruginosa* with 100%, 98% and 93% blast identity, respectively. Phylogenetic tree of the three bacterial strains and closely correlated strains are illustrated in Fig. 5.

Production of exopolysaccharide by selected isolates and its antioxidant activity

The EPS produced by the chosen strains and the total activity of antioxidant of the extracted EPS were shown in Fig. 6. The EPS production by the selected isolates ranged from 0.85 to 1.24 g/100ml. *K. oxytoca* produced the highest EPS (1.24 g/100ml). The total activity of antioxidant of the extracted EPS ranged from 1.464 to 1.827%. EPS produced by *K. oxytoca* had the highest antioxidant capacity (1.827%) while the EPS produced by *S. marcescens* had the least one (1.464%). This could be due to the fact that the antioxidant activity of a polysaccharide is a function of a combination of several factors since the antioxidant capacity of EPS depends primarily on its structural distinguishing and configuration of the glycosidic linkage as reported by Li *et al.* (2015). It could also be due to other antioxidant constituents in the crude EPS extract such as peptides, proteins and microelements (Li *et al.*, 2015).

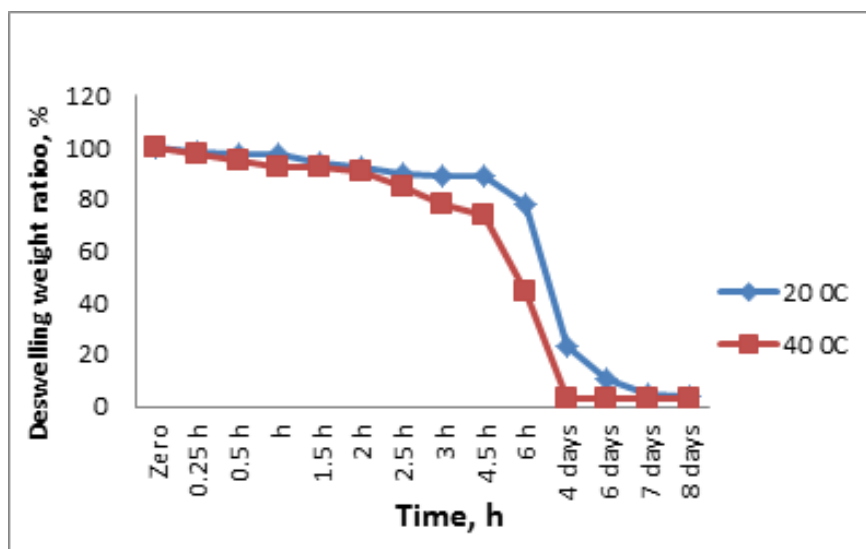


Fig. 3. De-swelling water ratio of polymer at 20°C and 50°C

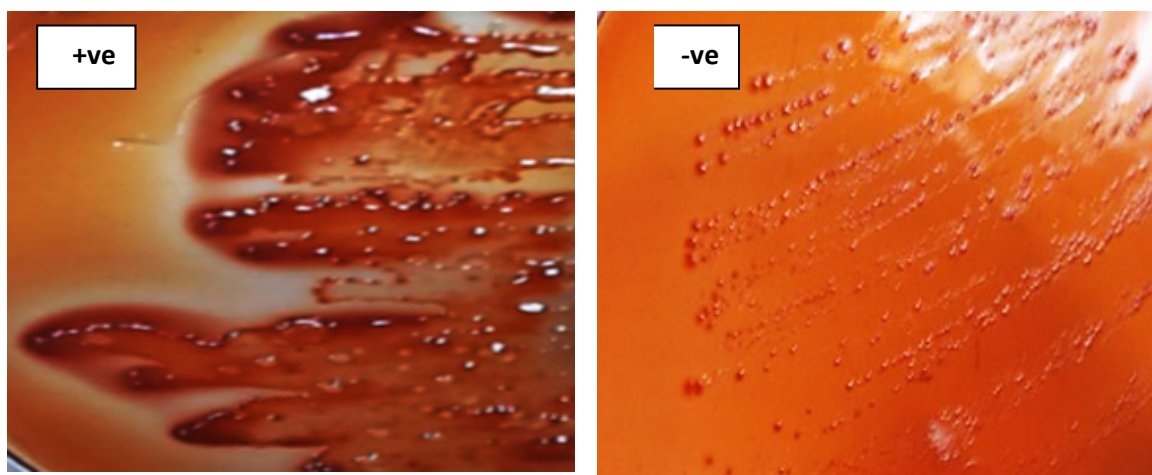


Fig. 4. Congo red agar media for testing Exopolysaccharide production

TABLE 3. Morphological and biochemical characters of selected bacterial isolates.

Characteristic	Bacterial isolate		
	I1	I2	I3
Gram staining	Gram-negative	Gram-negative	Gram-negative
shape	Rod	Rod	Rod
Motility test	Non-motile	motile	motile
Spore forming	-ve	-ve	-ve
Methyl red test	-ve	-ve	-ve
Oxidase test	-ve	-ve	+ve
Catalase test	+ve	+ve	+ve
Citrate utilization	+ve	+ve	+ve
Gelatin hydrolysis	-ve	+ve	+ve
H ₂ S	-ve	-ve	+ve
Urease	+ve	+ve	-ve
Voges Proskauer	+ve	+ve	-ve

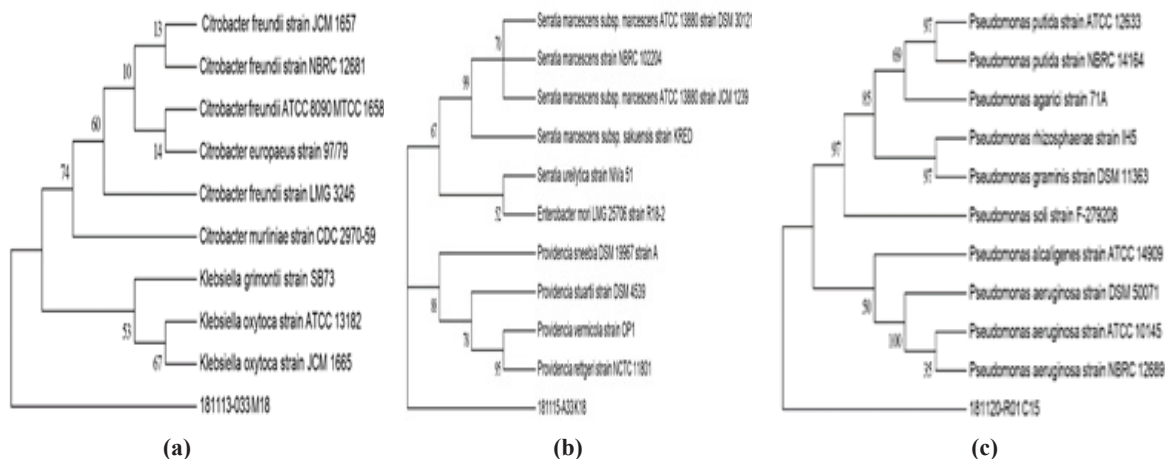


Fig. 5. Phylogenetic tree of partial 16S rRNA sequence for isolate (a) *Klebsiella oxytoca* (b) *Serratia marcescens* and (c) *Pseudomonas aeruginosa*. The scale bar represents 10% nucleotide substitutions. Percentages of bootstrap values recovered from 70 trees are presented on the nodes

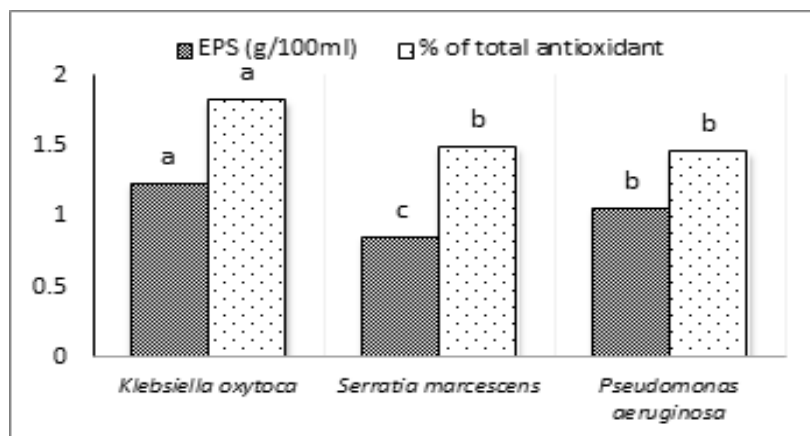


Fig. 6. Evaluation of exopolysaccharide production by selected strains and its antioxidant activity

Effect of drought stress on EPS produced bacteria

The effect of drought stress on growth and production of EPS by selected isolates were evaluated and illustrated in Fig. 7. All three selected isolates could tolerate up to -2.24 MPa of water stress, although the bacterial growth decreased gradually with the increase in osmotic potential value. The bacterial isolates were screened for EPS production under non-stressed (Control) and stressed conditions. The total yield of EPS produced by selected isolates was greatly influenced by drought stress. EPS production increased with the increase in drought stress (from -0.52 to -2.24 MPa) as compared to non-stressed conditions. The isolate *K. oxytoca* produced the highest amount of EPS under low osmotic potential of -2.24 MPa (19.2 mg/100ml), which is followed by *P. aeruginosa* and *S. marcescens*

(14.4 and 11.8 mg/100ml) respectively. According to Lin *et al.* (2014), the development of extracellular carbohydrates increased under different environmental stresses. An increase in the yield of EPS with increasing osmotic potential levels has been reported in *Pseudomonas aeruginosa* ZNP1 and *Bacillus endophyticus* J13 by Ghosh *et al.* (2019). Tsegaye *et al.* (2019) reported that the EPS producing isolates of *S. marcescens* and *K. oxytoca* could grow well at 5% salinity and at 40 °C. Furthermore, under stress conditions, microorganisms generate EPS as one of the stress responsive mechanism to protect their cells from outer unfavorable conditions. EPS act as a diffusion barrier between the cell wall and extreme environments (Collins and Margesin, 2019).

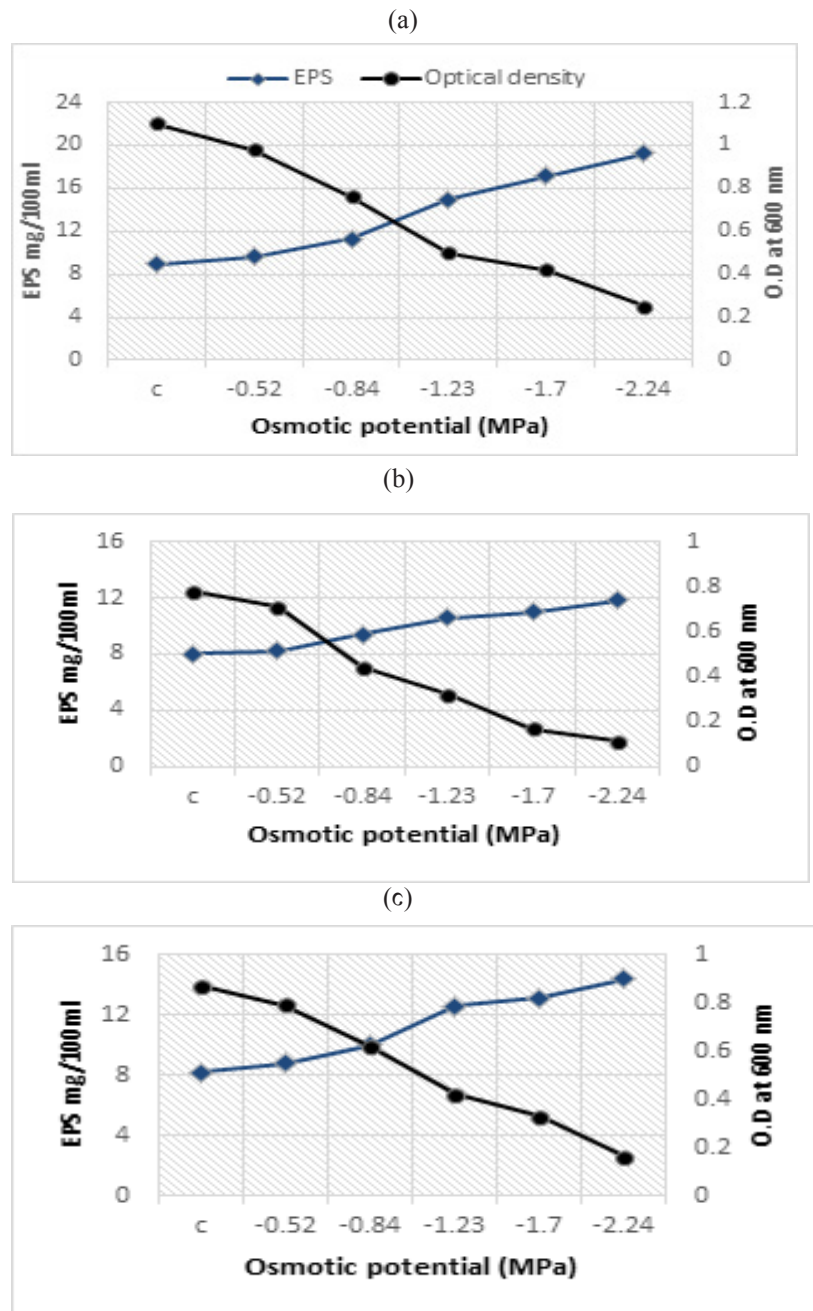


Fig. 7. Tolerance and EPS production of (a) *Klebsiella oxytoca* (b) *Serratia marcescens* and (c) *Pseudomonas aeruginosa*, under different osmotic potential. Values are mean of three replicates

Plant growth promoting properties of bacterial isolates

The selected isolates exhibited plant growth promoting properties as shown in Table 4. The three tested isolates had the capacity to dissolve phosphate and produce IAA, gibberellic acid (GA), hydrogen cyanide (HCN), organic acids and ammonia. These results suggest that the *K. oxytoca*, *S. marcescens* and *P. aeruginosa*, can be beneficial in improving growth of maize and other plants by providing nitrogen and phosphorous nutrition. They also produced considerable

amount of IAA and GA, which is a valuable trait of PGPR, as this growth regulator helps the plant to establish greatly organized root system, thus increasing the nutrients uptake and improving plant growth (Etesami and Beattie, 2017). In addition, the studied isolates gave promising results for HCN production by altering the filter paper color to dark brown. Rhizobacteria-based HCN development suggests their ability to inhibit phyto-pathogens growth and thus promote plant growth (Ali et al., 2018).

TABLE 4. Plant growth promoting activities of bacterial isolates

Characteristics	Bacterial isolates		
	<i>Klebsiella oxytoca</i>	<i>Serratia marcescens</i>	<i>Pseudomonas aeruginosa</i>
Nitrogen fixation	+	-	-
Phosphate dissolving	22.5 ug/ml	13.4 ug/ml	27 ug/ml
Indole acetic acid production	0.43 ug/ml	0.31 ug/ml	0.59 ug/ml
Gibberellic acid production	25.3 ug/ml	12.7 ug/ml	29.1 ug/ml
Hydrogen cyanide production	+	+	+
Organic acid production	+	+	+
Ammonia production	+	+	-

In vivo mesurment of maize growth and yield prameters

Result of combined analysis (Table 5 a,b) showed that under normal irrigation condition, plant height, ear diameter, ear length, number of rows/ ear, number of grains/row, ear weight, ear grain weight, 100 grain weight and grain yield were significantly more than drought stress condition at both seasons. Several researchers reported that maize yield decreased under water or drought stress condition (Daryanto *et al.* 2016 and Maqbool and Sadiq, 2017). In this experiment, grain yield augmented from 2993.2kg ha⁻¹ at a level of 50 % of water requirements to 4646.6kg ha⁻¹ at a level of 75 % of water requirements and to 6167kg ha⁻¹ under a level of 100 % of water requirements in the first season. However, yield increased in the second season from 2856 and 4972.8kg ha⁻¹ at the two water treatments (50 % and 75 %) respectively to 6370kg ha⁻¹ at 100 % of water requirements. The yield enhancement is due to the increasing of the numbers of grains for each row and ear under the same conditions. The rise in productivity of maize attributable to the growing volume of irrigation water can be related to the complete irrigation that enhanced the absorption of nutrients from the roots, which is a good representation of maize yield and its components. The efficiency of water use (WUE) as affected by different water treatments did not meet the same pattern as its impact on overall grain yield. Generally, WUE increased significantly with increasing irrigation from 50 up to 75 percent of water requirements and decreased significantly with increasing irrigation from 75 up to 100 percent of water requirements for both seasons. Over the two seasons respectively, the maximum mean values of WUE (87.19 and 93.21 percent) were reported at 75 percent of water requirements as a consequence of growing the ratio of grain yield to the quantity of water used. Such results concorded with Osman (2004)

observations on barley. Kuscü *et al.* (2012) also found that complete irrigation treatments at three growth stages (vegetative, flowering, and grain-filling) improved grain production, dry matter output, and other maize characteristics more than non-irrigated ones.

Regarding the treatments of SAP and EPS producing bacteria under different irrigation water levels, data in Table 5 (a,b) indicated that during both seasons, the combined application of SAP and EPS producing bacteria gave the highest growth parameters including plant height, ear diameter, ear length, number of rows/ ear, number of grains/row, ear weight, ear grain weight, 100-grain weight, grain yield and water use efficiency under normal and drought stress conditions when compared with the application of SAP or EPS producing bacteria alone. The maximum values of plant height (194.03 and 202.76 cm), ear weight (157.31 and 162.40 g), ear grain weight (136.19 and 138.05 g), 100-grain weight (27.95 and 26.9 g), grain yield (6959.4 and 7180.6 Kg ha⁻¹) and WUE (91.35 and 94.24 %) were observed by applying 100 % from irrigation water requirement and SAP and EPS producing bacteria in the two seasons respectively. The highest mean values of ear diameter and ear length were 3.98 cm and 14.05 cm in the first season respectively. In the second season the highest mean values (2.83 cm) for ear diameter were detected by using full irrigation and treating with SAP or EPS producing bacteria alone. The highest mean values for ear length (14.08 cm) were recorded by applying full irrigation and SAP without bacteria, on the other side, number of rows per ear and number of grains per row behaved the same trend under the same treatments. The maximum mean values of number of rows / ear (14.61) and number of grain / row (36.57) were recorded by 100 % from irrigation water requirement and bacterial inoculation in

the first season, but in the second season, the highest values of these traits (15.15 and 35.20 respectively) were recorded when maize plants irrigated by full irrigation with applying SAP without addition of bacteria.

These results are in line with those achieved by Zahra et al. (2011) who showed that applying SAP and PGPR increased the yield of maize grain in both stress and normal condition. In addition, the hormonal production by PGPR increased roots growth of maize, especially by increasing the density of roots hair in active physiological parts to absorb water and nutrition materials. Also, Li et al. (2019) showed that the application of SAP under drought stress resulted in an increase in grain yield and total dry weight of wheat and cucumber. Tohidi-Moghadam et al., (2009) stated that hydrogel can hold water efficiently and delivers this water to the plant under stress conditions. According to their idea, these materials prevent water and nutrition materials washing and there-fore increase canola yield. Lamochi, and

Sakinejad, (2019) demonstrated that inoculating maize plants with nitrogen fixers in water shortage conditions leads to an increase in plant growth and nutrient concentration, this because some strains of PGPR isolated from arid or semi-arid areas may help plants to survive drought stress via various mechanisms. Gholami et al. (2009) recorded that, as a result of inoculation of *Pseudomonas* and *Azospirillum* strains, the amount of grains per ear and dry weight of ear increased. Result in drought stress showed that bio-fertilizer did not have a considerable effect on the increase of the grains per row and grains per ear under stress condition. That is probably because of this case that drought affected the biofertilizer activity; but when the biofertilizer were used with superabsorbent polymer number of grains per row, number of grains per ear and grain yield increased. That is probably because of better activity of biofertilizer in soil by saving water capacity; hence better growth and higher yield of maize.

TABLE 5a. Effect of superabsorbent polymer and EPS producing bacteria on maize growth, yield parameters and water use efficiency of maize plant under drought stress conditions in 2018 season

Treatments	Plant height (cm)	Ear diameter (cm)	Ear length (cm)	Number of rows/ear	Number of grains/row	Ear weight (g)	Ear grain weight(g)	100-grain weight(g)	Grain yield (kg/ha ⁻¹)	Water use efficiency (%)
Con.	141.79j	2.79i	9.15d	11.29k	28.25fg	87.05l	65.71k	20.60k	2993j	78.56h
% 50 .W.R										
SAP	150.74h	2.89h	9.35c	11.43i	28.57f	91.74j	73.09i	21.40j	3280i	86.06d
Bio	146.76i	2.89h	9.28cd	11.04l	27.64h	88.75k	70.32j	22.40i	2980j	78.18h
SAP + Bio	150.64h	2.79i	9.37c	11.38j	28.46f	102.19i	82.35h	23.05h	3782h	99.34a
Con.	169.65g	3.28g	11.84b	12.43e	31.09d	112.82h	82.80h	23.40g	4646g	81.31g
% 75 .W.R										
SAP	170.63f	3.48e	11.84b	12.35g	30.86d	119.41f	91.43f	24.00f	4893e	85.65e
Bio	169.75g	3.38f	11.87b	12.06h	30.21de	117.92g	89.65g	24.60e	4800f	84.02f
SAP + Bio	173.63e	3.58d	11.96b	12.41f	31.02d	130.95e	101.41e	24.60e	5586d	97.78b
Con.	187.16d	3.68c	13.93a	13.98c	34.97c	141.36d	120.07d	25.45d	6167c	80.93g
100 % .W.R										
SAP	192.73b	3.68c	14.03a	14.01b	35.01b	147.95b	127.88b	26.05c	6517b	85.53e
Bio	189.85c	3.88b	13.96a	14.61a	36.57a	143.89c	124.94c	26.35b	6189c	81.26g
SAP + Bio	194.03a	3.98a	14.05a	13.96d	34.90c	157.31a	136.19a	27.95a	6959a	91.35c

WR: Water requirement for maize plant during growing season

Cont.: Control; no application

SAP: Superabsorbent Polymer

Bio: bio fertilizer (EPS producing bacteria)

SAP + Bio: polymer and bio fertilizer

TABLE 5b. Effect of superabsorbent polymer and EPS producing bacteria on maize growth, yield parameters and water use efficiency of maize plant under drought stress conditions in 2019 season.

Treatments	Plant height (cm)	Ear diameter (cm)	Ear length (cm)	Number of rows/ear	Number of grains/row	Ear weight(g)	Ear grain weight(g)	100-grain weight(g)	Grain yield (kg ha ⁻¹)	Water use efficiency (%)	
% 50 WR	Con.	152.51i	2.94d	9.45f	10.89j	28.30ij	83.98j	61.70k	20.05g	2856l	75.02h
	SAP	152.31k	2.94d	9.65f	11.10i	28.85h	89.35i	69.58i	20.05g	3357j	88.15e
	Bio	150.72l	2.74e	9.45f	10.89j	28.40i	88.56i	68.26j	21.70f	3166k	83.14g
	SAP + Bio	152.41j	2.88d	9.41f	10.81k	28.16jk	101.19h	78.43h	22.16e	3780i	99.25b
% 75 WR	Con.	167.93h	3.38c	12.09cd	12.75f	31.17e	119.29g	79.71g	24.15d	4972h	87.00e
	SAP	169.81g	3.38c	11.69e	12.31h	30.10g	123.08e	96.48e	24.20d	5392f	94.38c
	Bio	170.12f	3.38c	11.99d	12.64g	31.00f	121.69f	94.76f	24.30d	5170g	90.46d
	SAP + Bio	171.91e	3.52b	12.32c	13.00e	31.86d	134.12d	104.79d	24.30d	5773e	101.01a
100 % WR	Con.	195.89d	3.73ab	13.78b	14.82d	34.39c	147.21c	123.60c	25.30c	6370d	83.62
	SAP	201.36b	3.83a	14.08a	15.15a	35.20a	148.91b	128.78b	25.75b	6686b	87.77e
	Bio	200.67c	3.83a	13.98ab	15.04b	35.01b	148.40b	127.71b	26.05b	6512c	85.47f
	SAP + Bio	202.76a	3.77a	13.81ab	14.85c	34.52c	162.40a	138.05a	26.90a	7180a	94.24c

WR: Water requirement for maize plant during growing season

Cont.: Control; no application

SAP: Superabsorbent Polymer

Bio: bio fertilizer (EPS producing bacteria)

SAP + Bio: polymer and bio fertilizer

Determination of NPK uptake in maize grains

It is clear from data in Table 6 that the NPK content in maize grains significantly affected by drought stress in the two seasons. NPK uptake decreased significantly from (65.60, 83.34, 7.73, 9.49, 7.72 and 8.90g kg ha⁻¹) under normal condition to (25.04, 34.43, 2.79, 3.71, 2.42 and 3.021 kg ha⁻¹) under stressed conditions in both seasons, respectively. The decline in absorption of nutrients as a result to drought may be attributable either to the decrease in cell elongation due to water scarcity, which resulted in a decrease in each t Turgot cell, cell volume, and ultimately cell growth (Hu and Schmidhalter, 2005), or to the blockage of xylem and phloem channels. Application of SAP in combination with EPS producing bacteria significantly increased NPK uptake by maize under normal and drought stress condition at both seasons. Biofertilizers enhance root development and volume by producing growth regulators such as auxin and gibberellins, resulting in an increase in nutrient uptake from soil (Abdelhameid, 2020). PGPR improved the plants uptake of nutrients and water, thereby increased

the growth of plant shoot (Davoodifard et al., 2012). At the other side, Delshadi et al., (2017) observed that nutritional status of plant enhanced via application of biofertilizers at daily irrigation, while incorporating bacteria could not mitigate the harmful impact of drought on nutrient uptake.

Evaluation of soil microbial activities in the rhizosphere soil of maize plant

Microbial density as affected by application of different irrigation levels, SAP and EPS producing bacteria was shown in Table 7. Results indicated that counts of total microbe and EPS producing bacteria in addition to soil dehydrogenase activity were negatively affected by drought stress. The most restrictive factors that limit microbial growth in arid environments are drought and high salinity (Clark et al. 2009). Changes in microbial community composition may subsequently affect the dynamics of nutrient cycling and the rates of decomposition (Griffiths et al. 2003; Kloos et al. 1998). Many studies indicated that drought reduces microbial activity and biomass and changes the structure of microbial communities (Meisner et

al., 2018; Yaseen and Yossif, 2019). Application of halotolerant bacteria with PGP properties enhanced the soil microbial biomass through production of osmolyte (Yasin et al. 2018).

Co-application of SAP and EPS producing bacteria increased the microbial densities and their activities at both seasons under drought stress. It is notable that, soil dehydrogenase increased significantly with increasing irrigation from 50 up to 75 percent of field capacity and decreased significantly with increasing irrigation from 75 up to 100 percent of field capacity for the two seasons, the highest mean values (1.086 and 1.259 μmol triphenyl formazan /g dry soil) were recorded at 75 % of field capacity of water requirement in the two seasons respectively. This result confirmed the previous theory that microbial decomposition in wet soils may be triggered by drier climate change conditions (Freeman et al. 1996).

Evaluation of nutrient availability (NPK) in soil

Table 8 showed the availability of N, P and K in soil at the end of the experiment. The combined application of SAP and EPS producing bacteria increased the available nutrients in soil when compared with other treatments. The application of SAP with bio fertilizer under normal condition

increased significantly the available nutrients in the soil compared with that under drought stress by 57.57, 59.52, 15.34, 15.79, 202.33 and 212.45 mg/kg for available N, P and K, in two seasons, respectively. The previous results seemed to be supported by those obtained by other researchers (El-Kady and Borham, 2013 and Aly et al., 2016), who revealed that application of hydrophilic polymers reduced nutrient losses from soils and enhanced plant growth by allowing nutrients, incorporated into the hydrogel matrix, to release to the plant. These results showed that SAP aids to increase the capacity of soil cationic exchange and better absorption of water and nutrition materials; that is the result of not washing the water and fertilizers. Yaseen et al. (2018) also reported that PGPR was the most significant factor affected NPK availability in soil.

PGPR play an essential role in nutrient cycling in soils, they involved in processes such as oxidation, nitrification, ammonification and nitrogen fixation. Kurokura et al. (2017) stated that the formation of organic acids by PGPR contribute in the conversion of undissolvable form of phosphorus to dissolvable one.

TABLE 6. Nutrients uptake in maize grain as affected by superabsorbent polymer and EPS producing bacteria under drought stress conditions in 2018 and 2019 seasons

Treatments	N uptake (kg ha ⁻¹)		P uptake (kg ha ⁻¹)		K uptake (kg ha ⁻¹)		
	2018	2019	2018	2019	2018	2019	
50% of WR	Con.	25.04l	34.43j	2.79j	3.71k	2.42j	3.02l
	SAP	37.94j	48.66h	1.71i	5.06j	4.91h	5.72j
	Bio	35.80k	44.20i	1.80h	5.11j	3.83i	4.30k
	SAP + Bio	42.73i	56.12g	5.42g	7.09 h	6.48g	7.73h
75% of WR	Con.	46.22h	56.67g	5.58g	6.41i	5.79gh	6.46 i
	SAP	62.32g	74.20f	9.11e	10.48f	9.74d	10.53e
	Bio	64.93f	79.11e	9.42d	11.09e	9.220e	10.20f
	SAP + Bio	75.41d	95.76d	10.58c	12.99d	10.69c	12.33d
100% of WR	Con.	65.60e	83.34e	7.73f	9.49g	7.72f	8.90g
	SAP	90.70 c	116.00c	12.37b	15.42b	12.66b	15.09b
	Bio	94.89b	118.34b	12.47b	14.91c	12.98b	14.34c
	SAP + Bio	122.64a	156.01a	16.42a	20.18a	17.06a	19.72a

WR: Water requirement for maize plant during growing season

Cont.: Control; no application

SAP: Superabsorbent Polymer

Bio: bio fertilizer (EPS producing bacteria)

SAP + Bio: polymer and bio fertilizer

TABLE 7. Effect of superabsorbent polymer and EPS producing bacteria on microbial activities in maize rhizosphere during 2018 and 2019 seasons under drought stress.

Treatments	Total microbial counts×10 ⁵ cfu /g dry soil		EPS producing bacteria counts×10 ⁵ cfu /g dry soil		Dehydrogenase µg triphenyl formazan /g dry soil		
	2018	2019	2018	2019	2018	2019	
50% of WR	Con.	47j	50k	3.1h	5k	0.43k	0.457i
	SAP	57.2h	70i	6.2g	7i	0.513j	0.63h
	Bio	68.5g	80g	7f	9g	0.584i	0.717f
	SAP + Bio	94d	105d	9d	9g	0.611h	0.889c
75% of WR	Con.	47j	56j	3.4h	6j	0.689f	0.713f
	SAP	71f	70i	7.9e	8h	0.702e	0.742e
	Bio	80e	85f	8.7d	11e	0.785c	0.901c
	SAP + Bio	144b	146b	14.9c	19c	1.086a	1.259a
100% of WR	Con.	52.5	78h	7f	10f	0.611h	0.640h
	SAP	72.4f	96e	9d	13d	0.636g	0.671g
	Bio	119c	138c	17b	23b	0.744d	0.849d
	SAP + Bio	169a	180a	20a	25a	0.981b	1.036b

WR: Water requirement for maize plant during growing season

Cont.: Control; no application

SAP: Superabsorbent Polymer

Bio: bio fertilizer (EPS producing bacteria)

SAP + Bio: polymer and bio fertilizer

TABLE 8. Effect of superabsorbent polymer and EPS producing bacteria on nutrient availability in soil under drought stress conditions.

Treatments	Available N (mg kg ⁻¹)		Available P (mg kg ⁻¹)		Available K (mg kg ⁻¹)		
	2018	2019	2018	2019	2018	2019	
50% of WR	Con.	20.30j	22.30i	6.20i	6.39i	134.89l	141.63l
	SAP	23.68i	25.68h	6.70h	6.90h	162.77g	170.91g
	Bio	27.07h	29.07g	8.91f	9.17f	148.22j	155.63j
	SAP + Bio	30.45g	32.45f	10.10e	10.40e	170.56e	179.09e
75% of WR	Con.	23.68i	26.68gh	7.23g	7.42g	139.40k	146.37k
	SAP	33.83f	36.83e	8.753f	9.01f	166.87f	175.21f
	Bio	37.22e	40.22d	10.26e	10.56e	161.54h	169.62h
	SAP + Bio	50.75c	53.75b	13.267c	13.67	186.14d	195.45d
100% of WR	Con.	33.83f	35.83e	7.23g	7.44g	150.06i	157.56i
	SAP	43.98d	45.98c	12.89d	13.28d	195.16b	204.92b
	Bio	54.13b	56.13b	14.52b	14.96b	192.29c	201.90c
	SAP + Bio	57.57a	59.52a	15.34a	15.79a	202.33a	212.45a

WR: Water requirement for maize plant during growing season

Cont.: Control; no application

SAP: Superabsorbent Polymer

Bio: bio fertilizer (EPS producing bacteria)

Conclusion

Application of SAP and EPS producing bacteria improve maize grain productivity, the biological yield and increase the availability of nutrients in soil. Also, the co-application of SAP and EPS producing bacteria increased the positive effect of biofertilizer on the growth and yield of maize under drought condition.

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Compete of Interest

The authors declare no compete of interest.

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تأثير البوليمر فائق الامتصاص والتسميد الحيوى على انتاجية الذرة و خصوبة التربة تحت ظروف إجهاد الجفاف

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هدفت هذه الدراسة إلى دراسة استجابة الذرة لاضافة البوليمر فائق الامتصاص و البكتيريا المنتجة للسكريات المتعدده (EPS) تحت ظروف الإجهاد في منطقة بنجر السكر الإسكندرية، مصر. تم تحديد الجاميع الوظيفيه و محتوى البوليمر من المعادن الثقيلة. ازدادت قدرة البوليمر للتشبع بالماء مع زيادة وقت التحلل المائي حتى 30 دقيقة وتفاوتت نسب فقد الماء من البوليمر من 0.25 إلى 8 أيام. تم اختبار ثلاث عزلات بكتيرية من نوع *Klebsiella oxytoca* و *Serratia marcescens* و *Pseudomonas aeruginosa* بكتيريا منتجة للسكريات المتعدده وتم تعريفها على أساس معاييرها المورفولوجية والكيميائية الحيوية والجزئية. تراوح إنتاج السكريات المتعدده للعزلات المختارة من 0.85 إلى 1.24 جم / 100 مل وتراوحت نسبة النشاط المضاد للأكسدة للسكريات المتعدده من 1.464 إلى 1.827٪. يمكن للعزلات البكتيرية تحمل ما يصل إلى -2.24 ميغا باسكال من الإجهاد المائي كذلك ويزداد إنتاجها منالسكريات المتعدده مع زيادة مستويات الجفاف. كما تم تقييم معايير تعزيز نمو النبات للعزلات المختارة. تم إجراء تجربة حقلية خلال موسمي النمو الصيفي 2018 و 2019. أظهرت البيانات التي تم الحصول عليها أن الإجهاد الناتج عن الجفاف كان له آثار معاكسة كبيرة على معايير النمو . وقياسات المحصول والنشاط الميكروبي في منطقة الجذور لنبات الذرة . وامتصاص العناصر الكبرى وتوافر المغذيات في التربة. أدى تلقيح بذور الذرة باستخدام العزلات البكتيرية المنتجة للسكريات المتعدده مع البوليمر فائق الامتصاص إلى تحسين جميع الصفات المقاسة مقارنة باللقاح الميكروبي أو البوليمر فائق الامتصاص المستخدم بمفرده. أوضحت النتائج أن استخدام البوليمر فائق الامتصاص والبكتيريا المنتجة للسكريات المتعدده يمكن أن يعزز تحمل نبات الذرة للجفاف.