Effect of Phosphate Dissolving Bacteria on Physiological Behavior of Some Sesame Cultivars under Saline Conditions at Sahle Eltina- North Sinai

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ABSTRACT

Four highly efficient halotolerant phosphates solubilizing bacteria (PSB) were investigated for their activities under saline conditions. Increasing salinity over 3% NaCL concentration had a negative effect on both phosphatase activity and pH reduction resulting a net reduction in their phosphate solubilization activity. The four strains could secrete multiple organic acids under saline condition. Both *Pseudomonas geniculate* and *Alcaligenes faecalis* had achieved the maximum phosphate solubilization at 3% NaCL .

Green house experiment was conducted for evaluation of four sesame cultivars (Sohag ,Giza 32, Shandaweel and Zail Elgamal) and two phosphates solubilizing bacteria (*Alcaligenes faecalis* and *Pseudomonas geniculate*) and mixture of them under saline conditions. The results revealed that Giza 32 was the most resistant cultivar to salinity while Zail Elgamal cultivar was the lowest one, at the same time, mixture of two PSB was more efficient than single bacterial inoculation.

Two growing seasons were conducted at Sahle El-Tina, North Sinai to study the potential of PSB mixture and KCL foliar application on the growth and productivity of sesame cultivar Giza 32. The highest significant increase in sesame yield, oil content and chemical constituents of sesame seeds were recorded by the interaction of biofertilizer and foliar application in both seasons. Gas liquid chromatography analysis indicated that the unsaturated fatty acid constituents in sesame oil were oleic, linoleic and linolenic acid while the predominant saturated acids were palmitic and stearic acids. Dual inoculation of biofertilizer and foliar applications recorded the the highest bacterial counts and phosphatase enzyme activity in the rhizosphere regions.

Key words : ***Sesame ; Pseudomonas sp. ; Alcaligenes sp.* ;salinity.**

INTRODUCTION

Phosphorus (P) is often the most limiting macronutrient for plant growth required in relatively large amounts by plants. One of the main roles of P in living organisms is in the transfer of energy. It plays a significant role in several physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch, transporting of the genetic traits and supplying energy required for metabolic processes (Lal, 2002). Although phosphorus is abundant inboth organic and inorganic forms in the soil , it is one of the major plant growth–limiting nutrient that it is mostly present in an insoluble form that a large proportion of applied P in the soil becomes static (Rahmatullah*et al.*, 1994). The availability of this nutrient for plants is limited by different chemical reactions especially in arid and semi-arid soils as well as alkaline sandy soil which causes the conversion of phosphorus content to unavailable form, mainly as tricalcium phosphate. The efficiency of P fertilizer throughout the world is around 10 - 25 % (Isherword, 1998) and the concentration of bio available P in soil is very low reaching the level of 1.0 mg kg^{-1} soil (Goldstein,1994).

Salinization of soil is a serious problem which increased gradually in many parts of the world, particularly in arid and semiarid areas, nearly 40% of world's surface has salinity problems (Jadhav*et al.*, 2010). Expanding problems of soil salinity and water logging have become serious issues of concern that they affect productivity and sustainability of agriculture. The impact of soil salinity on the concentration of phosphorus in plants depends mainly on plant species, the type and level of salinity and concentration of phospohorus that is already present in the soil (Grattan & Grieve, 1999). In most cases, excess of salts in soil solution leads to a reduction in phosphorus concentration in the tissues of plants (Sonneveldand de Kreij, 1999, Kaya et al. 2001). The reduction of phosphorus availability in saline soils is the result of the activity of ions antagonists, which can reduce the activity of phosphate and phosphate transporters of both high and low affinity, which are necessary for the uptake of phosphorus (Kochian, 2000). Reduction of phosphorus uptake may be a result of the high effect of sorption processes which control the concentration of phosphorus in the soil and low solubility of Ca-P minerals (Marschner, 1995).

The growth of phosphate-solubilizing bacteria (PSB) are playing a key role in phosphorus solubilization in the soil(Abd-Alla , 1994) that they can transform the insoluble phosphorus to soluble forms $HPO^{-2}$and $H_2PO^{-4}$by acidification, chelation, exchange reactions and polymeric substances formation(Delvasto et al. 2006).Many PSB have been isolated including, for example, those in *Bacillus, Pseudomonas, Erwinia,*

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Agrobacterium, Serratia, Flavobacterium, Enterobacter, Micrococcus, Azotobacter, Bradyrhizobium, Salmonella, Alcaligenes, Chromobacterium, Arthrobacter, Streptomyces, Thiobacillus and *Escherichia*(Zhao and Lin, 2001).Therefore, the use of phosphate solubilizing microbes in agricultural practice would not only overcome the high cost of manufacturing phosphatic fertilizers but also could mobilize insoluble phosphorus in the fertilizers and soils to which they are applied. The salinity stress causes less effect on halotolerant bacteria since they have adapted during evolution to tolerate and optimally grow in hyper saline environments(Nautiyal et al.,2000).So, application of salt-tolerant or halophilic PSB will facilitate the development of saline-alkali soil-based agriculture.

Foliar application of potassium can also be used for alleviating stress on plants. Potassium is a key essential plant nutrient although it is not a constituent of any plant part. It acts as catalyst for many of the enzymatic processes which are necessary for plant growth. It also regulates the opening and closing of stomata which affect carbon dioxide uptake for photosynthesis (Somida, 2002). Also, potassium has an important role in translocation of metabolites from source to sink (Kramer, 1980).Adequate potassium levels in the plant help it to withstand water stress during periods of drought. Potassium regulates the osmotic turgor of the cells and the water balance. Crops grown with adequate potassium availability use less water per unit weight of plant biomass and are therefore better able to survive periods of drought(El-Latif*et al.* 2011).

Sesame (*Sesamum indicum L.)* is a crop grown in many parts of the world for production of high quality oil and its insecticidal and medicinal properties as well as for its cosmetic and ornamental values. Total area under sesame production in Egypt has increased from 11,264 ha in 1961 to 36,907 ha in 2010 and the productivity increased from 1,145.7kg/ha in 2005 to 1,250.3 kg/ha in 2010 (Faostat, 2012). It is grown in many governorates and ranks first among the cultivated oil cropsin Ismailia Governorate (El-Bramawy, 2006).The seed of sesame contains about 50 to 51% oil, 17 to 19% protein and 16 to 18% carbohydrate and that the oil is edible, odourless and semi-drying, containing oleic, stearic and palmitic acids. Sesame oil serves as antioxidant in the manufacture of margarine and salad creams, and as a fixative in the industries for making of perfumes and cosmetics (Yermanos*et al.* 1972).

The present investigation was designed for evaluation of four sesame cultivars under salinity stress and potential of different PSB as P solubilization at salt stress and their effect on the yield ,chemical composition and microbial activity of sesame cultivars.

MATERIALS AND METHODS

The present investigation consisted of two experiments at nursery and field experiments as follows:-

Evaluation of phosphates solubilizing bacteria (PSB) strains under saline condition : Four highly efficient halotolerant phosphates solubilizing bacteria previously isolated from saline soils and identified using partial 16S rRNA gene sequence technique as (*B. subtilis* ATCC 6633**,** *Pseudomonas geniculate* strain ATCC 19374, *Azotobacter chroococcum and Alcaligenes faecalis strain NBRC 13111*) were investigated for their activities as PSB under saline conditions ranged from 0 to 10 % NaCL concentrations. The phosphate solubilizing activities of bacterial strains included the following:

- Change in the pH of Pikovskaya broth media after three days of bacterial inoculation
- Quantitative amount of soluble phosphate in liquid cultures after three days of bacterial inoculation using molybdenum blue method of Watenabe and Olsen (1965).
- Phosphatase activity after three days of bacterial inoculation was estimated by Tabatabai and Brimner method (1969). One enzyme unit of phosphatase was defined as amount of enzyme that hydrolyzed 1mM of p-nitrophenol $hour^{-1}$.
- Organic acid analysis of Pikovskaya broth media at different concentrations of NaCL (The concentrations at which the selected strains exhibited the maximum solubilization activity) after three days of bacterial inoculation using HPLC equipped with a UV detector as described by Aktas*et al.,(* 2005).

Pot experiment

Pot experiment was conducted in the green house of microbiological unit of Desert Research Center to evaluate the four cultivars of sesame and two phosphates solubilizing bacteria under salt conditions. Sesame cultivars to be evaluated were : Sohag ,Giza 32, Shandaweel and ZE (Zail Elgamal) while PSB were : *Alcaligenes faecalis* and *Pseudomonas geniculate* and mixture of them. The Experimental design was a randomized complete block design with three replications. The main block was devoted to sesame cultivars and sub block to bio-fertilizers. Seeds of different sesame cultivars were planted into10 kg pot containing soil of Sahle El-Tina. For bacterial treatments, seeds were coated with bacterial inoculum

Table 1a. Soil mechanical analysis at two depths

Soil depth (cm)	Total sand (%)	Silt (%)	Clay (%)	Texture
0-15	31.0	9.5	59.5	Clay
15-30	29.0	11.0	60.0	Clay

Table 1b.Soil chemical analysis at two depths

Soil depth (cm)	EC Mmhos\ cm	pH	Soluble anions meq\L			Soluble cations meq\L			
			HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
0-15	12.1	7.7	19.5	52.6	10.0	22.1	15.3	60.1	0.46
15-30	11.0	7.5	17.4	50.5	9.4	20.0	15.0	52.3	0.81

using CMC solution (1%) before application to get a thin, uniform coating of bacterial inoculum on seeds. Inoculated seeds were dried in shade before sowing (Samasegaran*et al.*,1982),untreated control seeds were maintained. After seed germination, each pot was inoculated with 10 ml of microbial inoculum of an individual strain and mixture of them. Pots had been irrigated two times weekly. After 45 days of planting, the plants were harvested, and plant heights, fresh and dry weights were measured. A mechanical and chemical analysis of soil is presented in Table (1a,b).

Field experiment:-

Two growing seasons were conducted in 2015 and2016 at Sahle El-Tina North Sinai to study the potential of PSA mixture (*Alcaligenes faecalis* and *Pseudomonas geniculate*) and foliar application on the growth and productivity of selected sesame cultivar . Sahl El-Tina area is located within the extremely arid zone and suffering from mixed water and salinity problems. Organic manure and calcium superphosphate fertilizers were added during soil preparation at the rate of 20 m^3 and 33 Kg P_2O_5/fed respectively. Three equal doses of ammonium sulphate (20.5% N) were added at a rate of 66 kg N/fed. after thinning, 35 and50 days from sowing. Each experimental plot included 5 ridges, 60 cm apart with 3.5 m length, comprising an area of 10.5 m^2 (1/400feddan). This area is irrigated through El-Salam Canal (Nile water mixed with agricultural drainage water at a rate of 1:1), chemical analysis of irrigated water was represented in Table (2).

For bio-treatments, seeds were coated with bacterial inoculum as previously mentioned ,untreated control seeds were maintained. Three foliar application were applied (Two concentrations of KCL 1% , 2% and without as a control) . Foliar application treatments were carried out twice after 45 and 65 days from sowing using tween 20 as wetting agent.

The experiment included 6 treatments, which were the combination of three different foliar application treatments X two bio treatments. Treatments were arranged in a split plot design with three replications. Foliar application treatments occupied the main plots and bio treatments were in the sub plot .At harvest time, sesame plants were collected from each plot and the following characters were determined: plant height (cm), number of branches/ plant, number of capsules per plant, number of seeds / capsules,1000 seed weight, seeds weight / plant, and seeds yield /fed. were recorded. Oil yield / fed. was determined using method of British pharmacopoeia (1936).

For chemical analysis of sesame seeds: Nitrogen was determined by micro-Kjeldahl method according to (Bremner and Mulvaney 1982) .Phosphorus and potassium were estimated using spectrophotometer and flame photometer, respectively as described in (Page *et al*. 1982). Fatty acids were determined using gas liquid chromatography (GLC) as fatty acid methyl esters (FAME) after acidic esterification of oil samples according to the method of Were *et al.* (2006).

For microbiological analysis: Nutrient and modified Bunt and Rovira media were used for counting of total microbes and phosphate dissolving bacteria from rhizosphere samples, respectively .Phosphatase activity in the rhizosphere regions were also estimated by Tabatabai and Brimner method (1969).

Statistical analysis:

Data were subjected to statistical analysis using the method described by (Snedecor, 1990). The least significant difference (L.S.D) was used to differentiate means according to (Waller and Duncan, 1969).

Table 2. Chemical analysis of El-Salam canal water

Water sample	EC Mmhos\ cm	pH	Soluble anions dSm^{-1}			Soluble cations dSm^{-1}			
			HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
Average	1.44	7.5	9.1	17.3	6.4	6.0	8.0	18.6	28.0

RERSULTS

Evaluation of highly efficient phosphate dissolving bacteria under salt stress

In the medium without salts (0% NaCL), the selected four PSB were able to solubilize the insoluble form of phosphate in medium and achieve high concentration of soluble-P that ranged between 41.5 and 52.63 ppm among strains after 72 hours of incubation as in Fig.(1).The solubilization process at (0% NaCL), was accompanied by a drop in initial pH from (6.9 at zero time to 4.6 , 4.5, 4.5 and 4.37) and high phosphatase enzyme activities (0.38, 0.42, 0.58 and 0.61 EU) for *Azotobacter chroococcum* , *Alcaligene faecalis, Pseudomonas geniculate* and *B. subtilis* ,respectively. The inverse relationship observed between the pH and soluble-P concentration indicates that organic acid production by these PSB strains plays a significant role in the acidification of the medium facilitating the P solubilization (Chen *et al.*,2006).Studying the effect of salinity on solubilization process revealed that the maximum phosphate solubilization expressed as soluble-P concentration in the growth media was achieved at 1% NaCL for *Azotobacter chroococcum* (0.39 EU) and 3% for others which mean that *A. chroococcum* was more sensitive to salinity than other strains. For pH, the highest reduction were achieved at 3% NaCL for *Alc. faecales* and 1% for the rest strains. Concerning to phosphatase activity, increasing salinity from 0% to 9% NaCL had a negative effect on enzyme activity that the highest activity was recorded at 0% NaCL for all strains studied which decreased gradually with increasing salinity. The acidification does not seem to be the only mechanism of solubilization, as the ability to reduce the pH in some cases did not correlate with the ability to solubilize mineral phosphates (Subbarao, 1982).These mean that the solubilization is due to both reduction of pH and secretion of phosphatase enzymes. As a particular case, Gram-negative bacteria had shown to be capable of mobilize insoluble phosphate very efficiently; they produce gluconic acid during the extracellular oxidation of glucose catalyzed by quinoprotein glucose dehydrogenase (Goldstein,1994).Salinity up to 1% is very suitable for the growth, activity and effectiveness of phosphate solubilizing bacteria as *Pseudomonas fluorescens, Bacillus thuringiensis, B. megaterium, Nocardi amesentrica, Aerobacter aerogenes, Azospirillum lipoferum* and *Azotobacter indicus* while 4% salinity is still safe for their growth and activity (Sri,2015).

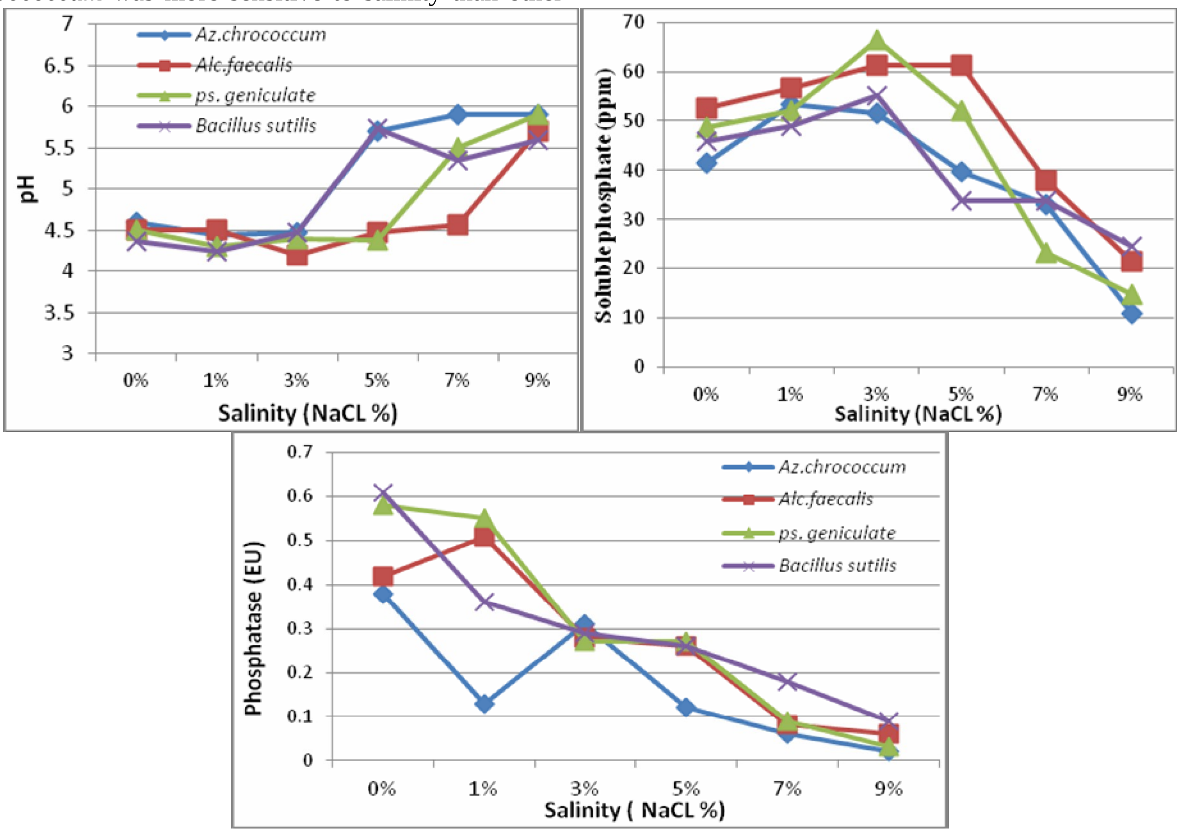

Figure 1.Effect of salinity on pH, phosphate and phosphatase activity of four PSB

At 3% NaCL concentration ,*Pseudomonas geniculate* and *Alcaligenes faecalis* gave the maximum phosphate solubilization and so, they were selected as most efficient phosphates solubilizing bacteria under salt stress for further pot and field investigations. As recorded by Bikash*et al*. (2017), *Alcaligenes faecalis* exhibited maximum phosphate solubilizing activity to be 48 µg/ml with decreasing in the pH of the growth medium from 7.0 to 3.2 and high alkaline phosphatase activity.

Concerning to organic acids, HPLC analysis of bacterial broth grown at 1% NaCL for *Azotobacter chroococcum* and 3% for others (The concentrations at which the selected strains exhibited the maximum solubilization activity) revealed that the four strains secreted multiple organic acids as malic acid, formic acid , oxalic acid , lactic acid and shikimic acid with different quantities, while *Alcaligenes faecalis* and *Pseudomonas geniculate* also produce citric and succinic acids, only *B .sutilis* produce tartaric acid. In addition , all strains except *Azotobacter chroococcum* produced ascorbic acid as presented in Table (3). Alam *et al*. (2002) and Kumari *et al.* (2008) reported that malic, tartaric, oxalic, and citric acids have high capability to release soluble phosphate from insoluble tricalcium phosphate. During phosphate solubilization, *Alcaligenes faecalis* secreted various organic acids, such as oxalic acid (289 mg/L), citric acid (0.2 mg/L), malic acid (0.3 mg/L), succinic acid (0.5 mg/L) and acetic acid (0.4 mg/L) in the broth culture (Bikash *et al*. 2017).

Pot Experiment:-

Evaluation of sesame cultivars under salt stress revealed significant differences among all cultivars for all traits recorded. While Giza32 cultivar recorded the highest plant height, fresh and dry weight (34.16 cm, 24.6 and 7.07gm), respectively, Zail Elgamal cultivar recorded the lowest one (34.16 cm , 24.6 and 7.07 gm) as in Table (4). This means that Giza32 was the most resistant cultivar to salinity followed by Shandaweel and Sohag while ZE cultivar was the lowest one. These results may be due to the differences in genetic background between the four studied cultivars. Difference in sensitivity and response of plants to NaCl at cultivars level largely depends upon genetic constitution of the plants (Yeo and Flowers, 1986;Garcia-Reina *et al*., 1988).

For biotreatments studied, *Ps. geniculate* was more efficient than *Alc. faecalis* for enhancing all sesame growth traits. Also, mixture of *Pseudomonas geniculate* and *Alcaligenes faecalis* gave the highest results for all traits recorded compared to each one separately as indicated in Table (4). *Pseudomonas geniculate* characterized as plant growth-promoting significantly enhanced shoot and root weight of chickpea (Subramaniam*et al.* 2015). *Alcaligene faecalis* showed significantly taller plant height, more number of leaves, higher numbers of pods and plant dry weight of soybean compared to un-inoculated ones(Nandini*et al.* , 2014). From previous data, both Giza31 cultivar and mixed bio-fertilizers were selected for further investigation in two successive field experiments.

Field experiments

Application of mixed bio treatment as seed coating increased the seeds yield /fed. reaching 452.6 and 472.5 Kg/fed. compared to untreated plants in first and second season, respectively. This might be attributable to the increase in the number of branches, number of capsules /plant as tabulated in Table (5), number of seeds / capsules and 1000 seed weight (g) compared to untreated ones. Sabannavarand Lakshman (2009) mentioned that bio fertilization increased plant growth, number of capsules/plant and seed yield of sesame.

Table 3. Organic acids secreted by four phosphate solubilizing strains under salinity condition

Standard organic acids (mg/100ml)	**RT (min)**	**Organic acids (mg/100ml)**			
		Alcaligenes faecalis	***Bacillus subtilis***	***Azotobacter chroococcum***	***Pseudomonas geniculate***
Ascorbic acid	6.569	0.38	0.365	-	0.46
Citric acid	11.153	8.2	-	-	8.05
Formic acid	5.346	3.2	9.465	4.437	3.9-
Lactic acid	7.471	4.65	3.722	3.015	4.27
Malic acid	8.717	5.7	4.3	5.1	6.33
Oxalic acid	4.413	10.98	0.771	2.435	9.96
Shikimic acid	6.342	0.14	0.046	0.045	0.09
Succinic acid	12.610	4.53	-	-	3.9
Tartaric acid	5.013	-	4.077	-	-

Table 4.Evaluation of different sesame cultivars and biofertilizer on different growth traits of sesame plant in pot experiment

Treatments		Growth Characteristics		
Cultivar	**Biotreatments**	**Plant height (cm)**	**Fresh weight (gm)**	**Dry weight (gm)**
Effect of Cultivar				
Sohag		32	31.42	6.34
Giza32		34.16	24.6	7.07
Shandaweel		32.25	18.55	5.82
Zail Elgamal		28.66	18.41	5.16
LSD		1.46	0.953	0.317
Effect of biofertilizer				
Without		28.58	17.25	5.31
Alc. faecalis		32.41	22.96	5.84
Ps. geniculate		32.75	25.45	6.4
Mixture		33.4	27.4	6.85
LSD		1.54	1.52	0.14
Effect of interaction				
Sohag	Without	29.5	16.7	5.3
	Alc. faecalis	33	27.1	6.4
	Ps. geniculate	32.8	25.9	6.4
	Mixture	33.1	28.8	7.1
Giza32	Without	30.8	22.2	6.0
	Alc. faecalis	34.8	29.2	6.4
	Ps. geniculate	35.7	37.1	7.8
	Mixture	35.8	37.2	7.9
Shandaweel	Without	28.1	13.8	5.4
	Alc. faecalis	33.4	17.5	5.7
	Ps. geniculate	33.2	19.5	6
	Mixture	34.8	23.3	6.1
Zail Elgamal	Without	26.4	16.2	4.4
	Alc. faecalis	28.7	17.3	4.7
	Ps. geniculate	29.3	19.2	5.2
	Mixture	30.4	21.2	6.2
L.S.D 0.05		1.32	1.03	0.16

Concerning to foliar spraying of potassium, significant increases in yield and all of its components i.e. number of branches, number of capsules /plant, number of seeds / capsules, 1000 seed weight (g), seeds weight / plant (g) ,seeds yield /fed. (kg) were recorded with spraying different concentrations of KCL (1 and 2 %) compared with the control. Khan *et al.* (2006) carried out experiments to evaluate the response of wheat to foliar application of N and K and observed that both KNO_3 and KCL used as foliar spray were proven to be equally effective in increasing wheat yield. Abaye (1998) showed that supplementing potassium by any method increased lint yields compared with that of untreated control.

Concerning the interaction between bio-fertilizers and foliar application, plant height number of branches, number of capsules /plant, number of seeds / capsules, 1000 seed weight (g), seeds weight / plant (g) ,seeds yield /fed. (kg) and oil% of sesame plants were significantly increased with applying both bio treatment and foliar application under saline soil conditions. Elwan (2010) mentioned that spraying of di-potassium hydrogen orthophosphate (K_2HPO_4) ameliorated the negative effects of salinity on plant growth, fruit yield and fruit total sugar content of egg plant.Hussain *et al.* (2015) indicated that the effects of salinity were ameliorated by the application of K and P fertilizers resulting in higher yield. Shehu *et al*. (2010) observed that number of capsules/plant and seed weight/plant were increased by adding potassium fertilization (22.5 and 45 kg K_2O/ha). Under farm field conditions, *Pseudomonas geniculata* significantly enhanced nodule number and weight, shoot, and root weight, stover and grain yield and total dry matter of chickpea (Subramaniam*et al.*, 2015).

Table 5. Effect of Bio-fertilizer and foliar application treatments on the yield of sesame plant and its components

Treatments		Plant height (cm)	Number of branches / plant	Number of capsules /plant	Number of seeds/ capsules	1000 seeds weight (g)	Seeds weight / plant (g)	Seeds yield /fed. (Kg/fed.)	Oil Yield (Kg/fed)
Season 2015									
Effect of Biofertilizar:-									
Control		120.6	3.2	52.7	49.7	3.5	13.1	371.1	190.0
Bio.1		132.5	3.9	58.2	55.8	4.2	16.0	452.6	225.4
L.S.D.		1.57	0.36	0.23	0.70	0.06	0.44	33.15	15.56
Effect of foliar application									
Control		112.85	2.8	49.00	45.55	3.1	12.05	360.85	166.05
KCL 1 %		129.60	3.7	56.35	53.50	4.0	14.85	416.15	213.60
KCL 2 %		137.35	4.2	60.95	59.20	4.4	16.70	458.6	243.50
L.S.D.		1.26	0.05	0.62	0.78	0.14	0.44	30.09	12.38
Effect of interaction:-									
Bio.	Foliar								
Without	Control	109.2	2.6	46.4	42.6	2.6	11.5	325.2	145.6
	KCL1 %	120.6	3.2	53.2	50.2	3.8	13.2	385.5	198.6
	KCL2 %	132.2	3.8	58.5	56.2	4.0	14.6	402.6	225.8
Mixture	Control	116.5	3.0	51.6	48.5	3.6	12.6	396.5	186.5
	KCL1 %	138.6	4.2	59.5	56.8	4.2	16.5	446.8	228.6
	KCL2 %	142.5	4.6	63.4	62.2	4.8	18.8	514.6	261.2
L.S.D.		1.43	0.03	0.21	0.80	0.10	0.38	29.55	17.43
Season 2016									
Effect of Biofertilizar:-									
Control		118.6	3.3	54.8	51.2	3.53	14.7	383.8	195.7
Bio.1		135.6	4.1	59.3	57.8	4.30	16.7	472.5	233.1
L.S.D.		1.22	0.20	0.34	0.65	0.26	0.30	31.16	11.07
Effect of foliar application									
Control		109.00	3.0	48.15	46.85	3.05	12.9	371.0	168.6
KCL 1 %		133.05	3.8	59.10	56.40	4.00	16.1	437.1	222.4
KCL 2 %		139.30	4.4	63.80	60.25	4.70	18.1	476.5	252.2
L.S.D.		1.19	0.13	1.04	1.50	0.22	1.09	26.40	22.26
Effect of Interaction :-									
Bio.	Folair								
Without	control	98.60	2.8	47.5	43.5	2.6	12.6	336.5	148.2
	KCL1 %	122.5	3.2	55.6	53.6	3.8	15.4	392.6	204.6
	KCL2 %	134.8	4.0	61.2	56.5	4.2	16.0	422.5	234.2
Mixture	control	119.4	3.2	48.8	50.2	3.5	13.2	405.5	189.0
	KCL1 %	143.6	4.4	62.6	59.2	4.2	16.8	481.6	240.2
	KCL2 %	143.8	4.8	66.4	64.0	5.2	20.2	530.5	270.2
L.S.D.		1.35	0.31	1.00	1.21	0.11	0.80	8.02	20.22

Similar trends were observed for oil content in sesame seeds that interaction between mixed bio treatment and foliar application of 2% KCL recorded the highest results reaching 261.2and 270.2 Kg/fed. for the first and second season compared to controls (190 and 195.7 Kg/fed.) , respectively.

As indicated in Table (6), mixed biofertilizer was found to be highly significantly effective in increasing NPK values of seed (3.42 ,0.55 and 1.07%) respectively comparing to untreated ones in second season. Quesni *et al.* (2010) concluded that biofertilizers decreased the hazard effect of salinity and exerted a favorable effect on growth and N, P and K concentration in *Schefflera arboricola* L. seedlings irrigated with saline water.

For foliar application, spraying plants with 2% KCL resulted a significant increase in chemical contents of seeds, recorded highest values (0.55and 1.105 %) for P and K, respectively, comparing to control in the second season. On other hand, application of K had no significant increase in N content of seed in two seasons .Foliar feeding of a nutrient may actually promote root absorption of the same nutrient (Oosterhuis, 1998) or other nutrients through improving root growth and increasing nutrients uptake (El-Fouly and El-Sayed, 1997). Furthermore, the highest significant increase in N, P and K concentrations of sesame seeds was recorded by the interaction of biofertilizer and foliar application in both seasons. Generally the increases in macronutrient concentrations in seeds may be due to the availability of them in the soil as a result of decreasing soil pH and salinity caused by the action of organic materials or biofertilizer (Antoun *et al.* 2010).Under field condition, *Pseudomonas geniculate* significantly enhanced grain yield, total nitrogen and available phosphorus of chickpea (Subramaniam *et al.,* 2015).

Fatty-acids of sesame oil :

Composition of sesame oil fatty acids under different treatments are presented in Table (7). Results indicated that the highest fatty-acid percentage was as follows: Oleic, linoleic and palmitic acids. Data also indicated that the unsaturated fatty acid constituents in sesame oil were oleic, linoleic and linolenic acid while the predominant saturated ones were palmitic and stearic acids. As illustrated by Nzikou *et al.* (2009), the main saturated fatty acids in sesame seed oil were palmitic (8.67%), stearic (5.56%) acids with small arachidic acid (0.8%). Oleic and linoleic acids were the major fatty acids of sesame oil which reported in large amounts in the oils of all genotypes, palmitic and stearic acids were reported as predominant saturated fatty acids while arachidic acid as minimum ones (Kurt *et al*., 2016). Application of mixed bacterial treatment showed slightly increase in all saturated fatty acids compared to the control (without biofertilizer) while contents of Linoleic and Linolenic acids were decreased under the same conditions. Concerning to foliar application of KCL, increase in oleic acid and all saturated fatty acids compared to the control were detected. In addition, the decrease of linoleic and linolenic acid were detected under the same foliar application.

Table 6. Effect of biofertilizer and foliar application treatments on chemical components of sesame seeds

Treatments		Chemical constituents of sesame seeds					
		Season 1			Season 2		
		N%	P%	K%	N%	P%	K%
Effect of Biofertilizar:-							
Control		3.2	0.47	1.02	3.23	0.5	1.03
Biotreatment		3.12	0.546	1.05	3.42	0.55	1.07
L.S.D.		0.1	0.021	0.06	0.051	0.021	0.011
Effect of foliar application							
Control		3.15	0.48	0.975	3.31	0.495	0.98
KCL_1 %		3.16	0.52	1.05	3.33	0.53	1.07
KCL_2 %		3.175	0.53	1.08	3.34	0.55	1.105
L.S.D.		0.11	0.026	0.074	0.063	0.025	0.014
Effect of interaction:-							
Biotreatment	Folair						
	Control	3.1	0.45	0.94	3.23	0.46	0.94
Without	KCL_1%	3.13	0.49	1.05	3.24	0.51	1.06
	KCL_2 %	3.14	0.49	1.08	3.24	0.53	1.09
	Control	3.2	0.51	1.01	3.4	0.53	1.02
Mixture	KCL_1%	3.2	0.56	1.06	3.43	0.55	1.08
	KCL_2 %	3.21	0.57	1.08	3.44	0.57	1.12
L.S.D.		0.12	0.021	0.076	0.068	0.024	0.012

Table 7. Effect of Bio fertilizer and foliar application treatments on fatty acids composition of sesame seeds

Treatments		Fatty acids composition (%)										
		Saturated					Unsaturated					
Bio.	Foliar	Myristic	Palmitic	Stearic	Arachidic	Total	Oleic	Linoleic	Linolenic	Total	TotalUnsat.: Total Sat.	Linoleic :Linolenic
Without	Control	0.23	11.2	3.8	0.62	15.85	45.74	36.9	0.64	83.28	5.25	1.23
	KCL 1%	0.32	11.6	4.2	0.73	16.85	46.12	36.4	0.37	82.89	4.91	1.26
	KCL2 %	0.33	11.9	4.3	0.74	17.27	46.20	36.1	0.39	82.69	4.78	1.27
Mixture	Control	0.25	11.4	4.0	0.64	16.29	45.63	36.9	0.41	82.94	5.09	1.23
	KCL 1%	0.37	12.2	4.5	0.77	17.84	45.77	35.8	0.36	81.93	4.59	1.27
	KCL2 %	0.37	12.5	4.6	0.76	18.23	46.25	35.1	0.38	81.73	4.48	1.31

Table 8. Effect of Bio fertilizer and foliar applications on microbiological characteristics of sesame rhizosphere

Treatments		Season 1			Season 2		
Bio	Foliar	Total microbial count x 10^5 CFU g^{-1} dry soil	Phosphate Dissolving Bacteria x 10^3 CFU g^{-1} dry soil	Phosphatase enzyme (EU)	Total microbial count x10^5 CFU g^{-1} dry soil	Phosphate Dissolving Bacteria x 10^3 CFU g^{-1} dry soil	Phosphatase enzyme (EU)
Without Bio	Control	82	2.5	0.52b	98	2.8	0.54b
	KCL 1%	102	2.6	0.56b	104	3.4	0.54b
	KCL 2 %	104	2.5	0.56b	1.6	3.5	0.56b
Mixture	Control	180	7.3	0.67a	265	9.1	0.69a
	KCL 1%	192	7.5	0.66a	269	9.4	0.69a
	KCL 2 %	191	7.5	0.68a	268	9.5	0.71a
L.S.D				0.039			0.035

- Initial total microbial counts was 63 ×10^5 cfu g^{-1} dry soil
- Initial Phosphate Dissolving Bacteria 1.9 x 10^3 cfu g^{-1} dry soil
- Initial phosphatase enzyme was 0.34 (EU)

Microbiological activities

Both total microbial populations and phosphate dissolving bacterial counts had increased in all treatments over the initial population prior to field experiments. Mixed bio-treatment with *Pseudomonas geniculate* and *Alcaligenes faecalis* recorded the highest counts of total and phosphate dissolving bacteria compared to that without bacterial inoculation in both seasons as indicated in Table (8) . Foliar application of KCL have no remarkable increase on total microbial community or phosphate dissolving bacteria in the rhizosphere region. Ashrafuzzaman*et al.* (2009) reported that inoculation with the plant growth promoting rhizobacteria had stimulation effect on the population of rhizosphere microorganism (RMO) and increased their numbers by more than 50% at the end of the experiment comparing with the number recorded before planting.

Concerning to phosphatase enzyme, a dual effect of bacterial and foliar applications produced the highest significant activity of the enzyme in the rhizosphere regions reaching 0.71 EU followed by bacterial inoculation only which recorded 0.69 EU in second season of planting. On the other hand, application of KCL at both concentrations have no significant effect on the enzyme activity at the rhizosphere regions. Many enzymes as phosphatase are produced constitutively by microbes and generate low concentrations of microbially-available products that induce additional enzyme synthesis (Koroljova *et al.*, 1998; Klonowska *et al.*, 2002).

CONCLUSION

It could be concluded that, while increasing salinity had a negative effect on phosphatase activity enzyme activity which decreased gradually with increasing salinity, solubilization expressed as soluble-P concentration in the growth media had achieved at 1% NaCL and 3% for bacterial strains under investigation and this may be attributed to the reduction in the pH of the growth media. Giza32 cultivar of sesame was the most resistant cultivar to salinity followed by

Shandaweel and Sohag while ZE cultivar was the least one. Both mixed biofertilizer of *Pseudomonas geniculate* and *Alcaligenes faecalis* and foliar application of KCL had significant positive effect on the sesame yield, oil content and chemical constituents of sesame seeds under saline condition.

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الملخص العربي

تأثيرالبكتيريا المذيبة للفوسفات على السلوك الفسيولوجى لبعض اصناف السمسم تحت ظروف الملوحة بسهل الطينة- شمال سيناء

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تم دراسه نشاط اربعه من السلالات البكتيريه المتحملـه للملوحه والمذيبه للفوسفات تحت الظروف الملحيه. وقد كان لزيادة الملوحه لاكثر من ٣% كلوريد صوديوم تأثير سلبى على كل من نشاط انزيم الفوسفاتيز والنقص فى الاس الهيدروجينى لبيئه النمو مما يؤدى الى حدوث نقص واضح فى قدره البكتيريا على اذابة الفوسفات . وقد تبين قدره الاربع سلالات على افراز الاحماض العضويه المختلفه تحت ظروف الملوحه . وقد سجلت كل من بكتيريا *Pseudomonas geniculate* و *Alcaligenes faecalis* اعلى اذابه للفوسفات عند تركيز ٣% كلوريد صوديوم.

تم تطبيق تجربة الاصص بالصوبة وذلك لتقييم اربع اصناف من السمسم (سوهاج ، جيزه ٣٢، شندويل ، ذيل الجمل) بجانب تقييم السلالات البكتيريه *Pseudomonas geniculate* و *Alcaligenes faecalis* منفرده اوكمخلوط بكتيرى تحت ظروف الملوحة . وقد أظهرت النتائج أن صنف جيزة ٣٢ كان أكثر الاصناف قدره على تحمل الملوحه بينما صنف ذيل الجمل أقل الاصناف تحملا وفى نفس الوقت كان المخلوط البكتيرى افضل من التلقيح الفردى.

تم اجراء تجربتين زراعيتين بمنطقة سهل الطينة (شمال سيناء) وذلك بهدف تقييم قدرة المخلوط البكتيرى والرش الورقى من كلوريد البوتاسيوم على نمو وانتاجية نبات السمسم جيزة ٣٢ . وقد وجد أن التلقيح بمخلوط البكتيريا والرش الورقى يزيد من كميه المحصول، كميه الزيت والتركيب الكيميائى لبذور السمسم فى الموسمين. وقد أوضحت التحاليل ان الاحماض الدهنيه الغير مشبعه فى زيت السمسم هى الاوليك ،اللينوليك واللينولينيك بينما اعلى نسبه للاحماض الدهنيه المشبعه كانت للبالميتيك والسيتريك. وكان التلقيح بالمخلوط البكتيرى والرش الورقى قد انتج زيادة فى اعداد ونشاط انزيم الفوسفاتيز فى منطقة الريزوسفير.