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Multifaceted Potentialities of Some Rhizobacteria Associated With Sorghum Plants on Their Growth and Development

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ABSTRACT

The present study deals with the potential of rhizobactria isolated from soils rhizosphere associated with Sorghum [*Sorghum bicolor* (L.) Moench] roots. The isolates were characterized for production of indole-3-acetic acid (IAA), phosphate solubiliztion ability and some lytic enzyme activity as functional potentialities correlated with plant growth promoting activities. Consequently, the isolates were identified by 16S ribosomal RNA by sequencing analysis. The result appeared that most isolates produced IAA and the highest amount of IAA was detected from *Bacillus megaterium* which produced 0.453 µg/ml and *Pseudomonas hibiscicola* that produced 0.370 µg/ml.

Sorghum plants inoculated with selected rhizobacterial strains were significantly enhanced specially by *Pseudomonas geniculat* (SC), *Rhizobium pusense* (SD), *Bacillus cerues* ATCC 14579 (S4) and *Bacillus cerues* strain X3 (S2) *Lysinibacillus* sp (S3). Whereas, the mineral contents (Mn, Fe, Cu, Zn, N, P and K) were significantly higher values in the shortest plant shoot and lowers in the higher shoot of sorghum plants. Meanwhile, sorghum parameters of photosynthetic pigments, amino acids and N, P, and K concentrations were increased significantly as compared with its untreated control. Consequently, the more efficient isolates were identified as *Rhizobium pusense*, *Bacillus cerues* strain X3 and *Bacillus cerues* ATCC 14579 respectively. Further, the isolate may be used as plant growth promoting rhizobacteria and could use as abiocontrol agents based on the production of lytic enzymes like protease, amylase, lipase and chitinase which are the key enzyme for lyses fungal cell wall.

It could be concluded that the isolated strains have the ability for production of phytohormons and phosphate solubilization which can be used as abiofertilizers due to enhancing the tested crop plant growth parameters. Finally the rhizospheric isolated strains could used as bioinculant to increase plant tolerance against biotic and a biotic stress and providing a step forward toward sustainable agriculture.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is fifth among six principal cereal crops in the world. It is a food staple in large portions of Africa and Asia which gluten-free, and contains a high concentration of beneficial phytochemicals whereas, the grain is important for livestock and poultry feed (Asif *et al*. 2010).

Microorganisms play a vital role in maintaining soil fertility and plant health. They can act as biofertilizers and increase the resistance to biotic and abiotic stress. A total of 136 bacteria were isolated, with 83 of them presenting some plant growth mechanism: 47 % phosphate solubilizers, 26 %nitrogen fixers and 57 % producing IAA, 0.7 % HCN and chitinase, 45 % ammonia, 30 % cellulose and 8 % pectinase. The seven best isolates were tested for their ability to promote plant growth in maize (Rodrigues *et al*. 2016). Rhizosphere is a rich niche of microbes and should be explored for obtaining potential plant growth promoting rhizobacteria (PGPR), which can be useful in developing bio-inoculants for enhancement of growth and yield of crop plants. Indole acetic acid is one the most physiologically active auxin and a common product of L-Tryptopohan metabolism by several microorganisms inducing plant growth promoting bacteria (PGPR) such as rhizobium strains (Ambika *et al*. 2014). Plant-growth-promoting rhizobacteria (PGPR) are associated with plant roots and augment plant productivity and immunity; however, recent work by several groups shows that PGPR also elicit so-called 'induced systemic tolerance' to salt and drought. PGPR might also increase nutrient uptake from soils, thus reducing the need for fertilizers and preventing the accumulation of nitrates and phosphates in agricultural soils Yang, *et al*., (2009). Plant growth promoting microorganisms (PGPM) and biological control agents (BCA) are shown to possess secondary beneficial effects that would increase their usefulness as bio-inoculants, regardless of the need for their primary function. Indeed, PGPM, such as *Rhizobium* and *Glomus* spp., can promote plant growth and productivity (primary effect) but have now been shown to also play a role in reducing disease (secondary effect). Conversely, BCA, such as *Trichoderma* and *Pseudomonas* spp., can control disease (primary effect) but have recently demonstrated stimulation of plant growth (secondary effect) in the absence of a pathogen Avis *et al*., (2008). The use of naturally existing plant-microbe symbiosis for plant growth and biocontrol reduces synthetic fertilizer and pesticide treatments leading to cost-effectiveness and less impact by nutrients (Boddey *et al.* 2003) and pesticides (Whipps and Gerhardson, 2007) on surrounding fauna and flora. Plants are under constant attack by a vast array of pathogens. To impede their attackers they use both broad-spectrum and pathogen-specific defence mechanisms. The arms race between plants and fungal pathogens is fascinatingly varied, and what might be elicited as a plant defence mechanism against a pathogen could promote or enhance the virulence of other pathogens. Several lines of evidence indicate a co-evolutionary arms race in which both plants and fungi can respond to changes that occur in their opponents (Maor and Shirasu, 2005).

Nowadays, chemical fertilizers are used to boost the crop production. However, its application affects the total productivity of the crops and in the long run the soil becomes sterile and unfit for cultivation practices. Hence in order to enhance the fertility status of the soil, the natural way of feeding the soil with different types of organic inputs (composts, vermicomposts, Biofertilizers, farmyard manure etc.) has been developed so as to ensure sustained productivity. As plant root grow through soil they release water-soluble compounds such as amino acids, sugars and organic acid that supply food for the microorganism (Sivasankari and Pradeep, 2016). The present study aimed to isolate rhizospheric soils bactria associated to sorghum roots. In addition to, evaluate their characteristics in plant growth and developments.

MATERIALS AND METHODS

Isolation and purification of associated rhizobacteria to sorghum roots:

Soil rhizosphere and non-rhizosphere soil associated to sorghum roots were collected in October 2015, when the plant

were three month old (200-150 centimeter tall), soil cores (5 cm X 15 cm) (30 total cores) from around all 10 plants immediately after collection and sieve pooled soil through a 2 mm screen to remove coarse fragments and roots. These samples kept in refrigerators until microbial investigation, using dilution method with nutrient agar medium for bacterial isolation (Jacobs and Gerstein, 1960). One gram of soil rhizosphere were homogenized and aseptically transferred to 9 ml of blank sterile water. Then the solutions were prepared to the dilution level of 10^{-1} to $1o^{-5}$. Thereby, 0.1ml of each of diluted solution was pipetted out into sterile petri dishes by using 1 ml sterile graduate. The sterilized NA media were cooled to the temperature of 40^{o}C and about 15-20 ml of the medium was poured into each petri dish. The randomly selected bacterial colonies were sub-cultured on the same medium slants and were kept at a temperature of 4°C in nutrient broth supplemented with 30% glycerol.

Characterization of bacterial isolates

Morphological characteristics, such as shapes, Gram reaction (Hucker and Conn, 1923), motility, and catalase activity (Whittenbury, 1964) of all isolates, were performed by standard procedures. Motility of bacteria was observed by hanging drop method as described by Bertrand *et al*., (2001).

Bacterial Identification using 16 S rRNA sequences;

The most efficient bacterial isolates were completely identified by using 16S rRNA sequences technique as the following: The isolate was grown in nutrient broth medium and incubated on a rotary shaker (120 rpm) at 28^{0}C for 24 hrs. Bacterial genomic DNA extracted by use protocol of GeneJet genomic DNA purification Kit (Thermo K0721) according to SIGMA company instructions. PCR made by using Maxima Hot Start PCR Master Mix (Thermo K1051). The universal 16S primers used were as follows:

F: AGA GTT TGA TCC TGG CTC AG,
R: GGT TAC CTT GTT ACG ACT T

Discard the Gene JET™ purification column and store the purified DNA at -20°C. PCR product sequenced by GATC Company using ABI 3730xl DNA sequencer by using forward and reverse primers. 16S rRNA gene sequences were compared with the other bacterial sequences using NCBI mega Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi)

Screening of bacterial isolates for plant growth promoting (PGP) activities:

Quantitative estimation of indole-3-acetic acid (IAA) production:

Production of auxin by PGPR strains was assayed based on the method described by Patten and Glick (1996). Briefly, each inoculum was cultured in Erlenmeyer flask (250 ml in volume) containing 100 ml sterilized nutrient broth medium and incubated at 28^{0}C for 48 hrs. in a shaker incubator at120 rpm. Then 50 µL of each bacterial suspension were transferred to sterilized nutrient broth containing 50 µg mL^{-1} L-tryptophan. After 48 hrs., the suspensions were centrifuged at 8000 rpm for 10 min. Consequently, 1 mL of supernatant was mixed with 4 mL Salkowski reagent (2 mL 0.5 mol L^{-1} $FeCl_3$ + 98 mL 35% $HClO_4$). After 20 min, the samples that turned red were considered as positive and the absorbance of the mixture was measured at 535 nm with a spectrophotometer, tryptophan concentration was detected through preparation stander curve.

Evaluation of phosphate solubilizing ability of bacterial isolates on agar plates:

Bacterial ability to solubilize soluble phosphate was assayed on Pikovskaya medium (Pikovskaya, 1948) containing yeast extract 0.5 g, glucose 10 g, Ca_3 $(PO_4)_2$ (5.oo g) , $(NH_4)_2SO_4$ (0.5 g), KCl (0.2 g) , $MgSO_4$ $7H_2O$ (0.1 g), $MnSO_4 \cdot H_2O$ (0.0001 g), $FeSO_4$ $7H_2O$ (0.0001 g) and agar (15 g). After 5 days of incubation, phosphate solubilization was verified by clear halo zone appearance around colonies. Diameters of bacterial colony and zone of clearance was measured and according to their values the relative efficiency of phosphate solubilization was evaluated according to Nguyen *et al.,* (1992) as follows:

$$SE = \frac{\text{Solubilization diameter}}{\text{Growth diameter}} \times 100$$

Screening of bacterial isolates for hydrolytic enzymes activities:

In vitro assays of lipase enzyme activity on agar plats

Determination of lipase enzyme implemented: by using nutrient agar as described with (Omidvari, 2008): Peptone 10 g, calcium chloride 0.1 g, sodium chloride 5 g, Agar 15 g, distilled water 1 Liter and 10 mL sterile Tween 20. All of bacterial isolates were streaked on plats of nutrient agar medium and incubated at 28^{o}C for 48 hrs. Depositions around the bacterial colonies indicted activity of lipase enzyme. The Relative Enzyme activity was measured according to Bradner *et al.,* (1999) using formula:

$$\frac{\text{Clear zone diameter-Colony diameter}}{\text{Colony diameter}}$$

Assay of Protease activity upon SMA medium:

Determination of protease enzyme: bacterial isolates were spotted on plates of Skime milk agar (SMA) media (casein agar medium (g/l) (peptic digest of animal tissue, 5.0; beef extract, 1.5gm; yeast extract, 1.5gm; sodium chloride, 5.0gm, agar, 15gm , skim milk powder 15 gm and incubated at 28 ±2°C for 48 hrs. hrs. The diameters of colorless halo zone around the bacterial colonies were measured to determine the ability of protease production. The Relative Enzyme activity was measured according to Bradner *et al.,* (1999) using formula**:**

$$\frac{\text{Clear zone diameter-Colony diameter}}{\text{Colony diameter}}$$

Assay of chitinolytic activity on colloidal chitin agar medium:

All bacterial strains were spotted on colloidal chitin agar medium plates and incubated for 3 days at 30°C. Appearance of clearance zone around bacterial colony indicated on chitinolytic activity. The Relative Enzyme activity was measured according to Bradner *et al.,* (1999) using formula:

$$\frac{\text{Clear zone diameter-Colony diameter}}{\text{Colony diameter}}$$

Assay of starch hydrolysis (amylase activity) upon agar plates:

All bacterial strains were streaked on sterilized starch agar medium poured in sterilized petri plates (9 cm) and incubated at 28 -30^{o}c for 3 days and activity of bacterial enzyme were detected by adding iodine solution, halo zone around bacterial colony indicated activity of amylase enzyme. The Relative Enzyme activity was measured according to Bradner *et al.,* (1999) using formula**:**

$$\frac{\text{Clear zone diameter-Colony diameter}}{\text{Colony diameter}}$$

Effect of soil microorganisms on sorghum seeds germination and seedling growth under the greenhouse conditions:

Preparation of culture:

Seven bacterial isolates were isolated from the rhizosphere of cultivated sorghum in Balloza Research Station of Desert Research Center. Erlenmeyer flasks (250 ml volume) containing previously sterilized nutrient broth medium for bacilli and king' B media for pseudomonas isolates were inoculated with loop of active culture of the tested organisms. Then inoculated flasks were incubated at 28^{0}c on a rotary shaker incubator (120 rpm) for 48hrs.,at stationary phase of growth culture were centrifuged at 4000 rpm for 15min., precipitates were added to 100 ml sterilized distilled water to final OD reached 10^{8} cfu/ml.

Treatment:

Sorghum seeds (Shadwell 1) obtained from Agricultural Research Center, Egypt were surface sterilized using sodium hypochlorite (0.3% v/v) for 1-2 min and 70% ethyl alcohol and then washed four times in sterile double-distilled water These sterilized seeds were soaked in bacterial suspension for each bacterial strains containing about 10^{8}cfu/ml for 30 min either with 5% (CMC) carboxy methyl cellulose and in uninoculated media as a control. Seeds were air dried for 2hrs. at 25-28^{0}C, then seeds were cultivated in pots containing autoclaved soil obtained from Balloza Research Station. Ten seeds were sown in each pot, the pots were divided to seven groups each of four pots (20 cm in

diameter and 14cm in height) While, the eight group of sterilized seeds without bacterial inoculation were performed as a control. All groups were arranged in a Complete-Randomized Block Design and irrigated as needed. All pots were kept under greenhouse conditions until four and eight weeks growth parameter in term of germination, shoot and root length, number of leaves, fresh and dry weight of shoot were recorded .

Pigment extraction:

Fresh samples were homogeneous with 10 mL 80% Acetone and filtrated with what man No 1. Then the samples measured at 662 and 664 nm and 440 for chlorophyll a, b and carotinoides and the concentrations calculated by mg/g fresh leaves.

Measurement of minerals concentration:

Samples of vegetative parts dried and 0.2 gm were ground, then placed in a beaker, 10 ml of concentrated H_2SO_4 was added, the beaker with sample move to the hot plate till boiling, After boiling 3 ml of Hydrogen peroxide H_2O_2 were add, Samples left to boil at the hot plate till clearly appearance, Then solution was transferred to a majoring flask 50 ml. The concentration and total uptake of micronutrients and macronutrients in sorghum were determined by Atomic Absorption (UNICAM, 929 AA spectrometer).

Amino acids composition by Amino Acid Analyzer

Total amino acids were estimated according to the method of (Block *et al.*, 1958) by acid hydrolysis (Fig.1). A known weight of sorghum (leaves) was transferred into a tube containing 10 ml of 6 N hydrochloric acid, the tube sealed and hydrolysis was continued for a period of 24 hours in an oven at 110^{o}C. At the end of this period, hydrolsate was transferred quantitatively to a porcelain dish and the hydrochloric acid evaporated to dryness a 50-60^{o}C on a water bath. Distilled water 5 ml was added to the hydrolsate and evaporated to dryness to remove the excess of hydrochloric acid and the final residue was dissolved in 10 ml of glass – distilled water added. The hydrolsate sample dried a second time. One ml of 0.2 N sodium citrate buffers (pH 2.2) was added and the samples stored frozen in a sealed vial until separation of the amino acids by Amino Acid Analyzer amino (Sykum (S 7130). The peak area and percentage of each amino acid were calculated:

Calculation of amounts (ppm) =

$$\frac{\text{Dilution of sample x Amount (ppm)}}{\text{Volume take}} \times 1000$$

Condition of amino acid analyzer for hydrolysate program:

Column: Hydrolysate column amino acid analyzer Sykum (S 7130) (4.6 * 150 mm) and its temperature 57°C, Sample: 100 µl, Buffer system: Sodium acetate. Buffer A (pH 3.45), Buffer B (pH 10.85), Regeneration solution, Sample dilution buffer (PH 2.20). Flow rate: 0.25 ml /min. for Ninhydrin pump. 0.45 ml /min. for quaternary pump.

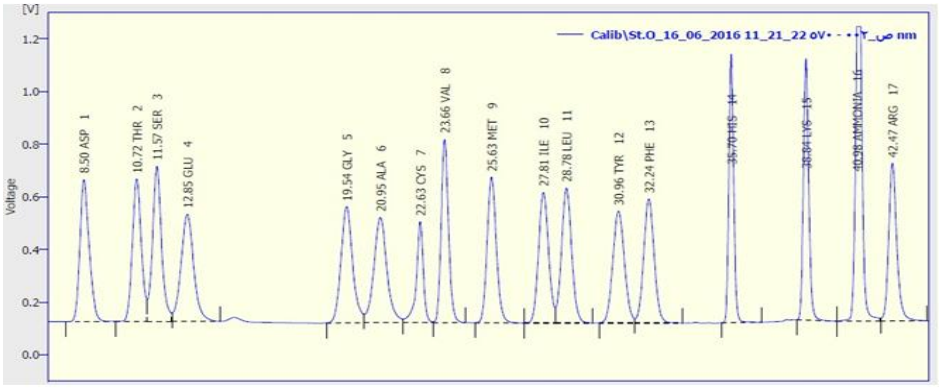

Fig. 1: Stander curve of amino acids compounds.

Detection : Ninhydrin is used for the detection of amino acids at 440 nm for Proline and 570 nm for the other amino acids through and oxidative decarboxylation reaction of the amino acids with ninhydrin to give ruhemann's purple a compound detected by AAA Spectro-photometer at the above mentioned wave length.

Statistical analysis

The treatments were arranged in a complete randomized block design. Data were subjected to statistical analysis by ANOVA using the method described by Snedecor and Cochran. (1990). The least significant difference (L.S.D) and Duncan letter at 5% level of probability was used to differential between the means (Waller and Duncan, 1969). The reduction (R %) was calculated as follow:

R% = $C-T/C\ x100$ Where the growth trait value is (C), in control and (T) in treatments.

RESULTS

Morphological characterization of isolates

The morphological features of the obtained isolates were illustrated in Table (1). Accordingly; SA, S2, S3 and S4 strains were gram positive, however, SB, Sc and SD stains were produced negative reactions to gram stain. For the shape under microscopes, most isolated strains have rode shape; consequently the strains had different colors on agar plats. While SA, SD, S2 and S3 strains were white color, also, S4 strain was appeared cream. But, SB and SC strains were yellowish color. For the motility, all the obtained strains were motile, the motility of obtained strains may confer the ability of isolates to attached plant roots. For Catalase; SB, SC and S4 strain were produced catalase. Moreover, SA, SD, S2 and S3 strains were catalase negative.

Table 1: Some morphological characteristic of obtained isolates

Bacterial strains	SA	SB	SC	SD	S2	S3	S4
Gram reaction	+	-	-	-	+	+	+
shape	rode	rode	rode	rode	rode	rode	rode
color	white	yellowish	yellowish	white	white	white	cream
Motility	motile	motile	motile	motile	motile	motile	motile
Catalase	-	+	+	-	-	-	+

Identification of selected rhizobacterial strain with molecular analysis:

Molecular sequencing of the isolates were performed according to Polymerase Chain Reaction (PCR) techniques by SIGMA Services Company, on GATC Company using ABI 3730xl DNA sequencer, whereas, the 16S rRNA gene sequences were compared with the other bacterial sequences using NCBI mega Blast (http://blast.ncbi.nlm.nih.gov/ Blast. cgi). These 16S rRNA sequence for SA, SB, SC, SD, S2 and S4 isolates showed that the identification percentage reached; 98, 98, 98,97, 86 and 97% to the sequence of, *Bacillus megaterium* strain ATCC 14581, *Pseudomonas hibiscicola ATCC19867*, *Pseudomonas geniculata* strain ATCC19374, Rhizobium pusense strain NRCPB10, *Bacillus cereus* strain X3, *Lysinibacillus sp* and Bacillus cereus ATCC 14579, respectively (Table 2).

Table 2: Molecular identification to isolated bacteria.

Code of isolated bacterial strains	Strain name	16S ribosomal RNA gene sequence
SA	*Bacillus megaterium* strain ATCC 14581	TAGCGTCCGACGGGTGANTAACACGTGGGCAACCTGC CTGTAAGACTGGGATAACTTCGG
SB	*Pseudomonas hibiscicola* strain ATCC 19867	GAAGGTTAANCTACCTGCTTCTGGTGCAACAAACTCC CATGGTGTGACGGGCGGTGTGTA
SC	*Pseudomonas geniculata* strain ATCC19374	CTACCTGCTTCTGGTGCAACAAACTCCCATGGTGTGA CGGGCGGTGTGTACAAGGCCCGG
SD	Rhizobium pusense strain NRCPB10	CTTCNGGT-ANACCAACTCCCGTGGTGTGACGGGCGGTGTGTACAA GGCCCGGGAACGTA
S2	*Bacillus cereus* strain X3	GATAACTCCGGGAAACCGGGGCTAATACCGAATAATC TGTTTCACCTCATGGTGAAATAT
S3	*Lysinibacillus sp*	TGAAAGACGGTTTCGGCTGTCGCTATAGGATGGGCCC GCGGCGCATTAGCTAGTTGGTGA
S4	*Bacillus cereus* ATCC 14579	TGANTAANCACGNGGGTAACCTGCCCATAAGACTGGG ATAACTCCGGGAAACCGGGGCTA

Quantitative Determination of Indole Acetic Acids

The colorimetric determination of indole 3-acetic acids (IAA) conducted with sequence concentration from the authentic IAA samples based on the natural quantity in the selected microbes. The obtained results revealed that all bacterial isolates were produced IAA with varied levels under laboratory conditions. Whereas, the IAA quantities ranged from 0.083 to 0.453 µg ml^{-1}. The highest level of IAA production was observed from *Bacillus megaterium* strain (SA) strain by 0.453 µg ml^{-1} followed by *Pseudomonas hibiscicola* (SB) strain by 0.370 µg ml^{-1} and S3 by 0.274 µg ml^{-} respectively. While, the lowest production detected from *Bacillus cereus* (S4) strain was 0.083µg ml^{-1} (Table 3).

Table 3: The quantitative determination of indole 3-acetic acids for the isolated bacteria.

IAA concentration ppm (µg/ml)	Standard curve Reading (OD)	Bacterial strains	IAA concentration (µg/ml)
0.25	0.012	SA	0.453
0.50	0.039	SB	0.370
1.00	0.068	SC	0.374
2.00	0.312	SD	0.114
5.00	0.784	S2	0.255
10.00	1.359	S3	0.274
20.00	1.600	S4	0.083
LSD (0.05)			0.211

Enzyme activities of isolated rhizobactria:

Data in Table (4) revealed that protease enzyme activity was studied under laboratory conditions using skim milk agar medium selective media. The obtained results based on the ability of tested organisms to produce the enzyme through formation of halo zone around colony growth. The diameters of both colony and halo zone were measured. The result indicated that the highest relative enzyme activity achieved from (SA) strain which reached 42.550% Followed by strains SB, SC, SD, S2 and S3 isolates caused activity reached by 19.4., 16.63, 7.19, 6.708 and 4.74 %, respectively. Finally, the lowest relative enzyme activity was obtained from S4 strain by 2.726%.

Table 4: Assessment of bacterial isolates ability for protease enzyme activity on agar plats

Strains	Colony dim (cm)	Halo zone diameter (cm)	Enzyme activity	Relative enzyme activity (%)
SA	0.333 ± 0.15	1.900 ± 0.10	4.758	42.550
SB	0.233 ± 0.06	0.733 ± 0.015	2.174	19.443
SC	0.433 ± 0.06	1.233 ± 0.21	1.860	16.639
SD	0.467 ± 0.06	0.833 ± 0.015	0.804	7.194
S2	0.400 ± 0.17	0.700 ± 0.20	0.750	6.708
S3	0.567 ± 0.12	0.867 ± 0.15	0.530	4.740
S4	0.867± 0.06	1.133 ± 0.06	0.305	2.726
LSD (0.05)		0.1869		

± Standard division

Data in Table (5) showed the ability of the selected bacterial strains for lipase enzyme production within the nutrient agar which supplemented with surfactant (Tween 20). Based on the growth and the formation of clear zone around bacterial colony, the result appeared SD strain produced the highest relative enzyme activity by 36.739%. Followed by S2 and S3 isolates were caused activity reached 25.05 and 19.67 %, respectively. The third enzyme activity was achieved by using SC strain which reached 10.912%. Finally, the lowest enzyme activity observed from SB strain by 7.621%. However SA and S4 did not have the ability for production.

Table 5: Assessment of bacterial isolates ability for lipase enzyme activity on agar plats

Strains	Colony dim (cm)	Halo zone diameter (cm)	Enzyme activity	Relative enzyme activity (%)
SA	ND	ND	ND	ND
SB	1.733 ± 0.40	3.300 ± 0.36	0.904	7.621
SC	1.700 ± 0.43	3.900 ± 0.20	1.294	10.912
SD	0.467 ± 0.058	2.500 ± 0.20	4.357	36.739
S2	1.167 ± 0.15	4.633 ± 0.23	2.971	25.054
S3	0.600 ± 0.10	2.000 ± 0.0	2.333	19.674
S4	ND	ND	ND	ND
F value		7.3		
LSD (0.05)		0.414		

± Standard division ND not detected

Data represented in Table (6) showed the production ability of bacterial strains for chitinase enzyme when tested on colloidal agar medium at 30 ^{0}C, whereas clear halo zone around their colonies described this ability. The result indicated that SC and S2 strains did not have this ability as observed from the experiment under laboratory condition. The SB and S3 isolates caused activity reached 14.59 and 19.15%, respectively. The highest relative enzyme activity was achieved from SA strain by 43.782%. While the moderate enzyme activity detected from SD strain by 13.181%. Finally, the lowest activity was 9.287% observed from S4 strain.

Table 6: Assessment of bacterial isolates ability for chitinase enzyme activity on agar plats.

Strains	Colony dim (cm)	Halo zone (cm)	Enzyme activity	Relative enzyme activity (%)
SA	0.467 ± 0.115	3.267 ± 0.25	6.000	43.78227
SB	1.000 ± 0.20	3.000 ± 0.36	2.000	14.59409
SC	ND	ND	ND	ND
SD	1.033 ± 0.25	2.900 ± 0.17	1.806	13.18176
S2	ND	ND	ND	ND
S3	0.800 ± 0.20	2.900 ± 0.26	2.625	19.15474
S4	0.367 ± 0.058	0.833 ± 0.058	1.273	9.287147
F value		8.57		
LSD (0.05)		0.258		

± Standard division ND: Not detected

Table (7) showed the production ability of bacterial strains for amylase enzyme when tested on starch agar medium as a selective medium at 30 ^{0}C, whereas clear halo zone around their colonies after addition of iodine described this ability. The results showed that SA, SB, S4, SD and S3 strains produced amylase enzyme which had the ability to hydrolyzed starch, while strains SC and S2 were non detected. The highest relative activity achieved from S4 strain reached 46.146%.

Table 7: Assessment of bacterial isolates ability for starch hydrolysis on agar plats.

Strains	Colony dim (cm)	Halo zone (cm)	Enzyme activity	Relative enzyme activity (%)
SA	0.267 ± 0.058	0.600 ± 0.100	1.250	15.38212
SB	0.667 ± 0.115	1.400 ± 0.100	1.100	13.53627
SC	ND	ND	ND	ND
SD	0.667 ± 0.289	1.667 ± 0.351	1.500	18.45855
S2	ND	ND	ND	ND
S3	0.633 ± 0.115	0.967 ± 0.058	0.526	6.476684
S4	0.267 ± 0.058	1.267 ± 0.23	3.750	46.14637
F value		21.45		
LSD (0.05)		0.266		

± Standard division ND= Not detected

While, the moderate activity observed from SD strain was estimated by 18.458%. Finally, the lowest activity was observed from S3 strain reached 6.476%.

Data in Table (8) clearly indicated the efficiency of SA, SB, SC, S2, S3 and S4 strains to phosphate solubilizing using Pikovskaya(PVK) agar medium for three days at 30 ^{0}C. The revealed results represented that SA strain achieved the highest phosphate solubilization efficiency reached 450%. Followed by S2 strain was recorded Solubilization index by 238.46%. Consequently, SC and SB achieved activity by 194.5 and 209.37%, respectively. Finally, S4 strain recorded the lowest phosphate solubilization efficiency by 179.592%. However, SD and S3 did not have this ability.

Table 8: Assessment of phosphate solubilizing ability of bacterial isolates using agar plates

Strains	Colony dim (cm)	Solubilization dim (cm)	Enzyme activity	Solubilization index	Relative enzyme activity (%)
SA	0.600±0.100	2.700±0.200	4.500	450.000	35.38
SB	1.067±0.115	2.233±0.058	2.094	209.375	16.46
SC	1.233±0.058	2.400±0.100	1.946	194.595	15.30
SD	ND	ND	ND	ND	ND
S2	0.867±0.058	2.067±0.115	2.385	238.462	18.75
S3	ND	ND	ND	ND	ND
S4	1.633±0.321	2.933±0.306	1.796	179.592	14.12
LSD (0.05)		0.181			

± Standard division ND= Not detected

Effect of bacterial inoculation on sorghum growth parameters:

Data in Table (9) presented the promoting activity of bacterial isolates on sorghum crop parameters (root length, shoot length, number of leaves, fresh weight and dry weight) after four weeks of cultivation. The obtained data showed the varied response to sorghum growth parameters. The activation in root length was shown from SD, S2, S3, S4 and SB by 2.07, 20.0, 2.0, 8.0% and 7.33%, than its control. Consequently, shoot length increased from treating with SC, SD, S2, S3 and S4, these activation reached 11.76, 17. 65, 41.18, 11.76, and 11.76% comparing with its control.

Table 9: Effect of isolated microbes in sorghum seedling growth parameters after 4 weeks of cultivation.

Growth parameters		Microbes strains								
		Control	SA	SB	SC	SD	S2	S3	S4	LSD (0.05)
Root length (cm)	Mean	50.00	46.33	46.00	47.67	51.03	60.00	51.00	54.00	4.094
	R%	0.00	7.33	8.00	4.67	-2.07	-20.00	-2.00	-8.00	
shoot length (cm)	Mean	17.00	16.00	11.00	19.00	20.00	24.00	19.00	19.00	1.261
	R%	0.00	5.88	35.29	-11.76	-17.65	-41.18	-11.76	-11.76	
Number of Leaves	Mean	3.33	2.33	3.00	2.00	3.00	3.00	4.00	3.33	0.549
	R%	0.00	30.00	10.00	40.00	10.00	10.00	-20.00	0.00	
fresh Weight (gm/pot)	Mean	1.82	1.31	1.08	1.22	2.19	3.14	2.33	3.05	3.327
	R%	0.00	28.14	40.42	32.82	-20.59	-72.74	-28.52	-68.17	
Dry Weight (gm/pot)	Mean	0.57	0.50	0.39	0.40	0.53	0.93	0.86	0.90	0.333
	R%	0.00	11.68	31.15	29.91	6.90	-64.96	-52.74	-59.65	
Fresh Weight (gm/plant)	Mean	0.26	0.25	0.21	0.24	0.26	0.44	0.36	0.41	0.0820
	R%	0.00	4.77	18.43	6.44	-1.80	-69.90	-38.66	-56.70	
Dry Weight (gm/plant)	Mean	0.09	0.08	0.06	0.16	0.09	0.13	0.11	0.10	0.0566
	R%	0.00	5.75	26.05	-81.61	-6.90	-47.51	-22.99	-17.24	

(-)= Means activation than the control R%= Reduction Percentage

As for total biomass, fresh weight was achieved from SD, S2, S3 and S4 activation by 20.59, 72.74, 28.52 and 68.17% respectively, than the control. While, total biomass dry weight increased with S2, S3 and S4 reached 64.96, 52.74 and 59.65%, respectively over its control. It could be concluded that SD, S2, S3 and S4 strains showed clearly increasing in all vegetative parameters as compared to control. Whereas strain S2 achieved the highest activation value than control in all growth parameters root length (20%), shoot length (41%), fresh weight (72.74%) dry weight (64.96%) compared with its control.

Data in Table (10) showed a significant increasing in all vegetative growth parameters (shoot length, root length, fresh and dry weight) as compared to control after eight weeks of cultivation, SA, SB, SC, S2, S3 and S4 strains showed a significant increasing in root length reached 3.90, 5.19, 1.30, 7.14, 3.90, 8.44 and 9.74%respectively as the highest root length was detected with strain S4, while the lowest root length was detected with SC strain. In this trend, SC, SD, S2,S3 and S4 strains indicted increasing in shoot length by 6.06,13.13,11.11, 9.09 and 22.22% respectively, over the control. The highest shoot length was observed with strain S4 and the lowest shoot length was observed with strain SC. While SA, SB, SD, S2, S3 and S4 strains were recorded increasing in fresh weight reached 14.61, 6.22, 13.26.69.43, 14.88 and 88.15%over the control. Whereas, S4 strain treatment was achieved the highest fresh weight. On the other hand, the lowest fresh weight obtained with SB strain. For the dry weight for treating with SA SB,SC, SD, S2, S3 and S4 strains which recorded increasing in dry weight by (12.89,3.77,14.11, 60.39, 40.31, 14.86 and71.32%) over control. The highest dry weight was achieved from S4 strain, however, the lowest dry weight detected with SB strain, whereas strains SA, SD ,S2 and S4 caused germination rat by (3.45%) over control. On the other hand, S4 strain showed significant increasing in all growth parameters when compared with untreated control.

Table 10: Effect of isolated microbes in sorghum seedling growth parameters after 8 weeks of cultivation

Growth parameters		Microbes strains								
		Control	SA	SB	SC	SD	S2	S3	S4	LSD (0.05)
Root length (cm)	Mean	51.33	53.33	54.00	52.00	55.00	53.33	55.67	56.33	5.308
	R%	0.00	-3.90	-5.19	-1.30	-7.14	-3.90	-8.44	-9.74	
shoot length (cm)	Mean	33.00	30.67	32.00	35.00	37.33	36.67	36.00	40.33	4.852
	R%	0.00	7.07	3.03	-6.06	-13.13	-11.11	-9.09	-22.22	
Number of Leaves	Mean	3.33	3.00	3.00	3.33	3.33	3.33	3.33	3.67	0.225
	R%	0.00	10.00	10.00	0.00	0.00	0.00	0.00	-10.00	
fresh Weight (gm/pot)	Mean	3.70	4.24	3.93	3.21	6.26	5.34	4.25	6.96	0.673
	R%	0.00	-14.61	-6.22	13.26	-69.43	-44.36	-14.88	-88.15	
Dry Weight (gm/pot)	Mean	1.61	1.82	1.55	1.38	2.58	2.26	1.85	2.76	0.242
	R%	0.00	-12.89	3.77	14.11	-60.39	-40.31	-14.86	-71.32	
Germination Percentage	Mean	96.67	100.0	90.00	100.0	100.0	100.0	96.67	100.0	5.856
	R%	0.00	-3.45	6.90	-3.45	-3.45	-3.45	0.00	-3.45	

(-)= Means activation than the control R%= Reduction Percentage

Effect of bacterial inoculation on sorghum photosynthetic pigments.

Table (11) indicated that SC, SD, S2, S3 and S4 strains caused a significant increasing in chlorophyll A and chlorophyll B, total chlorophyll and total carotenes. The obtained result appeared that SA,SB,SC, S2, S3 and S4 strains showed differentiation in reduction effect chlorophyll A by (55.31,53.08,128.82, 44.62, 213.08 and 168.18%) respectively, over the control. Whereas the maximum deceased achieved from treated sorghum plant with SD strain. However, lowest value observed from treating with S2 strain. As for chlorophyll B, the reduction was ranged from (15.33 to 84.91%), on the other side the highest reduction value was observed from S4 strain

by (84.91%) and the lowest value detected from SD strain by (10.72%) than control. Regard to the total chlorophyll, the highest activation effect value observed with treating plant strain S3 which recorded by (144.76%) and the lowest value achieved from strain SD by (0.59%) over control. Finally, the estimated total carotenes were measured for all plant treated with the isolated strains. Whereas the highest value caused from S3strain by (134.48%) over control while the lowest value was observed from SD strain.

Table 11: Effect of isolated microbes in sorghum photosynthetic pigments after 8 weeks.

Strains	Chlorophyll A		Chlorophyll B		Total chlorophyll		Total carotenes	
	Mean	R%	Mean	R%	Mean	R%	Mean	R%
Control	4.41	0.00	2.94	0.00	7.35	0.00	4.21	4.21
SA	1.97	55.31	2.49	15.33	4.46	39.31	2.43	2.43
SB	2.07	53.08	2.93	0.49	5.00	32.04	2.85	2.85
SC	10.09	-128.82	4.34	-47.50	14.43	-96.27	5.39	5.39
SD	4.14	6.17	3.26	-10.72	7.40	-0.59	3.40	3.40
S2	6.38	-44.62	2.68	8.94	9.06	-23.18	1.99	1.99
S3	13.81	-213.08	4.19	-42.37	18.00	-144.76	9.88	9.88
S4	11.83	-168.18	5.44	-84.91	17.27	-134.85	8.28	8.28
LSD (0.05)	2.204		0.3207		1.920		1.823	

Effect of bacterial inoculation on sorghum minerals content.

Macro-element concentrations (N, P and K) in sorghum samples are represented in Table (12). Nitrogen, phosphorus and potassium had the highest concentration among the detected elements in SB. Nitrogen ranged from 1.56 to 2.10, while phosphorus concentrations in different treatment of sorghum ranged from 0.19 to 0.34. Whereas, the untreated control achieved values was 1.76, 0.21 and 1.91% for Nitrogen, phosphorus and potassium respectively.

Microelement concentrations (Fe, Mn, Zn and Cu) in sorghum samples are represented in Table (12). Fe values were ranged from160.2 to 256.1 ppm. The highest concentration of Fe among the treated sorghum was detected in SC treated, while the lowest Fe was detected in S2. Mn concentrations in different treatment of sorghum ranged from 51.8 to85.0. Whereas, treated with in Sc strain caused the highest concentration. For Zn, the content ranged from 15.9 to 32.5 ppm. Finally, Cu concentration was higher in S2 treatment by 9.17 and the lower concentration was achieved by 5.11 in S3 treatment (Table 12).

Table 12: Elemental analysis parts and minerals content concentrations of vegetative sorghum.

Treatments	N	P	K	Fe	Mn	Zn	Cu
	%			ppm			
Control	1.76	0.21	1.91	234.1	77.8	29.8	6.81
SA	1.81	0.25	1.86	203.8	64.1	24.1	8.23
SB	2.10	0.34	2.21	187.2	60.6	18.9	5.19
SC	2.0	0.30	1.96	256.1	85.0	32.6	7.89
CD	1.95	0.29	1. 52	172.9	73.4	23.5	6.03
S2	1.56	0.22	1.19	160.2	51.8	17.2	9.17
S3	1.57	0.19	1.35	183.7	57.9	15.9	5.11
S4	1.60	0.20	1.22	231.5	69.4	19.3	6.18

Effect of bacterial inoculation on sorghum shoots total amino acids.

Data in Table (13) revealed the variation of the total amino acids concentration and composition in shoot parts of sorghum plants affected with different microbes strains. 18 amino acids were detected in sorghum plants inoculated with SB, S2, SA ,SC, S3 ,S4 Strains and the control, while 17 amino acids were detected

in the plants inoculated with SD. Whereas, all microbes were increased the amount of total amino acids as compared with the untreated control. In this respect treating sorghum with SD recorded the higher amount of amino acids followed by S3and S4 strains as compared with other treatments reached; 59504.0, 47720.7 and 44278.1 µg/gm dry weights respectively.

Aspartic was the major protein amino acids in sorghum plants detected in the control, SB, S2, SA, SC, S3, SD and S4 with concentration, 804.8, 3326.4, 4770.0, 2917.4, 2627.4, 5129.3, 5686.3, 4651.6 µg/gm dry weights respectively. Meanwhile, Cystine was the lowest amino acids detected in the control, SA, SC, S3 and S4 recorded 16.3, 75.7, 35.9, 158.5 and 113.2 µg/gm dry weights, respectively. However, Cystine amino acid was absent in SD treatment and not detected. The amount of aspartic acid, threonine, serine, glutamic acid, glysine, alanine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine and proline was higher in plants inoculated with SD than in other plants. The major amount of proline amino acids was recorded in SD treatment by 2289.8 µg/gm dry weight. The greatest amount of cystine was recorded in SB treatment by 214.2 µg/gm dry weight.

Table 13: Amino Acids composition (µg/gm dry weight) in sorghum seedling regardless of bio-agents treatments.

Amino Acids	Control	SA	SB	SC	S D	S2	S3	S 4
Aspartic	804.8	2917.4	3326.4	2627.4	5686.3	4770.0	5129.3	4651.6
Threonine	329.8	1768.9	1752.9	1842.8	2745.1	2208.9	2330.2	2196.4
Serine	372.0	1801.1	1558.9	1866.3	3203.3	2491.7	2635.7	2422.9
Glutamic	757.9	2572.3	3685.3	3574.2	6354.0	5058.3	5468.9	5108.7
Glysine	406.9	1666.0	1795.2	1795.0	3835.6	2667.1	2804.8	2646.8
Alanine	525.1	2452.9	2423.3	2129.4	5127.9	3571.4	3761.5	3574.5
Cystine	16.3	75.7	214.2	35.9	0.000	104.0	158.5	113.2
Valine	376.5	1570.8	2013.1	1625.0	3657.7	2553.7	2707.6	2578.9
Methionine	32.2	201.7	54.0	147.5	48.6	144.2	274.8	118.2
Isoleucine	277.9	1285.9	1486.0	1272.8	2720.0	1929.2	2044.1	1959.5
Leucine	571.8	2801.5	3200.0	2482.2	5722.3	4106.2	4277.2	4151.2
Tyrosine	222.0	1094.3	1331.6	1297.1	1618.1	1191.7	1332.4	1270.2
Phenylalanine	306.6	1872.7	1818.9	1630.7	3124.0	2305.0	2405.0	2332.8
Histidine	181.8	920.9	1042.0	763.2	1862.3	1279.8	1450.9	1266.8
Histidine	413.3	1802.8	2398.7	1780.7	4136.5	2776.3	3063.3	2900.2
Amonia	1233.0	3909.4	2548.4	4480.1	4708.4	2894.5	4319.8	3509.3
Arginine	385.4	1822.0	1568.0	1993.0	2664.2	1892.9	2166.3	1997.8
Proline	228.1	758.6	1271.7	1096.4	2289.8	1502.7	1390.4	1479.1
Total	7441.5	31295.0	33488.7	32439.7	59504.0	43447.6	47720.7	44278.1

DISCUSSION

Sorghum bicolor commonly called sorghum and also known as great millet, *durra*, is a grass species cultivated for its grain, which is used for food, both for animals and humans, and for ethanol production. *Sorghum* is the world's fifth most important cereal crop after rice, wheat, maize and barley. Sorghum (*Sorghum bicolor*) are popular cereals consumed by both adults and infants in Africa (Asiedu, *et al*., 1993). This research deal with sorghum plant and their associated rhizobacteria, whereas, bacteria were isolated and exposed to number of investigation to determine its role on sorghum growth. In addition to, identify the cross signals between sorghum plant and their associated microbes though the underground interactions which reflected in positively or a negatively upon sorghum productivity. Rhizosphere, the layer of soil influenced by plant root and play pivotal role in plant growth and development (Hrynkiewicz and Baum, 2012). Lytic enzymes were indirect plant growth promoting traits. Some biocontrol bacteria produced many enzymes including

chitinases, cellulases, proteases, and lipase. These bacteria that could parasitize disease-causing fungi by the production of these enzymes. Some of these bacterial enzymes producing bacteria are able to destroy oospores of phytopathogenic fungi (El-Tarabily, 2006) and affect the spore germination and germ-tube elongation of phytopathogenic fungi (Sneh *et al*., 1984) Extracellular lytic enzymes act in different ways: many of them can affect the cell wall of pathogens, and this is documented for cellulases, chitinase, protease and lipase.

The obtained results of microbial biochemical revealed that protease enzyme was the most produced from the isolated strains, followed by chitinase, amaylase and lipase enzyme. Based on these results the obtained strains can be used as biocontrol agents. These resulted supported by (Fridlender *et al*., 1993; Singh *et al*., 1999 and El-Tarabily 2006) they indicated that production of extracellular cell wall degrading enzymes has been associated with biocontrol abilities of the producing bacteria.

The obtained strains were produced indole -3- acetic acid the direct plant growth promoting traits so these strains could use as bio inoculants to enhance plant growth and productivity these results are in agreement with (Tsavkelova *et al*., 2007and Idris *et al.*, 2007) indicated that IAA biosynthesis is wide spread in plant associated bacilli and considered to be directly involved in plant growth promotion. The property of synthesizing indole acetic acid is considered as an effective tool for screening beneficial microorganism (Lambrecht *et al.*, 2000; Bloemberg and Lugtenberg 2001).

IAA producing naturally by bacteria Like in plants, tryptophan is considered as IAA precursor in bacteria because its addition to IAA-producing bacterial cultures increases the IAA concentration in liquid medium (Patten and Glick 2002).

Root exudates of plants are the natural source of tryptophan for rhizosphere microorganisms, which may enhance auxin biosynthesis within the rhizosphere that can induce a physiological response in the host plant (Kamilova *et al*., 2006). PGPR based products mostly contain strains of Bacillus that may have direct agricultural application.

The result represented that all obtained strains produced IAA and the highest amount of IAA was detected with *Bacillus megaterium* which produced 0.453 µg/ml and *Pseudomonas hibiscicola* that produced 0.370 µg/ml. These results are in agreement with Harrison *et al*., 2005.they reported that growth in a biofilm is a developmental process that is in some regards analogous to differentiation in tissues of multicellular organisms, and likewise involves cell-to cell signals that regulate growth and coordinate cell behaviour.

Sorghum seeds were inoculated with the obtained isolates and growing in plastic pots after four and eight weeks the results were taken, according to these data Bacillus and Rhizobium strains increased sorghum shoot and root length, fresh and dry weight as compared to control. Research on Plant Growth-Promoting Rhizobacteria (PGPR) with non-legumes such as rice have shown beneficial effects through biological N 2 fixation (Malik *et al*., 1997), increased root growth (Mia *et al*., 2012) with enhanced nutrient uptake (Yanni *et al*., 1997), phytohormone production (Chabot *et al*., 1996), plant growth enhancement stimulation by other beneficial bacteria and fungi (Saharan and Nehra, 2011).

Peng *et al*., (2002) reported that Rhizobium inoculation known for their symbiotic relationship with legumes, could also increase rice grain yield, involved The beneficial effects of the selected rhizobial isolates could be due to their plant growth-promoting abilities namely biological N fixation, phosphate solubilization and plant growth regulator/phytohormone and siderophore production, similar to the known beneficial effects of PGPR (Boddey *et al*., 1997; Verma *et al*., 2001; Araujo *et al*., 2013; Kloepper *et al*., 2004), (Apastambh *et al*., 2016) and Ramesh *et al*., (2012).

the mechanism of activation of growth promoting IAA by microorganisms seems to vary according to their physiological properties and to the conditions of the environment in which they live. It is also presumed that microorganisms act on seedlings both through IAA production and through nutrient absorption. Release of IAA and free enzymes and their subsequent participation in the promotion of growth and nutrient regeneration would assess the potential fertility of the environment. Fluorescent *Pseudomonas spp* used as wheat inoculants to enhance growth and improve yield as growth promoter due to (Phosphate solubilization, IAA and ammonia production) and their biocontrol activities (siderophores production, HCN, and antagonistic effect) Arif *et al*., (2015).

Photosynthetic pigments of sorghum plants that inoculated with selected rhizobacterial strains were determined in Table (11), which strain *Pseudomonas geniculat* (SC), *Rhizobium pusense* (SD), *Bacillus cerues* ATCC 14579 (S4) and *Bacillus cerues* strain X3 (S2) increased sorghum chlorophyll a, chlorophyll b, carotenoids and total chlorophyll contents, these results supported with Eleiwa *et al.*, (2012) who found that inoculation of wheat grains with biofertilizers, *Bacillus polymyxa*,or *Azospirillum brasilinseas* that produced auxin significantly increased the chlorophyll a, chlorophyll b, and carotenoids as compared with uninoculated treatment.

The data of the present study shown that mineral content for Mn, Fe, Cu, Zn, N, P and K affected by all isolated bacterial strains. The mineral uptake in sorghum plant was significantly higher values in the shortest plant shoot and lowers in the higher shoot of sorghum plants. These result supported by Hao *et al*., (2007) they suggested that (Fe), zinc (Zn), copper (Cu), and manganese (Mn) are essential micronutrients for plants and humans.

Finally, sorghum plant could be well-matched with the associate bacteria in rhizosphere soils whereas the allopathic abilities of sorghum arranged through their interaction through the mediated allelochemicals as well as their growth and productivity. The role of each rhizospheric bacteria are very important to sorghum growth, whereas the transduction signals activity as appositive or negative as well as their concentrations should be undertaken and need further investigation especially under the stress condition. It could be concluded from the obtained result that the isolated *Rhizobium pusense* strain NRCPB10, *Bacillus cereus* strain X3, S3 and *Bacillus cereus* ATCC 14579 can be used as biofertilizers that achieved from enhancing sorghum growth and parameters as well as, the natural of the isolates characteristics which able to solublize phosphate and production of phytohormons.

Regarding the isolated strains may be used as plant growth promoting rhizobacteria and could use as a biocontrol agents against plant root pathogen, The most isolates able to produce lytic enzymes like protease, amylase, lipase and chitinase which are the key enzyme for lyses fungi cell wall. These capabilities can apply against some plant pathogens.

Finally the rhizospheric isolated strains could use as bioinculant to increase plant tolerance against biotic and a biotic stress.

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ARABIC SUMMARY

القدرات التنشيطية المتعددة لبعض بكتريا الجذر محيطيه المصاحبة لنبات السورجم على نموه وتطوره

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إستهدفت الدراسة الحالية تقييم قدرة بعض العزلات البكتيريه المعزولة من تربة الريزوسفيرالمرتبطة بجذور نبات السورجم (*Sorghum bicolor*) من خلال تقدير بعض النشاط الانزيمي المرتبط بالامكانات الوظيفية للتحكم الحيوى للتقليل من الاصابة بالامراض النباتية وكمحفذات حيوية تزيد من نشاط ونمو النبات وذلك من خلال تلقيح بذور النبات ثم زراعتة وتقييم تأثيرها على خصائص النمو.

حيث تميزت العزلات بإنتاج هرمون الإندول ٣-أسيتيك (IAA)، والقدرة على إذابة الفوسفات والانزيمات المحللة للجدار الخلوى للفطر .حيث تم تعريف العزلات بواسطة ١٦ S RNA و أظهرت النتائج أن معظم العزلات لها القدره علي انتاج الهرمون النباتى (IAA) وكانت أكثرالعزلات إفرازا للإندول ٣-أسيتيك (IAA) هى سلالة *Bacillus megaterium* التي تنتج ٠,٤٥٣ ميكروجرام / مل ثم *Pseudomonas hibiscicola* التي أنتجت ٠,٣٧٠ ميكروجرام / مل.

تم تلقيح بذور نبات السورجم بسلالات البكتريا المعزولة وأوضحت النتائج أن كل من السلالات البكتيريه *Pseudomonas geniculat* (SC) و *Rhizobium pusense* (SD) و *Bacillus cerues* ATCC 14579 (S4) و *Bacillus cerues* X3 (S2) و *Lysinibacillus sp* (S3) احدثت ذياده في نمو النبات المعامل وأن محتويات المعادن الصغرى من الحديد، النحاس، الزنك، نيتروجين، فوسفور و البوتاسيوم كانت أعلى بكثير فى النباتات قصيرة الطول فى حين كانت اقل فى النباتات الطويلة. وكذلك إرتفع المحتوى الكلورفيلى لأوراق السورجم، والأحماض الأمينية وذاد تركيزالعناصر الكبرى N ، P، K بشكل ملحوظ مقارنة بغير المعامل. ،وكانت السلات ألاكثر كفاءة هي *Rhizobium pusense* و *Bacillus cerues* X3 و *Bacillus cerues* ATCC 14579 على التوالي. علاوة على ذلك، يمكن أن تستخدم هذه السلالات الريزوبكتيريه التي تم فصلها كمستحثات طبيعيه لنمو النبات وايضا يمكن ان تستخدم كعناصر مكافحة حيوية على أساس إنتاج الإنزيمات المحللة للبروتيز والأميليز والليبيز والكيتينز والتي تعتبر من الانزيمات الرئيسيه المكونة لجدار الخلية الفطرية . كما أن السلالات المعزولة لديها القدرة على إنتاج هرمون النمو وذوبان املاح الفوسفات لذا يمكن استخدامها كأسمدة حيوية لتحسين خصائص نمو النبات المختبر. وايضا يمكن أن تستخدم لزيادة تحمل النبات ضد عوامل الإجهاد الحيويه والغير حيويه حيث تعتبر نقطة مهمة نحو الزراعة المستدامة.