# Synergistic Effect between Azotobacter *vinelandii* and *Streptomyces* sp. Isolated From Saline Soil on Seed Germination and Growth of Wheat Plant

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**Abstract:** Twenty-two bacterial isolates were obtained from rhizosphere of wheat plants, grown in saline soil in western region, Saudi Arabia. All the isolates were grown in broth media supplemented with 2 mg/ml L-tryptophan and screened for indole acetic acid production. Out of the isolated bacteria, 17 isolates showed positive results for IAA production. The isolates M1 and M10 were selected and identified using morphological, physiological and biochemical characters as *Azotobacter vinelandii* MM1 and *Streptomyces* sp. MM10. Soaking wheat seeds in either *Azotobacter vinelandii* (AZ) or *Streptomyces* sp. (ST) or both culture filtrates (AZ+ST) increased significantly wheat germination. Moreover, soil inoculations with the bacterial cells of AZ, ST or AZ+ST increased the growth and development of wheat in normal and saline conditions. There were significant increases in root depth, shoot length and shoot and root dry weights compared to the control. The amounts of phosphate, N, Mg, K and proteins present in wheat shoots, grown in normal and saline soil were also increased by soil inoculation. No significant effect on Ca was found by soil inoculation under non-saline conditions. Increasing NaCl concentration increased proline content but soil inoculation decreased the adverse effects of NaCl and decreased proline concentration compared to control at the same salinity level. In conclusion, results of this study indicated that *Streptomyces*, Azotobacter *vinelandii* or both could be utilized as biofertilizer in saline soils.
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## 1. Introduction

The soil contained millions of microorganisms and approximately more than 85% of them are important for plant life and provide precious life to soil systems. Moreover, soil microorganisms that are closely associated with roots play a vital role in stimulating plant **growth (Aly *et al*., 2001, Ebrahim and Aly, 2004, Merzaeva and Shirokikh, 2010, Shahzadi *et al*., 2012)** and the effects can be mediated by the direct or indirect mechanisms. The direct effects have been most commonly attributed to the production of plant hormones such as auxins, gibberellins and cytokines as by supplying biologically fixed nitrogen **(Ahmad *et al*., 2005, Babaloa, 2010)** and the indirect mechanisms including suppression of pathogens by production of antibiotics **(Mahmoud *et al*., 2004**). Soil microorganisms increased nutrient availability, seed germination and metabolic activities **(Mahmoud *et al*., 2004, Adesemoye and Kloepper, 2009,)**. *Azotobacter vinelandii* is Gram-negative cocci that fix nitrogen using nitrogenase holoenzyme, which possesses molybdenum iron-sulfido cluster cofactors (FeMoCo) as active sites **(Chiu *et al*., 2001).** It undergoes differentiation to form cysts resistant to desiccation and consists of a contracted cell known as the central body that is surrounded by a capsule made up of a thin laminated outer layer (exine) and a thicker inner layer **(Sadoff, 1975).** *Azotobacter* lives as free-living saprophyte in soil, fresh water, marine environments and many other natural habitats and have been used as effective inoculum to enhance plant growth and pest control **(Meshram, 1984, Kole and Altosaar, 1988, Aquilanti *et al*., 2004).** *Streptomyces* shows dry and smooth colonies with substrate and aerial mycelia of different colors **(Aly, 1997).** It is abundant in soils and produced a group of secondary metabolites, such as antibiotics and extracellular enzymes, which have a role in degradation of complex molecules especially, cellulose, xylan and lignin, that play an important role in decomposition of organic matter **(Aly *et al*., 2011, 2012).**

Soil inoculation by bacteria promoted growth of tomato, *Arabidopsis thaliana*, *Phaseolus vulgaris* plants due to phytohormones, which increased biomass production and lateral root growth and formation **(Azcon and Barea 1975, Lopez-Bucio *et al*., 2007, Ortiz-Castro *et al*., 2008).** Rhizosphere bacteria such as *Azotobacter*, *Arthrobacter* and

*Streptomyces* have strong beneficial effects on plant growth and integrity by nutrient dissolution, nitrogen fixation, and the production of plant hormones and vitamins **(Fiorelli *et al*., 1996, Revillas *et al*., 2000, El-Shanshoury, 1991).**

Salinity is an important environmental stress and posing threat to agriculture and food supply **(Munns, 2002; Flowers, 2004).** It affect plant growth by osmotic effect of salts in the outside solution and ion toxicity due to salt build-up in transpiring leaves in a second phase in addition to induction of nutrient deficiencies **(Wyn Jones, 1981)**. High salt stress disrupts homeostasis in water potential and ion distribution, leading to molecular damage, growth arrest, and even death **(Zhu, 2001).** Sodium toxicity under saline conditions is particularly common in graminaceous crops and results in a range of disorders in protein synthesis and enzyme activation **(Tester and Davenport, 2003)**. In culture medium, *Streptomyces* showed good solubilization of tricalcium phosphate and produced plant growth promoting substance especially indolyl-3-acetic acid (2.4 µg/ml) but under saline conditions of 300 mM NaCl the amount of IAA reached to 4.7 µg/ml **(Sadeghi *et al*., 2012). Aly *et al*. (2003)** stated that applying *Azotobacter chroococcum* and/or *Streptomyces niveus* to maize plants grown under NaCl, influenced the content of total-soluble sugars, total free amino acids, proline total soluble proteins, DNA and RNA in shoots and roots resulting in a higher salt tolerance of the plants. **Hamdia *et al.* (2004)** found that *Azospirillum* inoculation of two maize cultivars increased soluble and total saccharides, soluble protein in shoots and total protein in roots under salinity stress. Moreover, proline accumulation was higher at a lower salinity concentration in the salt sensitive maize compared to the salt tolerant and presence of *Azospirillum* declined proline significantly. Soil treatment with *Streptomyces* C increased growth and development of wheat plant in normal and saline conditions. In this treatment there were significant increases in germination rate, percentage and uniformity, shoot length and dry weight compared to the control. Applying the bacterial inocula increased the concentration of N, P, Fe and Mn in wheat shoots grown in normal and saline soil but had non-significant effect on other micro and macronutrients concentrations **(Sadeghi *et al*., 2012)**. The objectives of this project were to isolate and identify indole acidic acids bacteria from saline soil, selection and identification of the most active isolates. The use of the identified bacteria singly or in combination as biofertilizer of wheat plants grown under saline conditions was also carried out.

## 2. Material and Methods:

### Bacterial isolation:

The present investigation was carried out to isolate and identify IAA bacteria from saline soil samples collected from Western region. Twenty soil samples of 500 g each were collected randomly from 10 cm depth from the rhizosphere regions of wheat plants in polythene bag The samples were sieved through a 4.75 mm-mesh sieve and soil pH was measured. All the bacterial isolates were obtained after growing for 2-4 days at 30°C on either nutrient agar **(Green and Gray, 1950),** starch nitrate **(Shirling and Gottlieb, 1966)** for actinomycetes isolation or Ashby-Sucrose agar (Agar 1.5%, Sucrose 0.5%, $CaCO_3$ 0.5%, $MgSO_4$ 0.02%, NaCl 0.02%, $KH_2PO_4$ 0.02%, $FeSO_4$ 0.0005%), for free living nitrogen fixing bacteria. All the isolates were purified and screened for IAA production, *in vitro*.

### Extraction and detection of IAA

All the isolated were grown in nutrient broth except free living nitrogen fixing bacteria which grown in nitrogen free broth **(El-Essawy *et al*., 1984)** and actinomycetes which grown in production medium **(Aly,1997).** All the media were supplemented with 2 mg/ml L-tryptophan at a pH of 7.0. The supernatants were filtered using Milipore filter (0.45 mm) and the cell free filtrate IAA was extracted from the supernatants with ethyl acetate according to the method described by **Ahmad *et al.* (2005).** Ethyl acetate extract was applied to TLC plates (Silica gel, thickness 0.25 mm, Merck, Germany) and developed in butanone/ethyl acetate/ethanol/ water (3:5:1:1v/v/v/v). Spots with Rf values identical to authentic IAA were identified under UV light (254 nm) by spraying the plates with Ehmann's reagent **(Ehmann 1977).**

### Quantification of IAA production

The production of IAA by the bacterial isolates was determined according to the method of **Bano and Musarrat (2003)**. Production broth medium, 50 ml containing 2 mg/ml L-trytophan were inoculated with the tested bacterium and incubated at 30°C with shaking at 120 rpm for 7 days. After centrifugation at 5000 rpm for 15 min, one milliliter of the supernatant was mixed with 2 ml of Salkowski reagent and the appearance of a pink color indicated IAA production. The absorbance was measured at 530 nm and the quantity of IAA produced was estimated against the IAA standard.

### Growth determination of bacteria

Growth of the isolated bacteria was measured after 7 days by determining the optical density at 550 nm.

### Identification of the bacterial isolates

The best IAA producers were characterized through a number of microbiological, physiological

and biochemical tests. The selected nitrogen fixing bacteria was biochemically characterized for Gram reaction, $H_2S$ production, starch hydrolysis, carbohydrate fermentation and oxidase test as described in **Aly (1990)**. The selected actinomycete isolate was examined after incubation at 30°C for 14 days on International *Streptomyces* Project media. The color of aerial and spore masses, diffusible pigment production and spore chain morphology were estimated. Isomer of diaminopimelic acid, amino acids and phospholipids were determined by TLC according to **Hasegawa *et al.* (1983) and Hoischen *et al.* (1997).** Sugars in whole-cell hydrolysates and fatty acids **(Butte, 1983)** were analyzed using Gas chromatography.

**The effect of IAA in bacterial culture filtrates on seed germination of wheat**

The supernatant of the two bacteria was filtered (Milipore filter, 0.45 mm) and the cell free filtrate was used to determine IAA concentration. Wheat seeds (*Triticum aestivum* L. cv. Sakha 155) were surface-sterilized by soaking in a 10% sodium hypochlorite (NaOCl) for 5 min, followed by rinsing in sterile distilled-water. The surface-sterilized seeds were separately soaked in the culture filtrate or sterile distilled water incubated in the dark until the seedlings emerged (10 days) and germination percentage (%) and index were determined as described by **Dhamangaonkar and Pragati (2009).**

$$\text{Germination Index} = \frac{\text{Sum of germinated seed for a certain period}}{\text{Total days} \times \text{Total seeds}}$$

**Preparation of inoculums:**

*Azotobacter* and *Streptomyces* were grown on Ashby-Sucrose agar or starch nitrate agar respectively. The bacterial cells were scraped from seven-day-old culture into sterile saline solution to give a suspension containing $2xl0^6$ cells/ml.

**Plant growth studies**

This experiment was carried out during the period 2009-2010. The sterile seeds were germinated in the dark and one week-old seedlings were transferred to each pot containing 2kg of steam sterilized sandy soil. The pots were kept in a glasshouse with a temperature range of 20-22°C. Four groups of pots were established: the first one remained without any inoculation (control), the second was inoculated with *Azotobacter chroococcum*, the third was inoculated with *Streptomyces* and the last one was inoculated with the two tested organisms. When plants were grown for certain length, 15 ml of the bacterial suspension ($2x10^6$ CFU/ml) was used to inoculate each pot. In case of inoculation with mixed culture, 15 ml of each organism were mixed and used to inoculate the plants. For control, only water was added. The plants were irrigated by Hoagland nutrient solution (Hoagland and Arnon, 1950). The nutrient solution had the following composition, in mM: $KH_2PO_4$, 1.0; $KNO_3$, 5; $Ca(NO_3)_2$, 5; $MgSO_4$, 2; Fe- EDTA, 0.1; $H_3BO_3$, 0.005; $MnCl_2$, 0.010; $ZnSO_4$, 0.008; $CuSO_4$,0.004; $(NH4)Mo_7O_{24}$, 0.0002. NaCl addition to the saline treatment was started after 7 days of transplanting and each pot received only 200 ml two times / week and the plants were irrigated with distilled water when needed. 200 ml of sterile dist. water were used to wash each pot. After 3 months, the plants were harvested and the root depth, shoot length and dry weight of the plants were determined. The shoot and root systems were dried and weighted.

**Plant analysis**

The control and treated plants were analyzed for minerals and protein contents. Phosphorus and nitrogen concentration were estimated according to methods described by **Allen *et al.* (1974).** Mineral elements (Na, K, Ca and Mg) were determined after acid digestion using Shimadzu Atomic Absorption Flame Spectrophotometer (Model AA-640-12).

**Statistical analysis**

Data of the shoot and root length and dry biomass recorded was statistically analyzed by *t*-Test to determine whether the differences between control and treated samples were significant or not at $P<0.05$ using SPSS software 16

## 3. Results

About 22 bacterial isolates were obtained from saline soil samples and screened on liquid medium for IAA production. The quantities of IAA detected were ranged from 4.9 to 11.4 $\mu gml^{-1}$ and the two isolates named MM1 and MM10 were the most active isolates (Table 1). The two selected bacterial isolates were identified according to morphological, physiological, biochemical properties. The isolate MM1 was a rod-shaped Gram-negative motile bacterium. The colonies were smooth, glistening, opaque, low convex, with an entire edge and developed a yellowish green pigment that was visible under day and UV light (table 2). The best growth was at temperature range 20 -30°C. Nitrogen-fixing soil bacterium form cyst (Fig.1A). Indole, citrate, catalase and oxidase Methyl red and Vogus proskauer tests were positive. Thus, it was belonging to genus *Azotobacter* and identified as *Azotobacter vinelandii*. However the selected isolate MM10 was Gram positive bacteria has substrate and aerial mycelia bearing a straight chain of conidia with spiny surface (Fig 1,B and C). No zoospore, sporangium, sclerichia or fragment hyphae were present. Some physiological characters were recorded in Table 3. The isolate MM10 was resistant to a wide variety of antibiotics, grew aerobically and catalase and oxidase

positive. It utilizes glucose, glycerol, mannitol and sucrose, ammonium chloride, sodium nitrate and amino acids as carbon and nitrogen sources. The morphological and physiological characters in addition to the characteristics lipids, sugars and fatty acids (table 4), the isolate MM10 was belonging to genus Streptomyces and identified as *Streptomyces* sp. MM10. Soaking sterile wheat seeds in culture filtrate of *Azotobacter* or *Streptomyces* (ST) + *Azotobacter* (AZ) enhanced significantly % of seed germination and germination index was 0.34 and 0.47 respectively (table 5).

Inoculation of soil by AZ or ST or both significantly increased the root depth; shoot height, fresh and dry weights of roots and shoots (table 6). On contrast, increasing salinity decreases plant growth and dry weight especially at 60 mM. At 20, 40 and 60 mM, maximum root depth, shoot height, fresh and dry weights were recorded using dual inculcation with AZ+ST. Inoculation of soil with AZ at all saline concentration enhanced significantly nitrogen and protein content of shoot system (Table 7). Maximum P content was found in plants inoculated with either AZ or both AZ+ST at 20 and 40 and 60 mM but at 80 mM maximum P concentration was found in plants inoculated with either ST or AZ+ST. Microbial inoculation of plants enhanced K and Mg concentrations significantly compared to controls under normal and saline conditions. Inoculation of plants with either ST or both ST+AZ decreased Na concentration in plants grown in saline conditions. Increasing NaCl concentration increased proline content but soil inoculation decreased the adverse effects of NaCl and decreased proline concentration compared to control at the same salinity level.

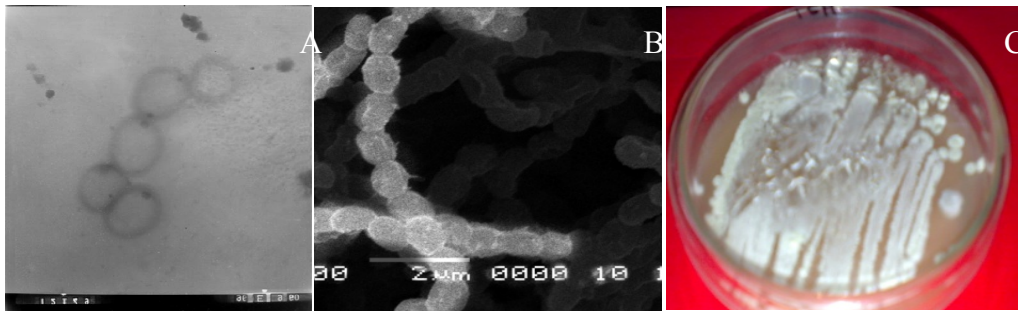


**Fig 1: A and B: The cyst and spore chain of the selected bacteria MM1 and MM10 under scanning electron microscope, C: the selected bacterium MM10 on starch nitrate agar.**

**Table 1: Growth and indole acetic acid (IAA) production of some bacterial isolates obtained from rhizosphere of wheat plant grown in saline soil**

| BACTERIAL ISOLATE | ISOLATION AGAR MEDIUM | GRAM REACTION | SHAPE | GROWTH ($A_{550}$NM) | I AA |
|---|---|---|---|---|---|
| MM1 | Ashby- Sucrose | -ve | Bailli | 0.55 | 11.4 ±4.0 |
| MM7 | Ashby- Sucrose | -ve | Cocci | 0.89 | 4.9±0.9 |
| MM10 | Starch nitrate | +ve | Filamentous | 0.65 | 9.8±1.0 |
| MM11 | Starch nitrate | +ve | Filamentous | 0.99 | 6.1±1.3 |
| MM14 | Nutrient agar | -ve | Bacilli | 1.67 | 5.8±1.0 |
| MM19 | Nutrient agar | +ve | Bacilli | 1.78 | 5.9±2.1 |
| MM20 | Nutrient agar | -ve | Bacilli | 1.55 | 7.9±1.3 |

**Table 2: Physiological properties of the isolate MM1, obtained from rhizosphere of wheat plant**

| TEST | RESULT | TEST | RESULT | |
|---|---|---|---|---|
| Gram reaction | Gram negative | Utilization of carbon sources | | |
| Growth | aerobic | Sucrose | ++ | |
| Cyst formation | Form cyst | D-Glucose | ++ | |
| Shape | Bacilli | D-Mannitol | ++ | |
| Source | Saline soil | Fructose | + | |
| Enzyme production | | Mannose | - | |
| Gelatinase | -ve | Sorbitol | - | |
| DNase | -ve | Inositol | - | |
| Lipase | -ve | Urea | - | |
| Amylase | -ve | Resistance to antibiotic | | |

| | | | |
|---|---|---|---|
| Protease | +ve | Vancomycin | S |
| Phosphatase | -ve | Erythromycin | S |
| Chitinase | -ve | Kanamycin | S |
| Oxidase | +ve | Tetracycline | S |
| Catalase | +ve | Bacitracin | S |
| Growth temperature | 10-55 °C | Chloramphenicol | S |
| Growth pH | 6-6.5 | Cotrimoxazole | S |
| Nitrate reduction | +ve | Gentamycin | S |
| Indole production | +ve | Cephalosporin | R |
| 5 % NaCl tolerance | +ve | Amoxicillin | S |
| Voges-Proskauer | +ve | Ciprofloxacin | S |

**++: Good utilization, +: Poor utilization +: positive results, -: on utilization, -ve: Negative results, +ve: Positive results, S: Sensitive**

**Table 3 : Physiological properties of the isolate MM10, obtained from rhizosphere of wheat plant**

| CHARACTERISTIC | RESULT | CHARACTERISTIC | RESULT |
|---|---|---|---|
| Melanin production on ISP 6, ISP7 | +ve | Galactose | + |
| Decomposition of hypothanthine, urea, xanthine, casien | +ve | L- Arabinose | + |
| Hydrolysis of chitin, gelatin, pectin | +ve | Mannitol | + |
| Tolerance to NaCl | 5-12% | D-Sorbitol | - |
| pH range | 6-9 | Glucose | + |
| Growth temperature | 10 - 45°C | Sucrose | + |
| Nitrate reduction | + | Lactose | + |
| $H_2S$ production | + | Na $NO_3$ | ++ |
| Resistance to Penicillin Cephalosporine | R | $NH_4Cl$ | ++ |
| Resistance to Kanamycin, Rifampin Tetracyclines, Gentamycin, | S | Utilization of valine, phenyl alanine, peptone, yeast extract | + |

**++: Good utilization, +: Poor utilization +: Positive results, -: Negative results, R: Resistant, S : Sensitive**

**Table 4: The biochemical tests (sugar, amino acid, phospholipids, and fatty acid composition of the cell wall or cell hydrolysate) of the selected isolate MM10**

| TYPE OF THE REACTION | RESULTS |
|---|---|
| Sugar in the cell hydrolysate<br>Glucose | <br>+ |
| Amino acids in the cell wall<br>Diaminopimelic acid (DAP)<br>Glutamic acid<br>Lysine | <br>L-Form<br>+<br>- |
| Phospholipids<br>Phosphatidylethanolamine<br>phosphatidylcholine<br>phosphatidylinositol | <br>+<br>+<br>+ |
| Fatty Acids | Iso and antiso fatty acids |

**+: Present, - : Absence**

**Table 5: Effect of bacterial culture filtrate on % and index of germination**

| TYPE OF CELL FREE FILTRATE | % OF GERMINATION | GERMINATION INDEX |
|---|---|---|
| Control | 90.7 | 0.22 |
| *Azotobacter* (AZ) | 94.8 | 0.34* |
| *Streptomyces* (ST) | 92.7 | 0.30 |
| AZ+ST | 97.1 | 0.47* |

***Significant results at P<0.05**

**Table 6: Effect of wheat inoculation with *Azotobacter, Streptomyces* or both on root depth (Cm), shoot length (Cm) and root and shoot dry weight ( g/plant) of plants grown in sterile soil under saline conditions.**

| NACL CONC. (MM) | INOCULUM | ROOT DEPTH (CM) | SHOOT LENGTH (CM) | ROOT DRY WEIGHT G/PLANT | SHOOT DRY WEIGHT G/PLANT |
|---|---|---|---|---|---|
| 0.0 | C | 32.4 | 60.4 | 0.39 | 2.9 |
| | AZ | 34.2 | 66.5* | 0.44* | 3.3* |
| | ST | 36.2* | 69.6 * | 0.33 | 3.4* |

| | | | | | |
|---|---|---|---|---|---|
| | AZ+ST | 40.2* | 73.1* | 0.55* | 4.0* |
| 10 | C | 37.0 | 60.0 | 0.30 | 2.8 |
| | AZ | 38.6 | 62.8* | 0.40* | 3.9* |
| | ST | 40.1* | 62.8* | 0.30 | 3.4* |
| | AZ+ST | 38.7 | 64.6* | 0.44* | 3.4* |
| 20 | C | 28.5 | 56.4 | 0.29 | 2.5 |
| | AZ | 25.7 | 58.4 | 0.34 | 2.7 |
| | ST | 28.6 | 58.6 | 0.33 | 2.8* |
| | AZ+ST | 29.4 | 60.4* | 0.50* | 2.8* |
| 40 | C | 25.3 | 50.5 | 0.23 | 2.2 |
| | AZ | 26.7 | 55.1 | 0.24 | 2.4* |
| | ST | 28.8* | 55.2 | 0.23 | 2.4* |
| | AZ+ST | 28.8* | 57.1* | 0.35* | 2.5* |
| 60 | C | 23.9 | 48.3 | 0.19 | 1.4 |
| | AZ | 25.8 | 52.3 | 0.24 | 1.6* |
| | ST | 25.3 | 54.3* | 0.23 | 1.8* |
| | AZ+ST | 26.2 | 55.5* | 0.25* | 2.0* |

*Significant results at P<0.05

**Table 7: Effect of wheat inoculation with *Azotobacter, Streptomyces* or both on shoot mineral content (mg/g), protein and proline of plants grown in sterile soil under saline conditions.**

| NACL CONC. (MM) | INOCULUM TYPE | P MG/G | NA MG/G | K MG/G | CA MG/G | MG MG/G | N MG/G | PROLINE µG/G | PROTEIN MG/G |
|---|---|---|---|---|---|---|---|---|---|
| 0.0 | C | 12.5 | 3.5 | 26.4 | 5.5 | 3.4 | 24.4 | 15 | 19.9 |
| | AZ | 13.7 | 3.6 | 28.3* | 6.0* | 4.0 | 25.6* | 15 | 28.4 |
| | ST | 13.0 | 2.6 | 27.6* | 6.8* | 4.4* | 22.0 | 17 | 24.2* |
| | AZ+ST | 13.9 | 3.6 | 27.0* | 7.0* | 5.5* | 23.9 | 15 | 24.2 |
| 10 | C | 15.3 | 5.5 | 27.8 | 6.0 | 4.6 | 23.1 | 44 | 19.4 |
| | AZ | 15.5 | 4.9 | 27.7 | 5.5 | 4.1 | 24.9 | 41 | 22.8* |
| | ST | 15.7 | 4.0* | 28.3 | 6.0 | 4.6 | 25.4 | 40 | 20.2 |
| | AZ+ST | 15.0 | 4.4* | 28.5 | 6.8* | 4.0 | 25.8 | 38 | 22.6* |
| 20 | C | 12.2 | 6.0 | 25.4 | 4.0 | 5.0 | 20.3 | 83 | 20.3 |
| | AZ | 13.3 | 5.5 | 26.8* | 4.8* | 5.6* | 25.5* | 63* | 28.5* |
| | ST | 12.5 | 5.0* | 25.8 | 4.5* | 5.4* | 23.6 | 44* | 22.5 |
| | AZ+ST | 13.7 | 4.5* | 26.6* | 4.6* | 5.0 | 24.0 | 48* | 22.6 |
| 40 | C | 12.0 | 6.5 | 20.0 | 3.8 | 3.4 | 17.9 | 100 | 20.8 |
| | AZ | 12.9 | 6.1* | 25.4* | 3.9 | 4.5* | 20.9* | 88* | 23.3* |
| | ST | 12.3 | 6.0* | 25.8* | 4.8* | 4.6* | 19.2 | 56* | 24.4* |
| | AZ+ST | 12.9 | 5.9* | 25.8* | 4.5* | 4.4* | 19.4 | 74* | 25.6* |
| 60 | C | 9.7 | 6.9 | 20.1 | 4.0 | 2.8 | 12.5 | 130 | 14.8 |
| | AZ | 10.0 | 6.0* | 24.3* | 4.8* | 3.4* | 18.6* | 111 | 19.9* |
| | ST | 11.7* | 6.4* | 24.9* | 4.7* | 3.5* | 12.8 | 84* | 16.7* |
| | AZ+ST | 11.9* | 6.0* | 24.8* | 4.8* | 4.6* | 12.8 | 90* | 20.3* |

*Significant results at P<0.05

## 4. Discussion

Indole acetic acid (IAA) is a common natural auxin and is a product of L-tryptophan metabolism in microorganisms. Saline soil is a rich source of IAA producing bacteria where 75% of the bacterial isolates were active in IAA production. **Bhavdish et al. (2003)** found that approximately 80% of rhizosphere bacteria can secrete IAA which may due to high input of organic materials derived from the plant roots and root exudates that are necessary for microbial growth **(Lynch 1990).** In this study, the quantities detected in liquid broth media were ranged from 4.9 to 11.4 $\mu gml^{-1}$. *Azotobacter vinelandii* MM1 and *Streptomyces* sp. MM10 were the most active isolates in IAA production and were identified based on morphological, physiological and biochemical characteristics which are useful and valuable tools in systematic and distinguish bacteria to species level **(Tchan, 1984, Williams *et al*., 1989, 1994)**. *A vinelandii* was more active in IAA production (11.4 $\mu gml^{-1}$) compared to *Streptomyces* sp MM10 (9.8 $\mu g/ml^{-1}$). Coryneform bacteria produced indolyl-3-acetic acid into the medium in the amount of 9.0-95.0 $\mu gml^{-1}$ and the isolates of actinomycetes in the amount of 39.5-83.0 $\mu gml^{-1}$. **El-Tarabilya and Sivasithamparamb (2006) and**

**Tsavkelova *et al.* (2006)** found that soil microbes have the ability to produce IAA and promote plant growth. Many authors confirmed production of IAA in *Azotobacter* (**Azcon and Barea, 1975, Harper and Lynch, 1979, Aly, 1990 and Ahmad *et al*., 2005)** and the IAA quantity increased with tryptophan to 5 mg/ml (Patil, 2011). Lower auxin production ranged from 0.60 to 3.0 $\mu gml^{-1}$ was obtained by *Bacillus* spp. in broth medium supplemented with L-tryptophan **(Ali *et al*., 2010).** Higher quantities of IAA from actinomycete isolates were recorded by **Gangwar *el al*. (2012)** (17-39 $\mu g/ml^{-1}$) and **Khamna *et al*. (2009**) (5.5–144 $\mu gml^{-1}$) where *Streptomyces* CMU-H009, isolated from lemongrass showed the highest ability. Furthermore, several *Streptomyces* species, such as *S. livaceoviridis, S. rimosus, S. rochei* and *Streptomyces* spp. from the tomato rhizosphere, have the ability to produce IAA and improve plant growth **(Aldesuquy *et al.*, 1998, Tokala *et al*., 2002, El-Tarabily, 2008).**

The cell free filtrates of AZ, ST or both enhanced seed germination due to the presence of plant growth regulators, vitamins, amino acids or secondary metabolites. **Azcon and Barea (1975)** reported that culture supernatants of *Azotobacter vinelandii* and *Azotobacter beijerinckii* contain auxins, at least three gibberellin-like substances and three cytokinin-like substances.

Our results also revealed that soil inoculation by AZ, ST or both enhanced root depth, shoot length, dry weights of root and shoot and mineral and protein content which may due to nitrogen fixation, auxins production or unidentified compounds. Similar results were obtained by **EL-Shanshoury (1995)** who found single and dual inoculations with *Azotobacter chroococcum, Azospirillum brasilense* or *Streptomyces mutabilis* stimulated plant growth, significantly increased the concentrations of indole-3-acetic acid, P, Mg, N and total soluble sugars in wheat shoots. **Brown (1974)** found that *Azotobacter* can release IAA to the soil and significantly raise the growth and dry weight of roots and leaves of different plants. Moreover, **El- Shourbagy *et al*. (1979)** found that the beneficial effect of *Azotobacter* on tomato plants might be due to nitrogen fixation and secretion of plant growth regulators. Similarly, **Ahmed *et al*. (2004)** found that bacterial inoculation improve the straw, seed yield, root weight, root length, phosphorus, nitrogen and potassium absorption in straw and seed compared to control. The results of **Araujo *et al*. (2005)** confirmed the potential use of *B. subtilis* in promoting soybean growth and biological control of seed pathogens. Growth promotion of wheat seedlings after soil inoculation with *Streptomyces atroolivaceus* was recorded by **El-Shanshoury (1989).** Many biologically active compounds such as antifungal and antibacterial compounds or plant growth promoting substances that have been developed for agricultural use were originated from *Streptomyces* **(Ilic *et al*., 2007)** and the substrates available in root exudates helps *Streptomyces* to synthesize and release IAA **(Frankenberger and Arshad, 1995). Alizadeh *et al* (2012)** reviewed that in China, many studies have been conducted on the yield increase after bacterial inoculation , wheat (8.5-16%), rice (8.1-16%), maize (6-11%), beans (7-16%), sugar beet (15-20%), sorghum (5-10%), sweet potato (15-19%), linen (6-13%), oily turnip (16-18%), peanut (10-15%) and vegetables (13-35%).

Saline soils are one of the major biotic stresses that adversely affect the overall metabolic activities and cause plant growth inhibition **(Roychoudury *et al*., 2008)**. The obtained results showed that increasing soil salinity to 80 mM decreased plant growth and increased proline concentration which may due to membrane leakage, ion imbalance or disequilibrium, enhanced lipid peroxidation and increased production of superoxide radicals, hydrogen peroxide and hydroxy radicals. Sugars, sugar alcohols, polyols, inositols, quarternary amino compounds like glycine-betaine, proline and higher polyamines, serve as osmoprotectants under stress conditions, maintain membrane structure and act as free-radical scavengers preventing lipid per oxidation or as regulators of K+ channels in stomata **(Hasegawa *et al.*, 2000)**. **Summart *et al.* (2010)** reported that at high level of Na+, rice cells accumulated high level of Na+ whereas the accumulation of K+ and Ca2+ was decreased in addition there is an increase proline accumulation which may play a crucial role in protecting the rice cells under salt stress. The increase in the proline content under stress condition may be due to breakdown of proline rich protein or *de novo* synthesis of proline **(Tewari and Singh, 1991).** It is generally assumed that proline is acting as a compatible solute in osmotic adjustment, an enzyme protectant, stabilizes membranes, as an organic nitrogen reservoir ready to be used after stress relief to sustain both amino acid and protein synthesis **(Larher *et al*., 1993, Hong *et al*., 2000, Sairam and Tygai, 2004).**

Our finding also confirmed that soil inoculation with AZ, ST or both enhanced plant growth under saline conditions. Several studies show successfully using the plant growth promoting rhizobacteria to increase the plant resistance against salinity and reduce the undesirable effects of salinity **(Alizadeh *et al*., 2012).** Evidence has accumulated that the bacteria including *Azotobacter* are essential elements in saline environment because of their activity such

as degradation of plant remains, nitrogen fixation and production of active metabolites **(Page and Shivprasad, 1991).** The majority of soil bacteria can osmoregulate by synthesizing specific compatible organic osmolytes such as glutamine, proline and glycine betaine and a few of them accumulate inorganic solutes such as Na+, $K^+$ and $Mg^{2+}$. Eight strains of salt-tolerant bacteria from the rhizosphere of wheat with potentially beneficial traits were isolated and characterized by **Egamberdieva *et al.* (2008)** and they initially have some of the following plant growth-beneficial properties: production of auxin, HCN, lipase or protease and wheat growth promotion. **Bacilio *et al.* (2004)** stated that *Azospirillum lipoferum* can reduce negative effects of salinity on wheat and increased dry weight of roots and leaves and height of inoculated plant. Furthermore, **Ashraf and Harris (2004)** found an increase in the root (149 to 522%) and shoot (85 to 281%) dry weights by inoculating wheat by polysaccharide-producing bacteria and the produced polysaccharides prevented sodium absorption by plant root. **Gravel *et al.* (2007)** stated that *Pseudomonas putida* increased tomato yield under saline condition in hydroponic environment. They attributed the increase to the ability of the bacteria to produce IAA. On contrast, **Woitke *et al.* (2004)** reported that inoculating tomato seeds with *Bacillus subtilis*, didn't have any significant effect on tomato yield cultivated in saline condition and the yield significantly decreased in high salinity treatment.

In conclusion, the results of this study show that *Azotobacter, Streptomyces* or both have potential to be utilized as biofertilizer in normal and saline soils.

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