

Effect of Some Soil Microorganisms on Soil Properties and Wheat Production under North Sinai Conditions.

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Abstract: About 77 different microbial isolates (24 *Azotobacter*, 14 *Bacillus*, 9 *Pseudomonas*, 14 Actinomycetes and 16 Fungi), isolated from different plant rhizosphere and compost from different localities in Egyptian governorates. The ability of microbial isolates in (N₂ fixation, production of phytohormone, phosphate solubilization, antimicrobial (antibacterial and antifungal) and enzyme production) were tested. The most powerful isolates in previous tests were selected and identified being *Azotobacter chroococcum*, *Bacillus megatherium*, *Pseudomonas fluorescense*, *Streptomyces fulvissimus*, *Aspergillus candidus*, *Lactobacillus lactis* and *Sacchromyces cerevisiae*. Selected effective microorganism showed high compatibility when mixed together. *Azotobacter chroococcum* recorded the highest values of carbohydrates and microbial gum production. Two field experiments for wheat were carried out in El-Sheikh Zowaied experimental station-El-Arish-North Sinai-DRC, Cairo, Egypt. Soil used was sandy received 1% chicken manure as organic matter and supplemented with the half dose of inorganic nitrogen, to evaluate the effect of employment of some effective chosen microorganisms in improving sandy soil properties and productivity of wheat yield. Physical properties of soil (Hydraulic conductivity, Bulk density and aggregation) chemical properties were improved by the product of organic matter decomposition during growth season, microbial gums producing and root growth promoting substances enhanced soil aggregation process, subsequently soil penetrability resistance decrease. The net result was less cohesion relation to adhesion forces between soil particles. Inoculation of wheat plant for two seasons with mixture of selected effective microorganisms significantly increased: total microbial counts, CO₂ evolution, *Azotobacter*, PDB, Fungi and Actinomycetes. Wheat growth criteria (shoot length, root length, shoot fresh and dry weight, root fresh and dry weight, chlorophyll content, number of leaves), yield parameters, mineral content (NPK) of wheat in soil rhizosphere and in plant were measured and, increased by inoculation. The highest effective of soil microorganisms treatment in improving sandy soil (El-Sheikh Zowaied) properties (physical and chemical) and productivity of wheat plant were by amending soil combined treatment with organic matter, half dose of mineral fertilizers and inoculation with the five selected microbes as seed+ soil +foliar, enhancing 87% increase of yield than control i.e., effect for agriculture production, improving soil properties, increasing soil fertility and reducing environmental pollution.

Key words: Microorganisms, sandy soil, *Bacillus*, *Pseudomonas*, *Streptomyces fulvissium*, *Lactobacillus lactis* and *Sachrromyces cerevisiae*.

INTRODUCTION

Egypt vitally needs sustained agricultural development to cope with the social and economic obligations that are the normal consequences of the continued high rates of population growth. This urgent need requires continuous scientifically based implementation of effective agricultural practices. On the limited cultivable land area it is essential to obtain the maximum yield per unit of irrigation water used. Wheat (*Triticum aestivum* L.) is the most important cereal crop in Egypt and increasing wheat production is an essential national target to fill the gap between production and consumption. Production could be increased through cultivation of high yielding cultivars

and appropriate agronomic practices^[1]. Use of soil microorganisms which can either fix atmospheric nitrogen, solubilize phosphate, synthesis of growth promoting substances or by enhancing the decomposition of plant residues to release vital nutrients and increase humic content of soils, will be environmentally begin approach for nutrient management and ecosystem function^[2]. Application of biofertilizer is considered today to limit the use of mineral fertilizers and supports an effective tool for desert development under less polluted environments, decreasing agricultural costs, maximizing crop yield due to providing them with an available nutritive elements and growth promoting substances^[3,4,5].

Effect of inoculation on soil: Soils are one of the most important resources a farmer has. Soil health is fundamental to profitable and sustainable production. Soils are the most basic and most important resource we use in agriculture. Proper management of the soil is a key to plant health and crop productivity. Soil structure has a strong impact on a range of processes influencing crop yield. It refers to the manner and stability with which individual sand, silt, and clay particles are bound together into units called aggregates. Soil aggregation is an important characteristic of soil fertility; the greater the degree of aggregation. Aggregates determine the mechanical and physical properties of soil such as retention and movement of water, aeration, and temperature^[6]. Aggregate formation is an important factor controlling germination and root growth^[7]. Several studies have shown that formation of stable aggregates strongly depends on both the nature and the content of organic matter^[8,9,10,11,12]. Unstable aggregates generally have a lower content of organic matter than do stable ones^[13]. Plant roots contribute to soil organic material, and thereby to soil aggregate stability, directly through the root material itself^[14] and indirectly through stimulation of microbial activity in the rhizosphere^[15]. It is generally believed that microbial action on soil aggregation is due to the production of exopolysaccharides (EPS)^[16]. This is supported by experimental observations demonstrating that the amendment of soil with microbial EPS results in an increased soil aggregation^[10,17].

Andrade *et al.*^[18] reported that soil aggregation is a dynamic process in which plants and the soil microbiota play a major role. It has been supposed that Vascular arbuscular mycorrhizal (VAM) fungi and some N₂-fixing bacteria (*Azotobacter* spp. and *Rhizobium* spp.) could play several important roles including the binding of the soil particles into stable aggregates. The influence of microbes on aggregate stability has largely been studied in bulk soil^[16,19]. Relatively little attention has been paid to the influence of microorganisms, particularly EPS-producing rhizobacteria, on the aggregation of root-adhering soil (RAS)^[14,20]. According to the model of Oades and Waters^[21] roots and fungal hyphae contribute to the formation of macro-aggregates (diameter > 0.25 mm), whereas formation of meso- and microaggregates (diameter < 0.25 mm) involves plant and microbial debris and bacteria. They suggest that bacteria, probably via their EPS production, also contribute to macro aggregate formation. Theoretically, new aggregates can be obtained from either breakdown of larger aggregates or accretion of meso aggregates^[22].

Ahmed *et al.*^[23] and Amara and Dahdoh^[24] reported that, treatments with different genera (*Azotobacter*, *Azospirillum*, *Pseudomonas* and *Rhizobium*)

individually or in mixtures improved grain yield and total plant dry weight. Inoculation with *Rhizobium* showed the highest value of grain yield representing 106.1% over control, while inoculation with mixture of *Azotobacter* and *Azospirillum* showed the highest value of straw yield representing 157.0% over control. Inoculation increased the uptake of N, P, K, Na, Fe, Zn, Mn and Cu. On the other hand, inoculation with mixtures of *Pseudomonas* and *Rhizobium* increased the uptake of N, Na, Fe and Cu, while inoculation with *Rhizobium* increased the uptake of N, K, Na, Zn, Fe, and Cu, but inoculation with *Azotobacter* increased the uptake of Mn. Khamis and Metwally^[25] investigated that, incorporation of organic materials inoculated with microbial decomposers and *Azotobacter* in the soil favorably increased the amounts of biomass-N, biomass-C. Yield of wheat and N uptake were increased by incorporation of organic materials inoculated with microbial decomposers and *Azotobacter* in the soil.

Hegazi *et al.*^[3] studied the effect of introducing single and multiple strains of diazotrophs to plant systems (wheat and barley) under gnotobiotic conditions. Single inoculation enhanced the efficiency of N₂-fixation in both plant systems, particularly with *Azospirillum* and *Azotobacter* spp. Increases in total dry weight of plants between 8-16 % were achieved in the wheat system and between 54.5 and 68% in the barley system, but the highest value being for *Azospirillum* spp and *Pseudomonas* spp. treatments. Khosravi *et al.*^[26] and Arafa *et al.*^[5] studied the effect of *Azotobacter* inoculation as a biological fertilizer on growth and yield of wheat and reported that, *A. chroococcum* had significant effects on shoot dry matter and root system development. A combination of *A. chroococcum* and compost enhanced number and weight of wheat heads. However it had no significant effect on N-uptake. The results also showed that *A. chroococcum* and farmyard manure can be applied instead of nitrogen fertilizer. Yadav *et al.*^[27] reported that, various strains of *Azotobacter chroococcum* were evaluated for their effect in wheat. Pot culture studies showed that, plant height, biomass and grain yield increased due to inoculation with *Azotobacter* strains with and without added nitrogen. Egamberdiyeva *et al.*^[28] studied the improvement of wheat and cotton growth and nutrient uptake by phosphate solubilizing bacteria and they recorded that, stimulatory effects of bacterial species such *Pseudomonas*, *Bacillus*, *Arthrobacter* and *Rhizobium* on growth of wheat, maize and cotton growth, yield, N,P-uptake, and soil p content. This result suggests that phosphate solubilizing bacteria are able to mobilize more P to the plants and improve plant growth compared with standard treatment without bacterial inoculation which resulted very low P uptake in plants. Neeru *et al.*^[29] reported that, inoculation of wheat varieties with the soil isolates and mutant strains

of *A. chroococcum* showed greater NPK uptakes compared with parent soil isolates. Mutant strains M15 and M37 were proved to be the most effective for all three wheat varieties with regard to NPK uptake as well as root biomass production under greenhouse conditions.

Kumar *et al.*^[30] reported that, seed inoculation of wheat varieties with phosphate solubilizing and phytohormone produced by *A. chroococcum* showed a better response over the control. Mutant strain of *A. chroococcum* showed a higher increase in grain (15.3%) and straw (15.1%) yields over the control and better survival (12-14%) in the rhizosphere compared to their parent soil. Amer *et al.* showed that, the inoculation with the most active strains of *Azotobacter*, *Azospirillum* and *Streptomyces* in a green house experiment increased the soil microbial activities. The used strains as a tri mixture also, exhibited high reduction of disease severity together with an increase of growth and yield of cucumber plants (plant height, root length, fresh and dry weight, number of flowers and fruits, weight of fruits, chlorophyll content and nitrogen percent in fruits. Bandel and Meisinger^[31] reported that soil fertility is very important for essential plant nutrients and for soil properties as texture, structure, organic matter, anion and cation retention, cation exchange capacity (CEC), base saturation (BS) and pH (acidity). Ali *et al.*^[32] showed that inoculation with *Bacillus* sp. OSU-142 significantly increased kernel number per spike in wheat, but no significant effect was determined in the other characteristics. Grain yields and yield components were also higher at all levels of nitrogen fertilizer in the inoculated plots as compared to the control.

Rai and Gaur^[33] and El-Sirafy *et al.*^[34] studied contribution of biofertilizers with (P)-solubilizing bacteria *Bacillus megatherium*, and Nitroben, containing a combination of nitrogen fixing bacteria (*Azotobacter chroococcum* and *Azospirillum liposum*) as well as nitrogen ammonium nitrate and polymer-coated urea fertilizers to nutrient uptake and yield of Egyptian winter wheat and they found that, the highest grain yield (5.76-6.74 Mg ha⁻¹) and straw yield (11.49-13.32 Mg ha⁻¹) occurred at the highest fertilizer rates with N fertilizer. In addition there was a slight additional increase in grain and straw yields when a biofertilizer was applied along with N fertilizer. Biofertilizer inoculations increased iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) concentrations in wheat tissue, but these higher levels did not influence grain or straw yield. Application of biofertilization generally raised the shoot fresh weight values in comparison with non biofertilized ones, although treatments using micronutrients in addition to 100 & 200 ppm antioxidant improved plant fresh

weight to reach those of the positive control (100% NPK). At high soil salinity levels a remarkable observation was found where the biofertilized treatments significantly overcame the non-biofertilized treatments and similar observation was estimated for plant shoot dry weight under the same previous mentioned conditions. Biofertilization with *Candida tropicalis* increased significantly the leaf area of tested wheat cultivars under and soil salinity levels. At high salinity level, it was found that treatments supplied by biofertilization with yeast mitigated the adverse effect of salinity. Application of yeast produced the highest spikes number irrespective of salinity level and cultivar type^[35].

The objective of this study was to evaluate the effect of employment of some effective soil microorganisms in improving sandy soil (El-Sheikh Zowaied) properties (physical and chemical) and productivity of wheat.

MATERIALS AND METHODS

Survey of Soil microorganisms in Egyptian soils:

Seventy seven samples (rhizosphere and soil samples) were collected from different locations in seven governorates of Egypt. These Samples were used for isolation of *Azotobacter*, Phosphate dissolving bacteria, Actinomycetes, Pseudomonas and Fungi isolates. They were grown separately on modified Ashby's medium^[36], Bunt and Rovira medium^[37], Starch nitrate medium^[38], Kings medium^[39] and Czabek's Dox agar^[40] respectively.

Isolation and Purification of Microbial Isolates:

Twenty four *Azotobacter* isolates, fourteen *Bacillus* isolates, nine *Pseudomonas* isolates, sixteen fungal isolates and fourteen Actinomycetes isolates were isolated from soil samples and compost. All isolates were subjected to purification trials by successive streaking on specific media for each isolate.

Isolation and Purification of Lactic Acid Bacteria:

Three isolates were taken from Ferm/bam Center Al-Azhar University. All isolates were subjected to purification trials by successive streaking on Nutrient Agar medium^[41].

Isolation and Purification of Yeast: *Sacchomyces*

cervisiae were used and grown on Yeast extract malt extract agar medium^[42].

Microbial activity:

Nitrogen fixation: The purified *microbial* isolates were tested for their N₂ fixation activity according to the microKjeldahl method described by Jackson^[43].

Phosphate Dissolving Efficiency: All microbial isolates were tested for phosphate dissolving capability qualitatively by inoculating all isolates on modified Bunt and Rovira medium^[37]. Estimating the clear zone around the developed colonies Their phosphate dissolving potency was also determined quantitatively according to method adopted by Watanabe and Olsen^[44].

Production of Antibiotic: the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism^[45].

Production of Phytohormones: All microbial isolates were tested for the promoting activity on plant seedlings by measuring elongation in shoots and roots^[46]. Phytohormones were also was also determined quantitatively for selected effective microorganisms using by high performance liquid chromatography (HPLC) according to the modified method of Rizzolo *et al.*^[47].

Production of Enzyme: Proteolytic assay technique: The protease enzyme activity was measured by the Gelatine Clearing Zone (GCZ) technique according to Ammar *et al.*^[48].

Lipolytic Assay Technique: The lipase enzyme productivity was measured by the Tributyrin clearing zone (TCZ) technique according to Barrow and Felthan^[49].

Amylase Assay Technique: The amylase enzyme activity was measured by the clearing zone technique as described by Barrow and Felthan^[49].

Pectinase Assay Technique: The pectinase enzyme activity was measured by the clearing zone technique. as described by Barrow and Felthan^[49].

Cellulase Assay Technique: The cellulose enzyme activity was measured by the clearing zone technique. as described by Barrow and Felthan^[49].

Determination of Total Carbohydrates: Total carbohydrates content was determined calorimetrically using UV/Visible Spectrophotometer, Unicam UV 300, Thermo Spectronic, USA by Nelson's reagent as reported by Cherry^[50].

Determination of Microbial Gum Production: Microbial gums produced by selected isolates were determine using method described by Hamilton^[51].

Identification of Selected Microbial Isolates:
Azotobacter isolates: One (A_{13}) of azotobacters isolates active in N_2 fixation, phosphate solubilization, enzymatic activity, hormonal production and antagonistic activity was subjected to complete identification according to its morphological and physiological characteristics using the methods described in Kerig and Holt^[52].

Phosphate Dissolving Bacteria: The most active isolates of *Bacillus* (B9) in phosphate solubilization, enzymatic activity, hormonal production and antagonistic activity was subjected to complete identification according to its morphological and physiological characteristics using the methods described in Bergy's Manual of Determinative Bacteriology^[53].

Pseudomonas: The most potent fluorescent pseudomonad isolate (Ps 9) with antagonistic activity, enzyme production, hormone production and phosphate dissolving activity was identified according to the methods described in Bergey's Manual of Determinative Bacteriology^[53,54].

Fungi: The most active isolate of *Aspergillus* sp. (F16) in antagonistic activity was completely identified according to Barnet and Hunter^[55] and Moubsher^[56].

Actinomycetes: The most active isolates of *Actinomycete*.(Act 14) in antagonistic activity was completely identified according to Bergey's Manual of Systematic Bacteriology^[54].

Field Experiments: Two field experiments were carried out at El-Sheikh Zowayed, Al-Arish, North Sinai Governorates Desert Research Center, Cairo, Egypt to study the effect of employment of some effective microorganisms in improving sandy soil properties and productivity. Chicken manure was air dried ground and milled using 2mm sieve to be analyzed for carbon and nitrogen content. Chicken manure was thoroughly mixed with soil before cultivation at the rate of 1%.

Mineral Fertilizer: Calcium super-phosphate (containing 15.5% P_2O_5) was added to all treatments at the rate of 200 kg /fed. and mixed with the soil 15 day latter before cultivation, Nitrogen fertilizer (calcium ammonium nitrate 33.3 %N) was added at a rate of 60 kg/fed. in two equal parts after 25 and 45days from sowing. Potassium sulphate (contains 48% K_2O) was added after 25 days of sowing at a rate equal to 40 kg/fed.

Grains of wheat were washed and immersed for 30 minutes in liquid culture of effective microorganisms (SEM containing *Azotobacter* for wheat). Carboxy methyl cellulose 0.5% was used as an adhesive agent. Seeds and grains were then dried at room temperature for two hour. Thus, all treatment used can be summarized as follows: Organic matter added to all treatments,

- 1-Uninoculated without mineral fertilizer (organic matter).
- 2-Uninoculated with mineral fertilizer.
- 3-O.M+Mf+ seed or grain inoculation.
- 4-O.M+Mf+ soil inoculation.
- 5-O.M+Mf+ foliar application.
- 6-O.M+Mf+ seed+ soil.
- 7-O.M+Mf+ soil+ foliar.
- 8-O.M+Mf+ seed+ soil+ foliar

Sampling and Determinations:

Physical and Chemical analysis of soil: Soil sample were mechanically analyzed according to the methods described by Piper^[57]. Bulk density, Hydraulic conductivity and aggregation according to Klute^[58]. The electrical conductivity (EC) was measured in saturated soil according to method described by Jackson^[43]. Soluble anions, cations and soil pH were determined in saturated soil according to the method described by Richard^[59]. Organic carbon was determined by the rapid titration method and total nitrogen was determined using Micro-Kjeldahl method^[60]. Phosphorus was determined according to Troug and Meyer^[61]. Potassium being evaluated flame photometrically.

Microbiological Determination: Microbiological analysis of soil included the determination of total microbial counts and phosphate dissolving bacterial counts by plating on modified Bunt and Rovira medium^[37] using the decimal plate count technique^[62]. The most probable number of Pseudomonads was determined after incubating the tubes at 30±2 °C for 48 hour on King's B medium^[39]. Estimates of number of pseudomonads by MPN technique were calculated using Cochran's table^[63]. The most probable number (MPN) of *Azotobacter* was determined after incubating the tubes at 28 ±2°C for 10 days on modified Ashby's medium^[36]. Total fungi counts on Martins agar^[64] and total actinomycetes counts on Starch nitrate medium^[38].

Parameters of wheat plant.

- a) Plant height (cm).
- b) Fresh weight of both shoots and roots (g/plant).
- c) Dry weight of both shoot and roots were recorded after oven drying at 70 °C until reaching a constant weight^[65].

d) Chlorophyll content was measured by using Minolta chlorophyll meter (SPAD-502) to determine the total chlorophyll in fresh leaves.

e) Leaf area cm² using leaf area meter (Li- COR 1200).

f) Yield characteristics (number of leaves, number of tillers, spickles features, weight of 100 grain and grain yield/fed.).

3.2.14. Statistical Analysis: Data were subjected to an analysis of variance (ANOVA), using a log transformation when necessary. When ANOVA generated a significant F-value (P < 0.05), treatment means were compared by Tukey's LSD-test. Experiment was carried out with three replicates per treatment.

RESULTS AND DISCUSSION

About 77 different microbial isolates (24 *Azotobacter*, 14 *Bacillus*, 9 *Pseudomonas*, 14 Actinomycetes and 16 Fungi), isolated from different plant rhizosphere and compost from different localities in Egyptian governorates. The ability of microbial isolates in N₂ fixation, production of phytohormone, phosphate solubilization, antimicrobial (antibacterial and antifungal) and enzyme production) Table (1) were tested . Selected effective microorganism showed high compatibility when mixed together Table (2). Total carbohydrates and microbial gums produced by selected microorganisms were determined (Table 3) and *Azotobacter chroococcum* was highest gums producing.

Identification of the Most Active Isolates: On the basis of pronounced plant growth promoting, antimicrobial activities, phosphate solubilization and enzyme production of the tested isolates, five efficient isolates that display strong activity towards previous tests were selected and identified as *Azotobacter chroococcum* Az. 13, *Bacillus megatherium* 9, *Pseudomonas fluorescens* 3, *Sterptomyces fulvissium*. Act. 14 and *Aspergillus candidus* F. 16, were chosen and used as a mixture with *Lactobacillus lactis* and *Sachrromyces cervisiae*. Their potential as biofertilizer agents to improve productivity of wheat and improve soil properties *A. chroococcum*, *Streptomyces* sp., *B. megatherium*, *P. fluorescens* and *A. candidus* isolated from rhizosphere of different plants and selected as biofertilizer agent possess many desirable properties, as well as they have potential for the biological control of plant pathogens. Besides the ability of *A. chroococcum* to fix nitrogen, all three species are able to produce the growth hormone IAA and other phytohormones, and all exhibited seedling growth promoting activities, as demonstrated. Therefore, the response of wheat to

inoculation with the five biofertilizer agents in combined treatment to seed, foliar and soil as triple, tri or single inoculation was evaluated in two field experiments.

Two field experiments were carried out in El-Sheikh Zowaied Experimental station-El-Arish-North Sinai-DRC, Cairo, Egypt to evaluate the effect of employment of effective microorganisms (*Azotobacter*, *Bacillus*, *Pseudomonas*, Actinomycetes, Fungi, Lactobacillus and yeast) on improving the productivity of wheat plant and also, improve soil properties. Soil used was sandy textured amended with 1% chicken manure and supplemented with the half dose of inorganic nitrogen. To evaluate the effects of biofertilization by using the selected effective microorganisms on the growth and yield of wheat. This was carried out to improve sandy soil properties by modifying its texture and water holding capacity. Also, organic matter influences the solubility of certain soil minerals and makes them more readily available for plants and microbial growth and increases the soil buffering capacity. In addition, organic matter also serves as a source of energy for the growth and proliferation of microorganisms and provides them with certain essential nutrients required for their growth and activity. The rhizosphere microorganisms i.e. (total microbes, *Azotobacter*, P-dissolvers, actinomycetes and fungi) were periodically enumerated to throw light on the effect of biofertilization with selected effective microorganisms on the growth of such important groups of microorganisms which known to play an important role in stimulating plant growth.

Total microbial counts: It is clear from the data provided in table (4) that, initial total microbial counts in El-Shiekh zowaied, sandy soil was 34×10^6 CFU/g soil. Generally, the counts at second season were significantly higher than those of first one. Also, the counts at heading stage were significantly higher than those of harvesting stage followed in descending order towards tillering stage of plant growth. All bioorganic treatments significantly increase microbial counts $>$ control₂ $>$ control₁. The highest significant increase recorded with (soil + seed+ foliar) applications followed in descending order by (soil+ foliar), (soil+ seed); soil ; seed applications being 339, 307 , 295, 279, 285 and 285×10^6 CFU/g soil for the second season at heading stage of wheat plant growth, respectively. The enhancement in microbial activity is a good parameter for many soil improvement indicis.

CO₂ evolution: Results in Table (4) show that CO₂ evolution is positively correlated with total microbial counts under different treatments.

Azotobacter: Initial densities of azotobacters was 17×10^4 cells/g dry soil However, their counts tended to increase in all treatments rather than control₂ $>$ control₁. It is clear from the data in Table (4) that the densities of azotobacters were affected by seasons, stages of wheat plant growth and kind of treatments. The highest significant increase for *Azotobacter* densities being 113×10^4 cells/g dry soil was recorded with soil+seed +foliar followed in descending order by soil + foliar $>$ soil+seed being 96 and 87×10^4 cells/g dry soil respectively, and the least significant densities were with seed treatment for bio-organic treatments being 61×10^4 cells/g dry soil in second season at heading stage of wheat plant growth.

Phosphate Dissolving Bacteria: Data presented in table (4) show that initial count of Phosphate dissolving bacteria 28×10^2 CFU/g dry soil. However their counts tended to increase in all treatments rather than control₂ $>$ control₁ and the counts were affected by bioorganic treatments, stages and season of plant growth. Counts of PDB increased significantly at heading if compared with harvesting and tillering growth stages and at second season if compared with first season of wheat plant growth. The highest significant counts were recorded with soil+ seed +foliar followed in descending order by soil+ foliar, soil+ seed, foliar, seed, soil application being $178,130,122,103,87,82 \times 10^2$ CFU/g dry soil at heading stage and second season of wheat plant growth.

Fungi Count: The data presented in table (4) illustrated that the initial count of fungi was 12×10^3 CFU/g dry soil. Generally, the counts increased under wheat growth reaching their maximum counts at heading stage. However, this trend was affected by the type of biofertilization, stage and season of plant growth. For bio-organic applications, the highest significant increase was recorded with soil+ seed +foliar being 33 and the least significant increase recorded with seed treatment being 24×10^3 CFU/g dry soil $>$ control₂ $>$ control₁ being 23. 21×10^3 CFU/g dry soil at heading and second season of wheat plant growth.

Actinomycetes: Table (4) show that actinomycetes count were affected by the different treatments under study, time and stage of plant growth. The initial total actinomycetes count was 10×10^3 CFU/g dry soils. With respect to stage and season of wheat plant growth, the counts tend to increase significantly towards heading stage then decreased towards harvesting whereas counts were significantly less than tillering stage of plant growth. Also, counts at second season were significantly higher than those of first season.

With respect to bioorganic treatments the least significant increase was recorded with seed inoculation being 23 followed in ascending order by foliar, soil, soil + seed, soil+ foliar and soil+ seed+ foliar being 23, 24, 28, 29, 33×10^3 CFU/g dry soil at heading stage and second season of wheat plant growth respectively.

Growth of Wheat Plants: Data in Tables (5) show that plant height (cm), root length (cm), shoot and root fresh and dry weights (gm), leaf area (cm^2), chlorophyll%, number of tiller and leave/plant increased significantly with bio-organic treatments and affected by stage, season cultivations and different treatments under study. For plant height the least height recorded at tillering stage and first season which significantly increased towards heading and harvesting stages of plant growth. Also, plant height significantly increased towards second season of wheat plant growth. The highest significant increase recorded with soil+ seed+ foliar inoculation and the least one with seed treatment being 93, 64 cm at second season and harvesting stage of wheat plant growth as presented in Table (5) respectively. Also, root length affected by different treatments and age of plant growth. The highest significant increase for root length recorded with mixed inoculation (soil + seed + foliar), (soil + foliar); (soil + seed) being 21.8, 20.6; 19.7 cm significantly decreased towards soil, foliar and seed inoculation being 19, 18.6 and 17.5 cm at harvesting stage at second season of wheat plant growth, respectively.

Shoot Fresh and Dry Weights: As presented in Table (5) data show that bioorganic treatments significantly increased shoot fresh weight from 11.9 to 30.8 gm and from 2.5 to 8.2 gm for dry weight at harvesting stage at second season of wheat plant growth. Uninoculated control treatments recorded the lowest shoot fresh and dry weights, control₂ > control₁. Also, shoot fresh or dry weights significantly increased towards harvesting stage

Application of soil + seed + foliar significantly increased shoot fresh and dry weight \approx 3 folds for fresh weight and 7-8 folds for dry weight if compared with control₁ or control₂.

Root weights: Concerning wheat root fresh and dry weight (Table 5), the lowest was 0.47 and 0.28 g for control₁ and 0.51 and 0.31 control₂, respectively. These significantly increased to 3.9 g and 0.92 g in treatments receiving soil + seed + foliar treatment at harvesting stage at second season of wheat plant growth for fresh and dry weight, respectively. On the other hand these treatments increased root fresh and dry weights as much as (3.9/0.93, 3.9/1.34) and (0.92/0.49, 0.92/0.57) comparing with control₁ and

control₂ for root fresh and dry weights respectively.

Leaf Area: Data presented in Table (6) showed that control₁ < control₂ gave less leaf area comparing with other treatments. Also, increased towards harvesting stage and second season of wheat plant growth and affected by type of fertilizers. Bio-organic treatments gave the best results as follows: (soil + seed + foliar) inoculation was highly significant being 9.79 cm^2 and the less significant seed inoculation being 3.34 cm^2 at harvesting stage and second season of wheat plant growth

Number of Tillers and Leaves/plant: The effect of treatments, age and stage of wheat plant growth on tillers/plant and number of leaves/plant could be seen from the set of values depicted in Table (6). The number of tillers and leaves / plant increased significantly towards harvesting at second season of plant growth. It is clear that control₂ recorded higher values than control₁. The bio-organic treatments significantly increased number of tillers and leaves/plant. The magnitude could be arranged descending as follow, soil + seed + foliar, soil + foliar, seed + soil, soil, foliar, seed inoculation being (7.53, 6), (7.12, 6), (6.5, 5.6), (6.14, 5.3), (5.69, 5.3) for tillers/plant and leaves/plant respectively.

Chlorophyll Content: Data represented in Table (6) showed that chlorophyll content recorded higher increase at heading and second season than harvesting > tillering stage of wheat plant growth, chlorophyll content affected by bio-organic treatments. These data also clarify the role of biofertilization type in increasing chlorophyll content, whereas the effectiveness order was as follows: soil + seed + foliar (61.7%) > soil + foliar (60.2%) > soil + seed (58.4%) and the least one seed inoculation (45.7%) at heading and second season of plant growth. This trend might be attributed to the enhancement of both microorganisms and plant roots in stimulating and producing humic materials which contribute in binding soil separates.

Total Nitrogen Content in Soil: Data represented in Table (7) clearly showed that, total nitrogen content of soil significantly reached their maximal levels at heading stage of wheat plants. The mixed application (seed+ soil+ foliar) represented the best treatment compared with the rest of treatments and the control group. The increase percentages over control₂ were (420%), (425%), for the two seasons respectively.

Total Phosphorus Content in Soil: The obtained data from Table (7) indicate that biofertilizer application increase the total phosphorus than control treatment. On

the other hand, data also revealed that biofertilization had a beneficial effect on total phosphorus in soil. Combination of selected effective microorganisms was more effective in increasing total p.

Total Potassium in Wheat Rhizosphere: Data in Table (7) demonstrate the total potassium in rhizosphere of wheat plant as affected by biofertilizer application to seed, soil and foliar. Data showed that total potassium in the rhizosphere significantly increased by addition of mineral fertilizer as recorded in control₂ if compared with control₁. The highest values were recorded with mixed biofertilizer application to both seed, soil and foliar in a mixed treatment, at heading stage being 2.86 and 2.92% in first and second season respectively.

Chemical Characteristics of Plant:

Total Nitrogen Content of Wheat Plant: Data reported in Table (7) clearly showed that, total nitrogen content of wheat plant reached their maximal level at heading then decreased again towards harvesting. Generally speaking inoculation of wheat plants gave higher records than uninoculated ones and affected by method of inoculation. The highest significant nitrogen content was recorded with triple application of selected effective microorganisms i.e., soil+ seed foliar being 2.98 and 3.0% at heading stage during first and second seasons respectively. However, mixed inoculation significantly increase N content for wheat by soil+foliar > soil+seed > soil > folia r>seed > cont₂ > control₁.

Total Phosphorous and Potassium Contents for Wheat Plant: Data in Table (7) clearly showed that total phosphorous and potassium contents of wheat plants reached their maximal levels at heading stage of wheat plant growth in the two seasons then decreased towards the end of the experiment at harvesting stage. Concerning the effect of biofertilizer application on phosphorus and potassium contents the results in Tables 4 revealed that biofertilizer application to seed, soil and foliar either individually or mixed treatments significantly increased total phosphorous and potassium contents than uninoculated treatments. Mixed inoculation with triple mixture of selected effective microorganisms was significantly superior to other treatments. However, mixed application by soil+foliar and soil+seed recorded higher figures if compared with control or single inoculation. The same trend of results was obtained during both seasons.

Effect of Different Types of Treatments on the Morphological Characteristics of Wheat Plants: Generally speaking data in Table (8) show significant increase over control for wheat yield and its component

in the two growing seasons by using biofertilizer. However, the magnitude of increase was affected by the method of inoculation. The highest significant increases for wheat yield and its components recorded with soil + seed+ foliar followed in descending order with soil+foliar, soil+seed, soil, foliar and the least one as seed inoculation. The mean value of the two growing seasons for spike/length, spike no., Fresh and dry weights of spike and n. of grain/spike for triple inoculation were 20.3, 7.2, 11.6, 7.1 and 56.5 respectively. The corresponding figures for the least one with seed inoculation were 13.1, 5.2, 7.7, 4.2 and 40. With respect to wheat yield Table (8) shows that the mean values of the growing seasons for grain and straw yield were 2.66, 2.4, 1.88, 1.8 and 3.34, 3.13, 2.93, 2.4, 2.17 and 1.95 T/fed inoculated with soil+foliar seed, soil+foliar, soil+seed, soil, foliar and seed respectively. The corresponding percentage increases over control₂ were 87, 68.1, 66, 34, 22.7 and 13.1 (Table 10).

Chemical Composition of the Whole Grain of Wheat Plant:

Data reported in Table (9) showed the effect of biofertilizer on Phosphorous, Nitrogen and Protein contents in wheat grains in the two growing seasons. Triple inoculation treatment recorded the highest figures while the least are with seed inoculations. The mean values of the two growing seasons were 0.16, 0.14 and 0.9 for p, N and protein respectively.

Physical Properties of Soil: The selected and tested soil physical properties include: Bulk density, hydraulic conductivity and soil aggregation

Bulk Density: Table (11) show the measured values of bulk density for the soil samples after each cultivation season for both pea nut and wheat crops for different applied treatments and the base soil as well, as the table also include the percentage of decrease relative to the base soil value. From the data it can be concluded the following:

- 1- General decreasing trend for all treatments relative to the base soil which is indicative to general physical improvement.
- 2- The second cultivation season for both crops indicates greater decrease in values which reflect the radical effect of treatments across the two seasons.
- 3- General trend of greater changes in bulk density values from the sole treatments which give indication of good interactions among the applied kinds of microbiological strains with the organic base treatment.

Table 1: General Microbial activity for selected effective microorganisms.

Parameters	Az13	B9	Ps3	F16	Act14	
N ₂ fixation	T.N(ppm)	114.6	0	0	0	
	Nitrogenase (µC2H4H-II-1)	423	0	0	0	
Phosphate solubilization	Qualitative Inhibition zone(cm)	1.2	3.8	0.9	0.7	0.5
	Quntitative Colorimetric (mgP/l)	1.44	4.75	1.1	1.3	0.8
Hormonal activity Quntitative(HPLC)/ Qualitative (bioassay)	Sh.L	13.4	15.2	12.4	9.3	9.2
	R.L	11.81	13.85	11.2	7.4	7.12
	Total Sh+R	25.21	29.05	23.61	16.7	16.32
	% of increase	101.68	66	69.86	64.5	69.2
	IAA	0.17	0.26	0.837	0.973	0.183
	GA3	3.2	1.37	2.54	15.46	4.16
	Cytokinine	26	12	13.9	60.7	16.2
Enzyme production	amylase	+++	+	-	+++	+++
	Cellulolase	-	++	+	+++	++
	Pectinase	-	++	+	+++	+++
	Protease	+	++	+	+	+
	Lipase	+	++	+	+	+
Antimicrobial activity Antifungal Antibacterial	<i>E.coli</i>	33	28	25	55	48
	<i>S.typhi</i>	29	32	31	34	31
	<i>S.aureus</i>	0	24	32	46	38
	<i>C.albicans</i>	17	30	33	39	28
	<i>B.subtilis</i>	21	23	19	41	40
	<i>F.oxysporum</i>	13	27	29	36	36
	<i>R.solani</i>	15	18	34	40	29
<i>Alt.solani</i>	0	15	25	45	32	

Table 2: Synergistic effect between selected microorganisms

(a) Synergistic effect between bacterial isolates

Microorganism	<i>B₉</i>	<i>Ps₃</i>	<i>L. Lactis</i>
<i>A₁₃</i>	+	+	+
<i>Bradyrhizobium</i>	+	+	+

(b) Synergistic effect between Fungi, Actino, Yeast and other bacterial isolates.

Microorganism	<i>A₁₃</i>	<i>B₉</i>	<i>Ps₃</i>	<i>L. Lactis</i>
<i>Fungi</i>	13	9	3	0
<i>Actino</i>	0	0	0	0
<i>Yeast</i>	0	0	0	0

Table 3: Determination of total carbohydrates and microbial gums produced by selected microorganisms.

Parameters	Reducing sugar mg/l	Disaccharide mg/l	Poly saccharide mg/l	Total carbohydrates mg/l	Gums mg/l
<i>Azotobacter</i>	310.59	29.67	17.61	357.87	750
<i>Bacillus</i>	103	78	51	232	365
<i>Pseudomonas</i>	89.18	64.37	17.96	171.51	207
Rhizobia	260.25	116.45	11.65	388.44	519
Actinomycetes	0.131	0.064	0.023	0.218	0.08
Fungi	237.6	86.4	6.29	330.29	65
Lactobacillus	293.7	189.86	119.01	601.94	136
Yeast	218.96	43.52	15.41	277.87	92

Table 4: Effect of inoculation with effective microorganisms on Microbial determinations of wheat plant at different stages of growth during two seasons.

Treatments	Total microbial count						CO ₂ evolution						Azotobacter count					
	First Season			Second Season			First Season			Second Season			First Season			Second Season		
	II	III		II	III		I	II	III	II	III		II	III		II	III	
Cont 1	49	161	138	55	173	142	15	22	18	16	22	20	18	34	28	19	35	30
Control2	66	210	171	76	220	196	18	25	20	20	25	24	25	47	37	26	49	40
Seed	80	255	203	94	272	209	22	28	24	24	33	30	31	52	43	35	61	48
Soil	138	273	227	152	285	232	26	34	29	28	38	34	39	67	52	44	67	60
Foliar	112	246	216	129	279	225	24	30	26	26	34	31	33	56	45	38	64	49
Soil+Seed	157	287	239	171	295	242	27	38	32	30	39	36	45	81	62	50	87	68
Soil+Foliar	173	291	246	186	307	249	32	44	39	34	47	43	54	91	75	59	96	84
Soil+Seed+Foliar	182	318	263	194	339	269	35	46	41	38	61	54	61	105	80	68	113	91
L.S.D.at 0.05%	0.95875			0.67			11.755			0.45667			0.6804			0.78		
Treatment	PDB counts						Fungi counts						Actinomycetes count					
	First Season			Second Season			First Season			Second Season			First Season			Second Season		
	II	III		II	III		II	III		II	III		II	III		II	III	
Cont 1	31	43	52	32	45	54	12	18	16	13	21	19	10	18	142	11	19	16
Control2	43	50	62	47	56	64	13	20	17	13	23	19	11	19	16	12	20	18
Seed	52	64	70	56	70	82	13	22	18	14	24	21	12	20	17	13	23	20
Soil	70	81	94	74	92	103	14	24	21	15	25	22	14	23	20	15	25	22
Foliar	61	68	84	65	76	87	14	23	20	14	23	21	12	21	18	13	23	21
Soil+Seed	84	93	104	86	110	122	15	25	22	16	27	23	15	27	22	15	28	24
Soil+Foliar	96	106	119	99	117	130	16	28	24	18	28	24	16	28	23	17	29	25
Soil+Seed+Foliar	108	135	159	32	45	54	18	29	24	18	33	29	17	30	25	18	33	27
L.S.D.at 0.05%	1.6			11.3			0.48			0.18			0.74			0.42		

Table 5: Effect of inoculation with effective microorganisms on Plant characteristic of wheat plant at different stages of growth during two seasons.

Treatments	Shoot length						Shoot fresh weight						Shoot dry weight					
	First Season			Second Season			First Season			Second Season			First Season			Second Season		
	II	III		II	III		II	III		II	III		II	III		II	III	
Cont 1	15	39	47	18	40	54	2	5.5	7.8	2.4	5.8	8.4	0.5	0.8	1	0.5	1	1.2
Control2	21	46	53	23	46	62	2.8	6.4	10.7	3.3	7.5	10.8	0.6	1.1	1.3	0.6	1.2	1.4
Seed	22	53	60	24	59	64	3.1	8.2	12.2	3.8	9	11.9	0.9	2.1	2.3	0.9	2.2	2.5
Soil	24	61	69	27	62	69	3.4	12.2	16.9	4.1	14.1	17.2	1.3	3.5	4.8	1.5	3.7	4.9
Foliar	24	59	63	25	61	67	3	11.3	14.2	3.5	10.4	14.7	1	2.6	3.7	1.2	2.9	3.9
Soil+Seed	26	64	74	28	65	72	4.3	12.9	17.8	5.1	15.4	18.6	1.9	4.3	6.1	2	5.8	6.3
Soil+Foliar	27	67	75	30	72	79	4.8	16.1	20.6	5.6	16.9	19.8	2.6	5.6	6.5	2.8	6.8	7.5
Soil+Seed +Foliar	28	69	83	33	78	93	4.9	18.2	26.4	6.3	18.9	30.8	2.9	6.5	7.6	3.2	7.1	8.2
L.S.D.at 0.05%	0.01125			0.05125			2.43			2			2.2			2.8		
Treatment	Root length						Root fresh weight						Root dry weight					
	First Season			Second Season			First Season			Second Season			First Season			Second Season		
	II	III		II	III		II	III		II	III		II	III		II	III	
Cont 1	6.9	13	16	8.5	13.9	15	0.47	0.8	0.85	0.52	0.87	0.93	0.28	0.38	0.43	0.3	0.47	0.49
Control2	7.4	13.4	16.8	8.9	14.5	17	0.51	1.05	1.2	0.54	1.29	1.34	0.31	0.45	0.49	0.34	0.51	0.57
Seed	8.5	13.9	15	9.2	15	17.5	0.58	1.39	1.56	0.65	1.78	1.9	0.35	0.52	0.57	0.37	0.55	0.6
Soil	9.8	14.6	17.2	10.1	16.3	19	0.71	1.54	1.86	0.85	2.1	2.4	0.4	0.59	0.65	0.45	0.64	0.74
Foliar	8.7	14.2	17	9.5	15.7	18.6	0.65	1.46	1.63	0.76	1.95	2.2	0.37	0.54	0.62	0.42	0.61	0.69
Soil+Seed	10.1	15	17.5	10.8	16.8	19.7	0.82	1.79	2.09	1.14	2.8	3.1	0.46	0.62	0.71	0.52	0.75	0.81
Soil+Foliar	10.6	16	18.4	11.4	17.2	20.6	0.89	1.85	2.17	1.46	3.2	3.5	0.49	0.7	0.78	0.57	0.8	0.85
Soil+Seed +Foliar	11	18.8	19.5	12.3	18.1	21.8	0.94	2.38	2.76	1.82	3.41	3.9	0.53	0.75	0.84	0.61	0.86	0.92
L.S.D.at 0.05%	1.34			0.68			0.15			0.32			0.03			0.02		

Table 6: Effect of inoculation with effective microorganisms on Chlorophyll %, Leaf area, number of leaves and number of tillers of wheat plant at different stages of growth during two seasons.

Treatments	Chlorophyll%						Leaf area					
	First Season			Second Season			First Season			Second Season		
	II	III		II	III		II	III		II	III	
Cont 1	28.1	37.4	36.1	29.5	38.2	36.9	0.43	0.48	0.56	0.45	0.53	0.62
Control2	31.4	39.5	37.8	32.8	42.6	40.3	0.5	0.74	1.69	0.57	0.84	2.29
Seed	33.8	43.5	41.7	35.4	45.7	44	0.63	1.23	2.87	0.79	1.72	3.34
Soil	36.4	51.84	50.1	37.1	52.1	51.9	1.25	4.29	6.52	1.49	5.1	7.13
Foliar	35.2	47.3	45.9	36.2	47.9	46.3	1.03	3.61	6.11	1.1	4.02	6.39
Soil+Seed	39.8	54.6	51.2	40.2	58.4	53.1	2.17	5.6	7.33	2.38	5.92	7.78
Soil+Foliar	40.1	55.4	53.7	41.9	60.2	54.8	2.59	5.84	8.28	2.65	6.29	8.56
Soil+Seed+Foliar	43.6	59.5	57.9	44.5	61.7	58.2	2.76	6.45	9.41	2.91	6.7	9.79
L.S.D.at 0.05%	0.38			0.28			0.02			0.06		

Table 6: Continue

Treatment	Number of leaves						Number of tillers					
	First Season			Second Season			First Season			Second Season		
	II	III		II	III		II	III		II	III	
Cont 1	3	4	4	3	4	4	2	3	3	2	3	3
Control2	4	4	4	4	4	4	3	3	4	3	3	5
Seed	4	4	5	4	5	5	3	4	5	3	5	6
Soil	4	4	5	5	5	5	4	5	6	5	5	6
Foliar	4	4	5	4	5	6	4	5	5	4	5	6
Soil+Seed	4	4	6	5	5	6	4	6	6	4	6	6
Soil+Foliar	4	5	6	5	5	6	5	6	6	5	6	7
Soil+Seed+Foliar	5	5	6	5	6	6	6	6	7	6	6	7
L.S.D. at 0.05%	0.2			0.14			0.7			0.3		

Table 7: Effect of inoculation with effective microorganisms on NPK in soil and plant of wheat plant at different stages of growth during two seasons.

Treatments	N in soil						P in soil						K in soil					
	First Season			Second Season			First Season			Second Season			First Season			Second Season		
	II	III		II	III		II	III		II	III		II	III		II	III	
Cont 1	0.02	0.03	0.024	0.027	0.041	0.036	0.64	0.92	0.76	0.68	0.65	0.81	15.1	21.9	17.9	15.7	22.17	18.3
Control2	0.045	0.061	0.052	0.049	0.078	0.067	0.71	1.2	0.79	0.73	1.3	0.9	19.4	24.8	21.5	20.1	25.9	22.6
Seed	0.072	0.081	0.074	0.084	0.13	0.092	0.72	1.5	0.8	0.76	1.8	0.93	20.1	26.1	22.7	20.5	26.5	24
Soil	0.096	0.14	0.11	0.1	0.16	0.14	0.73	1.6	0.81	0.79	1.8	0.95	23.9	33.5	26.2	24.1	33.8	27.8
Foliar	0.09	0.12	0.098	0.092	0.154	0.12	0.71	1.3	0.8	0.76	1.6	0.92	21.6	28	24	21.9	28.3	25.2
Soil+Seed	0.127	0.189	0.14	0.134	0.22	0.179	0.75	1.9	0.83	0.8	2.0	0.96	28.3	46.9	37.8	24.5	47.5	38
Soil+Foliar	0.144	0.216	0.178	0.165	0.29	0.21	0.78	1.9	0.82	0.82	1.8	0.95	31.8	51	42.7	32.8	52.7	43.2
Soil+Seed+Foliar	0.235	0.319	0.245	0.27	0.342	0.28	0.79	2.1	0.85	0.84	2.3	0.97	39.5	64.9	53.9	42.9	65.5	54.7
L.S.D. at 0.05%	0.004			0.007			1.3			3.7			0.19			0.21		
Treatment	N in plant						P in plant						K in plant					
	First Season			Second Season			First Season			Second Season			First Season			Second Season		
	II	III		II	III		II	III		II	III		II	III		II	III	
Cont 1	2.65	3.61	3.28	3.02	4.01	3.6	0.31	0.41	0.36	0.32	0.34	0.38	1.22	2.02	1.76	1.28	2.08	1.74
Control2	4.1	5.1	4.51	4.31	5.52	4.73	0.35	0.48	0.4	0.36	0.5	0.41	1.46	2.34	2.1	1.47	2.36	2.15
Seed	4.26	5.54	5	4.6	6.38	5.52	0.37	0.52	0.42	0.37	0.55	0.43	1.55	2.42	2.18	1.59	2.47	2.23
Soil	5.15	6.45	5.65	5.37	7.3	6.15	0.4	0.61	0.48	0.42	0.58	0.46	1.83	2.43	2.07	1.9	2.48	2.11
Foliar	4.81	5.9	5.22	5.03	6.92	5.69	0.38	0.57	0.44	0.4	0.6	0.46	1.85	2.46	2.12	1.92	2.47	2.15
Soil+Seed	5.49	7	5.91	6.36	7.83	6.55	0.43	0.66	0.52	0.45	0.71	0.56	2.08	2.52	2.21	2.12	2.53	2.26
Soil+Foliar	5.7	7.6	6.49	6.29	8.31	7.03	0.44	0.72	0.59	0.48	0.77	0.64	2.31	2.72	2.51	2.38	2.82	2.52
Soil+Seed+Foliar	5.86	8.12	6.95	6.18	8.6	7.43	0.47	0.78	0.64	0.52	0.81	0.69	3.42	4.11	3.62	3.44	4.16	3.76
L.S.D. at 0.05%	0.08			0.2			0.01			0.01			0.11			0.13		

Table 8: Effect of inoculation with effective microorganisms on yield of Wheat plant.

Treatments	Yield Wheat (first season)								
	Spike length (cm)	Spike No.	Grain yield T/f	Straw yield T/f	T. yield T/f	F.W of Spike	D.W. of spike	No of grain/ spike	Wt. of 1000 grain gm
Cont1	8.3	3.5	1.19	1.73	2.9	6.3	3.39	34	27.8
Cont2	11.1	4.7	1.47	1.78	2.99	7.4	3.75	36	30.3
Seed	13	5	1.63	1.92	3.5	7.6	4.03	39	32.1
Soil	17	5.3	1.83	2.4	4.2	8.1	4.4	42	34.6
Foliar	15	5.5	1.72	2.08	3.8	7.9	4.27	41	33.8
Soil+Seed	18.9	6.7	2.34	2.9	5.25	9.6	5.19	46	36
Soil+Foliar	19.6	6.7	2.38	3.03	5.42	9.82	5.8	51	36.5
Soil+Foliar+Seed	20.2	7	2.46	3.29	5.75	11.24	6.97	55	36.8
Treatments	Yield Wheat (Second season)								
	Spike length (cm)	Spike No.	Grain yield T/f	Straw yield T/f	T. yield T/f	F.W of Spike	D.W. of spike	No of grain/ spike	Wt. of 1000 grain gm
Cont1	8.7	3.8	1.38	1.79	3.17	6.5	3.42	37	28.4
Cont2	11.4	4.9	1.58	1.86	3.44	7.7	3.91	38	31.2
Seed	13.2	5.4	1.78	1.98	3.76	7.8	4.28	41	33.7
Soil	17.8	5.7	1.93	2.44	4.37	8.5	4.73	45	36.5
Foliar	15.2	5.6	1.92	2.16	4.08	8.2	4.51	42	35.4
Soil+Seed	19.1	6.9	2.46	2.96	5.42	9.8	5.34	49	38.2
Soil+Foliar	20.2	7.1	2.53	3.23	5.76	9.96	5.87	53	38.9
Soil+Foliar+Seed	20.3	7.3	2.86	3.4	6.26	10.95	6.39	58	39.5

Table 9: Chemical composition of the whole grain of wheat plant.

Treatments	First season			Second season		
	P. content of grain %	N. content of grain %	Protein content of grain %	P. content of grain %	N. content of grain %	Protein content of grain %
Cont1	0.263	0.24	1.49	0.265	0.242	1.51
Cont2	0.274	0.25	1.55	0.279	0.25	1.56
Seed	0.287	0.253	1.58	0.288	0.26	1.62
Soil	0.302	0.26	1.63	0.307	0.27	1.67
Foliar	0.296	0.258	1.61	0.298	0.26	1.64
Soil+Seed	0.311	0.26	1.65	0.315	0.275	1.72
Soil+Foliar	0.319	0.27	1.69	0.324	0.28	1.75
Soil+Foliar+Seed	0.325	0.28	1.73	0.332	0.285	1.78

Hydraulic Conductivity: Saturated hydraulic conductivity (K) refers to the steady improvement of water through the soil under a head of water. The larger the value of hydraulic conductivity means the faster the movement of water which may be described in some soils while not for the others. Table (11) show the hydraulic conductivity values of the experiments conducted in El-Sheikh Zowaied experimental station

after cultivating with Wheat crops with different applications of microbial inoculations. From the table it can be concluded the following:

- 1- General trend of decreasing K values with different application under the two cultivated crops which means movement of water through soil .
- 2- The greater decrease values are contributed to the high dose of inoculation, by means of soil +

seed + foliar > soil +seed or soil+ foliar. This could be ascribed by complementary utilization of organic base treatment by different ways as soil & seed treatments utilize directly from the soil, while the foliar application utilizes indirectly from the soil through enhancing plant growth.

3- The effect of complementary treatments also includes root growth enhancement. So, their exudates which could impede the water movement due to their viscous nature.

4- The second growth season shows greater decrease in K values than the first one which renders to residual effect of treatments.

5- For both seasons wheat crops show greater effect K values than Pea nut may be due to fibrous nature of wheat.

Dry Stable Aggregates: Table (12) shows the four measured dry stable aggregates in the two seasons with different treatments. From the table it can be concluded the following:

1- 1.00-0.84 mm aggregate: This size considers as the limit of erodible aggregates by wind, so the greater the amount of this size is the greater soil surface stability as well. The data indicate slight, but gradual increase in this size by increasing treatments complementary i.e., from sole to triple treatments. Increases are more sensible in the second season than first one.

2- 0.84- 0.50mm.aggregate: The same trends in the former aggregate size have been achieved with this size, but with greater increasing values. This size is responsible for the easy uptake of water with root system, so its increase is effective in the active growth of plants as a whole.

3- 0.50-0.25 mm Aggregate: The minimum increasing values are contributed with this size of aggregates with the same trends.

4- <0.25mm Aggregates: This size is responsible for reserving soil water near the wilting point WP, so increasing its value by any portion will participate in avoiding plant from dryness threaten. Similar trends are noticed for the values in this size but higher than former one but less than the first two sizes; i.e. 1-0.84 and 0.84-0.5mm.

From the aforementioned discussion it can be concluded that the complementary treatments are more effective than double or sole ones which could be described by enhancing the plant growth by different ways.

Chemical Analysis of the Experimental Soil: The data of soil chemical analysis are presented in Tables (13) and (14) soil that collected after wheat cultivation during two seasons for each crop. The pH value range of soil samples showed slight differences between treatments. pH for wheat first and second season recorded 7.04 to 7.66 and initial pH before cultivation 7.88 Also, electrical conductivity differed from season to other ranged between 0.34-1.29 for wheat compared with initial 1.63. Control2 recorded highest chloride contents being 5.32 compared with triple inoculation treatment recorded the lowest chloride content being 1.02.

The soil content for some minerals were presented in Table (14) such as, sodium cations detected in soil sample showed high level with control 1 treatment being 1.14. Treatment with triple inoculation recorded the highest concentration of potassium being 4.32, however the lowest concentration was determined in soil treated with control1 being 1.47. The lowest calcium content was determined in soil sample treated with triple application being 0.1. It is clear from table (14) that soil sample of wheat not detected any amount of carbonates. Sulphate content in soil samples ranged between 1.13-5.63 for two seasons.

Table 10: Mean Yield of wheat at two seasons.

Treatments	Grain yield T/fed.	Straw yield T/f	Total yield T/fed.	% of increase Over cont2
Cont1	1.3	1.76	3.06	-
Cont2	1.5	1.82	3.32	-
Seed	1.7	1.95	3.65	13.08
Soil	1.88	2.42	4.3	34
Foliar	1.8	2.12	3.94	22.7
Soil+Seed	2.4	2.93	5.33	66
Soil+Foliar	2.45	3.13	5.58	65.1
Soil+Foliar+Seed	2.66	3.34	6	87

Table 11: Effect of inoculation with effective microorganisms on Soil Bulk density and Hydraulic Conductivity.

Treatment	Bulk density				Hydraulic Conductivity cm/h			
	Wheat 1 st season		Wheat 2 nd season		Wheat 1 st season		Wheat 2 nd season	
	R	%	R	%	R	%	R	%
Cont 1	1.61	-9.7	1.52	-14.9	24.2	-9.7	22.8	-14.9
Cont 2	1.60	-10.4	1.51	-15.3	24	-10.4	22.7	15.3
Seed	1.59	-14.2	1.50	-18.3	23	-14.2	21.9	-18.3
Soil	1.58	-19.4	1.48	-23.5	21.6	-19.2	20.5	-23.5
Foliar	1.59	-14.9	1.51	-26.9	22.8	-14.9	19.6	-26.9
Soil+ Seed	1.57	-22.8	1.47	-31	20.7	-22.8	18.5	-31
Soil+ Foliar	1.56	-20.1	1.48	-28.7	21.4	-20.1	19.1	-28.7
Soil+Seed+Foliar	1.53	-25.4	1.44	-31.3	20	-25.4	18.4	-31.3
L.S.D at 0.05	0.02		0.04		0.37		0.17	

Initial Bulk density: 1.66, Initial Hydraulic conductivity: 26.8

Table 12: Effect of inoculation with effective microorganisms on dry stable aggregates.

Treatments	Dry Stable aggregates1.0-0.84		0.84-0.5	
	Wheat 1 st season	Wheat 2 nd season	Wheat 1 st season	Wheat 2 nd season
Cont 1	0.49	0.54	0.76	0.82
Cont 2	0.49	0.54	0.79	0.83
Seed	0.5	0.55	0.81	0.86
Soil	0.53	0.57	0.82	0.89
Foliar	0.51	0.55	0.81	0.87
Soil+ Seed	0.54	0.57	0.83	0.92
Soil+ Foliar	0.55	0.58	0.84	0.92
Soil+Seed+Foliar	0.55	0.61	0.87	0.94
Treatments	Dry Stable aggregates0.5-0.25			
	Wheat 1 st season	Wheat 2 nd season	Wheat 1 st season	Wheat 2 nd season
Cont 1	76.95	77.31	21.8	21.33
Cont 2	77.03	77.35	21.69	21.28
Seed	77.05	77.35	21.62	21.24
Soil	77.13	77.38	22.51	21.16
Foliar	77.08	77.35	21.58	21.23
Soil+ Seed	77.18	77.43	21.45	21.08
Soil+ Foliar	77.18	77.41	21.43	21.09
Soil+Seed+Foliar	77.23	77.48	21.35	20.97

Table 13: Chemical analysis of the experimental soil.

pH	E.C.	Cation				anion				Microelement			
		Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻	Fe	Mn	Zn	Cu
7.88	1.63	1.5	3.58	8.72	2.49	-	4.37	5.53	6.41	0.61	0.91	0.32	0.24

Table 14: Chemical analysis after Wheat cultivation (first season).

Treatment	pH	E.C.	Cation				anion				Microelement			
			Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻	Fe	Mn	Zn	Cu
1	7.49	1.29	1.14	2.76	7.5	1.5	-	4.51	2.73	5.63	0.73	0.97	0.39	0.28
2	7.33	0.69	0.65	2.73	2.52	1	-	2.8	1.85	2.25	0.75	0.97	0.4	0.28
3	7.48	0.64	0.44	2.62	2.33	1	-	2.7	1.8	1.9	0.75	0.98	0.4	0.29
4	7.62	0.62	0.45	2.64	1.2	2	-	2.4	1.5	2.3	0.77	0.99	0.41	0.31
5	7.66	0.56	0.42	2.88	1.5	0.8	-	2	1.25	3.35	0.75	0.98	0.4	0.29
6	7.63	0.43	0.28	2.82	0.1	1.1	-	1.13	1.2	1.97	0.78	1.00	0.42	0.31
7	7.65	0.61	0.39	4.16	0.35	1.2	-	2.99	1.11	2.5	0.78	0.99	0.41	0.31
8	7.4	0.34	0.21	2.06	0.4	0.73	-	1.25	1.02	1.13	0.78	1.02	0.43	0.33
Wheat cultivation (second season)														
1	7.26	0.89	0.95	2.56	2.85	2.51	-	3.7	2.3	2.9	0.77	0.98	0.46	0.31
2	7.35	0.78	0.64	3.47	1.26	2.39	-	3.3	1.78	2.72	0.79	0.99	0.47	0.31
3	7.31	0.75	0.54	3.79	1.15	2.02	-	3.12	1.9	2.48	0.8	0.99	0.48	0.31
4	7.04	0.68	0.51	3.47	0.91	1.89	-	2.82	1.62	2.35	0.82	1.03	0.48	0.33
5	7.25	0.61	0.42	3.39	0.55	1.74	-	2.54	1.48	2.08	0.79	1.01	0.48	0.32
6	7.62	0.56	0.4	3.45	0.5	1.19	-	2.5	1.24	1.86	0.84	1.05	0.49	0.36
7	7.38	0.53	0.33	3.34	0.4	1.23	-	2.1	1.18	2.02	0.83	1.03	0.48	0.34
8	7.18	0.49	0.28	3.37	0.1	1.14	-	1.8	1.15	1.95	0.84	1.06	0.5	0.36

Discussion: Soil fertility and plant nutrition are important components in crop production. In addition to providing basic physical support for plants, productive, fertile soils also supply moisture and air to the roots and act as a reservoir for available plant nutrients. In the present study, different changes in the growth and proliferation of the microbial counts in wheat rhizosphere during all stages of plant growth was determined. The present data showed also that total microbial counts as well as *A. chroococcum*, actinomycete, *fungi and Bacillus* population increased over a relatively long period of time during plant growth reaching the maximum values at second sampling period (Heading stage) then slightly decreased at the harvesting. This may be due to the shortage of biological nitrogen during the maturing stage of plant growth. In this respect similar conclusion were recorded by Nelson^[66], Ishac *et al.*^[67], Visser and Dennis^[68] and Kumar *et al.*^[69]. On the other hand addition of selected effective microorganisms to soil significantly stimulated the population densities of all microbial counts and more prominently by treatment with mixture of the five biofertilizer agents as seed, soil and foliar. The mechanism used by microbes to stimulate plant growth include biofertilization (increasing the supply of mineral nutrients to the plant).

Biological control (elimination of the plant enemies including microbial pathogens, and insects) and direct plant growth promotion (e.g., by delivering plant growth hormones to plants)^[70].

Plant growth yield parameter (shoot length, root length, fresh and dry weights, total leaf area, chlorophyll content, number of leaves, number of tillers, spicks features were determined. In addition to the percent of nitrogen as affected by inoculation with selected effective microorganisms as a mixture were also determined. All these parameters were evaluated at different stages of wheat growth i.e., germination, heading and harvesting stages. The highest significant increase by amending soil with organic matter, half dose of mineral fertilizers and inoculation with selected microbes as seed+soil+foliar. From the present results, it has been found that seed bacterization with selected effective microorganisms, gave higher values. However, the percentage increase over uninoculated treatment for the previous parameters of plant growth were higher at soil > seed > foliar due to the response of wheat plant to biofertilization grown in the low fertile sandy soil. Many investigators showed that, symbiotic nitrogen-fixing bacterium (*Azotobacter chroococcum*) were used to increase the nitrogen content of wheat straw. A 13% increase in N content occurred following bacterial

inoculation. Nitrogen addition to the residual straw was 8.35-8.55 mg N/g of straw consumed. Seed inoculation with *A. chroococcum* at 1.5 kg/ha increased the yields from 1.53 to 1.71 t in 1979-80 and from 1.72 to 1.81 t in 1980-81. Applied N and/or seed inoculation increased the plant N content, grain protein content and soil N content at harvest^[71].

Pati *et al.*^[72] and Rabie *et al.*^[73] reported that, wheat inoculation with diazotrophs (*Azotobacter chroococcum*) increased germination, seedling growth, root growth, shoot length and crop yield. they elucidated that, inoculation with *Azotobacter chroococcum* increased plant height, shoot and root DW, total number of tillers, number of productive tillers, number and weight of speckles, grain yield/plant and grain P content.

The stimulating effect observed in this study due to inoculation with selected effective microorganisms on the wheat plants crop and microbial communities and activities in the rhizosphere can be explained by the capability of such microorganisms to produce growth promoting substances and nitrogen fixation which improve the plant growth and grain yield^[74,75,76]. The plant growth promoting ability of biofertilizer agents isolated from rhizosphere has been reported in previous studies^[77,78,79]. The beneficial effects of antagonistic biocontrol microorganisms including *Azotobacter* sp, *Streptomyces* and *Cheatomium* on tomato have been reported^[80,81,82,83].

Several investigators used biofertilizers to improve soil properties to the most convenient ones for the growth of different plants and their rhizospheric microorganisms, and they also indicated that, rhizobacteria can produce plant growth promoting substances^[84,85]. Similar results were obtained by Kundu and Sharma^[86] who stated that, the plant growth hormones and N₂- fixation generally increased by inoculation of wheat and sunflowers with bacteria isolated from the rhizosphere.

Data reported that hydraulic conductivity and bulk density decreases with addition of chicken manure and biofertilizer application to soil, seed and foliar. For hydraulic conductivity, this trend of decreasing soil (K) may be rendered to several reasons, (1) The soil became under cultivation. (2) formation of small aggregates as a result of organic manure and biofertilizer application. (3) subsequently large pores declined against the increasing of medium, small and very small pores, (4) the root growth through the soil profile and its root hairs combined with soil separates which caused slowing in water flow; (5) the attraction and to some extent the expansion of the organic materials led to hold water and reduce its movement into the soil, and(6) the migration of fine sand from the soil surface to underneath can be contributed in

partial blocking of drainable pores. These concomitant with those obtained by El-Dawwey^[87], El-Sersawy^[88], Abd El-Hamid *et al.*^[89] Khalil *et al.*^[90] and Mohamed and Awad^[91] where they concluded that increasing of cultivation period caused a marked decrease in soil hydraulic conductivity. In addition, by using different soil amendments such as FYM sludge, sheep dung, bentonite and town refuse, the K of light textured soil decreased.

Soil bulk density significantly decreased with addition of organic matter, mineral fertilizers and selected effective microorganisms this can be attributed to the low specific gravity of organic materials and the role of organic products in enhancing soil aggregation which increase the apparent soil volume and consequently decrease bulk density this results in agreement with many workers^[92,93]. The general improve in plant growth include healthy and active root system which imply an efficient sole in improving the physical properties of soil as well both root and microbial exudates which contains several organic compounds accumulated with those resulted from organic manure decomposition play together an enhancement role of physical properties especially with sandy soil such as the soil of experimental site. The main target of bioorganic farming technique is to improve the whole soil properties. Relatively, the improving of biological properties (like microbial counts) is easier than chemical, while both are easier compared with physical properties. So that, improving of physical properties of soil include automatically the improvement of biological and chemical properties as well. In these work, physical properties of the experimental soil was measured five times before cultivation and after each season of cultivation for both Peanut and Wheat crops.

For soil aggregation, the product of organic matter decomposition during growth season, microbial gums and root growth promoting substances enhanced soil aggregation process, subsequently soil penetrability resistance decrease. The net result was less cohesion relation to adhesion forces between soil particles.

Abd El-Ghany *et al.*^[94] studied the effect of composted garbage, dry sludge and sheep wastes on some physical properties of the sandy calcareous soil and found that manuring of Wadi Sudr soil south Sinai Egypt produced favorable conditions for the formation of dry stable aggregates. They found that additions of theses wastes increased the dry stable aggregates and their effectiveness can be arranged as follows: composted garbage=sheep wastes > sludge man.

Application of a mixed culture of selected effective microorganisms active in fixing N₂, producing plant growth promoting substances, antimicrobial activity and enzyme p roduction resistant to adverse conditions is

prevailing in desertic soil environments. So, we recommend using a mixture of selected effective microorganisms active in nitrogen fixation, hormonal production, phosphate solubilization, antibiotic production and enzyme production in cultivation of plants under desert soil conditions. Artificial inoculation with selected effective microorganisms cause enhancing effect for agriculture production process improving soil properties, increasing soil fertility and reducing environmental pollution.

REFERENCES

1. Tawfik, K.P., A. Amany, Bahr and A.K.M. Salem, 2006. Response of Kaller grass (*Leptochloa fuscata* L.) to biofertilizer inoculation under different levels of sea water irrigation. *J. Appl. Sci. Res.*, 2(12): 1023-1211.
2. Wu, S.C., Z.H. Cao, z.g. Li, K.C. Cheung and M.H. Wang, 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma*, 125(1-2): 155.
3. Hegazi, N.A., M. Fayez, G. Amin, M.A. Hamza, M. Abbas, H.I. Youssef, M. Monib, K.A. Malik, 1998. Diazotrophs associated with non-legumes grown in sandy soils. Nitrogen fixation with non-legumes. Proceedings of the 7th International Symposium on Nitrogen Fixation with Non-legumes, Faisalabad, Pakistan, 16-21. October, 209-222.
4. Amer, M.M., M.A. Swelim, F. Bouthaina AbdEl-Ghany and Amal M. Omar, 2002. Effect of N₂ fixing bacteria and actinomycetes as biofertilizers on growth and yield of cucumbers in sandy soil in Egypt. *Egyptian J. of Desert Research*, 52(1): 113-126.
5. Arafa, Rawhia A.M. AbdEl-Ghany, F. Bouthaina Sidkey, M. Nagwa and ElShazly, M. Mona, 2009. The Beneficial Use of Biofertilizers on Growth and yield of Wheat Plants Grown on Sandy Soil with or Without Nitrogen fertilization. *Egyptian J. of biotechnology*, 32: 127-146.
6. Dickson, E.L., V. Rasiah and P.H. Groenevelt, 1990. Comparison of four prewetting techniques in wet aggregates stability determination. *Can. J. Soil Sci.*, 71: 67-72.
7. Diné, H., P.E.M. Levsque, P. Jambu and D. Righi, 1992. Microbial activity and long chain aliphatics in the formation of stable aggregates. *Soil Sci. Soc. Am. J.*, 56: 1455-1463.
8. Chaney, K. and R.S. Swift, 1984. The influence of organic matter on aggregate stability of some British soils. *J. Soil Sci.*, 35: 223-230.
9. Chenu, C., 1993. Clay or sand polysaccharide associations as models for the interface between micro-organisms and soil: water-related properties and microstructure. *Geoderma*, 56: 143-156.
10. Cheshire, K.V., 1979. Nature and origin of carbohydrates in soil. Academic Press, Inc., New York, N.Y.
11. Flustondo, J., D.A. Angers, M.R. Laverdiere and A.N. Dayegamiye, 1990. Etude comparative de l'aggregation et de la matiere organique associee aux fraction granulometriques de sept sols sous culture de maïs en prairie. *Can. J. Soil Sci.*, 70: 395-402.
12. Ried, J.B. and M.J. Goss, 1982. Effect of living roots of different plant species on the aggregates stability of two arable soils. *J. Soil. Sci.*, 32: 521-541.
13. Haynes, R.J. and R.S. Swift, 1990. Stability of soil aggregates in relation to organic constituents and soil water content. *J. Soil Sci.*, 41: 73-83.
14. Watt, M., M.E. McCully and C.E. Jeffre, 1993. Plant and bacterial mucilages of the maize rhizosphere: comparison of their soil binding properties and histochemistry in a model system. *Plant Soil*, 151: 151-165.
15. Angers, D.A. and Mehuys, G.R. 1989. Effects of cropping on carbohydrate content and water stable aggregation of a clay soil. *Can. J. Soil. Sci.*, 69: 373-380.
16. Lynch, J.M. and E. Bragg, 1985. Microorganisms and soil aggregates stability. *Adv. Soil Sci.*, 2: 133-171.
17. Martens, D.A. and W.R.T. Frankenberger, Jr. 1993. Soil saccharide extraction and detection. *Plant Soil*, 149: 145-147.
18. Andrade, G., K.L.Mihara, R.G. Linderman and G.J.Bethlenfalvay, 1998. Soil aggregation status and rhizobacteria in the mycorrhizosphere. *Plant and Soil*, 202(2): 89-96.
19. Tisdall, J.M. and J.M. Oades, 1980. The effects of crop rotation on aggregation in a red-brown earth. *Aust. J. Soil Res.*, 18: 423-434.
20. Amellal, N., G. Burtin, F. Bartoli and T. Heulin, 1998. Colonization of Wheat Roots by an Exopolysaccharide-Producing *Pantoea agglomerans* Strain and Its Effect on Rhizosphere Soil Aggregation Applied and Environmental Microbiology, 64: 3740-3747.
21. Oades, J.M. and A.G. Waters, 1991. Aggregate hierarchy in soils. *Aust. J. Soil Res.*, 29: 815-828.
22. Tespara, R., 1990. Formation of new aggregates and weed seed behaviour in a coarse and in a fine-textured loam soil: a laboratory experiments. *Soil Tillage Res.*, 15: 285-296.
23. Ahmed, A.T., K.W. Khalil, B.F.A. El-Ghany and Awadalla, S.Y. 1996. Ameliorating the properties of virgin sandy soil and its impact on wheat production. *Desert Institute Bulletin, Egypt, publ.*, 46(2): 319-339.

24. Amara, M.A.T. and M.S.A. Dahdoh, 1997. Effect of inoculation with plant growth promoting rhizobacteria (PGPR) on yield and up take of nutrients by wheat grown. Egyptian of Soil science, 37(4): 467-484.
25. Khamis, M.A. and S.M. Metwally, 1998. Utilization of organic materials inoculated with microbial decomposers as a nitrogen source for wheat in presence of *Azotobacter* in a sandy soil. Egypt. J. Soil. Sci., 38(1-4): 81.
26. Khosravi, H., N.S. Rastin and M. Mohammadi, 1998. Effect of *Azotobacter* inoculation as a biological fertilizer on growth and yield of wheat. Soil and Water Journal, 12: 6(1-8): 111.
27. Yadav, K.S., D.P. Singh, S. Sunita, N. Neeru, K. Lakshminarayana, S. Suneja and N. Narula, 2000. Effect of *Azotobacter chroococcum* on yield and nitrogen economy in wheat (*Triticum aestivum*) under field conditions. Environment-and-Ecology, 18(1): 109-113.
28. Egamberdiyeva, D.D., S. Juraeve, O. Poberejskaya, P.T. Myachina, L. Eryuhova, Seydaliyeva and A. Aliev, 2000. Improvement of wheat and cotton growth and nutrient uptake by phosphate solubilizing bacteria. 26th Southern Conversation Tillage Conference.
29. Neeru Narula, Vivek Kumar, Rishi K. Behl, Annette Deubel, Andreas Gransee and Wolfgang Merbach 2000. Effect of P-solubilizing *Azotobacter chroococcum* on N, P, K uptake in P-responsive wheat genotypes grown under greenhouse conditions. Journal of Plant Nutrition and Soil Science, 163: 393-398.
30. Kumar, V., R.K. Behl and N. Narula, 2001. Effect of phosphate-solubilizing strains of *Azotobacter chroococcum* on yield traits and their survival in the rhizosphere of wheat genotypes under field conditions. Acta Agronomica Hungarica, 49(2): 141-149.
31. Bandel, A.V. and J.J. Meisinger, 2002. Basic Principles of Soil Fertility II: Soil Properties Agriculture and Natural Resources University of Mary Land.
32. Ali, A.A.G. and S.A.E. Mowafy, 2003. Effect of different levels of Potassium and Phosphorus Fertilizers with the foliar Application of Zinc and Boron on Peanut in Sandy Soils. Zagazig J. Agric. Res., 30(2): 335-358.
33. Rai, S.N. and A.C.Gaur, 2005. Characterization of *Azotobacter* spp. and effect of *Azotobacter* and *Azospirillum* as inoculant on the yield and N-uptake of wheat crop plant and soil journal, 109(1): 131-134.
34. El-Sirafy, Z.M., H.J. Woodard and E.M. El-Norjar, 2006. Contribution of Biofertilizers and Fertilizer Nitrogen to Nutrient Uptake and Yield of Egyptian Winter Wheat. Journal of Plant Nutrition, 29(4): 587.
35. Gomaa, A.M., T. El-Mesiry and M. Rady, 2008. The Potential Impact of Biofertilization, Antioxidant and Micronutrients Application in Reducing Salinity Stress on Two Wheat Cultivars Australian Journal of Basic and Applied Sciences, 2(3): 575-582.
36. Abdel-Malek, Y. and Y.Z. Ishac, 1968. Evaluation of methods used in counting *Azotobacter*. J. Appl. Bacteriol., 31: 26.
37. Abdel-Hafez, A.M., 1966. Some studies on acid producing microorganisms in soil and rhizosphere with special reference to phosphate solubilizers. Ph. D. Thesis, Fac. Agric., Ain Shams Univ. Cairo, Egypt.
38. Waksman, S.A. and H.A. Lechevalier, 1962. The Actinomycetes Vol. III. Antibiotics of Actinomycetes, Baltimore, the Williams and Wilkins Company USA. 430.
39. King, E.O., M.K. Ward and O.E. Raney, 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med., 44: 301-307.
40. Oxoid, 1982. Oxoid manual of culture media ingredient and other laboratory surface, Turner graphic Ltd. England. Fifth Ed.
41. Shirling, E.B. and D. Gottlieb, 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol., 16: 313-340.
42. Pridham, A.P., C. Foley, L.A. Lindenfelser, C.W. Hesseltine and R.G. Benedict, 1956. A selection of media for maintenance and taxonomic study of *Streptomyces*. Annual (1956-1957). Medical Encyclopedia. Inc. New York, 947- 953.
43. Jackson, M.L., 1958. Soil chemical analysis. Prentice-Hall, Inc., Engl. and Cliffs, 219-221.
44. Watanabe, F.S. and S.R. Olsen, 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO₃ extracts from soil. Soil Sci. Soc. Am. Proc., 29: 677-678.
45. Irob, O.N., Moo-Young, M. and Anderson, W.A. 1996. Antimicrobial activity of Annato extract. International J. Pharmacog., 34: 87-90.
46. Zimmer, W., K. Roeben and H. Bothe, 1988. An alternative explanation for plant growth promotion by bacteria of the genus *Azospirillum*. Planta, 176: 333-342.
47. Rizzolo, A.C., Baldo and A. Polesello, 1991. Application of high performance liquid chromatography to the analysis of niacin and biotin in Italian almond cultivars. J. of Chromatography, 553(1-2).

48. Ammar, M.S., S. Louboudy and U.M. Abdul-Raouf, 1991a. Distribution, total viable bacteria and identification of the most potent proteolytic bacteria strains isolated from Aswan City. *Az. J. Microbiol.*, 11: 224-238.
49. Barrow, G.I. and R.K.A. Feltham, 1993. *Cowan & Steel's, Manual for the identification of medical bacteria.* Cambridge University press.
50. Cherry, J.H., 1973. *Molecular biology of plants test-Manual*. Columbia Univ. Press, New York and London, 68-71.
51. Hamilton, W.B., 1931. Gum production by *Azotobacter chroococcum* of Beijerinck and its composition. *J. Amer. Chem. Soc.*, 46: 249-254.
52. Krig, N.R. and J.G. Holt, (Eds.) 1986. *The Bergey's manual of systematic bacteriology.* Vol. I and II.
53. *Bergey's Manual of Determinative Bacteriology* 1994. John G. Holt, Noel R. Krieger, Peter H.A. Sneath, James T. Staley T. Williams (Ed.) (Ninth edition) Williams and Wilkins, Baltimore London.
54. *Bergey's Manual of Systemic Bacteriology* 1984. Gram Negative Aerobic Rods and Cocci. Vol. 1, section 4 Krieg N.R. and J.G. Holt (Eds.), Williams and Wilkins, Baltimore, 141-199.
55. Barnett, H.L. and B.B. Hunter, 1972. *Illustrated genera of imperfect fungi.* Burgess. Pub. Co., Minneapolis, Minnesota, USA, pp: 241.
56. Moubasher, A.H., 1993. *Soil fungi in Qatar and other Arab Countries.* Doha university of Qatar, Center for Scientific and applied research.
57. Piper, C.S., 1950. *Soil and plant analysis.* 1st Ed. Int. Publishers. New York, 47-79.
58. Klute, A.A., 1986. "Methods of Soil analysis" 2nd edition American Society of Agronomy Inc., Publish, Madison Wisconsin, USA.
59. Richards, L.A., 1954. *Diagnoses and improvement of saline and alkaline soils.* USA. Dept. Agric. Handbook, 60: 17-133.
60. Chapman, H.D. and P.F. Pratt, 1961. "Methods of analysis for soils, plants and waters". Riverside Univ., California. Div. Agric. Sci., California, USA, pp: 150-152.
61. Troug, E. and A.H. Meyer, 1939. Improvement in deioness colorimetric method for phosphorus and arsenic. *Ind. Eng. Chem. Anal. Ed.*, 1: 136-139.
62. Timinon, M.I., 1940. The interaction of higher plants and soil microorganisms. II. Study of the microbiological population of the rhizosphere in relation to resistance of plant to soil borne diseases. *Can. J. Res. B.* 18: 144-456.
63. Cochran, W.G., 1950. Estimation of bacterial densities by means of the "Most Probable Number". *Biometrics*, 6: 105-115.
64. A.T.C.C., 1984. *American type culture collection.* 13 Ed., Parklawn Drive Rockville Maryland, USA, 433-477.
65. Black, C.A., D.D. Evans, L.E. Ensminger, J.L. White and F.E. Clarck, 1965. *Methods of soil analysis part 1.* chap. 18. Water capacity by Klute A, ASA Monograph series No. 9, Amer. Soc. Agron. Madison, Wisconsin, USA.
66. Nelson, L.M., 1983. Variation in ability of *Rhizobium leguminosarum* isolates to fix dinitrogen symbiotically in the presence of ammonium nitrate. *Can. J. Microbiol.*, 29(12): 1626-1633.
67. Ishac, Y.Z., El-Haddad M.A. El-Borollosy, M.I. Mostafa, 1983. Effect of organic amendments on nitrogen fixation by asymbiotic bacteria associated with wheat and maize plants. 5th Int. Symp. On N₂-Fixation Noordwijkerhout, Netherlands, August 28- September 3.
68. Visser, S. and P. Dennis, 1992. Soil biological criteria as indications of soil quantity : Soil microorganisms. *American J. of Alternative Agriculture*, 7(1 and 2): 33-37.
69. Kumar, V., N.K. Aggarwal and B.P. Singh, 2000. Performance and persistence of phosphate solubilizing *Azotobacter chroococcum* in wheat rhizosphere. *Folia Microbiologica*, 45(4): 343-347.
70. El-Shazly, M.M., 2003. Studies on some factors affecting productivity of growth regulators by *Azotobacter* in desert soil. M.Sc. Thesis, Fac. Sci., Al-Azhar Univ., Cairo, Egypt.
71. Kulkarni, S.M. and R. Nagaraj, 1988. Microbial increase of nitrogen in wheat straw. *Biological Wastes*, 25(2): 145-147.
72. Pati, B.R., S. Sengupta and A.K. Chandra, 1995. Impact of selected phyllospheric diazotrophs on the growth of wheat seedlings and assay of the growth substances produced by the diazotrophs. *Microbiological Research*, 150(2): 121-127.
73. Rabie, K.A.E., S.A. Nasr and M.A. Amara, 1995. The effect of symbiotic nitrogen fixers on the growth and endogenous growth substances of wheat plants. *Annals of Agricultural Science .Cairo*, 40(1): 11-32.
74. Harber, S.H.T. and J.M. Lynch, 1979. Effect of *Azotobacter chroococcum* on barley seed germination and seedling development. *J. Gen. Microbiol.*, 112: 45-51.
75. El-Haddad, S.A., 1986. Studies on *Phytophthora* sp. of tomato plants. M.Sc. Thesis, Faculty of Agric., Cairo Univ.
76. Ishac, Y.Z., M.A. Ahmed, F. Aboul-Abbas and R.S. Abd El-Aziz, 1989. Interaction between VA-micorrhizae, *Fusarium solani* sp. phaseoli and/or soybean mosaic virus (SMV). Proc. 5th Int. Symp. on Microbiol Ecology, Kyoto, Japan, August 27-September 1.

77. Kloepper, J.W., G.W. Zehnder, S. Tuzun, J.F. Murphy, G. Wei, C. Yao and G.S. Raupach, 1996. Toward agriculture implementation of PGPR-mediated induced systemic resistance against crop pests. Pages 165-174 in: *Advances in Biological Control of Plant Diseases*. T. Wenhua, R.J. Cook, and A. Rovira. Eds. China Agriculture University Press, Beijing.
78. McCullagh, M., R. Utkehde, J.G. Menzies, Z.K. Punja and T.C. Paulitz, 1996. Evaluation of plant growth-promoting rhizobacteria for biological control of pythium root rot of cucumber grown in rockwool and effects on yield. *Eur. J. Plant Pathol.*, 102: 747-755.
79. Raupach, G.S. and J.W. Kloepper, 2000. Biocontrol of Cucumber Disease in the Field by Plant Growth-Promoting Rhizobacteria With and Without Methyl Bromide Fumigation. *The American Phytopathological Journal*, 88: 1164-1185.
80. Gupta, S., D.K. Arora and A.K. Srivastava, 1995. Growth promotion of tomato plants by rhizobacteria and imposition of energy stress on *Rhizoctonia solani*. *Soil Biol. Biochem.*, 27(8): 1051-1058.
81. El-Abyad, M.S., M.A. El-Sayed, A.R. El-Shanshoury and S.M. El-Sabbagh, 1996. Antimicrobial activities of *Streptomyces pulcher*, *S. canescens* and *S. citreofluorescens* against fungal and bacterial pathogens of tomato *in vitro*. *Folia Microbiologica.*, 41(4): 321-328.
82. Larkin, R.P. and D.R. Fravel, 1998. Efficacy of various fungal and bacterial biocontrol organisms for control of *Fusarium* wilt of tomato. *Plant Disease*, 82(9): 1022-1028.
83. Monaco, C.I., A.I. Nico, M. Rollan and M. Urrutia, 2001. *In vitro* effect of fungicides used for controlling tomato early blight on mycoflora-antagonists to phylloplane. *Investigation Agraria, Production & Protection Vegetables*, 16(3): 325-332.
84. Kloepper, J.W. and M.N. Schroth, 1981. Relationship of *in vitro* antibiosis of plant growth promoting rhizobacteria to plant growth and the displacement of root microflora. *Phytopathology*, 7: 1020-1024.
85. Hassouna, M.G., M.A. Madkour and T. Mahmoud, 1990. Growth promotion of sorghum by the N₂-fixers in Egyptian poorlands of the northwestern coast. 2nd Int. Workshop on PGPR. Interlaken, Switzerland., 68-72.
86. Kundu, B.S. and P.K. Sharma, 1994. Plant response to phytohormones release by diazotrophs. *Environment and Ecology*, 12(4): 749-751.
87. El-Dawwey, G.M., 1993. Evaluation the changes in Physical and chemical properties of newly reclaimed soil throughout years of cultivation. *Assiut. J. Agric., Sci.*, 24: 155-168.
88. El-Sersawy, M.M., F. Bouthaina, Abd El-Ghany and M.S. Barsoum, 1993. Effects of organic fertilization and integration on soil physical, microbiological properties and Sunflower production in the North Western Coastal Zone of Egypt. *Desert Ins. Bull. Egypt*, 43(2): 73.
89. Abdel-Hamid, W.M., A.O. El-Hady, A.S. Hammad and M.T.A. Kotlo, 1995. Effect of incorporating organic manure and/or hydrogels in sandy soil structurization and stabilization. *Egyptian Soil Sci. Soc. (ESSSJ) 5th Nat. Congress. Nov.*, 20-21, Cairo, Egypt.
90. Khalil, K.W., M.M. El Sersawy, F. Bouthaina, Abd El-Ghany and F.A. Hashem, 1991. Profitability of using some organic wastes with P. fertilization on wheat production under saline irrigation water and Wadi Sudr conditions. *Egypt. J. Appl. Sci.*, 6(7): 267- 284.
91. Mohammed, S.A. and Y.H. Awad, 1998. Evaluation of the physical and chemical properties of soil with application of wheat straw pulp waste water sludge. *Arab univ. J. of Agric., Sci.*, 6: 597-605.
92. Metzger, L. and Yaron, 1987. Influence of Sludge organic matter on soil physical properties. *Adv. Soil. Sci.*, 7: 141-163.
93. Hall, J.E. and E.G. Coker, 1983. Some effects of Sewage Sludge on soil physical conditions and plant growth in the influence of Sewage sludge application on physical and biological properties of soils (G. Catrou, P.L. Hermit, 6 Guess and E. Susseds., D. Reidel Publishing company. Dardrecht. Holland, 43-60.
94. Abdel-Ghany, F., Bouthaina K.W. Khalil, M.M. El-Sersawy, S.Y.A.D. Awadalla, 1979. Improvement of Wadi Sudr soil properties using modern bio-organic techniques and their effects on desertification combat and barley production. *Desert-Intitute-Bulletin- Egypt*, 47(1): 69-100.