

Effect of Biofertilization and Silicon Foliar Application on Productivity of Sunflower (*Helianthus annuus* L.) under New Valley Conditions

A.M. Abd El-Gwad* and E.M.M. Salem**

*Soil Fertility and Microbiology Department and **Agronomy Unit, Plant Production Department, Desert Research Center, Cairo, Egypt.

TWO field experiments were carried out at Desert Research Center (D.R.C.), Agricultural Experimental Station at EL-Kharga, New Valley Governorate, during the two summer growing seasons of 2010 and 2011, these experiments aimed to study the effect of biofertilization (*Azotobacter chroococcum*, *Bacillus megatherium* (PDB) and mixture of two isolates) and silicon spraying rates (200, 400, 600,800 and 1000 mg/L.) against control on the productivity of Sunflower (*Helianthus annuus* L.) by using cultivar Sakha 53. The experiments were laid out in a split plot design with four replicates. Foliar application of silicon treatments were arranged in the main plots and biofertilization treatments in the sub-plots.

Results showed that both spraying silicon and biofertilization treatments had enhancement effect on plant height, number of leaves, leaves surface area, fresh and dry weight of leaves/plant and stem diameter, also head diameter, seeds number/head and 100-seed weight as well as seed and straw yields. Moreover, seed oil percentage and oil yield. The enhancement effect of all abovementioned traits with inoculation of *Azotobacter chroococcum*, PDB individual or mixed compared with the control treatment (without biofertilization). Also, remarkable influence of the interaction between silicon foliar application and biofertilization treatments on all yield and yield components. Results also indicated significant microbial activity in rhizosphere soil expressed by total microbial counts, CO₂ evolution, *Azotobacter* and Phosphate dissolving bacteria counts and Enzymatic activities (Dehydrogenase, Nitrogenase and Phosphatase) exhibited positive response in all treatments compared to uninoculated control.

Keywords: Sunflower, Silicon, Biofertilization, *Azotobacter chroococcum*, *Bacillus megatherium*, Yield and its components and Oil yield.

New Valley governorate, is one of the most promising newly reclaimed lands in Egypt, one and represents large land resources for agriculture expansion. Weather in this region is hot and dry, and cultivation depends mainly on ground water. So agriculture expansion in this region needs application of special practices for the best use of land and water resources.

Sunflower (*Helianthus annuus*, L.) considered one of the major sources of edible vegetable oil in the world and also in Egypt due to its high unsaturated fatty acids content (Leland, 1996). So, there is need to increase the oil yield to enhance food security.

Nitrogen deficiency in Egyptian soils is one of the most limiting factors for Sunflower production. Therefore fertilizer application either organic or inorganic becomes a major practice towards yield increase. Phosphorus (P) has similar importance for the growth of Sunflower, its deficiency results in stunted growth, purplish discoloration of leaves. It also affects flowering, fruit formation and seed production (Aduayi *et al.*, 2002). Uptake of major nutrients elements by sunflower has also been reported to be facilitated on P was application (Fagbayide and Adeoye, 1999). Several investigators showed the effect of mineral and organic fertilizers application on sunflower as, Abou khadrah *et al.* (2002), Mohamed (2003), Awad (2004), Mohamed and Ayman (2009).

The biofertilizers considered one of the sources for supplying nutrients for the crops and conserve the environment from pollution by excessive use of mineral N fertilizer. The beneficial effect of biofertilizers, viz. *Azospirillum* and *Azotobacter* inoculation on sunflower has been reported by several investigators. Saleh *et al.* (2004) studied the response of some sunflower cultivars to Rhizobacterien as biofertilizers as comparing with mineral nitrogen, they reported that all studied characters were significantly increased by increasing nitrogen levels up to 30 kg N /fed or inoculation of sunflower seed with Rhizobacterien plus application of 20 kg N/fed. Mohamed (2003) and Abou khadrah *et al.* (2002) revealed that the inoculation of sunflower seed with (N_2 -fixing) bacteria (Cerealin) or with phosphate dissolving bacteria (Phosphorine) or with combined of the two biofertilizers significantly enhanced all the studied traits over the control (dry matter accumulation/plant in some growth stages, head diameter, number of seeds/head, seed oil content, seed yield/plant as well as seed and oil yields/fed). Nawar (1994) and Radwan (1996) reported that inoculation of sunflower seed with phosphate dissolving bacteria (phosphorine) significantly increased number and weight of seeds/head and head diameter in addition to growth attributes.

The results obtained by Keshta and El-Kholy (1999) indicated that application of inorganic nitrogen and biofertilizers as a source of N_2 fixing bacteria for sunflower increased plant height, head diameter, 100- seed weight, seed yield/fed and seed oil content.

Some free living microorganisms in soil have capability to produce extracellular enzymes such as phosphatase (George *et al.*, 2002), this enzyme able to mineralize organic phosphates into inorganic phosphates that provides high P for plant. Soil phosphatases play a major role in the mineralization processes (dephosphorilation) of organic P substrates. The use of phosphate solubilizing bacteria as inoculants simultaneously increases P uptake by the plant and crop yield.

Silicon is required as a nutrient for normal growth in wetland species of the families Gramineae, Equisetaceae and some Cyperaceae but in dicotyledons and other grasses, its role remains elusive (Chen and Lewin, 1968, Kaufman *et al.*, 1985, Takahashi *et al.*, 1990, Lewin and Reimann, 1969 and Fawe *et al.*, 1998). There are several hypotheses concerning the role of Si in dicots and nonaccumulator grasses including a positive effect on reproduction, alleviation of metal toxicity and nutrient imbalance, provision of structural rigidity and increased resistance to fungal diseases such as powdery mildews and root rots (Epstein, 1994 and Belanger *et al.*, 1995). Recent work contended that Si may act by stimulating the natural defense mechanisms of the plant (Belanger *et al.*, 1995).

It has been reported that adding silicon to monocots, especially Gramineae plants, not only promotes growth and development but also promotes photosynthesis, reduces pest infection, maintains the shoot in an erect position and alleviates salt stress (Ahmad *et al.*, 1992, Epstein, 1999, Korndorfer and Lepsch, 2001 and Ma, 2004). It is found in the soil in the form of silicic acid (Chen *et al.*, 2010) and all plant species take it in the form of silicic acid (Ma *et al.*, 2001). Different studies indicated positive effect of silicon application on the plant growth and development including enhanced pollination, increase dry biomass and final yield (Korndorfer and Lepsch, 2001) and resistance against various diseases (Gillman *et al.*, 2003). Application of Si could alleviate the oxidative stress of wheat and regulate activities of antioxidant enzymes, which contributed to improvement of growth of plants under drought (Gong *et al.*, 2008).

Silicon considered to be important element under stress because it increased drought tolerance in plants by maintaining leaf water potential, assimilation of CO₂ and reduction in transpiration rates by adjusting plant leaf area (Hattori *et al.*, 2005). Maintenance of higher leaf water potential under stress is one of remarkable feature which silicon nutrition does for plants as reported by Lux *et al.* (2002). Silicon was reported to enhance growth of many plants particularly under biotic and abiotic stresses (Epstein, 1999). A number of possible mechanisms have been proposed by which Si would increase resistance of plants against salinity stress which is a major yield limiting factor in arid and semiarid areas. (Al-Aghabary *et al.*, 2004).

The objective of this investigation was to study the effect of the application of silicon foliar and biofertilization on Sunflower production under New Valley conditions.

Material and Methods

Two field experiments were conducted at the Agriculture Experimental station at El-Kharga Oasis (30.53 longitude, 24.45 latitude and elevation 78.8), New Valley Governorate, Desert Research Center (DRC), Egypt during the two summer growing seasons of 2010 and 2011 to study the effect of biofertilization (*Azotobacter chroococcum* and *Bacillus megatherium*) and silicon foliar

application on productivity of Sunflower. Representative soil samples were taken from the experimental sites at depth from 0 to 30 cm from soil surface and were prepared for both mechanical and chemical analysis.

The experiment was laid out in a split plot design with four replicates. Foliar application of silicon treatments were allocated the main plots and biofertilization treatments occupied the sub-plots. Each plot was 12 m² contained five ridges (4 m length and 60 cm width with hills 20 cm apart).

Each experiment included twenty four treatments which were the combinations of six silicon spraying rates (control, 200, 400, 600, 800 and 1000 mg/L) and four biofertilization treatments (control, *Azotobacter chroococcum*, *Bacillus megatherium* (PDB) and mixture of the two isolates).

Seeds of sunflower (*Helianthus annuus*, L.) cultivar Sakha 53 were sown on May 22 and 26 in the first and second seasons, respectively. Also, sheep manure of 20 m³/feddan as organic manure containing O.C 25%, N 2.17%, C/N ratio 11.52 and O.M 43 % . Calcium super phosphate (15.5% P₂O₅) was applied at the rate of 31 kg P₂O₅/feddan during land preparation, before sowing, N and K fertilizers were added at the rate of 60 Kg N/fed as (NH₄)₂SO₄ and 75 Kg K₂O/fed as K₂SO₄ with three equal doses.

Bacterial culture preparation

The systematic biotechnology was used taking fresh liquid cultures 48 hr old from pure local strains of *A. chroococcum* and *B. megatherium* var. *phosphaticum*, previously isolated from the rhizosphere soils of New Valley Governorate, purified and identified according to Bergey's Manual (1984) as biofertilizers in the form of single or mixed inoculations at the rate of ~10⁸ cfu/ml.

Application methods

Bacterial strains were applied separately or in combination as soil drench. Sunflower seeds were soaked in a single or mixture of bacterial isolates suspensions (10⁸cfu/ml) for 3 hr before planting (carboxyl methyl cellulose 0.5% was used as an adhesive agent). Seeds of the control plots were soaked in water only. An additional dose was applied twenty one days later once again to soil.

Silicon application

Silicon was applied as a foliar spray at a concentration of 200, 400, 600, 800 and 1000 mg/L at 40 days from planting. Knapsack sprayer with water volume of 300 L /fed were used.

Soil was directly irrigated after planting to provide suitable moisture for the inoculants. Thinning practices were conducted 21 days after planting to secure one plant per hill. Other practices for growing sunflower were conducted as recommended.

The physical and chemical analysis of soil and irrigation water were presented in Tables 1 and 2.

TABLE 1. Some physical and chemical properties of the experimental soil.

| Mechanical analysis | | | | | | | | | | |
|-----------------------|-------------|------------|------------------|------------------|----------------|-----------------|-------------------------------|--------------------------------|-----------------|-------------------------------|
| Sand | | | Clay | | | Silt | | Soil Texture | | |
| 50.2% | | | 31.5% | | | 18.3% | | Sandy clay loam | | |
| Chemical analysis | | | | | | | | | | |
| pH | EC dS/cm | T.N ppm | Cations (meq/L) | | | | Anions (meq/L) | | | |
| | | | Ca ⁺² | Mg ⁺² | K ⁺ | Na ⁺ | CO ₃ ⁻² | HCO ₃ ⁻³ | Cl ⁻ | SO ₄ ⁻² |
| 8.32 | 4.35 | 100 | 9.1 | 2.45 | 1.98 | 29.11 | 0.00 | 8 | 26.6 | 8.04 |
| Trace elements (mg/l) | | | | | | | | | | |
| Zn | | Mn | | Cu | | Fe | | B | | |
| 5.93 | | 4.04 | | 1.82 | | 33.1 | | 0.58 | | |

TABLE 2. Chemical analysis of irrigation water.

| pH | E.C D S/cm | Soluble ions (ppm) | | | | | | | |
|-----|------------------|--------------------|------------------|-----------------|----------------|-------------------------------|-------------------------------|-----------------|-------------------------------|
| | | Cations | | | | Anions | | | |
| | | Ca ⁺² | Mg ⁺² | Na ⁺ | K ⁺ | CO ₃ ⁻² | HCO ₃ ⁻ | Cl ⁻ | SO ₄ ⁻² |
| 7.4 | 730 | 19.61 | 11.91 | 88 | 22 | - | 230.15 | 74.2 | 29.38 |

Assessments

A. Growth traits

After 60 days from sowing, five guarded plants were chosen randomly from each experimental unit of four replicates to estimate plant height, number of leaves/plant, fresh and dry leaves weight /plant, stem diameter and leaf surface area computed as described by Bremner and Taha (1966).

B. Yield and its attributes

Ten guarded plants at harvest were randomly taken from each plot and the following characters were determined: head diameter (cm), number of seeds/head and 100-seeds weight (g). Moreover, all plants of the experimental unit (12 m²) were harvested to evaluate seed and Straw yields.

C. Seed chemical composition

Samples of sunflower seeds were dried at 70°C for 24 hr and seed oil content was determined according to A.O.A.C. (1980) using soxhlet apparatus and diethyl ether as a solvent, then oil yield (kg/fed.) was calculated by multiplying seed yield (kg/fed) by seed oil content.

D. Microbial determinations

Soil samples of sunflower rhizosphere were collected at flowering and harvesting stage of sunflower plant growth and analyzed for total count of microorganisms according to Nautiyal (1999) using the decimal plate method technique. For counting and growing phosphate dissolving bacteria using Bunt and Rovira medium as described by Abd El- Hafez (1966). For *Azotobacter* densities, nitrogen deficient medium was used as described by Abd El-Malek and Ishac (1968). CO₂ evolution according to Anderson (1982).

Soil samples were analyzed for: Dehydrogenase activity according to method described by Casida *et al.* (1964). Nitrogenase activity was measured using a standard acetylene reduction assay as described by Haahtela *et al.* (1981). For determination of phosphatase activity Disodium phenylphosphate served as enzyme substrate (Öhlinger, 1996), alkaline phosphatase activity was measured in reaction mixture treated with borax buffer (PH4.9). The reaction mixtures consisted of 2.5g soil, 2 ml toluene (antiseptic), 10 ml buffer solution and 10 ml 0.5% substrate solution. Reaction mixtures without soil or without substrate solution were the control. All reaction mixtures were incubated at 37⁰C for 2 hr. After incubation, the phenol released from the substrate under the action of phosphatases was determined spectrophotometrically (at 614 nm) based on the colour reaction between phenol and 2,6-dibromoquinone-4-chloroimide. Phosphatase activity is expressed in mg phenol/g soil/2 hr.

Statistical analysis

All the obtained data from each season were exposed to the proper statistical analysis of variance according to Gomez and Gomez (1984). LSD at 0.05 level of significance was used for the comparison between means.

Results and Discussion

1. Growth traits

Growth parameters values during 2010 and 2011 seasons were shown in Table 3. Results indicated that gradual increase in sunflower plant height, number of leaves, leaves surface area, fresh and dry weight of leaves/plant and stem diameter were observed with increasing concentrations of silicon up to 1000mg/l and biofertilization using *Azotobacter chroococcum* as nitrogen fixer and *Bacillus megatherium* as phosphate dissolving bacteria (individual and mixed treatments). Interaction of biofertilization with silicon foliar application resulted in increasing abovementioned growth parameters. The highest increase was recorded with mixed biofertilization treatment and silicon foliar application (1000 mg/L.), where the highest % of increase than control was recorded being 43% and 45% for plant height, 62% and 68% for No of leaves, 23% and 39% for leaf area, 67% and 75% for fresh weight, 116% and 126% for dry weight, 62% and 65% for stem diameter at first and second season, respectively.

TABLE 3. Effect of silicon foliar application and biofertilization on growth traits of sunflower after 60 days from sowing (2010 growing season).

| bio Silicon | Plant height (cm) 1 st season | | | | | Plant height (cm) 2 nd season | | | | |
|---------------------------|---|-------|-------|------|-------|---|-------|-------|-------|---------|
| | Cont. | Azoto | PDB | Mix | Mean | Cont. | Azoto | PDB | Mix | Mean |
| 0 | 140 | 149 | 145 | 158 | 148 | 142 | 153 | 149 | 163 | 151.8 |
| 200 | 143 | 152 | 148 | 165 | 152 | 148 | 156 | 151 | 172 | 156.8 |
| 400 | 151 | 159 | 154 | 171 | 158.8 | 153 | 162 | 159 | 177 | 162.8 |
| 600 | 156 | 162 | 158 | 179 | 163.8 | 159 | 167 | 166 | 184 | 169 |
| 800 | 160 | 169 | 165 | 183 | 169.3 | 162 | 173 | 170 | 195 | 175 |
| 1000 | 165 | 174 | 170 | 188 | 174.3 | 168 | 178 | 173 | 206 | 181.3 |
| Mean | 152.5 | 160.8 | 156.7 | 174 | 161 | 155.3 | 164.8 | 161.3 | 182.8 | 166.1 |
| L.S.D | 1.388 | | | | | 1.647 | | | | |
| Silicon | 1.133 | | | | | 1.345 | | | | |
| 0.05% Bio. interaction | 4.161 | | | | | 4.027 | | | | |
| | No. of leaves /plant 1 st season | | | | | No. of leaves /plant 2 nd season | | | | |
| 0 | 21 | 26 | 23.8 | 27.8 | 24.7 | 21.9 | 29.5 | 25.4 | 29.8 | 26.7 |
| 200 | 22.9 | 26.4 | 24.1 | 28.5 | 25.5 | 24.5 | 28.1 | 25.6 | 30.2 | 27.1 |
| 400 | 24.6 | 26.7 | 25.3 | 29.8 | 26.6 | 26.3 | 28.4 | 26.8 | 31.9 | 28.4 |
| 600 | 25.3 | 27.7 | 26.4 | 31.5 | 27.7 | 26.8 | 29.3 | 28.2 | 33.4 | 29.4 |
| 800 | 26.8 | 28.2 | 27.2 | 33.1 | 28.8 | 27 | 29.7 | 28.8 | 35.8 | 30.3 |
| 1000 | 27 | 29.6 | 28.3 | 34 | 29.7 | 28.6 | 32.4 | 30.6 | 37.4 | 32.3 |
| Mean | 24.6 | 27.4 | 25.9 | 30.8 | 27.2 | 25.9 | 29.6 | 27.6 | 33.1 | 29 |
| L.S.D | 0.408 | | | | | 0.581 | | | | |
| Silicon | 0.333 | | | | | 0.474 | | | | |
| 0.05% Bio. interaction | 2.462 | | | | | 2.034 | | | | |
| | Leaves surface area (cm ²) 1 st season | | | | | Leaves surface area (cm ²) 2 nd season | | | | |
| 0 | 66.5 | 71.5 | 70 | 72.8 | 70.2 | 67 | 72.3 | 72 | 74 | 71.325 |
| 200 | 69 | 72.5 | 72.4 | 76.3 | 72.6 | 69.8 | 73.2 | 73.6 | 77.8 | 73.6 |
| 400 | 69.8 | 75 | 73 | 79 | 74.2 | 70.9 | 75.6 | 74.1 | 81 | 75.4 |
| 600 | 72.3 | 78 | 75.7 | 81.4 | 76.9 | 73.4 | 79.2 | 76.5 | 84 | 78.275 |
| 800 | 73.1 | 79.8 | 77.6 | 85.6 | 79 | 75.2 | 80.4 | 79 | 87 | 80.4 |
| 1000 | 74.6 | 81.1 | 79.2 | 88.4 | 80.8 | 76.8 | 85.7 | 80.6 | 93 | 84.025 |
| Mean | 70.9 | 76.3 | 74.7 | 80.6 | 75.6 | 72.2 | 77.7 | 76 | 82.8 | 77.1708 |
| L.S.D. (%0.05) | 0.533 | | | | | 0.505 | | | | |
| Silicon | 0.435 | | | | | 0.567 | | | | |
| con. Bio. interaction | 0.915 | | | | | 2.404 | | | | |
| | Stem diameter (cm) 1 st season | | | | | Stem diameter (cm) 2 nd season | | | | |
| 0 | 1.42 | 1.69 | 1.6 | 1.73 | 1.61 | 1.54 | 1.85 | 1.74 | 1.94 | 1.7675 |
| 200 | 1.51 | 1.69 | 1.63 | 1.75 | 1.65 | 1.72 | 1.87 | 1.79 | 2.11 | 1.8725 |
| 400 | 1.59 | 1.7 | 1.65 | 1.89 | 1.71 | 1.79 | 1.93 | 1.8 | 2.29 | 1.9525 |
| 600 | 1.62 | 1.73 | 1.69 | 2.08 | 1.78 | 1.85 | 1.97 | 1.84 | 2.45 | 2.0275 |
| 800 | 1.67 | 1.84 | 1.73 | 2.26 | 1.88 | 1.92 | 2.03 | 1.89 | 2.49 | 2.0825 |
| 1000 | 1.75 | 1.9 | 1.78 | 2.3 | 1.93 | 1.98 | 2.25 | 2.01 | 2.54 | 2.195 |
| Mean | 1.6 | 1.8 | 1.68 | 2 | 1.76 | 1.8 | 1.98 | 1.85 | 2.30 | 1.98292 |
| L.S.D. (%0.05) | 0.038 | | | | | 0.035 | | | | |
| Silicon | 0.031 | | | | | 0.029 | | | | |
| con. Bio. interaction | 1.122 | | | | | 0.019 | | | | |

TABLE 3. Contd.

| bio Silicon | Fresh weight of leaves/ plant (g)1 st season | | | | | Fresh weight of leaves/plant (g)2 nd season | | | | |
|--|---|-------|-------|-------|-------|--|-------|-------|-------|-------|
| | Cont. | Azoto | PDB | Mix | Mean | Cont. | Azoto | PDB | Mix | Mean |
| 0 | 104.2 | 109.1 | 107 | 114 | 108.6 | 106 | 111.3 | 110.2 | 116.9 | 111.1 |
| 200 | 106.1 | 112.8 | 109.6 | 117 | 111.4 | 108.1 | 115.3 | 113.8 | 119.7 | 114.2 |
| 400 | 109.1 | 117.6 | 115 | 120.4 | 115.5 | 111.4 | 121 | 117.4 | 123.9 | 118.4 |
| 600 | 111.6 | 123 | 117.8 | 130.8 | 120.8 | 114.3 | 125.5 | 120.1 | 135 | 123.7 |
| 800 | 112.9 | 126 | 122.4 | 148 | 127.3 | 115.8 | 133 | 125.6 | 154 | 132.1 |
| 1000 | 117.3 | 149 | 134 | 174 | 143.6 | 119.5 | 175 | 138 | 181 | 153.4 |
| Mean | 110.2 | 122.9 | 117.6 | 134 | 121.2 | 112.5 | 130.2 | 120.9 | 138.4 | 125.5 |
| L.S.D. (%0.05) Silicon con. Bio. interaction | 1.64 0.858 1.69 | | | | | 0.503 0.411 0.376 | | | | |
| | Dry weight of leaves/ plant (g)1 st season | | | | | Dry weight of leaves/plant (g)2 nd season | | | | |
| 0 | 22.9 | 24.6 | 23.7 | 26.2 | 24.4 | 23.1 | 25.3 | 24.2 | 28 | 25.2 |
| 200 | 23.9 | 26.3 | 24.8 | 28.2 | 25.8 | 25 | 28 | 25.3 | 30 | 27.1 |
| 400 | 25.4 | 29 | 26.8 | 32.7 | 28.5 | 26.1 | 31.2 | 27.6 | 34 | 29.7 |
| 600 | 25.8 | 36.2 | 31.8 | 40.2 | 33.5 | 27.3 | 37.4 | 34.5 | 41.8 | 35.2 |
| 800 | 27.2 | 37.5 | 33.9 | 46.8 | 36.4 | 27.6 | 39.7 | 35.5 | 48.2 | 37.8 |
| 1000 | 29.3 | 40.8 | 38 | 49.6 | 39.4 | 30.8 | 42 | 40.7 | 42.3 | 39 |
| Mean | 25.8 | 32.4 | 29.9 | 37.3 | 31.3 | 26.7 | 33.9 | 31.3 | 37.4 | 32.3 |
| L.S.D. (%0.05) Silicon con. Bio. interaction | 0.46 0.376 0.314 | | | | | 0.34 0.277 0.171 | | | | |

Si conc.: Silicon foliar application.

Bio. : Biofertilization Azoto: *Azotobacter chroococcum*,
PDB: *Bacillus megatherium*.

The stimulatory effects might be attributed to the activation of the growth of microflora including many plant growth stimulators, biological nitrogen fixation and increasing available phosphorous which improve plant growth (Shehata and El-Khawas, 2003). Moreover, among the advantages of using silicon in agriculture are a reduction in water stress, since this element reduces transpiration, an increase in photosynthetic efficiency by maintaining leaves more erect and rigid and with more light interception; and an increase in the resistance to diseases, pests, cold, salinity and toxicity caused by an excess of Al, Mn and Fe. Many of these benefits are attributed to a layer of silicon accumulating beneath the cuticle (Epstein, 1999, Mauad *et al.*, 2003 and Tahir *et al.*, 2006). The highest significant effects on growth parameters were recorded with mixing biofertilization and silicon foliar application (1000 mg/L.) interaction treatments.

The stimulative effect of both biofertilizers used and silicon foliar application on growth parameters of sunflower are in accordance with the results obtained by Mahmoud and Amara (2000), Shaukat *et al.* (2006) and Yasari & Patwarahan (2007).

2. Yield and yield attributes

The data in Table 4 showed that head diameter, weight of 100-seed, seed and straw yields (Kg/fed) were significantly influenced by the biofertilization, silicon foliar application and their interaction treatments.

Yield and yield attributes increased ascendingly with increasing silicon foliar application up to 1000 mg/l. (Fig.1,2) Similar results were obtained by Shengyi *et al.* (1999), Kumbhar and Saavant (1999), Filho *et al.* (2005), Singh *et al.* (2007), Gunes *et al.* (2008) and Muhammad *et al.* (2013).

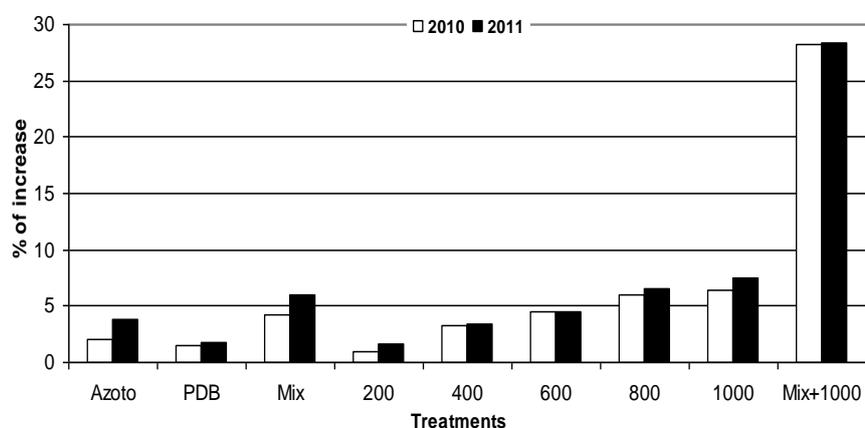


Fig.1. Effect of silicon foliar application and biofertilization on % of increase for sunflower seed yield (2010 and 2011 growing seasons).

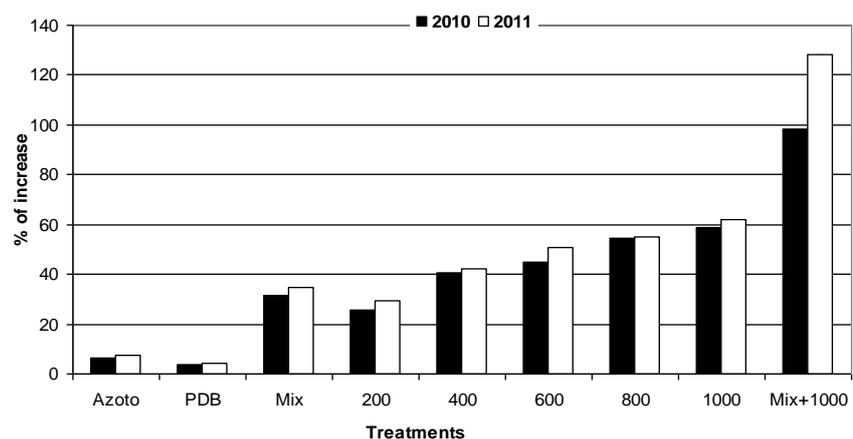


Fig. 2. Effect of silicon foliar application and biofertilization on % of increase for sunflower straw yield (2010 and 2011 growing seasons).

In this respect, biofertilization treatments had significant effects on the studied yield criteria. Mixed biofertilization treatment showed synergistic effect compared with single treatment. It was clearly noticed that, *A.chroococcum* treatment recorded higher values compared with *B. megatherium* treatment. The % of increase than control with *A.chroococcum* treatment was 6% and 7% for seed yield and 2% and 4% for straw yield compared with PDB treatment was 3.8% and 4% for seed yield and 1.5% and 1.8% for straw yield at first and second season respectively. The superiority of *A.chroococcum* may be due to its important role in Sunflower generative growth and therefore a significant increase in 100-seed weight which reflected on seed and straw yields. Kader *et al.* (2000) reported that *A.chroococcum* increase the available nitrogen in the soil which could enhance seed number in plant.

Remarkable influence of the interaction between silicon foliar application levels and biofertilization treatments with all yield and yield components was obtained (Table 4 and Fig.1,2). In this respect, interaction of silicon at 1000 mg/L with mixed biofertilization treatment recorded the highest values being (23.5 and 24.9), (6.39 and 6.56), (983 and 1143) and (2072 and 2164.1) each for head diameter, weight of 100-seed, seed and straw yields (Kg/fed) through 1st and 2nd season respectively. This significant increase in yield and yield components due to biofertilization along with silicon application treatments had synergistic effects on subsequent plant growth and stimulate microbial activities beneficial to plant growth and yield.

The stimulatory effects of biofertilizers on yield and yield components might be attributed to its efficiency in supplying the growing plant with biologically fixed nitrogen, dissolved immobilized phosphorus and produced phytohormones, which could stimulate nutrient absorption as well as photosynthesis process which subsequently increased plant growth and yield. Additionally, these results may be due to silicon generally stimulated leaves surface area, number of leaves/plant and dry matter of sunflower plants (Table 3 and 4) and this in turn increased photosynthetic areas and activity also, dry matter accumulation in seeds which were reflected in yield and yield attributes. The increments in sunflower yield and its components are in agreement with those of Saleh *et al.* (2004), Awad (2004) and Gunes *et al.* (2008).

TABLE 4. Effect of silicon foliar application and biofertilization on growth traits of sunflower after 60 days from sowing (2010 growing season).

| bio Silicon | Head diameter (cm) 1 st season | | | | | Head diameter (cm) 2 nd season | | | | |
|---|---|--------|--------|-------|--|---|--------|--------|--------|--------|
| | Cont. | Azoto | | Cont. | Azoto | | Cont. | Azoto | | Cont. |
| 0 | 12.7 | 13.6 | 13.2 | 15.1 | 13.7 | 12.9 | 14.3 | 13.7 | 16 | 14.2 |
| 200 | 13.2 | 15.8 | 14.9 | 16.2 | 15 | 13.8 | 16.5 | 15.7 | 18.7 | 16.2 |
| 400 | 14.6 | 16.5 | 15.8 | 18.3 | 16.3 | 14.9 | 17.2 | 16.5 | 20 | 17.2 |
| 600 | 14.9 | 17.9 | 17 | 19.6 | 17.4 | 15.5 | 19.4 | 18.2 | 21.2 | 18.6 |
| 800 | 15.3 | 18.9 | 17.6 | 21 | 18.2 | 15.9 | 19.9 | 19.3 | 23.2 | 19.6 |
| 1000 | 15.8 | 20.4 | 19.5 | 23.5 | 19.8 | 16.4 | 22 | 21.7 | 24.9 | 21.3 |
| Mean | 14.4 | 17.2 | 16.3 | 19 | 16.7 | 14.9 | 18.2 | 17.5 | 20.7 | 17.8 |
| L.S.D. (% 0.05) Silicon con. Bio. interaction | 0.305 0.248 2.130 | | | | | 0.246 0.201 2.404 | | | | |
| Weight of 100 seed (g) 1 st season | | | | | Weight of 100 seed 2 nd season | | | | | |
| 0 | 4 | 4.2 | 4.1 | 4.5 | 4.2 | 4.1 | 4.2 | 4.3 | 4.7 | 4.3 |
| 200 | 4.1 | 4.5 | 4.3 | 4.9 | 4.5 | 4.4 | 4.8 | 4.5 | 5.4 | 4.8 |
| 400 | 4.6 | 5.1 | 4.7 | 5.2 | 4.9 | 4.6 | 5.3 | 4.9 | 5.7 | 5.1 |
| 600 | 4.7 | 5.3 | 4.8 | 5.5 | 5.1 | 4.9 | 5.6 | 5.1 | 5.9 | 5.4 |
| 800 | 5 | 5.6 | 5.2 | 6.2 | 5.5 | 5.2 | 5.9 | 5.6 | 6.5 | 5.8 |
| 1000 | 5.3 | 5.9 | 5.7 | 6.4 | 5.8 | 5.7 | 6.1 | 6.2 | 6.6 | 6.2 |
| Mean | 4.7 | 5.1 | 4.8 | 5.5 | 5 | 4.9 | 5.3 | 5.1 | 5.8 | 5.3 |
| L.S.D Silicon 0.05% Bio. interaction | 0.0305 0.028 0.074 | | | | | 0.062 0.050 0.098 | | | | |
| Seed yield kg/fed. 1 st season | | | | | Seed yield kg/fed. 2 nd season | | | | | |
| 0 | 495 | 527 | 514 | 650 | 546.5 | 513 | 538 | 522 | 674 | 561.8 |
| 200 | 622 | 745 | 709 | 793 | 717.3 | 649 | 765 | 731 | 829 | 743.5 |
| 400 | 696 | 813 | 781 | 844 | 783.5 | 713 | 838 | 815 | 875 | 810.3 |
| 600 | 718 | 825 | 794 | 886 | 805.8 | 756 | 849 | 822 | 911 | 834.5 |
| 800 | 766 | 837 | 723 | 920 | 811.5 | 778 | 864 | 839 | 965 | 861.5 |
| 1000 | 785 | 852 | 841 | 983 | 865.3 | 811 | 885 | 862 | 1143 | 925.3 |
| Mean | 680.3 | 766.5 | 727 | 846 | 755 | 703 | 789.8 | 765.2 | 899.5 | 789.5 |
| L.S.D silicon 0.05% Bio. interaction | 1.962 1.602 5.171 | | | | | 1.765 1.441 4.625 | | | | |
| Straw yield kg/fed. 1 st season | | | | | Straw yield kg/fed. 2 nd season | | | | | |
| 0 | 1612 | 1644 | 1636 | 1680 | 1643 | 1690 | 1754 | 1721 | 1791 | 1739 |
| 200 | 1628 | 1672 | 1652 | 1728 | 1670 | 1717 | 1804 | 1783 | 1862 | 1791.5 |
| 400 | 1664 | 1716 | 1704 | 1796 | 1720 | 1747 | 1849 | 1824 | 1940 | 1840 |
| 600 | 1684 | 1780 | 1752 | 1872 | 1772 | 1766 | 1870 | 1845 | 1990 | 1867.8 |
| 800 | 1708 | 1836 | 1804 | 1932 | 1820 | 1800 | 1930 | 1912 | 2114 | 1939 |
| 1000 | 1716 | 1944 | 1900 | 2068 | 1907 | 1816 | 2041 | 2002 | 2169 | 2007 |
| Mean | 1668.7 | 1765.3 | 1741.3 | 1846 | 1755.3 | 1756 | 1874.7 | 1847.8 | 1977.7 | 1864 |
| L.S.D silicon 0.05% Bio. interaction | 0.039 0.285 0.037 | | | | | 0.056 0.033 0.069 | | | | |

Si conc.: Silicon foliar application.
PDB: *Bacillus megatherium*.

Bio. : Biofertilization Azoto: *Azotobacter chroococcum*,

3. Oil percentage and oil yield

Data in Table 5 clarified that oil percentage and oil yield of sunflower were significantly affected by silicon foliar application, biofertilization treatments and their interaction in both seasons. It was noticed that 2nd season surpassed 1st season.

TABLE 5. Effect of silicon foliar application and biofertilization on yield , yield components, Oil% and Oil yield of sunflower (2010 and 2011 growing seasons).

| Silicon | Oil % 1 st season | | | | | Oil% 2 nd season | | | | |
|---|------------------------------|-------|-------|------|---|-----------------------------|-------|------|------|--------|
| | Cont. | Azoto | PDB | Mix | Mean | Cont. | Azoto | PDB | Mix | Mean |
| 0 | 25.6 | 31.2 | 34.8 | 35.6 | 31.8 | 29.2 | 31.8 | 35.2 | 36.2 | 33.1 |
| 200 | 30.4 | 31.9 | 36.5 | 37.2 | 34 | 31.6 | 32.1 | 37.6 | 38.1 | 34.85 |
| 400 | 31.8 | 32.1 | 37.5 | 37.9 | 34.825 | 32.1 | 32.4 | 37.9 | 38.2 | 35.15 |
| 600 | 32.1 | 33 | 38.1 | 38.5 | 35.425 | 32.5 | 33.7 | 38.3 | 38.9 | 35.85 |
| 800 | 32.6 | 34.2 | 38.5 | 38.9 | 36.05 | 33 | 34.9 | 38.9 | 39.3 | 36.525 |
| 1000 | 32.8 | 35.9 | 39.3 | 39.7 | 36.925 | 33.3 | 36.1 | 39.5 | 39.8 | 37.175 |
| Mean | 30.9 | 33.1 | 37.5 | 38 | 34.8 | 32 | 33.5 | 37.9 | 38.4 | 35.4 |
| L.S.D | 0.212 | | | | | 0.237 | | | | |
| Silicon | 0.173 | | | | | 0.194 | | | | |
| 0.05% | 0.629 | | | | | 0.773 | | | | |
| Bio. interaction | | | | | | | | | | |
| Oil yield kg/fed 1 st season | | | | | Oil yield kg/fed 2 nd season | | | | | |
| 0 | 310 | 34.9 | 367 | 374 | 271.5 | 319 | 358 | 375 | 389 | 360.3 |
| 200 | 341 | 378 | 394 | 412 | 381.3 | 359 | 383 | 403 | 418 | 390.8 |
| 400 | 365 | 391 | 426 | 438 | 405 | 376 | 410 | 435 | 445 | 416.5 |
| 600 | 391 | 415 | 448 | 492 | 436.5 | 397 | 421 | 456 | 528 | 450.5 |
| 800 | 413 | 424 | 456 | 514 | 451.8 | 421 | 435 | 481 | 539 | 469 |
| 1000 | 422 | 433 | 472 | 548 | 468.8 | 429 | 442 | 496 | 573 | 485 |
| Mean | 373.7 | 346 | 427.2 | 463 | 402.5 | 383.5 | 408.6 | 441 | 482 | 428.7 |
| L.S.D | 1.711 | | | | | 1.765 | | | | |
| Silicon | 1.397 | | | | | 1.44 | | | | |
| 0.05% | 4.347 | | | | | 4.625 | | | | |
| Bio. interaction | | | | | | | | | | |

Oil % and oil yield had a gradually increasing as silicon concentration increased. The promoting effect of biofertilization treatments (Single or mixed) extended to both oil yield and oil %, As a result to the ability of phosphate dissolving bacteria (*B.megatherium*) to solubilize phosphate and increase its availability for plant metabolism, it exhibited superiority effect in oil yield and oil % compared with *A.chroococcum*, this results in accordance with Ogbo (2010).

In this respect, sunflower plants which received the different concentrations of silicon up to 1000 mg/l with mixtures of *A.chroococcum* and *B. megatherium* showed superiority in oil % and oil yield as compared with other concentrations of silicon and single biofertilization treatments.

Moreover, the highest values of seed oil percentage and oil yield (Kg/fed) were recorded from sunflower plants spraying with silicon at 1000 mg/L in combination with mixed biofertilization treatment being 39.7, 39.8 % and 548, 573 (Kg/fed) each for oil % and oil yield respectively, in both seasons as shown in (Table 5, Fig. 3), such significant increase due to improvement in translocation of assimilates. Different studies indicated positive effect of silicon application on the plant growth and development including enhanced pollination, increase dry biomass and final yield (Korndorfer and Lepsch, 2001 and Muhammad *et al.*, 2013).

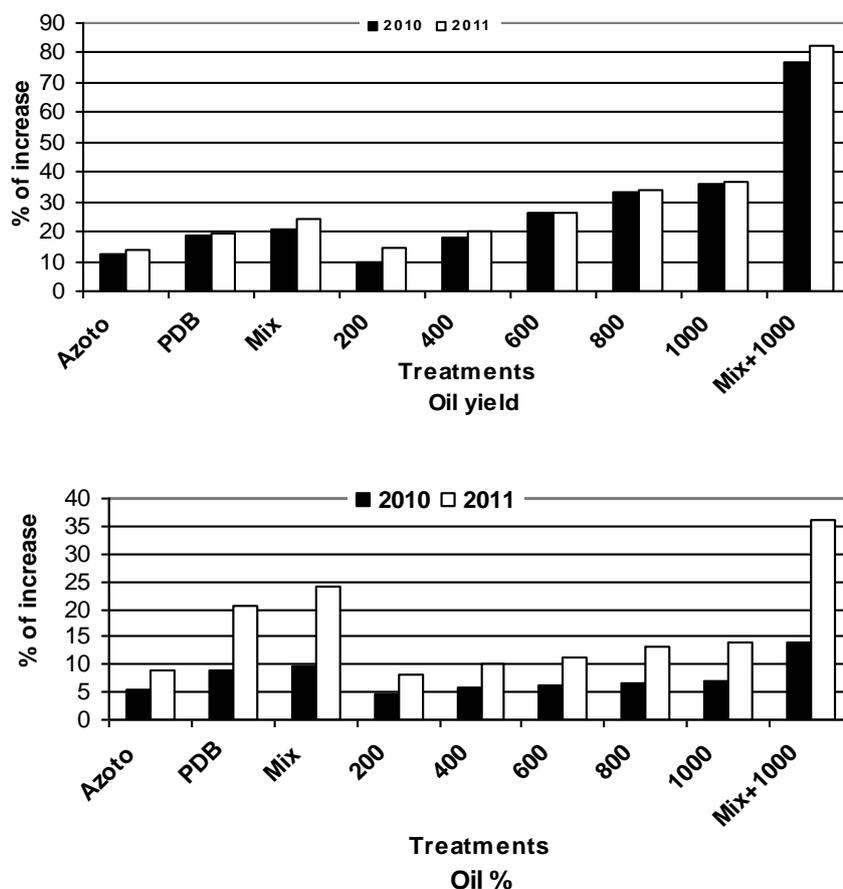


Fig. 3. Effect of silicon foliar application and biofertilization on % of increase sunflower oil yield and Oil % (2010 and 2011 growing seasons).

4. Effect of biofertilization and silicon on soil microbial analysis

4.1. General microbial activities

4.1.1: *Total microbial counts:* Initial total microbial counts before cultivation were 19 and 23 $\times 10^5$ cfu/g dry soil during two seasons, respectively (Table 6).

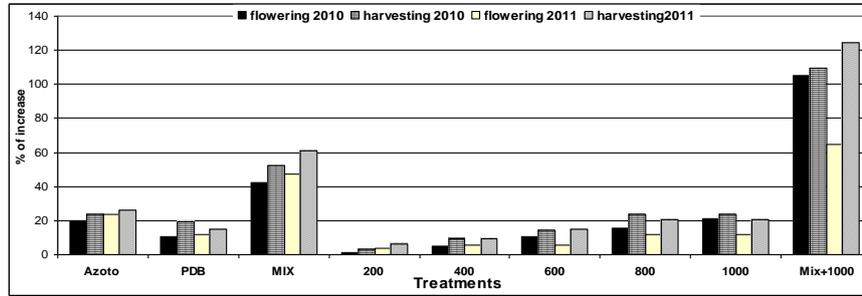
TABLE 6. Effect of silicon foliar application and biofertilization on CO₂ evolution (mg CO₂/100g dry soil/24 hr), total microbial counts ×10⁵ cfu/g dry soil, in sunflower rhizosphere (2010 , 2011 growing season).

| Bio Si | CO ₂ 1 st season | | | | | | | | | |
|---|--|-------|-----------------------------|-------|-------|-------|-------|-------|--------|-------|
| | Cont. | | Azoto | | PDB | | Mix | | Mean | |
| | flow | Harv. | flow | Harv. | flow | Harv. | flow | Harv. | flow | Harv. |
| 0 | 20 | 17 | 23 | 21 | 22 | 19 | 28 | 25 | 23.25 | 20.5 |
| 200 | 20 | 17.6 | 25 | 22 | 24 | 20 | 30 | 25 | 24.75 | 21.2 |
| 400 | 21 | 18 | 28 | 22 | 25 | 21 | 33 | 27 | 26.75 | 22 |
| 600 | 22 | 18 | 30 | 23 | 27 | 21 | 36 | 28 | 28.75 | 22.5 |
| 800 | 23 | 19 | 31 | 24 | 28 | 23 | 39 | 28 | 30.25 | 23.5 |
| 1000 | 24 | 19 | 31 | 25 | 28 | 22 | 40 | 28 | 30.75 | 23.5 |
| Mean | 21.7 | 18.1 | 28 | 22.8 | 25.7 | 21 | 34.3 | 26.8 | 27.4 | 26.8 |
| L.S.D. silicon 0.05% Bio. interaction | Flow: 0.67 2.13 0.82 | | Harv.: 0.69 0.88 0.84 | | | | | | | |
| CO ₂ 2 nd season | | | | | | | | | | |
| 0 | 21 | 17.4 | 26 | 22 | 25 | 20 | 32 | 28 | 26 | 21.9 |
| 200 | 21 | 18.5 | 28 | 23 | 26 | 21 | 33 | 29 | 27 | 22.9 |
| 400 | 23 | 19 | 30 | 25 | 27 | 23 | 37 | 31 | 29.25 | 24.5 |
| 600 | 24 | 20 | 33 | 28 | 29 | 25 | 39 | 34 | 31.25 | 26.8 |
| 800 | 26 | 21 | 34 | 30 | 30 | 26 | 42 | 37 | 33 | 28.5 |
| 1000 | 26 | 21 | 35 | 32 | 31 | 28 | 44 | 39 | 34 | 30 |
| Mean | 23.5 | 19.5 | 31 | 26.7 | 28 | 23.8 | 37.8 | 33 | 30.1 | 25.7 |
| L.S.D. silicon 0.05% Bio. interaction | Flow: 0.75 2.03 0.91 | | Harv.: 0.67 2.9 0.82 | | | | | | | |
| Total microbial counts 1 st season | | | | | | | | | | |
| 0 | 37 | 32 | 72 | 66 | 65 | 62 | 78 | 74 | 63 | 58.5 |
| 200 | 41 | 37 | 87 | 83 | 82 | 73 | 96 | 92 | 76.5 | 71.3 |
| 400 | 46 | 41 | 93 | 86 | 84 | 80 | 108 | 103 | 82.75 | 77.5 |
| 600 | 49 | 45 | 98 | 94 | 91 | 86 | 128 | 122 | 91.5 | 86.8 |
| 800 | 52 | 48 | 111 | 105 | 103 | 97 | 139 | 135 | 101.25 | 96.3 |
| 1000 | 57 | 52 | 134 | 125 | 115 | 108 | 150 | 141 | 114 | 106.5 |
| Mean | 47 | 42.5 | 99.2 | 93.2 | 90 | 84.3 | 116.5 | 111.2 | 88.2 | 82.8 |
| L.S.D. silicon 0.05% Bio. interaction | Flow: 0.84 1.56 1.02 | | Harv.: 1.55 1.38 3.52 | | | | | | | |
| Total microbial counts 2 nd season | | | | | | | | | | |
| 0 | 39 | 35 | 77 | 76 | 69 | 71 | 92 | 85 | 69.25 | 66.8 |
| 200 | 43 | 43 | 92 | 92 | 87 | 82 | 110 | 106 | 83 | 80.8 |
| 400 | 49 | 46 | 99 | 97 | 96 | 93 | 132 | 128 | 94 | 91 |
| 600 | 59 | 54 | 118 | 113 | 103 | 98 | 147 | 142 | 106.8 | 101.8 |
| 800 | 59 | 56 | 132 | 128 | 118 | 115 | 154 | 135 | 115.8 | 108.5 |
| 1000 | 61 | 52 | 150 | 125 | 137 | 108 | 161 | 141 | 127.3 | 106.5 |
| Mean | 51.7 | 47.7 | 111.3 | 105.2 | 101.7 | 94.5 | 132.7 | 122.8 | 99.3 | 99.5 |
| L.S.D. silicon 0.05% Bio. interaction | Flow: 0.67 1.06 0.82 | | Harv.: 1.8 1.47 4.83 | | | | | | | |

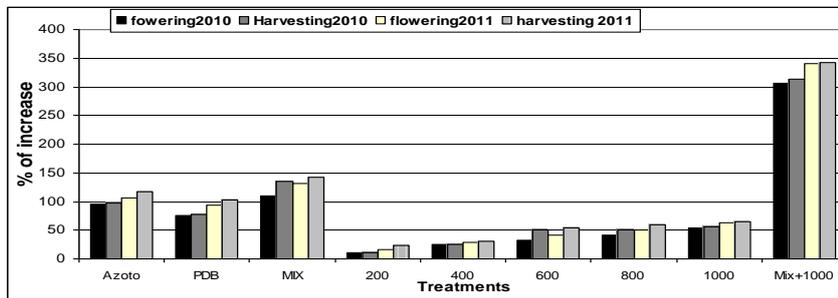
Si conc.: Silicon foliar application. Bio. : Biofertilization. Initial total microbial counts: 19×10⁵ cfu/g dry soil, Initial CO₂ evolution: 10.82mg CO₂/100g dry soil/24 hr.

Generally the counts at flowering stage of sunflower growth were higher than those of harvesting stage and all the treatments exceeded the control. Total microbial counts slightly increased with increasing silicon concentrations which might be due to silicon foliar spray enhance plant growth, the stimulative effect of plant rhizosphere on the adjacent microorganisms leads to increase total microbial counts. Another increase in counts was associated with the use of biofertilizers either in the form of single or mixed treatment as shown in Fig.4. The enhancement effect in microbial activity is a good parameter for many soil improvement indicators. For example *A.chroococcum* and *B. megatherium* produce growth promoting substances, biological nitrogen fixation, organic acids production and other enzymatic activities which enhance plant growth and proliferate lateral roots and root hairs which increase nutrient absorbing surface (El-Shazly, 2010). The highest counts were associated with mixed treatment (*A.chroococcum* and *B. megatherium*) and silicon foliar application at 1000 mg/L to be 154 and 161×10^5 cfu/g dry soil at flowering stage of sunflower during two seasons, respectively. These results are compatible with those obtained by (Ashrafuzzaman *et al.*, 2009) who reported that, inoculation with the plant growth promoting rhizobacteria (*Azotobacter*, *Bacillus megatherium*) had stimulation effect on the population of rhizosphere microorganism and increased their numbers by more than 50% at the end of the experiment comparing with the number recorded before planting.

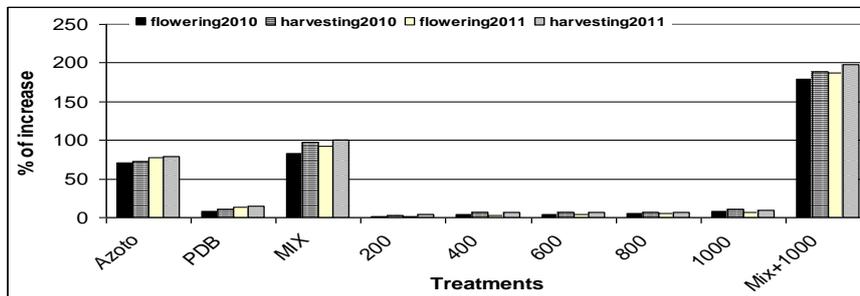
4.1.2: CO₂ evolution : The generation of carbon dioxide (CO₂) was determined as an indication of the biological activity in plant rhizosphere. Results in Table 6 clearly showed that a slight increase in microbial activity as a result of increasing foliar application of silicon up to 1000 mg/l due to indirect enhancement effect on microbial activity in rhizosphere of cultivated plant. Inoculation with both biofertilizers (*A.chroococcum* and *B. megatherium*) individual or mixed encourage the microbial activity in rhizosphere of sunflower plant. Interaction of biofertilization with silicon foliar application gave higher rate of CO₂ evolution than single treatment. The highest % of increase than control for mixed biofertilization and silicon foliar application (1000 mg/l) treatment being 105% and 109% and 67% and 124% at flowering stage of sunflower during two seasons, respectively (Fig.4). Data of CO₂ evolution were almost in harmony with those of total microbial counts discussed before (Visser and Dennis, 1992).



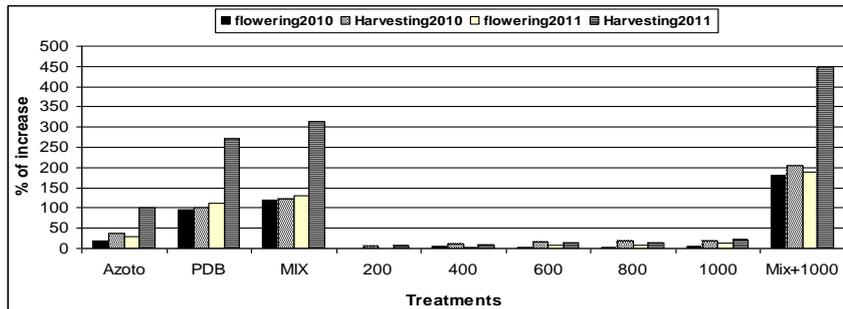
A



B



C



D

Fig. 4. % of increase than control for A-CO₂ evolution , B-total microbial counts × C- Azotobacter counts D- Phosphate dissolving bacteria .

4.2. Specific microbial activities

4.2.1 *Azotobacter densities*: Represented data in Table 6 recorded improvement in azotobacters counts by different treatments as compared with control. The indirect role of silicon foliar application on microbial activity in rhizosphere of treated plant reflected on *Azotobacter* densities in soil. Inoculation with biofertilizers with *A.chroococcum* and *B. megatherium* (individually or mixed) had stimulating effect on *Azotobacter* counts in rhizosphere. Synergistic effects of biofertilizers application and silicon spray enhances *Azotobacter* counts in soil (Fig. 4).

Interaction of *A.chroococcum* and *B. megatherium* with silicon foliar application at 1000 mg/L in mixed treatment recorded the highest counts and highest % of increase than control to be 67 and 70×10^4 cfu/g dry soil for counts and 187% and 189% for % of increase at flowering stage of sunflower during two seasons, respectively. The promoting effect due to application of *A. chroococcum* not only due to the nitrogen fixation but also to the production of plant growth promoting substances, production of amino acids, organic acids, vitamins and antimicrobial substances as well, which increase soil fertility, microbial community and plant growth (Revillas *et al.*, 2005).

4.2.2. *Phosphate dissolving bacteria (PDB)*: Initial counts of PDB before cultivation were 4.2 and 6.0×10^2 cfu/g dry soil during two seasons, respectively (Table 7). However their counts tended to increase in all treatments rather than the control. Significant increases were recorded at flowering compared to harvesting stages of plant growth. It was noticed that, the enhancement effect with silicon foliar application up to 1000 mg/l on counts of PDB was slightly compared with biofertilizers application (single or mixed). The highest counts and % of increase than control were recorded in mixed biofertilization treatment and silicon foliar application at 1000 mg/L to be 25.9 and 29×10^2 cfu/g dry soil for counts and 181.5% and 208% for % of increase at flowering stage of sunflower during two seasons, respectively (Fig. 4). A similar trend was recorded by Khan *et al.* (2006).

4.3. Enzymatic activity

4.3.1. *Dehydrogenase enzyme*: Data in Table 8 showed the determination of enzymatic activities in rhizosphere of sunflower plants. Dehydrogenase activity (DHA) represents the energy transfer, therefore, it is considered as an index of overall microbial activity in the soil. Represented data recorded that silicon foliar application recorded lower values for DHA activity compared with biofertilization treatments. Interaction treatment of biofertilization with silicon at concentration 1000 mg/l recorded the highest DHA activity. This may be due to that *A.chroococcum* and *B.megatherium* played an important role as plant growth promoting rhizobacteria via N_2 fixation and P-solubilization (El-Howeity *et al.*, 2003 and Muthukumar & Udaiyan, 2006). This might led to accumulate available nutrients and stimulate the microorganisms in soil rhizosphere.

TABLE 7. Effect of silicon foliar application and biofertilization on *Azotobacter* densities $\times 10^4$ cfu/g dry soil and phosphate dissolving bacteria counts $\times 10^2$ cfu/g dry soil, in sunflower rhizosphere (2010, 2011 growing season).

| Bio Si | <i>Azotobacter</i> densities 1 st season | | | | | | | | | |
|---|---|-------|-----------------------------|-------|------|-------|------|-------|--------|-------|
| | Cont. | | Azoto | | PDB | | Mix | | Mean | |
| | flow | Harv. | flow | Harv. | flow | Harv. | flow | Harv. | flow | Harv. |
| 0 | 24 | 22.3 | 41 | 39.5 | 26 | 25.2 | 44 | 43 | 33.8 | 32.5 |
| 200 | 24.3 | 22.6 | 45 | 44 | 28 | 25.8 | 51 | 49 | 37.1 | 35.4 |
| 400 | 25 | 23 | 51 | 50 | 29 | 27 | 57 | 56 | 40.5 | 39 |
| 600 | 25 | 23.2 | 56 | 54 | 31 | 29 | 62 | 59 | 43.5 | 41.3 |
| 800 | 25.3 | 23.5 | 57 | 55 | 31 | 29 | 64 | 62 | 44.3 | 42.4 |
| 1000 | 26 | 23.9 | 59 | 57 | 33 | 32 | 67 | 64 | 46.3 | 44.2 |
| Mean | 24.9 | 23.1 | 51.5 | 49.4 | 29.7 | 28 | 57.5 | 55.5 | 40.9 | 39.1 |
| L.S.D. silicon 0.05% Bio. interaction | Flow: 0.67 1.06 0.83 | | Harv.: 1.35 1.10 2.71 | | | | | | | |
| <i>Azotobacter</i> densities 2 nd season | | | | | | | | | | |
| 0 | 24.3 | 22.5 | 42 | 40.2 | 27 | 25.9 | 48 | 45 | 35.3 | 33.4 |
| 200 | 25 | 23.3 | 49 | 48 | 29 | 26.1 | 56 | 52 | 39.8 | 37.6 |
| 400 | 26 | 23.9 | 53 | 51.6 | 30 | 27.7 | 63 | 59 | 43 | 40.6 |
| 600 | 26 | 24 | 59 | 56 | 33 | 30 | 67 | 63 | 46.3 | 43.3 |
| 800 | 26 | 24.1 | 59 | 57 | 33 | 31.2 | 69 | 65 | 46.8 | 44.3 |
| 1000 | 27 | 24.7 | 62 | 59 | 35 | 32.5 | 70 | 67 | 48.5 | 45.8 |
| Mean | 25.7 | 23.8 | 54 | 52 | 31.2 | 28.9 | 62.2 | 58.5 | 43.3 | 40.8 |
| L.S.D. silicon 0.05% Bio. interaction | Flow: 0.68 1.01 0.83 | | Harv.: 1.34 1.09 2.66 | | | | | | | |
| PDB counts 1 st season | | | | | | | | | | |
| 0 | 9.2 | 8.3 | 11 | 10.8 | 18 | 17.5 | 20.2 | 19.2 | 14.6 | 14 |
| 200 | 9.3 | 8.4 | 13 | 11.5 | 20.3 | 18.7 | 22.1 | 20.1 | 16.175 | 14.7 |
| 400 | 9.4 | 8.5 | 13.2 | 12.4 | 22.9 | 19.3 | 24 | 21.2 | 17.45 | 15.4 |
| 600 | 9.5 | 8.9 | 14 | 12.9 | 22.7 | 20.8 | 25 | 23 | 17.775 | 16.4 |
| 800 | 9.7 | 8.9 | 15.4 | 13 | 23 | 21 | 25.6 | 23.8 | 18.375 | 16.7 |
| 1000 | 9.8 | 9.3 | 15.9 | 13.5 | 23.6 | 21.4 | 25.9 | 24 | 18.8 | 17.1 |
| Mean | 9.5 | 8.7 | 13.8 | 12.3 | 21.8 | 19.8 | 23.8 | 21.9 | 17.2 | 15.7 |
| L.S.D. silicon 0.05% Bio. interaction | Flow: 0.35 0.28 0.43 | | Harv.: 1.34 1.2 2.63 | | | | | | | |
| PDB 2 nd season | | | | | | | | | | |
| 0 | 9.5 | 8.5 | 13.1 | 12.1 | 19 | 18.3 | 21 | 19.8 | 15.65 | 14.7 |
| 200 | 10 | 8.7 | 13.7 | 11.9 | 21 | 19.1 | 23 | 20.3 | 16.925 | 15 |
| 400 | 10.6 | 8.8 | 14 | 12.9 | 24 | 20.8 | 26 | 22.6 | 18.65 | 16.3 |
| 600 | 11 | 9 | 14.6 | 13 | 25.1 | 21.3 | 26.7 | 23.4 | 19.35 | 16.7 |
| 800 | 11.2 | 9 | 16 | 13.2 | 26 | 21.3 | 27.3 | 24.1 | 20.125 | 16.9 |
| 1000 | 11.2 | 9.3 | 16.8 | 13.9 | 27.5 | 22 | 29 | 24.7 | 21.125 | 17.5 |
| Mean | 10.6 | 8.9 | 14.7 | 12.8 | 23.8 | 20.5 | 25.5 | 22.5 | 18.6 | 16.2 |
| L.S.D. silicon 0.05% Bio. interaction | Flow: 0.35 0.28 0.43 | | Harv.: 0.24 0.19 1.82 | | | | | | | |

Si conc.: Silicon foliar application. Bio.: Biofertilizati. Initial *Azotobacter* counts: 9.8×10^4 cfu/g dry soil. Initial Phosphate dissolving bacteria: 4.2×10^2 cfu/g dry soil.

TABLE 8. Effect of silicon foliar application and biofertilization on dehydrogenase activity ($\mu\text{DHA/g}$ dry soil), nitrogenase activity ($\mu\text{MC}_2\text{H}_4\text{kg/hr}$) and phosphatase ($\text{mgphenol/g soil/24hr}$) in sunflower rhizosphere (2010 , 2011 growing season).

| Bio Si | Dehydrogenase ($\mu\text{DHA/g}$ dry soil) 1 st season | | | | | | | | | |
|--|--|-------|-----------------------------|-------|-------|-------|-------|-------|-------|-------|
| | Cont. | | Azoto | | PDB | | Mix | | Mean | |
| | flow | Harv. | flow | Harv. | flow | Harv. | flow | Harv. | flow | Harv. |
| 0 | 19.51 | 16.25 | 30.9 | 24.21 | 28.03 | 22.4 | 33.26 | 41.5 | 27.9 | 26.1 |
| 200 | 20.3 | 18.62 | 31.14 | 24.8 | 28.25 | 23.8 | 33.7 | 41.73 | 28.3 | 27.2 |
| 400 | 21.15 | 18.95 | 33.7 | 27.22 | 28.3 | 24.33 | 34.08 | 41.49 | 29.3 | 28 |
| 600 | 21.6 | 19.2 | 34.2 | 27.61 | 29.1 | 24.81 | 34.2 | 42.18 | 29.8 | 28.5 |
| 800 | 22.53 | 20.06 | 34.88 | 28.97 | 29.53 | 25.12 | 34.58 | 43.16 | 30.4 | 29.3 |
| 1000 | 22.93 | 20.34 | 35.1 | 29.86 | 29.75 | 25.75 | 35.22 | 43.8 | 30.8 | 29.9 |
| Mean | 21.3 | 18.9 | 33.3 | 27.1 | 28.8 | 24.4 | 34.1 | 42.3 | 29.4 | 28.2 |
| L.S.D. silicon 0.05% Bio. interaction | Flow: 0.21 0.59 1.03 | | Harv.: 0.82 0.61 1.33 | | | | | | | |
| Dehydrogenase ($\mu\text{DHA/g}$ dry soil) 2 nd season | | | | | | | | | | |
| 0 | 22.4 | 20.1 | 31.8 | 26.64 | 31.14 | 25.4 | 35.2 | 43.61 | 30.1 | 28.9 |
| 200 | 23.2 | 20.9 | 32.6 | 28.49 | 31.6 | 25.7 | 35.4 | 43.8 | 30.7 | 29.7 |
| 400 | 24.1 | 22.4 | 31.53 | 29.8 | 32 | 26.4 | 35.6 | 43.8 | 30.8 | 30.6 |
| 600 | 24.3 | 23.11 | 35.14 | 30.4 | 30.8 | 26.5 | 35.9 | 43.4 | 31.5 | 30.9 |
| 800 | 25.1 | 23.9 | 35.52 | 30.42 | 32.1 | 26.8 | 36.61 | 43.9 | 32.3 | 31.3 |
| 1000 | 25.6 | 24.2 | 35.94 | 30.59 | 32.53 | 26.9 | 37.3 | 44.4 | 32.8 | 31.5 |
| Mean | 24.1 | 22.4 | 33.8 | 29.4 | 31.7 | 26.3 | 36. | 43.8 | 31.4 | 30.5 |
| L.S.D. silicon 0.05% Bio. interaction | Flow: 0.15 0.38 1.19 | | Harv.: 0.41 0.93 1.86 | | | | | | | |
| Nitrogenase ($\mu\text{MC}_2\text{H}_4\text{kg/h}$) 1 st season | | | | | | | | | | |
| 0 | 43.8 | 40 | 92.9 | 81.7 | 35.6 | 32.2 | 132 | 119 | 76.1 | 68.21 |
| 200 | 43.2 | 42 | 103.4 | 83.1 | 43.9 | 35.8 | 138 | 124 | 82.11 | 71.21 |
| 400 | 44.8 | 42.5 | 108 | 83.9 | 50.7 | 37.3 | 146 | 125 | 87.4 | 72.2 |
| 600 | 45.9 | 43 | 116.3 | 85.2 | 51.6 | 40.4 | 151 | 137 | 91.2 | 76.4 |
| 800 | 46.2 | 43.1 | 112.9 | 88 | 53.4 | 40.9 | 160 | 139 | 93.1 | 77.8 |
| 1000 | 46.8 | 44 | 118.1 | 88.6 | 54.1 | 41.5 | 163 | 144 | 95.5 | 79.5 |
| Mean | 45.1 | 42.4 | 108.6 | 85.1 | 48.2 | 38 | 148.3 | 131.3 | 87.6 | 74.2 |
| L.S.D. silicon 0.05% Bio. interaction | Flow: 0.17 0.24 2.04 | | Harv.: 0.39 0.16 1.04 | | | | | | | |
| Nitrogenase ($\mu\text{MC}_2\text{H}_4\text{kg/h}$) 2 nd season | | | | | | | | | | |
| 0 | 45.1 | 41 | 96.3 | 85.1 | 36.2 | 32.7 | 149 | 131 | 81.7 | 72.5 |
| 200 | 46.6 | 42.1 | 109 | 85.4 | 44.1 | 35.2 | 153 | 135 | 88.2 | 74.4 |
| 400 | 48.7 | 42.5 | 113 | 85.4 | 50.8 | 38 | 157 | 138 | 92.4 | 76 |
| 600 | 55.1 | 42.8 | 122 | 86 | 51.3 | 41.2 | 168 | 141 | 99.1 | 77.8 |
| 800 | 57.3 | 44.6 | 127 | 86.1 | 53.9 | 43 | 176 | 142 | 103.6 | 78.9 |
| 1000 | 61.3 | 54 | 129 | 86.7 | 54.6 | 43.8 | 183 | 149 | 107 | 83.4 |
| Mean | 52.4 | 44.5 | 116.1 | 85.8 | 49 | 39 | 164.3 | 139.3 | 95.34 | 77.2 |
| L.S.D. silicon 0.05% Bio. interaction | Flow: 0.72 0.34 1.17 | | Harv.: 0.47 0.25 1.67 | | | | | | | |

TABLE 8. Contd.

| Bio Si | Phosphatase (mgphenol/g soil/24hr) 1 st season | | | | | | | | | |
|--|---|-------|-----------------------------|-------|------|-------|------|-------|------|-------|
| | Cont. | | Azoto | | PDB | | Mix | | Mean | |
| | flow | Harv. | flow | Harv. | flow | Harv. | flow | Harv. | flow | Harv. |
| 0 | 0.41 | 0.39 | 0.57 | 0.52 | 0.48 | 0.46 | 0.71 | 0.69 | 0.5 | 0.51 |
| 200 | 0.43 | 0.4 | 0.66 | 0.6 | 0.55 | 0.54 | 0.8 | 0.78 | 0.61 | 0.58 |
| 400 | 0.44 | 0.4 | 0.71 | 0.63 | 0.58 | 0.58 | 0.84 | 0.81 | 0.64 | 0.61 |
| 600 | 0.4 | 0.42 | 0.78 | 0.66 | 0.62 | 0.63 | 0.87 | 0.82 | 0.67 | 0.63 |
| 800 | 0.46 | 0.43 | 0.83 | 0.68 | 0.71 | 0.65 | 0.9 | 0.85 | 0.73 | 0.65 |
| 1000 | 0.48 | 0.47 | 0.83 | 0.68 | 0.75 | 0.68 | 0.93 | 0.86 | 0.75 | 0.67 |
| Mean | 0.44 | 0.42 | 0.73 | 0.63 | 0.62 | 0.59 | 0.84 | 0.8 | 0.66 | 0.601 |
| L.S.D. silicon 0.05% Bio. interaction | Flow:0.35 0.28 0.43 | | Harv.: 0.24 0.19 1.82 | | | | | | | |
| Phosphatase (mg phenol/g soil/24hr) 2 nd season | | | | | | | | | | |
| 0 | 0.46 | 0.42 | 0.64 | 0.58 | 0.53 | 0.49 | 0.83 | 0.77 | 0.62 | 0.57 |
| 200 | 0.46 | 0.43 | 0.69 | 0.63 | 0.57 | 0.53 | 0.88 | 0.81 | 0.65 | 0.6 |
| 400 | 0.48 | 0.44 | 0.73 | 0.64 | 0.61 | 0.59 | 0.9 | 0.83 | 0.68 | 0.63 |
| 600 | 0.49 | 0.46 | 0.77 | 0.69 | 0.67 | 0.62 | 0.91 | 0.84 | 0.71 | 0.65 |
| 800 | 0.51 | 0.47 | 0.85 | 0.71 | 0.74 | 0.67 | 0.91 | 0.87 | 0.75 | 0.68 |
| 1000 | 0.53 | 0.47 | 0.89 | 0.75 | 0.79 | 0.7 | 0.95 | 0.9 | 0.79 | 0.71 |
| Mean | 0.49 | 0.45 | 0.76 | 0.67 | 0.66 | 0.6 | 0.9 | 0.84 | 0.7 | 0.64 |
| L.S.D. silicon 0.05% Bio. interaction | Flow:0.24 0.57 1.09 | | Harv.: 0.51 0.27 1.49 | | | | | | | |

4.3.2. *Nitrogenase activity*: Concerning nitrogenase activity was more pronounced at flowering stage and 2nd season than harvesting stage and 1st season. Similarly, lower variation in nitrogenase activity were recorded with silicon foliar application up to 1000 mg/l while, biofertilization treatments (individually or mixed) recorded significant differences in nitrogenase activity, synergistic effect of mixed biofertilization with silicon foliar application 1000 mg/l showed highest figure for nitrogenase activity (Table 8). Many investigators demonstrated the positive effect of dual inoculation with N₂-fixer and P-solubilizer on N₂-ase activity (El- Komy, 2005).

4.3-3-*Phosphatase activity*: Some free living microorganisms in soil have capability to produce extracellular enzymes such as phosphatase (George *et al.*, 2002). This enzyme is able to mineralize organic phosphates into inorganic phosphates that provides high phosphate for plant. The results in Table 8 clearly showed that, phosphatase activity recorded significant increase at flowering stage compared to harvesting stage of plant growth. Silicon foliar application caused slight increase in phosphatase activity. While, biofertilizers (single or mixed) recorded significant differences in phosphatase activity. Mixed

biofertilization with silicon at concentration 1000 mg/l recorded the highest phosphatase activity (Table 8).

5. Economic evaluation

In order to evaluate the results obtained, the investment ratio for every treatment was calculated by the ratio of total gain to its total costs/fed. The total costs in L.E. for different agricultural practices under the experimental conditions recorded 1740 LE/fed. For treatment with Silicon and Biofertilizers, 1700LE/fed. For treatment with silicon only, 1690 for biofertilization treatment only and 1650 for control (Table 9).

The investment ratios (IR) of sunflower crop were recorded in Table 10. The results showed that silicon foliar spray with biofertilizers application increased IR. The highest ratio was obtained under with mixed treatment (*A.chroococcum* and *B. megatherium*) and silicon foliar application at 1000 mg/L. compared with control.

From Table 10 it could be noticed that, for biofertilization treatments mixed treatment with *A.chroococcum* and *B.megatherium* increased IR followed by individual treatment with *A.chroococcum* only, then PDB only. In order to evaluate the effect of silicon foliar spray, IR increased with increasing silicon concentration. In general, mixed treatment with both biofertilizers and silicon foliar spray 1000mg/L gave the highest values for seed, straw and IR as well.

TABLE 9. The price in L.E. for different agriculture inputs under experimental conditions.

| Items | Price (L.E.) |
|----------------------|--------------|
| Land Preparation | 150 |
| Seed and Cultivation | 300 |
| Irrigation | 200 |
| Organic manuring | 300 |
| Silicon | 50 |
| Biofertilizers | 40 |
| Harvest Crop | 200 |
| transportation | 200 |
| Rent | 400 |
| Total | 1840 |

TABLE 10 . Investement ratios of sunflower crop under silicon and biofertilization treatments.

| Treatments | Gain LE./fed | | | Cost | IR |
|------------------|--------------|-------|--------|------|--------|
| | Seed | Straw | Total | | |
| Control | 1089 | 644.8 | 1733.8 | 1750 | -0.991 |
| Azotobacter | 1159.4 | 657.6 | 1817 | 1750 | 1.038 |
| PDB | 1130.8 | 654.4 | 1785.2 | 1790 | -0.997 |
| Mix | 1430 | 672 | 2102 | 1790 | 1.174 |
| Silicon 200 | 1368.4 | 651.2 | 2019.6 | 1800 | 1.122 |
| Silicon 200+Az | 1639 | 668.8 | 2307.8 | 1840 | 1.254 |
| Silicon 200+PDB | 1559.8 | 660.8 | 2220.6 | 1840 | 1.207 |
| Silicon 200+mix | 1744.6 | 691.2 | 2435.8 | 1840 | 1.324 |
| Silicon 400 | 1531.2 | 665.6 | 2196.8 | 1800 | 1.22 |
| Silicon 400+Az | 1788.6 | 686.4 | 2475 | 1840 | 1.345 |
| Silicon 400+PDB | 1718.2 | 681.6 | 2399.8 | 1840 | 1.304 |
| Silicon 400+mix | 1856.8 | 718.4 | 2575.2 | 1840 | 1.4 |
| Silicon 600 | 1579.6 | 673.6 | 2253.2 | 1800 | 1.251 |
| Silicon 600+Az | 1815 | 712 | 2527 | 1840 | 1.373 |
| Silicon 600+PDB | 1746.8 | 700.8 | 2447.6 | 1840 | 1.33 |
| Silicon 600+mix | 1949.2 | 748.8 | 2698 | 1840 | 1.466 |
| Silicon 800 | 1685.2 | 683.2 | 2368.4 | 1800 | 1.356 |
| Silicon 800+Az | 1841.4 | 734.4 | 2575.8 | 1840 | 1.4 |
| Silicon 800+PDB | 1810.6 | 721.6 | 2532.2 | 1840 | 1.376 |
| Silicon 800+mix | 2037.2 | 772.8 | 2810 | 1840 | 1.527 |
| Silicon 1000 | 1727 | 686.4 | 2413.4 | 1800 | 1.34 |
| Silicon 1000+Az | 1874.4 | 777.6 | 2652 | 1840 | 1.441 |
| Silicon 1000+PDB | 1850.2 | 760 | 2610.2 | 1840 | 1.418 |
| Silicon 1000+mix | 2162.6 | 828.8 | 2991.4 | 1840 | 1.625 |

Conclusion

It could be concluded from the abovementioned results that silicon and biofertilization contribute a considerable enhancement effect on sunflower plant growth as well as IR. For N-fixing bacteria as biofertilizers, *Azotobacter chroococcum* enhance the straw production of sunflower. On the other hand, The biofertilization with PDB (*B.megatherium* var. phosphaticum) showed better results on plant growth when added in combination with *Azotobacter chroococcum* than when applied alone. So, it could be recommended for enhancing sunflower productivity by mixed biofertilization (*Azotobacter chroococcum* plus *B.megatherium*) in conjunction with spraying silicon at rate of 1000 mg/L.

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تأثير التسميد الحيوي والرش بالسيلكون على إنتاجية دوار الشمس تحت ظروف الوادي الجديد

عمرو محمود عبد الجواد* و عماد محمد محمد سالم**

*وحدة ميكروبيولوجى الأراضى – قسم خصوبة وميكروبيولوجيا الأراضى
**وحدة المحاصيل – قسم الانتاج النباتى – مركز بحوث الصحراء – القاهرة –
مصر.

أجريت تجربتان حقليتان بمحطة التجارب الزراعية بالخارجة التابعة لمركز بحوث الصحراء ، محافظة الوادي الجديد ، أثناء موسمى الزراعة الصيفية (مايو – سبتمبر) لموسمى 2010 ، 2011 وذلك لدراسة تأثير ستة مستويات للرش بالسيلكون (بدون ، 200 ملجم/لتر سيلكون ، 400 ، 600 ، 800 ، 1000 ملجم/لتر سيلكون) و أربعة معاملات من التسميد الحيوى (بدون تسميد حيوى ، التلقيح باستخدام الازوتوباكتر ، التلقيح باستخدام البكتريا المذيبة للفوسفات ، التلقيح باستخدام مخلوط من الازوتوباكتر+ البكتريا المذيبة للفوسفات) وذلك على نبات دوار الشمس صنف سخا 53 ، و قد نفذت التجريبتين لدراسة المحصول و مكوناته و النسبه المئوية للزيت و محصول الزيت للفدان . و قد أستخدم تصميم القطع المنشق مره فى أربع مكررات حيث احتلت مستويات الرش بالسيلكون القطع الرئيسيه فى حين وزعت معاملات التسميد الحيوى فى القطع الشقيه.

و يمكن تلخيص النتائج المتحصل عليها فيما يلى:

1. وجدت اختلافات معنويه بين مستويات الرش بالسيلكون للصفات المدروسه. و قد أظهرت النتائج المتحصل عليها أن إضافة 1000 ملجم/لتر من السيلكون رشا على نباتات دوار الشمس بعد 60 يوم من الزراعه كانت المعامله الفعاله فى زيادة قيم الصفات المدروسه : طول النبات (سم) ، عدد الاوراق/نبات، مساحه سطح الاوراق/نبات(سم²)،الوزن الغض والجاف للاوراق/نبات (جم) ، و قطر الساق (سم) ، قطر القرص (سم) ، عدد البذور/قرص ، ووزن 100 بذره (جم) ، و محصول البذور و القش (كجم/فدان) ، و النسبه المئوية للزيت و محصول الزيت (كجم/فدان) متفوقه على جميع مستويات الرش الاخرى.

2. كما أشارت النتائج المتحصل عليها الى وجود اختلافات معنوية بين معاملات التسميد الحيوى مقارنة بالكنترول (بدون تسميد حيوى) ، و قد تفوقت معاملته التلقيح باستخدام مخلوط من الازوتوباكتري و البكتريا المذيبة للفوسفات على الاضافة المنفرده لكل منهما و ذلك لجميع الصفات تحت الدراسه ، بينما تم الحصول على اعلى قيم لكل من النسبه المئوية للزيت و محصول الزيت (كجم/فدان) و الذى تفوقت فيه معاملة التلقيح باستخدام البكتريا المذيبة للفوسفات مقارنة بمعامله الكنترول (بدون تسميد حيوى).
3. و أظهرت النتائج المتحصل عليها أن التفاعل بين مستويات الرش بالسيليكون و معاملات التسميد الحيوى أعطت اختلافات معنويه على صفات المحصول و مكوناته و كذلك النسبه المئوية للزيت و محصول الزيت (كجم/فدان) و أن أضافه معاملة التفاعل بين التسميد الحيوى (التلقيح باستخدام مخلوط من الازوتوباكتري و البكتريا المذيبة للفوسفات) مع معاملة الرش بالسيليكون بمعدل 1000 ملجم/لتر سجلت اعلى قيم لجميع الصفات تحت الدراسه.

و قد خلصت الدراسه للتوصيه بأضافه التسميد الحيوى (التلقيح باستخدام مخلوط من الازوتوباكتري و البكتريا المذيبة للفوسفات) مع الرش بالسيليكون بمعدل 1000 ملجم/لتر بعد 60 يوم من الزراعه للحصول على اعلى انتاجية لدوار الشمس تحت ظروف منطقة الدراسه (الوادى الجديد) و أيضا تشجيع المزارعين على استبدال التسميد الحيوى بالاسمده المعدنيه و لو جزئيا .