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RESPONSE OF SUNFLOWER TO BIOFERTILIZATION AND DIATOMS UNDER SALINE CONDITION.

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ABSTRACT

The study's purpose was to identify and isolate a salinity-tolerant *Azotobacter chroococcum* strain that would be used as a biofertilizer. Different salinity-affected sites in North Sinai, Ras Sudr and Sahle El-Tina, were sampled for soil. Under saline conditions, two extremely effective halotolerant Azotobacters were tested for their nitrogen fixing, phosphate dissolving, and synthesis of indol, gibrillin, and siderophores at (3 % NaCl). A field experiment in Sahle El-Tina, North Sinai, looked at the effects of biofertilization and Ditoms foliar spray on the development and production of sunflower cultivar Sakha 53. Sunflower yield, oil content, and chemical content were increased significantly when biofertilizer and diatoms interacted. Also, the interaction between diatom foliar spray and biofertilization treatments had a significant impact on all yield and yield components. In addition, total microbial counts, CO_2 evolution, and azotobacter counts in the rhizosphere soil indicated significant microbial activity, and enzymatic activity (Dehydrogenase) revealed a good response in all treatments compared to the uninoculated control.

Key Words: Sunflower, Salinity tolerant, Biofertilization, *Azotobacter chroococcum*, Ditoms.

INTRODUCTION

The sunflower plant (*Helianthus annuus L.*) is one of Egypt's most important oil crops **(Abdel Razik *et al.*, 2021; Ahmed *et al.*, 2022).** The seed includes 25-48 percent oil and 20-27 percent protein, and it has a high percentage of polyunsaturated fatty acids and a low cholesterol level. Sunflower is fairly susceptible to soil salinity, with an E.C of 1.7 dS/m tolerance limit. **(Zulfiqar *et al.*, (2021).** It attracted a lot of attention since it could be grown in a variety of soils and climate conditions in recently reclaimed soils, and it could supply the growing need for vegetable oil. **(Islam *et al.*, 2022; Ait Kaci Ahmed *et al.*, 2022).**

Salinity is a primary abiotic stress or that affects plant health and well-being among the different abiotic stresses **(Sumbul *et al.*, 2020).** Salinity disrupts the water and ionic movement of plant cells, hampering plant growth, morphology, physiology, and other activities, ultimately resulting in plant death **(Hailu & Mehari 2021)**.

It is required to generate stress-tolerant crop kinds by genetic engineering and plant breeding, but this is a long drawn out process, whereas microbial inoculation to relieve stress in plants could be a more cost-effective, ecologically friendly alternative that could be available sooner. **(Mashabela *et al.*, 2022)**. Plants are frequently subjected to many pressures in nature, and their ability to survive is determined on how they respond to these challenges. Plant growth promoting microorganisms (PGPM) that improve plant stress tolerance are a promising new technique for long-term agriculture production **Schmidt *et al.*, (2022).** Plant-associated microbes may play a key role in giving abiotic stress resistance. Rhizosphirc bacteria, and symbiotic fungus are among these creatures. They work through a diversity of methods, including new genes in plants and stimulating the osmotic response. **(Gamalero & Glick 2022).**

Beneficial bacteria, for example, are being considered as a possible treatment. They have an impact on plant growth and biochemical indicators, as well as helping to the production of organic compounds that protect plants from abiotic stress. Plant growth-promoting beneficial bacteria, or PGPB, has also been shown to improve plant health by reducing biotic and abiotic stress. (**Sumbul *et al.*, 2020**). Various mechanisms that affect plant development and yield performance are thought to occur at the soil-plant-microbe interfaces. The use of PGPB is thought to be highly essential in improving plant health by minimizing stresses in hostile environments **(Jabin 2016 and Sumbul *et al.*, 2020).** Currently, *Azotobacter spp.* (nitrogen-fixing bacterial strains) are being used successfully in large-scale sustainable agriculture. **(Mahanty *et al.*, 2017; Kour *et al.*, 2020 and Gogoi *et al.*, 2021).** Nitrogen fixation, siderophore generation, IAA and exopolysaccharide production are all characteristics of *Azotobacter spp.*, which improve plant health and produce indol-3-acetic acid and exopolysaccharides (EPS) **(Naitam & Kaushik 2021).** Apart from the main traits that increase the plant's tolerance index in hostile environments, *Azotobacter spp*. has a variety of other characteristics **(Shahid & Khan 2022).**

Diatomites de Mozambique (DDM) is a sedimentary rock discovered in Mozambique that is primarily composed of fossilised freshwater diatoms. It's largely made up of soluble silicat oxide (SiO_2) 86 to 89 % that plants may use, as well as a few trace elements. Because it improves the physical structure of soil, aerates the root zone of plants, lowers leaching and runoff, and so increases soil water retention and reduces watering, it is considered a complete, long-lasting, recyclable, reusable, and ecologically friendly soil fertilizer and enhancer. As a result, diatomite promotes the growth of stronger, healthier, higher-yielding plants that mature faster and acquire self-resistance to both

abiotic and biotic stresses. **Abdalla, 2011a ; Safwat, *et aL.*, 2016 and Gokavi *et al.*, (2021).**

This research is conducted to evaluate the application of *Azotobacter spp.* As a biofertilizer and diatoms to enhance the productivity of sunflower oil plant in growth in saline soil.

MATERIALS AND METHODS

1- Isolation and characterization of Azotobacter isolates:

The target isolates were isolated from the rhizosphere soil of wheat and barley plants grown in Ras Sudr and Sahle El-Tina, North Sinai, with electrical conductivity (EC: 10.8 and 12.1 dS/m) and identified according to **Bergey,s manual of systematic bacteriology** classification of **Brenner *et al.*, (2005) and Vos *et al.*,(2009).** Cell morphology, motility, gram reaction, catalase production, starch hydrolysis, nitrate reduction, sucrose, mannitol, benzoate as a sole carbon source, ammonium sulphate, KNO_3 and yeast extract as a sole nitrogen source, production of non diffusible pigment, and pH values were among the most important tests.

Different growth promoter traits for two *Azotobacter* isolates were investigated at 3% NaCl concentration including:

- Nitrogen fixation ability by investigating the nitrogenase enzyme activity (acetylene reduction technique) in Ashby's medium as described by **Hardy *et al.*, (1973).**
- Indole acetic acid (IAA) production using spectrophotometer as described by **Ehmann, (1977).**
– Gibberellins production in ethyl acetate extraction using HCl and Folin reagent according to the method described by **Graham and Henderson (1960).**
- The phosphorus solubilization index (PSI) and soluble phosphorus quantity were calculated on Pikovskaya medium using the methods of **Page *et al.*, (1982)** and **Kumar (1999). Tabatabai and Brimner (1969)** method was used to calculate phosphatase activity after three days of bacterial inoculation . Phosphatase enzyme units were defined as the quantity of enzyme that hydrolyzed 1mM of p-nitrophenol hour-1.
- Siderophores were produced using the procedures described by **(Neilands, 1981)**.

Effect of Salinity on proline production of the two Azotobacter isoletes:

The two bacterial isolates were investigated for their proline production at different concentrations of salt ranging from (1%, 2%, 3%,4% and 5% NaCl).

Preparation of Azotobacter inocula and seed inoculation*:*

Synergistic effect between the two selected effective Azotobacter isolates was done according to **Shirling and Gottlieb, (1966).**

Fresh liquid cultures 48 h old from pure local strains of *A. chroococcum* (*Az1*) and *A.chroococcum* (*Az2*) from each strain were prepared to obtain the desired concentration ($1.8x10^8$ cells/ml) and were kept at 4˚C until used. A mixture of the both strains was prepared just before inoculation by adding equal volum of the culture of each strain (1:1v/v) to inoculate the seeds of sunflower.

Seeds were successively washed and immersed for 30 minutes in heavy cell suspensions of the two strains of *Azotobacter chroococcum* (10^8 cells ml^{-1}) at the ratio of (1:1v/v) , carboxy methyl cellulose solution 0.5 % was used as an adhesive agent. Seeds of control treatments were soaked in water only. The inoculated seeds were air dried at room temperature for 2 hours before sowing. An additional dose was applied twenty one days later once again to soil.

Diatoms application

DDM diatomite is a natural diatomaceous earth originated from fossilized remains of fresh water diatoms with cell wall impregnated with silica. It is pHneutral and composed mainly of SiO_2 (86-89%) in asoluble form beneficial to plants. After 40 days from planting, diatoms were administered as a foliar spray at a rate of 1 kg/fed. We used a knapsack sprayer with a 300 L/fed water capacity. Major Chemical Elements for diatoms SiO_2 (89.00%), Al_2O_3 (5.95%), Fe_2O_3 (0.88%), CaO (0.10%), K_2O (0.63%), MgO (0.20%), Na_2O (0.32%), TiO_2 (0.29%) and H_2O (‹3%).

Field Experiments

The field experiment was done in Sahle El-Tina, North Sinai, during (2020/21) to evaluate the effect of two biofertilization bacteria on sunflower Sakha 53 growth and productivity. The experiment was designed with three replicates and was totally randomised.

Organic manure and calcium superphosphate fertilizers were added during soil preparation at the rate of 20 m^3 and 80 kg/fed (15.5% P_2O_5) respectively, 35 kg of potassium sulphate (50.0% K_2SO_4) was added at flowering stage, whereas nitrogen fertilizer was applied as ammonium sulfate (20.5% N) at rate of 120 kg/feddan for full of recommended dose and 60 kg/feddan for half of recommended dose (1/3 of the amount was incorporated in dry soil before sowing, 1/3 was added one month after sowing and the rest was added one week pre flowering stage). This area is irrigated through El-Salam Canal (Nile water mixed with agricultural drainage water at a rate of 1:1), 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 treatments used could be summarized as following:

1. Control without bacterial inoculation + full dose of (N,P,K).

2. Biofertilizer (mix of two *Azotobacter spp.*) + (half dose of N, full dose of P,K).
3. Diatoms + (half dose of N, full dose of P,K).
4. Biofertilizer + Diatoms + (half dose of N, full dose of P,K).

Soil mechanical and chemical analysis was represented in Table (1a,1b). Chemical analysis of irrigated water was represented in Table (2).

Table 1a. Soil mechanical analysis at two depths.

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

Table 1b.Soil chemical analysis at two depths.

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

Table 2. Chemical analysis of El-Salam canal water.

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

Measurement of plant parameters:

The following traits were estimated after harvesting: Plant height, fresh weight, dried weight, head diameter, 1000-grain weight, seed weight/plant, and seeds yield /fed. Oil yield / fed. was calculated using a **British pharmacopoeia (1936).** Oil extraction follows the IUPAC standard procedure **(Anonymous, 1987)**, oil was extracted from the ground seed material using 500 mL n- hexane in a Soxhlet device. Oil content (%) = Oil content (g)/seed weight (g) x 100

For chemical analysis of sunflower seeds: Nitrogen was evaluated using the micro-Kjeldahl method **(Bremner and Mulvaney 1982)** for sunflower seed chemical analysis. As mentioned in, phosphorus and potassium were measured using a spectrophotometer and a flame photometer, respectively **(Page et al., 1982).**

Protein content of seeds: Seed nitrogen content was evaluated using the Kjeldahl method, and protein content was estimated using the formula: Protein (%) = Nitrogen content x 6.25. The content of proline in leaf tissue was determined using the method of **(Bates et al., 1973).** Total antioxidant activity was measured in milligrammes of ascorbic acid equivalents (AAE) per gramme of extract (Prieto et al., 1999).While Ca, Mg and other micronutrients content, were determined by the Ionic Coupled Plasma according to **(Ure, 1995)**

For microbiological analysis soil samples from the sunflower rhizosphere were collected at harvesting stage and analysed for total count of microorganisms using the decimal plate method technique as described by **Nautiyal (1999).** Azotobacter densities were determined using nitrogen deficient medium as described by **Abd El-Malek and Ishac (1968). Casida et al., (1964)** established a method for determining Dehydrogenase activity in soil samples

Statistical analysis

The results of this study were statistically examined using Statistix version 9 computer software, and differences between treatment alternatives were declared significant when they were more than the least significant differences (LSD) at the 5% stage. **(Analytical software, 2008).**

RESULTS AND DISCUSSION

Isolation and identification of Azotobacter.

Two Azotobacter (nitrogen-fixing bacterium) isolates were recovered and coded (Az-1, Az-2) based on their isolation location. These isolates were identified according to **Holt *et al.*, (1994).**Some biochemical and morphological characteristics of Azotobacter isolates were shown in Table(3). The two isolates were ovoid to rod-shaped and formed cysts in pairs. Gram negative, aerobic, nitrogen fixer in aerobic conditions, oxidase positive microbial strain. These isolates were identified as *Azotobacter chroococcum* based on the following characteristics and could be distinguished from other Azotobacter species. The use of various carbon sources and the synthesis of yellow brown colours

Table (3): Azotobacter isoletes morphological and physiological characteristics:

Morphological and biochemical test	***Az1***	***Az2***
Cell morphology	**Ovoid shape**	**Ovoid shape**
motility	**-**	**-**
Gram reaction	**negative**	**negative**
Catalase production	+	+
Starch hydrolysis	+	+
Nitrate reduction	+	+
Sucrose as a sole carbon source	+	+
mannitol as a sole carbon source	+	+
benzoate as a sole carbon source	+	+
Ammonium sulphate as sole nitrogen source	+	+
KNO_3 as sole nitrogen source	+	+
Yeast extract as sole nitrogen source	+	+
Production of non diffusible pigment	**Yellow brown pigment**	**Yellow brown pigment**
pH	**7-7.4**	**7-7.4**

2- The ability of Azotobacter strains as plant growth promoters:

Only data of the highly potential bacteria were recorded as shown in table (4). The two most successful Azotobacter strains were selected for their ability to promote plant development. Strain(*Az-1*) was more active in nitrogenase activities, indol acitic acids and gibberellins production which recorded 93.6 n.mole C_2H_4 /ml/h at 3% NaCl, 153 µg/ml and 26.3 µg/ml, respectively.

Strain (*Az-2*) recorded 80 µmole C_2H_4 /ml/h at 3% NaCl, 51 µg/ml and 17 µg/ml respectively, On the other hand, isolate(*Az-2*) was more active in Phosphate solubilization and siderophores production which recorded 430.83 ppm and 59.0 (µg/ml, 410nm) than isolate*(Az-1)* which recorded 293.13ppm and 35.2(µg/ml, 410nm), respectively.

Table (4) Biochemical and microbial activities for selected N_2-fixers *Azotobacter chroococcum*

Code	Plant rhizosphere	Location of isolates	Nitrogenase (n.moleC2 H4/ml/h)	Phosphate solubilization (ppm)	Phosphatase (EU)	IAA (µg/ml	Gibberellins (µg/ml)	Siderophores production (µg/ml, 410nm)
Az-1	Wheat	Ras Sudr	93.6	293.13	0.36	153	26.3	35.2
Az-2	Barley	Sahl Eltina	80	430.83	0.31	51	17	59.0

3- Proline synthesis by selected strains in response to increased NaCl concentration.

To evaluate the effect of increased osmotic stress on proline production, the selected strains were grown in varied concentrations of sodium chloride (1 %, 2 %, 3 %, 4 %, and 5%).

Increased NaCl concentration greatly increased proline synthesis as showen in fig (1); these findings were consistent with those reported by **Mahmoud, *et al.*, (2020).** Proline accumulation, which is linked to improved plant growth, may result in increased leaf water potential as well as protection from salt-induced oxidative stress **(Farouk and Al-Huqail 2022).**

Synergistic effect between selected strains:

Both selected strains compatible to grow together, where each strain grew well in the presence of the other strain without any restrictions, resulting in a synergistic behaviour that maximised the benefits gained from selected strains over each microbe.

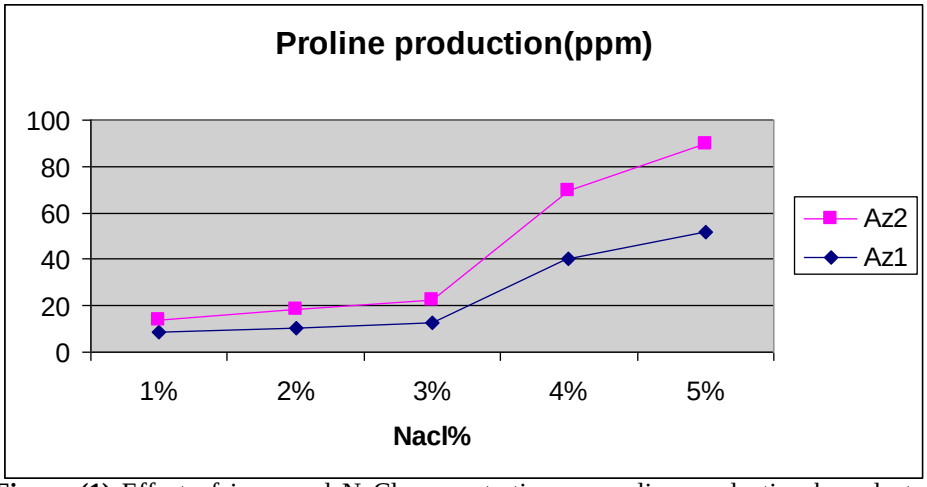

Figure (1) Effect of increased NaCl concentration on proline production by selected strains.

Growth traits:

Growth parameters values during 2020 and 2021 season were shown in table (5,6).

Results indicated that gradual increase in sunflower plant height, shoot fresh weight, shoot dry weight , head diameter, seed weight and Weight of 1000 seed.

The combined application of mixed treatment of biofertilization and diatoms foliar followed by biofertilization and diatoms individually yielded the maximum plant height, the plants in the control group are the tiniest ones.

The stimulatory effects could be related to the stimulation of microflora growth, which includes various plant growth stimulators, biological nitrogen fixation, and increased accessible phosphorus, all of which help plants grow faster as stated by **Sehrawat *et al.*, (2022).** The benefits of using silicon in agriculture include increased tolerance to diseases, pests, cold, salt, and toxicity produced by excess Al, Mn, and Fe. A layer of silicon collecting behind the cuticle is responsible for many of these advantages as mentioned by **HuaSun *et al.*, (2021) and Christian *et al*., (2022).** The biofertilization and silicon foliar application combination treatments had the greatest impact on growth parameters (**Hassan *et al*., 2021).**

Hafez *et al*., (2021) found that both biofertilizers and silicon foliar spray have a stimulative effect on sunflower growth parameters. Combination of biofertilization and silicon foliar treatment increased the growth. The highest increase was recorded with mixed biofertilization treatment and diatom foliar application, which recorded 191 cm in plant hight, 187 (gm) in shoot fresh weight and 25.8 (gm) in shoot dry weight

while control recorded 145cm for plant height, 87(gm)in shoot dry weight and 10,2(gm)in shoot dry weight, respectively.

Table (5) Effect of biofertilization and diatoms foliar application on plant hight, Shoot fresh weight and shoot dry weight of sunflower and halfe dose of N fertilizer at the harvest.

Treatments	Growth parameters		
	Plant hight(cm)	Shoot fresh weight(gm)	Shoot dry weight(gm)
Control	145d	87d	10.2d
Bio	176b	155b	17.9b
Diatoms	159c	136c	15.2c
Bio+Diatoms	191a	187a	25.8a

The data in Table (6) showed that plant growth parameters were significantly influenced by the biofertilization, diatoms foliar application and their interaction treatments. In this respect, the superiority of *A. chroococcum* may be due to its important role in sunflower generative growth and therefore a significant increase in 1000 seed weight which reflected on seeds yield, according to **Moradzadeh *et al.*, (2021),** *A. chroococcum* increases accessible nitrogen in the soil and potentially increasing seed number in plants. In this respect, interaction of diatoms with mixed biofertilization treatment recorded the highest values being (20 cm, 22.3 g, 1130 kg and 60 Kg/fed) for head diameter, seed weight/plant, seed weight /fed and weight of 1000 seed respectively. Followed by individual biofertilization and individual diatom foliar application while control gave the lowest values. This significant increase in yield and yield components due to biofertilization along with diatoms application treatments had synergistic effects on subsequent plant growth and stimulate microbial activities beneficial to plant growth and yield.

The efficiency of biofertilizers supplied to the plant grown with biologically fixed nitrogen, solubilized phosphorus, and produced plant hormones, may stimulate nutrient uptake and photosynthesis, resulting in increased plant growth and yield, may be attributed to their stimulatory effects on yield and yield components. Furthermore, as seen in fig (2) yield and yield characteristics, ditoms may have increased the surface area, number of leaves per plant, and dry matter of sunflower plants, resulting in greater photosynthetic areas and activity, as well as dry matter accumulation in seeds. The increases in sunflower yield and its components are consistent with those seen in other crops as reported by **Semida *et al.*, (2021).**

Table (6) Effect of biofertilization and diatoms foliar application on head diameter, seed weight per plant, seed weight per fed, percent increase, and weight of 1000 sunflower seeds with half dose of nitrogen fertilizer at the harvest.

Treatments	Growth parameters				
	Head diameter (cm)	Seed weight /plant (gm)	Seed weight /fed (kg)	% of increase on seed Yield (kg/fed)	Weight of 1000 seed (gm)
Control	12d	14.32d	721.56d	-	32d
Bio	19b	19.05b	960.12b	33.1b	52b
Diatoms	17c	17.6c	887.04c	23c	38c
Bio+Diatoms	20a	22.3a	1130.22a	56a	60a

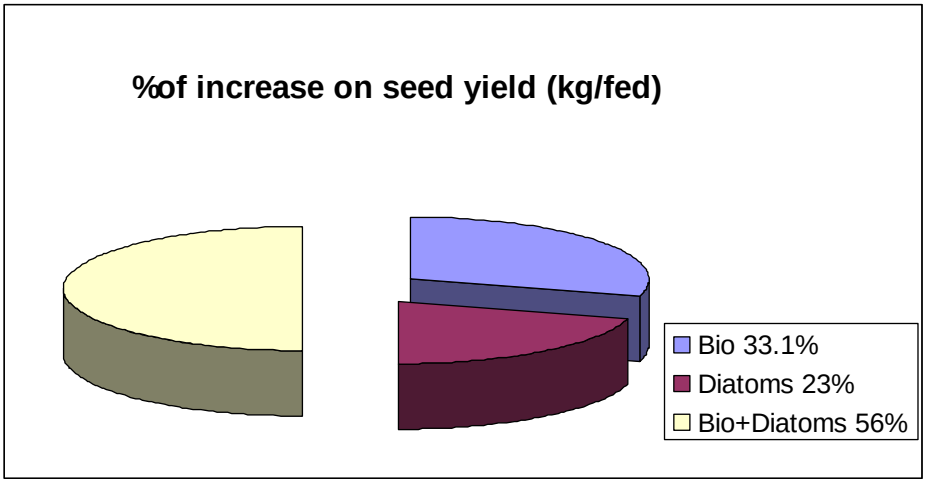

Figure (2) Effect of biofertilization and foliar diatom application on sunflower seed yield (kg/fed) at harvest.

Data in Table (7) clarified that oil percentage and oil yield of sunflower were significantly affected by silicon foliar application, the promoting effect of mixed biofertilization and diatoms treatments extended to both oil % and oil yield as a result to the ability of phosphate dissolving by the mixure of azotobacters 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 **Moradzadeh *et al*., (2021)**.

In this respect, sunflower plants which treated with mixtures of *A. chroococcum* and diatoms showed superiority in oil % and oil yield as compared with other concentrations of diatoms and single biofertilization treatments.

Moreover, the highest values of seed oil percentage and oil yield (Kg/fed) were recorded from sunflower plants spraying with diatoms in combination with mixed biofertilization treatment being 42.012 % and 474.828 (Kg/fed) for oil % and oil yield respectively, 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 **(Soleymanifard *et al*., 2022).** Diatoms lowered salinity stress by either coping with salinity in the rooting medium or inhibiting the mechanism of sodium transport to the leaves **(Omer & Abd-Elnaby (2017) and Garg *et al*., 2020).** Moreover, Si could stimulate growth and yield under saline conditions by increasing plant water status, cell wall thickness, elasticity and strength thus preventing lodging and providing leaf erectness. It also increases the synthesis of RNA and DNA. This situation reduces trans- piration and chlorophyll destruction, whereas it increases CO_2 assimilation rates which eventually resulted in an elevated rate of growth and yield **(Abdalla 2011 a). Consentino *et al*., (2022)** stated that the soluble proteins are increased with better nitrogen supply and favorable growth condition. **Kurmanbayeva *et al*.,. (2022).** In photosynthetic active leaf tissue, high levels of the reduced N fraction (protein fraction) were discovered. This is especially true when nitrate supplies are plentiful. These findings show that a high N-rate enhances amino acid synthesis in the leaves, which stimulates protein accumulation rather than oil content in the seed. In comparison to the untreated control, diatom biofertilizer considerably enhanced seed protein content by (18.625%), (12.062 %) as seen in Table (7)

Table (7) Effect of biofertilization and diatoms foliar application on Oil%, Oil Yield and Protein % of sunflower seeds using halfe dose of N fertilizer at the harvest.

Treatments	Oil%	Oil yield (Kg/fed	Protein %
Control	40.6165d	293.072d	12.062
Bio	41.522b	398.661b	18.562
Diatoms	41.0474c	364.107c	15.313
Bio+Diatoms	42.012a	474.828a	18.625

To understand the protective action of antioxidants under salinity stress, sunflower plants were treated with PGPR producing strains and diatoms followed by measurement of total antioxidant activity. The results presented in table (8) revealed that PGPR strains (*Az1* and *Az2*) under salinity soil stress significantly increased total antioxidant activity of sunflower leaves compared to the control. The highest increase was recorded with mixed biofertilization treatment and diatoms foliar application (252.7 µg/g FW) followed by individual biofertilization (190

µg/g FW.), and individual diatoms (189.7 µg/g FW.) compared with control (77.7 µg/g FW.). Proline accumulation is an adaptive response by plants to both general stress and salinity, since it mediates osmotic adjustment at the cellular level, thereby protecting intracellular macromolecules from dehydration, and also because it serves as a hydroxyl radical scavenger **(Mukarram *et al*., 2021 and Ghosh *et al*., 2022).**

In the present study, we found that the contents of proline and antioxidant were enhanced in the PGPR-inoculated sunflower plants under saline conditions. Therefore, it is likely that the PGPR strains promoted plant growth under salinity stress by enhancing metabolic defense strategies. On the other hand, diatoms enhanced salt tolerance in plants by enhancing the activity of antioxidant enzymes which, in turn, decreases the permeability of plasma membrane and in the mean time increases its integrity, stability and functioning **(Rawat *et al*., 2021)**. Plants commonly respond to stress by increasing the production of amino acids and proline whereas Si treatment reduced them **(Haghighi & Saharkhiz 2022).**

Table (8) Effect of biofertilizer and diatoms foliar application treatments on antioxidant and proline in the leaf tissues of sunflower plants.

Treatments	Antioxidant (ug/g freshwt.)	Proline content (ug/g fresh wt.)
Control	**77.7**	**90**
Bio	**190**	**152**
Diatoms	**189.7**	**121**
Bio+Diatoms	**252.7**	**192**

Nutrient contents in sunflower seeds:

Data of the studied macro and micronutrients (N, P ,K, Ca, Mg, B, Mn, Fe and Zn) contents in sunflower seeds are presented in Table (9), mixed biofertilizer and diatoms were found to be highly significantly effective in increasing NPK values of seed (2.98 ,0.882 and 0.860%) respectively comparing to untreated control. Same results were observed with the rest of the studied elements since the mixed treatment significantly influenced the uptake of micronutrients.

The PGPR strains could improve production of plant growth regulators or increase plant nutrient uptake. **Omer & Abd-Elnaby (2017)** concluded that biofertilizers decreased the hazard effect of salinity and exerted a favorable effect on growth and N, P and K concentration in sunflower seeds. On other hand **Quigley *et al*., (2020)** claimed that a great benefit of Si application is that it can balance nutrient element in plant tissue, the highest significant increase in N, P and K concentrations

of sunflower seeds was recorded by the interaction of biofertilizer and diatoms foliar application. Generally the increases in macronutrient concentrations in seeds may be due to the availability of them in the soil as a result of decreasing soil pH and salinitv caused by the action of organic materials or biofertilizer **(Abdalla 2011 b).** The improving effect of the combined treatments attained biofertilizer or diatoms was commonly achieved may be due to lowering soil pH that improve nutrients availability, mobility and ability to uptake by plant roots. In addition, the superiority of applied treatments attained (Biofertilizer+Diatoms) were more attributed to their richness in organic substances that ameliorate soil-moisture regime and the biological soil condition. **Cozzolino *et al.*, (2021)** pointed out that phytohormones producer bacteria causes pronounced increases for plant root elongation by then uptake of more nutrients via the root system, and hence utilization of N as a result of bio-inoculation.

Table (9) Effect of biofertilizer and diatoms foliar application treatments on chemical components of sunflower seeds.

Treatments	Seeds content of macro and micro nutrients								
	Macronutrients (%)					Micronutrients (mg kg^{-1})			
	N%	P%	K%	Ca%	Mg%	B	Mn	Fe	Zn
Control	1.93d	0.278d	0.615d	0.599d	0.450c	3.400d	22.1c	31.2d	63.3c
Bio	2.97b	0.746b	0.857b	0.747c	0.447c	4.700c	28.4a	37.6c	63.4c
Diatoms	2.45c	0.552c	0.715c	0.776b	0.486b	6.875b	23.1b	47.8b	65.1b
Bio+Diatoms	2.98a	0.882a	0.860a	0.831a	0.772a	17.6a	29.0a	53.3a	67.1a

Effect of biofertilization and diatoms on soil microbial analysis:

General microbial activities:

Azotobacter densities: Represented data in Table (10) recorded improvement in azotobacters counts by different treatments as compared with control. Inoculation with the two strains of azotobacters had stimulating effect on Azotobacter counts in rhizosphere. Synergistic effects of biofertilizers application and diatoms spray enhances Azotobacter counts in soil . Interaction of *Az1* and *Az2* with diatoms foliar application in mixed treatment recorded the highest counts than control to be 70 and 20×10^4 cfu/g dry rhizosphere soil for counts respectively. The indirect role of diatoms foliar application on microbial activity in rhizosphere of treated plant reflected on Azotobacter densities in soil. The promoting effect of *A. chroococcum* application is due not only to nitrogen fixation, but also to the production of plant growth promoting substances, amino acids, organic acids, vitamins, and antimicrobial substances, all of which contribute to increased soil fertility, microbial community, and plant growth **(Aasfar *et al.*, 2021 and Kumar & Brar 2021).** Total microbial counts slightly increased with

adding diatoms which might be due to diatoms foliar spray enhance plant growth, the simulative 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 in the form of mixed treatment as shown in Table (10) The enhancement effect in microbial activity is a good parameter for many soil improvement indicators. For example *A. chroococcum* 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 (**Nosheen *et al.*, 2021)**. The highest counts were associated with mixed treatment biofertilization and diatoms foliar application to be 98×10^5 cfu/g dry soil followed by individual biofertilizer being 77followed by individual diatoms foliar application bieng 77 and control recorded the lowest values being 39×10^5 cfu/g dry soil at harvest stage of sunflower. These results are compatible with those obtained by **Kumar *et al*., (2021) and Fasusi *et al*., (2021)** who reported that, inoculation with the plant growth promoting rhizobacteria (*Azotobcter chroococcum*) 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.

Table (10) Effect of biofertilization and diatoms foliar application on Azotobacter densities, total microbial counts and dehydrogense activity in sunflower rhizosphere using half dose of N fertilizer at the harvest.

Treatments	Azotobacter densities$\times10^4$ cfu/g dry soil	Total microbial counts$\times10^5$cfu /g dry soil	Dehydrogenase (µg) triphenyl formazan /g dry soil
Control	20d	39d	0.09d
Bio	55b	77b	0.338b
Diatoms	42c	56c	0.182c
Bio+Diatos	70a	98a	0.65a

Bio= Biofertilization. Initial total microbial counts: 13×105 cfu/g dry soil, Initial Azotobacter counts: 5.8×10^4 cfu/g dry soil.

Concerning the dehydrogenase enzyme: Data in Table (10) 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. Ref Represented data recorded that diatoms foliar application recorded lower values for DHA activity compared with biofertilization treatments. Interaction treatment of biofertilization with diatoms recorded the highest DHA activity. This may be due to that *A.chroococcum* played an important role as plant growth promoting

rhizobacteria via N_2 fixation and P-solubilization **(Aasfar, *et al.*, 2021).** This might led to accumulate available nutrients and stimulate the microorganisms in rhizosphere soil.

CONCLUSION

The above-mentioned findings suggest that biofertilization with Azotobacters and diatoms has a significant enhancing effect on sunflower plant growth and oil yield. On the other hand, mixed biofertilization in combination with diatom spraying may be recommended for increasing sunflower productivity. By fixing nitrogen, solubilizing phosphate, and generating exopolysaccharides, siderophore, and phytohormone, Azotobacters can help plants grow and develop under salt stress.

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استجابة نبات عباد الشمس للتسميد الحيوي والدياتومات تحت ظروف الملوحه

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كان الغرض من الدراسة هو تحديد وعزل سلالة *Azotobacter chroococcum* التي تتحمل الملوحة والتي يمكن استخدامها كسماد حيوي. تم أخذ عينات تربة من المواقع المتأثرة بالملوحة في شمال سيناء ورأس سدر وسهل الطينة ، تم اختبار افضل اثنين لسلالاتي ال Azotobacter من حيث تحمل ظروف الملوحه عند 3٪ كلوريد الصوديوم في تثبيت النيتروجين ، وإذابة الفوسفات ، وانتاج الإندول ، والجبريلين ، ومخلبيات الحديد. صممت تجربة حقلية في سهل الطينة بشمال سيناء لدراسة تأثير التسميد الحيوي ورذاذ الدياتومات الورقي على تطوير وإنتاج صنف دوار الشمس سخا 53. زاد محصول عباد الشمس ومحتوى الزيت والمحتوى الكيميائي بشكل ملحوظ عند استخدام الأسمدة الحيوية مع الدياتومات باستخدام نص الجرعه من السماد المعدني النيتروجيني . أيضا ، كان للتفاعل بين رش الدياتوم الورقي ومعاملات التسميد الحيوي تأثير معنوي على جميع مكونات المحصول. بالإضافة إلى ذلك ، وقد وجد ان هناك زيادة ملحوظه في كل مكونات المحصول والمحتوي الكميائي للحبوب عند التلقيح بخليط البكتريا مع الدياتومات مقارنة بالكنترول وقد اظهرت التحاليل الميكروبيولوجيه ان هناك زياده في الاعداد الكليه للبكتريا وعدد الآزوتوباكتر في تربة الريزوسفير و انبعاث ثاني اوكسيد الكربون في منطقه الريزوسفير ، كما أظهر النشاط الإنزيمي (ديهيدروجينيز) استجابة جيدة في جميع المعاملات مقارنة بالكنترول. وهكذا فقد اوضحت النتائج ان التلقيح ببكتريا *Azotobacter chroococcum* مع الدياتومات يمكن ان يكون بديل واعد واقتصادي لمقاومة النبات للملوحة.