



**ORIGINAL ARTICLE**

## **EFFECT OF BIO-FERTILIZATION WITH *AZOTOBACTER* SPP. and NITROGEN FERTILIZER ON GROWTH OF SUNFLOWER IRRIGATED WITH DIFFERENT LEVELS OF SALINE WATER**

**Huda A. Yaseen Aljanabi**

Department of Soil Science and Water Resources, College of Agriculture, Basrah University, Iraq.

E-mail: [hudaaa979@gmail.com](mailto:hudaaa979@gmail.com)

**Abstract:** The agricultural experiment was conducted in the greenhouse of the Research Station of the College of the Agriculture, University of Basrah in the spring season 2018, using soil taken from Abu Al-Khasib area alluvial clay soil (Silty clay). The study included several local isolates of the bacteria *Azotobacter chroococcum* seeds and used in the agricultural experiment. Sunflower seeds (*Helianthus annuus* L.) were inoculated with bacterial isolates (*Azotobacter chroococcum*), with (6 potted seeds<sup>-1</sup>) nitrogen added in three levels (0, 100 and 200) kg N ha<sup>-1</sup> in the form of urea fertilizer (46% N) dissolved with irrigation water. Phosphate fertilizer added at one level 80kg P ha<sup>-1</sup>. In contrast, added potassium at a level of 120 kg k ha<sup>-1</sup> in the form of potassium sulfate fertilizer in one batch before planting mixing with the soil, the sunflower seeds, inoculated with nitrogen-fixing bacteria, planted by six seeds pot<sup>-1</sup> and irrigated liquefaction water to the limits of the field capacity. After germination, they have reduced the plants to one plant. After growth, watered the plants with water of different salinity levels (2.5, 5, 7.5) ds.cm<sup>-1</sup> and after 60 days of growth, measured the plant's height. It was harvested from the area near the soil surface and dried and took its dry weight for the vegetative part, then grind the green part and estimated the concentration in them. The results showed a significant increase in plant height and dry weight, nitrogen concentration and the amount of nitrogen absorption in the vegetative fraction when adding different levels of nitrogen fertilizer and inoculating with *Azotobacter chroococcum* under different salt levels in irrigation water compared to untreated soil.

**Key words:** Bio-fertilization, *Azotobacter*. spp., Saline water, Sunflower.

### **Cite this article**

Huda A. Yaseen Aljanabi (2021). Effect of Bio-fertilization with *Azotobacter* spp. and Nitrogen fertilizer on growth of sunflower irrigated with different levels of saline water. *International Journal of Agricultural and Statistical Sciences*. DocID: <https://connectjournals.com/03899.2021.17.1125>

### **1. Introduction**

One of the modern techniques to reduce the excessive use of mineral fertilizers are bio-fertilizers as they have great importance in the field of agriculture by increasing the absorption of some necessary nutrients such as phosphorous and nitrogen or through their ability to decompose the components of organic waste and increase the availability of nutrients as well as their role in the excretion of some growth regulators [Al-Haddad (1998)]. Research on *Azotobacter chroococcum* spp. in crop production has manifested its significance in plant nutrition and its contribution to soil fertility. The possibility of using *Azotobacter*

*chroococcum* in research experiments as microbial inoculant through the production of growth substances and their effects on the plant has markedly enhanced crop production in agriculture. Being a free-living N<sub>2</sub>-fixer diazotroph, the *Azotobacteria* genus synthesizes auxins, cytokinins and GA-like substances. These materials regulate and enhance growth by stimulating rhizospheric microbes to protect the plants from phytopathogens by improving nutrient uptake and ultimately boosting the biological nitrogen fixation process. These hormonal substances originate from the rhizosphere or root surface, affecting the closely associated higher plants' growth. Inoculants with

microbiological fertilizers increased effectiveness of inoculants and microbiological fertilizers. It is necessary to find the compatible partners, *i.e.* a particular plant genotype and a particular *Azotobacter* strain that will form a good association [Sartaj *et al.* (2016)].

The most important organisms used in biological fertilization are *Azotobacter* bacteria, one of the free-living bacteria with a high ability to fix atmospheric nitrogen. That expanded its use as a bio-fertilizer with many crops and its ability to secrete some hormones, enzymes, vitamins and growth regulators. These compounds have an essential role in plant growth [Abdel-Gawad *et al.* (2009)].

The use of useful microorganisms in agriculture also increases the production by providing them with elements such as nitrogen and phosphorous by adding them directly to the soil or treating the seeds with them or adding them with irrigation water, or indirectly by loading them on a suitable substance called (carrier) [Wani *et al.* (2016)].

Nitrogen is also one of the essential nutrients and it is the most important for plant growth and development. Its concentration varies in plant tissues according to the plant's part, plant's age and crop's type, as its percentage decreases with the plant's age, contribute in photosynthesis and enzyme reaction focuses on developing the quality and quantity of dry matter in vegetable leaves and protein in cereal crops [Al-Shammari (2011)].

Nitrogen in soils losses in several ways, like washing, reversing nitrification, volatilization and soil erosion, the addition of nitrogen through chemical fertilizers is the primary source of nitrogen's contribution to the crop production systems prevailing around the world, that 50% of human societies depend on nitrogen fertilizer for food production and 60% is from fertilizer, therefore, chemical fertilizers have become imperative to secure the crop's need for nutrients. Nitrogen, phosphate and potassium fertilizers have taken the lead in terms of quantities used from them.

Nitrogen is essential for the growth and production of crops, so chemical fertilization is associated with many problems, especially in Iraqi soils, characterized by their high content of calcium carbonate and relatively high values of the degree of reaction of pH. The necessity went beyond the intensification of efforts in

the agricultural field to increase production. One of the most critical inputs to achieving this increase was the use of chemical fertilizers. Still, this increase accompanied by environmental risks and the economic cost spent on chemical fertilization prompted researchers to move towards biological fertilization because it is the least expensive. and safer for the environment [Dey *et al.* (2017), Alalaf (2020)].

Also, competition for freshwater between the agricultural, industrial and civil sectors led to a reduction in the farm share of freshwater and to fill the large water deficit, the need to use less quality water for irrigation purposes supplemented by water from drains, wells and salt lakes [Oster and Grattan (2002)] and the use of saltwater for irrigation purposes, it has adverse effects on the physical and chemical properties of the soil, the biological activity in the soil, the accumulation of salts that are readily soluble in the soil and at levels affecting the plant and negatively affect its growth and many factors contribute to increasing the salinity of the soil, including the quantity and quality of irrigation water used in agriculture and all these factors effect on plant growth and yield [Shin *et al.* (2016)].

Modern techniques used to reduce the effect of salinity on plants, including inoculation with bacteria that encourage plant growth, including the *Azotobacter* bacteria, promote plants to tolerate salinity by producing multiple types of hormones and increasing the readiness of the nutrients present in the soil solution. It also works to create osmotic resistance materials such as external polysaccharides that it works to reduce salt stress on plants growing in saline soils [Omer *et al.* (2016)].

The study aims to determine biological fertilization in reducing the saltwater effect, absorption of nitrogen element and sunflower yield growth.

## 2. Materials and Methods

The agricultural experiment of the spring season 2018 was conducted in the wooden canopy of the College of the Agriculture, University of Basra to study the effect of biological fertilization with *Azotobacter* bacteria and nitrogen fertilization under different salt levels of irrigation water and its impact on the growth of the sunflower plant. Local isolates obtained from the *Azotobacter* bacteria through the isolation process for different soil models obtained from Abu Al-Khasib, Al-Hartha and Al-Madina. Nutritional media was used to grow the *Azotobacter* bacteria on Jensens media

(sucrose mineral salt agar) [Sharma (2003)].

*Azotobacter* bacteria diagnosed to species and type belong to the *Azotobacter chroococcum* by studying its biochemical characteristics in the Department of Soil Sciences and Agricultural Resources of the Agriculture College laboratories.

The most efficient isolates were selected to pollinate the sunflower's seeds (*Helianthus annuus* L.) and used in the agricultural experiment. The soil brought from Abu Al-Khasib district in Basra governorate was air-dried, milled and sifted with a sieve of 2 mm holes. The physical and chemical characteristics estimated and part of it is preserved in the refrigerator to conduct biological analyses. The soil's chemical and physical properties were evaluated according to the standard methods of analysis mentioned in Black (1965) and shown in Table 1.

The experiment carried out during the spring season of 2018. Using plastic pots containing 3kg soil<sup>-1</sup> and sunflower seeds (*Helianthus annuus* L.) were used

**Table 1:** Some chemical, physical and biological properties of the study soil.

| Properties                   | Unit                          | Value                  |
|------------------------------|-------------------------------|------------------------|
| pH(1:1)                      |                               | 7.8                    |
| EC                           | ds m <sup>-1</sup>            | 10.6                   |
| CaCO <sub>3</sub>            | Gm kg <sup>-1</sup>           | 389.0                  |
| CEC                          | Cnti mol kg <sup>-1</sup>     | 14.3                   |
| O.M                          | Gm kg <sup>-1</sup>           | 3.2                    |
| N                            | Mg kg <sup>-1</sup>           | 32.4                   |
| P                            | Mg kg <sup>-1</sup>           | 13.2                   |
| K                            | Mg kg <sup>-1</sup>           | 157.7                  |
| Ca                           | Ml mol L <sup>-1</sup>        | 24.5                   |
| Mg                           |                               | 18.5                   |
| Na                           |                               | 55.9                   |
| K                            |                               | 2.3                    |
| Cl                           |                               | 75.3                   |
| SO <sub>4</sub>              |                               | 34.0                   |
| CO <sub>2</sub>              |                               | 0.0                    |
| HCO <sub>3</sub>             |                               | 3.8                    |
| Clay                         |                               | 414.7                  |
| Silt                         |                               | 538.9                  |
| Sand                         |                               | 46.4                   |
| Soil type                    |                               | Silty Clay             |
| Preparing the total bacteria | CFU gm <sup>-1</sup> dry soil | 4.8 × 10 <sup>-6</sup> |
| Preparing the total          |                               | 3.8 × 10 <sup>-3</sup> |
| fungi <i>Azotobacter</i>     |                               | 3.3 × 10 <sup>-5</sup> |

in the Complete randomized design (CRD) with three replicates. Nitrogen was added in three levels (0, 100 and 200) kg N ha<sup>-1</sup> in the form of urea fertilizer (46% N) as it added in two batches, the first when planting mixed with the soil and the second batch after a month of germination dissolved with irrigation water. Phosphate fertilizer added at one level 80 kg P ha<sup>-1</sup> while potassium added at level 120 kg k ha<sup>-1</sup> in the form of potassium sulfate fertilizer in one batch before planting mixing with the soil. Sunflower seeds, inoculated with nitrogen-fixing bacteria, sown by six seeds, pot<sup>-1</sup> and rinsed with tap water to the limits of the field capacity. After germination, the plants reduced to 1 plant<sup>-1</sup>. After the plant grew for 60 days from planting, first filled it from the area near the pot's soil surface.

The shoot parts washed first with plain water, then with distilled water to remove the dust stuck to them; they dried in the oven at a temperature of 65°C for 48 hours until the weight fixed the required chemical.

### 3. Results and Discussion

#### Plant height

Table 2 showed a significant increase in plant length for the green part, which reached 135.10cm. Increased plant height in the triple interaction between the inoculated with *Azotobacter* bacteria, levels of nitrogen fertilization and irrigation water salinity levels compared to the non-inoculated treatment at the same fertilization and salinity.

In the treatment, with 200kg Nh<sup>-1</sup> of fertilizer and a salinity level of 2.5 ds m<sup>-1</sup> irrigation water. and a percentage increase in the inoculated soil was 56% compared with the non-inoculated treatment with 0 Kg Nh<sup>-1</sup> fertilizer and 2.5 ds m<sup>-1</sup> in which the length of the green part of the plant reached 86.33 cm.

This increase in the length of the plant is because the *Azotobacter* bacteria has an essential role in the production of substance like gibberellins, cytokines, auxins and vitamins that stimulant growth and increase the size of the root system and the development of the root capillaries, this lead to an increase in the plant's nitrogen content, *Azotobacter* bacteria has proven effective in fixing atmospheric nitrogen, which led to an increase in plant height for the green part of the plant [Suhail *et al.* (2010)].

As for the interaction between nitrogen fertilization levels and inoculation with *Azotobacter* bacteria only, the treatment at the fertilization level 200kg h<sup>-1</sup>, the

**Table 2:** The effect of the interaction of nitrogen fertilization levels and different levels of irrigation water and inoculation with Azotobacter bacteria on the average length of the vegetative part of the sunflower plant (cm).

| Levels of nitrogen fertilization Kg N h <sup>-1</sup>   | The salinity level of irrigation water ds m <sup>-1</sup> | Non-Inoculation Soil | Inoculation Soil |              |
|---|---|----------------------|------------------|--------------|
| N0  | W1  | 86.336±0.621         | 91.183±1.165     |              |
|   | W2  | 81.920±2.166         | 84.213±1.277     |              |
|   | W3  | 77.343±0.586         | 79.000±0.100     |              |
| N1  | W1  | 108.406±0.901        | 123.473±0.557    |              |
|   | W2  | 97.760±1.339         | 118.603±0.943    |              |
|   | W3  | 78.353±0.722         | 87.776±0.440     |              |
| N2  | W1  | 132.540±0.811        | 135.100±0.556    |              |
|   | W2  | 124.910±0.324        | 128.956±0.166    |              |
|   | W3  | 94.216±0.908         | 95.353±4.659     |              |
| RLSD <sub>0.05</sub>  |   | 2.351                |                  |              |
| *Bilateral interaction between levels of nitrogen fertilization and inoculation with Azotobacter bacteria |   |                      |                  |              |
| Levels of nitrogen fertilization Kg N h <sup>-1</sup>   | N0  | 86.336±0.621         | 91.183±1.165     |              |
|   | N1  | 108.406±0.901        | 123.473±0.557    |              |
|   | N2  | 132.540±0.811        | 135.100±0.556    |              |
| RLSD <sub>0.05</sub>  |   | 0.820                |                  |              |
| *The interaction between inoculation with Azotobacter bacteria, salinity levels of irrigation water       |   |                      |                  |              |
| The salinity level of irrigation water ds m <sup>-1</sup>   | W1  | 86.336±0.621         | 91.183±1.165     |              |
|   | W2  | 81.920±2.166         | 84.213±1.277     |              |
|   | W3  | 77.343±0.586         | 79.000±0.100     |              |
| RLSD <sub>0.05</sub>  |   | 1.216                |                  |              |
| The interaction between nitrogen fertilization and salinity levels of irrigation water                    |   |                      |                  |              |
| Levels of nitrogen fertilization Kg N h <sup>-1</sup>   | The salinity level of irrigation water ds m <sup>-1</sup> |                      |                  |              |
|   |   | W1                   | W2               | W3           |
|   | N0  | 86.336±0.621         | 81.920±2.166     | 77.343±0.586 |
|   | N1  | 108.406±0.901        | 97.760±1.339     | 78.353±0.722 |
| N2  | 132.540±0.811   | 124.910±0.324        | 94.216±0.908     |              |
| RLSD <sub>0.05</sub>  |   | 0.786                |                  |              |

average length was 135.10 cm for inoculated soils in compared with non-inoculated soils in which the length of the green part of the plant was 86.33 cm without adding nitrogen fertilizer.

Also, the interaction between inoculation with Azotobacter bacteria and the salinity levels of irrigation water showed the best significant increase in plant height for the green part in inoculated soils compared to non-inoculated soils was at the salinity level of irrigation water 2.5 ds m<sup>-1</sup>.

While the interaction between nitrogen fertilization levels and salinity levels of irrigation water showed the lowest rate of increase, which is 77.34 cm at the level of nitrogen fertilization 0 kg N h<sup>-1</sup> and at the level of salinity of irrigation water 7.5 ds m<sup>-1</sup> compared to the level of nitrogen fertilization of 0 kg N h<sup>-1</sup> and at the level

of salinity of irrigation water 2.5 ds m<sup>-1</sup> the height of the plant in it reached 86.33 cm and this is due to the increase in soil salinity reduces the photosynthesis process, reduced leaf growth, partial closure of stomata and nutritional imbalance resulting from increased chlorine and sodium ions [Raheem (2019)].

#### Dry weight for plant

The dry weight of the green part showed a significant increase of 31.40 g Kg<sup>-1</sup> pot due to the effect of the 200 Kg N h<sup>-1</sup> of fertilizer treatment and the salinity level of irrigation water 2.5 ds m<sup>-1</sup> in inoculated soils with an increased percentage (28%) when compared with the non-inoculated comparison treatment 0 Kg N h<sup>-1</sup> of fertilizer and 2.5 ds m<sup>-1</sup> which amounted to 24.49 g pot<sup>-1</sup> (Table 3).

This increase in dry weight is attributed to the

**Table 3:** The effect of the interaction of nitrogen fertilization levels and different levels of irrigation water and inoculation with *Azotobacter* bacteria on the dry weight of the sunflower plant (g.Potted<sup>-1</sup>).

| Levels of nitrogen fertilization Kg N h <sup>-1</sup>  | The salinity level of irrigation water ds m <sup>-1</sup> | Non-Inoculation Soil | Inoculation Soil |             |
|--|---|----------------------|------------------|-------------|
| N0   | W1  | 24.49±0.869          | 26.12±0.383      |             |
|  | W2  | 23.07±0.167          | 23.43±0.550      |             |
|  | W3  | 21.61±0.654          | 22.36±0.945      |             |
| N1   | W1  | 26.09±0.415          | 28.36±0.734      |             |
|  | W2  | 24.90±0.434          | 28.30±1.249      |             |
|  | W3  | 22.40±0.556          | 24.99±1.121      |             |
| N2   | W1  | 30.67±0.923          | 31.40±0.396      |             |
|  | W2  | 29.11±1.03           | 30.25±1.62       |             |
|  | W3  | 26.97±0.469          | 28.54±1.124      |             |
| RLSD <sub>0.05</sub>   |   | 1.417                |                  |             |
| *Bilateral interaction between levels of nitrogen fertilization and inoculation with <i>Azotobacter</i> bacteria |   |                      |                  |             |
| Levels of nitrogen fertilization Kg N h <sup>-1</sup>  | N0  | 24.49±0.869          | 26.12±0.383      |             |
|  | N1  | 26.09±0.415          | 28.36±0.734      |             |
|  | N2  | 30.67±0.923          | 31.40±0.396      |             |
| RLSD <sub>0.05</sub>   |   | 0.791                |                  |             |
| *The interaction between inoculation with <i>Azotobacter</i> bacteria, salinity levels of irrigation water       |   |                      |                  |             |
| The salinity level of irrigation water ds m <sup>-1</sup>  | W1  | 24.49±0.869          | 26.12±0.383      |             |
|  | W2  | 23.07±0.167          | 23.43±0.550      |             |
|  | W3  | 21.61±0.654          | 22.36±0.945      |             |
| RLSD <sub>0.05</sub>   |   | 0.678                |                  |             |
| The interaction between nitrogen fertilization and salinity levels of irrigation water                           |   |                      |                  |             |
| Levels of nitrogen fertilization Kg N h <sup>-1</sup>  | The salinity level of irrigation water ds m <sup>-1</sup> |                      |                  |             |
|  | W1  | W2                   | W3               |             |
|  | N0  | 24.49±0.869          | 23.07±0.167      | 21.61±0.654 |
|  | N1  | 26.09±0.415          | 24.90±0.434      | 22.40±0.556 |
| N2   | 30.67±0.923   | 29.11±1.03           | 26.97±0.469      |             |
| RLSD <sub>0.05</sub>   |   | 0.673                |                  |             |

microorganism's ability represented by the *Azotobacter* to stabilize atmospheric nitrogen contributes to the rise in the rate of absorption of nutrients. It also plays an essential role in building proteins and amino acids, which leads to an increase in the dry weight of the green mass [Hanoon *et al.* (2020)].

The interaction effect between nitrogen fertilization treatment and inoculation with *Azotobacter* bacteria only, at nitrogen fertilizer level 200 kg h<sup>-1</sup>, showed that the green part's dry weight was 31.40 g pot<sup>-1</sup> inoculated soils. In contrast, non-inoculated soil without Nitrogen fertilizer was 24.49 g Potted<sup>-1</sup>.

The interaction between inoculation with *Azotobacter* bacteria and the salinity levels of irrigation water showed a significant increase in the green part's dry weight (26.12 g pot<sup>-1</sup>) in the inoculated soils non-

inoculated soils was 24.49 g pot<sup>-1</sup> at salinity level 2.5 ds m<sup>-1</sup>.

The interaction between nitrogen fertilization levels and the salinity levels of irrigation water showed the lowest average of dry weight in fertilizer level 0 Kg N h<sup>-1</sup> and the salinity level of irrigation water 7.5 ds m<sup>-1</sup>, which reached 21.61 pot<sup>-1</sup>.

The best significant increase in dry weight was in the fertilization level of 200 kg h<sup>-1</sup>. The salinity level of irrigation water was 2.5 ds m<sup>-1</sup>, which amounted to 30.67 g of pot<sup>-1</sup> compared to the comparison treatment reached an increased percentage (25%). increase in the readiness of the nutrients in the fertilization treatment and then the increase in their absorption by the plant, which in turn reflected positively on the green growth in a general manner and plant height in a special manner

**Table 4:** The effect of the interaction of nitrogen fertilization levels and different levels of irrigation water and inoculation with Azotobacter bacteria on the sunflower plant for nitrogen concentration (mg kg<sup>-1</sup>).

| Levels of nitrogen fertilization Kg N h <sup>-1</sup>   | The salinity level of irrigation water ds m <sup>-1</sup> | Non-Inoculation Soil | Inoculation Soil |            |
|---|---|----------------------|------------------|------------|
| N0  | W1  | 4.97±0.282           | 5.08±0.793       |            |
|   | W2  | 5.03±0.167           | 5.16±0.181       |            |
|   | W3  | 5.06±0.450           | 5.09±0.095       |            |
| N1  | W1  | 5.10±0.527           | 5.62±0.080       |            |
|   | W2  | 5.17±0.825           | 5.23±0.221       |            |
|   | W3  | 5.10±0.273           | 5.33±0.330       |            |
| N2  | W1  | 5.16±0.783           | 5.80±0.233       |            |
|   | W2  | 5.22±0.220           | 5.79±0.446       |            |
|   | W3  | 5.15±0.361           | 5.27±0.032       |            |
| RLSD <sub>0.05</sub>  |   | 0.588                |                  |            |
| *Bilateral interaction between levels of nitrogen fertilization and inoculation with Azotobacter bacteria |   |                      |                  |            |
| Levels of nitrogen fertilization Kg N h <sup>-1</sup>   | N0  | 4.97±0.282           | 5.08±0.793       |            |
|   | N1  | 5.10±0.527           | 5.62±0.080       |            |
|   | N2  | 5.16±0.783           | 5.80±0.233*      |            |
| RLSD <sub>0.05</sub>  |   | 0.435                |                  |            |
| *The interaction between inoculation with Azotobacter bacteria, salinity levels of irrigation water       |   |                      |                  |            |
| The salinity level of irrigation water ds m <sup>-1</sup>   | W1  | 4.97±0.282           | 5.08±0.793       |            |
|   | W2  | 5.03±0.167           | 5.16±0.181       |            |
|   | W3  | 5.06±0.450           | 5.09±0.095       |            |
| RLSD <sub>0.05</sub>  |   | 0.277                |                  |            |
| The interaction between nitrogen fertilization and salinity levels of irrigation water                    |   |                      |                  |            |
| Levels of nitrogen fertilization Kg N h <sup>-1</sup>   | The salinity level of irrigation water ds m <sup>-1</sup> |                      |                  |            |
|   |   | W1                   | W2               | W3         |
|   | N0  | 4.97±0.282           | 5.03±0.167       | 5.06±0.450 |
|   | N1  | 5.10±0.527           | 5.17±0.825       | 5.10±0.273 |
| N2  | 5.16±0.783  | 5.22±0.220           | 5.15±0.361       |            |
| RLSD <sub>0.05</sub>  |   | 0.392                |                  |            |

[Kareem *et al.* (2012)].

#### Nitrogen concentration in the plant

Table 4 referred to the effect of the interaction between levels of nitrogen fertilization and different levels of salinity of irrigation water and inoculation with Azotobacter bacteria for nitrogen concentration in the green part of sunflower plants. The result showed a significant increase in nitrogen concentration in the green part of the plant is reached to 5.80 mg kg<sup>-1</sup> in inoculated soils in comparison to non- inoculated soil with a 5.16 mg kg<sup>-1</sup> at the level of 200 kg N h<sup>-1</sup> fertilization and the level of 2.5 ds m<sup>-1</sup> salinity in inoculated soils.

Compared to comparison treatment at the level of fertilization 0Kg N h<sup>-1</sup>, irrigation water salinity level 2.5ds m<sup>-1</sup>, the nitrogen concentration reached 4.97 mg kg<sup>-1</sup> with the percentage of increased estimated (17%).

Bilateral interaction between nitrogen fertilization levels and inoculated with Azotobacter bacteria result showed a significant increase in green part nitrogen concentration percentage by (12%), which reached 5.80 mg kg<sup>-1</sup> for inoculated soil in contrast to non-inoculated treatment, the concentration got 5.16 mg kg<sup>-1</sup> in the fertilization treatment 200Kg Nh<sup>-1</sup> and at the lowest salinity level of irrigation water 2.5 ds m<sup>-1</sup>.

Increased nitrogen absorption is attributed to microorganisms' ability to fix the atmospheric nitrogen in free form, which meets the plant's need for the vital nutrient's component.

The positive effect of microorganisms in the rootstock's growth stimulates them to produce phytohormones, leading to the enhancement of the absorbed nitrogen component and its transfer to the

**Table 5:** The effect of overlapping levels of nitrogen fertilization and different levels of irrigation water and inoculation with *Azotobacter* bacteria on the sunflower plant of the absorbed nitrogen (mg kg<sup>-1</sup>).

| Levels of nitrogen fertilization Kg N h <sup>-1</sup>  | The salinity level of irrigation water ds m <sup>-1</sup> | Non-Inoculation Soil | Inoculation Soil |              |
|--|---|----------------------|------------------|--------------|
| N0   | W1  | 124.51±0.697         | 136.53±1.087     |              |
|  | W2  | 116.36±0.737         | 122.41±0.653     |              |
|  | W3  | 110.32±0.942         | 115.59±0.726     |              |
| N1   | W1  | 137.20±0.529         | 160.61±0.950     |              |
|  | W2  | 136.26±0.918         | 156.84±0.982     |              |
|  | W3  | 115.76±0.735         | 134.51±1.107     |              |
| N2   | W1  | 162.38±0.786         | 185.25±0.725     |              |
|  | W2  | 160.61±0.940         | 174.63±0.865     |              |
|  | W3  | 140.82±1.050         | 149.71±0.825     |              |
| RLSD <sub>0.05</sub>   |   | 1.486                |                  |              |
| *Bilateral interaction between levels of nitrogen fertilization and inoculation with <i>Azotobacter</i> bacteria |   |                      |                  |              |
| Levels of nitrogen fertilization Kg N h <sup>-1</sup>  | N0  | 124.51±0.697         | 136.53±1.087     |              |
|  | N1  | 137.20±0.529         | 160.61±0.950     |              |
|  | N2  | 162.38±0.786         | 185.25±0.725     |              |
| RLSD <sub>0.05</sub>   |   | 0.818                |                  |              |
| *The interaction between inoculation with <i>Azotobacter</i> bacteria, salinity levels of irrigation water       |   |                      |                  |              |
| The salinity level of irrigation water ds m <sup>-1</sup>  | W1  | 124.51±0.697         | 136.53±1.087     |              |
|  | W2  | 116.36±0.737         | 122.41±0.653     |              |
|  | W3  | 110.32±0.942         | 115.59±0.726     |              |
| RLSD <sub>0.05</sub>   |   | 0.924                |                  |              |
| The interaction between nitrogen fertilization and salinity levels of irrigation water                           |   |                      |                  |              |
| Levels of nitrogen fertilization Kg N h <sup>-1</sup>  | The salinity level of irrigation water ds m <sup>-1</sup> |                      |                  |              |
|  |   | W1                   | W2               | W3           |
|  | N0  | 124.51±0.697         | 116.36±0.737     | 110.32±0.942 |
|  | N1  | 137.20±0.529         | 136.26±0.918     | 115.76±0.735 |
| N2   | 162.38±0.786  | 160.61±0.940         | 140.82±1.050     |              |
| RLSD <sub>0.05</sub>   |   | 0.964                |                  |              |

plant and the microorganism. It colonizes the rhizosphere area and helps the plant absorb some elements, including nitrogen [Salman and Abdel-Wahab (2016)].

As for the interaction between inoculation with *Azotobacter* bacteria, salinity levels of irrigation water and between nitrogen fertilization and salinity levels of irrigation water, both did not give any significant increase.

#### Nitrogen absorbed in the plant

Bilateral interaction between nitrogen fertilization and inoculation with *Azotobacter* bacteria showed a substantial increase in the absorption of nitrogen in the fertilization with 200 kg h<sup>-1</sup> in inoculated soils compared to the comparison treatment 0Kg N h<sup>-1</sup> fertilizer, which reached 185.25 mg kg<sup>-1</sup> and with percentage was (49%).

Compared to the same fertilization level but with the lowest salinity level of irrigation water, it reached 162.38 mg kg<sup>-1</sup>, which was a percentage increase of (14%).

The significant increase in the nitrogen absorbed level was in the interaction between inoculated with *Azotobacter* bacteria with salinity water levels reached 136.25 mg kg<sup>-1</sup> to the non-inoculated soils at the irrigation water level 2.5ds m<sup>-1</sup> and by percentage increase (10%).

As for the interaction between nitrogen fertilization levels and salinity levels of irrigation water, the highest level of nitrogen absorbed by the plant was at 200KgN h<sup>-1</sup> and at 2.5 ds m<sup>-1</sup> salinity, which was 162.38 mg plant<sup>-1</sup> when compared with the comparison treatment, while the lowest average of nitrogen absorption was 110.32

mg plant<sup>-1</sup> at the nitrogen fertilization level 0KgN<sup>-1</sup> and at the salinity level of irrigation water 7.5 dsm<sup>-1</sup> compared to the nitrogen fertilization level.

At levels of nitrogenous mineral fertilization reduction in the nitrogen absorption due to decreases the activity of bacteria in nitrogen fixation and inhibition of the nitrogenase enzyme.

Many researchers have indicated that the bacteria positively affect the plant's nitrogen content even at high levels of metallic nitrogen. This is due to the Azotobacter bacteria excretion of Regulator substances for growth in environments that contain and free from nitrogen. It is known that these materials improve and form a dense root system, which leads to an increase in the absorption of nutrients, including the nitrogen element [Al-Shammari (2011)].

The amount of nitrogen absorbed by the sunflower plant in Table 5 showed a significant effect of the triple interaction between inoculation with Azotobacter bacteria and different levels of nitrogen fertilization and salinity of irrigation water, which showed the best increase in the amount of nitrogen absorbed by 185.25 mg plant<sup>-1</sup> in the treatment with 200Kg N<sup>-1</sup> of fertilizer and salinity level of irrigation water is 2.5 ds m<sup>-1</sup> in inoculated soils when compared with the non-inoculated treatment at the level of fertilization 0Kg N<sup>-1</sup> and irrigation water with salinity level 2.5 ds m<sup>-1</sup> which reached 124.51 mg plant<sup>-1</sup> with an increase percentage (49%).

Increasing the amount of nitrogen absorption in the plant by the nitrogen-fixing bacteria through atmospheric fixation of nitrogen, especially under nitrogen deficiency due to bacterial biological fertilizer [Al-Shammari (2011)].

#### 4. Conclusion

The result appeared that the optimal fertilizer and irrigation water level for good results was 200kg.h<sup>-1</sup> and 2.5ds.m<sup>-1</sup> inoculated sunflower seed with *Azotobacter chroococcum* showed a significant increase in plant height, dry weight, nitrogen concentration and nitrogen absorption in the green part when treated with different nitrogen levels fertilizer under different levels irrigation water salinity compared to untreated soil.

#### Acknowledgement

Author thankfully acknowledge the Editor-in-Chief

and anonymous referee for their constructive and fruitful comments for the much improvement on the earlier version of this paper.

#### References

- Abd El-Gawad, A.M., M.H. Hendawey and H.I.A. Farag (2009). Interaction between bio fertilization and canola genotypes in relation to some biochemical constituent under Siwa oasis conditions. *Res. J. Agric. and Biol. Sci.*, **5(1)**, 82-96.
- Alalaf, A.H. (2020). The Role of Biofertilization in improving Fruit productivity: A Review. *Int. J. Agricult. Stat. Sci.*, **16(1)**, 107-112.
- Al-Haddad, Muhammad Al-Sayed Mustafa (1998). The role of bio-fertilizers in reducing agricultural costs, reducing environmental pollution and increasing crop productivity. National training course on the productivity of biological fertilizers, Arab Organization for Agricultural Development AOAD. *Jordan*, **5**, 16-21.
- Al-Shammari, Asma Salim Hussein (2011). The effect of biological (Azotobacter), organic and mineral fertilization on growth and yield of bread wheat and its nutrient content. *Master Thesis*, College of Agriculture, University of Baghdad, Iraq.
- Black, C.A. (1965). Method of soil analysis. Part 2, Chemical and Microbiology properties. Am. Sco. Agron. Inc. Madison. Wisconsin. USA.
- Dey, R., K. Sarkar, S. Dutta, S. Murmu and N. Mandal (2017). Role of Azotobacter sp. isolates as a plant growth-promoting agent and their antagonistic potentiality against soil-borne pathogen (*Rhizoctonia solani*) under in vitro condition. *Int. J. Curr. Micro biol. App. Sci.*, **6(11)**, 2830-2836.
- Hanoon, M.B., M.S. Haran and M.K. Sahi (2020). Effect Of Rhizobium inoculation and different levels of organic and Nitrogen fertilizers on Growth and Production of Broad Bean (*Vicia faba* L.) and Nitrogen readiness In Soil. *Int. J. Agricult. Stat. Sci.*, **16(1)**, 229-236.
- Kareem, A.N., Adil F. Hadawee and Amer H. Hamzah (2012). The effect of organic, bio and chemical fertilizer in growth and yield of sunflower (*Helianthus annuus* L.) *Al Furat Journal of Agricultural Sciences*, **4(2)**, 130-137.
- Omer, A.M., H.M. Emara, R.A. Zaghloul, M.A. Monem and G.E. Dawwam (2016). Potential of *Azotobacter sailinestr* as plant growth-promoting rhizobacteria under saline stress conditions. *RJPBCS*, **7(6)**, 2572-2582.
- Oster, J.D. and S.R. Grattan (2002). *Drainage water reuse Irrigation and Drainage Systems*, 297-310.
- Raheem, N.S. (2019). Effect of inoculation with Azotobacter chroococcum and nitrogen fertilization on growth parameters of corn grown in different salinity levels. *Master Thesis*, College of Agriculture, University of Basrah.



- Salman, N.D. and N.R. Abd Al-Wahab (2016). Effect of bacterial biofertilizer and compost on the growth of tomato plant, *Journal of Agricultural Sciences*, **47(6)**, 1520-1527.
- Sartaj, A. Wani, Subhash Chand, Muneeb A. Wani, M. Ramzan and Khalid Rehman Hakeem (2016). *Azotobacter chroococcum* - Potential bio-fertilizer in Agriculture: An Overview. DOI: 10.1007/978-3-319-34451-5
- Sharma, A.K. (2003). *Bio-fertilizer for sustainable agriculture*. Agrobios, India.
- Shin, W.A., M.M. Siddikee, A.K. Joe, A. Benson, K. Kim, G. Selvakumar, Y. kang, S. Samaddar, P. Chatterjee, D. Walitang, M. Chanratana and T. Sa (2016). Halotolerant plant growth-promoting bacteria salinity stress amelioration in plants. *Korean J. Soil. Sci. Fert*, **49(4)**, 355-367.
- Suhail, Faris Muhammad, Mahdi Imad Adnan and Fahmy Alaa Hassan (2010). The corn plant response to inoculation with bacteria - *A. chroococcum* and *R. Tharzianum* and nitrogenous fertilizer. *Diyala Journal of Agricultural Seeds*, **2(1)**, 162-170.
- Wani, S.A., S. Chand, M.A. Wani, M. Ramzan and K.R. Hakeem (2016). *Azotobacter chroococcum* a potential biofertilizer in Agriculture, *Soil Sci. J.*, **15(1)**, 313-348.