Alleviation of Drought Stress in Zea Mays Seeding by Extracellular Polysaccharides Produced by Azotobacter

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ABSTRACT
Azotobacter bacteria are Gram-negative, rod-shaped soil bacteria that can fix atmospheric nitrogen and promote plant growth. They are known to produce exopolysaccharides (EPS), which can help plants to retain water and tolerate drought stress. In this study, 20 Azotobacter isolates were isolated from dry and semi-dry soil samples. The isolates were characterized morphologically and microscopically and tested for their tolerance to drought using PEG6000 at concentrations of 0%, 5%, 10%, 15%, 20%, and 25%. They were also tested for their ability to produce EPS. The isolates that were most tolerant to drought and produced the most EPS were used as biofertilizers to improve the drought tolerance of maize seedlings. The seedlings were irrigated every 24, 48, or 72 hours. The results showed that the biofertilizers containing the two most drought-tolerant and EPS-producing Azotobacter isolates (Azotobacter 1 and Azotobacter 2) significantly improved the germination rate, dry weight, stress tolerance index, chlorophyll stability, and membrane damage index of the maize seedlings.

1. Introduction

Water scarcity is a major issue in the face of climate change and limited water availability. This has forced researchers and farmers to find alternatives and strategies to achieve food security for the growing population, which is considered one of the main current risks 51. Thousands of hectares of land are left fallow each year worldwide due to the effects of drought. In Iraq, drought is one of the most important factors affecting crop production due to poor water management and drought conditions. One of the strategies used to improve plant growth and tolerance to drought stress is the use of plant growth-promoting bacteria (PGPB). Riwbacteria are considered one of the groups of bacteria that efficiently colonize plant roots and improve plant growth and production through several direct and indirect mechanisms 52. The first is a symbiotic relationship with the plant, such as nitrogen-fixing Azotobacter, and the other is free-living 53, which stimulates plant growth-promoting compounds such as hormones (auxins, gibberellins, and cytokinins), which help improve the ability of plants to tolerate stresses such as drought stress54. Azotobacter is one of the bacterial genera found in the soil, rod-shaped, Gram-negative. Some species of this genus have the ability to produce exopolysaccharide (EPS) in addition to biological nitrogen fixation.Bacteria secrete EPS to survive and live under stress conditions55. It allows them to maintain a higher water content, which helps them survive and live in conditions of reduced soil water content.The use of this type of bacteria as a bacterial inoculant or biofertilizer to reduce the use of chemicals, which are one of the main environmental pollutants of the ecosystem 56. Therefore, this study aims to improve the drought tolerance of maize seedlings, which is one of the important crops in Iraq, using a relatively inexpensive and easy method, namely inoculating seeds with two bacterial strains of Azotobacter to determine the ability of the isolates to alleviate drought stress.

2. Materials And Methods

2.1 Sample preparation

Twenty strains of *Azotobacter* bacteria were isolated from a soil sample from the root zone of

cultivated lands in semi-arid areas. The soil characteristics are presented in Table 1. The samples were collected according to the method described by 16. where a small shovel was used to clean the soil. The soil was dug to a depth of 15 cm from the ground surface, and the samples were placed in clean, sterile tubes and transported to the laboratory as described by (17**).**

Table (2-1) : shows the soil characteristics

Test	**EC ds/m**	**T.D.S PPM**	**PH**	**O.M %**	**N-NO3- ppm**	**N-NH4+ ppm**	**CL+ ppm**	**K+ ppm**	**Na++ ppm**	**Mg++ ppm**	**Ca++ ppm**
Sample	3.1	1985	8.1	1.8	20.1	40	2130	176	414.2	70.5	**360**

Isolation and diagnosis of bacteria

Azotobacter was isolated from soil samples according to the method of Becking (1981), where Winogrdsky medium was used, and the bacteria were purified using the method of Garrity *et al.,* (2005). The appearance of colonies, pigmentation, Gram stain, cell morphology, and cyst formation were evaluated. (18) (19)

Estimation of exopolysaccharide

The method described by Kanmani *et al* (2011) was followed in extracting exopolysaccharides with some modifications. After the end of the incubation period (48 hours), the resulting medium was placed in a water bath at 91 degrees Celsius for 11 minutes, and the cells were separated from the fermentation medium using a centrifuge at 8000 speed. RPM for 11 minutes. The cells were dried for the purpose of to calculate the dry weight of the biomass, while the filtrate was used. Trichloroacetic acid (TCA) was added to it at a concentration of 8% (volume/volume) and left for 3 hours at 4°C. Then it was quickly centrifuged. 8000 rpm for 11 minutes in to precipitate the protein present in the medium. After that, the sediment was discarded and the filtrate was taken. Two volumes of refrigerated ethanol at a concentration of 95% were added to it and left at 4°C for 24 hours. Then the EPS was separated by centrifugation at a speed of 12000 rpm for 12 minutes. The filtrate was discarded and the sediment was dried at 41°C for 24 hours for the purpose to calculate calculating the dry weight. **(**20**)**

Drought tolerance test

Polyethylene glycol (PEG 6000) was used at different concentrations in NB medium to evaluate the ability of bacterial isolates to grow under drought - stress stress conditions. Different concentrations of 0% PEG6000, 10%, 15%, 20%, and 25% (wt/vol) were added to the NB medium to adjust the water potential respectively, and then these solutions were inoculated with 1% bacterial isolates grown overnight according to method Joshi's *et al.* (2020). **(**Joshi's *et al* 2020**)** After 24 h of incubation in a shaking incubator (200 rpm) at 30 °C, the OD density was measured at a wavelength of 600 nm using a spectrophotometer. The growth of the strains at different stress levels was recorded by measuring the optical density OD at 600 nm and comparative growth rates (compared to the PEG-free control) were calculated at different water potentials. A direct relationship between water potential and its growth inhibitory activity was determined according to the method of Sati *et al.* (2023). **(**22**)**

The bacterial isolates that produce the most exopolysaccharide and are tolerant to drought were selected and their ability to tolerate drought conditions was estimated.

Preparing the bacterial inculated

The bacterial isolates preserved on Slant were activated by growing them on a suspension of bacterial inoculum prepared for the isolates for seed treatment. They were activated by mixing one colony of each bacterial strain in nutrient N.B medium and incubating the culture for 24 hours at 37°C. (23)

Seed inoculation with bacterial isolates

The bacterial isolates found on Slant, preserved, isolated and previously characterized, whose xopolysaccharides and their ability to tolerate drought were previously determined, were activated. They were activated on N.B. After 24 hours, the isolates were taken and inoculated with lurian bacteria (L.B) and then placed in a vibrating incubator at a temperature of 30 for 72 hours. Then the isolates were centrifuged at (300 revolutions/minute for 10 minutes). After that, the filtrate is discarded and the sediment (pellet) is taken. Then we add small amounts of water to the sediment in order to obtain a reading (OD = 1) at 660 nano, which is equivalent to (10-10) CFU ml-1 (colony forming unit) . The sterilized seeds were then soaked in this bacterial inoculum for 2-3 hours and planted in soil. **(**24**) (**25**) (**26**) (**Khan et al 2012**)**

Extraction of chlorophyll:

Chlorophyll was estimated by Hiscox and Israelstam method (1979), which involves the estimention of plant chlorophyll without maceration. 100 mg of leaves were washed with DW and chopped. These chopped leaves were taken in test tubes in triplicates and 10 ml DMSO was added to each test tube. Test tubes were incubated in a water bath at 60 0 C for 15 min. The absorbance of the solutions was recorded at 663, 645 nm on UV-visible double - beam beam spectrophotometer. (Model. ELIT, BioEra Ltd.) For evaluation of chlorophyll stability index, chlorophyll extract was prepared from 100 mg fresh plant material as well as 100 mg fresh plant material kept in an oven at 600 C for 1 hour using DMSO as a solvent. It was also calculated periodically using the following formula: **(**32**) (**33**)**

$$\text{Chlorophyll Stability Index} = \frac{Total\ chl.content\ of\ heated\ plant\ material}{Total\ chl.content\ of\ fresh\ plant\ material} \times 100$$

Cell membrane stability index

Cell membrane stability index was determined based on the number of ions contained in ddH2O according to Guo *et al.* (2007) and Singh *et al.* (2017). Two hundred mg of leaf were cleaned and cut with a length of 5mm and put in a tube containing 20 ml ddH2O. The tubes were incubated for 12 hours at room temperature with constant lighting The value of the conductivity of the solution is measured with an Electro-conductivity meter (EC-meter CM-21P, TOA Corp, Japan) as the initial conductivity (EC1), then the solution is boiled to 100ºC for 15 minutes and cooled to 25 ºC then the EC is measured as EC2 and ISM determined by the following formula : **(**31**) (**32**)**

$$\text{ISM (\%)} = \left[1 - \frac{\text{EC1}}{\text{EC2}}\right] \times 100\%$$

Stress tolerance index

The Stress Tolerance Index (STI) was calculated for both seedlings and cuttings according to the following equation: **(**29**)**

$$\text{STI} = (\text{Ypi} \times \text{Ysi}) / \text{Ypi}^2$$

- Ysi \ Dry weight of cuttings.

- Ypi \ Dry weight of cuttings treated.

2.2 Stress intensity

The severity of stress was measured according to the method of Fischer & Murrer, (1978) and based on the dry weight of plant samples and was calculated from the following equation:

$$\text{Stress intensity} = 1 - (\text{Ysi} / \text{Ypi})$$

The Statistical Analysis

For statistical analysis, the analysis of variance (ANOVA) had was utilized in to compare different variables; values were presented as the mean ± S.E. of triplicate measurements.

3. Results and Discussion

3.1 Bacterial isolation

20 bacterial isolates of Azotobacter were obtained from 20 soil samples suffering from drought in Karbala Governorate, through the process of isolating and purifying these isolates and cultivating them on selective culture media in the laboratories of the College of Science at the University of Karbala.

3.2 Microscopic and biochemical diagnosis

Azotobacter showed on SMS medium spherical colonies varying in size, smooth and shiny, some of which produced a mucus layer, and their color ranged from white to orange.

When conducting biochemical tests on the isolates of Azotobacter bacteria obtained in this study, it turned out that all of the isolates were positive for the catalase, oxidase, and indole tests. It has the ability to grow in 1% of both NaCl and Glycerol. While it was not able to grow in 0.1% phenol.

3.3 Estimated of exopolysaccharide

All bacterial isolates were examined. Azotobacter spp to test their ability and determine the most efficient among all 20 isolates to produce EPS by calculating the dry weight of EPS as shown in Table No. (3-1)

The results of the dry weight of EPS mg/L showed variation in its ability to produce exopolysaccharides, as production ranged from (131) mg/L for isolate AZ8 to (880) mg/L for isolate

AZ4. The ten samples that produced the largest amount of EPS (1.86, 2.25, 1.88, 1.8, 2.2, 1.94, 2.06, 1.93, 2.7, 2.13) were processed to test their ability to withstand drought conditions, and the other samples were neglected.

Table (3-1) : Screening of bacterial isolates. . *Azotobacter spp* to produce extracellular polysaccharides

Symbol of isolation	AZ 1	AZ 2	AZ 3	AZ 4	AZ 5	AZ 6	AZ 7	AZ 8	Az 9	Az 10	AZ 11	AZ 12	AZ 13	AZ 14	AZ 15	AZ 16	AZ 17	AZ 18	AZ 19	AZ 20
Dry weight of EPS mg/L	420	720	320	880	700	380	442	131	350	660	120	335	370	252	270	230	211	270	239	330
Dry weight of cells mg/L	225	320	170	710	500	210	195	96	180	320	100	215	320	130	100	150	126	190	112	250
Dry weight of EPS/dry weight of cells	1.86	2.25	1.88	1.23	1.4	1.8	2.2	1.36	1.94	2.06	1.2	1.55	1.15	1.93	2.7	1.53	1.67	1.42	2.13	1.32

Drought tolerance

10 isolates that produced the greatest amount of EPS in terms of their ability and efficiency to resist drought were selected by exposing them to five successive concentrations of PEG (0%, 10%, 15%, 20%, 25%). The results showed that isolate (A2) and isolate (A15) were significantly more tolerant than the rest of the isolates, with values reaching (0.582) and (0.586), respectively, as shown in Table No. (3-2). From the results obtained, it is evident that there was a significant decrease in the production of EPS under drought conditions when compared to the control, as well as a significant decrease when the intensity of stress increased in the bacterial growth medium. The bacteria's production of EPS decreased to its lowest level when treated with drought (25%). The decrease reached (0.27).

Table (3-2) : shows the results of the efficiency of *Azotobacter spp* isolates to tolerate drought

Isolate No.	**Polyethylene glycol concentration**					
	0%	10%	15%	20%	25%	**Average**
Az6	0.880	0.660	0.440	0.380	0.270	0.526
Az2	0.770	0.680	0.610	0.540	0.310	0.582
Az7	0.700	0.670	0.620	0.580	0.320	0.578
Az15	0.730	0.680	0.540	0.530	0.450	0.586
Az10	0.740	0.660	0.540	0.490	0.310	0.548
Az19	0.730	0.660	0.590	0.520	0.380	0.576
Az1	0.670	0.660	0.600	0.410	0.330	0.534
Az3	0.690	0.560	0.400	0.400	0.310	0.534
Az14	0.820	0.720	0.510	0.350	0.180	0.534
Az9	0.840	0.720	0.360	0.320	0.180	0.534

L.S.D	0.058					0.026
Average	0.757	0.667	0.521	0.452	0.304	
L.S.D	0.018					

Effect of Azotobacter inoculum on maize plants

After obtaining the results of EPS production tests and estimating their tolerance to drought (PEG) for the 10 isolates, the two isolates AZ1 and AZ2 were selected, as these isolates were the highest in EPS production and most tolerant to drought, to determine the effects of these isolates on the following criteria:

The effect of Azotobacter inoculum on drought tolerance of maize seedlings

Table No. (3-3) shows a clear decrease in the seedling strength mg cm-1 for all yellow maize plants when exposed to drought conditions, while treating the seeds with biofertilizers led to a slight increase in the seedling strength of the yellow maize plants under drought conditions, with a clear difference according to the isolates of Azotobacter bacteria. The greatest effect was most likely after 48 hours of watering, then 24 hours of watering, and then 72 hours of watering, as there was an increase in seedling strength by bacterial fertilizers of the type Azotobacter 1 and Azotobacter 2.

Table (3-3) : Results of mg cm-1 seedling potency of maize plants after treating the seeds with two isolates of Azotobacter

treatment	**Control uninculated**	***Azotobacter* 1**	***Azotobacter 2***	**P value**
watering 24 hours	0.16	0.24	0.28	0.0007
watering 48 hours	0.09	0.26	0.29	0.0007
watering 72 hours	0.01	0.12	0.19	<0.0001

The effect of azotobacter inoculum on the rate of transpiration

The results of this study, measuring the transpiration rates g/hour/plant of yellow corn plants after treating the seeds with different types of biobacterial fertilizers, showed that all types of bacteria contributed to a decrease in the transpiration rate when the plant was exposed to drought conditions compared to the plant that was treated but without drought, with a difference. Significant <0.0001 = p. The effect of two days of watering on transpiration rates was greater than the effect of watering for four or six days, and no significant difference was observed, as the p-value was = 0.22. When watering rates were increased to six days, there was a sharp and variable decrease in the transpiration rate in the presence of bacterial fertilizers. The transpiration rate after watering for six days was the lowest possible when treating the yellow corn plant with the bacterial isolate Azotobacter 1.

Table (3-4) : Transpiration rate g/hour/plant of maize plants after treating the seeds with isolates of Azotobacter bacteria

treatment	**Control uninculated**	***Azotobacter* 1**	***Azotobacter* 2**	**P value**
watering 24 hours	0.09	0.35	0.41	0.22
watering 48 hours	0.04	0.11	0.16	0.004
watering 72 hours	0.01	0.14	0.17	0.0003

The effect of azotobacter inoculum on stress intensity

All yellow maize plants, after being treated with biofertilizers, showed that they suffered less stress intensity when exposed to drought conditions than they did after watering (Table 3-5), as treating the seeds with biofertilizers led to a reduction in stress intensity in yellow maize plants under drought conditions. In cases of watering for different periods of time, the greatest effect was at 72 hours of watering, then 24 hours of watering, then 48 hours of watering. When comparing the stress rate of corn plants treated with bacteria and watered for only 48 hours. *Azotobacter* 2 isolate was more effective in reducing stress intensity, with a significant difference of 0.003. These results indicate that seed treatment with biofertilizers is effective in reducing stress intensity in maize plants under drought conditions. This effect may be due to the ability of bacteria to improve the absorption of water and nutrients by plants, as well as stimulate the production of antioxidants that help protect plants from stress. The period in which yellow corn plants are watered after seed treatment may also play a role in reducing stress intensity. The effectiveness of different types of bacteria in reducing stress intensity.

Table (3-5) : The stress intensity of maize plants after treating the seeds with two isolates of *Azotobacter*

treatment	**Control uninculated**	***Azotobacter* 1**	***Azotobacter* 2**	**P value**
watering 24 hours	0.34	0.72	0.97	0.4
watering 48 hours	0.61	0.58	0.31	0.003
watering 72 hours	0.90	0.66	0.75	<0.0001

The effect of azotobacter inoculum on stress tolerance index

Table No. (8) shows the difference in the effectiveness of biofertilizers and their effect on plant stress tolerance. Stress tolerance increased for all yellow maize plants when exposed to drought conditions. treating seeds with biofertilizers led to a significant increase of (0.001) in stress tolerance under drought conditions. The results also indicated that the greatest effect came after 48 hours of watering, then 24 hours of watering, then 72 hours of watering. *Azotobacter* 1 and *Azotobacter* 2 isolates showed little effectiveness in increasing stress tolerance.

Table (3-6) : Stress tolerance index of maize plants after seed treatment with two isolates of *Azotobacter*

treatment	**Control uninculated**	***Azotobacter* 1**	***Azotobacter 2***	**P value**
watering 24 hours	2.5	2.4	2	0.0003
watering 48 hours	1.8	4.6	2	0.002
watering 72 hours	1	4.4	1.5	0.006

The effect of azotobacter inoculum on membrane stability

Table No. (3-6) shows that all maize plants suffered from a decrease in the stability of the plasma membrane when exposed to drought conditions, and treating the seeds with bacteria led to the stability of the plasma membrane being lower depending on the type of bacteria. The rate of effect of bacteria on the stability of the plasma membrane was greatest at 24 hours of watering, then 48 hours of watering, then 72 hours of watering.

The Azotobacter 2 bacterial isolate was the best in raising membrane stability to the highest extent and improving plasma membrane stability, with a significant difference of 0.005.

These results indicate that seed treatment with biofertilizers is effective in improving plasma membrane stability in maize plants under drought conditions. This effect is due to the ability of bacteria to improve the absorption of water and nutrients by plants, as well as stimulate the production of antioxidants that help protect the plasma membrane from damage.

Table (3-7) : Stability of the plasma membrane of maize plants after treating the seeds with two isolates of *Azotobacter*

treatment	**Control uninculated**	***Azotobacter* 1**	***Azotobacter 2***	**P value**
watering 24 hours	61.1	67.93	83	0.005
watering 48	47.6	58.27	47.43	<0.001

hours				
watering 72 hours	33.8	44.14	42.5	<0.001

The effect of azotobacter inoculum on chlorophyll stability

The results of the current study showed that chlorophyll stability increased with the two bacterial isolates depending on the watering rate (whether 24 hours, 48 hours, and 72 hours) compared to untreated seeds or treated seeds without watering. (Table No. 3-7)

The increase in growth was greatest at 24 hours of watering, then 48 hours of watering, and then 72 hours of watering. The results indicate that the bacterial isolate *Azotobacter* 2 contributed to significantly increasing the stability of chlorophyll, especially after watering for two days.

Table (3-8) : Results of chlorophyll stability in maize plants after treating the seeds with two isolates of *Azotobacter*.

treatment	**Control uninculated**	***Azotobacter* 1**	***Azotobacter* 2**	**P value**
watering 24 hours	72.7	82.2	86.11	< 0.0001
watering 48 hours	56.2	71.35	69.05	< 0.0001
watering 72 hours	42.3	68.54	60.62	< 0.0002

Drought is a prominent abiotic stress that affects global food production, leading to economic losses in crops, especially cereals such as wheat, rice, and maize. This study investigated the abilities of strains of Azotobacter to mitigate the effects of drought stress on maize varieties to enhance their growth. The presence of Azotobacter bacteria in the root area leads to a beneficial exchange between the plant and the bacteria and leads to enhanced plant growth. Due to the richness of secretions released by plants, the rhizosphere constitutes a suitable place for abundant microbial activities. The use of beneficial microbes, especially rhizobacteria within the rhizosphere of plants that possess multifunctional growth-promoting factors and the ability to withstand abiotic stresses, is a cheap and alternative way to increase plant growth under abiotic stresses. Previous researchers indicated that application of PGPR reduces drought stress in plants. (34) (35) (36) (37)

Azotobacter insulation was obtained from the soil around the roots of the yellow corn and its development in the center of SMS, where it showed sticky, convex, high and soft colonies It also increased its remains on the middle of growth. These results are in addition to what was found by Matloub (2012), and (39) that the bacteria Azotobacter. It was one of the dominant species in Iraqi agricultural soils. Between the results of the journey of the journey, the ritual parties, after the installation of the chemical mechanism The size of the size and a profile of the generosity. Often, they are in the form of marriage, and these characteristics are affected by the cultivation and the public jerseys, and this is what is confirmed by the numbers of the previous studies. She was

interested in the study of these platforms. (1998, Madhav Rao and Shankrappa) Beneficial microorganisms from areas experiencing drought can help host plants cope with stress. The corn was inoculated with two types of drought-resistant Azotobacter. Strains under drought stress improved some plant growth parameters such as biomass. Inoculation with the isolated bacteria improved the water use efficiency of the plant . (Marasco et al., 2013) (Shirinbayan et al ., 2019)

Azotobacter isolates enhanced plant growth under drought stress conditions by increasing soil moisture content. This has been made possible thanks to the ability of rhizobacterial strains to produce exopolysaccharides that maintain soil moisture by increasing their ability to retain water and thus protecting bacteria and plant roots from drying out (Ahmed et al., 2022). Thus, EPS production by these microbes may have increased the ability of the soil to balance its water potential and maintain soil aggregation which enhanced nutrient uptake with the resulting growth of maize plants and protection from drought (Subramaniam et al., 2020; Ahmad et al., 2022).). In terms of alleviating abiotic stresses such as drought, EPS-producing microbes are indispensable because they increase the water holding capacity of soil, thus alleviating plants from stress (Ojuederie et al., 2019).

The data in the table show that the Az4 strain is more tolerant to drought conditions, with a score of (0.586), followed by the Az2 strain, which scored (0.582). The strains ranged from (0.526) to (0.583), and among (Shirinbayan etal 2019). out of 20 Azotobacter isolates out of 77 A soil sample from different regions showed the growth of these isolates under conditions of drought induced by PEG 600. It was found that three isolates, az70, az69, and az63, were the most growing and tolerant in the medium containing PEG 600. (Gnosh etal 2019) (مصدر)reported that bacteria produced EPS from drought-tolerant isolates in The growth medium prepared with high concentrations of PEG increases its ability to survive and grow under drought conditions, and the two isolates AZ1 and AZ2 are chosen because they produce more EPS and are more tolerant to drought. PEG 600 in maize seed inoculation experiments, which tolerated drought.

It is a well-known fact that in drought conditions the plant implements a strategy to increase the length of the root system in order to obtain moisture more efficiently. Consequently, the plant will suffer from the consumption of mineral elements, nutrients, and stored water surrounding the root. If using rhizobacteria, a balance must be achieved between the amount of growth stimulants they release and the need for external biologically active substances for the plants themselves. Bacteria maintain the moisture content of the plant to enhance plant growth through a balance in drought stress, transpiration rates, stress tolerance indicators, and chlorophyll and plasma membrane stability, and thus lead to an increase in the root mass of plants and improved growth and is a more useful strategy for plant development under drought stress .(Kudoyarova et al., 2019) (Vinnikova et al ., 2023)

Chlorophyll concentration is reduced under drought stress conditions, which may be because chlorophyll degradation occurs at greater rates than chlorophyll synthesis (Zarei et al., 2020). The concentration of chlorophyll a is generally higher than that of chlorophyll b and carotenoids under drought stress and good water conditions. This can be attributed to the fact that chlorophyll a is the primary pigment while the other pigments are basically secondary pigments. Inoculation of maize plants with Azotobacter significantly enhanced total chlorophyll contents compared to the uninoculated control treatment. Singh NB et al. (2015), reported a significant increase in total chlorophyll content in sunflower plants under water stress upon co-inoculation with Azotobacter, compared to non-inoculated plants. Likewise Saikia et al. (2018) reported a significant improvement

in leaf chlorophyll contents of chlorophyll a and b in plants suffering from drought stress and inoculated with Azotobacter bacteria. The results of this study are consistent with the findings of (Bakry, 2001) when using a bacterial extract Azotobacter on yellow corn plants to increase chlorophyll, which provides additional amounts of nitrogen, which is reflected in an increase in chlorophyll. Increasing nitrogen increases the formation of chlorophyll, as 70% of the nitrogen in the leaf enters the synthesis of chlorophyll, in addition to iron, which has an effective role in increasing the chlorophyll content through the effect of increasing the numbers and sizes of chloroplasts. There was a significant decrease in the concentration of chlorophyll and an increase in the severity of drought stress. The highest levels of chlorophyll in the leaves (82.2) reached the control treatment from decline to (68.54) when the treatment (72 hours) day. The decrease may be attributed to the increase in the effectiveness of the chlorophyllase enzyme due to the decrease in the concentration of (N, Mg) which enter In the structure of the chlorophyll molecule.

Rot stress increases the production of substances such as MDA malon di aldehyde, which is a high-volume peroxide product, an indicator of oxidative damage to the muscle cell membrane by some ROS and accumulates in plant tissues when exposed to the influence of salt, which may increase the amount of carbon dioxide in them, causing an increase in permeability. Important, exposed to electrolytes, and smaller in size for vital systems. (Li et al., 2017) narrowed inoculation with Azotobacter to significantly reduce the amount of MDA in fortified maize, indicating that Azotobacter reduces oxidative stress and thus significantly reduces MDA. This ensured control over their integrity and leakage of important ions . (Abdel Latif et al. 2019).

4. Conclusions

Drought stress had a negative and significant effect on the decrease in the index of tolerance, an increase in the severity of the stress, and an increase in the rate of damage to plasma membranes and the stability of chlorophyll. The use of the vital extract of Azoto bacteria to reduce the severity of stress on maize seedlings in conditions of drought stress by increasing both the tolerance index and stability of chlorophyll and reducing the severity of stress and the percentage of membrane damage under conditions of drought stress.

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