



LEAF AREA, PROLINE AND RELATIVE WATER CONTENT IN THE LEAVES OF MAIZE PLANT UNDER THE EFFECT OF SOIL BACTERIA, ORGANIC MATTER AND WATER STRESS

O.A.R. AL-Khazrji¹, S.A. Al-Saedi² and B.A.A.H. Alkhateb¹

¹ Department of Soil and Water Resource, College of Agriculture, University of Anbar, Iraq.

² Ministry of Science and Technology, Agriculture Research Directorate, Baghdad, Iraq.

Abstract

The objective of this study was to determine the effect of soil bacteria, soil organic matter and water stress on corn (*Zea mays* L.) growth and development as indicated by leaf area, Proline content, in cell sap, and relative water content in the leaves. The experiment was carried out in agricultural research station of Agricultural research directorate, Ministry of Sciences and Technology during the fall of 2018. The studied factors were randomly distributed in a factorial experiment in of Randomized Complete Block Design (RCBD) with three Replicates. First factor was added with/without bacterial inoculate, second factor (Organic matter) was added at three levels viz. 0, 1.5 and 3% of the soil weight, and the third factor (Irrigation) at the water depletion of 50, 60 and 70% of available water. Results showed that both treatments of bacterial inoculation and the addition of organic matter at 3% level showed the highest significant values for the objective studied above at the rate of 5305 and 5438 cm² plant⁻¹ and 79.06 and 80.95% and 56.17 and 59.84 μmol g⁻¹ respectively. Relative water content and leaf area were reduced by 68.75% and 4194 cm² plant⁻¹, under water stress respectively. Proline concentration in the leaves, was increased by 56.56 μmol g⁻¹ with the same increase of water stress. The interference was significant between bacterial inoculate treatments and the 3.0% level of organic matter addition in reducing the effects of water stress on relative water content, leaf area and increasing the proline content in the plant leaves. as it gave a significant difference from the other factors of interference at a rate of 86.35% and 6060 cm² plant⁻¹ on succession and under water depletion 50%, these values did not differ significantly with the treatment of interference and for the same study factors and under 60% water depletion, which gave an average of 85.81% and 5983 cm² plant⁻¹ respectively. The treatment of bacterial inoculation and organic fertilization at the level of 3% and below the level of water depletion 70 and 60% of the available water resulted in recording the highest proline content in the leaves of the plants amounted to 74.12 and 64.22 μmol gm⁻¹, respectively.

Keywords: Azotobacter, pseudomonas, water depletion, Diviner – 2000.

Introduction

In recent years, there has been an increase in global temperatures and irregular rainfall as a result of climate change of large areas within dry and semi-dry areas which lead to the occurrence of droughts and increased their intensity and frequency. These coupled with the increase in population growth rates tremendously increased the demand for water resources to be provided for agriculture and the emergence of environmental challenges related to phenomena Desertification and water deficiency and considering it an obstacle to the development of plant growth and lead to a loss in the outcome of more than 50% of agricultural crops (Farooq *et al.* 2014). As a response to abiotic stress conditions, plants usually develop several physiological and cellular mechanisms to raise the water use efficiency to maintain optimal growth and production (Farooq *et al.*, 2009). The accumulation of proline in cytoplasmic with concentration of 10-25 times of normal level in the plant is considered as one of the molecular defense mechanisms of stressful environmental conditions, including drought (Al-Hilali, 2005). Many studies have shown the positive role of proline and his contribute to the stability of cellular membranes and protection of the enzymes, fats and nucleic acids from the negative effect of free radicals, as it acts as a sniper for these free radicals (Okuma *et al.*, 2000; Yassin, 2001). In addition to his role in regulating the osmotic pressure in the cytoplasm, which would raise the cellular water content and preserve the cell swelling, thus opening the stomata and flow of CO₂ and raise the efficiency of the effectiveness of the process of carbon representation during Droughts. On the other hand, proline is non-toxic and stored nitrogen, carbon and energy which is used by the plant when exposed to environmental stress, as

the oxidation of each molecule of proline produces 30 ATP (Zhang and Becker, 2015; Anjum *et al.* 2014). Mutlak (2018) noted that proline content has increased exponentially in the maize plant exposed to water stress levels. It was found that exposing plants to the depletion rates of 75% of the available water recorded proline content of 86 μmol g⁻¹ compared to the treatment exposed to the rates of 50% of the available water, which recorded content of 71 μmol g⁻¹. Under drought conditions. the relative water content of plant is related to the mechanism of osmotic modification current in the cells of the leaf, including the accumulation of proline, which leads to a decrease in osmotic pressure Accordingly, any disturbance in this mechanism will in return reduce the accumulation of dry matter and then the leaf area, which is the most effective factor in the carbon representation process (Abdul Azim, 2017; Alfalahi *et al.*, 2015; Ali *et al.*, 2011; Farhad *et al.*, 2011). Therefore, recently the researchers interested in adopting low cost, environmentally friendly and it is economically feasibility methods such as the use of bacterial inoculate, interlocks with organic fertilization as one of the alternative solutions for chemical compounds, and the fact that the soil bacteria support plant growth and increases its ability to hold out water stress and it maintains high productivity (Mickan, 2016). By increasing the soil's organic content it will increase the activity of microorganisms as a source of important energy and nutrients to plants and bacteria, in addition it will improve the physical and chemical soil characteristics, which is reflected in plant promotion under drought conditions. The maize crop was used in this study as a field biological indicator because it is one of the most important cereal crops after wheat and rice and that its economic product affected by water stress (Kuscu *et al.*, 2013).

The aim of this study was to evaluate the role of both soil bacteria (*Azotobacter* spp and *Pseudomonas* spp) and organic fertilization under different levels of water stress in growth characteristics, the relative water content and proline in plant leaves.

Material and Methods

A field experiment was carried out during the fall 2018 at the Plant Research Station - Tuwaitha / Agricultural Research Directorate, Ministry of Science and Technology, which is (40) km southeast of Baghdad within coordinates of 33.21° north, 44.52° east and at an a height of 34 degrees Celsius above sea level, in calcareous sedimentary soil of clay texture classified to a level under Typic Torriflu according to the American classification (Soil Survey Staff, 2006). To study the role of plant growth-promoting bacteria (*Azotobacter* spp and *pseudomonas* spp) and organic fertilization under water stress levels in some of characteristics of growth and some biochemical characteristics of maize crop (*Zea mays* L). Plant growth-promoting bacteria were isolated from 25 rhizosphere soil samples with a depth of 30 cm and for different agricultural crops from different areas in Iraq. The field experimental design was randomly complete block design (RCBD) with

three replicates as the bacterial inoculants was the first factor where add with tow levels (with and without adding), the second factor was organic fertilization (characteristics in table 1.), which added with three levels (0, 1.5 and 3.0%) of the soil weight in depth 30 cm and mixing until homogenous. The third factor represented by three levels of water stress, which is (50, 60 and 70%) of available water. Many soil samples were taken for depth (30 cm), the samples were mixed, dried and then grinded by wooden hammer and passed from a sieve with 2 mm diameter holes, for physical and chemical analysis shown in (table 2).

Table 1 : Chemical properties of organic matter

Property	Quantity	Units
pH	7.2	-
EC _e	2	dS m ⁻¹
Organic matter content	41.4	%
C/N	15/1	
Available nitrogen	1.79	
Available Phosphorus	0.62	
Available Potassium	1.66	
Calcium	1.77	
Magnesium	0.93	

Table 2 : Some physical and chemical properties of the experimental field soil

Property	Quantity	Units	Property	Quantity	Units		
Sand	215	g kg ⁻¹	pH	7.15	—		
Silty	378		EC _e	1.5	dS m ⁻¹		
Clay	407		Positive Dissolved Anion	Ca ²⁺	16	meq l ⁻¹	
Texture	Clayey Loam	Mg ²⁺		9.45			
Bulk density	1.33	Na ⁺		10.35			
hydraulic conductivity	1.01	K ⁺		0.74			
Porosity	49.2	%	Negative Dissolved Anion	SO ₄ ²⁻	343.7		
soil moisture	33	0.29		Cm ³ .cm ³	Cl ⁻		2.46
	1500				0.19		HCO ₃ ⁻
available water	0.1	g kg ⁻¹	CO ₃ ²⁻		8.34		
Organic matter content	1.58		Cmol kg ⁻¹				
CEC	21.12	mg kg ⁻¹ soil					
Available nitrogen	66.14						
Available Phosphorus	13.9						
Available Potassium	46.34						

Field preparation and experimental lay out

Experimental area was plown and was carefully rotavated. Entire area was divided into three blocks with eighteen experimental units in each. Units were of 3×3 meter dimensions. Each unit was dividing into four lines 0.75 m apart, each line planted with local maize seeds type (masra) on 17/7/2018 for plant density 53,000 plants ha⁻¹. Diammonium phosphate (DAP) was used as a source of Nitrogen and phosphorus. DAP was added in an amount to provide 199 kg P₂O₅ ha⁻¹ and 36 kg N ha⁻¹ before seeding, while nitrogen requirement is supplemented by addition of urea fertilizer (46% N) at a rate of 183 kg N ha⁻¹ with three equal batches, the first batch was added a week after the start of the vegetative growth, the second batch is two weeks after the first batch and the third batch at the beginning of the flowering. The plants were harvested after full maturity at 10 December 2018.

Method, scheduling and fellow-up of water depletion

The irrigation process was carried out using a drip irrigation system with T-Tape type, after the evaluation process and the determination of the operational pressure which gives the required discharge of 3.84 liter hour⁻¹ at the operational pressure of 60 kPa. Changes in soil moisture content according to the rates of moisture depletion were monitored and determination of irrigation time according to the growing stages of the maize plant by adopting a moisture sensor device type Diviner-2000, which is one of the efficient devices for estimating moisture content after installing its access tube in the middle of each experimental unit (up to 100 cm in depth) and calibrate it in the study soil. All experimental units were irrigated with 50% moisture content of available water at a depth of (0-10) cm until the plant becomes in the vegetative growth stage. The depth of irrigation water was calculated by adopting moisture depletion ratios for each treatment in order to amends for the drained moisture from the soil until the last irrigation and

before the plant reaches the physiological maturity stage according to the equation described by (Kovda *et al.*, 1973):

$$d = [\theta_{f.c} - \theta_w] \times D$$

Where:

d: depth of water added (cm)

$\theta_{f.c}$: volumetric moisture content at field capacity $\text{cm}^3 \text{cm}^{-3}$

θ_w : volumetric moisture content before irrigation $\text{cm}^3 \text{cm}^{-3}$

D: Soil depth at the root system cm^3 .

The relative water content in the leaves % :

The method described by (Barr and Weatherly, 1962) was adopted according to the equation:

$$\text{RWC}(\%) = \left[\frac{(\text{FW} - \text{DW})}{\text{TW} - \text{DW}} \right] \times 100$$

Where:

RWC = relative water content in the leaves (%)

FW = wet weight (g).

DW = dry weight (g).

TW = full weight (g).

The proline content in plant's leaves $\mu\text{mol g}^{-1}$:

The method described by (Bates *et al.*, 1973) was followed according to the following equation:

$$\text{Proline } \mu\text{mol g}^{-1} \text{ FW} = \frac{(\text{ug proline/ml Toluene})}{(115.5 \text{ ug } \mu\text{mol})} \times \text{g sample/5}$$

The leaf area $\text{cm}^2 \text{plant}^{-1}$:

It was estimated by taking ten plants randomly and apply according to (Elsahookie, 1990) equation as follows:

$$\text{LSA} = 0.65 L^2$$

Where:

LSA = leaf area cm^2

L = length of leaf cm

Statistical analysis:

Experimental data and the adjectives studied were statistically analyzed by contrast analysis method (ANOVA) according to randomly complete block design (RCBD), the means were compared by using the less significant difference (LSD) at 5% level of significance and using the SAS 2000 (Statistical Analysis System) program.

Results and discussion

The leaf area of the plant

The results indicated table 3. that the high values of the leaf area of the maize crop was obtained under addition

Table 3 : The effect of inoculate bacterial, organic matter and water stress on leaf area

Treatment		Leaf area $\text{cm}^2 \text{plant}^{-1}$
<i>inoculate bacterial</i>	uninoculate Bacterial	4518
	Inoculate Bacterial	5305
LSD _{0.05} B		51.11
<i>organic matter</i>	Organic matter 0	4291
	Organic matter 1.5%	5005
	Organic matter 3%	5438
LSD _{0.05} O		62.6
<i>water stress</i>	Water Stress 50 %	5393
	Water Stress 60 %	5148
	Water Stress 70 %	4194
LSD _{0.05} S		62.6

bacterial inoculants treatment, with the mean of 5305 $\text{cm}^2 \text{plant}^{-1}$ while the leaf area decreased with the mean of 4518 $\text{cm}^2 \text{plant}^{-1}$ under un-inoculants treatment. This may be due to the improvement of the nutritional status of the plant by increasing availability and absorbing nutrients elements from soil solution such as nitrogen and phosphorus and increasing the plant resistance to extreme environmental conditions. Moreover, the ability of bacterial inoculate production of growth hormones such as Oxins and Geberlins, which was reported by (Gholami *et al.*, 2009; Khan *et al.*, 2009) may be other important factor. Gradual significant increase was observed in the leaf area of the maize plants that added 1.5% and followed by the organic fertilizer treatment at level 3% with mean of 5005 and 5438 $\text{cm}^2 \text{plant}^{-1}$ respectively. However leaf area in treatment with no organic fertilizer addition was 4291 $\text{cm}^2 \text{plants}^{-1}$ as an average. Addition of organic fertilizer has led to increase the leaf area of the plant as an important physiological characteristic in the process of carbon or nutritional representation of the plant compared to the plants with not add organic fertilizer (control). The increase is due to the effect of organic fertilization in increasing plant efficiency to absorb nutrients from the soil as a result of the increased soil susceptibility to water retention due to the high retaining capacity of organic matter and then increase of available elements in the soil solution. This was positively reflected in Increasing the processes of cell division and expansion of leaf cells, as well, and then increasing the leaf area of the plant. These results are consistent with those of (Uwah *et al.*, 2014; Kumar *et al.*, 2012). The increase in water depletion levels (table 3) led to a significant decrease in the leaf area values of the maize plant at a significant level of 5%, as it recorded the highest mean of area at water depletion levels of 50% was 5393 $\text{cm}^2 \text{plant}^{-1}$ and then significant gradually decreased with increased levels of water depletion. However the treatment with high water stress 70% was recorded of the lowest mean of 4194 $\text{cm}^2 \text{plant}^{-1}$. The reason for the reduction of leaf area with increased levels of water stress to the occurrence of reduction in the growth of the leaves due to the decrease in the process of division and elongation of cells due to the decrease in the relative water content of leaves. This is was attributed to, increase the leaf water pressure and then reducing the opening of stomata and reduce the process of carbon representation. Accordingly production of plant pigments, including chlorophyll reduces the carbohydrates produced which negatively effects on leaf area, (Abdul Azim, 2017; Alfalahi *et al.*, 2015).

The results are shown in figure 1. That the values of the maize leaf area were affected by the interference between the study factors. It should be noted that the addition of bacterial inoculate under the levels of organic fertilization has resulted in a significant increase in the leaf area, especially under the treatment with water depletion 50%. The treatment of bacterial inoculate and organic fertilizer with level of 3% and below the level of water depletion 50% showed 6060 cm²

plant⁻¹, which is the highest leaf area. However, this treatment did not significantly differ with the same above study factors and under the water depletion level 60%, which gave a mean of 5983 cm² plant⁻¹ with increase rate of 40.34 and 57.15% compared to the treatment that did not adding study factors under the level of water depletion 70% which reported a mean of 3114 cm² plants⁻¹.

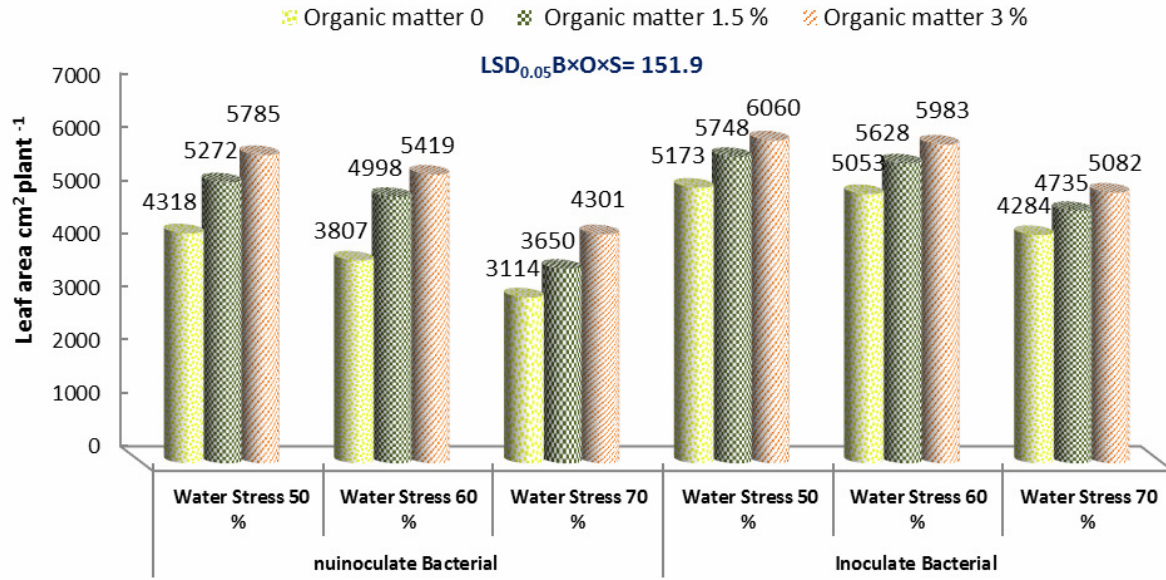


Fig 1 . The effect of inoculate bacterial , organic matter and water stress on leaf area

Relative water content in leaves

The results in Figure 2. show the effective impact of plant growth-promoting bacteria on the relative water content in leaves of the maize plant. It is clear that the values of the relative water content achieved the highest mean of 79.06% when adding the bacterial inoculants compared to the non-adding treatment (control) which reported a decrease in the water content of the leaves with a rate of 71.88%. The higher relative water content in the maize leaves is due to the role of bacteria in improving the water state of the plant by secretion the growth-promoting hormones such as indole acetic acid, which stimulates the growth of the plant's root system (Dilfuza, 2011). The increases in the surface may be due to increase of absorption water from the soil or may be due to role of these bacteria in increase the availability of nutrients (Martin,2011) which stimulates the growth of plant root system by increasing their solubility through the release of organic acids. Therefore, both steps will increase the relative water content of the leaf and this result is in agreement with those of (Chakraborty *et al.*, 2013; Vardharajula *et al.*, 2011). The addition of organic fertilization at 1.5% and 3% levels has improved the water state of the plant, as there was a variation in water content values as shown in (Figure 3). At1.5 % fertilizer, a mean water content in plant was 75.78%

and the treatment of 3% fertilizer was reported the highest mean 80.95% of water content compared to 69.68% water content under the non-fertilization treatment (control). , This increase may be attributed to the role of organic matter, which helped to improve soil construction and the stability of its concentrations, which in return increased the ability of the soil to hold water (Leu, 2010). In addition to considering organic matter as an important source of many nutrients, which play a role in encouraging plant growth, especially the root system (Al-Khateb,2018) this positively reflected on the relative water content in the leaves of the plant . Results in Figure 4. show the effect of water stress levels on the values of the relative water content of the leaf was observed when the water depletion 50% increase in the relative water content of the leaves, which recorded a mean of 80.29% with increase rate of 3.77 and 16.78% compared to the levels 60% and 70% which recorded a significant decrease in the values of The relative water content it was 77.37 and 68.75%, respectively. The reason for this decrease in the relative water content for plants exposed to water stress is due to the inability of the plant to absorb water due to the lack of soil moisture, which is reduced the turgor pressure and transpiration of plant cells. This result is consistent with (Kebede *et al.*, 2014 and Morales, 2009).

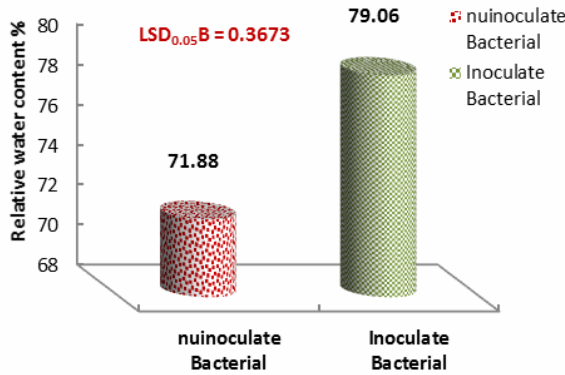


Fig 2 . The effect of inoculate bacterial on Relative water content

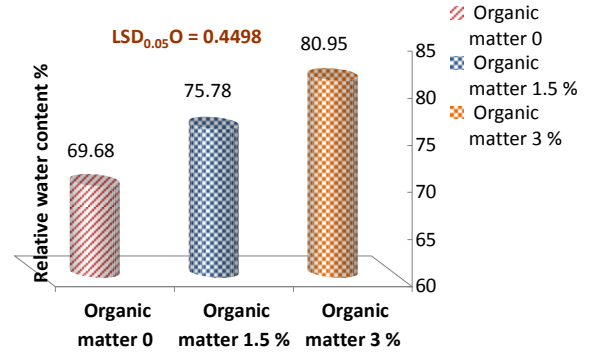


Fig 3. The effect of organic matter on Relative water content

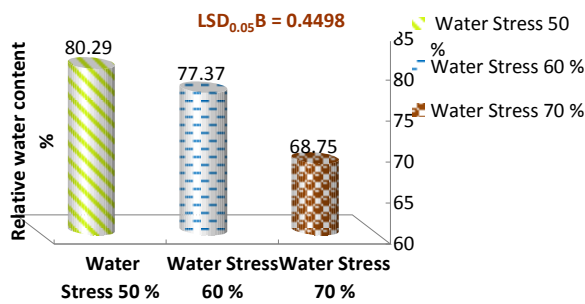


Fig 4 . The effect of water stress on Relative water content

Results in Figure 5. interact effect of this study factors in the values of the relative water content in the leaves, apparently, there is a direct relationship in increasing of the relative water content in the leaves with increasing of organic matter levels. On the other hand, there is inverse relationship with increased levels of water stress, but when the bacterial inoculate interferes with organic matter and water stress levels then means of water content significantly exceeded their means without the addition of bacterial inoculate. The

treatment of bacterial inoculate with organic matter at the level of 3% under depletion level of 50% has significantly exceed their other interference factors at a mean of 86.35%, which did not differ as a result from the same interference of study factors for the previous treatment and under the water depletion level 60%, which gave a mean of 85.81%, while the treatment of interference, which did not add the study factors and under the rate of water depletion 70%, gave the lowest mean of 57.37%.

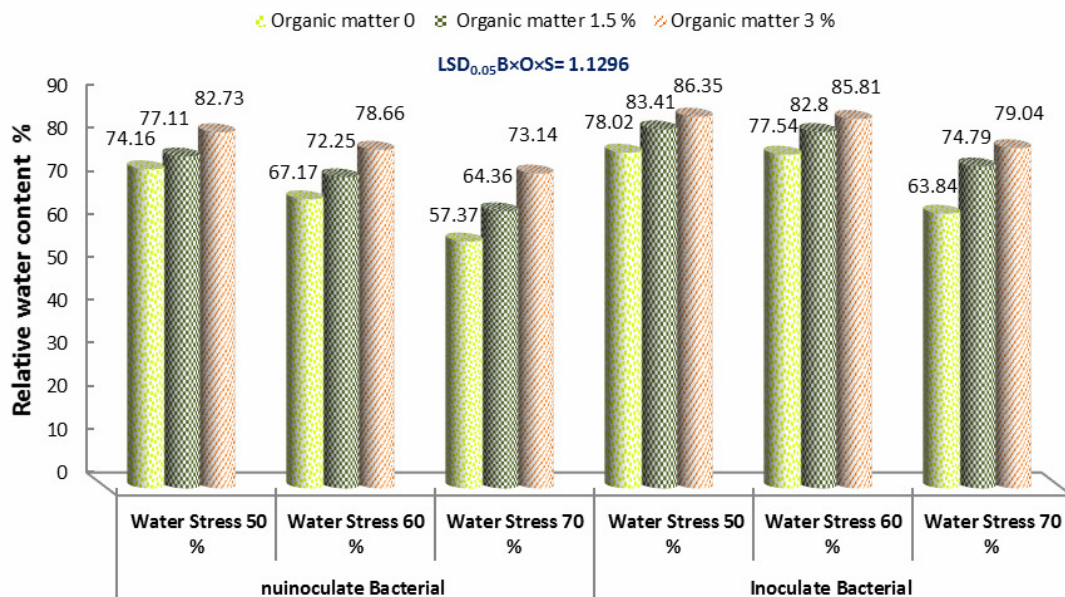


Fig 5 . the effect of inoculate bacterial , organic matter and water stress on Relative water content

Proline content in plant leaves

Figure 6. shows the effect of the bacterial inoculate, organic matter and water stress on the proline content of the leaves, proline content was increased in leaf content at bacterial inoculate treatment of $56.17 \mu\text{mol g}^{-1}$, while rate of proline content decreased to $42.83 \mu\text{mol g}^{-1}$ with un-inoculate treatment (control). This result is due to the use of the bacterial inoculate for its beneficial effect in increasing the efficiency of plant growth and enhancing its water and nutrition condition by increasing the accumulation of proline within the plant. These results can be explained by increasing the supply of the proline synthesis mechanism with nitrogen source in the form of ammonium NH_4 is available and ready to be absorbed by the plant roots and without spending any vital energy, and thus enhances the plant's tolerance for water stress which is in contrary to the nitrate form that are provide to the soil from chemical sources which may be supported by the findings of (Ding *et al.* 2018; Guo *et al.* 2007). It should be The increase in the levels of organic matter added to the soil from 0% to 3% (Fig. 7) leads to a significant increase in the proline content in the leaves and showed highest rate of $59.84 \mu\text{mol g}^{-1}$ followed by the treatment at the organic level 1.5% which recorded a rate of $48.06 \mu\text{mol g}^{-1}$ compared to the non-organic matter treatment, which gave the lowest rate of $40.61 \mu\text{mol g}^{-1}$, the high proline content in maize leaves with increased levels of organic matter it may be due to the main role of organic matter in improving the physical, chemical and fertility soil properties, in addition to considered as a substance help reserve water because of its high absorption capacity. Thus increase the capacity of soil retention of available water and provide it to the plant during the period of fluctuating soil of moisture content (Alinezhad

et al. 2013). This, on the other hand can explaining the role of organic matter in raising the proline content in plant leaves which may be due to increase in the accumulation of osmotic activity elements such as potassium K^+ inside the stress plant tissue. This was reflected in the improvement of the plant's ability to absorb water from the soil and ultimately leads to increase the proline content in the leaves (Mannan *et al.* 2016; Zain, 2016; Ali *et al.* 2014). As noted in (fig. 8) when increasing levels of water stress to 60% and 70% it will stimulate accumulation of amino acid (proline) in the leaves, where the treatment of 50% water depletion was the lowest proline content reached $43.88 \mu\text{mol g}^{-1}$ and then it rises to $56.56 \mu\text{mol g}^{-1}$ in the treatment of 70% water depletion. A finding which may be attributed to the amino acid (proline) is becoming more concentrated due to the inability of the stressed plant to protein syntheses as a result of stimulating proteinase enzyme (protein analyzer). Results in increased proline concentration with increased levels of water stress were also observed by (Hassan, 2014). proline also plays a role in reducing the Negative effects of oxidative stresses by regulation and raising the osmotic pressure in the plant cell cytoplasm, which would raise the cellular water content by maintaining the water pressure gradient towards the entry of water from the soil into the cells of plant root tissue. Which keeps the cell bulging, expanding and elongation, thus maintaining the opening of the stomata, flow of CO_2 and increasing the efficiency of the carbon representation process during drought periods, proline also contributes to the stability of cellular membranes and protect enzymes, fats and nucleic acids from the negative effect of free radicals (ROS), Being a sniper of this free radicals (Salehi *et al.* 2016).

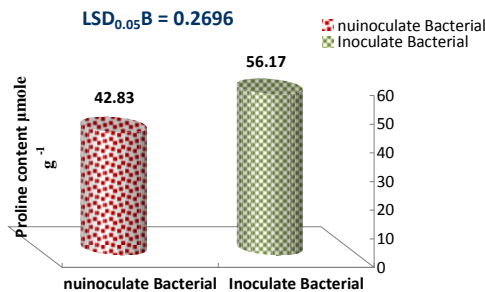


Fig 6 . The effect of inoculate bacterial on proline content

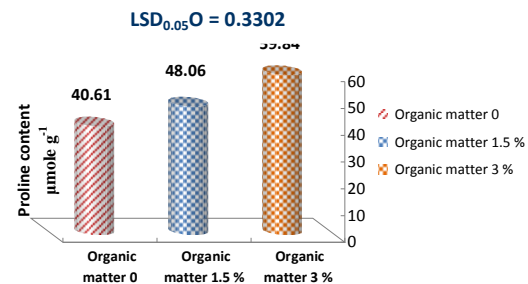


Fig 7 . The effect of organic matter on proline content

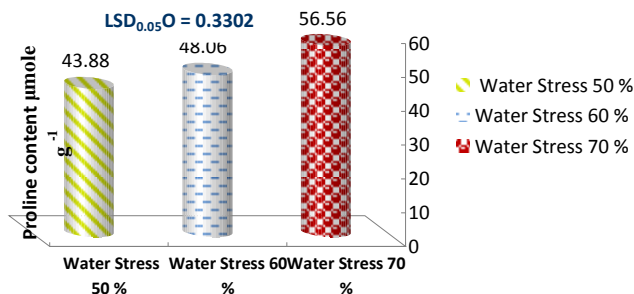


Fig 8 . The effect of water stress on proline content

The results in Figure 9. show that the proline content in the leaves increases significantly with increased levels of both organic matter and water stress, whether by adding or not adding bacterial inoculate , but the effect of adding bacterial inoculate on raising the proline content in the leaves was significantly exceed to non-addition treatments and under all levels of organic matter and water stress. The results indicate that the treatment of bacterial inoculate with

3% organic matter and under the 70% water depletion was significantly exceed on the other interference treatments at a rate of $74.12 \mu\text{mol g}^{-1}$ followed by the treatment of interference bacterial inoculate and 3% organic matter under 60% water depletion at a rate of 64.22 g^{-1} compared to the interference treatments without added to the study factors and under the 70% and 60% water depletion at a rate of 40.31 and $34.49 \mu\text{mol g}^{-1}$ respectively.

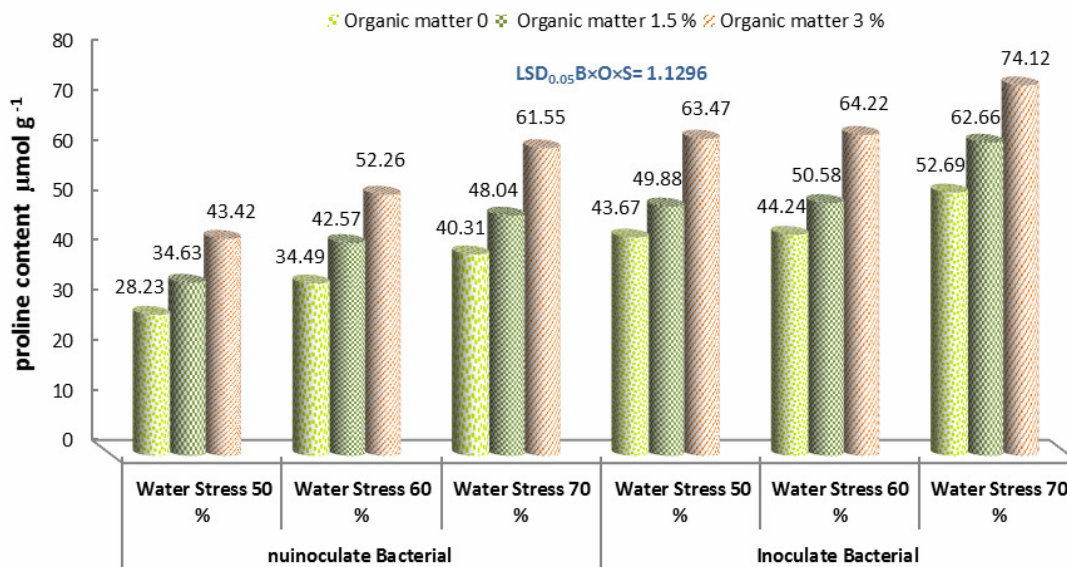


Fig 9 . The effect of inoculate bacterial , organic matter and water stress on proline content

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