

## Amelioration of waterlogging conditions on the growth *Zea mays* L. by marine algae extracts as biofertilizers and salicylic acid application

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**Abstract:** *Zea mays* L. tolerated drought stress up to 150 % water field capacity (F.C.). Water stress (waterlogging condition) decreased maize growth parameters especially at high drought stress level 150 % water field capacity (F.C.%). Marine algae extracts as biofertilizers and salicylic acid foliar application increased height plants and leaf-area at 200 % water field capacity compared with absolute control treatment. Marine algae extracts as biofertilizers and salicylic acid foliar application increased maize dry shoot-mass 200 % field capacity as compared with control plant. Marine algae extracts as biofertilizers and salicylic acid foliar application increased maize chlorophyll a, b and carotenoids at 100 % field capacity as compared with control plant. Marine algae extracts as biofertilizers and salicylic acid foliar application highly significant increased shoot soluble carbohydrates up to 200 % field capacity as compared with the control plant. Calcium, Potassium and Phosphorus accumulation was decreased by decreasing water field-capacity in maize plants of control-plant. Phosphorus accumulation was increased by Marine algae extracts as biofertilizers and salicylic acid foliar application in the maize plants and recorded increases compared with control, respectively. Salicylic acid application with or without marine algae extracts as biofertilizers reported increases in proline in the shoot system. Hydrogen peroxide  $H_2O_2$  generation was increased in flooding treatments and increases at 150 and 200 % field-capacity level, respectively. Marine algae extracts as biofertilizers or salicylic acid reduced  $H_2O_2$  concentration as compared to control plant.

**Key word :** maize plants, marine algae extracts as biofertilizers, salicylic acid foliar application, field capacity

### Introduction

The word *Zea mays* comes from two languages. *Zea* comes from ancient Greek and is a generic name for cereal and grains. Some scientists believe that *Zea* stands for "sustaining life". *Mays* comes from the language Taino, meaning "life giver." Maize or corn (*Zea mays* L.) is the world's third leading cereal crop, after wheat and rice ( Parle and Dhamija, 2013 ). Maize (*Zea mays* L.) plays a significant role in human and livestock nutrition worldwide. In global it is an important cereal crop ranks third and first position in terms of acreage and production, respectively. Due to high yield potentiality, versatile uses, and almost year round growth ability and higher per acre yield compare to other cereals, area and production of maize is increasing day by day in our country. Its production also has increased significantly in the country because of the fast growing poultry and poultry feed industry, and price hike of food materials. The maize crops grown during the summer season occasionally face extreme climatic conditions and biotic/abiotic pressure that limits crop growth and development, and eventually limits yield potential. Among the abiotic stresses, excessive soil moisture, caused by flooding, water logging or high water table, is one of the most important constraints for maize production and productivity. More than 5-10% of the total maize growing area is affected by floods and water logging problems in global. However, considerable genetic variability has been observed in maize for tolerance to excess moisture. That variability may

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be exploited to develop maize varieties tolerant to excess soil moisture condition. Inability of non-wetland crop species, including maize, to with stand excessive soil moisture conditions in the rhizosphere, caused by water-logging or any other factor, results in substantial yield losses. Maize crops grown during the summer-rainy season in the tropics occasionally face extreme climatic conditions and a variety of biotic and abiotic pressures that limit yield potential. Among abiotic stresses, water-logging, caused by contingent flooding, continuous rainfall coupled with inadequate drainage or a high water table, is one of the most important constraints for maize production in Asia and many other parts of the world. In South and Southeast Asia alone, over 18% of the total maize growing areas are frequently affected by floods and water-logging problems (Zaidi *et al.*, 2009). Excessive moisture or submergence leads to reduced gas exchange between root tissues and the atmosphere because the diffusion rate of gases in flooded soil is approximately 100 times lower than in air (Kennedy *et al.*, 1992). Respiration by plant roots, soil micro-flora and fauna leads to a rapid exhaustion of soil oxygen, resulting in hypoxia followed by anoxia. Unlike rice plants, maize plants have no naturally occurring air spaces in their roots. Therefore, as a result of the gradual decline in oxygen, plant roots suffer hypoxia (low oxygen) followed by anoxia (no oxygen) when faced with prolonged (>3 days) excess soil moisture (Dennis *et al.*, 2000; Zaidi and Singh, 2002). However, the extent of damage due to water-logging stress varies significantly with the developmental stage of the crop. Previous studies have shown that maize is comparatively more susceptible to water-logging from the early seedling stage to the tasseling stage (Mukhtar *et al.*, 1990; Zaidi *et al.*, 2004). However, significant genetic variability has been observed in the tolerance of maize to water-logging stress (Torbert *et al.*, 1993; Rathore *et al.*, 1996; Zaidi *et al.*, 2002, 2003, 2007a). This variability could be exploited to develop maize varieties tolerant to contingent/ intermittent water logging stress during the summer-rainy season in the tropics. An effective breeding strategy for developing water-logging tolerant cultivars primarily depends on a sound knowledge and understanding of the inheritance mechanism of the stress tolerance in tropical maize. Studies on the combining ability of water-logging stress tolerance in Indian maize have been attempted (Khera *et al.*, 1990; Hossain, 2001), however, only limited information on some location-specific germplasm is available. We selected maize inbred lines from wide genetic background, including Indian maize program and CIMMYT lines from diverse sources. Biofertilizers drew the attention as a partial part goal alternative to N fertilizer application. In addition, biofertilizers have many advantages i.e. supply part of plant N. requirement by 25%, increase the availability of nutrients, reduce the environment pollution, control the vegetative growth and improve the yield potential (Inderjit and Dakshini, 1997; Chunchun *et al.*, 1998; Saad and Ahmed, 2002; Cocking, 2003 and Gomaa, 2008). Inoculation of corn seeds with VAM mycorrhizae could supply the plants with apart of nitrogen required and could increase grain yield, its attributes and chemical composition (Radwan, *et al.* 2008; Ahmed *et al.* 2003; Virendra and Ahlawat, 2004 ; Mekail *et al.*, 2005). Microalgae are agriculturally significant source of biofertilizers, predominantly for the tropical rice fields. Cyanobacterial biofertilizers served for a variety of purposes including soil enrichment in fixing atmospheric nitrogen and essential microelements for the growth of crop plants such as rice and wheat. Additionally, algae produce bio-active compounds (secondary metabolites) that inhibit the growth of plant pathogenic bacteria and fungi ( Boddey and Dobreiner, 1988 ; Bohnert, *et al.*, 1995 ; Bashor and Dalton, 1999 ; Badr and Authman, 2006 ) increase growth and development of some plant species. The microalgae also provide organic matter for plant growth ( Bray, 1997 ; Bray *et al.*, 2000 ) . Thus the objective of present study was to investigate the effect of marine algae extracts as biofertilizers and salicylic acid foliar application on growth and some metabolic activities of maize grown under waterlogging conditions.

### Material and Methods

The experiment was conducted on the research green house of the department of agronomy, faculty of agriculture, Omar Al-Mukhtar university, Libya during in the summer season of 2017 (from April to July). The study was investigated the effect of marine algae extracts as biofertilizers and salicylic acid foliar application on growth and some metabolic activities of maize (*Zea mays* L. var. local) grown under flooding conditions. Seeds of Maize were obtained from Department of Agronomy, Faculty of Agriculture, Omar El-Mukhtar University, Libya. Seeds were selected and surface sterilized with a mixture of ethanol (90%) and H<sub>2</sub>O<sub>2</sub> (25%) in a ratio of 1:1 (V:V) for 3 minutes, followed by several washings with sterile distilled water. Sterilized seeds were germinated in sterile Petri dishes containing damp sterile filter paper. Sterile water was added at intervals to keep the paper and germinated seeds wet. Dishes were incubated at 30°C for 2-3 days or until the radicals were 2-3 cm long. Randomized Complete Block Design (RCBD) in three replicates was used in this pots experiments included in this study were carried out during the convenient in summer seasons of the years 2016-2017 (from April to July). The study was done to investigate the effect of deleterious effects of flooding conditions (100%, 150% and 200% field capacity (F.C.) on growth and some morphological and physiological changes of maize (*Zea mays* L. var. local cultivar) grown in pot experiments. Pots were kept in the wire proof greenhouse. Five kilograms of dried soil was put into each pot. When the growing plants were about 12 cm length, they were thinned down to three per pot, and pots were divided into three groups: seedlings of the first pots group were inoculated with marine algae extracts as biofertilizers and their soil moisture content was adjusted to 100%, 150% and 200% field capacity as control and flooding conditions treatments. The second group of pots, seedlings (2 weeks old) were sprayed 3 times with 1 mM salicylic acid (SA) (10 ml per pot) and their moisture content was adjusted to the corresponding water field capacity (100%, 150% and 200%). Pots of the third group were adjusted to the corresponding water field capacity but left without algae inoculation or SA application (as control treatments). Pots were then irrigated with tap water to maintain the required field capacities. After 60 days of sowing, plants were harvested, shoot and root system were separated for further analysis.

#### Treatments :

The marine algae extract used in the research study is a liquid solution in a ready-made package imported by the Agricultural Research Center - the Libyan Ministry of Agriculture during the year 2017 and it contains the following ingredients :

No.	Ingredients	Quantity	Quantity
1	Organic matter Seaweed extract	375 g / L	3.75 %
2	Proteins	125 g / L	1.25 %
3	Nitrogen	67 g / L	6.7 %
4	Phosphor	67 g / L	6.7 %
5	Potassium	67 g / L	6.7 %
6	Iron	550 g / L	550 ppm
7	Zinc	450 mg/L	450 ppm
8	Manganese	160 mg/L	160 ppm
9	Cobalt	10 mg / L	10 ppm
10	Molybdenum	120 mg / L	120 ppm
11	Magnesium	540 mg / L	540 ppm
12	Boron	150 mg / L	150 ppm
13	Calcium	100 mg / L	100 ppm
14	Copper	60 mg / L	60 ppm

**Field Capacity (F.C.)** is the amount of soil moisture or water content held in the soil after excess water has drained away and the rate of downward movement has decreased. This usually takes place 2–3 days after rain or irrigation in pervious soils of uniform structure and texture.



**Salicylic acid**, a natural compound extracted from Willow bark, is an anti-inflammatory inhibitor of activity cyclooxygenase. Salicylic acid (SA) is a phenolic phytohormone involved in plant defence against pathogens.

Linear Formula  $2\text{(HO)C}_6\text{H}_4\text{CO}_2\text{H}$   
Molecular Weight 138.12

### Growth characters

Observations were made from selected plant for each experiment after 60 days. The effect of treatments were noticed on morphological and yield parameters such as : Plant height / cm, No. of leaves / plant, Fresh weight g / plant, Dry weight g / plant, Leaf area /  $\text{cm}^2$ , No. of grains / ear, Ear weight / g, 100 Grain weight / g, Grain yield ( t / h ), Bio yield ( t / h ) and harvest index ( % ), the harvest index was accounted with follow :  $\text{HI} = (\text{Economical yield} / \text{Biological yield}) \times 100$ . Leaf area was determined according to Norman and Campbell (1994) by measuring leaf length and maximum leaf width according to the formula: Leaf area = K (leaf length  $\times$  leaf maximum width), Where the coefficient K = 0.7 for monocot plants. For determination of phosphorus (P), calcium (Ca), potassium (K), samples (0.5 g) were dried at 70 °C, ground and digested by mixture of  $\text{H}_2\text{SO}_4\text{-H}_2\text{O}_2$  as the procedure described by Lachica *et al.*, (1973). The extract was used for elements determination. The phosphorus content was determined calorimetrically according to the method of Rodriguez and Fraga, (1999). potassium, and calcium ions were measured by flame emission photometry according to Brown and Lillie (1946). The photosynthetic pigments were extracted from a known fresh weight of leaves ( 0.2 g ) in 85% aqueous acetone to certain concentration for spectrophotometric measurements. The photosynthetic pigments (chlorophyll a, b and carotenoids) were determined spectrophotometric method as described by Metzner *et al.*, 1965 .

$$\text{Chlorophyll a} = 10.3 E_{663} - 0.918 E_{644} = \text{mg/ml}$$

$$\text{Chlorophyll b} = 19.7 E_{644} - 3.87 E_{663} = \text{mg/ml}$$

$$\text{Carotenoids} = 4.2 E_{452} \begin{cases} 0.0264 \text{ chl. a} \\ + \\ 0.4260 \text{ chl. b} \end{cases} = \text{mg/ml}$$

Finally these pigment fractions were calculated as mg/g fresh matter.

Free proline amount was measured according to Bates *et al.* (1973). To estimate soluble carbohydrates, 2N HCl in a water bath hydrolysed a known weight of the dried tissue material for one hour. After cooling, the hydrolysate was filtered and then completed to a defined volume. The total carbohydrates were determined by the method of anthrone sulphuric acid that was carried out by Fales (1951), A.O.A.C. (1995). Hydrogen peroxide levels were determined according to the method of Sergiev *et al.*, (1997). 0.5g of fresh leaf or root was homogenized with 5 ml 0.1% (w:v) Trichloroacetic (TCA). The homogenate was then centrifuged for 15 min at 4000 rpm. and 0.5 ml of the supernatant was added to 0.5 potassium phosphate buffer (PH 7.0) and 1 ml of 1.0 M potassium iodide (KI). The absorbance of the supernatant was read at 390 nm. The content of  $\text{H}_2\text{O}_2$  was expressed as absorbance. Total nitrogen were determined according to the Association of official Agricultural Chemistry (A.

O. A. C.,1995), Data obtained during season was exposed to the proper method of statistical analysis of variance ( ANOVA ) as described by Steal and Torrie, (1960); Duncan's new multiple range test was used to differentiate between means as described by Duncan,( 1955 ) at 5% probability level.

### Results and Discussion

In this study, Results presented in (Tables 1 to 3) show that in control non-treated maize plants *Zea mays* L. increasing soil water content than the field capacity (flooding conditions) resulted in decreased plant growth parameters ( Plant height / cm, No. of leaves / plant, leaf area cm<sup>2</sup>, Fresh weight g / plant, Dry weight g / plant, Leaf area / cm<sup>2</sup> ). On the other hand, marine algae extracts as biofertilizers and salicylic acid foliar application resulted in significant increases in these growth parameters at all levels of soil moisture content as compared with both the corresponding and absolute ( control of 100 % F.C. ) control treatments. For example, the growth parameters recorded, Results showed that, the effect of marine algae extracts as biofertilizers and salicylic acid foliar application on growth of maize grown under flooding conditions on Plant height / cm and No. of leaves / plant were significant (Table 1). The comparison of the mean values of the Plant height / cm and No. of leaves / plant for interaction between marine algae extracts as biofertilizers, salicylic acid foliar application and field capacity (100 %) had the highest (201.2 cm, 216.4 cm and 12.5, 14.4) compared with the absolute control treatment had the

**Table 1:** Effect of marine algae extracts as biofertilizers and salicylic acid application on Plant height / cm, No. of leaves / plant and leaf area cm<sup>2</sup> of *Zea mays* L. grown at waterlogging conditions

Variable tested	Field capacity	Plant height / cm	No. of leaves/ plant
Control	Control	247.1 <sup>a</sup>	15.3 <sup>a</sup>
	100 %	212.4 <sup>b</sup>	13.1 <sup>b</sup>
	150 %	203.6 <sup>c</sup>	12.0 <sup>c</sup>
	200 %	198.2 <sup>d</sup>	11.2 <sup>d</sup>
Salicylic acid	Control	249.1 <sup>a</sup>	16.1 <sup>a</sup>
	100 %	216.4 <sup>b</sup>	14.4 <sup>b</sup>
	150 %	209.6 <sup>c</sup>	13.1 <sup>c</sup>
	200 %	201.2 <sup>d</sup>	11.9 <sup>d</sup>
Marine algae extracts as Biofertilizers	Control	246.5 <sup>a</sup>	15.9 <sup>a</sup>
	100 %	201.2 <sup>b</sup>	12.5 <sup>b</sup>
	150 %	198.2 <sup>c</sup>	11.8 <sup>c</sup>
	200 %	187.6 <sup>d</sup>	10.7 <sup>d</sup>

Mean having the same small letters in the same row are not significantly differed at p: 0.05

lowest Plant height and No. of leaves / plant (212.4 cm and 13.1) and the differences were significant, respectively. The analysis of variance showed that, the effect of marine algae extracts as biofertilizers and salicylic acid foliar application on growth of maize grown under flooding conditions on Fresh weight g / plant and Dry weight g / plant were significant (Table 2), The comparison of the mean values of the Fresh weight g / plant and Dry weight g / plant for marine algae extracts as biofertilizers and salicylic acid foliar application and field capacity (100%) had the highest (560.1 g, 562.2 g and 129.7 g, 134.5 g) compared with the absolute control treatment had the lowest Fresh weight g / plant and Dry weight g / plant (553.6 g and 122.1 g) and the differences were significant, respectively. The analysis showed that, the effect of marine algae extracts as biofertilizers and salicylic acid foliar application on growth of maize grown under flooding conditions and interaction between them on Leaf area



/ cm<sup>2</sup> and No. of grains / ear were significant (Table 3), The comparison of the mean values of the Leaf area / cm<sup>2</sup> and No. of grains / ear for interaction between marine algae extracts as biofertilizers, salicylic acid foliar application and field capacity (100 %) had the highest (411.6 cm<sup>2</sup>, 423.1 cm<sup>2</sup> and 459.5, 460.1) compared with the absolute control treatment had the lowest Leaf area / cm<sup>2</sup> and No. of grains / ear (397.1 cm<sup>2</sup> and 454.6) and the differences were significant, respectively.

**Table 2:** Effect of marine algae extracts as biofertilizers and salicylic acid application on Fresh weight g / plant and Dry weight g / plant of *Zea mays* L. grown at waterlogging conditions

Variable tested	Field capacity	Fresh weight g / plant	Dry weight g / plant
Control	Control	745.3 <sup>a</sup>	151.3 <sup>a</sup>
	100 %	553.6 <sup>b</sup>	122.1 <sup>b</sup>
	150 %	447.2 <sup>c</sup>	98.6 <sup>c</sup>
	200 %	288.4 <sup>d</sup>	67.3 <sup>d</sup>
Salicylic acid	Control	751.0 <sup>a</sup>	157.1 <sup>a</sup>
	100 %	562.2 <sup>b</sup>	134.5 <sup>b</sup>
	150 %	453.2 <sup>c</sup>	102.6 <sup>c</sup>
	200 %	301.5 <sup>d</sup>	88.2 <sup>d</sup>
Marine algae extracts as Biofertilizers	Control	746.5 <sup>a</sup>	159.2 <sup>a</sup>
	100 %	560.1 <sup>b</sup>	129.7 <sup>b</sup>
	150 %	451.0 <sup>c</sup>	104.3 <sup>c</sup>
	200 %	299.3 <sup>d</sup>	98.5 <sup>d</sup>

Mean having the same small letters in the same row are not significantly differed at p: 0.05

While, the comparison of the mean values ( Tables 4 and 5 ) of the Ear weight (341.1 and 336.5 g), 100 Grain weight (20.09 and 20.21 g), Grain yield (2.69 and 2.71 t / h ) , Bio yield (6.50 and 6.48 t / h ) and harvest index (41.4 and 41.8 % ) was increased and the differences were significant for interaction between marine algae extracts as biofertilizers, salicylic acid foliar application and field capacity (100 %), respectively. Previous studies on the reduction in plant growth due to water stress has been widely reported (El-Komy *et al.*, 2003). In the present study, biofertilizers inoculation and (or) salicylic acid (SA) application improved wheat growth compared to non-treated plants which indicated that these treatments helped wheat plants to mitigate adverse effects of water stress. biofertilizers that are beneficial to plants are of two general types: those form a symbiotic relationship, which involves formation of specialized structures or nodules on host plant roots, and those that are free-living in the soil (El-Komy *et al.*, 2003). Numerous free-living soil organism are considered to be plant growth-promoting bacteria (PGPB). Moreover fluorescent biofertilizers are well known for their ability to colonize the root tissues of wide crop plants and promote the plant growth . There are several ways in which plant growth promoting organism can directly facilitate plant proliferation.

**Table 3 :** Effect of marine algae extracts as biofertilizers and salicylic acid application on Leaf area / cm<sup>2</sup> and No. of grains / ear of *Zea mays* L. grown at waterlogging conditions

Variable tested	Field capacity	Leaf area/ cm <sup>2</sup>	No. of grains / ear
Control	Control	455.2 <sup>a</sup>	543.2 <sup>a</sup>
	100 %	397.1 <sup>b</sup>	454.6 <sup>b</sup>
	150 %	291.7 <sup>c</sup>	833.2 <sup>c</sup>
	200 %	277.9 <sup>d</sup>	642.3 <sup>d</sup>
Salicylic acid	Control	463.5 <sup>a</sup>	549.9 <sup>a</sup>
	100 %	423.1 <sup>b</sup>	460.1 <sup>b</sup>
	150 %	313.6 <sup>c</sup>	845.8 <sup>c</sup>

	200 %	287.6 <sup>d</sup>	647.0 <sup>d</sup>
Marine algae extracts as Biofertilizers	Control	468.3 <sup>a</sup>	551.3 <sup>a</sup>
	100 %	411.6 <sup>b</sup>	459.5 <sup>b</sup>
	150 %	299.4 <sup>c</sup>	843.7 <sup>c</sup>
	200 %	290.2 <sup>d</sup>	649.4 <sup>d</sup>

Mean having the same small letters in the same row are not significantly differed at p: 0.05

They may fix atmospheric nitrogen, synthesize siderophores which solubilize minerals such as phosphorus, and synthesize some less well characterized low molecular mass compounds or enzymes that can modulate plant growth and development (El-Komy, 2005; Jaleel *et al.*, 2007). Moreover, results of the present study on the role of salicylic acid (SA) in amelioration the adverse effects of flooding stress on plant fresh biomass are in accordance with other earlier reports. For example, Ejaz *et al.*, (2012) reported the ameliorative effect of Ascorbic acid (0.5 mM on fresh biomass of saccharum spp. when exposed to water-stress (Singh *et al.*, 2001 and Malik and Ashraf, 2012). Results presented in Tables (6) indicated that in control non-treated plants Ca<sup>+</sup>, K<sup>+</sup> and P<sup>+3</sup> accumulation was decreased by increasing soil moisture content both in the maize plants systems. However, the effect of marine algae extracts as biofertilizers and salicylic acid foliar application significantly enhanced K<sup>+</sup> and P<sup>+3</sup> but Ca<sup>+</sup> accumulation especially in the shoot-system compared with the absolute control or the corresponding control treatments. Waterlogged stress play an important role in the uptake and internal accumulation of minerals in different plant species (Zahran, 1999 and Abdel-Samad, 2005). The change in K<sup>+</sup>, Ca<sup>+2</sup>, Mg<sup>+2</sup> and P<sup>+3</sup> accumulation may play a role in the difference of water stress tolerance among plant species. Several species tend to take up more Ca<sup>+</sup> and exclude K<sup>+</sup> with increasing water stress (Werner and Finkelstein, 1995). Drought tolerant species of *Triticum* had lower Ca<sup>+</sup> accumulation than the sensitive species (Sultana *et al.*, 2002). However, active sequestration of Ca<sup>+</sup> in plant tissues grown in extreme water drought conditions may be one of the responses determinately to the tissue (Fortmeir and Schuber, 1995). Potassium accumulation could be replaced by Na<sup>+</sup> at the sites of uptake of alkali cations at the plasmalemmae of root cortical cells with increasing NaCl in the medium. The inhibition of Ca<sup>+2</sup> transport and accumulation was reported previously under water-stress conditions (El-Komy *et al.*, 2004).

**Table 4:** Effect of marine algae extracts as biofertilizers and salicylic acid application on Ear weight / g and 100 Grain weight / g of *Zea mays* L. grown at waterlogging conditions

Variable tested	Field capacity	Ear weight / g	100 grain weight / g
Control	Control	347.6 <sup>a</sup>	23.41 <sup>a</sup>
	100 %	332.2 <sup>b</sup>	20.13 <sup>b</sup>
	150 %	248.1 <sup>c</sup>	24.15 <sup>c</sup>
	200 %	197.0 <sup>d</sup>	14.34 <sup>d</sup>
Salicylic acid	Control	349.3 <sup>a</sup>	23.48 <sup>a</sup>
	100 %	336.5 <sup>b</sup>	20.21 <sup>b</sup>
	150 %	252.1 <sup>c</sup>	24.19 <sup>c</sup>
	200 %	211.6 <sup>d</sup>	14.31 <sup>d</sup>
Marine algae extracts as Biofertilizers	Control	351.0 <sup>a</sup>	23.40 <sup>a</sup>
	100 %	341.1 <sup>b</sup>	20.09 <sup>b</sup>
	150 %	250.6 <sup>c</sup>	24.14 <sup>c</sup>
	200 %	203.7 <sup>d</sup>	14.31 <sup>d</sup>

Mean having the same small letters in the same row are not significantly differed at p: 0.05

Results of this study are also in accordance with findings of several investigators in respect to deficiency of mineral uptake under flooding-stress conditions. On waterlogged soil, plants show chlorosis and necrotic spots on older leaves. Both  $Mn^{+2}$  toxicity and N deficiency may be induced by the low redox potential in waterlogged soils that produces plant-available  $Mn^{+2}$  and promote denitrification of  $NO_3^-$ . Under these anaerobic conditions,  $N^+$ ,  $P^{+3}$ ,  $K^+$  and  $Ca^{+2}$  uptake was decreased by *Brassica napus* (El-Komy *et al.*, 2004). On the other hand, water logging changes the available ion concentration of the soil solution. Due to electron excess, Fe and Mn are reduced to Fe and Mn, respectively. Rice roots can avoid uptake of the accumulated Fe and

**Table 5:** Effect of marine algae extracts as biofertilizers and salicylic acid application on grain yield (t / h), bio yield (t / h) and harvest index (HI %) of *Zea mays* L. grown at waterlogging conditions

Variable tested	Field capacity	Grain yield (t / h)	Bio yield (t / h)	HI (%)
Control	Control	3.34 <sup>a</sup>	8.12 <sup>a</sup>	41.3 <sup>a</sup>
	100 %	2.67 <sup>b</sup>	6.41 <sup>b</sup>	41.7 <sup>b</sup>
	150 %	2.31 <sup>c</sup>	5.30 <sup>c</sup>	43.6 <sup>c</sup>
	200 %	2.29 <sup>d</sup>	3.66 <sup>d</sup>	62.6 <sup>d</sup>
Salicylic acid	Control	3.37 <sup>a</sup>	7.87 <sup>a</sup>	42.8 <sup>a</sup>
	100 %	2.71 <sup>b</sup>	6.48 <sup>b</sup>	41.8 <sup>b</sup>
	150 %	2.34 <sup>c</sup>	5.39 <sup>c</sup>	43.4 <sup>c</sup>
	200 %	2.33 <sup>d</sup>	3.71 <sup>d</sup>	62.8 <sup>d</sup>
Marine algae extracts as Biofertilizers	Control	3.32 <sup>a</sup>	8.10 <sup>a</sup>	40.9 <sup>a</sup>
	100 %	2.69 <sup>b</sup>	6.50 <sup>b</sup>	41.4 <sup>b</sup>
	150 %	2.30 <sup>c</sup>	5.41 <sup>c</sup>	42.5 <sup>c</sup>
	200 %	2.28 <sup>d</sup>	3.69 <sup>d</sup>	61.8 <sup>d</sup>

Mean having the same small letters in the same row are not significantly differed at  $p: 0.05$

Mn ions by release of oxygen into the rhizosphere for Fe and Mn oxidation. Plants such as wheat and barley are not able to oxidize Fe and Mn so that a toxicity of these minerals may occur under waterlogged conditions. Reported that under water logged conditions oxygen deficiency did not induce nutrient toxicity of Mn and Fe, but caused sub-optimum nutrient supply of N, P, K, Mn and Zn of wheat and Barley plants. Results of this study indicate that the physiological status of marine algae extracts as biofertilizers inoculated plants was changed including mineral ions accumulation. The increases of  $K^+$  and  $P^{+3}$  accumulation was accompanied by reduction in  $Ca^+$  concentrations. The explanation of the increased nutrients uptake after marine algae extracts as biofertilizers inoculation under water-stress conditions based mainly on the stimulation of root development and root hairs proliferation (Bashan *et al.*, 2004; El-Komy *et al.*, 2004 and Rejli *et al.*, 2008). Some benefit rhizobacteria for example *Pseudomonas*, *Bacillus* and *Azospirillum* species can solubilize insoluble inorganic phosphate in vitro, and enhance phosphorus mobilization into plant tissue (El-Komy, 2005). Recently, Baniaghil *et al.*, (2013) reported that maximum amount of Mn accumulation was related to plant growth promoting rhizobacteria (PGPR) inoculation under water-stress conditions. The authors showed that PGRR inoculation facilitate microelements uptake. Fe, Mn and Zn uptake may be related to ability to produce plants siderophores or microbial siderophores.



**Table 6:** Effect of marine algae extracts as biofertilizers and salicylic acid application on the accumulation of  $Ca^{+2}$ ,  $K^+$  and  $P^{+3}$  (mg / g) of *Zea mays* L. grown at waterlogging conditions

Variable tested	Field capacity	$Ca^{+2}$ (mg / g)	$K^+$ (mg / g)	$P^{+3}$ (mg / g)
Control	Control	35.1 <sup>a</sup>	61.3 <sup>a</sup>	2.5 <sup>a</sup>
	100 %	24.0 <sup>b</sup>	60.7 <sup>b</sup>	2.1 <sup>b</sup>
	150 %	22.6 <sup>c</sup>	59.3 <sup>c</sup>	1.0 <sup>c</sup>
	200 %	20.2 <sup>d</sup>	57.4 <sup>d</sup>	1.1 <sup>d</sup>
Salicylic acid	Control	36.8 <sup>a</sup>	62.4 <sup>a</sup>	2.8 <sup>a</sup>
	100 %	26.2 <sup>b</sup>	61.3 <sup>b</sup>	2.4 <sup>b</sup>
	150 %	23.1 <sup>c</sup>	60.7 <sup>c</sup>	1.9 <sup>c</sup>
	200 %	21.0 <sup>d</sup>	58.6 <sup>d</sup>	1.5 <sup>d</sup>
Marine algae extracts as Biofertilizers	Control	37.7 <sup>a</sup>	64.1 <sup>a</sup>	2.7 <sup>a</sup>
	100 %	27.4 <sup>b</sup>	62.5 <sup>b</sup>	2.2 <sup>b</sup>
	150 %	25.8 <sup>c</sup>	61.0 <sup>c</sup>	1.6 <sup>c</sup>
	200 %	22.5 <sup>d</sup>	59.6 <sup>d</sup>	1.7 <sup>d</sup>

Mean having the same small letters in the same row are not significantly differed at  $p: 0.05$

Siderophores are organic compounds with low molecular weight and high affinity to complex with some cations such as Fe siderophores production in PGPR such as *pseudomonas*, *Azospirillum* and *Azotobacter* has been demonstrated (Arzanesh *et al.*, 2009). The biochemical parameter such as chloroplast pigments, chlorophyll a, b and carotenoid play an important role in phytochemical reactions, were also increased in the present study showed that flooding stress (at 100% F.C.) significantly reduced the leaf pigment content (Table 7 and 8). This is in line with what has been earlier reported in many researches (Hamdia and El-Komy, 1998; Jaleel *et al.*, 2009 and Yiu *et al.*, 2009). The decrease of chlorophyll content under water-stress conditions is reported to take place because of its photo-oxidation and degradation by the activity of chlorophyllase enzyme (Abdel-Samed, 2005), as well as due to the production of reactive oxygen species (ROS) in the thylakoids (Sairam *et al.*, 2005). Results of this study also showed that the effect of marine algae extracts as biofertilizers and salicylic acid foliar application significantly elevated the photosynthetic pigments especially chl. b and carotenoid at all levels of soil moisture-content compared with both the corresponding and absolute control treatments ( Tables 7 and 8 ). Exogenous application of

**Table7 :** Effect of marine algae extracts as biofertilizers and salicylic acid application on Chlorophyll a (mg/g) and Chlorophyll b (mg/g) of *Zea mays* L. grown at waterlogging conditions

Variable tested	Field capacity	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)
Control	Control	3.44 <sup>a</sup>	3.32 <sup>a</sup>
	100 %	3.69 <sup>b</sup>	3.53 <sup>b</sup>
	150 %	3.31 <sup>c</sup>	3.38 <sup>c</sup>
	200 %	3.23 <sup>d</sup>	3.58 <sup>d</sup>
Salicylic acid	Control	3.49 <sup>a</sup>	3.38 <sup>a</sup>
	100 %	3.78 <sup>b</sup>	3.57 <sup>b</sup>
	150 %	3.38 <sup>c</sup>	3.41 <sup>c</sup>
	200 %	3.27 <sup>d</sup>	3.55 <sup>d</sup>
Marine algae extracts as Biofertilizers	Control	3.51 <sup>a</sup>	3.40 <sup>a</sup>
	100 %	3.76 <sup>b</sup>	3.59 <sup>b</sup>
	150 %	3.35 <sup>c</sup>	3.43 <sup>c</sup>
	200 %	3.30 <sup>d</sup>	3.60 <sup>d</sup>

Mean having the same small letters in the same row are not significantly differed at p: 0.05 salicylic acid (SA) helped plants maintaining the chlorophyll pigments and hence mitigated the adverse effects of flooding stress. These finding are in line with some earlier reports on Cassia, Okra, wheat and maize. Reactive oxygen species (ROS) produced under stress conditions have been reported to cause pigment degradation (Anjum *et al.*, 2011). However, salicylic acid (SA) being an antioxidant activity scavenges these ROS, thereby reducing the chlorophyll degradation under stress (Ashraf, 2009). Similarly, several reports show that the exogenous application of brassinolide, spermidine (diamine precursor) and methyl jasmonate improved flooding water stress with increased activities of SOD, CAT and APX enzymes, and total improved carotenoid contents in maize (Li *et al.*, 1998) and welsh onion (Yiu *et al.*, 2009). The highest value of proline content was showed by was increased and the differences were significant for interaction between marine algae extracts as biofertilizers, salicylic acid foliar application and field capacity (100 %) and the lowest proline content was showed by compared with the absolute control treatment (Table 8), Proline is an organic compound that most accumulated in plant when experience to drought stress The function of proline in the plant cell was to keep the stability or turgidity of the cell, and protect the cell from damage due to drought . With the accumulation of proline inside the plant cell,

**Table 8 :** Effect of marine algae extracts as biofertilizers and salicylic acid application on Carotenoids (mg/g) and Proline content ( $\mu$  mole/g FW) of *Zea mays* L. grown at waterlogging conditions

Variable tested	Field capacity	Carotenoids (mg/g)	Proline ( $\mu$ mole/g FW)
Control	Control	4.21 <sup>a</sup>	13.3 <sup>a</sup>
	100 %	4.30 <sup>b</sup>	17.2 <sup>b</sup>
	150 %	4.30 <sup>c</sup>	17.0 <sup>c</sup>
	200 %	4.29 <sup>d</sup>	18.1 <sup>d</sup>
Salicylic acid	Control	4.25 <sup>a</sup>	13.9 <sup>a</sup>
	100 %	4.33 <sup>b</sup>	17.5 <sup>b</sup>
	150 %	4.37 <sup>c</sup>	17.1 <sup>c</sup>
	200 %	4.31 <sup>d</sup>	18.7 <sup>d</sup>
Marine algae extracts as Biofertilizers	Control	4.23 <sup>a</sup>	13.0 <sup>a</sup>
	100 %	4.29 <sup>b</sup>	16.8 <sup>b</sup>
	150 %	4.27 <sup>c</sup>	16.4 <sup>c</sup>
	200 %	4.28 <sup>d</sup>	17.5 <sup>d</sup>

Mean having the same small letters in the same row are not significantly differed at p: 0.05

it is expected to give a positive effect to the physiological process wich lead to the increase of plant yield (Yiu *et al.*, 2009). The results also indicated that proline was accumulated sharply by salicylic acid or marine algae extracts as biofertilizers inoculation. Proline accumulation was used as an index of plant resistance to stress-conditions in several studies (Hamdia and El-Komy, 1998). Shifting was also recorded in Agrinine and Glutamic acid accumulation to proline by rhizobacteria inoculation or ascorbic acid-application was previously reported in several studies (Zahran, 1999; Abdel-Samad, 2005). Results of Table ( 9 ) indicate that total soluble carbohydrates in the maize plants was increased by increasing flooding stress level compared with the absolute control plant. Maximum increasing in carbohydrate accumulation in control plants was reported at 100 % field-capacity which recorded increasing in the plants . Marine algae extracts as biofertilizers, salicylic acid foliar application further enhanced the accumulation the total soluble carbohydrate. Previous studies on the carbohydrate contents in plants grown under water-stress indicated that stress induced profound changes in both total

and relative components of carbohydrate pool. Some authors have reported carbohydrate accumulation in various plants grown under water stress conditions (Arafat, 2003; El-Komy *et al.*, 2003 and Sairam *et al.*, 2005). Others observed that at low and moderate water-stress level, sugars and total carbohydrates were decreased (Tattini *et al.*, 2002 and Abdel-Samad, 2005). The accumulation of sugar was attributed to the raised synthesis of carbohydrates more than to their utilization in new cells and tissues formation. In addition, the monosaccharides glucose and fructose might be of general important in osmotic adjustment at high levels of water stress (Hamdia and El-Komy, 1998; Geigenberger, 2003 and Sairam *et al.*, 2005). Sugar accumulation may be responsible for the relative maintenance of turgidity during plant growth under drought stress. Moreover, some authors concluded that sugar accumulation depends on the plant species and the level and duration of stress (Abdel-Samad, 2005 and Zahran, 1999).

**Table 9 :** Effect of marine algae extracts as biofertilizers and salicylic acid application on Soluble carbohydrates contents (mg/g) and H<sub>2</sub>O<sub>2</sub> content of *Zea mays* L. grown at waterlogging conditions

Variable tested	Field capacity	Soluble carbohydrates c (mg/g)	H <sub>2</sub> O <sub>2</sub> content
Control	Control	3.4 <sup>a</sup>	0.16 <sup>a</sup>
	100 %	3.1 <sup>b</sup>	0.12 <sup>b</sup>
	150 %	3.2 <sup>c</sup>	0.13 <sup>c</sup>
	200 %	3.0 <sup>d</sup>	0.14 <sup>d</sup>
Salicylic acid	Control	3.8 <sup>a</sup>	0.18 <sup>a</sup>
	100 %	3.7 <sup>b</sup>	0.14 <sup>b</sup>
	150 %	3.5 <sup>c</sup>	0.12 <sup>c</sup>
	200 %	3.1 <sup>d</sup>	0.13 <sup>d</sup>
Marine algae extracts as Biofertilizers	Control	3.9 <sup>a</sup>	0.17 <sup>a</sup>
	100 %	3.5 <sup>b</sup>	0.15 <sup>b</sup>
	150 %	3.6 <sup>c</sup>	0.13 <sup>c</sup>
	200 %	3.2 <sup>d</sup>	0.12 <sup>d</sup>

Mean having the same small letters in the same row are not significantly differed at p: 0.05

The observed increases in sugar accumulation by rhizobacterial inoculation and (or) Ascorbic acid application was recorded previously in our laboratory (El-Komy *et al.*, 2003 and Abdel-Samad *et al.*, 2005). These authors attributed the increase in growth parameters following rhizobacterial inoculation to general improvement of physiological status of the inoculated plants including saccharides content. Recently, El-Refaey *et al.*, (2011) indicated that the increase levels of soluble carbohydrates under *Azospirillum* inoculation was perhaps due to the necessity of its protective role on chloroplast integrity leading to enhanced photosynthesis under water-stress condition.

**Table 10 :** Effect of marine algae extracts as biofertilizers and salicylic acid application on total nitrogen (%) and crude protein (%) of *Zea mays* L. grown at waterlogging conditions

Variable tested	Field capacity	Total Nitrogen %	Crude-protein %
Control	Control	1.5 <sup>a</sup>	9.3 <sup>a</sup>
	100 %	1.3 <sup>b</sup>	8.1 <sup>b</sup>
	150 %	1.4 <sup>c</sup>	8.7 <sup>c</sup>
	200 %	1.2 <sup>d</sup>	7.5 <sup>d</sup>
Salicylic acid	Control	1.7 <sup>a</sup>	10.6 <sup>a</sup>
	100 %	1.6 <sup>b</sup>	10.0 <sup>b</sup>
	150 %	1.5 <sup>c</sup>	9.3 <sup>c</sup>

	200 %	1.4 <sup>d</sup>	8.7 <sup>d</sup>
Marine algae extracts as Biofertilizers	Control	1.6 <sup>a</sup>	10.0 <sup>a</sup>
	100 %	1.4 <sup>b</sup>	8.7 <sup>b</sup>
	150 %	1.5 <sup>c</sup>	9.3 <sup>c</sup>
	200 %	1.3 <sup>d</sup>	8.1 <sup>d</sup>

Mean having the same small letters in the same row are not significantly differed at p: 0.05

Our results (Table 1) with regards to leaf-area increasing by Marine algae extracts as biofertilizers and salicylic acid foliar application, revealed improved conductance by these treatments which may allow better gas exchange and enhancement of photosynthesis. Reported that tomato plants grown under flooding condition and inoculated with bacteria with ACC-deaminase activity (*Enterobacter cloaceae*, *Pseudomonas putida*) stimulated plant growth, and leaf chlorophyll A and B content compared to flooded non-bacterized plants. El-Refaey *et al.*, (2011), indicated that bacterization of tomato seeds lead to lower the amount of ACC(1-aminocyclopropane-1-carboxylate) available for oxidation to ethylene in the shoots of flooded plants by the bacterization of roots by plant growth promoting bacteria expressing ACC-deaminase gene, which is transcriptionally controlled by the anaerobically regulated promoter that normally regulates the expression of ACC deaminase in these bacteria. Thus, under flooding-condition ACC-synthase genes are induced in the root of tomato plants, while ACC oxidation (to ethylene by ACC oxidase) is arrested because of root hypoxia . ACC is therefore transported by the xylem to shoots where it is oxidized to ethylene . However, plants treated with ACC-deaminase containing bacteria exhibited a significant higher tolerance to flooding than did non-bacterized control plants. Thus, protection against flooding was achieved as a result of the presence of roots treated with ACC-deaminase bacteria which significantly increased overall plant growth, leaf chlorophyll content and subsequently decreased ethylene production in leaf tissues . Data shown in Table ( 9 ) indicate the results of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) accumulation in maize root-tissues at harvesting. In control untreated plants, H<sub>2</sub>O<sub>2</sub> increased by increasing the level of water flooding-stress, and the highest H<sub>2</sub>O<sub>2</sub> concentration was recorded at field-capacity At 100 % and 200 % field-capacity, H<sub>2</sub>O<sub>2</sub> accumulation was not affected by marine algae extracts as biofertilizers inoculation and salicylic acid foliar application. However, at field-capacity the accumulation of H<sub>2</sub>O<sub>2</sub> was decreased significantly by these treatments, and the lowest values of H<sub>2</sub>O<sub>2</sub> was decreased recorded by SA and marine algae extracts as biofertilizers treatment. These results are in accordance with several reports that under flooding-conditions (Hypoxia) H<sub>2</sub>O<sub>2</sub> is recorded. Hydrogen peroxide accumulation under flooding-condition (Hypoxia) has been shown in the roots and leaves of *Hordeum vulgare* and in wheat roots . The presence of H<sub>2</sub>O<sub>2</sub> in the apoplast and in association with the plasma membrane has been visualized by transmission electron microscopy under hypoxic conditions in four plant species (El-Refaey *et al.*, 2011). H<sub>2</sub>O<sub>2</sub>, OH<sup>-</sup> and other reactive oxygen species (ROS) are responsible for a state of oxidative stress in the whole plant tissues . H<sub>2</sub>O<sub>2</sub> is a strong oxidant and its higher concentration is injurious to cell, resulting an oxidative damage, lipid peroxidation and disruption of metabolic function and losses of cellular integrity at sites where it accumulates (El-Refaey *et al.*, 2011). In the present study and other studies, the decreased levels of H<sub>2</sub>O<sub>2</sub> by salicylic acid (SA) and (or) biofertilization application by the action of activation of antioxidant enzymes (Athar *et al.*, 2009). These higher levels of antioxidant enzymes e.g. POD and SOD might be attributed to their property to help develop the plant's resistance against oxidative damage. Athar *et al.*, (2009) reported an increase in antioxidant enzymes in wheat plants after salicylic acid (SA) application. Thus, earlier work suggested that an increase in the activity of antioxidant enzymes (as indicated by the reduction in H<sub>2</sub>O<sub>2</sub> level) helps the plants to maintain their growth under stress conditions and can be used as an

indicator of stress conditions and can be used as an indicator of stress tolerance (Ejaz *et al.*, 2012). Data presented in Table (10) show that the observed increases of saccharides in the grains of control non treated maize plants was accompanied by increasing in the percent of crude protein. marine algae extracts as biofertilizers inoculation and salicylic acid foliar application further increased plant grains protein content as compared with the corresponding control treatments. These results are in accordance with previously reported findings of Dolatabadian *et al.*, 2009. Thus biofertilizers might play an important role in the protein biosynthesis either directly (through fixation of nitrogen) or indirectly by enhancing the uptake of soil nitrogen due to their hormonal effects or nitrate-reductase activities (El-Komy *et al.*, 2003). Salicylic acid (SA) had variable effect on plant protein content at water stress conditions and it depends on salicylic acid (SA) concentration (Dolatabadian *et al.*, 2010). The application of 50 mg/L SA had no effect on number of leaves, height and protein percentage at the vegetative stage of corn plant compared with non-treated plants. However, protein percentage increased due to 150 mg/L salicylic acid (SA) foliar application at the same mentioned stage of growth (Dolatabadian *et al.*, 2010). It is reported previously that Ascorbic acid scavenges reactive oxygen species and prevent protein oxidation and degradation. Moreover, it was reported that by SA application under flooding stress condition, about 20 anaerobic proteins were synthesized in maize roots, while synthesis of the normal proteins were drastically repressed (El-Komy *et al.*, 2003). Many of these induced proteins were identified as enzymes of the glycolytic and fermentation pathways (Dolatabadian *et al.*, 2010). The identified anaerobiosis induced proteins (ANP) such as sucrose-synthase, phosphohexose isomerase, and pyruvate decarboxylase were reported (El-Komy *et al.*, 2003).

#### Conclusion :

Water is the most abundant constituent of living things . The living tissues of plants usually contain more than 70 % by weight of water and maintenance of satisfactory water content is essential for the plant-tissue water content can markedly influence processes of growth and metabolism . All land plants are to some degree adapted to the unfavorable water regime of their habitat. But some species can tolerate far more unfavorable draught stresses than can other species . Generally there are three basic types of adaptation which can occur, (a) The control of water loss from the plant may be more efficient, ( b ) The uptake of water may be more efficient and (c) The plant may have a greater ability to grow and metabolize or survive when claimed that water stress influences enzyme activity and in this way can influence all metabolic processes . Moreover, lowering water potential often synthetic processes are reduced more than breakdown processes . He mentioned also, the level of Auxin and Cytokinin in the tissue are reduced while the level of abscisic acid and ethylene are raised . The Auxin change is due to at least partly to increased IAA oxidase activity . The period of flooding conditions often cause yellowing and later browning of leaves, symptoms similar to senescence . On the other hand, the tolerance to flooding stress under field conditions was studied in barely varieties . It was found that higher proline accumulation during flooding stress were the more tolerant to water loading . Claimed that the unfavorable growth conditions such as flooding conditions or even heat stress can be tolerated by plants in juvenility rather than those at maturity . This is because plants in juvenile have high concentration of growth promoters such as IAA, GA and CKs . It helps significantly in compensating any decrease happen in photosynthesis pathway, mineral absorption and production of inhibitors such as ethylene and ABA when stressed occurred . On the other hand, Reported that plants at maturity generally have high concentrations of the inhibitors comparing with the promoters this encourages assimilates transportation from sources to sinks accompanied with fruity parts . The previous discussion clarify the results obtained in this study, taking into consideration the hazard effects of water stress on maize plants growth, chemical composition and hence yield and its components especially at the end of the



juvenility compare with the early juvenile growth period . These results are in concomitant we can state that all mentioned factors together led to produce higher yield as a result of incurring plant growth and this led to improve plant chemical composition and metabolism . The superior results obtained from algae-biofertilizer either under normal irrigation or water stress conditions, were because of the algae development of the Mycorrhiza which play a big role in improving the soil mechanical texture out of the nature growth; also it plays as lateral roots exchanging the carbohydrate and the amino acids from the co-operated plant to the algae, on the other hand, phosphate and other minerals from the algae to the co-operated plant . Under waterlogging conditions, the algae-biofertilizers play as additional lateral root system providing the water from long distances away from the root system to the plants . Moreover, claimed that the increase in nutrient uptake may be due to the physical increase in the surface area for nutrient uptake . This is partly due to the biofertilizers in the soil having a much greater surface area and extends more than root hairs .In addition, the algal-biofertilizers infection prolongs the life of lateral rootlets . Algal biofertilizers, helped in increasing nitrogen soil content through non-symbiotic nitrogen fixation pathway . This led to the production of plant growth promoting phyto-hormones such as IAA, GA<sub>3</sub> and CKs, which helped in encouraging plant growth and organic acids therefore reducing soil pH, thus release the unavailable soil nutrients particularly zinc and phosphate especially under calcareous soil conditions . All these factors together led to enhance the photosynthetic pigments accumulation thus increase photosynthesis pathway as well as increased yield and its components . This can illustrate the result observed under all studied water regime conditions . But once discussing the sever water loading stress condition, plants faced complex challenges which seriously defend against implementing plant life cycle . Biofertilization remains alone capable to overcome the water stress hazard effects through producing growth promoting phyto-hormones such as IAA, GA<sub>3</sub> and CKs, beside the organic acids.

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