

## Microbial Gibberellins Impact on *Zea mays* (L.) Plants under Different Levels of Water Salinity

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**T**HESSE days green advancements of phytohormones have a critical place in industrial food process displace the chemical manufacturing which causes genuine dangers by gathered in the environment, from these hormones; gibberellin has an industrial significance as it's a plant development controller hormone. Fungi produced several important substances with biological impact; from these substance plant hormones production involves great interest in agriculture. From different fungal isolates tested for gibberellins production, *Fusarium oxysporum* represented as a hopeful fungus for microbial gibberellin generation conducted 268.9±6.27 mg/l gibberellic acid with productivity rate 38.42 mg/l/day with succeeded capacity to apply in the field. In vivo application of microbial gibberellic acid occurred in pot experiment on *Zea mays* (L.) plants under water salinity stress. Synergetic development in maize plants exhibited with expanding microbial gibberellin concentrations until 150 ppm under water salinity stress and then no huge diverse was cleared. Increasing in plant weight and height observed in compared to the non-treated plants.

**Keywords:** Phytohormones, *Fusarium*, Fungi, Salinity stress

### Introduction

Fungi produced a critical group of natural bioactive compounds (secondary metabolites) (Quang et al., 2014), gibberellins represent small molecules of a large group of tetra-cyclic diterpenoid carboxylic acids, defined ent-gibberellane carbon skeleton. Gibberellins were identified first as in 1930s as phytohormones depends on an high-growth rice seedling due to infections by *Fusarium fujikuroi* (teleomorph *Gibberella fujikuroi*) a pathogenic rice fungus. There are 136 known gibberellins types produced by fungi, plants and even bacteria only GA1, GA3, GA4 and GA7 are prominent bioactive (Cross et al., 1959 and Davies, 2004). Gibberellic acid produced in elevated levels by fungi (*Gibberella fujikuroi*) compared to plants (Hedden et al., 2001).

Plant hormones assume a basic job in plant development and improvement, for example, seed

germination, stem extension, blooming and fruit advancement. Gibberellins help plants to change their physiology to the ecological changes in quick response and adapt to fluctuating environmental conditions to meet the requests against expanding of food demand especially over the most recent couple of years (Olszewski et al., 2002; Fahad et al., 2014 and Hussain et al., 2014). Most familiar producer genera of gibberellins were *Penicillium*, *Fusarium* and *Aspergillus* (Tansakul et al. 2014). Gibberellins hormones combined by fungal strains such as *Fusarium sacchari*, *F. konzum*, *F. glutinans*, *Aspergillus fumigatus*, *Penicillium janthinellum* and *P. resedanum* (Troncoso et al., 2010 and Khan et al., 2015a,b).

Salinity represented major ecological elements restricting plant development and productivity; it causes huge decrease in the plant development parameters (Hamada and Al-Hakimi, 2002). High groupings of water salinity cause genuine severe yield decreasing, around 20 % of the

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world's developed fields influenced by salinity as it's in charge of plant cell ions disturbance. Salinity action decreased photosynthesis by shut down the stomata and decreasing carbon dioxide accessibility (Sairam and Tyagi, 2004 and Jacoby et al., 2011). High salinity repress root and shoot development other than the high tension in canalized to the perfect solutes (betaine, trehalose and proline, phytohormone (abscisic, brassinosteroid and gibberellic), proteins (ascorbate peroxidase, glutathione peroxidase and catalase) biosynthesis and has extra negative impacts on the cell energy supply, redox homeostasis, and photosynthesis (Gao et al., 2004; Zhu et al., 2010; Chen and Murata, 2011; Jacoby et al., 2011; Nounjan et al., 2012; Muller et al., 2014; Leitão and Enguita, 2016).

Gibberellins have been accounted to advance the plant development (rice seedling) and a few halophytes in saline condition (Kaur et al., 1998 and Dheeba et al., 2015). Salt pressure prompted the repression of the gibberellin pathways signaling performing lower cell cycle which appeared to be essential amid the late periods of the salt reaction to promote recovery (West et al., 2004; Geng et al., 2013; Leitão and Enguita, 2016). If we assumed that in 2050 (As per FAO) the number of population will increment 2.3 billion, speaking to an expansion of 70% of crops production requests another new methodology for threatening the security of worldwide food is basic and essential (FAO, 2009). A maize crop is exceptionally delicate to salinity, germination percentage and rates of corn grains would diminish by expanding salinity concentrations (Maas, 1986). Treatment with gibberellins had no impact on seed germination in wrong concentrations, however when using the write one expanded in shoot length, root length, dry weight, fresh weight and tissue water content occurred (Khan et al., 2010 and Ghodrati & Roustai, 2012).

The principle goal of this research is to test the capacity of various *Fusarium* species to produce gibberellic acid on basal medium, furthermore test the application of microbial gibberellins extracted on *Zea mays* plant under various levels of salinity stress.

## **Materials and Methods**

### *Microorganisms and inoculums preparation*

Diverse *Fusarium* species, isolated from Egyptian clover, garlic, maize and onion plants on potato dextrose agar medium (PDA) at  $28 \pm 1$  °C with incubation time 7 days were used in this research. *Fusarium* species were identified based on their macroscopic development on various media and microscopic characteristics under light microscope instrument (Leslie and Summers, 2006). Fresh and pure cultures moved into pure PDA slants and kept up at  $4 \pm 1$  °C. For inoculums preparation, *Fusarium* sp. developed on PDA medium at  $28 \pm 1$  °C for 7 days. Fungal hyphae scraped from growth plates suspended in sterilized distilled water fortified with 0.01% (v/v) tween 80 to avoid spore gathering and stirred for half hour until get  $3 \times 10^5$  spore/ml (Mahmoud and Mostafa, 2017).

*Screening for gibberellin production by Fusarium sp.*

Czapek's dextrose fluid medium was utilized as production medium containing (g/l): glucose, 30.0; yeast extract, 5;  $\text{NaNO}_3$ , 3.0;  $\text{KH}_2\text{PO}_4$ , 1.0;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5; KCl, 0.5,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.01 and 1000 ml distilled water with initial pH regulate to 5.5. After sterilization in an autoclave at 121 °C for 20 min. chloramphenicol, 250 mg/ml include independently as bacteriostatic agent sterilized by membrane filtration (0.22 µm). The cultures incubated at  $28 \pm 1$  °C on a rotary shaking (150 rpm) for 7 days. All the experiments were done independently in triplicates (Shahzad et al., 2016).

### *Screening for gibberellin production by Fusarium sp.*

*In vivo application of microbial gibberellin*

Pots experiment was executed in the Experimental Farm of Arab El- Awammer Research Station, ARC, Assiut, Egypt (which, lies between latitude 27°, 11' N and longitude 31°, 06' E and the altitude of the area is 71 m) to evaluate the impacts of soaking *Zea mays* grains in five levels of microbial gibberellins extended from 0 to 200 ppm (soaked for 12 hours) under three levels of irrigation water salinity (500, 1500 and 2500 ppm). Germination percentage and seedling development (Plant high (cm), Shoot fresh weight (g) and Root weight (g)) of *Zea mays* (at 20 day age) were assessed. The experiment included 15 treatments with three replicates; all treatments were organized in spilt plots design. Soil physical and chemical properties were estimated and recorded in Table (1).

### *Analytical analysis*

Fungus mycelia recovered by filtration through dried and weighed Whatman filter paper (No. 113), washed three times with distilled water, dried in oven at 70 °C overnight for dry mass (DM) estimation. The supernatants were

centrifuged at 4,000 rpm for 15 min., sterilized by membrane filtration using a membrane of pore size 0.22 µm to evacuate any residual spores for quantitative estimation of gibberellic acid (GA3).

For gibberellins extraction, culture medium was acidified to pH 2.5 with HCl (1M) and extracted using liquid-liquid (ethylacetate/NaHCO<sub>3</sub>) extraction three times. Extracts were filtrated on filter papers contains sodium sulfate anhydrous to remove any water (Cho et al, 1979). Gibberellic acid in the ethyl acetate phase was measured by UV spectrophotometer at 254 nm against substrate-free blank using T60 UV with a split beam UV visible spectrophotometer covers a wavelength range of 190–1100 nm (Bruckner and Blechschmidt 1991 and Berrios et al., 2004). The amount of gibberellic acid was calculated from the standard curve (1-10 mg/L) using slandered gibberellic acid. The concentrate extract was stored at 4± 1 °C for crystallization.

Thin Layer Chromatography (TLC) was used as confirmation test for the presence of gibberellin. Gibberellic acid dissolved in ethanol and spotted on the silica gel (SiO<sub>2</sub>) plates using mobile phase containing isopropanol: ammonia: water (10:1:1). After the solvent reach to the end line, the plates were removed, sprayed with 3% sulphuric acid containing 50 mg FeCl<sub>3</sub> and heated in oven at 80°C for 10 min. The gibberellic acid appeared as greenish black spot under UV light (Cavell et al, 1976).

#### Statistical analysis

All obtained data were subjected to statistical analysis of variance and treatment means were compared for significant differences using the LSD at  $p = 0.05$ . The MSTAT-C (version 2.10) computer program was used to perform all the analysis of variance in agreement with the procedure outlined by Steel and Torrie (1982).

**TABLE 1. Physical and chemical characteristics of representative composite soil sample from the field experimental site**

Chemical properties									
pH (1 : 1)	EC dS/ m (1 : 1)	Soluble cations (meq / L)				Soluble anions (meq / L)		Available Phosphorus (ppm)	Total nitrogen (%)
		Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>-</sup> +HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>		
8.37	0.33	1.43	1.16	0.19	0.75	1.68	1.47	8.31	0.003
Physical properties									
Particle size distribution (%)		Texture		Moisture content (Volumetric %)			O.M	CaCO <sub>3</sub> (%)	bulk density
Sand	Silt	Clay	class	S. P.	F.C.	W.P.	(%)		
89.9	7.1	3.0	sandy	23.3	10.9	4.5	0.19	30.9	1.63

## Results

### Gibberellic acid production by *Fusarium sp.*

Seven *Fusarium* species, obtained from Egyptian clover, garlic, maize and onion plants on PDA medium used in this experiment. A wide variation in gibberellic acid production on the screening medium ranged from 0 to 268.96.27± mg/l, productivity from 0 to 38.42 and dry mass varied between 2.75 ±0.05 and 9.648±.33 g/l as shown in Fig. 1. The highest gibberellic acid producers were *Fusarium oxysporum* I, *F. solani* and *F. oxysporum* II giving 268.9 ± 6.27, 252.17±7.29 and 204.98± 5.437 mg/l GA3 (with

productivity 38.42, 36.03 and 29.28 mg/l/day) and 9.648 ± 0.33, 7.2±0.57 and 8.58±0.63 g/l dry mass, respectively. Brief description of gibberellic acid high producer; *Fusarium oxysporum* Schlechtendal was performed; growth on potato dextrose agar plates for 7 days showing growth rate 80 mm, with white mycelium and violet undersurface (Fig. 2A &B). The fungus formed monophialidic and polyphialidic conidiogenous cells (Fig. 2C). Microconidia abundant and oval in shaped, usually not septate microconidia, macroconidia present, curved and septate (Fig. 2D).

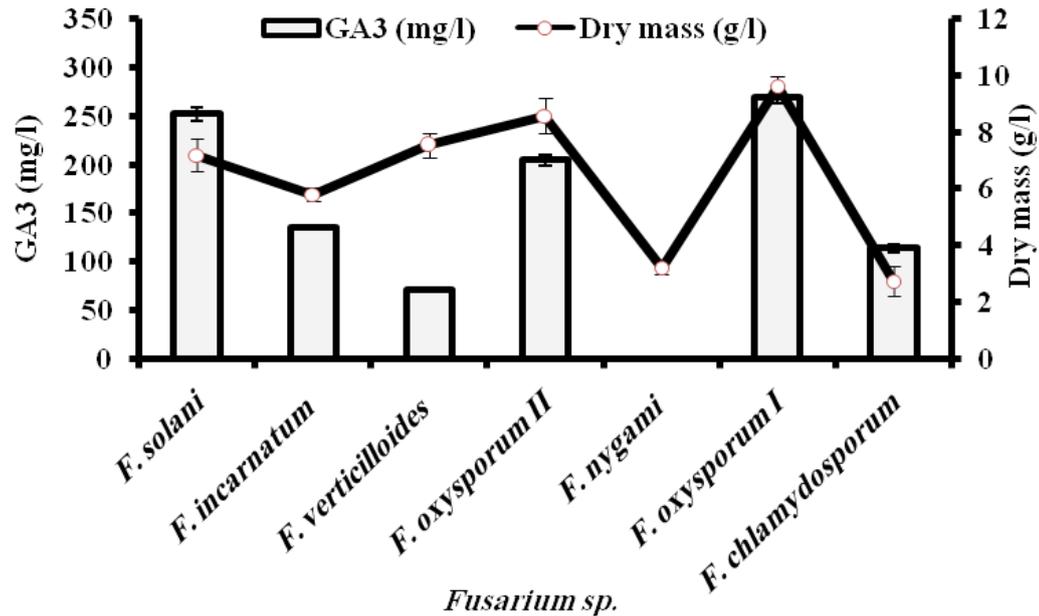


Fig. 1. Screening for GA3 production on Czapek's liquid medium using different isolates of *Fusarium sp*

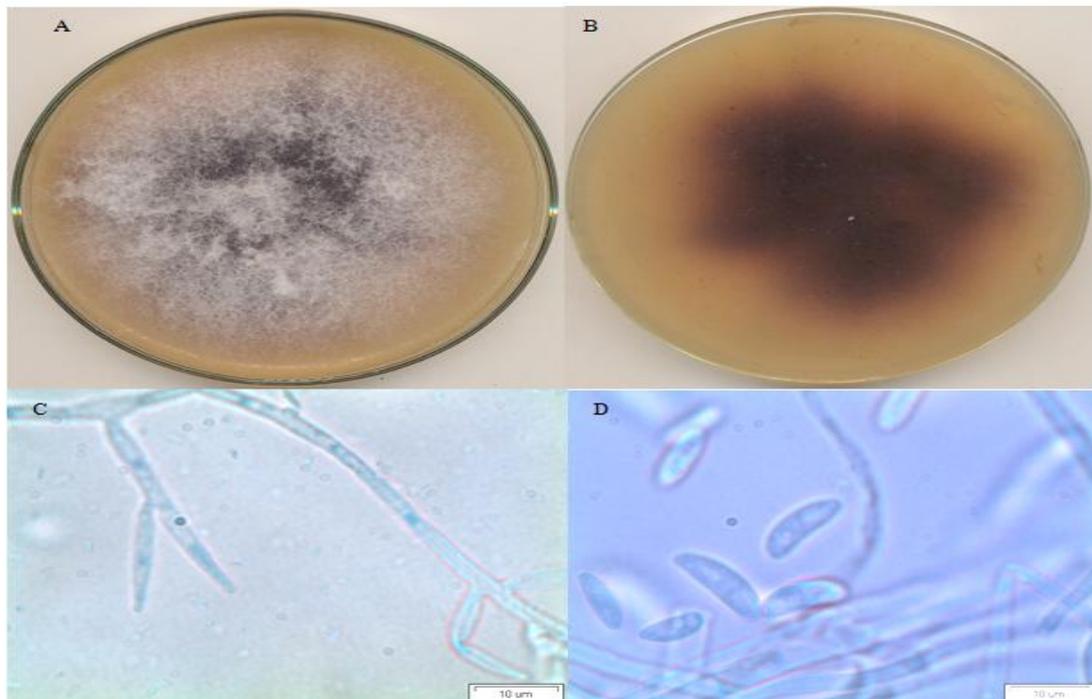


Fig. 2. *Fusarium oxysporum*, A, B: Fungus growth on potato dextrose agar medium and its reverse; C: Monophylaidic and polyphylaidic conidiogenous cell; D: Microconidia (Mi) and Macroconidia (Ma); Bars, 10 µm

#### *In vivo* application of gibberellic acid production by *Fusarium oxysporum*

Data in Fig. 3 - 6 showed the effect of soaking *Zea mays* grains in five levels of microbial gibberellins ranged from 0-200 ppm under three levels of water salinity (500, 1500 and 2500 ppm) on germination percentage and seedling growth (Plant high (cm), Shoot fresh weight (g) and Root

weight (g)) of *Zea mays* (at 20 day age). The results showed that the effect of water salinity on plant high (cm), shoot fresh weight (g), root weight (g) was significant ( $p < 0.05$ ); however no significant effect observed in germination percentage. The highest values of plant high (cm), shoot fresh weight (g), root weight (g) obtained by irrigation with 500 ppm of salinity water with

14 % 34.9 % and 33.7 % increasing respectively, rather than 2500 ppm salinity water.

The effect of gibberellin concentrations on seedling growth was significant, but was no significant ( $p < 0.05$ ) for germination rate. Soaking grains in 150 ppm gibberellins achieved the

highest values of plant high (cm), shoot fresh weight (g) and root weight (g) with 17.5 % 47.3 % and 42.9 % respectively increasing higher than control (zero ppm gibberellins). This might be due to enhancements in cell enlargement and division.

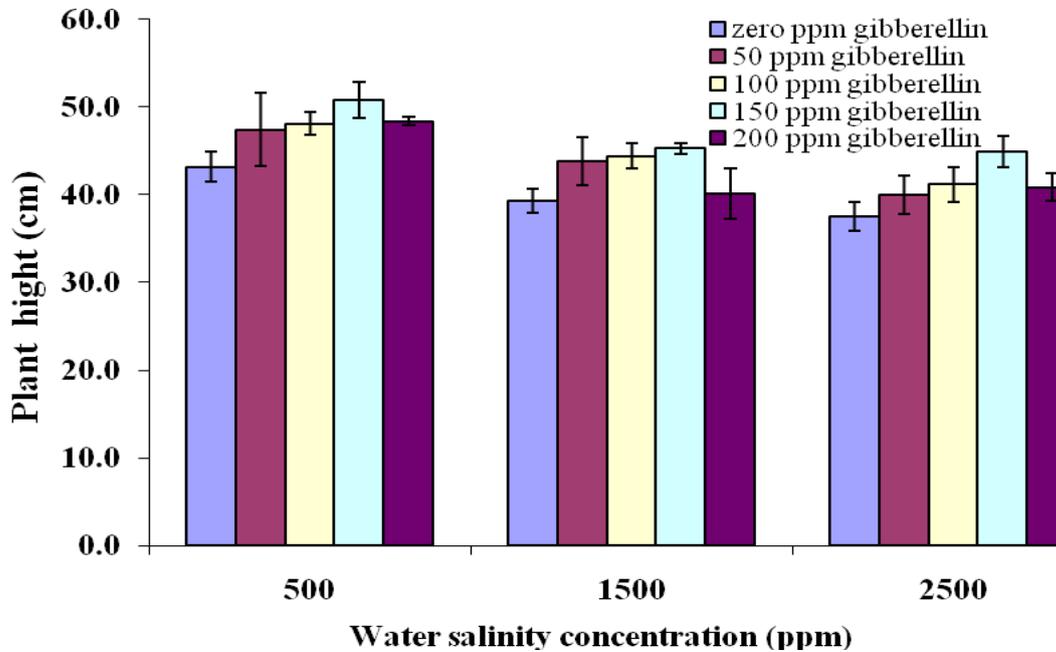


Fig. 3. Plant high (cm) of *Zea mays* (at 20 day age) as influenced by water salinity concentration levels and soaking grains in different levels of gibberellin

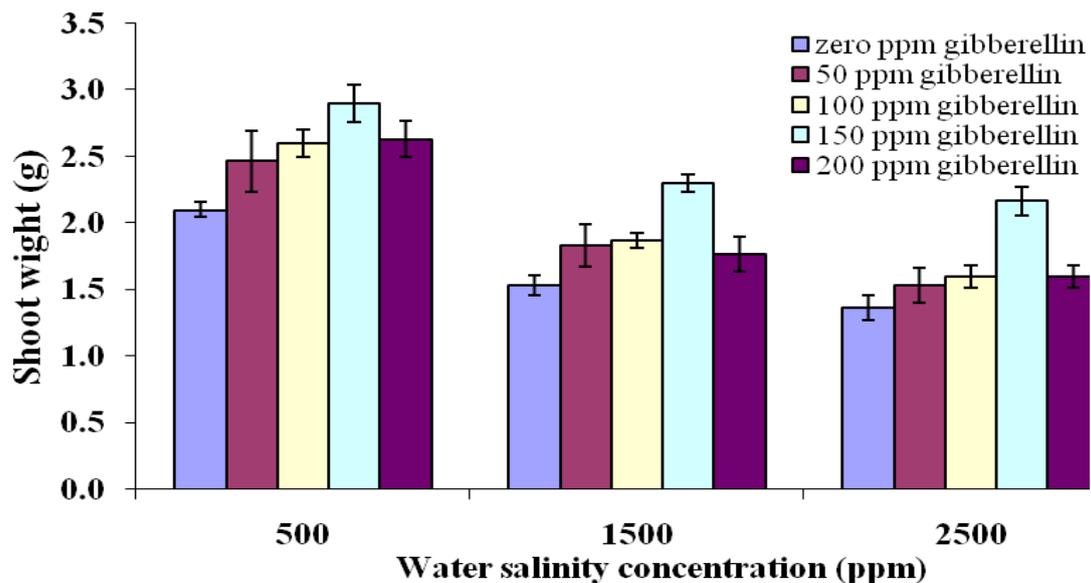


Fig. 4. Shoot fresh weight (g) of *Zea mays* (at 20 day age) as influenced by water salinity concentration levels and soaking grains in different levels of gibberellin

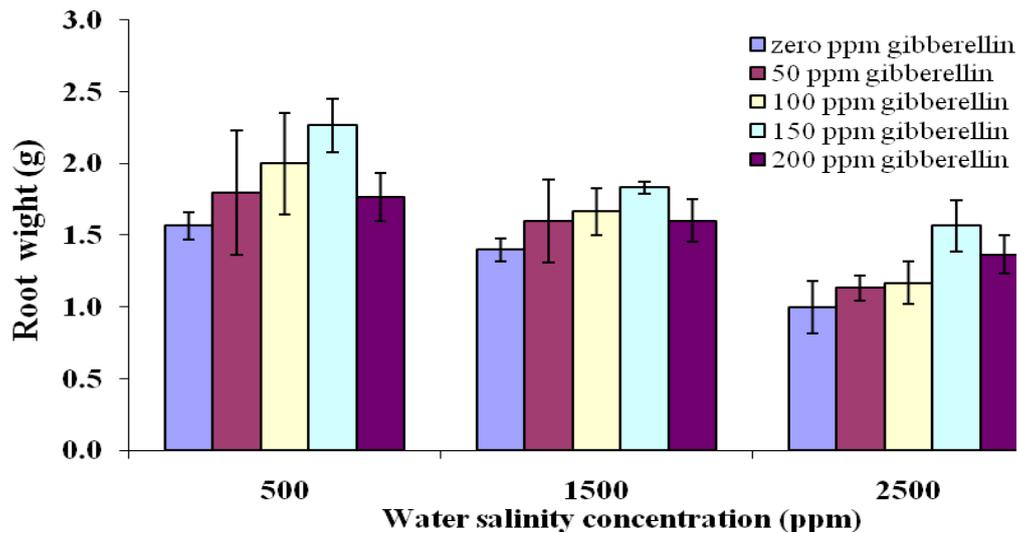


Fig. 5. Root weight (g) of *Zea mays* (at 20 day age) as influenced by water salinity concentration levels and soaking grains in different levels of gibberellin

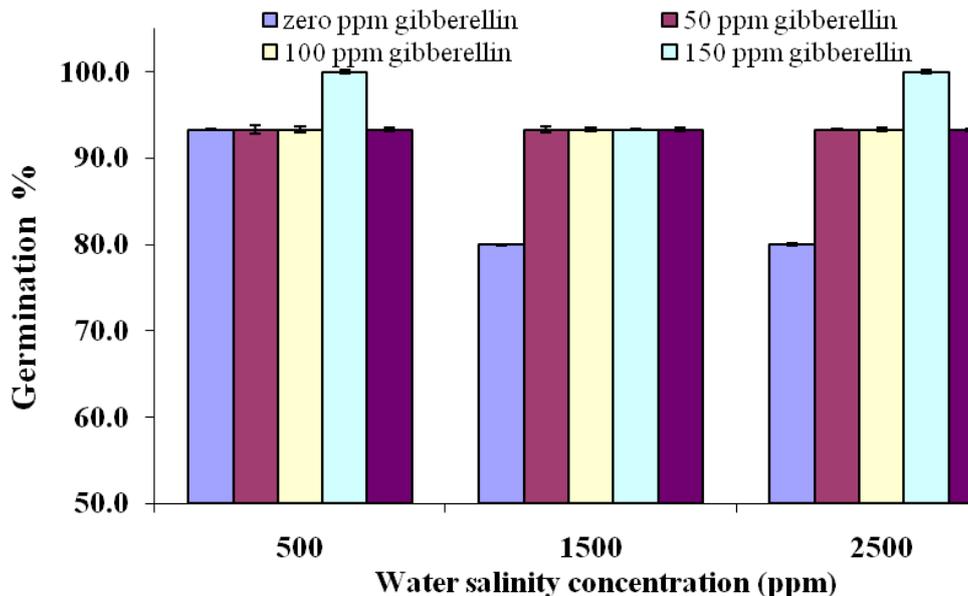


Fig. 6. Germination percentage of *Zea mays* as influenced by water salinity concentration levels and soaking grains in different levels of gibberellin

The interaction between water salinity and gibberellins concentrations on seedling growth was significant (Fig. 7). The highest synergetic effect of water salinity and gibberellins produced in 500 ppm water salinity and 150 ppm gibberellins. Irrigation with water salinity 2500 ppm with gibberellic acid soaking (150 ppm) chive remarkable increasing plant high and shoot fresh weight comparative with 500 ppm water salinity and zero ppm gibberellins this means the application of gibberellins increasing the

tolerance of plant to water salinity during their growing period.

#### Discussion

*Fusarium* species has a great capacity to produce various amounts of gibberellin as a plant hormone particularly *Fusarium oxysporum* which considered as a hopeful isolate for microbial gibberellin production with the capacity to apply in *vitro* and *vivo*. Several scientists in agreement with our outcomes; Curtis (1957) inferred that

*Gibberella fujikuroi* was the microorganism that can produce gibberellins. Sanchez-Marroquin (1963) tested around 43 strains of *Fusarium* sp. and detailed that *F. moniliforme* could give higher yields of GA3 on variety media. Other fungi additionally produced gibrellins e.g. *Fusarium sacchari*, *Fusarium konzum*, *Aspergillus fumigatus*, and *Penicillium resedanum* (Troncoso et al., 2010 and Khan et al., 2015 a, b). Jefferys (1970) revealed that the ideal temperature for development of the strain *Gibberella fujikuroi* is between 31-32 °C while the production of GA3 was maximized at 29 °C. Shukla et al. (2005) who's discovered that pH 5.0 was the ideal for gibberellic acid production. Meleigy and Khalaf 2009 recorded critical increase of gibberellic acid (2.25gl<sup>-1</sup>) at pH 5 after 6 days of incubation.

Salinity represents one of major environmental issues in charge of constraining in plant development, productivity and critical decrease in plant weight. *Zea mays* plant exceptionally delicate to salinity and numerous losses in the crop yield caused because of the stress (Maas, 1986; Hamada and Al-Hakimi, 2002). Ghodrat et al (2013) demonstrated that salinity stress considered as a serious issue cause diminished in plants root, shoot weights as well as germination rate and percentage of *Zea mays*. Application

of GA3 treatment on *Zea mays* plants indicated increase in the parameters which diminished by salt water stress. Microbial gibberellin raised the plant resistance to salinity stress synergistically with concentration until the most effective concentration of gibberellin 150 ppm (demonstrated the most increasing in shoot fresh weight, dry weight, plant length and roots weight compared to control under the three levels of irrigation water salinity). Gibberellic Acid (GA3) considered as the most essential development regulator promotes germination, hypocotyls development and increases the size of leaves. It stimulates hydrolytic enzymes essential for the degradation of cells around radical resulting speeds in germination (Boucaud and Ungar, 1976; Rood et al., 1990; Hisamatsu et al., 2000 and Tuna et al., 2008). The appropriate concentration of GA assumes an important role in the induction of plant salinity tolerance particularly when applied in environment (Jamil and Rha, 2007; Kaya et al., 2010). Ghodrat and Rousta (2012) test the priming of the corn by GA3 (0-5 mg/L) and found that it offer resistance to corn under salinity stress. Dheeba et al. (2015) revealed that application of gibberellin takes out the adverse impact of salinity stress on black gram seedlings.



**Fig. 7. Impact of microbial gibberellin on germination of *Zea mays* plant A; *Zea mays* germination under 1500 ppm water salinity with two levels of gibberellin G1 (50 ppm) and G0 (zero), B; *Zea mays* germination under 2500 ppm water salinity with two levels of gibberellin G3 (150 ppm) and G0 (zero)**

## Conclusion

*Fusarium oxysporum* considered as the highest potential gibberellic acid producer. The fungus has high efficiency to utilize glucose as a carbon source for production of gibberellic acid. Application of microbial gibberellin proved its ability to enhance *Zea mays* growth under different levels of water salinity. This application of microbial hormone pointed to the importance of the green technology of phytohormone instead of chemical once.

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### تأثير الجبرلين الميكروبي علي نباتات الذرة الشامية تحت تأثير مستويات مختلفة من ملحية المياه

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يحتل استخدام التكنولوجيا الخضراء في الوقت الحاضر لإنتاج الهرمونات النباتية مكاناً هاماً في الإنتاج الصناعي كبديل عن الإنتاج الكيميائي الذي يسبب العديد من المخاطر عند تراكمه في البيئة. من هذه الهرمونات يحتل الجبرلين مكانة صناعية هامة حيث انه هرمون منظم لنمو النباتات. وعند إختبار مقدرة عزلات فطرية مختلفة علي إنتاج الجبرلين كانت فطره فيوزاريوم أوكسيسبوريم عزلة واعدة في انتاج الجبرلين الميكروبي منتجة  $6.27 \pm 268.9$  مللجرام لكل لتر جبرلين بمعدل  $38, 42$  مللجرام لكل لتر لكل يوم. وقد تم تطبيق الجبرلين الميكروبي في تجربة أصص علي بادرات الذرة الشامية تحت تأثير الإجهاد الملحي. وقد وجد تحسن طردي في نمو البادرات مع زيادة تركيز الجبرلين حتى تركيز  $150$  ملليجرام/لتر تحت تأثير مستويات ملحية مياة الري المختلفة حيث حدثت زيادة في وزن و معدل نمو البادرات المعاملة بالجبرلين الميكروبي مقارنة بالغير معاملة.