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### Growth, Yield and Chemical Constituents of Hot Pepper Var. (Fire Bomb) As Affected By Biostimulants Extract Application under Salt Stress Conditions



Ibrahim, M. F.<sup>1\*</sup> and A. A. S. Abd-El Raheem<sup>2</sup>

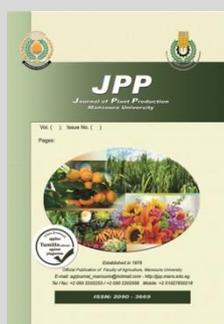
<sup>1</sup>Dept. Horticulture (Ornamental, Medicinal and Aromatic Plants), Fac. Agric., Al-Azhar Univ., Assiut branch, Egypt.

<sup>2</sup>Dept. Horticulture (Olericulture), Fac. Agric., Al-Azhar Univ., Assiut branch, Egypt.

#### ABSTRACT

A pot experiment was carried out at Faculty of Agriculture Farm, Al-Azhar Univ, Assiut, during the two successive seasons 2018 and 2019 aiming to study the effect of three rates of saline water at (0.0, 30 mM and 60 mM NaCl+CaCl<sub>2</sub>) and foliar spraying by biostimulants namely, (control, pigeon manure extract at 4g/L, pigeon manure extract at 8g/L, yeast extract at 4g/L, yeast extract at 8g/L, licorice extract at 4g/L and licorice extract at 8g/L ) as well as, their interaction on growth, yield and chemical constituents of hot pepper var. (Fire Bomb). The obtained results showed that both concentrations (30 mM and 60 mM NaCl+CaCl<sub>2</sub>) caused a significant reduction in all vegetative growth, yield components and chlorophyll content measurements of hot pepper. While, there was a significant increase in capsaicin content as a result of concentrations (30 mM and 60 mM NaCl+CaCl<sub>2</sub>) over control. With regard to foliar spray with biostimulant treatments at all concentrations, these treatments led to significantly increase in growth parameters, yield components, as well as, total chlorophyll content and total capsaicin content. The high concentration of pigeon manure extract and licorice extract at 8g/L gave the highest values of these previous traits over control. On the other side, foliar spray with these studied biostimulants at all concentrations caused a reduction in proline content, Na and Ca % in leaves. Concerning to the combined between the two examined factors, foliar spray by biostimulant treatments alleviated adverse effect of salt stress, in most cases.

**Keywords:** hot pepper var. (Fire Bomb), salt stress, biostimulants and total capsaicin content.



#### INTRODUCTION

Pepper, chilli, chile, or chili belongs to the Solanaceae family genus *Capsicum* and is closely related to tomato, eggplant and tobacco. The genus *Capsicum* represents a diverse plant group and includes twenty-seven species; five domesticated and twenty-two un-domesticated (Bosland, 1993). *Capsicum annuum*, *C. frutescens*, *C. chinense*, *C. baccatum* and *C. pubescens* are considered domesticated species of peppers. The cultivated pepper has a spicy flavor of the genus *Capsicum*, *C. annuum* L. var. *annuum* principally and *C. frutescens* L. to a lesser extent (Govindarajan and Sathyannarayana, 1991).

Hot pepper is a high value cash crops in many countries of the world and in Egypt, not only because of its economic importance, but also due to nutritional and medicinal value of its fruits. These are the excellent sources of natural colors and antioxidant compounds (Howard *et al.*, 2000). A wide spectrum of antioxidant vitamins, carotenoids, capsaicinoids and phenolic compounds are present in hot pepper fruits. The intake of these compounds in food is an important health-protecting factor by prevention of widespread human diseases. As consumption continues to increase, hot peppers could provide important amounts of nutritional antioxidants to the human diet (Daood *et al.*, 1996), but maturation affects the synthesis of these compounds, which influence hot pepper quality e.g.,

differences in hot pepper color, shape and capsaicin level changes continuously during maturation. An increase in important nutrients occurs like ascorbic acid and vitamin A increased from green to red stage (Howard *et al.*, 1994 and Sidonia, 2012) Therefore, stage of harvest is an important factor affecting the content of these antioxidants in hot pepper fruits.

The heat in chillies comes from the chemical capsaicin, which is concentrated in inner membranes and the seeds, which play an effective role in stopping sensory nerve pain, which may explain the role of capsaicin in treating places affected by rheumatism, joint and muscle pain (Hirt and Bindanda, 2008). The hottest chilli in the world is the Naga Jolokia chilli (loosely, the name likens the 'bite' to that of a King Cobra snake) (www.cogs.asn.au summer 2009). & According to the Guinness book of records, the BhutJolokia of Assam is the world's hottest chilli pepper (http://www.worldofchillies.com 2010) Chilli plants love heat. They are closely related to capsicums/bell peppers, but chillies prefer their growing conditions a lot hotter. Chilli seeds need 20°C to germinate and it should be 30°C or more for the fruit to ripen. Night temperatures should not drop below 15°C.

Salinity in soil or water is one of the most damaging abiotic stress factors limiting crops (Debez *et al.*, 2006). It declines yield for most major crop plants by more than 50% and affects more than 10% of arable land (Bray *et al.*, 2000).

\* Corresponding author.

E-mail address: mostafa.fathy104@gmail.com - fathimostafa420@gmail.com

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Salt load in irrigation water, overuse of fertilizers and lack of proper drainage can be major factors contributing to this problem. Thus, high concentration of salts in the soils immediately imposes on plants the osmotic stress effect due to lower soil water potential leading to retardation of water uptake (Niu *et al.*, 1995). When exposed for a longer period and to a higher level of salts, salinity entails ionic stress when plants absorb and accumulate toxic level of Na<sup>+</sup> and Cl<sup>-</sup> in the cytoplasm especially Na<sup>+</sup> known by its impact on membrane disorganization, inhibition of cell division and expansion (Deivanai *et al.*, 2011).

Bio-fertilizers are environmentally friendly for sustainable agriculture and clean agriculture. They positively affect plant growth. Bio-fertilizers can promote plant growth and productivity. They are internationally accepted as an alternative source of N-fertilizer. Also Bio-fertilizers are formulations of beneficial microorganisms, which upon application can increase the availability of nutrients by their biological activity. Yeast is a natural source of cytokinins and has stimulatory effects. Accordingly, spraying yeast extract was used to participate in a beneficial role during the vegetative and reproductive growth stages because it contains a high percentage of auxin and cytokinin and enhancement carbohydrate accumulation (Barnett *et al.*, 1990). From previous studies, it was found that yeast is one of the richest sources of high quality protein, especially the essential amino acids like lysine and tryptophan, essential minerals as calcium and trace elements as cobalt and iron. Yeast is the best source of the B-complex vitamins and a valuable source of bio-constituents especially cytokinins (Amer 2004). Where, Mahmoud *et al.*, (2013) found that foliar spray with yeast extracts (2%) improved vegetative growth, pods quality and green pods yield of pea plants.

Due to the global trend of a clean environment, one of the directions is an alternative agriculture, in which no chemicals are used in agriculture. Recently, considerable attention has been given to the use of plant extracts in order to reduce plant and soil contaminations with different elements and also to reduce the application of mineral fertilizers. Licorice is a plant of the legume family, which is one of the wild plants that are known by common names, including (licorice) or (licorice root), which is famous in the Arab countries, since the earliest times. Turkey and Iran are considered one of the richest regions in the world for the cultivation and production of licorice, there are about 30 species of licorice plant, belongs fifteen families of it are under study, and the family, whose scientific name is *Glycyrrhiza glabra* is considered the most important and best because it is rich in the name of licorice fertilizer, which has a sweet taste and this substance is glycyrrhizin. Recent studies indicated the possibility of using some plant extracts, such as licorice extract (a perennial weed) to enhance the growth and productivity of plants (Droche, 1976). This plant contains many chemical compounds such as gleserezin,

licorice acid and flavonoids. Furthermore, Moses *et al.* (2002) stated that it contains a wide variety of elements and nutrients. Zuhair (2010) investigated the effect of licorice root extract at rates 0, 2, and 4 g/L as a foliar spray on the growth and flowering agents of two types of strawberry.

Organic manures contain higher levels of relatively available nutritional elements, which are essentially required for plant growth. Moreover, using organic fertilizers such as, cattle manure, chicken manure, pigeon manure, rabbit manure and farmyard manure play an important role in improving soil physical properties. It has been stated that, organic manures are slow release nitrogen fertilizers, where natural organic materials are broken down slowly by soil microorganisms (Kolbe *et al.*, 1995, Marschner, 1995, Rizk, 2001 and El-Gamal and Selim, 2005). Pigeon manures, among other organic materials containing comparatively higher contents of plant nutrients, which grouped under concentrated organic manures (Chandra, 2005). Pigeon manure is an organic waste, it is used as a source of organic fertilization as a result of its high nitrogen content, it has long been recognized as one of the most desirable manures. Besides fertilizing crops, the ancient Egyptians had knowledge of the value of using pigeon dung, when they planted vines they filled the plant hole with a mix of Nile mud and pigeon dung and with the flower plants it makes the colors more intense. Pigeon dung not only has a record in nitrogen amounts but also in phosphorus, potassium, magnesium, calcium and the highest organic matter, compared to other bird wastes in addition it is rich in humic and fulvic acids (Villa-Serrano *et al.* (2010).

Accordingly, the main goal of this work is to determine the response of hot pepper var. (Fire Bomb) plants to foliar spray by different concentrations of pigeon manure extract, yeast extract and licorice extract on growth, yield components and chemical constituents under salinity conditions.

## MATERIALS AND METHODS

A pot experiment was conducted at the Experimental Farm, Faculty of Agriculture, Al Azhar University, Assiut, Egypt during the two successive seasons 2018 and 2019. The objective of this work was to examine the impact of salinity levels and foliar spraying with some biostimulants, as well as, their interaction on plant growth, yield components and some chemical constituents of hot pepper var. (Fire Bomb) plants. The seeds were obtained from Kanza Group for Projects Development and Services 16 El-Falah st. Mohandeseen. Giza, Egypt. The seeds were sown in the 1<sup>st</sup> February in the two experimental seasons in seedling trays inside plastic greenhouse. The seedlings were transplanted 45 days after sowing in polyethylene bags (20 X 30 cm.), each bag filled with 8kg soil. Physical and chemical properties of the used soil are shown in Table 1.

**Table 1. Physical and chemical properties of the used soil .**

Texture	pH (1:2.5)	EC (m.mohs/cm)	CaCO <sub>3</sub> %	OM %	Total N %	Available		Water soluble ions (meq/l) in the soil paste				
						P ppm	K(mg/100g soil)	Ca	Mg	CO <sub>3</sub> +HCO <sub>3</sub>	Cl	SO <sub>4</sub>
Loamy	7.4	2.1	2.49	0.54	0.11	0.13	3.2	3.4	1.9	2.9	2.2	6.2

Before planting, phosphorus fertilizer as calcium superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>) was added at a rate 30 kg P<sub>2</sub>O<sub>5</sub>/fed. potassium sulphate fertilizer (48% K<sub>2</sub>O) was used

at a rate of 48 kg K<sub>2</sub>O/fed., while ammonium sulphate fertilizer (20.5% N) was applied at a rate 80 kg N/fed. was

divided into five small quantities and applied at one month intervals.

Current work prepared in a split plot design with three replicates. Each replicate included 5 plants grown in 5 pots. The main plots (A) contain three levels of salinity levels, while seven biostimulant treatments were considered in the sub-plots (B), so that the interaction (A x B) were 21 treatments. Thus, each replicate included 35 plants grown in 35 pots, each 5 pots were assigned for one treatment, to be the number of pots in the experiment 315 pots. The levels of salinity (main plots) were as follows:

- 1- Control (plants were irrigated with tap water).
- 2- 30 mM
- 3- 60 mM

The levels of salinity in irrigation water were salt mixture of (NaCl + CaCl<sub>2</sub>, 2:1 W/W). Each level of saline water was added regularly (750 ml/pot/week), after one month from transplanting of plants during the duration of the experiment (six months from transplanting).

**The bio stimulant treatments (sub plots) were as follows:**

- 1- Control treatment (Tap water).
- 2 – Pigeon manure extract (4g/L).
- 3 - Pigeon manure extract (8g/L).
- 4 – Yeast extract (4g/L).
- 5 - Yeast extract (8g/L).
- 6 – Licorice extract (4g/L).
- 7 - Licorice extract (8g/L).

These concentrations of biostimulants were applied as foliar spray five times at 21day intervals, the first was 30 days after planting.

**Preparation of biosteamulants.**

Pigeon manure was prepared by selecting a good manure and completely dry, then cleaned from remains of feathers, then grinded and soaking in 500 ml tap water for each rate for a week, then filtered and supplement the filtrate with the appropriate size for spraying.

Active dry yeast (*Sacchaomyces cerevisiae*) was prepared by dissolving in warm water (36° C) followed by adding sugar at a ratio 1:1 to active growth and reproduction of yeast and left stand for 3 hours before spraying.

Root licorice extract (RLE) was prepared from dry roots of *Glycyrrhiza glabra* L., which were soaked in water at concentrations of 4 and 8 g L<sup>-1</sup> and kept 24 hours at temperature room, then filtered through the filter paper type 12 Whatman to obtain an intense brown liquid extract.

**Experimental Measurements**

**(A)- Vegetative growth:**

- 1- Plant height (cm).
- 2- Branch number.
- 3- Herb fresh weigh (g/plant).
- 4- Herb dry weight (g/plant).
- 5- Leaf area (cm<sup>2</sup>) was measured by using this equation:  
 $0.53 (\text{length} \times \text{width}) + 1.66$  mentioned by Ahmed and Morsy (1999).

**(B)- Yield components:**

- 1- Pods number.
- 2- Pod length (cm).
- 3- Pods yield/plant (g)

**(C)- Chemical constituents:**

**1 – Sodium and calcium percentages:**

Two studied elements Na and Ca% in dried leaves were determined according to Jackson (1962).

**2- Free Proline content:**

Free proline content ( $\mu$  mole/g DW) was determined in dry leaves according to Bates *et al.* (1973).

**3- Total chlorophyll content**

Total chlorophyll in plant leaves was measured as SPAD units using Minolta chlorophyll meter (model SPAD 502) according to Markwell *et al.* (1995).

**4- Total capsaicin content (mg/g):**

Total capsaicin content in dry fruits was measured according to HPLC conditions. Where, chromatography conditions were based on the validated method reported by González-Zamora *et al.* (2013). The analyses of capsaicinoids were performed by HPLC-DAD (Agilent 1200, Agilent Technologies Palo Alto, CA, USA) employing a reversed phase column Kromasil Eternity-5-C18 (4.6 × 150 mm) with Precolumn (SUPELCO Analytical, Sigma-Aldrich) at 25 °C. Elution was performed with an isocratic mixture of water: acetonitrile 50: 50. Detection was set at 280 nm. Injection volume was 20  $\mu$ L. All peaks related to capsaicinoids were eluted in about 15 min. Quantitative analysis was performed following the external standard method. After extraction, HPLC analyses allowed identification and determination of capsaicin which was the main capsaicinoids in samples.

The Analysis of Variance (ANOVA) and LSD multiple range tests at 0.05 % level of probability were used to test the significance of differences between the treatments. Statistical data analyses were performed using Costat soft-ware.

## RESULTS AND DISCUSSION

### Results

#### Vegetative growth parameters

The data listed in Table 2 point out that the application of irrigation with saline water by all concentrations (30 mM and 60 mM NaCl+CaCl<sub>2</sub>) significantly decreased plant height (cm), number of branches, herb fresh weight (g/plant), herb dry weight (g/plant) and leaf area (cm<sup>2</sup>) of hot pepper var. (Fire Bomb) plants during the two experimental seasons. The only exception was on leaf area in the second season compared to control (without saline water). Obviously, the greatest reduction of vegetative growth parameters was obtained under high concentration (60 mM) in both seasons. The largest reduction percentage of plant height, number of branches, herb fresh weight, herb dry weight and leaf area as ranged 14.0 and 18.7, 20.2 and 22.9, 21.7 and 19.6, 24.8 and 23.2 and 11.0 and 18.1% less than control in the first and second seasons, respectively.

With respect to biostimulant treatments, data in Table 2 indicated that biostimulant treatments at all concentrations caused a significant augmentation in all vegetative growth parameters. Apparently, the best results were detected when foliar spray with both, pigeon manure extract and licorice extract at 8g/L in most vegetative growth parameters by 10.4 and 17.4, 16.1 and 22.3, 21.7 and 19.6, 25.1 and 24.0 and 9.13 and 14.65% over no sprayed ones during the two consecutive seasons, respectively.

Regarding to the interaction between saline water irrigation and foliar spray with biostimulant treatments on plant height, number of branches, herb fresh weight, herb

dry weight and leaf area of hot pepper var. (Fire bomb) during the two growing seasons, clearly, the plants irrigated by tap water (control) plus foliar spray by biostimulants at all concentrations, during the two seasons gave the highest values of such traits. On the other side the first concentration of saline water (30mM) with most biostimulants treatments

had a significant effect in all parameters, as shown in Table 2. These results mean that biostimulant treatments gave a positive effect on growth parameters, where reduced the harmful effect of salinity with regarding to first rate of saline water.

**Table 2. Influence of foliar spray with biostimulants on vegetative growth characteristics of hot pepper var. (Fire Bomb) plants, grown under levels of salinity during the two seasons of 2018 and 2019.**

Biostimulant treatments (B)	Salinity levels (A)							
	Plant height (cm)							
	The first season				The second season			
	Control	30 mM	60 mM	Mean (B)	Control	30 mM	60 mM	Mean (B)
Control	74.1	71.3	67.9	71.1	77.8	74.4	69.1	73.8
Pigeon manure extract at 4g/L	83.9	80.3	76.1	80.1	89.7	85.5	76.5	83.9
Pigeon manure extract at 8g/L	87.4	85.1	78.7	83.7	95.7	86.9	78.7	87.1
Yeast extract at 4g/L	83.6	78.1	73.4	78.4	88.4	82.4	71.1	80.6
Yeast extract at 8g/L	85.2	79.8	74.7	79.9	93.7	84.1	73.9	83.9
Licorice extract at 4g/L	84.7	78.9	75.8	79.8	90.5	83.1	73.2	82.3
Licorice extract at 8g/L	86.1	83.9	77.7	82.6	94.1	84.7	77.8	85.5
Mean (A)	83.6	79.6	74.9		90.0	83.0	74.3	
L.S.D. 0.5%	A=2.1		B=1.5	AB=3.1	A=2.4		B=1.6	AB=3.2
Branch number								
Control	10.8	10.3	9.1	10.1	11.3	10.6	9.4	10.4
Pigeon manure extract at 4g/L	12.6	11.8	10.5	11.6	13.2	12.4	10.9	12.2
Pigeon manure extract at 8g/L	13.1	12.8	11.8	12.6	14.0	13.0	11.3	12.8
Yeast extract at 4g/L	12.3	11.7	9.9	11.3	12.8	12.2	9.4	11.5
Yeast extract at 8g/L	12.8	12.4	10.1	11.8	13.2	13.0	9.9	12.0
Licorice extract at 4g/L	12.3	12.2	10.3	11.6	12.9	12.8	9.7	11.8
Licorice extract at 8g/L	12.6	12.3	11.0	12.0	13.3	12.9	10.4	12.2
Mean (A)	12.4	11.9	10.4		13.0	12.4	10.1	
L.S.D. 0.5%	A=0.4		B=0.6	AB=1.2	A=0.6		B=0.7	AB=1.3
Herb fresh weight (g/plant)								
Control	111.5	97.4	86.3	98.4	112.8	102.4	83.1	99.4
Pigeon manure extract at 4g/L	128.5	121.3	100.8	116.9	133.8	122.6	104.6	120.3
Pigeon manure extract at 8g/L	136.8	124.7	117.9	126.5	143.5	126.2	122.9	130.9
Yeast extract at 4g/L	120.4	116.4	90.8	109.2	119.8	118.9	95.0	111.2
Yeast extract at 8g/L	123.8	119.8	92.1	111.9	122.4	121.3	97.9	113.9
Licorice extract at 4g/L	122.7	120.7	91.3	111.6	125.1	121.5	98.4	115.0
Licorice extract at 8g/L	128.4	123.2	103.3	118.3	130.6	126.1	112.2	123.0
Mean (A)	124.6	117.6	97.5		126.9	119.9	102.0	
L.S.D. 0.5%	A=1.2		B=1.3	AB=2.7	A=1.4		B=1.4	AB=2.8
Herb dry weight (g/plant)								
Control	28.8	24.5	19.8	24.4	30.6	26.9	22.5	26.7
Pigeon manure extract at 4g/L	37.8	32.8	28.6	33.1	41.9	35.1	28.6	35.2
Pigeon manure extract at 8g/L	40.1	35.4	31.3	35.6	43.1	36.3	34.9	38.1
Yeast extract at 4g/L	33.1	31.5	24.6	29.7	35.2	34.1	27.1	32.2
Yeast extract at 8g/L	36.4	35.2	27.1	32.9	37.2	35.1	28.3	33.5
Licorice extract at 4g/L	36.2	33.6	25.7	31.8	37.1	34.9	28.2	33.4
Licorice extract at 8g/L	37.9	35.6	30.5	34.7	39.9	35.9	32.3	36.0
Mean (A)	35.8	32.6	26.8		37.9	34.0	28.8	
L.S.D. 0.5%	A=0.8		B=1.3	AB=2.4	A=0.6		B=1.4	AB=2.7
Leaf area (cm <sup>2</sup> )								
Control	9.92	9.73	9.37	9.67	10.49	9.89	9.77	10.05
Pigeon manure extract at 4g/L	11.71	10.66	10.24	10.87	12.58	10.97	10.52	11.36
Pigeon manure extract at 8g/L	12.63	11.49	11.07	11.73	13.70	11.88	10.64	12.07
Yeast extract at 4g/L	10.57	10.75	10.22	10.51	11.82	10.90	10.08	10.93
Yeast extract at 8g/L	12.04	11.07	10.66	11.26	12.79	11.69	11.21	11.90
Licorice extract at 4g/L	11.40	10.41	10.42	10.74	12.01	11.43	10.34	11.26
Licorice extract at 8g/L	11.47	10.90	10.44	10.94	12.16	11.84	10.44	11.48
Mean (A)	11.39	10.72	10.35		12.22	11.23	10.43	
L.S.D. 0.5%	A=0.51		B=0.54	AB=0.94	A=1.16		B=0.69	AB=1.19

**Yield components:**

The recorded data in Table 3 indicate that pod number, pod length (cm) and pods yield/plant (g) significantly decreased as a result of irrigation with saline

water at all concentrations over control (un-salinized water) in the two seasons, except for the first concentration (30 mM) with regard to pod length (cm) in the first season only over control. It is clear that, the largest decrease in yield

components was due to the highest concentration of saline water (60 mM) during the two growing seasons. The largest reduce percentage of pod number, pods length (cm) and pods yield/plant (g) was by 14.8 and 15.5, 7.55 and 8.22 and 24.85 and 25.31% less than un-salinized plants in the first and second seasons, respectively.

With regard to biostimulant treatments, the recorded data in Table 3 proved that pod number, pod length (cm) and pods yield/plant (g) of hot pepper var. (Fire bomb) significantly affected by the application of the foliar spray of bio stimulants during the two consecutive seasons. Clearly, foliar spray with bio stimulants at all concentrations caused a significant augment in all yield components, during both seasons, as compared to control. The best results of pod number, pod length (cm) and pods yield/plant (g) were given by foliar spray with pigeon manure extract at 8g/L

followed by licorice extract at 8g/L in both seasons, except for 4g/L yeast and licorice extract, with regarding pods length (cm) in the second season. The increment in yield components were by 27.6 and 26.9, 14.72 and 17.01 and 28.52 and 30.93% over control.

Concerning to the combined effect between the two study factors, irrigation with saline water + bio stimulant treatments on hot pepper var. (Fire bomb) plants, apparently, the foliar spraying with most concentrations of biostimulants relieves the negative impact of salinity stress, can be clearly seen on both number of pod and pods yield/plant (g) with regard to the first concentration (30 mM) from saline water in the two growing seasons. While, there was no significant effect on pod length (cm) in the two seasons, as shown in Table 3.

**Table 3. Influence of foliar spray with biostimulants on yield components of hot pepper var. (Fire Bomb) plants, grown under levels of salinity during the two seasons of 2018 and 2019.**

Biostimulant treatments (B)	Salinity levels (A)							
	Pod number							
	The first season				The second season			
	Control	30 mM	60 mM	Mean (B)	Control	30 mM	60 mM	Mean (B)
Control	40.5	37.8	32.4	36.9	42.9	40.1	34.0	39.0
Pigeon manure extract at 4g/L	45.4	42.8	38.6	42.3	48.1	45.4	41.6	45.0
Pigeon manure extract at 8g/L	51.9	45.6	43.9	47.1	55.2	48.2	45.0	49.5
Yeast extract at 4g/L	44.1	41.0	39.3	41.5	47.2	42.8	40.7	43.5
Yeast extract at 8g/L	47.1	44.9	40.8	44.2	49.1	45.8	42.7	45.9
Licorice extract at 4g/L	47.2	42.7	40.7	43.5	48.8	45.0	41.0	44.9
Licorice extract at 8g/L	49.1	45.4	41.4	45.3	51.0	46.9	43.8	47.2
Mean (A)	46.5	42.9	39.6		48.9	44.9	41.3	
L.S.D. 0.5%	A=0.7		B=1.1	AB=1.9	A=0.9		B=1.2	AB=2.0
Pod length (cm)								
Control	5.53	5.20	4.97	5.23	6.03	5.77	5.49	5.76
Pigeon manure extract at 4g/L	6.07	5.90	5.57	5.84	6.49	6.38	6.14	6.34
Pigeon manure extract at 8g/L	6.17	6.03	5.80	6.00	7.11	6.72	6.39	6.74
Yeast extract at 4g/L	5.97	5.89	5.50	5.79	6.36	5.93	5.99	6.09
Yeast extract at 8g/L	6.30	6.10	5.73	6.04	6.97	6.42	6.17	6.52
Licorice extract at 4g/L	5.83	5.67	5.40	5.63	6.17	6.12	5.92	6.07
Licorice extract at 8g/L	5.87	5.80	5.60	5.76	6.89	6.17	6.13	6.40
Mean (A)	5.96	5.80	5.51		6.57	6.22	6.03	
L.S.D. 0.5%	A=0.24		B=0.37	AB=N.S	A=0.31		B=0.45	AB=N.S
Pods yield/plant (g)								
Control	21.76	20.85	17.44	20.02	22.45	21.18	17.96	20.53
Pigeon manure extract at 4g/L	27.24	22.60	20.37	23.40	28.47	23.57	20.64	24.23
Pigeon manure extract at 8g/L	31.14	24.08	21.95	25.73	32.59	25.10	22.95	26.88
Yeast extract at 4g/L	26.48	21.64	20.41	22.84	27.87	22.23	20.76	23.62
Yeast extract at 8g/L	28.15	23.61	21.51	24.42	29.00	23.81	22.20	25.00
Licorice extract at 4g/L	28.30	22.52	21.11	23.98	28.86	23.40	21.62	24.63
Licorice extract at 8g/L	29.36	23.75	21.82	24.98	30.20	24.40	22.79	25.80
Mean (A)	27.49	22.72	20.66		28.49	23.38	21.28	
L.S.D. 0.5%	A: 0.19		B:0.41	AB: 0.71	A: 0.46		B:0.51	AB: 0.88

**Chemical constituents:**

**1- Free Proline (µ mole/g), Sodium and Calcium%:**

Given data in Table 4 reveal that the obtained values of saline water irrigation treatments significantly affected proline (µ mole/g), Na and Ca% in leaves of hot pepper var. (Fire bomb) during the two experimental seasons. Where, there was a gradual increment in these traits with increasing saline water levels up to a concentration of 60mM. Clearly, the highest values of proline (µ mole/g), sodium and calcium% were given by plants irrigated with the high level of saline water (60mM), which increased proline (µ mole/g) by 18.25 and 17.74, Na % by 66.04 and 66.67% and

increased Ca% by 54.90 and 66.66% over un-salinized plants in the two seasons, respectively.

With respect to biostimulant treatments, data in Table 4 indicate that all concentrations caused a significant reduction in the three examined parameters, as proline (µ mole/g), sodium and calcium% in leaves of hot pepper var. (Fire bomb) as compared to untreated plants during the two growing seasons. The lowest values of these parameters were given by yeast extract at 8g/L, which reduced proline (µ mole/g) by 10.47 and 9.77%, Na % by 36.26 and by 38.37% and reduced Ca% by 28.40 and by 31.58% than control in the two seasons, respectively.

With regard to the interaction between the two examined factors, proline ( $\mu$  mole/g), sodium and calcium% in leaves of pepper var. (Fire bomb) had a significant effect in the two experimental seasons. From the obtained data, it could be noticed that the highest values of proline ( $\mu$  mole/g), sodium and calcium% were obtained in plants

irrigated with saline water at 60 mM without biostimulant treatments in both seasons. Whereas, the lowest values of proline ( $\mu$  mole/g), Na and Ca% were obtained from plants irrigated with control (tap water) plus sprayed by yeast extract at 8g/L in the two experimental seasons, respectively, as shown in Table 4.

**Table 4. Influence of foliar spray with biostimulants on free proline ( $\mu$  mole/g), sodium and calcium% of hot pepper var. (Fire Bomb) plants, grown under levels of salinity during the two seasons of 2018 and 2019.**

Biostimulant treatments (B)	Salinity levels (A)							
	Free proline ( $\mu$ mole/g D.W)							
	The first season				The second season			
	Control	30 mM	60 mM	Mean (B)	Control	30 mM	60 mM	Mean (B)
Control	2.69	2.98	3.21	2.96	2.76	3.18	3.27	3.07
Pigeon manure extract at 4g/L	2.57	2.86	3.03	2.82	2.69	2.95	3.20	2.95
Pigeon manure extract at 8g/L	2.53	2.83	2.98	2.78	2.67	2.94	3.19	2.93
Yeast extract at 4g/L	2.44	2.74	2.90	2.69	2.59	2.85	3.01	2.81
Yeast extract at 8g/L	2.40	2.71	2.85	2.65	2.57	2.79	2.95	2.77
Licorice extract at 4g/L	2.52	2.81	2.96	2.76	2.66	2.89	3.13	2.89
Licorice extract at 8g/L	2.45	2.75	2.89	2.70	2.59	2.84	3.07	2.83
Mean (A)	2.52	2.81	2.98		2.65	2.92	3.12	
L.S.D. 0.5%	A=0.02 B=0.12 AB=0.22				A=0.01 B=0.11 AB=0.23			
Sodium%								
Control	0.71	0.93	1.11	0.91	0.66	0.89	1.02	0.86
Pigeon manure extract at 4g/L	0.60	0.79	0.94	0.78	0.53	0.73	0.86	0.71
Pigeon manure extract at 8g/L	0.54	0.76	0.89	0.73	0.49	0.70	0.83	0.67
Yeast extract at 4g/L	0.45	0.63	0.78	0.62	0.42	0.56	0.71	0.56
Yeast extract at 8g/L	0.42	0.58	0.75	0.58	0.39	0.52	0.68	0.53
Licorice extract at 4g/L	0.52	0.71	0.86	0.70	0.46	0.63	0.78	0.62
Licorice extract at 8g/L	0.47	0.67	0.82	0.66	0.43	0.61	0.73	0.59
Mean (A)	0.53	0.72	0.88		0.48	0.66	0.80	
L.S.D. 0.5%	A=0.01 B=0.12 AB=0.21				A=0.02 B=0.11 AB=0.19			
Calcium%								
Control	0.62	0.84	0.97	0.81	0.57	0.80	0.92	0.76
Pigeon manure extract at 4g/L	0.54	0.74	0.85	0.71	0.49	0.70	0.82	0.67
Pigeon manure extract at 8g/L	0.52	0.73	0.83	0.69	0.46	0.68	0.80	0.65
Yeast extract at 4g/L	0.46	0.66	0.69	0.60	0.40	0.57	0.66	0.54
Yeast extract at 8g/L	0.44	0.63	0.67	0.58	0.37	0.54	0.64	0.52
Licorice extract at 4g/L	0.49	0.71	0.78	0.66	0.45	0.64	0.74	0.61
Licorice extract at 8g/L	0.47	0.68	0.73	0.63	0.42	0.60	0.69	0.57
Mean (A)	0.51	0.71	0.79		0.45	0.64	0.75	
L.S.D. 0.5%	A=0.01 B=0.09 AB=0.16				A=0.01 B=0.08 AB=0.14			

**2- Total chlorophyll content:**

Data in Table 5 indicated that the addition of saline water significantly decreased total chlorophyll in leaves of hot pepper var. (Fire bomb) as compared to un-salinized plants, during the two experimental seasons. The maximum decrease of total chlorophyll was due to the largest concentration of saline water (60 mM) during the two growing seasons. The maximum decrease of total chlorophyll content (SPAD) was by 9.48 and 7.24% less than un-salinized plants in the first and second seasons, respectively.

It worthy that foliar spray with biostimulant treatments significantly affected total chlorophyll in leaves of hot pepper var. (Fire bomb), during the two experimental seasons. Apparently, total chlorophyll was significantly increased as a result of foliar spray with biostimulant treatments at all concentrations, compared to no sprayed ones, in both seasons. The highest value of total chlorophyll was given using pigeon manure extract and licorice extract at 8g/L reached 17.19 and 13.58% over no sprayed plants in the first and second seasons, respectively, as clearly declared in Table 5.

As for the combined effect, it was significant in total chlorophyll of leaves of hot pepper var. (Fire bomb), during the two seasons. Apparently, most combined treatments, in both seasons, significantly augmented in such traits. Plants grown under saline water treatments at zero with foliar spray with biostimulants at all concentrations, during the two seasons gave highly values of total chlorophyll. On the other side, the intermediate of saline water (30mM) + all concentrations of biostimulants caused a significant effect of total chlorophyll, except with respect to yeast extract at 4g/L in the second season, as shown in Table (5).

**3- Total capsaicin content (mg/g):**

The obtained results in Table 6 reveale that saline water irrigation treatments positively affected total capsaicin (mg/g) of hot pepper var. (Fire bomb) plants during the two experimental seasons. It is appear, both concentrations of saline water 30 mM followed by 60 mM led to a significant increase in total capsaicin (mg/g) over control (un-salinized plants) in both seasons. However, the maximum value of total capsaicin (mg/g) was obtained due to the medium concentration of saline water (30 mM) as ranged 60.12 and 41.77% over un-salinized ones in the first and the second seasons, respectively.

With respect to biostimulant treatments, data in Table 6 show that all concentrations caused significant augmentation in total capsaicin (mg/g) of hot pepper var. (Fire bomb) in the two consecutive seasons. The maximum values of capsaicin (mg/g) were detected through the foliar spray with both, pigeon manure extract and licorice extract at 8g/L as ranged 24.50 and 17.75% over control in the two seasons.

With regarding to the interaction between the two factors, total capsaicin (mg/g) of hot pepper var. (Fire Bomb) had a significant effect in the two experimental seasons. The most effective treatments were detected due to treating the plants with saline water by medium concentration (30 mM) + pigeon manure extract and licorice extract at 8g/L. compared to other treatments in the two seasons, as clearly in Table 6.

**Table 5 and 6. Influence of foliar spray with biostimulants on total chlorophyll content (SPAD) and total capsaicin content (mg/g) of hot pepper var. (Fire Bomb) plants, grown under levels of salinity during the two seasons of 2018 and 2019.**

Biostimulant treatments (B)	Salinity levels (A)							
	Total chlorophyll content							
	The first season				The second season			
	Control	30 mM	60 mM	Mean (B)	Control	30 mM	60 mM	Mean (B)
Control	56.77	54.47	50.73	53.99	59.60	58.47	55.80	57.96
Pigeon manure extract at 4g/L	63.47	61.47	58.57	61.17	67.66	64.41	62.11	64.73
Pigeon manure extract at 8g/L	66.33	63.37	60.10	63.27	68.33	65.90	63.27	65.83
Yeast extract at 4g/L	62.73	59.67	55.90	59.43	65.21	61.68	61.07	62.65
Yeast extract at 8g/L	64.87	60.53	57.83	61.08	66.32	63.77	61.96	64.01
Licorice extract at 4g/L	62.37	61.07	57.27	60.23	65.76	65.33	60.72	63.94
Licorice extract at 8g/L	65.10	62.13	59.40	62.21	67.41	65.70	62.08	65.06
Mean (A)	63.09	60.39	57.11		65.76	63.61	61.00	
L.S.D. 0.5%	A=0.98 B=1.06 AB=1.83				A=1.11 B=1.26 AB=2.19			
Total capsaicin content (mg/g)								
Control	1.60	2.22	2.20	2.00	2.36	2.98	2.96	2.76
Pigeon manure extract at 4g/L	1.73	2.84	2.54	2.37	2.49	3.60	3.30	3.13
Pigeon manure extract at 8g/L	1.85	3.01	2.61	2.49	2.61	3.77	3.37	3.25
Yeast extract at 4g/L	1.64	2.70	2.50	2.28	2.40	3.46	3.26	3.04
Yeast extract at 8g/L	1.75	2.78	2.53	2.35	2.51	3.54	3.29	3.11
Licorice extract at 4g/L	1.77	2.86	2.53	2.39	2.53	3.62	3.29	3.15
Licorice extract at 8g/L	1.80	2.99	2.58	2.46	2.56	3.75	3.34	3.22
Mean (A)	1.73	2.77	2.50		2.49	3.53	3.26	
L.S.D. 0.5%	A=0.02 B=0.27 AB=0.47				A=0.03 B=0.29 AB=0.54			

**Discussion**

This work was conducted to explore the influence of saline water irrigation and some biostimulants namely pigeon manure extract, yeast extract and licorice extract, as well as, their interactions on plant growth traits, yield components and chemical constituents of hot pepper var. (Fire bomb) plants. The obtained results can be discussed physiologically and biologically as follows:

**The harmful effects of salinity on plant growth and yield can be explained in two ways:**

- The first phase, impacts of salinity on water relations can be important, causing leaf stomatal closure and the inhibition of leaf expansion (Munns and Termaat, 1986).
- The second phase, the ion dependent response to salinity, develops over a longer time (days to weeks) and involves accumulation of ions in the shoot to toxic concentrations, especially in old leaves, causing premature aging of leaves and ultimately reduced yield or even plant death (Munns and Tester, 2008). Salinity affects plant growth because the salts at the high concentration in soil solution interferes with equiponderant absorption of essential nutritional ions by plants (Tester and Devenport, 2003). Oxidative stress may also resulted from Imbalances of osmotic stress, metabolism triggered by ion toxicity and deficiency of nutrients under saline conditions (Zhu, 2002).

Proline accumulation is a known physiological response in many plants in response to a wide range of stresses (Nathalie and Christian, 2008). Azevedo Neto and Silva (2015) postulated that increased enzymatic activity,

cell redox homeostasis maintenance, membranes of proteins, protein stabilization, the stocks of carbon and nitrogen, Regulating cellular acidity and removing free radicals are the functions resulting from proline accumulation. Santos (2004) showed that inhibition of chlorophyll synthesis, along with activation of its decomposition by the enzyme chlorophyllase, led to reduction of chlorophyll levels in salt-treated plants. This is not the only explanation for photosynthesis inhibition in the presence of salinity whereas, NaCl also inhibits main enzymes such as Rubisco and PEP carboxylase that are responsible for this process (Soussi *et al.*, 1998). Free proline content is relies on the osmotic stress degree and positively associated with (Na<sup>+</sup> Cl<sup>-</sup>) content in plant after a certain period, depending on age of plant and proline molar to (Na<sup>+</sup> Cl<sup>-</sup>) becomes constant. (Flowers *et al.*, 1977). On contrary, the obtained results pointed out that the use of saline water at 30 and 60 mM led to an increase in total capsaicin in fruits of hot pepper (Fire Bomb) plants. In this regard, similar results were found by Arrowsmith *et al.*, (2012) they use of saline water at (0.0, 0.5, 1 and 1.5% Nacl solution) of *Capsicum annum*. They found that two highest salinities (1 and 1.5% Nacl solution) had higher levels of capsaicin compared to control.

The useful effect of biostimulants on growth, yield and chemical constituents of hot pepper plants might be due to the important role of these materials in biological and physiological process.

**- As for pigeon manures:**

Ahmed and Elzaawely (2010) investigated the effect of pigeon manure combined with chicken manure on cowpea plants. They demonstrated that these treatments were superior and significantly increased plant height, number of leaves, number of branches, leaf area and number of pods. Saadawy and Abdel-Moniem (2015) applied pigeon manure, as foliar spray at (4 and 6g/L) on *Euphorbia milii* and showed that number of branches/plant, number of leaves/plant, leaf length, leaf width and total chlorophyll significantly increased by application pigeon manure extract at concentrations 4 or 6 g/L. Bidabadi *et al.*, (2017) on pomegranate seedlings affected by salt stress, they applied vermicompost tea as foliar spray and indicated that plant height, shoot fresh and dry weight and leaf area in these seedlings significantly augmented as a result of salt tolerance by reducing the accumulation of Na<sup>+</sup>. Hassan *et al.* (2017) fertilized Chili Pepper (*Capsicum frutescens* L.) plants with filter mud cake at 0, 8, 16 and 24 m<sup>3</sup>/fed. The obtained data confirmed that plant height, number of branches, fresh and dry weight, pods yield/plant and fed and total capsaicin content were generally increased as a result of applying filter mud cake at high level (24 m<sup>3</sup> /fed.).

**- Regarding yeast extracts:**

Active dry yeast is safety natural bio-fertilizer causes various stimulation effects on plants. Yeast is a rich source for enzymes, cytokinines, amino acids, vitamins and minerals. (Khedr and Farid, 2002). Also, it has been stimulatory effects on cell division and hypertrophy and synthesis of proteins, nucleic acids, chlorophyll and vitamin B. (Castelfrance and Beale,1983). treatments affect growth and volatile oil production of rosemary plant. Nassar *et al.* (2016) showed that the application on of active dry yeast (ADY) at 100ml/L on leucaena plants significantly affected plant height, stem diameter, fresh stem weight/plant and chloroplast pigments. Whereas, stated that yeast extract had the ability to induce significant recovery for the reduction occurred in vegetative growth of leucaena plants which exposed to salinity stress. Matter and Elsayed (2015) studied the effect of spraying active dry yeast at 0, 2 and 4 g L<sup>-1</sup> on growth, seed yield, essential oil and its main components of caraway plants grown under newly reclaimed soils. The obtained results showed that the treated caraway plants with active dry yeast led to improve plant growth characters expressed as plant height, number of branches, fresh and dry weights of herb plant, fresh and dry weights of umbels plant and weight of fruits plant, total chlorophylls, carotenoids, total carbohydrates and essential oil %. Heikal (2005) showed that foliar spraying of (ADY) increased growth and essential oil yield of thyme plant.

**-Concerning with Licorice extracts:**

Newall, *et al.*, (1996) stated that licorice root extract (LRE) is contributed to improving plant growth and production. Where, contain of a substance namely "glycyrrhizin"; the calcium and potassium salts, glycyrrhizic acid and trihydroxy acid. Thanaa (2016) showed that licorice extract contains plant performance enhancing capabilities due to that it is a rich source in antioxidants such as amino acids, soluble sugars, proline, salicylic acid,  $\alpha$ -tocopherol, glutathione, ascorbic acid, some vitamins (i.e., A, E, and B group) and selenium. Also, it is rich in phytohormones including significant amounts of auxins,

gibberellins, cytokinins (zeatin-type) and nutrient elements. Desoky *et al.* (2019) studied the potential effects of licorice root extract (LRE) at (0.5%) as, foliar spray on growth and green yield, physio-biochemical attributes and antioxidant defense systems of *Capsicum annuum* L plants grown under salinity stress. The results showed that plant growth, yield, proline and chlorophylls content were significantly increased by application of licorice root extract under this conditions.

## CONCLUSION

From above mentioned results, could be noticed that hot pepper var. (Fire bomb) plants can grow healthy under medium concentration of saline water (30 mM), it could be recommended to foliar spray these plants with pigeon manure extract and licorice extract at rate 8 g/L, in most cases to enhance growth, yield, chlorophyll content and capsaicin content, as well as, reducing sodium, calcium % and proline content in leaves which they are harmful affect plants under this experiment conditions.

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## النمو والمحصول والمكونات الكيميائية في الفلفل الحار صنف (قنبلة النار) المتأثر بتطبيق مستخلص المنشطات الحيوية تحت ظروف الإجهاد الملحي

مصطفى فتحي إبراهيم<sup>1</sup> و أحمد عبد الرحيم سيد عبد الرحيم<sup>2</sup>

<sup>1</sup>قسم البساتين (زينة ونباتات طبية وعطرية) كلية الزراعة، جامعة الأزهر، فرع أسسيوط، مصر.

<sup>2</sup>قسم البساتين (خضص) كلية الزراعة، جامعة الأزهر، فرع أسسيوط، مصر.

تجربة أصص أجريت في مزرعة كلية الزراعة جامعة الأزهر بأسسيوط خلال موسمين متتاليين 2018 و 2019 بهدف دراسة تأثير ثلاثة معدلات من المياه المالحة بتركيزات (0.0، 30 ملليمول، 60 ملليمول كلوريد الصوديوم + كلوريد الكالسيوم) والرش الورقي بالمحفزات الحيوية (كنترول (ماء الصنوبر)، مستخلص سماد الحمام بمعدل 4 جم / لتر، مستخلص سماد الحمام بمعدل 8 جم / لتر، مستخلص الخميرة بمعدل 4 جم / لتر، مستخلص الخميرة بمعدل 8 جم / لتر / لتر مستخلص العرق سوس بمعدل 4 جم / لتر ومستخلص العرق سوس بمعدل 8 جم / لتر) وكذلك التفاعل بينهما علي النمو والمحصول والمكونات الكيميائية في الفلفل الحار صنف (قنبلة النار). أظهرت النتائج المتحصل عليها أن كلا التركيزين (30 ملليمول و 60 ملليمول كلوريد الصوديوم + كلوريد الكالسيوم) قد تسببا في انخفاض معنوي في كل صفات النمو الخضري ومكونات المحصول ومحتوى الكلوروفيل في نبات الفلفل الحار. لقد لوحظ أقصى انخفاض عند تركيز 60 ملليمول كلوريد الصوديوم + كلوريد الكالسيوم، والذي أظهر أعلى زيادة في محتوى البرولين والنسبة المئوية لكلا من عنصري الصوديوم والكالسيوم. بينما كان هناك زيادة معنوية في محتوى الكابيسيسين نتيجة تركيزات (30 ملليمول و 60 ملليمول كلوريد الصوديوم + كلوريد الكالسيوم) بالمقارنة بالكنترول. أفضل النتائج لمحتوى الكابيسيسين كانت مع الري بالمياه المالحة بالتركيز المتوسط (30 ملليمول كلوريد الصوديوم + كلوريد الكالسيوم). فيما يتعلق بالرش الورقي مع معاملات المنشطات الحيوية بجميع التركيزات، أدت هذه المعاملات إلى زيادة معنوية في صفات النمو الخضري ومكونات المحصول، محتوى الكلوروفيل الكلي ومحتوى الكابيسيسين الكلي. لقد أعطي التركيز المرتفع لمستخلص سماد الحمام ومستخلص العرق سوس بتركيز 8 جم / لتر أعلى قيم لهذه الصفات السابقة بالمقارنة بالكنترول. من ناحية أخرى، تسبب الرش الورقي بهذه المنشطات الحيوية المدروسة بجميع التركيزات في انخفاض محتوى البرولين، الصوديوم والكالسيوم في الأوراق. فيما يتعلق بالجمع بين العاملين تحت الدراسة، فإن الرش الورقي بمعاملات المنشطات الحيوية يخفف التأثير الضار للإجهاد الملحي، في معظم الحالات، على وجه التحديد، المعدل المتوسط للملوحة (30 ملليمول كلوريد الصوديوم + كلوريد الكالسيوم) وبالتالي فإن خصائص النمو الخضري ومكونات المحصول والمواد الكيميائية تحسنت في الموسمين التجريبيين.

**الكلمات الدالة:** الفلفل الحار (قنبلة النار)، إجهاد الملح، المنشطات الحيوية ومحتوى الكابيسيسين الكلي.