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# Reducing the harmful effect of salinity on lettuce productivity by foliar application of plant extracts under greenhouse conditions



Tarek A. A. M. El-Masry, Nevein A. El-Sawah, Ashraf S. Osman, Noha A. A. Abo-Arab\*

Horticulture Department, Faculty of Agriculture, Fayoum University, Fayoum 63514, Egypt

## ABSTRACT

Two greenhouse experiments were conducted during 2019/2020 and 2020/2021 at the Fayoum University of Egypt's Agricultural Test Station, Faculty of Agriculture, to study effect of leaf Nitrogen, Phosphorus, Potassium, Calcium and Sodium contents, inorganic and organic osmotica substances contents, productivity and quality of lettuce plants (Lactuca sativa L) to elucidate their potential to reduce the harmful effect of soil salinity (ECe =  $7.00 \pm 0.20$  dS m<sup>-</sup> <sup>1</sup>). Treatments comprised with twelve of plant growth promoters; palm pollen grains (100 and 200 mg  $L^{-1}$ ), corn grain extract (100 and 200 mg  $L^{-1}$ ), soybean seed extract (100 and 200 mg  $L^{-1}$ ) <sup>1</sup>), mannitol (1500 and 3000mgL<sup>-1</sup>), sorbitol (1500 and 3000 mg L<sup>-1</sup>) and calcium phosphate (800 and 1000  $Ca_3(PO_4)_2$  mg L<sup>-1</sup>), as well as, tap water. Three times of the thirteen treatments were made as foliar applications to runoff; 25, 40 and 55 days after transplanting. A randomized complete blocks design with five replications comprised the experimental setup. All the foliar application of the twelve treatments (plant extracts, mannitol, sorbitol and calcium phosphate) were better and considerably greater mean values were obtained of leaf N, P, K, and Ca contents, inorganic and organic osmotica substances contents as well as decreased of leaf Na comparing to control. As a result, the physio-biochemical components of plants were improved, which reflected on improve productivity and quality of lettuce plants (cv. Big Bell) under the salinized soil stress of the Favoum Governorate and other related regions. Generally, foliar application of soybean seed extract at a 200 mg L<sup>-1</sup> concentration or palm pollen grains extract at at a 100 mg L<sup>-1</sup> concentration were superior and substantially higher mean values were recorded of data recorded comparing to other treatments.

**Keywords**: Bio-stimulants, Calcium phosphate, Osmotic substances, Mannitol, Sorbitol, Yield and quality.

\*Corresponding author's e-mail address: naa07@fayoum.edu.eg Submit date: 27-03-2024 Revise date: 13-01-2025 Accept date: 13-01-2025

## **1. INTRODUCTION**

One of the most widely farmed vegetable crops is head lettuce, Capoutshi (Lactuca sativa L.) and one of the important leafy vegetable crops that are eaten fresh, and considered the world's most popular salad crop (Rubatzky et al., 1997). Vitamin A and minerals like calcium and iron are rich in lettuce. Protein. carbohydrates, and vitamin C are also present. For example, a 100 g edible portion of lettuce has 93.4 g of moisture, 2.1g of protein, 0.3 g of fat, 1.2 g of minerals, 0.5 g of fiber, 2.5 g of carbohydrates, 310 mg of calcium, 80 mg of phosphorus, 2.6 mg of iron, 1650 I.U. of vitamin A, 0.09 mg of thiamine, 0.13 mg of riboflavin, and approximately 10.0 mg of vitamin C (Gopalan and Balaraman, 1966). It has been determined that lettuce is a fairly sensitive crop to salt, having a negative slope that rises by 13% for every unit of increased salinity above the limit of conductivity of electricity (ECe) of 1.3 dS m<sup>-1</sup>. (Ayers et al., 1951). In Egypt, one of the main issues with agriculture is saline soil, because salty conditions lowers soil water's osmotic potential, it decreases plant availability. (Asik et al., 2009). Additionally, because saline soils have a weak structure, it reduces the availability of critical nutrients for plants (Osman and Rady, 2012). Reactive oxygen species (ROS) build up because of a variety of physiological alterations and metabolic process impairments brought on by it (Qaseem et al., 2019) these negative consequences have an adverse effect on the growth of plants, productivity and yield quality (Mansour et al., 2021; Mannan et al., 2022). However, plants use intricate defense mechanisms, such as the antioxidant defense system, to withstand saline soil stress (Khalvandi et al., 2021). The protective antioxidant system consists of two primary components: antioxidants without enzymes, glutathione. like

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ascorbate, and  $\alpha$ -tocopherol, antioxidants derived from enzymes like glutathione peroxidase, superoxide dismutase, and ascorbate peroxidase (Mishra et al., 2023). Thus, it is advised to increase plant's resistance to soil salinity stress by using applications exogenous (such as antioxidants and plant extracts) (Schäfer et al., 2020). Because of the high ROS production that prevents the antioxidant components from scavenging, prolonged exposure to saline soil stress results in significant damage and eventually kills plant cells (Taha et al., 2020). Thus, it is crucial to develop some of the methods for helping plants grown under stress (Rady et al., 2016; Merwad et al., 2018). The use of naturally occurring biostimulant substances with growth-promoting properties to reduce the negative The negative effects of stress from salinized soil are thus attracted a lot of attention. Plant development and growth can be promoted by the bioactive compounds found in plant extracts, which can enhance many physiological processes by Bulgari et al. (2015). Current research has concentrated on using plant extracts to enhance vegetative growth and boost plant production because these extracts include essential nutrients and growth regulators, such as vitamins and organic acids, which differ in quantity and quality in different plant parts and are also inexpensive, easily absorbed by the plant, and readily available (Ibriham and AL-Hussain, 2009).

Because salinized soil is thought to pose a risk to agricultural output. The primary focus of this work was on strategies to reduce the detrimental impact of salinity stress on the biochemical traits, inorganic and organic osmotica substance contents, productivity, and quality of lettuce plants grown under two sites with varying salinity levels (EC=  $7.00\pm0.20$  dS m<sup>-1</sup>). These strategies included the foliar application of plant extracts (date palm pollen grains, maize, and soybean seeds), as well as

mannitol, sorbitol, and calcium phosphate, to determine their capacity to modify plant responses to saline soil stress.

### 2. MATRIALS AND METHODS

Two greenhouse Experiments were performed in the Agriculture Test Station, Faculty of Agriculture, Fayoum University, Egypt (29° 17' N; 30° 53' E) during the winter seasons of 2019/2020 and 2020/2021.

#### 2.1. Sources of search materials used

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## **2.1.1 Preparation of maize, soybean date palm pollen grains extracts**

The extracts was prepared from maize, soybean date palm pollen grains using the procedure reported by **Rady** *et al.* (2019) Chemical characteristics of maize, soybean date palm pollen grains extracts, which are included in **Table 1** and were ascertained and recognized at the National Research Center's specialized laboratory for GC/MS.

**Table 1.** Chemical chracteristics of the tested maize, soybean and date palm grains extract based to dry weight as determined by GC/MS during the seasons of 2019/2020 and 2020/2021

Properties	Maize	Soybean	Date palm pollens
Osmoprotectants (mg g <sup>-1</sup> dry weight)			
Soluble sugars	70.4	9.05	60.50
Proline	4.89	2.37	2.80
Mineral nutrients (mg g <sup>-1</sup> dry weight)			
Nitrogen	25.5	56.0	54.1
Phosphorus	3.16	7.04	6.60
Potassium	27.1	17.97	17.5
Magnesium	2.52	2.80	3.18
Calcium	3.27	2.70	5.60
Iron	1.22	0.15	2.41
Manganese	0.76	0.25	2.84
Zinc	0.48	0.48	2.81
Copper	0.24	0.16	3.19
Antioxidants and vitamins (mM g <sup>-1</sup> dry weight)			
Total B-group vitamins	128	53.0	119
Ascorbic acid; vitamin C	1.57	2.93	1.97
Glutathione	0.84	0.92	0.23
Phytohormones (µg g <sup>-1</sup> dry weight)			
Total indoles	3.20	3.20	9.00
Indole-3-acetic acid	1.73	3.40	4.92
Gibberellic acid	1.89	2.29	6.74
Trans-Zeatin and trans-Zeatin riboside	2.81	1.80	2.33

## 2.1.2. Mannitol, sorbitol and calcium phosphate monobasic

Mannitol and sorbitol  $C_6H_{14}O_6$ produced by Techno Pharmchem, Bahadurarh, Haryana (India) AN ISO 9001: Certified Company. Made by 2008 chemical makers Hopkin & Williams Ltd. (St Davids Court, Union Street, Wolver Hampton, West Midlands, United Kingdom, WV1 3JE), calcium phosphate monobasic  $Ca(H_2PO_4)_2$ .

#### 2.2. Soil physical and chemical properties

Samples of soil were taken down to a depth of 25 cm at the start of each season's treatment to determine the properties, both physical and chemical of the trial site. In accordance with the

accepted published protocols (Wilde *et al.*, **1985**), soil samples were examined at the Soil Testing Laboratory, Faculty of Agriculture, Fayoum University. The findings are shown in **Table 2**.

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2.3. Experimental work

#### 2.3.1 Greenhouse experiments

Imported lettuce hybrid seeds cv. Big Bell (Agent MECCA Trade; Produced promoters; Palm pollen grains (100 and 200 mg  $L^{-1}$ ), Corn grain extract (100 and 200 mg  $L^{-1}$ ), Soybean seed extract (100 and 200 mg  $L^{-1}$ ), Mannitol (1500 and 3000 mg  $L^{-1}$ ),

**Table 2.** Some physical and chemical characteristics of the experimental site during the seasons of 2019 /2020 and 2020/2021

Properties		2019/2020	2020/2021	
Physical properties				
Clay	(%)	12.59	12.21	
Silt	(%)	12.01	12.53	
Coars Sand	(%)	49.79	50.33	
Fine Sand		25.61	24.93	
Soil Texture		Sand loamy	Sand loamy	
Chemical properties				
РН		7.70	7.59	
ECe	(dS m <sup>-1</sup> )	7.12	7.08	
Organic matter	(%)	0.84	0.92	
CaCO <sub>3</sub>	(%)	14.01	13.97	
N%		0.09	0.11	
Availbe elements ( mg	kg <sup>-1</sup> soil)			
P		65.3	66.7	
<b>K</b> <sup>+</sup>		168.5	171.3	
Exchangeable cations	(meq L <sup>-1</sup> )			
Ca <sup>+2</sup>		9.87	9.97	
Na <sup>+</sup>		27.5	26.57	
$Mg^{+2}$		7.11	7.21	

by EC-System St. Naktuinbouw 21, China) were hand sown on October 25th, 2019 and October 20th, 2020. Each season, thirty days after the seeds were sown, the seedlings were moved onto the field in rows on both sides of the ridges, with a 25-cm gap between each row. Transplanting date was November 28 and 23 in both seasons 2019 and 2020, respectively. Every experimental unit consisted of five rows, each measuring 2.4 meters in length and 1.5 meters in width, for an overall space of 18 meters. To mitigate bordering consequences, a 0.5 m alley was placed between each pair of adjacent experimental units. Treatments comprised with twelve of plant growth

Sorbitol (1500 and 3000 mg  $L^{-1}$ ) and Calcium phosphate (800 and 1000  $Ca_3(PO_4)_2$  mg L<sup>-1</sup>), as well as, tap water. Following transplanting, the thirteen treatments were applied foliarly to run-off three times, at 25, 40, and 55 days. A small amount of conspicuous film was added as a wetting agent to the spraying fluid. In both of the trials that were done, there were five replications using a randomized complete blocks system, and each replicate had thirteen treatments. Every experimental unit received recommended doses according to MLAR (Market- Led Agrarian Reform) Table 3.

Table 3. Chemical fertilization program as a ratio among N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O

Fertilization period	From total amount (%	⁄o) N	P2O5	K <sub>2</sub> O					
Frist month	25	3.0	1.0	2.0					
Second month	35	3.0	1.5	3.0					
Thread month	25	3.0	1.5	3.0					
2.3.2. Plant sampling	n	norphological	traits,	membrane					

The first row of plants in each experimental unit was assigned to

morphological traits, membrane permeability measurements (RWC and MSI), and leaf photosynthetic pigments. In

contrast, the yield and its components calculation was done on the second row.

### 2.4. Data Recorded

## 2.4.1. Contents of leaf N, P, K, Ca, and Na

After 65 days from the date of lettuce transplantation, leaf samples were taken from five randomly chosen plants in each experimental unit. The plants were then cleaned with tap water, rinsed three times with distilled water, and dried at 70 °C in a forced-air oven until their weight remained constant. Samples of dry leaves were pulverized finely. Samples of fine leaf powder weighing 0.2 g were broken down with a solution of sulfuric and perchloric acids. The ensuing assessments were made: **2.4.1.1. Leaf N (mg g**<sup>-1</sup> **dry weight)**; colorimetrically assessed with the method of **Hafez and Mikkelsen (1981)**.

**2.4.1.2.** Leaf P (mg g<sup>-1</sup> dry weight); according to the stannous molybdate chloride method as illustrated in A. O. A. C (1995).

**2.4.1.3.** Leaf K and Na (mg g<sup>-1</sup> dry weight); measured using Flame photometer as noted by Wilde *et al.* (1985).

**2.4.1.4.** Leaf Ca (mg g<sup>-1</sup> dry weight); measured by atomic absorption spectrophotometer (Analyst 300, Perkin-Elmer, Germany) by Chapman and Pratt (1961).

## 2.4.2. Antioxidants assays

Leaf samples from ten randomly selected plants, in each experimental unit, after 65 days from transplanting were collected, washed with tap water, rinsed three times with distilled water. The following parameters were considered:

2.4.2.1. Total soluble sugars in leaves (mg  $g^{-1}$  dry weight)

Total soluble sugars in leaves and total soluble sugars in roots (after 102 days from seeds sowing) were extracted and determined as described by **Irigoyen** *et al.* (1992).

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**2.4.2.2.** Free proline in leaves ( $\mu g g^{-1} dry$  weight)

Free proline contents were measured using the colorimetric method as outlined by **Bates** *et al.* (1973).

**2.4.2.3.** Ascorbic acid (AsA) in leaves  $(\mu mol g^{-1} \text{ fresh weight})$ 

Ascorbic acid content was determined using the 2,6-dichloro-indophenol method (**Helrich, 1990**).

**2.4.2.4.** Glutathione (GSH) in leaves  $(\mu mol g^{-1} fresh weight)$ 

Determinations of glutathione levels were performed essentially as described by **De Kok** *et al.* (1986).

2.4.2.5. Total flavonoid content (mg quercetin Eq 100g<sup>-1</sup> fresh weight)

Total flavonoid content was colorimetrically determined using the detailed method of **Kim** *et al.* (2002).

2.4.2.6. Nitrate (NO<sub>3</sub><sup>-</sup>) and nitrite (NO<sub>2</sub><sup>-</sup>) contents (mg g<sup>-1</sup> dry weight)

Nitrate and nitrite concentrations estmated by **Radojevic and Bashkin** (**1999**). The NO<sub>3</sub><sup>-</sup> ion concentration (in mg  $g^{-1}$  dry weight) of each leaf sample was calculated using the formula:

NO<sup>-3</sup> content (in mg g<sup>-1</sup> dry weight)= (C × V)/M

where, C was the concentration of  $NO_3^-$  in the sample (in mg g<sup>-1</sup> dry weight), V was the total volume of the sample solution (100 ml), and M was the dry weight of the sample (1.0 g) by **LaMotte (2000).** 

The nitrite ion  $(NO_2^-)$  content (in mg g<sup>-1</sup> dry weight) of each leaf sample was calculated using the formula:

 $NO_2^-$  content (in mg g<sup>-1</sup> dry weight) = (C × V)/M

where, C was the concentration of  $NO_2^-$  in the sample (in mg g<sup>-1</sup> dry weight), V was the total volume of the sample solution (100 ml), and M was the weight of the sample (1.0 g) by **Radojevic and Bashkin (1999). 2.4.2.7. Leaf DPPH radical-scavenging** activity (%) Leaf antioxidant activity was based on **Lee** *et al.* (2003). Following is the calculation of scavenging activity:

DPPH radical-scavenging activity  $(\%)=[(Acontrol-Asample)]/(Acontrol)] \times 100$ , where A is the absorbance at 515 nm.

## **2.4.2.8.** Leaf total phenolic content (mg g<sup>-1</sup> dry weight)

The method estimated by **Makkar** *et al.* (1997) was used to determine the total phenolic content of the ethanol extract of dry lettuce leaves. 2.4.4. Yield

The lettuce yields were harvested and measured 90 days after transplanting from second row in each experimental unit. The following data were recorded:

**2.4.4.1 Head weight plant**<sup>-1</sup> (**kg**); average weight of heads.

**2.4.4.2 Heads yield per square meter (m<sup>2</sup> kg<sup>-1</sup>)** theoretically calculated 16 plants (every square meter has 4 rows, and inside each row there are 4 plants).

## 2.5. Statistical analysis

After utilizing the InfoStat statistical program (2016) to test for uniformity of the variances of errors in accordance with the protocol described by **Gomez and Gomez** (1984), Analysis of variance (|ANOVA)

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was used to all the data in order to create a randomized complete blocks system. Using Duncan's multiple range test, significant differences between treatments were compared at P < 0.05.4.

## 3. RESULTS AND DISCUSSION

The gained results of two greenhouse experiments were conducted during two winter seasons of 2019/2020 and 2020/2021 seasons to identify effects of foliar applications of plant extracts (date palm pollen, maize and soybean seeds) and mannitol, sorbitol and calcium phosphate as well as control (tap water) are presented in following the topics; morphological characteristics, membrane permeability, leaf photosynthetic pigments content, leaf N, P, K, Ca, and Na contents, enzymatic antioxidants assays, and yield of lettuce plants grown on saline soil.

## 3.1. Contents of leaf N, P, K, Ca, and Na

Generally, all the foliar application of the twelve treatments (plant extracts, mannitol, sorbitol and calcium phosphate) were superior and obtained noticeably greater mean values of contents of leaf N, P, K and Ca of lettuce plants comparing to control (tap water), and the trend was parallel in both years. According to **Table 4.** 

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**Table 4**. Effect of some plant growth promoters (PGP) applications on leaf N, P, and K content of lettuce plants grown on saline soil during two winter seasons of 2019/2020 (1<sup>st</sup>) and 2020/2021 (2<sup>nd</sup>)

Treatment	(mg I -1)	$(mg L^{-1})$ $\frac{N (mg g^{-1} dry weight)}{N (mg g^{-1} dry weight)} P (mg g^{-1} dry weight)$		lry weight)	K (mg g <sup>-1</sup> dry weight)		
Treatment	(ing L)	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
Control (tap water)		19.8 <sup>d</sup>	20.4 <sup>d</sup>	2.22 <sup>g</sup>	2.29 <sup>g</sup>	1.48 <sup>g</sup>	1.53 <sup>g</sup>
	100	$28.2^{ab}$	29.1 <sup>ab</sup>	2.55 <sup>cd</sup>	2.63 <sup>cd</sup>	2.43 <sup>bc</sup>	2.51 <sup>bc</sup>
Palm pollen grains extract	200	28.6 <sup>a</sup>	29.5ª	2.99 <sup>a</sup>	3.08 <sup>a</sup>	2.59 <sup>a</sup>	2.675 <sup>a</sup>
Corn grain extract	100	27.5 <sup>abc</sup>	28.3 abc	2.33 <sup>fg</sup>	$2.40^{\mathrm{fg}}$	2.30 <sup>cde</sup>	2.37 <sup>de</sup>
	200	27.4 <sup>abc</sup>	28.3 abc	2.49 <sup>def</sup>	$2.57^{de}$	2.33 <sup>bcde</sup>	$2.40^{\text{cde}}$
Comboon and ontro of	100	28.1 <sup>ab</sup>	29.0 <sup>ab</sup>	2.71 <sup>bc</sup>	2.79 <sup>bc</sup>	2.35 <sup>bcd</sup>	2.42 <sup>bcd</sup>
Soybean seed extract	200	29.1ª	30.0 <sup>a</sup>	2.83 <sup>ab</sup>	2.92 <sup>ab</sup>	$2.46^{ab}$	2.54 <sup>ab</sup>
Mannitol	1500	25.2°	26.0 °	$2.35^{efg}$	$2.42^{efg}$	$2.14^{f}$	$2.21^{f}$
	3000	27.9 <sup>ab</sup>	28.8 <sup>ab</sup>	2.51 <sup>de</sup>	2.59 <sup>de</sup>	2.21 <sup>ef</sup>	2.28 <sup>ef</sup>
Sorbitol	1500	27.8 <sup>abc</sup>	28.6 <sup>ab</sup>	2.51 <sup>de</sup>	2.59 <sup>de</sup>	2.29 <sup>de</sup>	2.36 <sup>de</sup>
Sorbitol	3000	28.2 <sup>ab</sup>	29.1 <sup>ab</sup>	2.76 <sup>b</sup>	2.84 <sup>b</sup>	2.35 <sup>bcde</sup>	2.42 <sup>bcd</sup>
	800	25.9 <sup>bc</sup>	26.7 <sup>bc</sup>	$2.46^{def}$	$2.54^{def}$	$2.15^{f}$	$2.22^{\mathrm{f}}$
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	1000	26.7 <sup>abc</sup>	27.6 abc	2.59 <sup>cd</sup>	2.67 <sup>cd</sup>	2.23 <sup>def</sup>	2.30 <sup>def</sup>

Values marked with the same letter(s) within the effects are statistically similar using Duncan's multiple range test at  $p \le 0.05$ .

Foliar application of palm pollen grains and soybean seed extract at concentration of 200 mg  $L^{-1}$  achieved substantially greater contents of N, P, and K values compared to the other plant growth promoters applications concentrations. While, the foliar The utilization of calcium phosphate at 1000 mg  $L^{-1}$  concentrations provided the most significant values for the calcium content of lettuce leaves, in the two growing seasons. On other hand, spraying with any twelve treatments of plant growth promoters applications with any level led to a significant decrease in leaf Na conten of lettuce plants comparing to control (tap water). In this regard, foliar application of soybean seed extracts at concentrations 200 mg  $L^{-1}$  reported the least significant data for the sodium content of lettuce leaves, and the trend was parallel in the two years. Shown in **Tables 4 and 5.** 

**Table 5.** Effect of some plant growth promoters (PGP) applications on leaf Ca and Na content of lettuce plants grown on saline soil during two winter seasons of 2019/2020 (1<sup>st</sup>) and 2020/2021 (2<sup>nd</sup>)

6	0							
Tucctment	$(m \alpha \mathbf{I} = 1)$	Ca (mg g <sup>-1</sup> c	lry weight )	Na (mg g <sup>-1</sup>	dry weight )			
Treatment	(mg L <sup>-1</sup> )	1 <sup>st</sup>	2 <sup>nd</sup>	$1^{st}$	$2^{nd}$			
Control (tap water)		1.12 <sup>h</sup>	1.16 <sup>h</sup>	4.05 <sup>a</sup>	4.17 <sup>a</sup>			
Palm pollen grains extract	100	$1.40^{\mathrm{fg}}$	$1.44^{\mathrm{fg}}$	$3.20^{bcde}$	3.30 <sup>bcde</sup>			
	200	1.56 <sup>cd</sup>	1.61 <sup>cd</sup>	3.00 <sup>cde</sup>	3.10 <sup>cde</sup>			
Corn grain extract	100	1.38 <sup>g</sup>	1.41 <sup>g</sup>	3.38 <sup>bcd</sup>	3.48 <sup>bcd</sup>			
	200	$1.44^{defg}$	1.49 <sup>efg</sup>	$3.22^{bcde}$	3.32 <sup>bcde</sup>			
	100	1.56 <sup>cd</sup>	1.60 <sup>d</sup>	2.95 <sup>de</sup>	3.04 <sup>de</sup>			
Soybean seed extract	200	1.67 <sup>c</sup>	1.72 <sup>c</sup>	2.90 <sup>e</sup>	2.99 <sup>e</sup>			
Mannital	1500	1.43 <sup>efg</sup>	1.47 <sup>efg</sup>	3.12 <sup>cde</sup>	3.22 <sup>cde</sup>			
Mannitol	3000	1.51 <sup>def</sup>	$1.55^{def}$	3.44 <sup>bc</sup>	3.55 <sup>bc</sup>			
Corbitol	1500	1.36 <sup>g</sup>	1.41 <sup>g</sup>	$3.20^{bcde}$	3.29 <sup>bcde</sup>			
Sorbitol	3000	1.52 <sup>de</sup>	1.57 <sup>de</sup>	3.08 <sup>cde</sup>	3.17 <sup>cde</sup>			
$C_{\alpha}(\mathbf{H}, \mathbf{PO})$	800	1.94 <sup>b</sup>	2.00 <sup>b</sup>	3.65 <sup>ab</sup>	3.76 <sup>ab</sup>			
$Ca(H_2PO_4)_2$	1000	2.10 <sup>a</sup>	2.17 ª	3.34 <sup>bcde</sup>	3.44 <sup>bcde</sup>			

Values marked with the same letter(s) within the effects are statistically similar using Duncan's multiple range test at  $p \le 0.05$ .

### **3.2.** Antioxidants assays

Generally, foliar application of plant growth promoters was superior and significantly recorded higher mean values of flavonoid, ascorbic acid, glutathione, total soluble sugars, and DPPH-radicalscavenging activity and free proline contents of lettuce plants comparing to control. On other hand, spraying with any

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twelve treatments of plant growth promoters applications with any level led to a significant decrease in nitrate, nitrite and phynolics conten of lettuce plants comparing to control, in both experimental seasons (**Tables 6-8**).

**Table 6.** Effect of some plant growth promoters (PGP) applications on leaf Flavonoids, Ascorbate and Glutathione content of lettuce plants grown on saline soil during two winter seasons of 2019/2020 (1<sup>st</sup>) and 2020/2021 (2<sup>nd</sup>)

Treatment (mg	g L <sup>-1</sup> )	(mg querc	Flavonoids (mg quercetin Eq 100 g <sup>-1</sup> fresh weight)		Ascorbate (mM g <sup>-1</sup> fresh weight )		thione esh weight )
		1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
Control (tap water)		0.298 <sup>j</sup>	0.322 <sup>i</sup>	2.244 <sup>i</sup>	2.712 <sup>f</sup>	0.515 <sup>k</sup>	0.552 <sup>g</sup>
	100	3.354ª	3.821ª	4.022 <sup>a</sup>	4.906ª	1.892ª	2.080 <sup>a</sup>
Palm pollen grains extract	200	2.884 <sup>d</sup>	3.242°	3.842 <sup>b</sup>	4.543 <sup>b</sup>	1.689 <sup>bc</sup>	1.855 <sup>b</sup>
Corn grain extract	100	3.052 <sup>c</sup>	3.213°	3.712 <sup>c</sup>	3.994°	1.701 <sup>bc</sup>	1.784 <sup>b</sup>
	200	2.608 <sup>e</sup>	2.860 <sup>d</sup>	3.046 <sup>e</sup>	3.344 <sup>d</sup>	1.503 <sup>e</sup>	1.655°
Combann and antes of	100	2.828 <sup>d</sup>	3.185°	3.342 <sup>d</sup>	3.922°	1.621 <sup>c</sup>	1.780 <sup>b</sup>
Soybean seed extract	200	3.145 <sup>b</sup>	3.518 <sup>b</sup>	3.902 <sup>b</sup>	3.999°	1.744 <sup>b</sup>	1.848 <sup>b</sup>
Mannital	1500	2.079 <sup>h</sup>	2.321 <sup>g</sup>	2.498 <sup>h</sup>	2.718 <sup>f</sup>	1.170 <sup>i</sup>	1.280 <sup>ef</sup>
Mannitol	3000	1.992 <sup>i</sup>	2.232 <sup>gh</sup>	2.285 <sup>i</sup>	2.714 <sup>f</sup>	1.028 <sup>j</sup>	1.220 <sup>f</sup>
Carb 4al	1500	2.524 <sup>f</sup>	2.674 <sup>e</sup>	2.996 <sup>e</sup>	3.282 <sup>d</sup>	1.422 <sup>ef</sup>	1.520 <sup>d</sup>
Sorbitol	300	2.112 <sup>h</sup>	2.159 <sup>h</sup>	2.721 <sup>g</sup>	2.997 <sup>e</sup>	1.256 <sup>hi</sup>	1.324 <sup>e</sup>
C <sub>2</sub> (II DO)	800	2.226 <sup>g</sup>	2.571 <sup>f</sup>	2.894 <sup>f</sup>	3.084 <sup>e</sup>	1.326 <sup>gh</sup>	1.528 <sup>d</sup>
$Ca(H_2PO_4)_2$	1000	2.459 <sup>f</sup>	2.708 <sup>e</sup>	2.989 <sup>ef</sup>	3.128 <sup>e</sup>	1.404 <sup>fg</sup>	1.611 <sup>cd</sup>

Values marked with the same letter(s) within the effects are statistically similar using Duncan's multiple range test at  $p \le 0.05$ .

The best foliar application treatments that presented the most significant values; the treatment of palm pollen grains extract at concentration of 100 mg  $L^{-1}$  in leaf flavonoid, ascorbate and glutathionem contents. While, spraying with palm pollen grains extract at concentration of 200 mg  $L^{-1}$  gave the highest significant value in leaf total soluble sugars and free proline contents. In addition, foliar application of

mannitol. The most significant value in leaf DPPH radical scavenging activity was obtained at 3000 mg  $L^{-1}$  in **Tables 6-8**.

On the other side, foliar application of control (tap water) gave the highest significant values in leaf nitrate, nitrite and phynolics contens, and the trend was parallel in the two years shown in **Tables 7** and **8**.

**Table 7**. Effect of some plant growth promoters (PGP) applications on leaf DPPH radical-scavenging activity, free proline, and phynolics content of lettuce plants grown on saline soil during two winter seasons of 2019/2020 (1<sup>st</sup>) and 2020/2021 (2<sup>nd</sup>)

Treatment	(mg L <sup>-1</sup> )		DPPH radical-scavenging activity (%)		Free proline in leaves $(\mu g g^{-1} dry weight)$		Phynolics (mg g <sup>-1</sup> dry weight )	
		1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	
Control (tap water)		46.9 <sup>f</sup>	48.3 <sup>f</sup>	1.29 <sup>h</sup>	1.33 <sup>g</sup>	3.44 <sup>a</sup>	3.54a	
Palm pollen grains extract	100	49.6 <sup>e</sup>	51.1 <sup>e</sup>	2.26 <sup>ef</sup>	2.33 <sup>ef</sup>	$2.66^{cdef}$	$2.74^{cdef}$	
	200	51.6 <sup>abcde</sup>	53.2 <sup>abcd</sup>	3.06 <sup>a</sup>	3.15 <sup>a</sup>	2.99 <sup>b</sup>	3.08 <sup>b</sup>	
Corn grain extract	100	53.3 <sup>ab</sup>	54.7 <sup>ab</sup>	2.11 <sup>fg</sup>	$2.17^{f}$	2.73 <sup>bcde</sup>	$2.80^{bcde}$	
	200	51.5 <sup>abcde</sup>	53.1 <sup>bcde</sup>	$2.96^{ab}$	3.05 <sup>ab</sup>	2.86 <sup>bc</sup>	2.95 <sup>bc</sup>	
G	100	49.5 <sup>e</sup>	51.1 <sup>e</sup>	2.48 <sup>cd</sup>	2.56 <sup>cd</sup>	2.35 <sup>g</sup>	2.42 <sup>g</sup>	
Soybean seed extract	200	50.8 <sup>cde</sup>	52.4 <sup>cde</sup>	2.87 <sup>b</sup>	2.96 <sup>b</sup>	$2.51^{defg}$	2.59 <sup>defg</sup>	
	1500	51.2 <sup>bcde</sup>	52.8 <sup>bcde</sup>	2.25 <sup>efg</sup>	2.32 <sup>ef</sup>	$2.48^{efg}$	2.55 <sup>efg</sup>	
Mannitol	3000	53.6ª	55.3ª	2.33 <sup>de</sup>	2.40 <sup>de</sup>	2.73 <sup>bcde</sup>	2.81 <sup>bcde</sup>	
Sorbitol	1500	49.9 <sup>de</sup>	51.4 <sup>de</sup>	2.20 <sup>efg</sup>	2.27 <sup>ef</sup>	$2.37^{\mathrm{fg}}$	$2.44^{\mathrm{fg}}$	
	3000	51.3 <sup>bcde</sup>	52.9 <sup>bcde</sup>	2.61°	2.69 <sup>c</sup>	2.79 <sup>bcd</sup>	2.88 <sup>bcd</sup>	
	800	52.1 <sup>abcd</sup>	53.7 <sup>abc</sup>	2.10 <sup>g</sup>	$2.17^{f}$	$2.49^{efg}$	$2.56^{efg}$	
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	1000	52.8 <sup>abc</sup>	54.5 <sup>abc</sup>	2.46 <sup>cd</sup>	2.54 <sup>g</sup>	2.81 <sup>bcd</sup>	2.89 <sup>bc</sup>	

Values marked with the same letter(s) within the effects are statistically similar using Duncan's multiple range test at  $p \le 0.05$ .

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**Table 8.** Effect of some plant growth promoters (PGP) applications on leaf Nitrate, Nitrite and TS sugars content of lettuce plants grown on saline soil during two winter seasons of 2019/2020 (1<sup>st</sup>) and 2020/2021 (2<sup>nd</sup>)

		Nit	rate	Nit	trite	TS sugars in leaves		
Treatment	$(mg L^{-1})$	(mg g <sup>-1</sup> dry weight)		(mg g <sup>-1</sup> dry weight)		(mg g <sup>-1</sup> dry weigh		
	_	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	$2^{nd}$	$1^{st}$	2 <sup>nd</sup>	
Control (tap water)		1.856ª	1.671ª	0.482ª	0.458ª	1.80 <sup>d</sup>	1.86 <sup>d</sup>	
Dolm nollon grains or tract	100	0.486 <sup>j</sup>	0.422 <sup>i</sup>	0.156 <sup>j</sup>	0.128 <sup>k</sup>	$2.07^{abc}$	2.13 <sup>bc</sup>	
Palm pollen grains extract	200	0.531 <sup>j</sup>	0.470 <sup>i</sup>	0.198 <sup>i</sup>	0.158 <sup>j</sup>	2.18 <sup>a</sup>	2.25ª	
Com anoin orthoat	100	0.892 <sup>g</sup>	$0.801^{f}$	0.264 <sup>g</sup>	0.251 <sup>h</sup>	2.03 <sup>bc</sup>	2.09 <sup>bc</sup>	
Corn grain extract	200	0.945 <sup>f</sup>	0.831 <sup>f</sup>	0.288 <sup>f</sup>	0.284 <sup>g</sup>	2.13 <sup>ab</sup>	2.20 <sup>ab</sup>	
Southoon good outroat	100	0.744 <sup>h</sup>	0.670 <sup>g</sup>	0.251 <sup>g</sup>	0.238 <sup>h</sup>	2.04 <sup>bc</sup>	2.10 <sup>bc</sup>	
Soybean seed extract	200	0.632 <sup>i</sup>	0.582 <sup>h</sup>	0.230 <sup>h</sup>	0.208 <sup>i</sup>	2.12 <sup>ab</sup>	2.19 <sup>ab</sup>	
Mannital	1500	1.310 <sup>c</sup>	1.188°	0.433 <sup>b</sup>	0.421 <sup>b</sup>	1.99°	2.05°	
Mannitol	3000	1.412 <sup>b</sup>	1.260 <sup>b</sup>	0.421 <sup>b</sup>	0.403 <sup>bc</sup>	2.05 <sup>bc</sup>	2.11 <sup>bc</sup>	
Soubital	1500	1.172 <sup>d</sup>	1.055 <sup>d</sup>	0.387 <sup>c</sup>	0.366 <sup>d</sup>	1.99 <sup>c</sup>	2.05 <sup>c</sup>	
Sorbitol	3000	1.204 <sup>d</sup>	1.064 <sup>d</sup>	0.400 <sup>c</sup>	0.391°	2.11 <sup>abc</sup>	2.18 <sup>ab</sup>	
$C_{\mathbf{a}}(\mathbf{H},\mathbf{PO})$	800	1.042 <sup>e</sup>	0.948 <sup>e</sup>	0.352 <sup>d</sup>	0.344 <sup>e</sup>	2.05 <sup>abc</sup>	2.12 <sup>bc</sup>	
$Ca(H_2PO_4)_2$	1000	1.004 <sup>e</sup>	0.904 <sup>e</sup>	0.321 <sup>e</sup>	0.316 <sup>f</sup>	2.13 <sup>ab</sup>	2.19 <sup>ab</sup>	

Values marked with the same letter(s) within the effects are statistically similar using Duncan's multiple range test at  $p \le 0.05$ . **3.3. Yield** Head weight and heads yield per 1

In both experimental seasons, foliar spraying of plant growth promoters was generally better and resulted in considerably higher mean values of head weight and heads yield per 1 m<sup>2</sup> of lettuce plants when compared to the control.

m<sup>2</sup> increased with foliar spraying soybean seed extract at 200 mg L<sup>-1</sup>, but in both seasons indicated in **Table 9**, there was no appreciable significant increase with foliar spraying palm pollen grain extract at 100 mg L<sup>-1</sup> (**Table 9**).

**Table 9.** Effect of some plant growth promoters (PGP) applications on Head weight and Total heads yield of lettuce plants grown on saline soil during two winter seasons of 2019/2020 (1<sup>st</sup>) and 2020/2021 (2<sup>nd</sup>)

Treatment	(m ~ <b>I</b> -1)	Head we	ight (kg)	Heads yie	ld (m <sup>2</sup> kg <sup>-1</sup> )
Ireatment	$(mg L^{-1})$	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
Control (tap water)		0.442 <sup>h</sup>	0.511 <sup>h</sup>	7.1 <sup>g</sup>	8.2 <sup>g</sup>
	100	0.835 <sup>ab</sup>	0.964 <sup>ab</sup>	13.4 <sup>ab</sup>	15.4 <sup>ab</sup>
Palm pollen grains extract	200	0.705°	0.815 <sup>c</sup>	11.3°	13.0 <sup>c</sup>
Corn grain extract	100	0.784 <sup>b</sup>	0.905 <sup>b</sup>	12.5 <sup>b</sup>	14.5 <sup>b</sup>
	200	$0.553^{\mathrm{fg}}$	0.639 <sup>fg</sup>	8.9 <sup>ef</sup>	10.2 <sup>ef</sup>
	100	0.621 <sup>ef</sup>	$0.718^{\text{ef}}$	9.9 <sup>de</sup>	11.5 <sup>de</sup>
Soybean seed extract	200	0.864a	0.999a	13.8ª	16.0 <sup>a</sup>
Mannitol	1500	$0.626^{def}$	0.723 <sup>def</sup>	10.0 <sup>de</sup>	11.6 <sup>de</sup>
Mannitol	3000	0.527 <sup>g</sup>	$0.609^{g}$	$8.4^{\mathrm{f}}$	$9.7^{\mathrm{f}}$
Sorbitol	1500	0.695 <sup>cd</sup>	0.803 <sup>cd</sup>	11.1 <sup>cd</sup>	12.8 <sup>cd</sup>
	3000	0.666 <sup>cde</sup>	0.770 <sup>cde</sup>	10.7 <sup>cd</sup>	12.3 <sup>cd</sup>
	800	0.517 <sup>gh</sup>	$0.597^{g}$	8.3 <sup>f</sup>	9.6 <sup>f</sup>
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	1000	0.536 <sup>g</sup>	0.619 <sup>g</sup>	$8.6^{\mathrm{f}}$	9.9 <sup>f</sup>

Values marked with the same letter(s) within the effects are statistically similar using Duncan's multiple range test at  $p \le 0.05$ .

The positive effect of foliar applications of plant extracts (date palm pollen, maize and soybean seeds) on the macro and microelements content of lettuce leaves may be due to the presence of nutrients in these extracts (**Table 4**). On the other side, the mannitol and sorbitol help to cover the entire surface of the leaves, which increases

the efficiency of nutrient absorption, it also works to extend the time required for the leaves to absorb macro and micronutrients before drying on the surface of the leaves **Bielski (2005)**. This led to enhancing uptake N, P, K and Ca leaf contents when a foliar applications of plant extracts comparing to control (**Tables 4-5**). The same results were observed by **Semida and Rady (2014)**, **Abd El-Mageed** *et al.* (2017), **Elzaawely** *et al.* (2017), **Rady** *et al.* (2019), **Abdulkadhim (2019)**, and **Nessem** *et al.* (2023).

The increased phosphorous and calcium content inside lettuce plant leaves may be the cause of the calcium phosphate's beneficial effect on P and Ca leaf content (Table 5). However, calcium phosphate had less of an impact on the amount of Na in lettuce plants' leaves; this could be due to antagonism between the elements' metabolisms. Ilias et al. (2007) discovered that applying calcium encouraged the buildup of potassium, boron, magnesium, calcium, and iron in the okra plant's leaves. Tejashvini Furthermore, and Thippeshappa (2017)showed that applying various calcium sources like leaf foliarly would improve the tomato crop's uptake nutritional and content. Additionally, every calcium source that was discovered to be useful also markedly improved nutritional content and uptake. On the same side, El-Masry et al. (2021) stated that the higher concentrations of pigments involved in photosynthesis and the positive effect of calcium phosphate on membrane permeability, MSI, and RWC may be the cause of the positive effect of calcium phosphate on p and Ca leaf content. In addition, the application of calcium phosphate boosted the amount of calcium and phosphorus inside the leaves of pepper transplants.

Its attributed to the enhanced effect of foliar applications of plant extracts, mannitol, sorbitol and calcium phosphate on the non-

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enzymatic antioxidants assays (**Tables 6-8**), It was demonstrated by an increase in performance efficiency. of lettuce plants under saline soils conditions.

The enhancing effect of foliar application of plant extracts (date palm maize and soybean pollen. grains) comparing to control on non-enzymatic antioxidants assays contents of lettuce plants compared to control (Tables 6-8) maybe due to the plant extracts containing a lot of osmoprotectants, mineral nutrients, antioxidants and vitamins (antioxidants and vitamins, ascorbic acid and glutathione) and phytohormones as shown in Table 1. Maybe too might be due to easier availability of optimum plant growth promoters applications dose to plant due to which might have betterextracts containing a lot of osmoprotectants, mineral nutrients, antioxidants and vitamins (antioxidants and vitamins, ascorbic acid and glutathione) and phytohormones as shown in Table 1. which may be absorbed and transformed into metabolic products, the accumulation of that strengthens plant responses to stress and can work indirectly by generally enhancing plant performance under stress from salinized soils. In the same side, when plants under the negative influence of stress are treated with plant extracts (palm pollen, maize grain and soybeans extracts), the plants acquire several effective mechanisms that raise their resistance, givin satisfactory growth and yield with high quality, such as, osmo-protector compounds, phenolics, flavonoids, a-tocopherol, glutathion, ascorbate. organic acids and phytohormones are among the effective That group mechanisms. of plant biostimulators causing increased tolerance to some investigated abiotic stresses. including salinity (Rady and Seif El-Yazal, 2013; Seif El- yazal, 2014; Seif Elyazal et al., 2018, 2019; Taha et al., 2020).

Many reports support our obtained results such as **Samy (2022), Dawood and** 

El-Awadi (2022), Rashid and Mosleh (2023), and Abdullah and Alabdaly (2023) when spraying of mannitol or sorbitol on many plants increased of chemical components, including total sugars, starch content, total soluble solids, and total free amino acids.

Lower soil temperatures than 13 °C result in less P being absorbed. Therefore, spraying plants, especially winter crops, such as lettuce plants, leads to an increase in the P content in the plant cells, which increases the vital processes in the plant, which leads to the accumulation of more of non-enzymatic antioxidants assays in plant cells (**Table 4**). Which increased the tolerance of lettuce plants to saline soil stress. In the same direction, Numerous reports substantiate our findings, including **Khani** *et al.* (2020), **Niazi** *et al.* (2021), and **Attallah and Abdalla** (2021).

DPPH and decrease Na under any levels of plant growth promoters applications (Tables 5-8) caused accumulation of organic and inorganic osmotica substances to accumulate in the lettuce plant, including suitable solutes such free proline, total flavonoids, and TSS levels, in order to regulate osmotic pressure. Additionally, as leaf glutathione and ascorbate concentrations rise (Tables 6–8), tissues are able to react with free radicals, particularly peroxyl radicals and singlet oxygen, thanks to these antioxidants (Sies and Stahl, 1995). Which reduced the harmful effect of soil salinity and led to increased absorption of nutrients and thus improved the head weight and heads yield of lettuce plants (Table 9).

Generally, foliar application of soybean seed extract at concentration of 200 mg  $L^{-1}$  or palm pollen grains extract at 00 mg  $L^{-1}$  work to improve the physiobiochemical component of plants., which reflected on improve productivity and quality of lettuce plants (*Lactuca sativa* cv. Big Bell) under saline soil stress in

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conditions of Fayoum Governorate and other similar regions.

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