





## The Potential of a mixture of Zeolite, Calcium, and Organic compounds on mitigating the salinity stress in bread wheat (Triticum aestivum L.)

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## ABSTRACT

Given the population growth, climate change, and salinity stress conditions particularly in arid and semi-arid regions, wheat crop production is significantly hampered by soil salinity. Zeolite, Calcium or organic acids may diminish the negative effects of salinity stress on plants. The purpose of this study was to illustrate the ameliorative effect of Zeolite, Calcium and Organic compounds (Ze-Ca-OC) mixture (100mg /L) on two bread wheat cultivars (Sakha 95 and Misr 3) grown under four salinity concentrations (0,50, 100, 150 mM NaCl). The present experiment was conducted in two consecutive seasons (2020/2021, 2021/2022) in sandy soil pots at Sakha agricultural research station, Kafrelsheikh, Egypt. The wheat cultivars were exposed to only salinity stress levels or treated with the Ze-Ca-OC mixture, and were evaluated by different physiological characteristics in the vegetative stage were recorded for both cultivars. Additionally, at the harvest stage, the plant height, grain yield and grain protein content were measured as well. Salinity stress inhibited the growth of Misr 3 cultivar more than Sakha 95 through much compartmentalization of Na+ content and low K+/Na+ ratio with low Ca++ content in the leaf and root tissues. Application of Ze-Ca-OC mixture improved their growth by decreasing the Na+ toxicity and enhancing the oxidative defense of both cultivars over the upregulating the POD enzyme activity. Supplementing the wheat cultivars with Ze-Ca-OC mixture under salinity stress levels led to a significant increase in the RWC by (≈ 20, 17%) under (100, 150 mM NaCl), respectively, in cultivar Misr 3. Furthermore, the proline content in the leaf of Sakha 95 and Misr 3 considerably up surged (≈ 28, 21.4% and 60, 88%) at 100 and 150 mM NaCl, respectively. In addition, the root proline content recorded a massive increase when Ze-Ca-OC mixture was added to salinity concentrations for both cultivars. The grain yield was similar in the two cultivars under the different treatments; however, the Ze-Ca-OC mixture significantly enriched their seeds with high protein content particularly under the highest concentration of 150 mM NaCl by 50.6% and 23.17% for the Sakha 95 and Misr 3 cultivars, respectively. Taken together, the findings imply that exogenous Ze-Ca supplemented with OC can reduce salt stress in bread wheat by increasing osmoprotectant accumulation, extruding toxic sodium, and compartmentalizing K and Ca in a useful way. Keywords: Calcium, Organic compounds, Salinity mitigation, Wheat, Zeolite.

## **INTRODUCTION**

Salinity is one of the main impediments to global food security; it is regarded as one of the most severe ecological stresses that inhibit agricultural production in many countries (Abdelmageed et al., 2019). More than 20% of soils on the planet are affected by salt, and the magnitude of these soils is steadily growing due to human activity and global warming (Seleiman et al., 2022). By the end of 2050, food production has to increase by 70% due to the quick upsurge in global population(Hassan et al., 2021). Wheat is considered the major food crop that dominates the world in grain production. More than 36% of the world's population eats it regularly, and it provides 20% of the calories and 55% of the carbohydrates consumed globally (Hasanuzzaman et al., 2017). Furthermore, wheat is a substantial source of both macronutrients and micronutrients that are essential for human health. The productivity of wheat crops is negatively affected by salinity stress (Seleiman et al., 2022). Wheat yield begins to decrease at a salt stress level of 6-8 dS m<sup>-1</sup>(Royo and Abió, 2003). Salinity stress is severely affecting 397 million hectares of wheat cultivation, according to the Food and Agriculture Organization (FAO, 2019), seriously posing a threat to food security.

Plants become water stressed when soil solutions with high salt concentrations have a reduced osmotic potential. Since Na<sup>+</sup> is not as easily sequestered into vacuoles as it is in halophytes, it also causes severe ion toxicity (Munns and Tester, 2008). The interactions between salts and mineral nutrition may lead to nutrient deficiencies and imbalances. Because salinity imposes osmotic stress as well as ionic stress, it makes plant salt tolerance more complicated. Sodium Na<sup>+</sup> toxicity is the main cause of the ionic stress on the plant(Minhas *et al.*, 2017). The uptake of high concentrations of soluble salts by plants under salt stress is well known to limit the uptake of water through the root system due to higher osmotic stress. Due to the lack of water in plant cells, the turgor and membrane stability are affected(Yassin *et al.*, 2019). Moreover, the high concentration of ions absorbed by the cells competes with the uptake of vital nutrients, which results in nutrient deficiency (Goudarzi and Pakniyat, 2008). The most common salt in saline soil, like NaCl, raises the concentration of Na<sup>+</sup> and Cllevel in the soil, which prevents plants from absorbing nutrients like Ca<sup>++</sup>, Mg<sup>++</sup>, and K<sup>+</sup> and instead promotes the uptake of Na<sup>+</sup> and Cline-susceptible plants (Tuteja, 2007). The ability of crops to sustain high levels of proline and various antioxidant enzymes like POD and CAT to inhibit lower levels of lipid peroxidation by functioning as an effective scavenger of malondialdehyde (MDA) and reactive oxygen species (ROS) concentrations, (Szabados and Savouré, 2010; Elsawy *et al.*, 2018); they can also maintain a reduced risk of stress-induced harm (Munns and Tester, 2008; Sharbatkhari *et al.*, 2013).

Zeolite (Ze) is used to increase the value of fertilizers and retain valuable Nitrogen to encourage better plant growth, and enhance the quality of the resulting manures and sludge (Polat *et al.*, 2004). Zeolites are added to fertilizers; by enhancing the capacity of the soil to absorb nutrients, help to retain nutrients and thus improve the soil long-term quality. It concerns the most crucial plant nutrients, including calcium, magnesium, and microelements as well as nitrogen (N) and potassium (K). These nutrients can be stored by zeolite in the root zone for use by plants as needed. As a result, N and K fertilizers are used more effectively by producing higher yields, extending their activity, or reducing their rates for the same yield. Sandy soils frequently experience significant leaching of fertilizers, which reduces the ability of the soil to hold onto high levels of nutrients (Mumpton, 1981 and Mumpton, 1999). Thus, using zeolites will improve plant growth and development by diminishing nutrient loss (Polat *et al.*, 2004).

Calcium ions (Ca<sup>++</sup>) can regulate both Na<sup>+</sup> and K<sup>+</sup> elements under salinity stress conditions by adjusting the integrity of the cell membrane and the selective transport of K<sup>+</sup> as opposed to Na<sup>+</sup> (Wu and Wang, 2012; Cheng et al., 2015). When calcium ions are removed from membranes by sodium ions, membrane permeability increases and intracellular sodium levels rise (Wang *et al.*, 2012; Li *et al.*, 2016).

Due to the environmental stress conditions, the alteration in the content of various organic compounds (OC) is accompanied by a change in the salinity tolerance in plant species. Different organic acids such as humic acid showed the greatest increases in response to long-term abiotic stress, demonstrating how significantly the comparative organic content varies depending on the specific metabolites in the given condition(Khan *et al.*, 2020). Organic acids like humic acid application to soil have received a lot of attention because it can reduce the inhibitory effects of soil salinity by enhancing the physical and chemical properties of soils, increasing soil water retention, and supplying nutrients for plant growth. It may also have anti-stress effects in salinity-prone environments (El-sayed, 2020).

In general, the effects of individual, Zeolite (Ze), calcium (Ca) or organic compounds(OC) on stress tolerance have also drawn a lot of attention, but little is known about the effects of a mixture of Ze and Ca<sup>++</sup> applications combined with OCon the plant growth, yield and seed quality and composition of wheat crop under salinity stress. Hence, the objective of our study is to reveal that, the potential use of the zeolite, calcium, and organic compounds mixture in mitigating the salinity stress effects on two Egyptian bread wheat cultivars.

#### MATERIALS AND METHODS

#### Plant materials and growth conditions:

The experiment was conducted in the greenhouse of the Seed Technology Research Department, Sakha Agricultural Research Station, ARC, Kafr Elsheikh, Egypt under ambient conditions. Two Egyptian wheat cultivars, Misr 3 and Sakha 95, were obtained from the wheat research department, Agricultural Research Center (ARC), Egypt, based on their behavior under salinity stress conditions, the Sakha 95 cultivar is considered more salinity tolerant than Misr 3cultivar. Seeds were germinated and grown during the two successful seasons of 2020/2021 and 2021/2022 in acid-washed quartz sand (saturation percent 20%). Six kilograms of sand were packed in plastic pots (25 cm in diameter and 30 cm in height) with an outlet at the bottom for free drainage. Full strength nutrient solution (75g/L ZnSO4.H<sub>2</sub>O, 45g/L MnSO4.H<sub>2</sub>O, 100g/L FeSO4.5H<sub>2</sub>O,55g/L CuSO4.7H2O, 25g/L MgSO4.5H<sub>2</sub>O, 5g/L

potassium fulvate, 5 g/L citric acid, and 20 g/L Potassium alginate), with the recommended dose of NPK fertilizers. Later on, 10 days after germination, seedlings were thinned to 6 plants in each pot and then watered with either only nutrient solution with 0 mM NaCl (control) or a nutrient solution supplemented with 50 mM NaCl (first salinity level), 100 mM NaCl (second salinity level) and 150 mM NaCl (third salinity level). After that, 20 days later of those 3 salinity treatments because of the symptoms of salinity stress effect on the plants, the pots of the plants that were irrigated with salt solutions were divided into two groups, one of them was provided with Zeolite (Ze), Calcium (Ca)and organic compounds (OC) mixture (Saigo cal. compound) (100mg/L) (Ze, Ca and OC mixture + salt treatments). The other group was continued to be irrigated as follows with different three salt solution treatments (50, 100 and 150 mM NaCl) without applying Zeolite, Ca<sup>+</sup> and organic compounds mixture during the vegetative stage. Each treatment was replicated 4 times.

# Preparation of Zeolite (Ze), Calcium(Ca)and organic compounds (OC) mixture solution and applying the salt treatments:

The Zeolite, Calcium and organic compounds mixture used in the current study were the active ingredients in a commercial compound (Saigo Cal). The chemical components in this compound are (10% Ca, 15% N, 5% Zeolite, 10% organic acids, 0.5% B, 0.5% amino acids and 1% Mg). The (Ze-Ca-OC) mixture was prepared by adding (100mg/L) to salt solutions for each salinity concentration (50mM NaCl, 100mM NaCl and 150mM NaCl). In addition, salinity solution treatments (NaCl treatments) were applied gradually (25, 50, and finally100mM) for the second salinity level, (25, 50, 75, 100 and finally 150mM) for the third salinity level over 2-day intervals to avoid osmotic shock to the plants.

## **Growth Parameters:**

After 30 days of Ze-Ca-OC mixture applying, group of plants (3 plants from each pot) were collected and separated into 2 parts (roots and leaves), and then were kept at -80 °C for physiological analysis. Salt stress conditions and Ze, Ca and OC mixture were applied to salinity treatments. Another group of plants (6 plants in each pot) was continued growing until the harvest time to collect the yield parameters.

#### Germination percentage:

The germination % was measured according to the International Seed Testing Association, ISTA (Leist *et al.*, 2003). **Seedling vigour index:** 

## It was calculated as described by Leist et al. (2003)by applying the formula:

(Seedling vigor index = Average seedling length ((shoot + root) (cm) \* Germination (%))

## Plant pigments content determination:

Total chlorophyll and total carotenoids' contents were extracted from 0.5 g of fresh leaf sample, soaked in 10 ml of 100% N, N-dimethyl formamide, and then measured the concentration by spectrophotometer using the methods of (Porra, 2002 and Allel *et al.*, 2017)

## Relative water content (RWC):

Known weights of Leaf samples were placed in vials and weighed to determine leaf sample weight (FW) and the samples were hydrated to full turgidity for 12h under lamp light and room temperature. After that time, the samples were taken out from the distilled deionized water and weighed instantly to obtain a fully turgid weight (TW). After weighing, samples oven-dried at 80°C for 24h and then weighed again (after being cooled in an incubator) to determine the dry weight (DW). The relative water content of leaf samples calculated by using the formula: RWC (%) =[(FW – DW) / (TW - DW)] × 100

Where FW represents sample fresh weight, TW represents sample turgid weight and DW sample dry weight (Gonzalez and Gonzalez., 2001).

## Electrolyte leakage rate (ELR):

Electrolyte leakage rate (ELR) was measured in tissue taken from the third leaf from the top of each plant and 0.5g well washed roots using the method of (Murray *et al.*, 1989) with some modifications. The leaf and root samples were drenched in distilled deionized water and kept shaking for 12 h. Then electrical conductivity EC (EC1) was measured with a portable EC meter (9V-1AmP, Thermo Electron Corporation, USA). The samples were later autoclaved and cooled to determine total EC (EC2). The ELR was then calculated by the following formula: ELR (%) =  $(EC1/EC2) \times 100$ .

## Minerals (Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>++</sup>) concentrations:

The oven dried samples (root and leaf) were fine ground in the sample mill (model Labo - Miser LM-Plus, Osaka Chemical Co., LTD, Japan), and the fine powder was used to determine Na<sup>+</sup> and K<sup>+</sup> concentration. The powder was

digested with sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (2:1, v/v), and the Na<sup>+</sup> and K<sup>+</sup> concentrations of the extracts were measured using a flame photometer (ANA 135, Tokyo Photoelectric, Tokyo, Japan). According to Ca<sup>+2</sup> concentration of the extracts were measured by atomic absorption spectrometry (GBC Avanta E, Victoria, Australia).

## Proline content:

Proline was extracted from freeze-dried powdered samples with 3% sulfosalicylic acid and measured following the method of Bates *et al.* (1973).

## Malondialdehyde concentration:

Malondialdehyde (**MDA**) content was determined by thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968) with some modifications. Fresh leaf and root samples were homogenized in an extraction buffer (10 mM HEPES pH7, 15% tricarboxylic acid, 0.375% thiobarbituric acid, 0.25 N HCl, 0.04% butylated hydroxytoluene and 2% ethanol). The absorbance of the reaction was read at 535 nm and 600 nm, and MDA content was calculated using an extinction coefficient of  $1.57 \times 10^{-5}$  mM cm<sup>-1</sup>.

#### Peroxidase enzyme activity:

The extraction was carried out using a 0.5 g fresh sample according to the method of Takagi *et al.* (2013). Fresh (leaf and root) samples were ground in liquid nitrogen with adding phosphate buffer (pH 7.0), the homogenate was centrifuged then sPOD activity was determined in the supernatant. 1 mL reaction mixture contained 15 mM guaiacol, 10 mM H2O2, 73 mM phosphate buffer and 2% enzyme extract. The absorbance was observed at 470 nm for 1 min and the activity of sPOD was calculated according to Chance and Maehly, (1955), one unit sPOD activity was defined as  $\mu$ mol tetra guaiacol/sec.

#### Post-harvest measurements:

At Harvest time, plant height (cm) was measured. The remained spikes were harvested and threshed to obtain seeds. grain yield  $pot^{-1}$  (g) were measured.

#### Crude protein content in the seeds:

Known weight of the fine powdered seeds (ca 0.1g) was digested using a micro kjeldahl apparatus by (98% H<sub>2</sub>So<sub>4</sub>) and (30% H<sub>2</sub>o<sub>2</sub>). The crude protein was calculated by multiplying the total Nitrogen by 5.85 (AOAC, 2000).

### Statistical analysis:

The collected data of the two seasons were subjected to combined analysis of variance (ANOVA) for the randomized complete block design to each experiment, as mentioned by Gomez and Gomez (1984) using MSTAT-C 1990 computer software and the means of genotypes were compared using the Duncan's Multiple Range Test at p < 0.05(Duncan, 1955).

## RESULTS

Salinity stress levels severely affect plant growth of both wheat cultivars as shown in (Figure1), however, applying the mixture of Zeolite, Calcium and organic compounds (Ze-Ca-OC) to salinity stress levels led to a considerable mitigation of the Sodium Chloride (Nacl) effect on the most characteristics. Sakha 95 and Misr 3 wheat cultivars did not show a significant response to (Ze-Ca-OC) application on the Leaf Malondialdehyde (MDA), root Ca<sup>+</sup> content, leaf and root potassium(K<sup>+</sup>) contents and their grain yield (Table2, 3 and 4). On the other hand, the remaining parameters of the two cultivars were significantly affected by (Ze-Ca-OC) application, as shown in Table1, 2, 3 and 4).



Fig. 1. Different response of two wheat cultivars, Sakha 95 and Misr 3 to various salinity concentrations (A) 0mMNaCl, adding Saigo-Cal. compound as a source of Zeolite, Calcium and organic compounds to salinity stress levels as follows (B) 50 Mm NaCl + Ze-Ca-OC, (C) 100mMNaCl + Ze-Ca-OC and (D) 150mMNaCl + Ze-Ca-OC, in addition, just salt concentrations (E) 50mMNaCl, (F) 100mMNaCl and (G) 150mMNaCl for 30 days of treatment.

**Table 1.** Means of relative water content (RWC), leaf pigments content (Total chlorophyll and Total carotenoids), the electrolyte leakage ratio (ELR) and Proline content in both leaf and root tissues of the two wheat cultivars (Sakha 95 and Misr 3) under different treatments (Control, Ze-Ca-OC + NaCl concentrations and NaCl levels.

Measured parameter							
Relative water content (RWC) (%)		Total chlorophyll content (µg mg <sup>-1</sup> FW)	Total carotenoids content (μg mg <sup>-1</sup> FW)	Electrolyte Leakage Ratio (ELR) (%) Root Leaf		<u>Proline</u> content (μmolg <sup>-1</sup> ) Root Leaf	
		1	Wheat cultivar	1		1	
Sakha 95	91.68 ± 1.19a	57.24 ± 0.52a	5.87 ± 0.29	62.92 ± 2.60b	51.41± 4.07b	3.59 ± 0.40a	0.23 ±0.01a
Misr 3	81.88 ± 3.33b	55.89 ± 0.80 b	5.9 ± 0.21	622.23±29.94 a	535.58±28.8 8a	1.03 ± 0.19b	0.11 ± 0.01b
F. test	*	**	N.S	*	*	**	**
			Treatment				
Control	95.44 ± 1.80a	93.55 ± 1.49a	6.66 ± 0.54ab	61.78 ± 4.54b	26.47 ± 2.15b	0.94 ± 0.20d	0.12 ± 0.02d
(Ze-Ca-OC) + 50 mM NaCl	93.55 ± 0.91a	92.57 ± 0.10a	6.76 ± 0.28a	66.44 ± 4.84b	35.01 ± 1.87b	4.99 ± 1.07a	0.19 ± 0.03bc
(Ze-Ca-OC) + 100 mM NaCl	92.57 ± 2.43a	89.76 ± 1.21a	6.28 ± 0.58ab	69.03 ± 3.55b	39.12 ± 2.05b	3.37 ± 0.56b	0.22 ± 0.03ab
(Ze-Ca-OC) + 150 <u>mM</u> NaCl	89.76 ± 1.50a	89.27 ± 1.41a	4.78 ± 0.41c	69.17 ± 2.35b	56.88 ± 5.35b	1.94 ± 0.09c	0.24 ± 0.02a
50 mM NaCl	89.27 ± 2.55a	79.44 ± 0.68b	5.78 ± 0.40b	69.38 ± 6.80b	64.09 ± 4.50b	2.29 ± 0.81c	0.18 ± 0.03c
100 mM NaCl	79.44 ± 5.74b	54.72 ± 0.85b	6.06 ± 0.15ab	69.12 ± 3.36b	124.28 ± 41.39b	1.57 ± 0.54cd	0.13 ± 0.03d
150 mM NaCl	67.46 ± 7.50c	52.80 ± 1.44c	4.92 ± 0.34c	199.31 ± 91.35a	170.86 ± 79.17a	1.06 ± 0.35d	0.13 ± 0.04d
F. test	**	**	**	**	**	**	**

\*, \*\* and N.S are symbols for significant, highly significant and Not significant differences respectively. The values are the means (± S.E) of four replicates, means followed by the same letter within each line are not significantly different (p<0.05)

**Table 2**. Means of Malondialdehyde (MDA) content and Peroxidase (POD) enzyme activity in leaf and root tissues, of the two wheat cultivars (Sakha 95 and Misr 3) under different treatments (Control, Ze-Ca-OC + NaCl concentrations and NaCl levels.

Measured parameter							
Malondia	Idehyde concentration (MDA) (nmolg-1FW)		Peroxidase enzyme activity (POD) (µmolg <sup>1</sup> sec <sup>-1</sup> )				
Root Leaf			Root Leaf				
Wheat cultivar							
Sakha 95	20.60 ± 1.27a	25.23 ± 2.31	6.92 ± 0.96b	12.33 ± 2.25a			
Misr 3	16.10 ± 0.90b	22.93 ± 2.62	11.94 ± 1.96a	5.58 ± 1.01b			
F. test	*	N.S	**	**			
Treatment							
Control	12.50 ± 1.11e	15.14 ± 1.79c	9.93 ± 2.48bc	3.15 ± 0.77c			
(Ze-Ca-OC) + 50 mM NaCl	14.52 ± 0.61e	15.82 ± 2.29c	6.65 ± 1.53cd	11.94 ± 2.34b			
(Ze-Ca-OC) + 100 mM NaCl	17.69 ± 1.66d	29.49 ± 2.88c	16.87 ± 4.69a	12.80 ± 1.97b			
(Ze-Ca-OC) + 150 mM NaCl	21.44 ± 2.16b	31.78 ± 2.36b	13.03 ± 3.72ab	19.29 ± 6.50a			
50 mM NaCl	15.92 ± 2.15e	17.12 ± 2.28c	3.46 ± 0.61d	2.34 ± 0.53c			
100 mM NaCl	19.86 ± 1.29c	24.09 ± 2.94c	9.14 ± 2.07bcd	3.46 ± 0.33c			
150 mM NaCl	26.54 ± 1.81a	43.12 ± 6.37a	6.92 ± 2.12bc	9.69 ± 2.57b			
F. test	**	**	**	**			

\*, \*\* and N.S are symbols for significant, highly significant and Not significant differences respectively. The values are the means (± S.E) of four replicates, means followed by the same letter within each lineare not significantly different (p<0.05)

**Table 3**. Means of ions contents Sodium (Na<sup>+</sup>), Potassium(K<sup>+</sup>), Calcium(Ca<sup>++</sup>) in leaf and root tissues, and the ratio of K<sup>+</sup>/Na<sup>+</sup> in both leaf and root tissues as well, of the two wheat cultivars (Sakha 95 and Misr 3) under different treatments (Control, Ze-Ca-OC + NaCl concentrations and NaCl levels.

			Measur	ed paramet	ter			
Na <sup>+</sup> content (mgg <sup>1</sup> DW)		K⁺ content (mgg <sup>-1</sup> DW)		Ca <sup>++</sup> content (mgg <sup>-1</sup> DW)		K⁺/Na⁺ ratio		
Root	Leaf		Root	Leaf	Root	Leaf	Root	Leaf
			Wheat cul	tivar				
	25.22 ±	51.83 ±	39.31 ±	63.35 ±	8.89 ±	5.81 ±	9.85 ±	119.88
Sakha 95	2.20a	10.71a	0.78b	4.07	1.36a	0.78a	4.08a	±59.53a
Misr 3	19.43 ± 1.98b	25.32 ± 2.15b	48.18 ± 0.94a	62.64 ±2.72	3.93 ± 0.29b	2.74 ± 0.24b	50.17 ± 22.56b	79.19 ± 36.49b
F. test	*	**	**	N.S	**	**	**	**
			Treatme		1			
	0.46 ±	0.17 ±	49.38 ±	79.52 ±	9.35 ±	4.77 ±	198.92 ±	685.26 ±
Control	0.12f	0.01f	2.25a	5.35a	1.90a-d	1.17b	52.42b	97.47c
	17.81 ±	22.71 ±	47.52 ±	82.11 ±	9.37 ±	7.85 ±	2.71 ±	3.63 ±
(Ze-Ca-OC) + 50 mM NaCl	0.53e	0.22d	2.12a	6.81a	4.08a-d	1.59a	0.19b	0.31c
(Ze-Ca-OC) + 100 mM	23.85 ±	31.33 ±	43.38 ±	65.55 ±	8.25 ±	6.30 ±	2.07 ±	2.12 ±
NaCl	2.43d	1.45c	1.55b	1.48b	1.35a-d	0.95b	0.30bc	0.11c
(Ze-Ca-OC) + 150 mM	29.55 ±	42.32 ±	41.51 ±	56.29 ±	7.02 ±	5.24 ±	1.41 ±	1.20 ±
NaCl	0.64b	28.80b	1.45b	2.65c	1.73a-d	1.19b	0.06bc	0.37cd
50 mM NaCl	22.56 ±	31.14 ±	43.85 ±	58.23 ±	4.56 ±	2.57 ±	2.13 ±	1.88 ±
	2.10d	1.92e	2.28b	5.87c	0.63cd	0.13c	0.29bc	0.17d
	25.63 ±	35.38 ±	42.41 ±	52.51 ±	3.51 ±	2.16 ±	1.77 ±	1.50 ±
100 mM NaCl	2.00c	1.19d	2.45b	5.51c	0.41cd	0.11c	0.22c	0.16d
150 mM NaCl	36.88 ±	107.03 ±	38.23 ±	46.77 ±	2.81 ±c	1.04 ±	1.05 ±	1.17 ±
150 mivi Naci	0.92a	3.70a	1.63c	4.38c	0.49d	0.18c	0.06c	0.14d
F. test	**	**	**	**	*	**	**	**

\*, \*\* and N.S are symbols for significant, highly significant and Not significant differences respectively. The values are the means (± S.E) of four replicates, means followed by the same letter within each line are not significantly different (p<0.05)

## Performance of wheat seedlings under the different treatments:

Although there was a substantial effect of salinity stress levels (50,100 and 150 mM Nacl) on the germination % for both cultivars, the application of Ze-Ca-OC mixture slightly improved the percentage of the germination for both cultivars under the salinity stress (Table 4). The germination of Sakha 95 cultivar reacted to the Ze-Ca-OC mixture application more fruitfully than Misr 3. Accordingly, the seedling Vigor Index significantly decreased under the three salt stress levels for both cultivars compared with the control condition (Figure2). On the other hand, adding the mixture of Ze-Ca-OC substantially improved the seedling vigor index by  $\approx$ 24.3, 19.1, 26.2% and  $\approx$ 15.7, 15.2, 6.6 % for both cultivars Sakha 95 and Misr 3 under the three salinity levels (50,100,150 mM NaCl), respectively. The high salinity level, 150 mM NaCl concentration reduced the plant height of both cultivars more than that the other two concentrations (Table 4). On contrast, the use of Ze-Ca-OC mixture resulted in a significant increase in Plant height under salinity stress levels (Table 4).

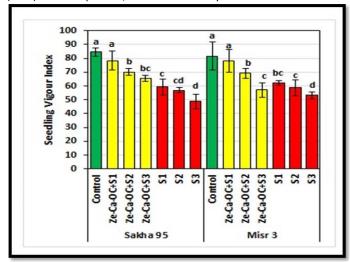
## Leaf pigments as affected by treatments:

The primary physiological process for plant survival is photosynthesis, which is severely influenced by salt stress. As shown in Figure (3B), by increasing the salinity levels, it led to a gradual decrease in the total chlorophyll content for Misr 3 cultivar, however adding the Ze-Ca-OC mixture increased the total chlorophyll content ( $\approx$ 5,  $\approx$ 10, and  $\approx$ 15 µg mg<sup>-1</sup>) significantly by rising the salinity from 50, 100 and 150 mM NaCl concentrations respectively. Although the salt stress levels led to a significant decrease in the total carotenoids content of cultivar Sakha 95, it increased by adding the mixture of Ze-Ca-OC under salinity stress levels 50 mM NaCl ( $\approx$  41%) and ( $\approx$  14, 23%) under 50,100 mM NaCl, respectively, for cultivar Misr 3 (Figure 3C).

**Table 4.** Means of Germination %, Seedling vigor index, Plant height, Grain yield  $pot^{-1}$  and the grain Protein content in the two wheat cultivars (Sakha 95 and Misr 3) under different treatments (Control, Ze-Ca-OC + NaCl concentrations and NaCl levels.

Measured parameter									
	Germination	Seedling vigor	Plant height	Grain yield Pot <sup>-1</sup>	Grain protein content				
	(%)	index	(cm)	( <u>gm</u> )	(%)				
Wheat cultivar									
Sakha 95	80.46 ± 1.90a	66.22 ± 2.65a	61.97 ± 1.03	18.01 ± 1.40a	13.43 ± 0.62b				
Misr 3	78.64 ± 1.81b	65.73 ± 2.77a	61.14 ± 1.12	12.97 ± 0.96b	14.91 ± 0.34a				
F. test	N.S	N.S	N.S	**	*				
Treatment									
Control	89 ± 2.59a	83.05 ± 4.91a	63.34 ± 1.80bc	16.58 ± 0.67bc	14.29 ± 0.59ab				
(Ze-Ca-OC) + 50 mM NaCl	86.75 ± 3.98a	78.05 ± 4.95b	69.55 ± 1.63a	26.78 ± 2.87a	14.72 ± 0.50ab				
(Ze-Ca-OC) + 100 mM	81 ± 1.96ab	69.69 ± 2.02b	64.61 ± 1.06b	17.6 ± 1.69b	15.29 ± 0.38a				
NaCl									
(Ze-Ca-OC) + 150 mM	76.65 ± 3.00abc	61.42 ± 3.04bc	59.66 ± 0.60cd	12.74 ± 0.16cd	16.07 ± 0.90a				
NaCl									
50 mM NaCl	76.5 ± 3.74bc	60.71 ± 2.78c	59.84 ± 1.25cd	13 ± 1.72cd	15.17 ± 0.44ab				
100 mM NaCl	77.25 ± 1.96bc	57.78 ± 2.90cd	59 ± 1.27cd	12 ± 1.38d	13.40 ± 0.96b				
150 mM NaCl	69.75 ± 1.67c	51.11 ± 2.76d	54.89 ± 1.28e	9.74 ± 1.36d	10.23 ± 1.17c				
F. test	**	**	**	**	**				

\*, \*\* and N.S are symbols for significant, highly significant and Not significant differences respectively. The values are the means (± S.E) of four replicates, means followed by the same letter within each line are not significantly different (p<0.05)



**Fig. 2.** Effect of different treatments on the average of the seedling vigor index of the two wheat cultivars Sakha 95 and Misr 3. Since S1 is 50 mM NaCl, S2 is100 mM NaCl, and S3 is 150 mM NaCl. Data represents the mean  $\pm$  SE (n = 4). The same letter indicates no significant difference (p ≤ 0.05).

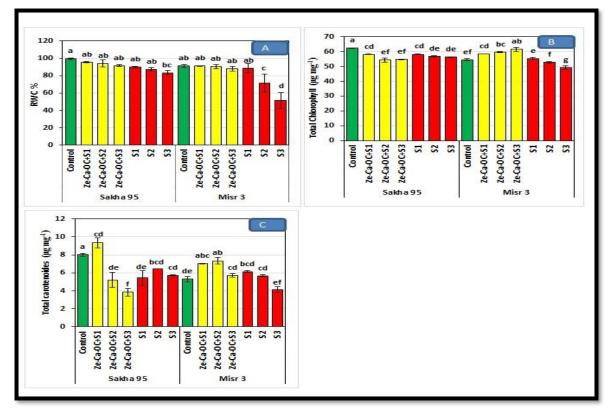
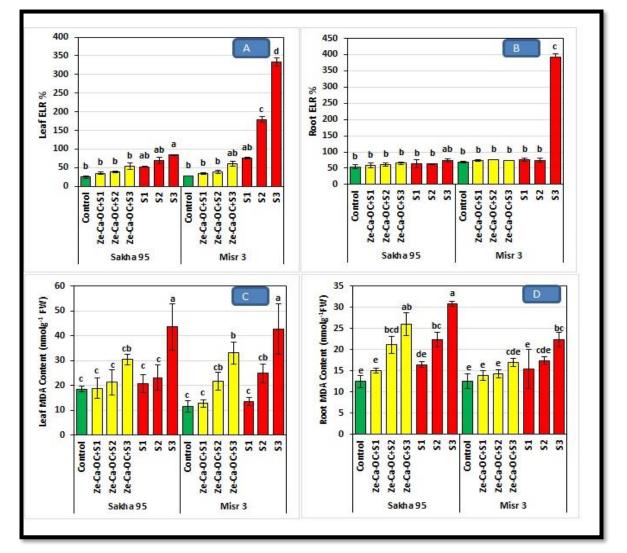
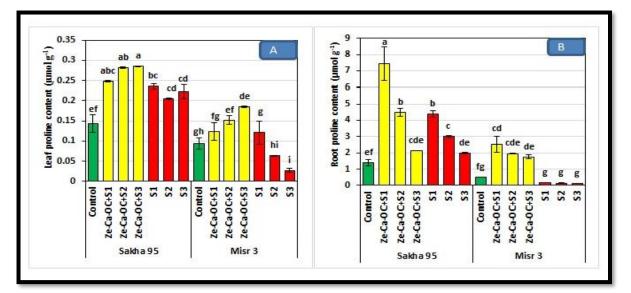


Fig. 3. Effect of different treatments on the average of (A) relative water content (RWC), (B) total chlorophyll content, and (C) total carotenoids content of the two wheat cultivars Sakha 95 and Misr 3. Since S1 is 50 mM NaCl, S2 is100 mM NaCl, and S3 is 150 mM NaCl. Data represents the mean  $\pm$  SE (n = 4). The same letter indicates no significant difference (p  $\leq$  0.05).

Water capacity and osmotic adjustment as affected by adding the Zeolite-Calcium-Organic compounds mixture: As illustrated in (Figure 3A) the two cultivars Sakha 95 and Misr 3 maintained their turgidity among the three salt levels by adding the Ze-Ca-OC mixture high RWC% in the leaf tissue. While under the highest salinity stress level 150mMNaC, Sakha 95 RWC declined to be 83.4%, cultivar Misr 3 RWC % exhibited a significant plunge under both salt levels (100 and 150mM NaCl) to be (71.69 and 51.49%), respectively (Figure3A) For the osmotic adjustment in both wheat cultivars, they produced different Proline contents in their root and leaf tissues. As shown in (Figure 5) under salinity stress levels (50, 100, 150mMNaCl), the leaf proline content of Cultivar Sakha 95 was higher than that of Misr 3 by 50, 70, 90%, respectively (Figure 5A). As well as, in the root tissues, Sakha 95 accumulated more massive amount of proline than that of Misr 3 (Figure 5B). Adding the mixture of Ze-Ca-OC to the salt affected plants enhanced the proline production in both cultivars. It was clear in that the leaf tissues of both cultivars Sakha 95 and Misr 3 proline content significantly increased (≈ 28, 21.4% and 60, 88%) under 100 and 150 mM NaCl, respectively by applying the Ze-Ca-OC (Figure 6A). Additionally, the root proline content exhibited an upsurge for the two cultivars particularly Misr 3 by approximately (93, 93, 92 %) under 50, 100,150 mM NaCl levels, respectively, (Figure 5B). Therefore, Ca in the Ze-Ca-OC mixture under salt stress levels retains the membrane of leaf and root for both cultivars without much leakage. While the salinity stress increased the leakage ratio (ELR) of leaf of cultivar Misr 3 more than that of Sakha 95 (≈ 2.5, 4 fold) under 100, 150 mM NaCl, respectively, the Ze-Ca-OC inhibited the salt stress for both of them (Figure4A). Electrolyte leakage was observed in the leaves of both cultivars and found to be impeded by the addition of the Ze-Ca-OC mixture to the high studied salinity levels (100 and 150 mM NaCl) in both cultivars leaves (Figure 4A). While adding the Ze-Ca-OC mixture to the salinity levels of Sakha 95 (50,100 and 150 mM NaCl) did not significantly reduce the leakage of root cells. However, at the highest salinity level, 150 mM NaCl, Ze-Ca-OC mixture maintained the stability of the root cell membrane of Misr 3 by reducing the ELR by  $\approx$  5.5-fold (Figure 4B).



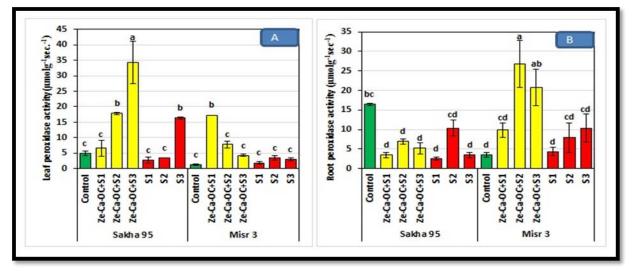
**Fig.4.** Effect of different treatments on the average of electric leakage ratio (ELR) of leaf (**A**), root (**B**), also Malondialdehyde (MDA) of both leaf (**C**), root (**D**) of the two wheat cultivars Sakha 95 and Misr 3. Since **S1** is 50 mM NaCl, **S2** is100 mM NaCl, and **S3** is 150 mM NaCl. Data represents the mean  $\pm$  SE (n = 4). The same letter indicates no significant difference (p ≤ 0.05).



**Fig.5.** Effect of different treatments on the average of the proline contents in both leaf (**A**) and root (**B**) of the two wheat cultivars Sakha 95 and Misr 3. Since **S1** is 50 mM NaCl, **S2** is100 mM NaCl, and **S3** is 150 mM NaCl. Data represents the mean  $\pm$  SE (n = 4). The same letter indicates no significant difference (p  $\leq$  0.05).

## The role of Ze-Ca-OC application in Oxidative stress management:

Salt stress caused an increase in MDA levels in the two cultivars of wheat leaves and roots, indicating concentration-dependent free radical generation compared to control ( $\approx$  3.8,1.8-fold and  $\approx$  2.4,2.5 fold at 150mMNaCl) in the leaf and root tissues of Misr 3 and Sakha 95 cultivars, respectively, (Figure4C, D). Applying Ze-Ca-OC mixture to salinity stressed plants caused that the MDA decreased in both cultivars leaf tissue up to ( $\approx$  30, 21%) at 150mMNaCl for cultivars Sakha 95 and Misr 3, respectively, (Figure 4C). Nevertheless, the mixture did not significantly decrease the MDA content in the roots under the three salinity levels and under 50 and 100mMNaCl concentrations in the leaves of both cultivars, as shown in Figure (4D). As shown in Figure (6A), the applying of Ze-Ca-OC mixture to the salinity stressed cultivars enhanced the activity of POD enzyme in the leaf of cultivar Sakha 95 by 82, 52% under 100, 150mMNaCl levels, respectively. On the other hand, cultivar Misr 3 leaf exhibited a massive increase (88%) in POD enzyme activity when Ze-Ca-OC was added under 50mMNaCl level with a negligible effect on 100 and 150mMNaCl levels Figure (6A). According to root tissues, in cultivar Sakha 95 there was no substantial change in POD activity under all treatments with a significant decrease compared with control condition, however cultivar Misr 3 exhibited embarked upregulation of POD enzyme activity (67.9% and 50.4%) by adding the mixture of Ze-Ca-OC under 100 and 150mMNaCl, respectively, (Figure 6B).

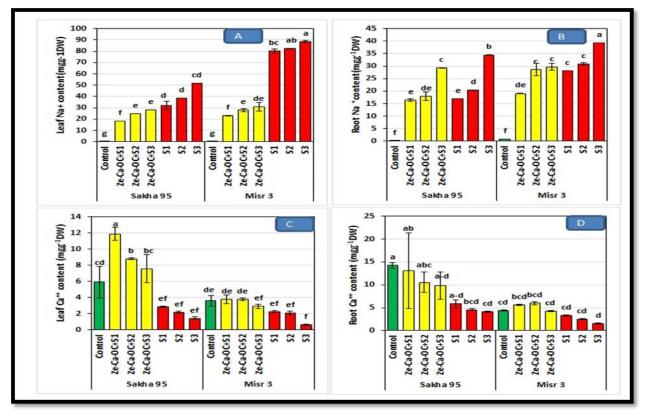


**Fig.6.** Effect of different treatments on the average of the Peroxide enzyme activity (POD) in both leaf (**A**) and root (**B**) of the two wheat cultivars Sakha 95 and Misr 3. Since, **S1** is 50 mM NaCl, **S2** is100 mM NaCl, and **S3** is 150 mM NaCl. Data represent the mean  $\pm$  SE (n = 4). The same letter indicates no significant difference (p  $\leq$  0.05).

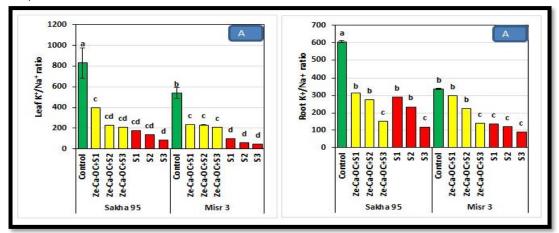
#### Minerals content and ion exchange as affected by applying the Ze-Ca-OC mixture under salinity stress:

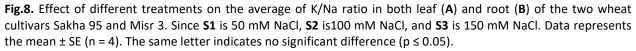
The results in Figure (7A, B) exhibits that, by increasing the salinity stress concentrations, the leaf and root tissues for both cultivars accumulate massively more Na<sup>+</sup> content than control conditions. Nevertheless, applying the mixture of Ze-Ca-OC simulated the wheat plant to extrude the excess Na<sup>+</sup> under salinity stress. The recorded decline in Na<sup>+</sup> content for leaf tissues was about 50% in cultivar Sakha 95 and more than 50% in cultivar Misr 3. Although the root Na<sup>+</sup> content under the three salinity levels in Sakha 95 was lower than that of Misr 3, the application of Ze-Ca-OC mixture plummeted the Na<sup>+</sup> content to be similar in both cultivars, except under 100mMNaCl concentration the root Na<sup>+</sup> content was higher in Misr 3 than in Sakha 95 by about 10 mgg<sup>-1</sup> DW (Figure7B). The Ze-Ca-OC application did not only change the Ca content in the root of both cultivars under salinity stress, but compared to the control conditions also, (Figure 7C, D). On contrast, the Ca content in leaf tissues which significantly decreased under the salinity stress levels (50, 100, 150 mM NaCl) by  $\approx$  52, 64, 76%, respectively, compared to control for Sakha 95 cultivar. Although in cultivar Misr 3 there was no significant decrease in Ca content of leaves under 50 and 100 mM NaCl levels, the concentration of 150 mM NaCl reduced the Ca content by about 48% compared to control condition (Figure 7C). Adding Ze-Ca-OC enhance the amount of Ca content in the leaf tissues of Sakha 95 (Figure 6C) under salinity levels 50, 100, 150 mM NaCl by approximately 50, 32, 25 %, respectively, but no significant change in that of Misr 3.

Under salt stress conditions, the cultivar Sakha 95 had a higher K<sup>+</sup>/Na<sup>+</sup> ratio than the cultivar Misr 3 in leaf and root tissues. Additionally, under the control condition, cultivar Sakha 95 had significantly higher leaf and root K<sup>+</sup>/Na<sup>+</sup> ratio than cultivar Misr 2 did (Figure 8). The two cultivars exhibited a significant reduction in K<sup>+</sup>/Na<sup>+</sup> ratio ( $\approx$ 78,83,89% and  $\approx$  81,88, 91%) under (50, 100, 150 mM NaCl) in the leaves of Sakha 95 and Misr 3 respectively, when compared to control plants (Figure 8A). similar results for root tissues with lower values of K<sup>+</sup>/Na<sup>+</sup> ratio in both cultivars as shown in Figure (8B). Applying Ze-Ca-OC mixture on the Misr 3 wheat cultivar led to a significant upsurge in the K<sup>+</sup>/Na<sup>+</sup> ratio under the three salinity stress levels for the leaf tissue (Figure 8A) and the two salinity levels 50 and 100mMNaCl for the root tissue by 100 and 159 percent, respectively, (Figure8B).



**Fig.7.** Effect of different treatments on the average of Na<sup>+</sup> ion content in leaf (**A**), root (**B**), also Ca<sup>++</sup> content in both leaf (**C**), root (**D**) of the two wheat cultivars Sakha 95 and Misr 3. Since, **S1** is 50 mM NaCl, **S2** is100 mM NaCl, and **S3** is 150 mM NaCl. Data represent the mean  $\pm$  SE (n = 4). The same letter indicates no significant difference (p  $\leq$  0.05).

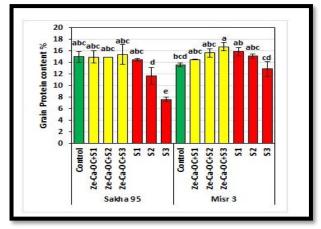




Grain Yeild and the crude protein content as influenced by application of Ze-Ca-OC under salt stress levels:

Salinity stress conditions led to a change in grain yield as shown in Table (4) and the grain protein content of both cultivars were affected as well (Figure9). The obtained results of the grain protein content that shown in Figure (9) illustrate that, cultivar Sakha 95 recorded a significant decrease under salinity stress levels 100 and 150 mM NaCl by around 22.6 and 50%, respectively, compared to control condition, however there was a slight change in the protein content of cultivar Misr 3 grains under the three NaCl levels compared to control. The addition of Ze-Ca-OC

moreover resulted in significant increases in the grain protein content of the Sakha 95 and Misr 3 cultivars, with 50.6% and 23.17% increasing, respectively, when the highest concentration of 150 mM NaCl was used (Figure 9).



**Fig. 9.** Effect of different treatments on the average of the grain protein content of the two wheat cultivars Sakha 95 and Misr 3. Since S1 is 50mMNaCl, S2 is 100mMNaCl and S3 is 150mMNaCl. Data presents the mean  $\pm$  SE (n=4). The same letter indicates no significant difference (P≤0.05)

## DISCUSSION

Adding the Ze-Ca-OC inhibited the salt stress on the plant by enhancing the soil's capacity to absorb nutrients, since zeolites added to fertilizers help to retain nutrients and thus improve the soil's long-term quality (Bybordi et al., 2018). Therefore, the plant keeps to grow up and increasing the seedling length resulting in higher seedling vigor index compared to under salinity stress. Similarly, on barley, Al-Busaidi et al, (2008) found that zeolite application inhibit the salinity stress by increasing length of the plant, which may improve the seedling vigor index in our study. Additionally, Ca<sup>++</sup> helps the plant to exchange with Na<sup>+</sup> ions so, it alleviates the toxic effect on the plant under salinity stress (Rashedy et al., 2022) which enhances the cell elongation and consequently enhance the seedling vigor index under salinity stress as shown in (Figure 2). The rate of photosynthesis is inhibited as salinity decreases water potential and increases the accumulation of Na<sup>+</sup> and Cl ions in the chloroplast (Tammam et al., 2008). The results in Table (1) and Figure (3B) showed a decrease in chl. content of the untreated plants with Ze-Ca-OC mixture, which may be due to salinity's harmful effects on photosynthesis. The highest salt level 150 mM NaCl revealed the lowest chlorophyll content with superior content for cultivar Sakha 95 (56.37 µgmg<sup>-1</sup>FW) compared to 49.23 µgmg<sup>-1</sup>FW for Misr 3 (Figure 3B), that is according to its ability to tolerate the salinity stress. This result is in harmony with Zou et al, (2016) who found that at 100 mM NaCl, wheat seedlings' chlorophyll (chl.) content significantly decreased. Surprisingly, adding Ze-Ca-OC mixture help the susceptible cultivar Misr 3 to overcome the salinity stress and enhance the plant to produce more chlorophyll by 61.49 µgmg<sup>-1</sup>FW compared to 49.23 µgmg<sup>-1</sup> <sup>1</sup>FW under salt stress 150 mM NaCl. Higher nutrient concentrations, including K, were caused by organic compounds like humic acid, and this resulted in an increase in chlorophyll (Rady, 2011). The Ze-Ca-OC application raises the photochemical efficiency of wheat seedlings by support the plant with OC that is important to protect it under the severe salinity stress. The carotenoids are no-enzymatic antioxidant which protect the plant from the oxidative stress resulted from salinity conditions (Bahari et al., 2013), which may help the plant to tolerate the salt stress conditions by decline the ROS production.

All the metabolic and physiological functions of the plant depend on the availability of water content. Low water potential is ultimately brought on by osmotic stress that higher salt concentrations cause in plants (Hasanuzaman *et al.*, 2017). Due to the high porosity of Zeolite crystalline structure, it can hold up to 60% of their weight in water (Polat *et al.*, 2004). The RWC is an indicator for the turgidity of tissues under normal conditions which usually decreased under salinity stress, similarly, the results of the (RWC) that dropped after 100 mM NaCl exposure (Mandhania *et al.*, 2006). Saline soils impede plant growth in a number of ways, including decreased water absorption, decreased metabolic activities due to salt toxicity, and nutrient deficiencies brought on by ionic disruptions (Pessarakli, 1994 and Al-Busaidi *et al.*, 2008).Proline is a substantial amino acid that is indispensable to plant responses to abiotic stress (Khan *et al.*, 2020). Under various environmental stresses, proline content rises in plants (Anjum *et al.*, 2011; Gill and Tuteja, 2012; Hayat *et al.*, 2012). The outcomes showed that Ca significantly

increased leaf proline content which is similar to results of pomegranate leaf affected by salt (Rashedy et al., 2022). Calcium controls various stress adaptation mechanisms, including membrane stability and the prevention of solute leakage from plant cell cytoplasm (El-Beltagy and Mohamed, 2013). The electrolyte leakage was measured in both cultivars' leaf and root tissues to investigate the damage to membranes because salt stress increased the amount of free radicals in plants. The obtained EIR results were similarly found by Elsawy et al., (2022) in barley tissues, the adding of Ca decreased the leakage of cells which was obvious in declining the ELR percentage compared with salinity stress conditions. The Lipid peroxidation produces Malondialdehyde (MDA), which builds up in stressed plant parts according to (Meloni et al., 2003). A high level of MDA concentration in plant parts is associated with oxidative damage to plant cell membranes (Zhang et al., 2007). A higher MDA level in plant cells demonstrates that the crop or plant is sensitive to salinity, whereas a lower MDA level demonstrates that the crop or plant is tolerant to oxidative stress. Elsawy et al, (2018) found that a small concentration of MDA in barley plant cells mainly corresponds to an increase in the activities of antioxidant enzymes in plant tissues, which aids the barley ability to grow well in salt stress environments. Thus, adding Ze-Ca-OC helped the wheat cultivars to protect themselves against the salinity stress levels by maintaining low MDA contents in the plant tissues according to the ability of Ca in mitigating oxidative stress on the plant tissues. Reactive oxygen species (ROS) are byproducts of the plants affected by salt stress, in addition, grave amounts of ROS, such as superoxide radicals ( $O_2^{-}$ ), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hydroxyl radicals (OH), are produced, which causes oxidative damage to plants (Tuteja, 2007). Under stress conditions, plants have developed sophisticated strategies with enzymatic and non-enzymatic mechanisms to maintain ROS homeostasis, and the enzymatic reactions of oxidation-resisting mechanisms heavily rely on antioxidative enzymes (Huang et al., 2017). Calcium (Ca) involved in Ze-Ca-OC mixture is capable in the regulation of the activities of Peroxidase (POD) enzyme related to ROS scavenging, which further fortify plants capability to tolerate the oxidative stress caused by salinity. Ahmad et al. (2015) investigated how Ca affected plant's ability to tolerate salt toxicity. The scientists concluded that adding Ca to the plants reduced Na uptake, increased proline content, avoided growth inhibition, and encouraged antioxidant enzymes activities such as POD. Those findings are comparable to our results and suggested that the role of Ca in enhancing the antioxidant enzymes activities was crucial in reducing NaCl toxicity on wheat cultivars.

Usually the higher Na<sup>+</sup> content in saline soils disturbs the nutritional balance and upsets the osmotic regulations of plant tissues (An et al., 2014). Zeolite is considered a perfect ion exchanger because, the positive cations like sodium, potassium, and calcium as well as positively charged groups like water and ammonia are easily captured by negatively charged zeolite. The negative charge of Zeolite attracts Ca<sup>++</sup>cations, and they can absorb water due to this. Furthermore, Zeolites are effective ion exchangers as the absorbed cations are relatively mobile due to their weak attraction and can be changed using this technique (Mumpton, 1999 and Polat et al., 2004). Calcium content in developing tissues under salinity stress conditions can be decreased yet is restored in the same area under elevated Ca levels (Cramer, 2002). By using electron-probe microanalysis, it has also been reported that salinity reduces Ca in the apical meristem and young lettuce leaves, as well as all along the elongation zone of sorghum leaves (Bernstein et al., 1995), this partial reversal of the growth inhibition and prevention of the decreasing growing zone length coincide with the reversal of the reduction in Ca level (Lazof and Bernstein, 1999). Calcium increasing in the plant tissues may help it to ameliorate the sodium toxicity, which lead to better growth under salinity stress conditions. Although Ca entry is predicted to come from the opposite side of the apoplast, the outward rectifying cation channels also appear to be permeable to Ca (apoplast) (Cramer, 2002). The observed transport of these ions from the root to the shoot in salt-stressed maize is consistent with these ion interactions with the outward rectifying cation channel (Cramer et al., 1994). It is indeed interesting to observe that channels precisely have different characteristics in root cortical cells, therefore applying the Ca to the wheat affected by the salinity stress did not affect the K<sup>+</sup>/Na<sup>+</sup> selectivity of K outward rectifying cation channels in two wheat cultivars Sakha 95 and Misr 3 which are differing in salt tolerance, similarly to the results of Schachtman et al, (1991). Although Cultivar Sakha 95 produced higher grain yield than that of Misr 3, there was no significant difference between them in the plant height, Cell elongation, cell division, membrane permeability, and nitrogen metabolism are all significantly influenced by Ca (Ahmad et al., 2018). Hence, adding such mixture Ze-Ca-OC that contains the calcium is responsible for increasing the plant height and enhance plant growth under salinity stress. With respect to grain yield, the organic acids such as humic and fulvic in the OC of the used mixture increase the grains filling by increase the N metabolism, and therefore enhance the quality of the grains as protein content % as shown in Figure (9). Zeolite concerns the most crucial plant nutrients, including calcium, magnesium, and microelements as well as

nitrogen (N) and potassium (K). These nutrients can be stored by zeolite in the root zone for use by plants as needed. As a result, N and K fertilizers are used more effectively by producing higher yields, extending their activity, or reducing their rates for the same yield. Applying Ze-Ca-OC with the involved Zeolite tends to increase nitrogen content, retain valuable nitrogen, and improve the quality of the seeds and helps plants absorb many nutrients which is responsible for the protein in the grains under salinity levels.

## CONCLUSION

It can be concluded that, salt stress severely affected the studied wheat cultivars growth even if Sakha 95 performance was better than Misr 3. The obtained results indicated that, the highest significant grain yield, protein content, and chemical constituents (antioxidant activity, proline content, chlorophyll, and carotenoids contents) of wheat crop were produced when zeolite, calcium, and organic compounds were applied as a fertilizer treatment under salinity stress conditions. Furthermore, the application of organic acids enhances plant defense against stress, leading to better plant performance in direct and indirect ways. Zeolite and calcium application plays a key role in the Na extrusion from salt-stressed wheat cultivars. By inhibiting sodium toxicity, more K and Ca influx improves wheat plant growth as it was clear in the declined ELR % and MDA content under treated wheat with Ze-Ca-OC mixture under salinity stress. Overall, using Ze-Ca-OC as an active and affordable Saigo-Cal ingredient to mitigate the salt stress in wheat plants and may also other crops enhance the growth and increases their ability to withstand this condition.

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# قدرة خليط من الزيوليت والكالسيوم والمركبات العضوية على تخفيف إجهاد الملوحة في قمح الخبز

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في ظل النمو السكاني والتغير المناخي وظروف إجهاد الملوحة خاصة فيالمناطق القاحلة وشبه القاحلة، تعرقل ملوحةالتربة إنتاج محصول القمح بشكل كبير. قد يقلل الزبوليت أو الكالسيوم أو الأحماض العضوية من آثار إجهاد الملوحة على النباتات. الغرض من هذه الدراسة هو توضيح التأثير التحسيني لخليط الزيوليت، الكالسيوم والمركباتالعضوية 100 مجم / لترعلي نمو قمح خبزالصنفان (سخا 95 ومصر 3) تحت ثلاثة تركيزات ملوحة (50، 100، 150ملي كلوريد الصوديوم). أجربت التجرية الحالية في موسمين متتاليين(2021/2020، 2022/2021) في قصاري التربة الرملية بمحطة البحوث الزراعية بسخا، كفر الشيخ، مصر. فأصناف القمح التي تعرضت فقط لضغط المستويات الملوحة وكذلك النباتات المعالجة بمزيج الزيوليت، الكالسيوم والمركباتالعضوية إلى مستويات الملح هذه تم تقييمها من خلال خصائص فسيولوجية مختلفة للنباتات في المرحلة الخضرية. وفي مرحلة الحصاد تم قياس ارتفاع النبات وحاصل الحبوب وكذلك المحتوى من بروتين في الحبوب.أدى إجهاد الملوحة إلى إعاقة نمو الصنف مصر 3 أكثر من سخا 95 من خلال تجزئة المحتوى من البوتاسيوم\الصوديوم ونسبة منخفضة من الكالسيوم في أنسجة الأوراق والجذور. تطبيق خليط الزيوليت، الكالسيوم والمركباتالعضويةادى الى تحسين نموها عن طريق تقليل سمية الصوديوم وتعزيز الدفاع المؤكسد لكلا من الصنفين من خلال المساعدة على تنظيم نشاط إنزيم POD .أدت الأصناف من القمح المزودة بخليط الزيوليت، الكالسيوم والمركباتالعضويةتحت مستويات إجهاد الملوحة إلى زبادة معنوبة في محتوى الماء النسبي بنسبة (≈ 20، 17٪) تحت (100، 150 ملى مولار كلوريد الصوديوم) على التوالي في الصنف مصر 3. علاوة على ذلك، فإن محتوى البرولين في ورقة سخا 95 ومصر 3 قد ارتفع بشكل كبير ارتفاع بما يقارب (≈ 28، 21.4٪ و60، 88٪) عند 100 و150 ملى مول كلوريد الصوديوم، على التوالى. بالإضافة إلى ذلك، سجل محتوى البرولين الجذري زبادة كبيرة عند إضافة خليط الزيوليت، الكالسيوم والمركباتالعضوية إلى تركيزات الملوحة لكلا الصنفين. كان محصول الحبوب متشابهًا في الصنفينتحت مُخصب العلاجات المختلفة، ومع ذلك، فإن خليط الزبوليت، الكالسيوم والمركباتالعضوية بشكل كبير تحتوى بذورها على نسبة عالية من البروتين خاصة تحت أعلى تركيز 150ملي مول كلوريد الصوديوم بنسبة 50.6٪ و23.17٪ من صنف سخا 95 ومصر 3 على التوالى.عند اخذ النتائج مجتمعه، تشير إلى أن خليط الزبوليت والكالسيوم الخارجية والمكملة بالمركبات العضوبة يمكنها تقليل إجهاد الملح على قمح الخبز عن طريق زبادة تراكم المواد الواقية من الضغط الأسموزي وطرد الصوديوم السام، واحتواء الكالسيوم والبوتاسيوم بطريقة مفيدة.

الكلمات المفتاحية: الكالسيوم، مركبات عضوية، تخفيف الملوحة، القمح، الزيوليت