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# Assessing the usefulness of *Moringa oleifera* leaf extract and zeatin in enhancing growth, phytohormones, antioxidant enzymes and osmoprotectants of wheat plant under salinity stress

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Salt stress is a main constituent of environmental stresses which causes a deterioration in crop development and yield. Fortunately, using leaves extract as *Moringa oleifera* leaf extract and zeatin have a real influence on lessening the harmful impacts of salt on crops. Two greenhouse studies were done in a complete randomized design with three levels of either *Moringa oleifera* leaf (MLE) extract or zeatin being applied to wheat plants without stress (0.23 dS $m^{-1}$) or with salinity stress (6.25 dS $m^{-1}$). Salinity led to increases in the contents of endogenous abscisic acid (ABA), total soluble sugars TSS and proline, malondialdehyde and hydrogen peroxide contents in addition to increasing antioxidant enzymes activities (peroxidase POX, superoxide dismutase SOD and catalase CAT). Moreover, salinity stress decreased the growth parameters, chlorophyll content and yield attributes of wheat plants. However, the foliar application of MLE (10% and 20%) and zeatin (200 and 400 mg $L^{-1}$) improved the growth of wheat plants under both normal and salt-stressed conditions through increasing endogenous phytohormones (IAA, $GA_3$ and zeatin), photosynthetic pigments, total soluble sugars and proline contents. Furthermore, the applicants further increased the activities of antioxidant enzymes beside the non-enzymatic antioxidants' phenolic compounds and antioxidant activity (DPPH%) which participate in reducing the excess accumulation of free radicals (ROS). The use of MLE and zeatin supports the salinity tolerance of wheat plants. Moringa oleifera leaf extract and zeatin are viable alternatives for optimizing agricultural production and enhancing plant tolerance in arid and semi-arid regions.

**Keywords:** Antioxidant enzymes, endogenous phytohormones, leaf extract, *Moringa oleifera,* salinity, zeatin

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## INTRODUCTION

Wheat (*Triticum aestivum L.*) is known as the most valuable dietary cereal plant. Wheat is a major feeding crop to about 36% of the world's humans, which is responsible for providing 20% of the calories as well as representing 55% of the needed carbohydrates in the world. (Bakry *et al*., 2019). Wheat grain is rich with mineral nutrients that are required for people´s health (Ragaey et al., 2022). Wheat is salinity sensitive (Quamruzzaman et al., 2021). The Food and Agriculture Organization (FAO) recorded that 397 million hectares of wheat cultivation were harmed by salinity stress, which poses a danger to food security (World Health Organization, 2019).

Salinity is one of the most serious environmental factors that causes various physiological impairments in crop plants and leads to less production. According to FAO reports, the effect of salinity covers one-third of the cultivated land in the world (Abdoli et al., 2013). Salinity problem has appeared by competition for high quality water in agricultural lands and industry thus landscape users have envcourged to use of various sources of irrigation water such as sea water. Salinity damage effects reflect on plant morphological, biochemical, physiological, and gene expression (Atkinson and Urwin, 2012; Abdelhamid et al., 2013).) that depending on species, plant growth phase, plant organs that are exposed to stress, ionic strength, salt solution components, concentration, and duration; (Robin et al., 2016 and Sadak, 2023).

Salinity stress significantly reduces plant growth and biomass, which is related to chlorophyll breakdown, instability of water status, imbalance in the ion ratio, stomata damage, and modifications in transpiration and respiration, (Al-Ashkar, et al. 2020; Khatab et al., 2021; Alshoaibi, 2021; Budran et al., 2023). Salinity also affects enzyme activities, plant hormonal equilibrium, cell membrane structure, water and nutrient absorption, and causes oxidative power and osmotic imbalances (Zhao et al., 2020). Osmotic stress reduced plant water potential and water uptake capacity induced several biochemical variations, like cell wall damage, influencing cell expansion, nutrient consumption, stomata movement, reducing assimilation of $CO_2$, and decreased both photosynthetic and detoxifying free radicals (ROS) rates (Quamruzzaman et al., 2021). Excessive uptake of harmful ions ($Na^+$ and $Cl^-$) leads to ionic stress. Besides that, oxidative stress is induced by free radical overproduction (ROS) such as singlet oxygen ($\cdot O_2$), superoxide radical ($O_2^{\cdot -}$), hydroxyl radical ($OH^-$), and hydrogen peroxide ($H_2O_2$). That caused a reduction in antioxidant system's ability, appearing as destruction in cell constituents such as proteins, DNA, and lipids, inducing destruction in plant physiological functions

(Emam 2013). ROS detoxification is achieved through enzymes and non-enzyme antioxidants. Enzymatic antioxidants include scavenging enzymes like peroxidase (POX), superoxide dismutase (SOD), and catalase (CAT) (Rout and Shaw, 2001). In addition to this, accumulation of solutes known as osmoprotectants like soluble sugars and proline, has essential impacts on saving plant cells (Munns, 2005).

A lot of attention has been paid recently to the prospect of using natural and healthy substances to increase plant tolerance and promote plant development and yield. Moringa oleifera leaf extract (MLE) is an example of those natural substances that do not hurt plants when introduced. The great nutrient contents of M. oleifera, which include antioxidants, vitamins, minerals, phytohormones, and proteins have earned it the name Miracle Tree. Furthermore, Moringa leaf extract is also utilized as an exogenous plant growth stimulator since it is a major zeatin source, ascorbate, carotenoids, phenols, and certain vital minerals (Foidl et al., 2001, Fuglie, 2001). Moringa leaf extracts, when applied to plants under drought or salt stress, could modify plant phenotypes and promote growth and productivity with modifications in plant metabolic processes (Yasmeen et al., 2013 a, b). Moreover, the extract from moringa leaves strengthens stems, increases longevity, increases the number of roots, stems, and leaves linked to increased fruit number and volume, and enhances biotic and abiotic tolerance, thus increasing plant yield (Foidl et al. 2001). Moringa leaf extract treatment of rocket plants increased growth traits, photosynthetic contents, stomatal conductance, carbohydrates, protein, ascorbic acid, total phenols, N, P, K, Fe, Ca, Mg, and plant hormones (Abdalla, 2013). Kanchani and Harris (2019) stated that okra plant growth and productivity were increased by using Moringa leaf extract (10%, 20%, and 30%). Furthermore, several researchers pointed out that moringa leaf extract (MLE) is a major source of plant regulators (Maishanu et al., 2017), and antioxidants (Makkar et al., 2007, Ezzo et al., 2018; and Abdalla et al., 2022).

Another solution for solving plant production challenges is plant growth regulators (PGRs) (Javid et al., 2011). Furthermore, the types of plants, the environment, their physiological and nutritional state, their developmental stage, and the equilibrium of their internal hormones can affect how they respond to PGRs (Naeem et al., 2011). One of these PGRs that control plant growth under both favorable and unfavorable environmental conditions is cytokinin. Cytokinin is typically produced by higher plants at the roots, then move to the aerial parts via xylem. Cytokinin has a significant influence on the regulation of chloroplast development, anthocyanin production, cell differentiation, and apical dominance (Hutchkinson and Kileber, 2002). Furthermore, it is well documented that exogenous cytokinin application delays the ageing process of leaves and fruits by preventing the degradation of chlorophyll and photosynthetic proteins (Pospíšilová et al., 2000). Zeatin is from this category, which encourages plant growth and production even under challenging conditions. Zeatin was known to have a wider concentration range than any other cytokinin (Al-Hussein and Rida, 2006). Additionally, zeatin is involved in the transportation and distribution processes of carbohydrates, which are required to meet the demands of rapid growth. Munoz et al., (2008) documented that zeatin riboside has a greater effect on the movement of carbohydrates than it does on the movement of protein.

As far as we are aware, not much study has examined how moringa leaf extract and zeatin treatments affect the morphological, and physiological properties of wheat plant under salinity stress. Hence, this investigation aimed to evaluate the impact of various moringa leaf extract and zeatin levels on different studied parameters of wheat plants grown at different salinity levels. So, it is imperative to understand in further detail how this plant responds to exogenous treatment of Moringa leaf extract and zeatin under different water salinity levels in order to boost the growth and yield of the wheat plant by counteracting the negative impacts of salinity stress on its endogenous phytohormones, photosynthetic pigments, antioxidant systems, and Osmo protectants. As a result, this investigation can help researchers choose the optimum Moringa leaf extract and zeatin levels to improve wheat salinity stress tolerance.

## MATERIALS AND METHODS

### Preparations of Moringa oleifera leaf extract (MLE)

*Moringa oleifera* plants were brought from experimental station of National Research Centre, Al Nubaria district El-Behira Governorate, Egypt Fresh leaves washed and frozen overnight (-5ºC) then MLE was extracted by water (10 Kg/L fresh leaves) in a blender (Yasmeen et al., 2012) then filtered using filter paper. Then centrifuged at 8,000 × *g* for 15 min then diluted using leaf extract to distilled water (V: V) to reach the required concentrations ratio of 1:10 and 1:20 (MLE1: 10% and MLE2: 20%). The diluted MLE were used for foliar treatment to wheat plants MLE was analyzed and its chemical components were recorded (mg/g dry wt.): Amino acids, 106.2, proline, 21.0, total soluble sugars, 248.0, calcium, 28.0, magnesium, 6.7, potassium, 25.1, phenols, 6.2 mg/g dry wt as well as, phytohormones as (µ g/g fresh wt), indole acetic acid IAA, 0.83, gibberellins GA, 0.74, zeatin, 0.96 and abscisic acid ABA 0.29. 0.1% (*v*/v) Tween-20 was used as a surfactant to achieve the best penetration to plant tissue.

**Experimental procedure**

This experiment was applied during the winter of 2018/2019 and was repeated in the winter of 2019/2020, at the National Research Centre's greenhouse in Dokki, Cairo Governorate, Egypt. Wheat (cv. Benisuef 5) grains were purchased from Egypt's Agricultural Research Centre in Giza conservative. November 25$^{th}$was the date of sowing in the two years, and wheat Benisuef 5 grains were chosen equal in size and have the same color. The soil had a clay-loam texture, with coarse sand 1.4%, fine sand 31.7%, silt 39.6%, and clay 27.3%, ECe 1.82 dS $m^{-1}$, pH 7.5, organic matter 1.93%, CaCO3 7.88%, and available N, P, and K accounting for 45.6, 7.8, and 415.0 mg $kg^{-1}$, respectively. Colorless granular ammonium sulfate $(NH_4)_2SO_4$ 20.5% N at 40 kg N $ha^{-1}$, and single superphosphate 15% P at 60 kg P $ha^{-1}$were mixed thoroughly into soil of each pot before wheat planting. Ten wheat grains were sown per pot at 30-mm depth. After ten days of planting, seedlings were then reduced to five per pot.

The experimental design was a factorial complete randomized design and replicated three times. The experiment comprised two factors. The first factor included two salinity levels namely S0 (0.23 dS $m^{-1}$) and S1 (6.25 dS $m^{-1}$). The second factor involved *Moringa oleifera* leaves extract and zeatin treatment namely MLE (0.0, 10% and 20%) and zeatin (0.0, 200 and 400 mg $L^{-1}$). Different treatments were applied as foliar treatments twice after 30 and 45 days from sowing. Pots were divided into two groups, every one watered by one of these salt levels (S0, 0.23 dS $m^{-1}$ and S1 = 6.25 dS $m^{-1}$). Plants under salinity stress irrigated three times with equal amounts (liter/pot) of the salt water (6.25 dS $m^{-1}$) followed by one time with tap water. Salinity water was added after 50 days from sowing. Salt water prepared as Stroganov (1962) equation as Table 1.

Plant samples were collected after 60 days of sowing to record the growth parameters data specially shoot length (cm), shoot fresh and dry weight (g/plant), number of leaves/tiller and root dry weight (g). Plant samples were subjected to biochemical analysis. After drying wheat leave samples, proline, total soluble sugars and phenolics were estimated. At full maturity (after 190 days from sowing) yield and its components: plant height (cm), spike length (cm), spikelet number per spike, grains no/spike, spike weight/plant (g), grains weight/plant (g) and 1000 grains weight (g). The protein percentage and carbohydrate percentage of the yielded grains were measured.

**Measurements**

Endogenous hormones: Depending on the recorded data of the first season, 20% MLE, 200 mg/L zeatin were chosen for estimation endogenous wheat plant hormones namely auxins (as indole acetic acid IAA), gibberellic acid (as $GA_3$), cytokinin (as zeatin), abscisic acid (as ABA) according to the method described by Kettner and Doerffling, (1995) and Pan et al., (2010), respectively. Photosynthetic pigments were determined by Lichtenthaler and Buschmann, (2001) method. Lipid peroxidation levels were expressed as malondialdehyde (MDA) level which was determined by method provided by Hodges et al., (1999). Method of Velikova et al., (2000) was applied to estimate Hydrogen peroxide content. Assay of enzymes activities: enzyme extractions according to Chen and Wang, (2006). Peroxidase (POX, EC 1.11.1.7) activity was evaluated according Bergmeyer (1974). Superoxide dismutase activity was measured according to Chen and Wang (2006). Catalase (EC 1.11.1.6) activity was measured according to Kong et al., (1999) Total phenolic was extracted and measured by Gonzalez et al., (2003). The method of Versluses, (2010) was used to estimate proline content. Total soluble sugars (TSS) were extracted and measured as Chow and Landhausser, (2004). Total carbohydrates were determined as Albalasmeh et al., (2013). Total protein content was estimated using the method of Pedrol and Tamayo, (2001).

**Statistical analysis**

Data were subjected to an analysis of variance for a factorial experiment in a completely randomized design (Gomez and Gomez 1984), after testing for the homogeneity of error variances using the Levene's test (1960), and after testing for normality distribution (Shapiro and Wilk, 1965). The Tukey's HSD (honestly significant difference) test was used to compare the differences between means at $p \leq 0.05$. The statistical analysis was conducted using Gen Stat 19th Edition (VSN International Ltd., Hemel Hempstead, UK).

## RESULTS

**Effect of Moringa Leaf extract (MLE) or zeatin on Growth parameters:**

Irrigation of wheat plants with 6.25 dS $m^{-1}$ saline water induced significant declines in shoot length (cm), shoot dry weight, and root dry weight compared to unstressed plants (0.23 dS $m^{-1}$). Table 2. The obvious negative impact of salt stress on wheat plants was shown in the same trend of the markedly significant reduction in shoot length and leaves number per tiller from 45.43 cm & 6.67 to 36.17 cm, and 6.00 respectively, showing a decline of 20.38% and 10.05% compared with the control plant. Moreover, shoot fresh and dry weights were attenuated from 4.47, 1.26 g (unstressed plant) to 3.20, 0.88 g (stressed plant), with reductions of 28.41% and 30.16%, respectively. Moreover, root dry weight decreased from 0.79 (control plant) to 0.39 (salinity - stressed plants), with 50.63 percentages of declines. On the other hand, foliar spraying of either MLE (10% and 20%) or zeatin (200 and 400 $mgL^{-1}$), significantly improved all the studied morphological features under salinity stress and natural conditions in wheat seedlings relative to their corresponding controls. Furthermore, Table 2 shows the superiority of MLE treatment in improving different vegetative growth parameters over zeatin treatments. Moreover, data clearly show that higher levels of 20% MLE and 400 $mgL^{-1}$ zeatin had more impact than lower levels. Maximum increases in growth indices were achieved at 20% of MLE, followed by 400 $mgL^{-1}$ of Zeatin under normal or saline conditions, (Table 2).

**Effect of Moringa leaf extract (MLE) or zeatin on endogenous phytohormones**

According to the first - season data, 20% MLE and 200 mg $L^{-1}$ zeatin were chosen for estimation of endogenous plant bioregulators (Table 3). It is obvious from Table 3 that irrigated wheat plants with saline solution caused marked decreases in the contents of various endogenous phytohormones (IAA, GA, and cytokinin) with a marked increase in endogenous ABA content. The obvious negative impact of salt stress on wheat plants was shown in terms of the significant reduction in IAA, GA, and cytokinin from 43.54, 42.64, and 56.68 to 29.28, 21.06, and 37.31, respectively, showing a decline of 32.75%, 50.61, and 34.17%, with an increasing in ABA contents from 20.97 to 38.38, reaching 80.02% increase percentages compared with the control plant. Additionally, different applications of 20% MLE, and 200 $mgL^{-1}$ zeatin increased IAA, GA and cytokinin contents, accompanied by a reduction in ABA contents in wheat plants. Additionally, 20% MLE was the best treatment for improving the studied endogenous phytohormones (IAA, GA, and cytokinin) while decreasing ABA content in comparison to corresponding controls in usual irrigation or salty conditions (Table 3).

**Effect of Moringa leaf extract (MLE) or zeatin on photosynthetic pigments**

The Obtained results in Fig. 1, demonstrated that salt water (6.25 dS $m^{-1}$) caused a substantial decrease in pigment content (chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), Chl *a* + Chl *b*, carotenoids, and total pigments) compared to control plants. The percentages of reductions were 38.9%, 20.3%, 32.7%, 30.8, and 32.9% in Chl *a*, Chl *b,* and carotenoids, respectively of wheat leaves as compared with unstressed controls. On the other hand, foliar treated with various levels of MLE (10% and 20%) or zeatin (200 and 400 mg $L^{-1}$) not only improved different photosynthetic pigment constituents under usual irrigation tap water but also restrained the decreases in the above-mentioned photosynthetic pigment content as compared with the untreated control plants under salt stress (Fig. 1). Meanwhile, Fig. 1 showed that MLE application with the mentioned levels was noticed to be more effective than zeatin in increasing Chl *a*, Chl b, and carotenoids in comparison to comparable untreated seedlings. In addition, higher levels of different treatments were more effective than lower ones under both conditions. Furthermore, 20% MLE was the superior treatment for improving photosynthetic components in the two tested salt levels (Figure 1).

**Effect of moringa leaf extract (MLE) or zeatin on osmolytes and phenolic contents:**

Osmolytes (total soluble sugars TSS and proline) and phenolic concentrations of salt- stressed wheat were significantly increased relative to control wheat (Table 4). The percentage of increases reached 55.8%, 59.5%, and 125.6% in TSS, proline, and phenolic, respectively, of wheat leaves as compared to unstressed plants. Meanwhile, foliar treated with the used levels of MLE (10% and 20%), or zeatin (200 and 400 mg $L^{-1}$) caused more significant increments in different studied parameters under usual tap water and salt stress (Table 4).

**Table 1**. Salt components of the saline solution (%).

| $MgSO_4$ | $CaSO_4$ | NaCl | $MgCl_2$ | $CaCO_3$ |
|---|---|---|---|---|
| 10 | 1 | 78 | 2 | 9 |

Component of certain anions & cation in salt mixture as percentage of total mill equivalents.

| $Na^+$ | $Mg^{+2}$ | $Ca^{+2}$ | $SO^{-2}$ | $Cl^-$ | $CO^{-2}$ |
|---|---|---|---|---|---|
| 38 | 6 | 6 | 5 | 40 | 5 |

**Table 2.** Effect of moringa leaf extract (MLE1: 10% and MLE2: 20%) and zeatin (Z1: 200 $mgL^{-1}$ and Z2: 400 $mgL^{-1}$) on growth parameters of wheat plants grown under (S0: 0.23 dS $m^{-1}$ and S1: 6.25 dS $m^{-1}$) salinity levels.

| Salinity ($dSm^{-1}$) | Treatment | Shoot length (cm) | Leaves number/tiller | Shoot fresh weight (g) | Shoot dry weight (g) | Root dry weight (g) |
|---|---|---|---|---|---|---|
| S0 | 0 | 45.43e±0.808 | 6.67b-d±1.145 | 4.47de±0.129 | 1.26d±0.011 | 0.79d±0.012 |
| | MLE1 | 49.13c±0.462 | 7.00a-c±0.000 | 4.89c±0.023 | 1.52b±0.046 | 0.98bc±0.035 |
| | MLE2 | 56.17a±0.462 | 7.67a±1.155 | 5.69a±0.035 | 1.94a±0.233 | 1.15a±0.092 |
| | Z1 | 46.17d±0.577 | 6.67b-d±1.555 | 4.66d±0.011 | 1.42c±0.023 | 0.94c±0.069 |
| | Z2 | 54.23b±0.503 | 7.33ab±1.555 | 5.21b±0.821 | 1.94a±0.042 | 1.04b±0.053 |
| S1 | 0 | 36.17h±0.577 | 6.00d±0.000 | 3.20g±0.023 | 0.88g±0.058 | 0.39g±0.012 |
| | MLE1 | 39.20f±0.692 | 6.33cd±1.57 | 4.03f±0.031 | 0.96fg±0.050 | 0.47f±0.042 |
| | MLE2 | 46.13d±0.462 | 6.67b-d±1.155 | 4.54de±0.058 | 1.24d±0.035 | 0.71e±0.012 |
| | Z1 | 37.83g±0.577 | 6.00d±0.00 | 3.90f±0.127 | 0.97f±0.092 | 0.45fg±0.023 |
| | Z2 | 45.27e±0.924 | 6.67b-d±1.555 | 4.43e±0.035 | 1.13e±0.139 | 0.65e±0.092 |
| LSD @ 5% | | 0.439 | 0.839 | 0.223 | 0.078 | 0.077 |

Data are means of three replicates ± SE; means with different letters within the same column are significantly different ($p \le 0.05$).

**Table 3.** Effect of moringa leaf extract (MLE2: 20%) and zeatin (Z2: 400 $mgL^{-1}$) on endogenous phytohormones: indole acetic acid (IAA), Gibberellic acid (GA), abscicic acid (ABA) and cytokinin (μg/100 g fresh weight) contents of wheat plants grown under (S0 = 0.23 dS $m^{-1}$ and S1 = 6.25 dS $m^{-1}$) salinity levels.

| Salinity (dS $m^{-1}$) | Treatment | IAA | GA | ABA | Cytokinin |
|---|---|---|---|---|---|
| S0 | Control | 43.536d±3.325 | 42.638c±2.325 | 20.969d±0.625 | 56.675c±4.325 |
| | MLE2 | 82.338a±2.412 | 78.463a±1.325 | 15.383f±0.638 | 77.356a±1.325 |
| | Zeatin 2 | 70.568b±1.362 | 72.424b±3.215 | 18.457e±0.354 | 64.612b±2.625 |
| S1 | Control | 29.277f±1.635 | 21.056f±0.752 | 38.376a±0.514 | 37.315f±1.625 |
| | MLE2 | 45.378c±1.514 | 35.734d±1.265 | 24.724c±0.524 | 53.677d±3.215 |
| | Zeatin 2 | 37.726e±1.425 | 33.374e±0.325 | 31.605b±0.325 | 48.944e±2.625 |

Data are means of three replicates ± SE; means with different letters within the same column are significantly different ($p \le 0.05$).

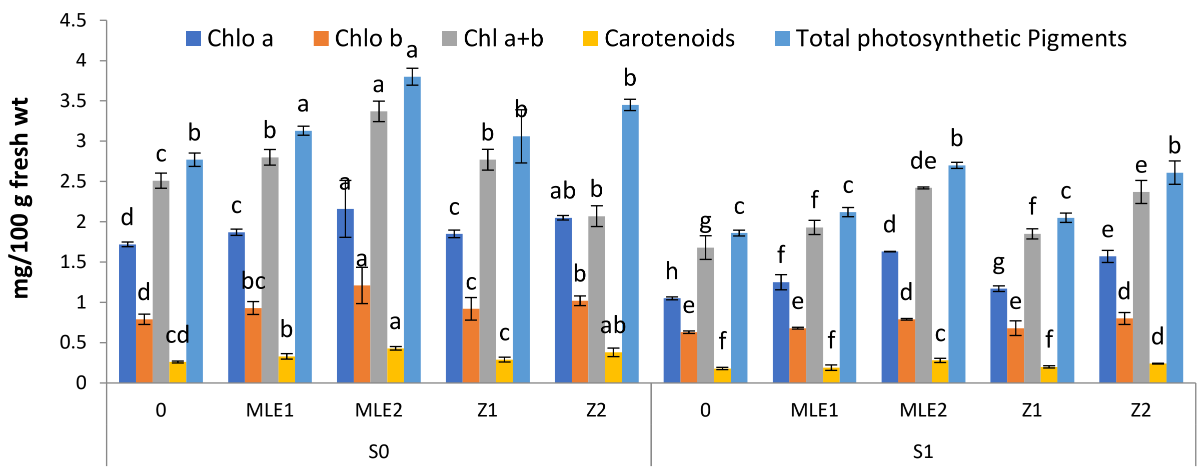


**Figure 1.** Effect of moringa leaf extract (MLE1: 10% and MLE2: 20%) and zeatin (Z1: 200 $mgL^{-1}$ and Z2: 400 $mgL^{-1}$) on photosynthetic pigments (mg/g fresh weight) of wheat tissue (Chl a, Chl *b,* Chl *a+b*, carotenoids, and total pigments) under (S0 = 0.23 dS $m^{-1}$ and S1 = 6.25 dS $m^{-1}$) salinity levels. Data are means of three replicates ± SE; means with different letters within the same column are significantly different ($p \le 0.05$).

In addition, higher levels of different used treatments were more effective than lower ones under both conditions. Furthermore, 20% MLE was the superior treatment in improving different osmolytes and phenolic contents in tap water or saline water (Table 4).

**Effect of moringa leaves extract (MLE) or zeatin on hydrogen peroxide ($H_2O_2$) and MDA contents**

Imposition of salt stress significantly ($p \leq 0.05$) improved leaf overproduction of $H_2O_2$ and MDA contents of wheat plants (Fig 2) compared with unstressed plants. While exogenous application of wheat plants by various levels of MLE or zeatin induced significant decreases in $H_2O_2$ and MDA contents under normal and stressed conditions compared to control. MLE was superior to zeatin treatments in decreasing $H_2O_2$ and malonaldehyde contents. Furthermore, higher levels of each MLE or zeatin treatment show lower $H_2O_2$ and malonaldehyde levels in wheat leaves in normal or salt conditions (Fig. 2). Among the different treatments, 20% MLE was the most effective, followed by 400 mg $L^{-1}$ zeatin compared with other treatments.

**Effect of moringa leaf extract MLE or zeatin on antioxidant enzymes**

Salt water (6.25 dS $m^{-1}$) which was used in irrigation of wheat plant caused significant increases in leaf accumulation of different antioxidative enzymes, including peroxidase POX, superoxide dismutase SOD, and catalase CAT, in wheat plants (Table 5). Furthermore, the applications of MLE and zeatin with different concentrations gave more accumulation in leaf antioxidant enzymes and antioxidant activity in the wheat plant not only in normal water irrigation but also under salinity stress (6.25 $\delta$S $m^{-1}$). It is obvious from Table 5 that there is a direct relationship between the concentrations of either MLE or zeatin and the increases in the activity of various enzymes compared to by untreated controls. Among the different treatments, 20% MLE was the most effective, followed by 400 mg $L^{-1}$ compared with other treatments.

**Effect of moringa leaf extract MLE or zeatin on grain yield and its components:**

Table 6 states that irrigation of wheat plants with 6.25 dS $m^{-1}$ salt water caused significant decreases in plant height, spike length, spikelet number/spike, grain number/spike, spike weight, and 1000 grains weight compared with unstressed control plants. The obvious negative impact of salt stress on wheat plant productivity was stated as significant decreases in spike weight and 1000 grain wt. (g) from 4.38 g, and 42.7 g to 3.14 g, and 34.53 respectively, showing a decline by a ratio of 28.31% and 19.13% compared with the control plant. Meanwhile, different applications of MLE (10% and 20%) and zeatin (200 and 400 mg $L^{-1}$) significantly increased yield parameters compared with untreated controls either under tap water irrigation or salt stress irrigation water (Table 6). Data clearly show that higher levels of either MLE or zeatin were superior to lower concentrations in increasing the grain yield of wheat plants. Furthermore, treatment with MLE was superior to eating application.

**Effect of moringa leaf extract MLE or zeatin on yielded Grain protein and carbohydrates contents**

The results presented in Table 7 showed that salt stress significantly decreased the protein content and carbohydrate contents of the yielded wheat grains in comparison with control plants. Meanwhile, different treatments of MLE and zeatin with different concentrations caused significant increases in the protein content and carbohydrate contents of the yielded wheat grains compared to their corresponding untreated plants. Results stated higher levels of either MLE or zeatin were superior to lower concentrations in increasing protein content and carbohydrate contents of wheat plants. Furthermore, treatment with MLE was more effective than zeatin treatment.

## DISCUSSION

Under stress circumference, plants used most of their resources for enhancing defense methods overgrowth and development (Kolbert *et al*. 2012). According to earlier research, salt stress is detrimental to plants's growth because it adversely impacts the various physiological and metabolic functions of plants (Gowayed et al., 2017; Sadak, 2022). Herein, salinity stress substantially reduced wheat growth (Table 2). Ragaey *et al*. (2022) confirmed the decreasing impact of salt water on the growth parameters of wheat plants. These decreases may result from the reduction of cell expansion due to low turgor pressure under salinity stress. Increasing salt stress resulted in increased formation of inhibitors such as abscisic acid and reduced biosynthesis of plant promoters as cytokinin, as shown in Table 3 (Liu *et al.* 2020). Different levels of moringa leaf extract as a putative source of cytokinin and zeatin induced increases in wheat growth parameters under normal water or salt stress conditions (Table 2). These obtained readings are in good similarity with those obtained via Latif and

**Table 4.** Effect of moringa leaf extract (MLE1: 10% and MLE2: 20%) and zeatin (Z1: 200 mgL$^{-1}$ and Z2: 400 mgL$^{-1}$) on proline (µg/g fresh weight) total soluble sugars (TSS) (mg/g dry wt) and phenolics (mg/g fresh wt) of wheat plants under salinity stress. S0 = 0.23 dS m$^{-1}$ and S1 = 6.25 dS m$^{-1}$.

| Salinity | Treatment | Proline (µmole /g fwt) | TSS (mg/dry wt) | Phenolics (mg/g f wt) |
|---|---|---|---|---|
| S0 | 0 | 3.42h±0.185 | 11.24j±0.017 | 1.17h±0.053 |
| | MLE1 | 3.82g±0.111 | 15.28h±0.088 | 2.10g±0.120 |
| | MLE2 | 4.70e±0.010 | 16.60f±0.053 | 2.25f±0.160 |
| | Z1 | 3.44h±0.047 | 11.43i±0.017 | 2.13fg±0..150 |
| | Z2 | 4.37f±0.021 | 15.84g±0.01 | 2.47e±0.13 |
| S1 | 0 | 5.33d±0.349 | 17.93e±0.017 | 2.64d±0.140 |
| | MLE1 | 5.94c±0.022 | 19.83c±0.017 | 2.75cd±0.151 |
| | MLE2 | 8.67a±0.019 | 28.62a±0.348 | 3.09b±0.167 |
| | Z1 | 5.46d±0.081 | 18.60d±0.07 | 2.80c±0.404 |
| | Z2 | 6.97b±0.2 | 28.00b±0.035 | 3.99a±0.190 |
| | | 0.138 | 0.084 | 0.123 |

Data are means of three replicates ± SE; means with different letters within the same column are significantly different (p ≤ 0.05).

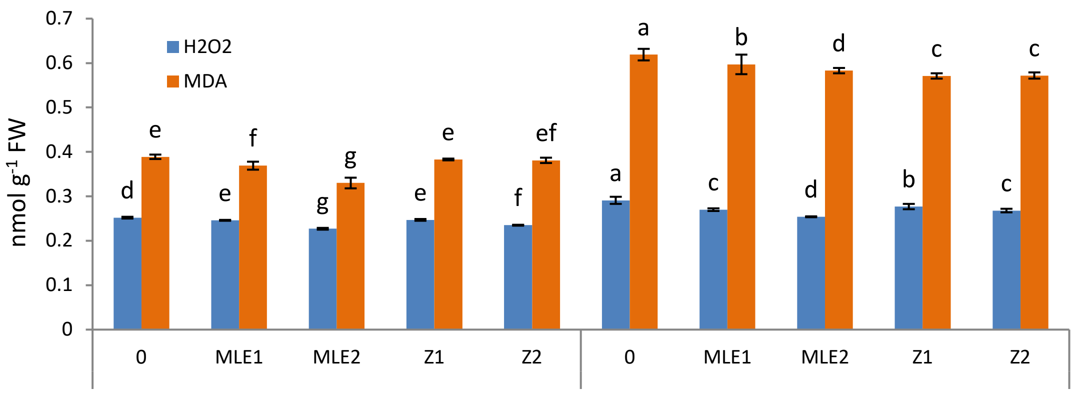


**Figure 2.** Effect of moringa leaves extract (MLE1: 10% and MLE2: 20%) and zeatin (Z1: 200 mgL$^{-1}$ and Z2: 400 mgL$^{-1}$) on hydrogen peroxide ($H_2O_2$) and malonaldehyde (MDA) (nmol g$^{-1}$ FW) of wheat plants under (S0 = 0.23 dS m$^{-1}$ and S1 = 6.25 dS m$^{-1}$) salinity levels.

**Table 5.** Effect of moringa leaf extract (MLE1: 10% and MLE2: 20%) and zeatin (Z1: 200 mgL$^{-1}$ and Z2: 400 mgL$^{-1}$) on antioxidant enzymes (Peroxidase POX, superoxide dismutase SOD, catalase CAT U/min/g Fresh wt) and antioxidant activity (DPPH%) of wheat plants under (S0 = 0.23 dS m$^{-1}$ and S1 = 6.25 dS m$^{-1}$) salinity levels.

| Salinity | Treatment | POX | SOD | CAT | DPPH |
|---|---|---|---|---|---|
| S0 | 0 | 61.5i±1.97 | 28.1g±1.20 | 26.8j±0.80 | 18.17j±1.155 |
| | MLE1 | 78.8g±0.00 | 32.9f±0.50 | 41.0h±0.93 | 22.00h±0.000 |
| | MLE2 | 84.7e±0.51 | 38.8c±0.20 | 50.9e±0.94 | 25.2f3±0.115 |
| | Z1 | 75.7h±0.27 | 34.3e±0.36 | 37.1i±0.77 | 21.33i±0.115 |
| | Z2 | 81.1f±0.10 | 37.1d±1.20 | 44.2g±3.22 | 24.13g±0.115 |
| S1 | 0 | 79.2g±0.59 | 33.9e±0.47 | 48.2f±1.00 | 32.23e±0.115 |
| | MLE1 | 91.9d±1.16 | 38.8c±1.17 | 62.3c±0.90 | 35.27c±0.115 |
| | MLE2 | 99.1a±1.48 | 43.3a±1.17 | 92.8a±0.61 | 40.70a±0.000 |
| | Z1 | 96.1c±0.83 | 36.7d±0.36 | 57.4d±0.89 | 34.53d±0.115 |
| | Z2 | 97.3b±1.00 | 42.1b±0.81 | 84.2b±1.07 | 39.50b±0.00 |

Data are means of three replicates ± SE; means with different letters within the same column are significantly different (p ≤ 0.05).

Mohamed (2017) on the common bean and Ahmed *et al*. (2021) on the wheat plant. Moringa leaf extracts are considered the most affordable and beneficial source of cytokinins, such as zeatin (Yang et al., 2006), while zeatin enhances the cell division process and chlorophyll biosynthesis, which finally increases wheat growth and development (Taiz and Zeiger, 2015). Sakhabutdinova et al. (2003) and Yasmeen et al. (2013) concluded that, by treating MLE, wheat plants achieved more growth by modifying their antioxidant defense pathway in salinity. Furthermore, the increases in shoot fresh and dry weight by MLE and zeatin treatments might result from seedling growth-induced dry matter accumulation in the elongating stem as well as stem weight (Ahmed et al., 2021). Additionally, that enhancement could be due to

**Table 6.** Effect of moringa leaf extract (MLE1: 10% and MLE2: 20%) and zeatin (Z1: 200 mgL$^{-1}$ and Z2: 400 mgL$^{-1}$) on yield and its components of wheat plants under (S0 = 0.23 dS m$^{-1}$ and S1 = 6.25 dS m$^{-1}$) salinity levels.

| Salinity | Treatment | Plant height (cm) | Spike length (cm) | Spikelet No/spike | Grains No/spike | Spike wt (g) | 1000 grains wt (g) |
|---|---|---|---|---|---|---|---|
| S0 | 0 | 58.7g±0.577 | 12.0e±0.000 | 16.3cd±2.309 | 16.7e±1.154 | 4.38d±0.029 | 42.7de±0.329 |
| | MLE1 | 69.3c±0.513 | 13.5c±1.000 | 18.3ab±2.309 | 19.3c±1.154 | 4.50c±0.022 | 47.53c±0.604 |
| | MLE2 | 75.7a±0.529 | 16.2a±0.577 | 19.0a±0.000 | 25.7a±1.155 | 5.87a±0.108 | 55.77a±0.361 |
| | Z1 | 67.5d±1.000 | 13.1d±0.231 | 18.3ab±2.309 | 19.0c±2.000 | 4.42cd±0.029 | 55.77a±0.361 |
| | Z2 | 74.7b±0.577 | 14.8b±0.577 | 19.0a±0.000 | 23.7b±1.155 | 5.36b±0.122 | 52.60b±0.441 |
| S1 | 0 | 50.2j±0.577 | 9.8g±0.577 | 12.3e±2.309 | 12.7g±1.155 | 3.14g±0.102 | 34.53h±0.211 |
| | MLE1 | 57.1h±0.231 | 11.8e±0.577 | 15.7d±2.309 | 14.7f±1.155 | 3.50f±0.129 | 38.72f±0.658 |
| | MLE2 | 62.3e±0.577 | 12.7de±0.577 | 17.7abc±2.309 | 18.3cd±1.154 | 3.83e±0.292 | 43.20d±0.476 |
| | Z1 | 54.7i±0.501 | 10.4f±0.653 | 13.7e±2.309 | 13.7fg±1.154 | 3.20g±0.168 | 36.65g±0.774 |
| | Z2 | 60.7f±0.577 | 12.2e±0.577 | 17.0bcd±0.00 | 17.3de±1.154 | 3.54f±1.208 | 41.61e±0.595 |

Data are means of three replicates ± SE; means with different letters within the same column are significantly different (p ≤ 0.05).

**Table 7.** Effect of moringa leaf extract (MLE1: 10% and MLE2: 20%) and zeatin (Z1: 200 mgL$^{-1}$ and Z2: 400 mgL$^{-1}$) on Grain protein% and grain carbohydrate% of wheat plants under S0 = 0.23 dS m$^{-1}$ and S1 = 6.25 dS m$^{-1}$) salinity levels.

| Salinity | Treatment | Protein (g/100 g dry wt) | carbohydrate% (g/100 g dry wt) |
|---|---|---|---|
| S0 | 0 | 10.55d±0.210 | 62.1d±0.236 |
| | MLE1 | 10.88c±0.009 | 64.0c±0.680 |
| | MLE2 | 11.44a±0.0.111 | 69.5a±1.162 |
| | Z1 | 10.85c±0.047 | 63.9c±0.707 |
| | Z2 | 11.05b±0.270 | 67.3b±0.235 |
| S1 | 0 | 8.95h±0.022 | 43.3i±0.136 |
| | MLE1 | 9.94f±0.084 | 47.8g±0.136 |
| | MLE2 | 10.2e6±0.006 | 58.3e±0.136 |
| | Z1 | 9.80g±168 | 45.7h±0.544 |
| | Z2 | 10.02f±0.010 | 57.4f±0.238 |

Data are means of three replicates ± SE; means with different letters within the same column are significantly different (p ≤ 0.05).

the high contents of vitamins, antioxidants, inorganic contents, and minerals in MLE. (Bakry et al., 2021). While the induced zeatin impact in control and salt conditions on plant growth might result from improved potentiality of photosynthesis and nutrient absorption (Farooq et al., 2019),

The previous data showed that salinity decreased levels of auxins, gibberellins, and cytokinins in wheat plants, while abscisic acid (ABA) content increased compared to unstressed control. The disruption of enzyme activities participating in phytohormone biosynthesis has an impact on their levels in plant cells (Vaseva-Gemisheva et al., 2005). Hence, disturbed hormonal equilibrium may be the cause of the decrease in plant growth induced by stressful circumstances. Meanwhile, the presented data in Table 3 stated increased levels of auxins, gibberellins, and cytokinins in response to MLE and zeatin, while decreasing ABA contents. Similar results were obtained by Abdalla (2013) and Latif and Mohamed (2016) on rocket and common bean plants, respectively. The promotive impact of MLE and zeatin might result from their stimulating impact on biosynthesis and/or reducing their breakdown. The promotive impact on auxin content might be achieved by decreasing its degradation by lowering IAA-oxodase, increasing the content of free IAA, and reducing the transformation of active free auxin to inactive form (Letham et al., 1978). Hopkins and Hünter (2004) stated that exogenous treatment of growth promoter has a potential impact on protecting cytokinins from cytokinin oxidase, and the exogenous treatment with zeatin might be converted into a stable cytokinins compound. Furthermore, MLE may promote earlier cytokinin biosynthesis (Rehman and Basra, 2010). Meanwhile, the decrease in ABA level might result from the shift of the precursor isopentenyl pyrophosphate to the formation of cytokinins and/or gibberellins instead of ABA (Hopkins and Hünter, 2004).

Total chlorophyll a, chlorophyll b, and carotenoids levels of wheat tissue were significantly reduced under salt stress conditions (Figure 1). The disorder of thylakoid membranes leads to the biosynthesis of proteolytic enzymes like chlorophyllase, causing chlorophyll degradation, damaging photosynthetic apparatus in plants (Rong-Hua et al., 2006), slowing down photosynthetic rate (Mafakheri et al., 2009), and hindering aggregated ions (Jaleel et al., 2008), thus leading to decreases in chlorophyll contents. Abd El-Hameid and Sadak (2020) and Sadak (2022) found that different photosynthetic pigment contents of sunflower and white termis plants were decreased under salinity stress levels. While exogenous treatment of wheat plants with zeatin or MLE increased photosynthetic pigment constituents (Fig. 1). The stimulative impact of MLE might be because MLE contains a considerable number of certain pigments due to the effectiveness of antioxidant properties such as chlorophyll and carotenoids (Owusu, 2008). Furthermore, MLE contains many macro-elements, like Mg (Yameogo et al., 2011), a component of Chlo. Moreover, the impact of zeatin on improving photosynthetic pigments might be due to chlorophyll breakdown retardation through inhibition of the chlorophyllase enzyme (Chernyad 2000).

Significant increases in different metabolites, such as total soluble sugars, proline, and phenolic levels, were observed in wheat subjected to salinity stress (Table 4). While osmotic adjustment activates the formation of different osmolytes like TSS (Ezzo et al., 2018) and proline (Sadak and Ahmed, 2016) to keep cell turgor and play various protective influences in different abiotic stresses (Wang et al., 2017), These increments of TSS and proline could help plant cells regulate the osmotic potential, leading to improved water uptake and translocation in salt conditions (Oraki et al., 2012). Furthermore, proline plays an important role in protecting different cell components and enzymes from oxidative destruction and acts as a scavenger of increased levels of free radicals. Proline has two techniques to reduce ROS: quenching singlet oxygen ($^1O_2$) and chemical interaction with OH radicals (Rady et al., 2015). Proline accumulation is considered a signal of stress, acting as an osmotic protective factor and helping in cell turgor stability (Lee et al., 2008). Additionally, the decrease in proline oxidase activity is responsible for the higher proline level. However, it is considered a C and N donor for rapid recovery and stabilization. The increases in TSS might be attributed to the high scientific and agricultural interest in TSS in plant cells. Furthermore, environmental stress encourages plants to respire more quickly, which is necessary to generate the ATP needed to activate stressed cells. They also release more osmotic-soluble sugars that lower the osmotic potential of cells and boost cell water intake (Khalil et al., 2012). Primary metabolites contribute to the creation of cellular structures and osmotic regulation. (Taiz and Zeiger., 2015). Phenolics are known as the main antioxidant scavengers of ROS due to their increased reactivity, like hydrogen or electron donors (Huang et al., 2005). The increased phenols due to salt stress could be caused by the disruption of various metabolic pathways (Keutgen and Pawelzik 2009). Treating wheat plants with either MLE or zeatin led to the accumulation of TSS, proline, and phenolic compounds in wheat leaves exceeding those of untreated plants in the two studied conditions (0.23 and 6.25 $dSm^{-1}$) (Table 4). Latif and Mohamed (2016), Pervez et al. (2017), and Bakry et al. (2021) confirmed the positive impact of MLE on common bean, maize, and flax plants by accumulating total soluble sugar levels.

Salt stress led to oxidative stress through elevated reactive oxygen species, such as hydrogen peroxide content in plant cells (Wang et al., 2016). $H_2O_2$ is one of these ROS molecules and is regarded as the primary ROS. Moreover, oxidative damage caused an imbalance between ROS formation and antioxidant defense in plant cells (Mittler, 2002). Excessive ROS causes cellular damage in the membrane via the oxidation of polyunsaturated fatty acids in the lipid bilayer, a process called lipid peroxidation. Malondialdehyde is an example of how oxidative damage is expressed. The increments in MDA may be caused by endogenous antioxidant's inability to completely scavenge all ROS generated by salt stress. Furthermore, MDA levels significantly increased under salinity conditions. This might be due to increased oxidative power in plants and the damaged structure of chloroplasts under salinity conditions (Singh et al., 2022). Plants evolved a variety of antioxidant systems to prevent the harm brought by oxidative stress. The primary antioxidant systems that deactivate and detoxify plant cells are POX, SOD, and CAT (Mushtaq et al., 2020). In our investigation, salt stress greatly improved those enzymes in wheat (Table 5). Plants must protect themselves from oxidative stress to alleviate the negative impacts of stress and help in scavenging ROS generated in plant cells (Sadak and Dawood, 2023). Thus, activation of those antioxidant enzymes under salinity conditions has a key impact on enhancing defense mechanisms through the decomposition of $H_2O_2$ into $H_2O$ (Zhu et al., 2004). Exogenous treatment of zeatin and MLE on non-stressed and salt-stressed wheat plants caused significant decreases in $H_2O_2$ and MDA contents, while improving different studied antioxidant enzymes (POX, SOD, and CAT). The obtained impact might be due to the fact the fact that MLE is a good source of enzymatic and non-enzymatic antioxidants as well as plant hormones, which enhance the defense system of plants (Khalofah et al*.,* 2020). Furthermore, moringa leaf extract and zeatin play a very important role in maintaining the oxidation equilibrium by decreasing $H_2O_2$ and MDA contents. Moreover, MLE has one of the most important antioxidant mechanisms (Kerdsomboon et al*.,* 2016). MLE extract as well as zeatin treatment might synthesize other substances that move and transport between cells and decrease ROS oxidative power to protect plants against stress (Cruz de Carvalho, 2008).

Our obtained results revealed that salt stress induced significant yield feature decreases in the tested plant compared to the control plant (Table 6). Those decreases might be due to growth (Table 2) and photosynthetic pigment reductions (Fig. 1). Additionally, the decreases in chlorophylls led to decreased photosynthesis activity, less carbohydrate synthesis, and reduced translocation from leaves to seeds (Anjum et al*.,* 2003). Salinity stress negatively impacted enzymatic activities, which decreased growth and yield attributes (Yap et al*.,* 2021). Furthermore, Table 7 shows that 6.25 $dSm^{-1}$ salt water caused significant decreases in protein and carbohydrate percentages. Sadak (2016), Bakhoum and Sadak (2016), Abd El-Hameid and Sadak (2020), and Ragaey *et al.* (2022) confirmed those data. While MLE and zeatin treatment caused improvements in different yield attributes as well as protein and carbohydrate contents of the grains (Tables 6 and 7). Those data are like the data of Zulfiqar *et al.* (2020) and Ngcobo *et al.* (2021). The positive effect of either MLE or zeatin treatment on yield and its parameters, protein and carbohydrate levels of the grains might be because moringa leaf extract is considered the most affordable and beneficial source of cytokinins in the form of zeatin (Yang et al*.,* 2006), while zeatin affects increasing cellular division in the apical meristem and chlorophyll biosynthesis, ultimately increasing plant growth traits and yield (Taiz and Zeiger, 2015).

## CONCLUSIONS

This investigation stated that irrigation of wheat plants with 6.25 $dSm^{-1}$ salt water reduced growth and yield parameters of wheat. Furthermore, treatment with MLE and zeatin at various levels improved the growth and yield attributes of wheat plants under tap water irrigation conditions. Meanwhile, under saline conditions, exogenous application of MLE and zeatin increased the growth and yield of wheat plant. Foliar treatment of MLE and zeatin increased phytohormones such as auxins, gibberellins, and cytokinins, photosynthetic pigment components in the leaves, chlorophylls A and B, total chlorophylls, carotenoids, phenolics, proline, total soluble sugars, and the antioxidant activity of some enzymes such as peroxidase, superoxide dismutase, and catalase. On the other hand, exogenous treatment with MLE and zeatin decreased hydrogen peroxide and lipid peroxidation as MDA contents. These results demonstrate that MLE and zeatin activate the enzymatic defense mechanism and osmoprotectant contents of wheat plants. Treatment with MLE and zeatin increased the protein and carbohydrates contents of the yielded grains, both with and without saline stress. So, MLE and zeatin can be used in wheat plants to induce tolerance to salt stress.

## LIST OF ABBREVIATIONS

FAO: Food and Agriculture Organization
ROS: Reactive oxygen species
$\cdot O_2$: Singlet oxygen
$O_2^{\cdot -}$ : superoxide radical
$OH^-$: hydroxyl radical
$H_2O_2$: hydrogen peroxide
POX: peroxidase
SOD: superoxide dismutase
CAT: catalase
MLE: moringa leaves extract
PGRs: Plant growth regulators
IAA: indole acetic acid
GA: gibberellins
ABA: abscisic acid
MDA: malondialdehyde

## STATEMENTS AND DECLARATIONS

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**Author´s Contributions:** SRE, designed and performed the experiment, responsible of physiological analysis of the manuscript and wrote and reviewed the manuscript, MSS, designed and performed the experiment, responsible of physiological analysis of the manuscript responsible of statistical analysis and also wrote and reviewed the manuscript. RSH, designed and performed the experiment, responsible of physiological analysis of the manuscript and wrote and reviewed the manuscript All authors have read and agreed to the published version of the manuscript.

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