

Research Article

Response of Wheat (*Triticum aestivum* L.) to Zinc Sulphate and Copper Sulphate under salt stress

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Abstract

Salt stress interrupts physiological and biochemical behaviors of plants by lowering the osmotic potential. The seed priming is an important technique which enhance the growth and yield by reducing the salt stress. The recent study was conducted to improve the salt tolerance in wheat plants. The seeds of wheat were primed with two concentrations (100ppm & 200ppm) of ZnSO₄ and CuSO₄ and then seed were grown under NaCl stress in both experiments i.e.; Invitro and Pot experiment. The results showed that seed priming with both ZnSO₄ and CuSO₄ increased the growth of wheat plants more than control under salt stress. The antioxidant enzymes (CAT, POD, SOD) are increased under salt stress. The seed priming with ZnSO₄ improved the growth and antioxidant enzymes activities more than CuSO₄ under all levels of salt stress.

Keywords; Antioxidant enzymes; NaCl; Photosynthetic pigments; Salinity; Seed priming

Introduction

Wheat (*Triticum aestivum* L.) is most important crop in Pakistan, which plays essential role in people's nutrition. The abiotic stress like salinity is the major cause which decreased the growth and yield of wheat by reducing water uptake and ionic imbalance ion toxicity.

Salinity proves more injurious factor in both arid and semi-arid regions. More than 800 million hectares land is salt affected. equating to more than 6% of the world's total land area. Salt stress is the major issue in Pakistan which decreased the plants growth and productivity. Salinity decreased the plants physiological

and biochemical behaviors of plants by lowering the osmotic potential as reported in previous literature by [1]. Plants have improved complex mechanisms systems for adaptation to osmotic and ionic stress caused by high salinity, under the salt stress. The adaptation is generally associated with osmoregulation adjustment by using some osmotic regulators, such as potassium, soluble sugar, proline and betaine [2]. Salt stress cause the production of reactive oxygen species (ROS) and which cause damage to cell by biotic and abiotic stresses [3]. However, the plants have evolved the protective mechanisms to reduce the ROS. Which are

effective to ameliorate the harmful effects of various levels of stress induced deterioration [4]. The antioxidant enzymes system is most important protective mechanisms including superoxide dismutase. However, the micronutrients have the potential to reduce the salinity. The most well-known strategies for micronutrient application are foliar application, seed treatment and soil application. The nutripriming is a basic priming strategy in which seeds are absorbed soaked in aerated solution of nutrients [1]. Copper and zinc are most abundant heavy metals in agriculture soils. Both heavy metals are involved in cellular metabolism of plants. Zinc and copper, mostly zinc is present in various protein [8]. Both are biological active and highly toxic at high concentration. Toxic effects of heavy metals control the germination and growth of seedlings [9]. Toxic effects of metals depend upon the species of plants as they showed genetic variations in their capability to tolerate the concentration of specific heavy metals [10].

Table 1. The detail of treatments

Treatments	Abbreviations
Control	T ₀
ZnSO ₄ 100ppm	T ₁
ZnSO ₄ 200ppm	T ₂
CuSO ₄ 100ppm	T ₃
CuSO ₄ 200ppm	T ₄

Chlorophyll (a & b) determination

The protocol of [14] and the following equation were used to calculate the chlorophyll a and chlorophyll b.

$$\text{Chl a} = 12.7 \times \text{OD}_{663} - 2.69 \times \text{OD}_{645}$$

$$\text{Chl b} = 22.9 \times \text{OD}_{645} - 4.68 \times \text{OD}_{663}$$

Antioxidant enzymes

The method of Beauchamp and Fridovich [11] was followed to determine the Superoxide dismutase (SOD) activity, protocol of [12] for Peroxidase (POD) activity and method of [13] was used for estimation of Catalase activity.

The recent study was conducted with the aim to investigate the effect of ZnSO₄ and CuSO₄ on growth, biochemical aspects and antioxidant enzymes activities under salt stress.

Materials and methods

Collection of seeds

Seeds of wheat (cv. Punjab-11) were collected from Ayub Agricultural Research Institute Faisalabad.

Experimental design

The surface of wheat seeds was sterilized with 70% ethanol and then seeds were washed with distilled water. The seeds were primed with following treatments mentioned in table 1 for one hour. The primed seeds were used for both experiments i.e.; invitro and Pot experiment. The seeds were grown under salt stress concentrations 0mM, 50mM, 75mM and 100mM. The length of shoot, root was measured with simple ruler, while the fresh and dry weight of root, shoot was measured with electronic measuring balance.

Statistical analysis

The findings were described as the mean of three replicates. The Analysis of variance (ANOVA) with two way completely randomized design was done by using the CoStat statistical program (CoHort software 1988).

Results

The findings of recent study showed that salt stress badly affected the germination percentage and the germination percentage of wheat plants reduced significantly ($P < 0.05$)

with the increase of salt stress. However, the nutripriming enhanced the germination percentage. The comparison of both concentrations (100ppm & 200ppm) showed that ZnSO₄ and CuSO₄ both enhanced the germination percentage at 100ppm. While the nutripriming with ZnSO₄ significantly (P<0.05) improved the germination percentage more than CuSO₄. The significant maximum germination percentage (95.23±4.26) were recorded at ZnSO₄ under 0 mM stress (Table 2).

The (Fig. 1) showed that shoot length was strongly affected by salinity. The shoot length

significantly (P<0.05) reduced under salt stress but nutripriming showed better result as compared to control. The nutripriming with ZnSO₄ at 200ppm improved the shoot length more than 100ppm. While nutripriming with CuSO₄ at 100ppm improved the shoot length. The comparison of both priming treatments showed that ZnSO₄ at 200 ppm improved the shoot length more than control and priming with CuSO₄. The significant (P<0.05) maximum shoot length (7.36±0.12) was observed with ZnSO₄ at 200ppm under 50mM NaCl stress.

Table 2. Effect of ZnSO₄ and CuSO₄ on germination percentage of wheat under NaCl stress (n=3, mean ± SE)

Treatments	0 mM	50 mM	75 mM	100 mM
Control	a90.47±4.76	b85.71±4.76	b76.18± 0.00	b71.42±4.76
ZnSO ₄ 100ppm	a95.23± 4.76	a90.47± 0.00	a85.71± 4.76	a76.18±4.76
ZnSO ₄ 200ppm	a90.47±0.00	a90.47± 4.76	ab80.94 ±8.25	b71.42±8.25
CuSO ₄ 100ppm	b85.71±4.25	bb80.94±	ab80.94±0.00	b71.42±8.25
CuSO ₄ 200ppm	b80.94±0.00	c76.18±	c71.42±8.25	c66.67±0.00

The (Fig.1) demonstrated that fresh weight of shoot was badly affected by salt stress. The ANOVA showed that shoot fresh weight significantly (P<0.05) reduced under salt stress. However, the nutripriming improved the fresh biomass of shoot under NaCl stress. The comparison of both (100ppm & 200ppm) priming concentration showed that ZnSO₄ at 100ppm showed best results while CuSO₄ showed more promotion to shoot fresh biomass at 200ppm. The comparison of both (ZnSO₄ & CuSO₄) showed that priming with ZnSO₄ increased shoot fresh biomass more than CuSO₄. The significant maximum value of fresh biomass (0.182±0.06) was found with ZnSO₄ at 100ppm under 50mM NaCl stress. The result mentioned in (Fig.1) showed that salinity strongly affected the dry biomass of shoot. The dry biomass of shoot significantly (P<0.05) reduced under salt stress but nutripriming showed better result as

compared to control. The nutripriming with ZnSO₄ at 100ppm improved this parameter more than 200ppm. While nutripriming with CuSO₄ at 200ppm improved the dry biomass of shoot. The comparison of both (ZnSO₄ & CuSO₄) showed that priming with ZnSO₄ increased dry biomass of shoot more than CuSO₄.

The (Fig.1) demonstrated that root length was badly affected by salt stress. The ANOVA showed that root length significantly (P<0.05) reduced under salt stress. However, the nutripriming significantly (P<0.05) improved the root length under NaCl stress. The comparison of both (100ppm & 200ppm) priming concentration showed that both ZnSO₄ & CuSO₄ showed more promotion to root length at 100ppm than control plants. The comparison of both (ZnSO₄ & CuSO₄) with control plants showed that priming with ZnSO₄ increased root length more than

CuSO₄ under all levels of salt stress. The significant maximum value of root length (4.20 ± 0.18) was found with ZnSO₄ at 100ppm under 0mM NaCl stress.

The results mentioned in (Fig.1) showed that fresh weight of root was significantly ($P < 0.05$) reduced under salt stress. However, the nutripriming improved the fresh biomass of root under NaCl stress. The comparison of both (100ppm & 200ppm) priming concentration showed that ZnSO₄ at 200ppm improved fresh biomass of shoot while CuSO₄ showed more promotion to root fresh biomass at 100ppm. The comparison of both (ZnSO₄ & CuSO₄) showed that priming with ZnSO₄ increased root fresh biomass more than CuSO₄ under all levels of salt stress. The significant maximum value of fresh biomass of root (0.032 ± 0.008) was found with ZnSO₄ at 100ppm under 0mM NaCl stress.

The result mentioned in (Fig.1) showed that dry biomass of root significantly ($P < 0.05$) reduced under salt stress but nutripriming showed better result as compared to control. The comparison of both (100ppm & 200ppm) priming concentration showed that both ZnSO₄ & CuSO₄ showed more promotion to root fresh biomass at 100ppm. The comparison of both (ZnSO₄ & CuSO₄) showed that priming with ZnSO₄ increased root fresh biomass more than CuSO₄ under all levels of salt stress. While nutripriming with CuSO₄ at 200ppm improve the dry biomass of root. The comparison of both (ZnSO₄ & CuSO₄) showed that priming with ZnSO₄ increased the fresh biomass of root more than CuSO₄ under all levels of salt stress.

The (Fig. 2) showed that salt stress strongly reduced the chlorophyll a content. The ANOVA showed that Chlorophyll a significantly ($P < 0.05$) reduced under salt stress. However, the nutripriming significantly ($P < 0.05$) improved the chlorophyll a under NaCl stress. The comparison of both (100ppm & 200ppm) priming concentration showed that both

ZnSO₄ & CuSO₄ showed more promotion to chlorophyll a at 100ppm. Overall priming with ZnSO₄ increased this parameter more than CuSO₄ under various levels (0-50-75-100 mM) of salt stress.

The findings of the recent study as mentioned in (Fig. 2) showed that under increasing levels of NaCl the chlorophyll b contents strongly reduced but nutripriming increased this parameter. The chlorophyll b contents in the leaves of plants emerged from primed seed with ZnSO₄ at 200ppm were significantly higher than 100ppm under all levels of NaCl stress but exception was found at 0mM NaCl where 100ppm showed best results. The significant maximum value of chlorophyll b contents was found at 200ppm under 50mM NaCl stress. While in case of CuSO₄ the comparison of both (100ppm & 200ppm) concentrations showed that 100ppm enhanced the chlorophyll contents more than 200ppm. Overall, the plants emerged from primed seeds with ZnSO₄ showed more chlorophyll b contents than CuSO₄ under salt stress.

The carotenoids contents were inversely proportional to salt stress. As under the increasing levels of salt stress the carotenoids contents decreased. The plants emerged from primed seed with ZnSO₄ at 100ppm showed significantly ($P < 0.05$) higher carotenoids contents than 200ppm under salt stress. The significant ($P < 0.05$) maximum value of carotenoids was found at 100ppm of ZnSO₄ under 0mM NaCl stress. Similar results were found in case of priming with CuSO₄. However, plants emerged from primed seeds with ZnSO₄ showed more carotenoids contents under all levels of salt stress (Fig. 2). The findings of recent study showed that under stress condition the catalase activity is increased in wheat plants. The both (ZnSO₄ & CuSO₄) primed treatments showed similar trends. The comparison of both concentrations (100ppm & 200ppm) showed that 100ppm proved beneficial and enhanced the CAT activity under various levels (0-50-

75-100 mM) of salt stress. The significantly ($P < 0.05$) maximum values were found at 100mM of $ZnSO_4$ under NaCl stress.

The findings of the recent study showed that peroxidase activity is directly proportional to salt stress. The (Fig. 2) showed that under stress condition the POD activity was increased in wheat plants. The POD activity in the leaves of plants emerged from primed seeds with $ZnSO_4$ and $CuSO_4$ at 100ppm showed more promotion to the POD activity than 200ppm. The significant ($P < 0.05$) maximum POD was observed in the leaves of plants emerged from primed seeds with $ZnSO_4$ at 100ppm under 120mM NaCl stress. While the comparison of both ($ZnSO_4$ & $CuSO_4$) priming treatments showed that plants grown from primed seeds with $ZnSO_4$ at both (100ppm & 200ppm) concentrations showed peroxidase activity higher than $CuSO_4$ under all levels of NaCl stress.

The ANOVA showed that under stress condition the Superoxide dismutase activity significantly ($P < 0.05$) increased in wheat plants. The both ($ZnSO_4$ & $CuSO_4$) primed treatments showed similar trends. The comparison of both concentrations (100ppm & 200ppm) showed that 100ppm proved beneficial and enhanced the SOD activity under various levels (0-50-75-100 mM) of salt stress. The significantly ($P < 0.05$) maximum values were found at 120mM NaCl stress. Moreover, nutripriming with $ZnSO_4$ showed more SOD activity than $CuSO_4$ under all levels of salt stress.

Discussion

The findings of recent study showed that germination percentage reduced under salt stress. However, nutripriming enhanced the germination percentage. Our results are in conformity with [8, 15]. Seeds germination includes activation or formation of enzyme systems, absorption of water, establishment or growth of seedlings. Due to salinity all these processes are badly affected [1]. Growth of young seedlings and germination

percentages decreased at higher concentration of salt in Beet root [5]. Main causes of salinity are limited water and hot dry climate in arid and semi-arid regions that reduces the crop production and it is also observed that germination is different among different crops or in different varieties of the same crop [6]. The differences in germination and seedling vigor among cultivars cause emergence variability [7].

The recent study showed that shoot length of wheat is significantly decreased under salt stress. Our findings are in conformity with [8, 15] they reported that higher concentrations of salt stress reduced the shoot length but seed priming with $ZnSO_4$ improved the shoot length. The recent findings are also in conformity with previously reported by [16, 17] they noted that stem length of hot pepper seedlings is increased by halo-priming and comprising. [18] also reported the reduction in shoot length of common beans under salt stress. This study shows the poor response of wheat to higher concentrations of copper sulphate. Growth parameters with Zn increased, while Cu has injurious effects on growth parameters as reported by [19]. The Zn is used as essential micronutrient to improve grain yield throughout the world [20].

In recent study the fresh and dry biomass of shoot strongly reduced. The higher levels of salt stress cause reduction in shoot length and in return fresh and dry weigh of shoot. Our findings are in consistence with [1, 21, 22]. However, the nutripriming with $ZnSO_4$ increased the fresh and dry biomass of shoot more than $CuSO_4$. The zinc sulphate plays essential role to mitigate the harmful effect of drought stress [23].

The roots are more affected by salt stress than shoot as roots are directly connected to soil. The findings of recent study showed that root length is more affected than shoot length because roots absorbs water from soil and then shoots empower supply in whole plant.

Due to this reason shoot and root length control critical indications of a plant's response to salt stress. These findings are correlated with [20, 24] on chickpea and wheat plants. The nutripriming with ZnSO₄ and CuSO₄ increased the root length under salt stress and similar findings were also reported by [1, 8, 15]. The recent study showed that Zn is an essential micronutrient that is important for plant growth. The plants treated with ZnSO₄ were less sensitive to salt stress. Similar findings were also reported by [15]. The higher levels of salinity may inhibit the root length by reduced uptake of water by plants. The salt stress inhibits root and shoot elongation by lowering water and essential mineral nutrients uptake from soil as reported in previous literature by [25, 26]. In the recent study the fresh and dry biomass of root significantly reduced under salt stress. Our findings are in conformity with previous reported by [8, 27].

The seedlings fresh biomass of shoot is more affected by salt stress as compared to fresh biomass of roots. Our findings are in consistence with previously reported in sugar beet, cabbage, amaranth and pak-choi [6]. The nutripriming with ZnSO₄ and CuSO₄ improved fresh and dry biomass of root under salt stress but ZnSO₄ showed more promotion to this parameter than CuSO₄ under stress. Our results are in conformity with previous findings reported in Oat by [8] and in *Brassica rapa* by [15]. The micronutrient like Zn activates the various metabolic enzymes in roots and plant body [28].

The chlorophyll (a & b) contents are directly affected by salt stress. The decreased in chlorophyll contents under salinity stress is might be because of injurious effects of accumulated (Na⁺ & Cl⁻) on biosynthesis of chloroplast structure. Salinity influences and causes swelling of membrane in chloroplast

and thus effects chlorophyll and excess ion in leaves induces loss of chlorophyll, as reported by [29]. While nutripriming with ZnSO₄ and CuSO₄ improved the chlorophyll contents in Wheat under salt stress. However, treatments of ZnSO₄ promoted the chlorophyll contents more than CuSO₄. The higher doses of CuSO₄ also lowers the chlorophyll contents. As The higher concentration of copper causes the determinantal effects such as pigment synthesis, damage to plasma membrane, permeability and inhibition of photosynthesis [30]. The positive effects of Zn to improve chlorophylls were also reported by [31, 33]. The recent study showed that carotenoids contents are directly proportional to salt stress. As the salt stress increases the carotenoids contents decreased. Our findings are in conformity with [33, 34].

The findings of recent study indicated that antioxidant enzymes activities like (CAT, POD, SOD) increased under salt stress. Our results are in conformity with the previous findings of [30, 35], who reported that the antioxidant enzymes like (SOD, CAT and POD) increased in wheat under salt stress. The CAT activity has the important role in the conversion of H₂O₂ to H₂O in the peroxisomes. In peroxisomes the H₂O₂ is produced from photorespiration and from β -oxidation of fatty acids. The higher activities of CAT reduced the H₂O₂ in cell and increase the membrane stability and carbon dioxide fixation due to many enzymes of Calvin cycle within the chloroplasts are sensitive to H₂O₂. As higher concentration of H₂O₂ directly inhibits CO₂ fixation [36].

However, in recent study the nutripriming with ZnSO₄ also improved the salt tolerance by increasing the reactive oxygen species scavenging and reduced the accumulation of Na⁺ in wheat. The similar findings were also reported by [37].

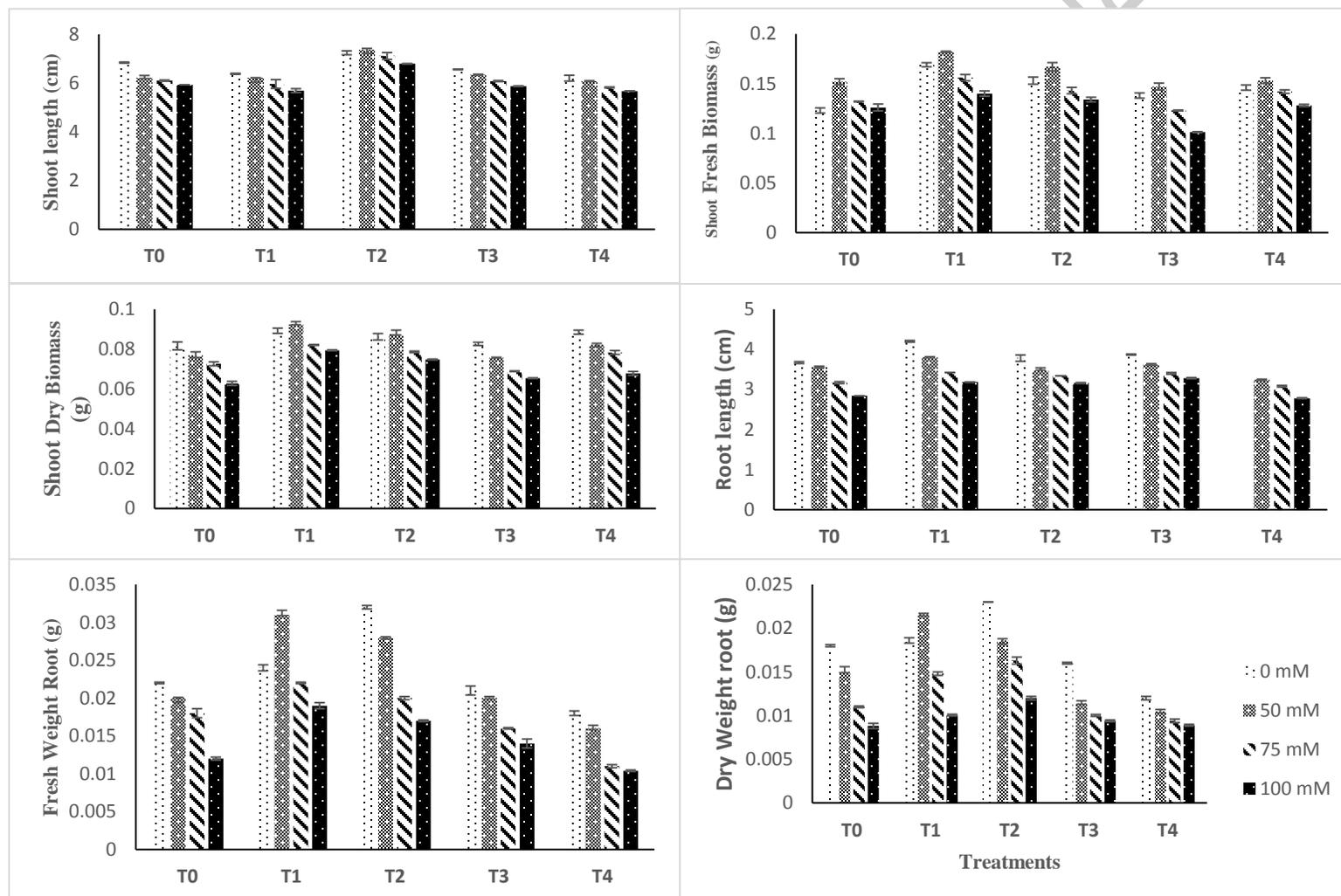


Figure 1. Effect of ZnSO₄ and CuSO₄ on Growth parameters (A) Shoot length (B) Fresh Biomass of Shoot (C) Dry Biomass of Shoot (D) Root length (E) Fresh weight of Root (F) Dry weight of Root

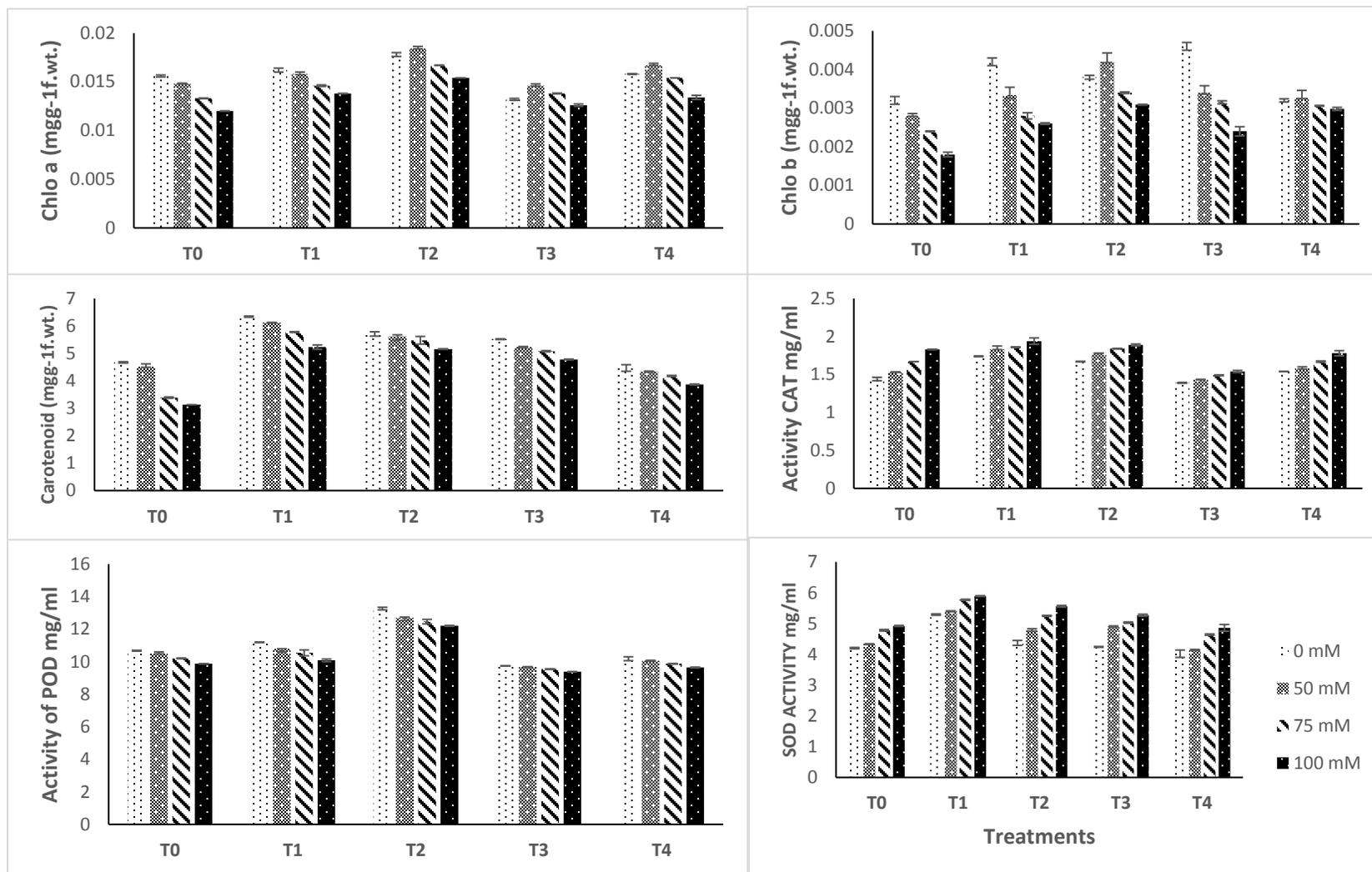


Figure 2. Effect of ZnSO₄ and CuSO₄ on (A) Chlorophyll a (B) Chlorophyll b (C) Carotenoids (D) CAT (E) POD (F) SOD

The nutripriming with ZnSO₄ and CuSO₄ improved and increased the antioxidant enzymes activities under salt stress. However, seed priming with excess of Zn has significantly increased the antioxidant enzymes. Our results are positively correlated with the previous findings of [38, 39].

In the recent study the SOD activity reduced under excess of Zn. Our results are in conformity with the previous findings of [40, 41]. In wheat, the expression and activity of the Cu/Zn SOD is dependent on Zn-efficiency of individual genotypes, as reported by [42]. The POD activity under stress can be a useful tool to reduce environmental stress and in wheat the increased POD activity is consistent with the previous findings of [43].

Conclusion

Salt stress negatively influenced the growth of *Triticum aestivum* L. but antioxidant enzymes (CAT, POD, SOD) increased due to salt stress. The nutripriming with ZnSO₄ and CuSO₄ improved the growth parameters (shoot, root length, root, shoot fresh weight and root, shoot dry weight) and biochemical aspects (chlorophyll a & b) under salt stress. The nutripriming also improved the antioxidant enzymes under salt stress. Nutripriming is important technique and can be used in future to increase growth and yield of wheat.

Authors' contributions

Conceived and designed the experiment work: RA Khan, Performed the experiments and wrote article: RA Khan & TA Qadri, Analyzed the data and helped in experiments: M Iftikhar & A Khan.

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