

## The developmental and metabolic effects of different magnesium doses in pepper plants under salt stress

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### Abstract

Morphological and biochemical effects of different magnesium (Mg) doses on pepper plants under salt stress were investigated in this study. Experiments were conducted under controlled conditions of a climate cabin at 25 °C temperature, 70% relative humidity and 16/8 hours light/dark photoperiod. The developmental and metabolic effects of different magnesium doses in plants under salt stress were investigated by examining leaf antioxidant enzyme activities, Malondialdehyde (MDA) levels and chlorophyll contents. Seedlings of 'Demre' pepper cultivar (*Capsicum annum* L. cv. 'Demre') were grown in Hoagland nutrient solution supplemented with 100 mM NaCl to generate salt stress. Besides salt treatments, different Mg doses (Mg 1 = 24.64 ppm, Mg 2 = 49.28 ppm, Mg 3 = 73.92 ppm, Mg 4 = 98.56 ppm, Mg 5 = 123.20 ppm) were applied to plants. On the 20<sup>th</sup> day of salt treatments, the total weight of the plants which is one of the growths and development parameters of pepper plants was measured, and plant samples were taken for analyses. A slight increase was observed in total weights of salt-treated plants with increasing Mg doses. The greatest plant weight was obtained from Mg 4 + salt treatments. It was observed that increasing Mg doses had positive effects on the development of plants under salt stress. Chlorophyll contents and antioxidant enzymes activities increased and MDA (malondialdehyde) levels, the product of lipid peroxidation, which indicates the amount of damage to plant cells, decreased with increasing Mg doses. Present measurements and analyses and resultant findings revealed that Mg treatments at increasing doses partially alleviated negative effects of salt stress on pepper seedlings.

**Keywords:** antioxidant enzyme activity; magnesium; oxidative stress; pepper (*Capsicum annum* L.); salt stress

### Introduction

Salt stress is an abiotic factor negatively influencing various metabolic processes of plants, reducing yield and quality of culture plants. Pepper is largely grown in open fields and greenhouses and salt stress has serious negative impacts also on pepper plants. Salinity is more effective in undercover production sites than open fields. Even in soilless cultures, low quality water resulted in salinity problems (Oztekin and Tuzel, 2011).

Salinity generally results in reduction in number of leaves and leaf sizes, plant heights, differentiation in tissues and organs, suppression of plant growth and development, unbalanced root and shoot weights, thus weak root system and recessed plant root and plant growth. Salinity also reduces plant fresh and dry biomass, chlorophyll content, fruit size and ultimately yield levels (Yu *et al.*, 2012). Salt stress recesses plant growth and development, slows down the photosynthetic activity, increases reactive oxygen species, decreases plant water potential, results in ion unbalance and stomal closure (Bartels and Sunkar, 2005; Yasar *et al.*, 2006a; Mahajan *et al.*, 2008; Yildirim *et al.*, 2008). As explained by Yasar (2003), stress conditions promote the synthesis of free radicals, which in turn damage plant cells and limit photosynthetic activity. Oxygen free radicals especially damage number of cellular components, including proteins, membrane lipids, nucleic acids and chlorophyll amount (Fridovich, 1986; Davies, 1987; Yasar *et al.*, 2006b; Yasar *et al.*, 2014). As it was in the other living organism, free radical levels of the plants are controlled by antioxidants and antioxidant enzyme activities, which convert oxygen free radicals into harmless compounds. A stressed plant will typically become more resistant to oxidative damage, and chloroplasts, which generate oxygen during photosynthesis, constitute key components of antioxidant defense systems that protect plants from destructions of toxic oxygen derivatives. The most effective enzymes that eliminate free oxygen radicals include superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), and catalase (CAT) (Cakmak and Manschner, 1992; Cakmak, 1994; Gosset *et al.*, 1994). Significant correlations were reported in previous studies between antioxidant activity and stress tolerance.

The adverse effect of high NaCl on chlorophyll concentration was previously shown in Yildirim *et al.* (2008). Magnesium ions are found in the center of chlorophyll molecules. Chlorophyll is a key component of the reaction of photosynthesis, which produces energy for growth. Mg ions are therefore essential components of photosynthesis. Magnesium also plays a substantial role in phosphorus transport in the plant; it assists in phosphate metabolism, plant respiration, protein synthesis, and activation of several enzyme systems (Marschner, 1995). It was shown that salt stress reduced Mg take of plants (Yildirim *et al.*, 2008). The application of KNO<sub>3</sub>, Mg (NO<sub>3</sub>)<sub>2</sub> and Ca (NO<sub>3</sub>)<sub>2</sub> significantly improved chlorophyll content (Yildirim *et al.*, 2009). Foliar applications of these elements could thus increase the chlorophyll content of plants under salt stress (Yildirim *et al.*, 2009).

Salt stress reduced leaf relative water content (LRWC) of plants as compared to non-salt stress treatments; plant LRWC was reduced by 15% at 40 mM NaCl. Such reductions were measured as 8.0% with 10 mM KNO<sub>3</sub>, 8.2% with Ca (NO<sub>3</sub>)<sub>2</sub> and 4.8% with Mg (NO<sub>3</sub>)<sub>2</sub> treatments (Yildirim *et al.*, 2008). It indicates a loss of turgor resulting in limited water availability for the cell-extension process. The application of KNO<sub>3</sub>, Mg (NO<sub>3</sub>)<sub>2</sub> and Ca (NO<sub>3</sub>)<sub>2</sub> significantly improved this parameter.

Yildirim *et al.* (2009) also explained that salinity reduced plant macro and micronutrient contents except for Na and Cl content of plant shoots and roots. The foliar application of KNO<sub>3</sub>, Mg (NO<sub>3</sub>)<sub>2</sub> and Ca (NO<sub>3</sub>)<sub>2</sub> increased N, K, Mg, Ca, S and P contents under salinity stress. It is not surprising that supplementary KNO<sub>3</sub>, Mg (NO<sub>3</sub>)<sub>2</sub> and Ca (NO<sub>3</sub>)<sub>2</sub> enhanced concentrations of N, K, Ca, and Mg; however, contents of these elements in plants receiving supplementary KNO<sub>3</sub>, Mg (NO<sub>3</sub>)<sub>2</sub>, Ca (NO<sub>3</sub>)<sub>2</sub> were still much lower than those of non-salt stress treatments. Plant inorganic ions were negatively related to salt doses. From the results of this experiment, it can be concluded that NO<sub>3</sub><sup>-</sup> with K, Ca and Mg counteracted the deleterious effects of salinity stress on the investigated parameters, helped the strawberry plants to avoid Na toxicity and improved cell membrane stability and nutrient uptake under salinity stress. Improvement of plant growth, water status of salt-stressed strawberry plants makes it possible to recommend the treatment of plants grown under saline conditions with the above chemicals.

Pepper (*Capsicum annuum* L.) is moderately tolerant to salt stress. Threshold salinity levels for pepper were reported as 1.0-1.5 dS/m and a salinity level of EC=3.4 dS/m may result in about 50% yield loss (Ayers, 1977). Salt resistant species and cultivars should be developed to minimize yield and quality losses or treatments reducing adverse effects of salinity on plants should be used or experimented.

Magnesium works as an activator of many enzymes involved in energy transfer and growth processes; it is also a key component of chlorophyll and is thus essential for photosynthesis (Bansal, 1989). Mg deficiency primarily affects the metabolism of carbohydrate resulting in reduced plant growth and a decreased transport rate of carbohydrates to sink organs, as reported by Gopikumar and Varghese (2004).

Based on this information, in this study, effects of magnesium (Mg) on antioxidative enzymes, chlorophyll and malondialdehyde (MDA) contents of pepper plants under salt stress were investigated. Besides salt treatments, Mg was applied at different doses and effects of Mg treatments under salt stress on plant salt tolerance were investigated.

## Materials and Methods

'Demre' pepper cultivar (*Capsicum annuum* L. cv. 'Demre') was used as the plant material of this study. Experiments were conducted in a climate cabin with split-air conditioner providing normal atmospheric conditions. Pepper seeds were sown in plastic germination cups filled with pumice and peat soil and irrigated with tap water. Seeds germination cups were placed into a climate cabin at  $25\pm 1$  °C temperature and 70% relative humidity. When the germinated seedlings had horizontal cotyledon leaves and the first true leaves, irrigations were initiated with the Hoagland nutrient solution (Table 1) (Hoagland and Arnon, 1938). The seedlings with the 2nd true leaves were surrounded with sponges and transplanted into aqua culture in plastic trays (25×25×18 cm) filled with nutrient solution. Aeration was provided with an aquarium pump. Seedlings were grown in aquaculture until they had 4-5 true leaves, then salt treatments were initiated. For salt-treated seedlings, NaCl was supplemented into nutrient solution (1/2 Hoagland) as to have 100 mM salt concentration. Nutrient solutions were replenished every week as to sustain the same salt concentration. Mg ratios of Hoagland nutrient solution in which salt-treated seedlings were grown were altered through MgSO<sub>4</sub> supplementations at 5 different doses (Mg 1=24.64 ppm, Mg 2 =49.28 ppm, Mg 3 = 73.92 ppm, Mg 4 = 98.56 ppm, Mg 5 = 123.20 ppm). Control plants were grown in standard Hoagland nutrient solution with salt treatments.

**Table 1.** Contents of the nutrient solution used (ppm)

Elements	App. 1 Control (ppm)	App. 2 Mg1+NaCl (ppm)	App. 3 Mg2+NaCl (ppm)	App. 4 Mg3+NaCl (ppm)	App. 5 Mg4+NaCl (ppm)	App. 6 Mg5+NaCl (ppm)
Nitrogen (N)	186	186	186	186	186	186
Phosphorus (P)	31	31	31	31	31	31
Potassium (K)	167	167	167	167	167	167
<b>Magnesium (Mg)</b>	<b>49.28</b>	<b>24.64</b>	<b>49.28</b>	<b>73.92</b>	<b>98.56</b>	<b>123.20</b>
Calcium (Ca)	200	150	200	250	300	350
Sulfur (S)	66	66	66	66	66	66
Iron (Fe)	3.3	3.3	3.3	3.3	3.3	3.3
Manganese (Mn)	0.031	0.031	0.031	0.031	0.031	0.031
Boron (B)	0.205	0.205	0.205	0.205	0.205	0.205
Copper (Cu)	0.015	0.015	0.015	0.015	0.015	0.015
Zinc (Zn)	0.023	0.023	0.023	0.023	0.023	0.023

At the 20th day of treatments, plant samples were taken and samples were weighed with a precise balance ( $\pm 0.0001$  g). Chlorophyll and MDA contents and antioxidative enzyme activities (Catalase, Ascorbate Peroxidase, Superoxide dismutase) were determined on plant leaves.

### *Chlorophyll analysis*

Leaf segments, either fresh or frozen at  $-40^{\circ}\text{C}$ , were placed in 5 ml of 80% ethanol and heated in a water bath at  $80^{\circ}\text{C}$  for 20 min. Total chlorophyll was evaluated in the alcohol extracts from absorbance readings, using the appropriate extinction coefficient. Chlorophyll content (mg/g fr wt) was calculated as  $1000 \times A_{654} / (39.8 \times \text{sample fr wt})$ , according to Luna *et al.* (2000).

### *Malondialdehyde analysis*

The method defined by Lutts *et al.* (1996) was employed for measuring the amount of malondialdehyde, which is produced as a result of the lipid peroxidation that causes stress-induced damage to cellular membranes. Malondialdehyde (MDA) concentration was determined by using an "extinction" coefficient, which is  $155 \text{ mM}^{-1} \text{ cm}^{-1}$ , expressed as  $\mu\text{mol/g}$  fresh weight. The following equation was used in the calculation:  $\text{MDA} = (A_{523} - A_{600}) \times \text{volume of the extract (ml)} / (155 \text{ mM/cm} \times \text{sample amount})$ .

### *Enzyme extraction and assay*

Fresh leaf samples were submersed for 5 min in liquid nitrogen. The frozen leaves were kept at  $-80^{\circ}\text{C}$  for further analyses. Enzymes were extracted from 0.5 g leaf tissue using a mortar and pestle with 5 ml extraction buffer containing 50 mM potassium phosphate buffer pH 7.6- and 0.1-mM Na-EDTA. The homogenate was centrifuged at 15,000 g for 15 min and the supernatant fraction was used for the various enzyme assays. All steps in the preparation of enzyme extracts were performed at  $4^{\circ}\text{C}$ .

SOD was assayed according to Cakmak and Marschner (1992), by monitoring the superoxide radical-induced nitro blue tetrazolium reduction (NBT) at 560 nm. One unit of SOD activity was defined as the amount of enzyme that causes 50% inhibition of the photochemical reduction of NBT. Catalase activity was determined by monitoring the disappearance of  $\text{H}_2\text{O}_2$  according to the method of Cakmak and Marschner (1992). APX activity was determined by measuring the consumption of ascorbate by following absorbance at 290 nm Cakmak and Marschner (1992). One unit of APX activity was defined as the amount of enzyme required to consume  $1 \mu\text{mol}$  ascorbate  $\text{min}^{-1}$ .

The experiment was designed as a completely randomized plot with three replicates. Data were analyzed statistically, and the means of each treatment were analyzed by Duncan's multiple range test using SAS software (1985).

## **Results and Discussion**

Total weights of salt-treated plants under NaCl stress were weighed at the end of 20<sup>th</sup> day and resultant values are provided in Table 2.

As compared to the control plants, significant decreases were observed in total weight of salt-treated plants at the end of 20-day salt stress. Among the salt-treated plants, the greatest plant weights were observed in Mg 4 + salt and Mg 3 + salt treatments. However, plant weights of these treatments were placed into the same statistical group with the control plants. The lowest plant weights were obtained from Mg 1 + salt and Mg 2 + salt treatments. Plant weights of Mg 1 + salt, Mg 2 + salt and Mg 3 + salt treatments were all placed into the same statistical group.

Munns and Termaat (1986) reported that plant growth was negatively influenced by salt stress. In present study conducted with the assumption of potential reduction in the effects of salt stress on plant growth, positive effects of Mg treatments were observed on plant growth under salt stress with increasing Mg doses.

Cell membrane damages and lipid peroxidation byproduct (MDA) contents are commonly used as an indicator of salt stress-induced oxidative damage. As compared to the control treatments, increases were observed in MDA content of salt-treated plants. The greatest MDA content was obtained from Mg 1 + salt treatments and the lowest value was obtained from Mg 4 + salt treatments (Table 3). In terms of negative effects

of salt treatments based on MDA contents, the greatest effects were observed in Mg 1 + salt treatments and the least effects were observed in Mg 4 + salt treatments.

**Table 2.** Effects of different Mg doses on total weight of pepper seedlings

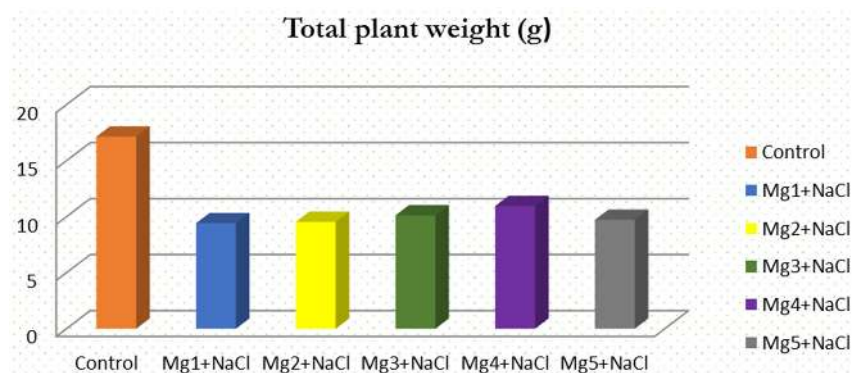
Treatments						
	Control	Mg 1+ NaCl	Mg 2 + NaCl	Mg 3 + NaCl	Mg 4+ NaCl	Mg 5+ NaCl
Total plant weight (g)	17.149 A	9.422 B	9.543 B	10.117 AB	10.953 AB	9.722 B

Means indicated with the same capital letter in the same line are not significantly different ( $P \leq 0.05$ ).

**Table 3.** Leaf MDA and chlorophyll contents ( $\mu$  mol/g F.W.) of plants

Treatments	MDA	Chlorophyll
Control	2.9950 D	4.9433 C
Mg1 + NaCl	6.4950 A	3.6963 D
Mg2 + NaCl	4.9033 B	5.5007 BC
Mg3 + NaCl	4.0553 B-D	6.7787 A
Mg4 + NaCl	3.6373 CD	6.8137 A
Mg5 + NaCl	4.5913 BC	6.1690AB

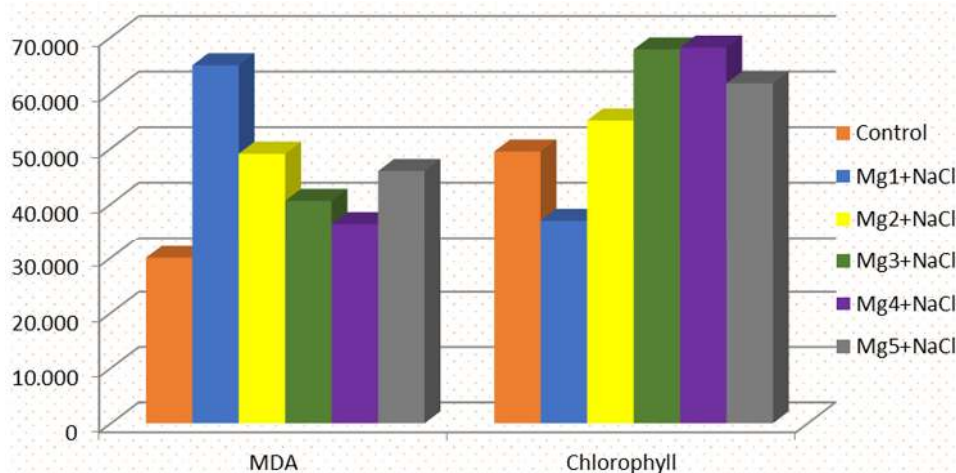
Means indicated with the same capital letter in the same column are not significantly different ( $P \leq 0.05$ ).



**Figure 1.** Effects of applications on the total weight of plants

Malondialdehyde (MDA) is a byproduct of lipid peroxidation and it is released when the cell membrane was damaged. High MDA levels indicate damaged cell membrane and low MDA levels indicate undamaged or slightly damaged cell membrane (Yaşar, 2003). Yaşar *et al.* (2007) conducted a study on watermelon and reported positive correlations between scale values and MDA contents and indicated high MDA values for genotypes with high scale values and low MDA values for salt-affected genotypes with low scale values. Similar findings were also reported by Shalata and Tal (1998) for tomato, by Aktaş (2002) for pepper and by Kusvuran *et al.* (2008) for *Cucumis* sp. genotypes. In present study, increasing Mg doses partially reduced the negative effects of salt stress on plants. Oxidative damage destroys the structure of chlorophyll and thus reduce leaf chlorophyll contents.

As compared to control plants, increase and decreases were observed in chlorophyll contents of salt-treated plants for 20 days. The greatest chlorophyll content was obtained from Mg 4 + salt treatments and the lowest chlorophyll content was obtained from Mg 1 + salt treatments (Figure 2).



**Figure 2.** Effects of applications on the MDA and chlorophyll contents

Mg functions as a central atom of chlorophyll molecule in photosynthesis (Papenbrock *et al.*, 2000). Therefore, it is essential for chlorophyll synthesis. Similar to Ca, it was reported that greater than normal Mg levels served a protective function against adverse environmental conditions in plant tissues (Hecht-Buchholtz and Schuster, 1987). Under salt stress, disruption of general metabolic processes, limited uptake of macro and micronutrients like Ca, K, N, P and Mg negatively influence chlorophyll formation. Negative effects of salt stress on chlorophyll contents were reported by Turhan *et al.* (2006) in sunflower and by Yaşar (2003) in eggplant. Salt stress-induced chlorosis on leaves is also resulted from chlorophyll degradation by free oxygen radicals (Üzal, 2009). Yakıt and Tuna (2006) reported that total chlorophyll and carotenoid contents significantly decreased with NaCl treatments, but Ca, K and Mg supplementations into nutrient solution alleviated such negative effects of NaCl on chlorophyll and carotenoid contents. Çiçek and Çakırlar (2002) and Gadallah (1999) also reported decreasing chlorophyll contents induced by distortions in general metabolic processes under salt stress. It was reported in another study conducted on peppers that external  $KNO_3$  treatments increased leaf and root K and chlorophyll contents of salt-treated plants and alleviated the negative effects of stress parameters on plant growth and development (Kaya and Higgs, 2003). The greatest chlorophyll contents were respectively observed in Mg 4 + salt, Mg 3 + salt and Mg 5 + salt treatments and they were all placed into the same statistical group. Increasing Mg doses also reduced the incidence of chlorosis and necrosis on the leaves of salt-treated plants and such a rehabilitating effect was revealed as an increase in chlorophyll content.

As compared to control plants, significant changes were observed in catalase (CAT) enzyme activity of salt-treated plants (Figure 3). In terms of catalase enzyme activity, Mg 1 + salt, Mg 2+ salt and Mg 3 + salt treatments were placed into the same statistical group. The greatest catalase enzyme activity was obtained from Mg 4 + salt and Mg 5 + salt treatments and the lowest value from Mg 2 + salt treatments (Table 4).

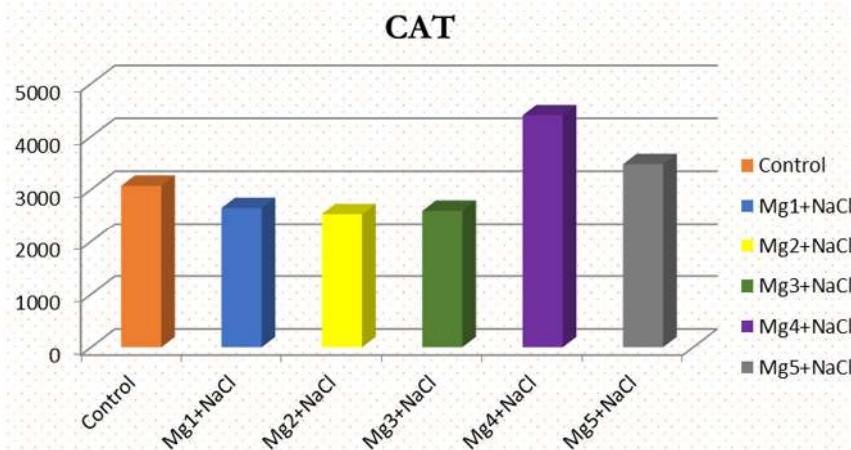
As compared to control plants, increases were observed in ascorbate peroxidase enzyme activity of salt-treated plants. The greatest ascorbate peroxidase enzyme activity was obtained from Mg 5 + salt treatments and the lowest value was obtained from Mg 1 + salt treatments (Table 4, Figure 4).

As compared to control treatment, significant decreases were observed in superoxide dismutase enzyme activity of salt-treated plants in Mg 1 + salt and Mg 2 + salt treatments. In terms of superoxide dismutase enzyme activity, Mg 3 + salt and Mg 4 + salt treatments were placed into the same statistical group. The greatest value was obtained from Mg 5 + salt treatments and the lowest value from Mg 2 + salt treatments followed by Mg 1 + salt treatments (Table 4, Figure 5).

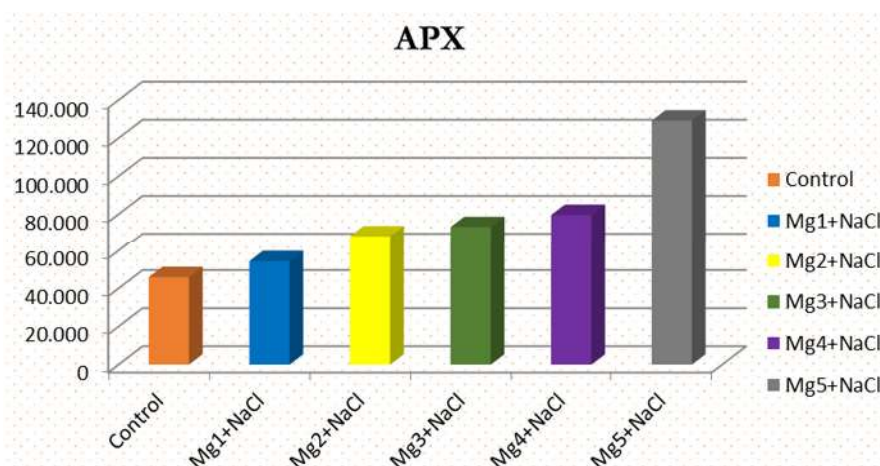
**Table 4.** Leaf catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) enzyme activities of plants (mol/min/mg F.W.)

Treatments	CAT	APX	SOD
Control	3071.5 C	4.5900D	113.333 B
Mg1 + NaCl	2651.8 D	5.4367CD	82.667D
Mg2 + NaCl	2533.9 D	6.8267 BC	68.333E
Mg3 + NaCl	2598.0 D	7.3200B	100.667 C
Mg4 + NaCl	4403.0 A	7.9600 B	107.333 BC
Mg5 + NaCl	3480.2 B	12.9200 A	141.333 A

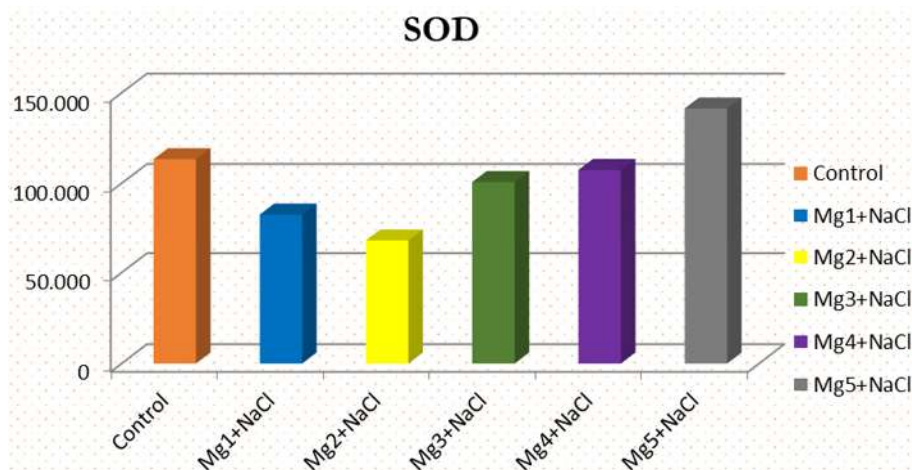
Means indicated with the same capital letter in the same column are not significantly different ( $P \leq 0.05$ ).



**Figure 3.** Leaf catalase (CAT) enzyme activities of plants



**Figure 4.** Leaf ascorbate peroxidase (APX) enzyme activities of plants



**Figure 5.** Leaf Superoxide dismutase (SOD) enzyme activities of plants

Significant correlations were reported between increase in antioxidant enzyme activities and decrease in oxidative stress-induced damages in plants under stress conditions (Yaşar *et al.*, 2006b; Yıldız *et al.*, 2010; Uzal *et al.*, 2019). Increasing antioxidant enzyme activities were also reported for different plant species under stress conditions and such increases generally varied based on plant genetics (Türkan *et al.*, 2005; Yasar *et al.*, 2008 a,b; Kuşvuran *et al.*, 2012; Yasar *et al.*, 2016; Uzal *et al.*, 2019). Kusvuran *et al.* (2008) investigated salt tolerance of some *Cucumis* sp. genotype and indicated that salt-tolerant genotypes had high enzyme activity and salt-sensitive genotypes had low enzyme activities. Similar findings were also reported by Aktaş (2002) and Karanlık (2001). In present study, it was observed that increasing Mg doses alleviated adverse effects of salt treatments on plants. Therefore, the least enzyme activity was measured in Mg 1 + salt treatment with the least Mg dose. On the other hand, the greatest enzyme activities were measured in Mg 4 + salt, Mg 5 + salt and Mg 3 + salt treatments with greater Mg doses.

## Conclusions

In conclusion; this study besides salt treatments, Mg was applied at different doses and effects of Mg treatments under salt stress on plant salt tolerance were investigated. In present study conducted with the assumption of potential reduction in the effects of salt stress on plant growth, positive effects of Mg treatments were observed on plant growth under salt stress with increasing Mg doses. In present study, Mg applied in different doses remarkably changed some antioxidant enzyme activities of salt-treated plants. Present measurements and analyses and resultant findings revealed that Mg treatments at increasing doses partially alleviated negative effects of salt stress on pepper seedlings.

## Authors' Contributions

The authors declare that they have contributed equally to the article. All authors read and approved the final manuscript.



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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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