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EFFECT OF FOLIAR-APPLIED AMINOPOLYSACCHARIDE CHITOSAN ON SEEDLING GROWTH CHARACTERISTICS, ANTIOXIDANT ENZYME ACTIVITY, AND CHLOROPHYLL AND CAROTENOID CONTENTS OF SAFFLOWER (*CARTHAMUS TINCTORIUS* L.) CULTIVARS UNDER SALINE CONDITIONS

MEHTAP GURSOY

Aksaray University Güzelyurt Vocational School Güzelyurt, Aksaray, Turkey
**Corresponding author's email: mehtapgrsoy@gmail.com*

Abstract

Salinity is a major concern in agricultural areas all over the world. Aminopolysaccharide chitosan is a biopolymer that is known to increase plant tolerance against salinity by increasing various antioxidant enzyme activities. Less studies are available on the effect foliar application of chitosan on safflower plants (*Carthamus tinctorius* L.) in saline conditions in the greenhouse. In this study, the effects of foliar aplication of 0-0.6% chitosan (4 concentrations) on the resistance to 0-150 mM salt (4 concentrations) in 3 safflower cultivars (Balcı, Linas and Remzibey) were investigated under greenhouse conditions. Chitosan applications played a role in reducing the negative effects of salt stress on the examined morphological features. In addition, the positive effect of chitosan application on enzyme activities in chlorophyll, carotenoid, SOD and CAT was determined by increasing salt doses. However, any positive effect of chitosan on the reduction of MDA content could not be determined. It was concluded that chitosan can be evaluated as an effective natural biopolymer material that can be used to increase resistance and tolerance of plants against salt stress.

Key words: Abiotic stress, Antioxidant enzymes, Environmental stress, Seedling development.

Introduction

Soil salinity is one of the biggest abiotic, environmental stress induce significant damages to plants that affects their yield and limit agricultural production (Yamaguchi & Blumwald, 2005; Bulgari *et al*., 2019; Jafari *et al*., 2019; Jamalian *et al*., 2019) by excessive ion intake resulting in increased uptake of Na^+ and Cl^- ions causing decreased and restricted availability of water in the soil to plants due to increased osmotic stress (Abogadallah, 2010).

There are some studies that report the development of increased salt tolerance using some external bioprotectors that improve working of some plant genetic mechanisms by increasing salt tolerance (Razzaq *et al*., 2020). The interest of researchers towards these protective treatments, to improve the resistance of plants to abiotic stresses has lead to an improvement in agricultural production and quality during last 3 decades (Boehme *et al*., 2008; Mahdavi *et al*., 2011; Du Jardin, 2015; Safikhan *et al*., 2018). Safflower is a an annual oilseed plant and high resistance against drought and salinity. (Moghadam & Mohammadi, 2014; Golkar & Taghizadeh, 2018; Gürsoy, 2019a; 2019b). These features with a high level of bioprotective and antioxidant activities, make it suitable for treating a number of diseases (Zhou *et al*., 2014). Besides these no literature has been found related to enzyme activities and behavior of chitosan treated safflower in greenhouse conditions under salt stress.

Chitosan is a bioprotector made up of natural, non-toxic biopolymers obtained by deacetylation of chitosan (Katiyar *et al*., 2015; Younes & Rinaudo, 2015). These are obtained from aquatic animals like crab, shrimp, crayfish, etc. of the Crustacean family (No *et al*., 2002; Gürsoy *et al*., 2018). It has been reported that chitosan is a natural amino polysaccharide that is abundant in nature (Kumar, 2000) and is soluble by alkali or enzymatic deacetylation (Asghari-Zakaria *et al*., 2009). It is biologically renewable, biodegradable, biocompatible, antigenic, non-toxic, and biofunctional structure that is frequently used in biomedical applications like dressing material and drug delivery systems (Kim *et al*., 2007; Hosseinnejad & Jafari, 2016; Muxika *et al*., 2017).

Chitosan has also attracted attention with its antioxidant properties (Guo *et al*., 2005). It is also known for extending the shelf life of many fruits and vegetables with thin bioprotective film coatings. Furthermore, it is known as a potential biotic inducer to improve resistance against pathogens (Katiyar *et al*., 2015).

Chitosan in agriculture is also used to check antimicrobial, antiviral, antifungal activities and allow stimulating plant growth and development by increasing or improving seed germination rates and crop yields (Tay *et al*, 1993; Tham *et al*., 2001; Vasyukova *et al*., 2001; Devlieghere *et al*., 2004). These features make it a very remarkable product in agriculture and therefore, can help in preventing environmental pollution. Some studies have shown chitosan has a positive effect on the development of roots, shoots, and leaves of various plants (Jabeen & Ahmad, 2013; Gürsoy, 2020).

Plant growth, seed germination, chlorophyll content, and ion uptake can be increased with chitosan applications (Ahmed *et al*., 2020) that promote healthy growth of plants.

Jabeen & Ahmad (2013) have reported that low dosed chitosan applications to safflower and sunflower seeds under salt stress improve seed germination parameters. Similarly, Cho *et al*., (2008) found improvement in weight, germination rate, and length of seedlings after chitosan applications to sunflower compared to control treatments. Whereas, Al-Tawaha *et al*., (2018) has reported a reduction in the effects of salt stress and effective increase in growth and yield of plants after chitosan treatments under saline conditions. All these studies suggest that chitosan treatments have high antioxidant activity and have free-radical destroying features that improve the resistance of plants against biotic and abiotic stresses.

The aim of this study was to investigate and found changes in morphologic and biochemical parameters like chlorophyll, carotenoid contents and antioxidant enzyme activities of safflower plants after chitosan treatments in greenhouse under salinity stress conditions.

Material and Methods

Research material and growth conditions: Three safflower (*Carthamus tinctorius* L.) cultivars (Balcı, Linas, Remzibey) were obtained from the Central Field Crops Research Institute, Ankara, Turkey. The research was carried out in a randomized plot design with 3 replications. They were treated with 0 (control), 50, 100, 150 mM NaCl (S1-S4) salt concentration and with 0 (control), 0.2, 0.4 and 0.6% chitosan (C1-C4) after dissolving in commercially available 0.1% acetic acid. The experimental pots were filled with peat moss followed by sowing the treated seeds of safflower cultivars in them using 10 seeds per pot for each treatment. The plants were watered with tap water until water saturation. Germination was observed in pots 5 days after planting. Thinning was made after 15 days of planting, by reducing them to 5 plants per pot. After thinning, the plants were watered with saline irrigation water every two days for 4 weeks. Different percentages of chitosan were sprayed once a week with a hand spray for 4 weeks. The pots in control group were spray irrigated with water only and the study was determined the end of 8th week after sowing seeds.

Measurements

Seedling length (cm): It was determined by measuring the lowest point on stems to the highest point on the germinating leaves.

Stem length (cm): The stems were separated from the plant, after removal from the soil without giving any damage. The adhering soil was removed by washing underwater followed by rinsing with distilled water. The stems were measured by drying them in a cool and shaded place.

Seedling fresh weight (g): It was determined by weighing seedling after cutting the stems.

Stem fresh weight (g): After the stem were separated from the seedling, they were weighed.

Biochemical parameters

Chlorophyll (mg/g): Safflower plants leaf samples (0.25g) homogenizing in acetone then the extracts were completed 25 ml with acetone. These extract were read at 645 and 663nm in spectrophotometer then by computation of chlorophyll using the formula (Lichtenthaler & Welburn, 1983).

Chlorophyll a (mg/g) = (12.7 * 663 nm) - (2.69 * 645 nm) * V / W * 10000

Chlorophyll b (mg/g) = (22.91 * 645 nm) - (4.68 * 663 nm) * V / W * 10000

Total Chlorophyll = Chlorophyll a + Chlorophyll b

Carotenoid (mg/g): The 0.25g samples taken from young leaves of safflower plants were homogenized in 80% acetone in a place not directly exposed to light, and then filtered. The amount of carotenoid will be determined according to the following formula by completing the obtained filtered extract with acetone to 25 ml and reading it at 450 nm wavelength (Lichtenthaler & Welburn, 1983).

Carotenoid = (4.07 x A450-(0.0435 x Chlorophyll a +0.367 x Chlorophyll b)

Lipid peroxidation (MDA): The 0.5 g young leaf sample taken the plants grown in the greenhouse was homogenized with 10 ml of 0.1% trichloracetic acid (TCA) and centrifuged at 15000 rpm for 5 minutes. 0.5 ml of thiobarbituric acid (TBA) was dissolved in 4 ml of 20% TCA that was taken from the upper phase or supernatant of the centrifuged samples. It was cooled and centrifuged for 10 minutes at 10000 rpm and its absorbance was determined at 532 nm and 600 nm wavelength spectrum by taking its clear part. The content of malondialdehyde (MDA) was calculated using the following (Heath & Packer, 1968).

$$MDA\ (nmol\ ml^{-1}) = [(A532-A600)/155\ 000]\ 10^6$$

Catalase (CAT) activity: For the determination of catalase (CAT) and superoxide dismutase (SOD) activities, after each 1 gr frozen leaf samples were homogenized with 5 ml cold 0.1M Na-phosphate, 0.5 mM Na-EDTA mixture (pH: 7.5), homogenate was centrifuged at 18000 rpm for 30 minutes at 4°C. Catalase activity was determined by monitoring the loss of H_2O_2 at 240 nm wavelength (Çakmak and Marschner, 1992).

Superoxide dismutase (SOD) activity: Na-phosphate buffer (50 mM) ($Na_2HPO_4 \times H_2O_2$), Na-EDTA (0.1 mM), NBT (33 μM), riboflavin (75 μM), methionine (13 mM) was used as the reaction solution (pH: 7.0). Then, reaction solution (2.5 ml) and plant extract (0.1 ml) solution were mixed. The control solution and reaction solution readings were taken at 560 nm (Rahnama & Ebrahimzadeh, 2005).

Statistical analysis

The experimental data obtained at the end of the research was subjected to analysis of variance using MSTAT-C computer software. Duncan Test was applied to determine the significance levels of the differences between the means of the applications.

Results

Analysis of variance for the examined traits showed the statistically significant differences between the cultivars except for stem fresh weight and total chlorophyll (seedling length $p<0.05$) another parameters ($p<0.01$). The difference between the salt doses was in all parameters ($p<0.01$) except the total chlorophyll. The cultivar×salt interaction; there was a statistical difference in seedling

length, seedling fresh weight, carotenoid, CAT ($p<0.01$) and MDA ($p<0.05$) enzymes. On the other hand, chitosan applications, caused a statistical difference in other properties ($p<0.01$) except stem length. There were significant differences in stem fresh weight, carotenoid, MDA, SOD, CAT parameters in terms of cultivar×chitosan interaction ($p<0.01$). In salt×chitosan interaction, seedling length, total chlorophyll, carotenoid, MDA, SOD, CAT parameters ($p<0.01$), and cultivar×salt×chitosan triple interaction; in carotenoid, SOD and CAT parameters were found at ($p<0.01$) significant levels.

When the examined (Table 1), cultivars × salt concentrations and salt × chitosan concentrations interaction was statistically significant in terms of seedling length. It was seen that the effect of chitosan application had increased even with increasing salt doses. However, in general, after the C3 application, it was seen that the seedling length stayed the same level. It is seen that chitosan application inhibited the effects of salt stress and played a role in promoting the growth of seedlings. The longest seedlings were obtained using C3 chitosan aplication and it was noted that chitosan was effective in eliminating the harmful effects of increasing doses of the salt in irrigation water. It was determined that the harmful effects of salt concentrations were alleviated by interaction with chitosan. Besides, salt treatments interacted with cultivars and showed different reactions for each cultivar.

In terms of stem length (Table 2), there was a statistical difference only between cultivars and doses. When the average values were examined, the longest stem length was determined in Linas variety. In terms of salt doses, it was seen that there was a 12% difference between the longest stem length and the shortest stem length. However, no effect of chitosan application on stem length was detected.

A statistical difference was determined between cultivars, salt doses, applied chitosan doses and cultivar×salt interaction in seedling fresh weight parameter (Table 3). Accordingly, when the Table 3 was examined, the S2 dose gave the highest results in terms of salt doses, and the lowest seedling fresh weight was obtained at the highest salt dose (S4). When the results were evaluated in terms of chitosan application, the highest seedling fresh weight was 8.52 g, obtained from the third dose (0.4%) of chitosan applications.

Table 1. Average values of the effect of chitosans at different concentrations applied to safflower cultivars under salt stress on seedling lenght (cm).

Salt doses	Seedling length (cm)							Mean
	Cultivars			Chitosan doses				
	Balcı	Linas	Remzibey	C 1	C2	C3	C4	
S1	13.47 d	14.97 b	14.19 bcd	13.57 d	13.64 d	15.37 ab	15.59 a	14.21 B
S2	13.70 cd	14.42 bcd	13.52 cd	13.50 d	13.48 d	15.03 abc	14.62 abcd	13.88 B
S3	14.68 bc	13.94 bcd	16.15 a	15.35 ab	14.57 abcd	15.23 ab	15.06 abc	14.92 A
S4	16.10 a	13.51 cd	15.06 ab	14.41 abcd	13.83 cd	14.04 bcd	14.30 abcd	14.89 A
Mean	**14.49 AB**	**14.21 B**	**14.73 A**	**14.54 AB**	**14.16 B**	**15.05 A**	**14.15 B***	

*Different letters in the same column indicate different groups

Table 2. Average values of the effect of chitosans at different concentrations applied to safflower cultivars under salt stress on stem lenght (cm).

Cultivars	Stem Length (cm)				Mean
	Salt doses				
	S1	S2	S3	S4	
Balcı	11.82	13.00	13.34	11.98	12.53 B
Linas	13.10	13.66	14.79	13.15	13.67 A
Remzibey	12.13	12.79	13.44	12.64	12.75 B
Mean	**12.35 C**	**13.15 B**	**13.86 A**	**12.59 C***	

*Different letters in the same column indicate different groups

Table 3. Average values of the effect of chitosans at different concentrations applied to safflower cultivars under salt stress on seedling fresh weight (g).

Cultivars	Seedling fresh weight (g)								Mean
	Salt doses				Chitosan doses				
	S1	S2	S3	S4	C1	C2	C3	C4	
Balcı	7.07 efg	8.72 ab	7.71 cd	7.49 cde	8.25	7.40	7.93	7.44	7.75 A
Linas	6.45 g	8.16 bc	6.66 fg	6.94 efg	7.50	6.95	7.05	6.74	7.06 B
Remzibey	9.08 a	9.02 a	7.30 def	6.60 fg	8.32	7.74	8.52	7.44	8.00 A
Mean	**7.54 B**	**8.64 A**	**7.22 BC**	**7.01 C**	**8.02 A**	**7.36 B**	**7.83 A**	**7.20 B***	

*Different letters in the same column indicate different groups

Table 4. Average values of the effect of chitosans at different concentrations applied to safflower cultivars under salt stress on stem fresh weight (g).

Cultivars	Salt doses			Chitosan doses					Mean
	S1	S2	S3	S4	C1	C2	C3	C4	
Balcı	1.53	1.87	1.79	1.79	1.78 a	1.73 a	1.77 a	1.69 a	1.74
Linas	1.64	1.78	1.85	1.89	1.79 a	1.74 a	1.88 a	1.74 a	1.79
Remzibey	1.62	1.66	1.84	1.75	1.45 b	1.74 a	1.89 a	1.76 a	1.71
Mean	**1.596 B**	**1.769 A**	**1.824 A**	**1.811 A**	**1.68 B**	**1.74 B**	**1.85 A**	**1.73 B***	

*Different letters in the same column indicate different groups

Table 5. Average values of the effect of chitosans at different concentrations applied to safflower cultivars under salt stress on total chlorophyll contents (mg g^{-1} FW).

Chitosan doses	Salt doses				Mean
	S1	S2	S3	S4	
C1	1.78 bc	1.78 bc	1.84 ab	1.85 ab	1.81 B
C2	1.50 d	1.67 cd	1.80 abc	1.65 cd	1.66 C
C3	1.97 a	1.94 ab	1.83 ab	1.84 ab	1.89 A
C4	1.60 d	1.60 d	1.60 d	1.61 d	1.60 C*
Mean	**1.72**	**1.75**	**1.77**	**1.74**	

*Different letters in the same column indicate different groups

There was no statistically significant effect of increasing salt doses on stem fresh weight (Table 4). Except for the control dose, the other doses were in the same statistical group. However, when the table including chitosan application was examined, it was seen that the third dose of chitosan (0.4%) made a difference. Stem fresh weight, which was the lowest 1.68 g at the control dose without chitosan, was 1.85 g at the 0.4% dose. It was observed that there was a 12% increase in stem fresh weight among chitosan applications.

The lowest results in terms of total chlorophyll were obtained from the 4th dose (0.6%) application of chitosan (Table 5). However, there was no statistical difference between the second dose (0.2%). The highest total chlorophyll value was obtained from the 3rd dose (0.4%) chitosan application. It is known that the chlorophyll content decreases with salt application. However, it was determined that chlorophyll increased with chitosan application. The positive effect of chitosan application was remarkable.

Carotenoids played a major role in inducing resistance of plants against high antioxidation stress under stress conditions. They were also effective in protecting cells and tissues from oxidative damage. There was a statistical difference in all parameters in terms of carotenoids, and the cultivar×salt×chitosan interaction was also significant at the 0.01 level. When Table 6 was examined, the highest average carotenoid 8.89mg/g was obtained from the third dose of chitosan (0.4%). The lowest carotenoid was obtained from the control application as 8.08mg/g. Comparing from the control, carotenoids increased with chitosan application under stress conditions, but this increase didn't occur at the 4th dose after the third dose. Chitosan was effective role in increasing the amount of carotenoid.

In the lipid peroxidation (MDA) parameter, all bilateral interactions (chitosan×salt, cultivar×chitosan, cultivar×salt) showed statistical significance. The highest MDA content was obtained in the highest salt application. However, chitosan application didn't have a reducing effect on lipid peroxidation (Table 7). However, it seems that the C2 dose gives better results than the C3 and C4 doses.

In the superoxide dismutase (SOD) enzyme, a statistically significant difference was found in the cultivar×salt×chitosan triple interaction. With the increase in salt doses, a parallel increase was observed in the SOD enzyme. The highest SOD value was determined at the 4th salt dose. However, there was no statistical difference between the 3rd dose and they are in the same group. In addition, in the 1st and 2nd salt doses, the 4th dose of chitosan caused higher enzyme activity, while the 3rd dose of chitosan was more effective with the increase in salt doses (Table 8).

When the average results (Table 9) of the cultivar×salt×chitosan triple interaction of the catalase enzyme were examined, the lowest CAT salt doses were found in the control and the highest in the 4th salt dose. In terms of chitosan doses, the application of the 3rd dose of chitosan at the 4th salt dose revealed the most advantageous result. In addition, the 4th dose of chitosan caused higher enzyme activity in the 1st and 2nd salt doses, while the 3rd dose of chitosan was more effective with the increase in salt doses.

Discussion

Morphological Characteristics: In this study, it was determined that different salt and chitosan concentrations applied to the 3 safflower cultivars under greenhouse conditions had a positive effect on the investigated characteristics. The results in the study are in line with the studies of Jabeen & Ahmad (2013) in safflower and sunflower, Jafari *et al*., (2019) in *Matthiola incana* and Sheikha & Al-Malki (2011). They reported that chitosan concentrations had a positive effect on plant growth parameters like seedling and root length, wet and dry weight. Al-Tawaha *et al*., (2018) applied chitosan to salt-affected plants and noted plant height of 81.94 cm in control treatments. A seedling height of 84.06 cm, 84.38 cm, and 84.81cm were noted after control, 30, and 60 mg l-1 chitosan treatment. Hasanah & Sembiring (2018) found that aerial application of chitosan and salicylic acid to leaves of soybean cultivars increased plant height, seedling, and root dry weights.

Table 6. Average values of the effect of chitosans at different concentrations applied to safflower cultivars under salt stress on carotenoid (mg g^{-1} FW).

Cultivars	Salt doses × Chitosan doses																Mean
	S1 C1	S1 C2	S1 C3	S1 C4	S2 C1	S2 C2	S2 C3	S2 C4	S3 C1	S3 C2	S3 C3	S3 C4	S4 C1	S4 C2	S4 C3	S4 C4	
Balcı	5.28 no	5.13 nop	8.31 ghı	11.5 bc	5.89 mn	6.61 klm	11.28 bcd	10.04 ef	3.47 r	7.69 h-l	13.17 a	10.44 c-f	3.52 r	7.78 h-k	13.66 a	9.31 fg	8.32 B
Linas	4.73 opq	6.71 klm	9.67 ef	10.71 cde	3.65 qr	7.39 h-l	11.91 b	9.88 ef	5.07 nop	8.05 hıj	13.15 a	10.34 def	3.67 qr	8.46 gh	13.28 a	9.56 ef	8.51 B
Remzibey	6.54 lm	6.91 j-m	10.7 cde	10.62 cde	4.70 opq	7.24 ı-l	13.61 a	10.03 ef	3.58 qr	7.97 hıj	13.39 a	10.29 def	4.10 pqr	8.03 hıj	13.67 a	9.67 ef	8.82 A
Mean	**8.07 C**				**8.52 B**				**8.89 A**				**8.73 AB**				

*Different letters in the same column indicate different groups

Table 7. Average values of the effect of chitosans at different concentrations applied to safflower cultivars under salt stress on MDA (nmol g^{-1} FW) activity.

Chitosan doses	Salt doses				Mean
	S1	S2	S3	S4	
C1	0.35 l	0.39 k	0.44 j	0.48 ı	0.41 C
C2	0.51 h	0.57 g	0.64 f	0.67 e	0.60 B
C3	0.70 d	0.73 c	0.77 b	0.80 a	0.75 A
C4	0.81 a	0.78 b	0.72 c	0.70 d	0.75 A
Mean	**0.59 D**	**0.62 C**	**0.64 B**	**0.66 A***	

Cultivars	Salt doses				Chitosan Doses				Mean
	S1	S2	S3	S4	C1	C2	C3	C4	
Balcı	0.57 e	0.60 d	0.63 c	0.66 a	0.39 g	0.58 e	0.74 c	0.76 ab	**0.62 B**
Linas	0.60 d	0.61 d	0.64 bc	0.66 ab	0.42 f	0.60 e	0.75 bc	0.74 c	**0.62 B**
Remzibey	0.61 d	0.63 c	0.66 a	0.67 a	0.43 f	0.62 d	0.77 a	0.75 abc	**0.64 A**
Mean	**0.59 D**	**0.62 C**	**0.64 B**	**0.66 A**	**0.41 C**	**0.60 B**	**0.75 A**	**0.75 A**	

*Different letters in the same column indicate different groups

Table 8. Average values of the effect of chitosans at different concentrations applied to safflower cultivars under salt stress on SOD (U g^{-1} FW) activity.

Cultivars	Salt doses × Chitosan doses																Mean
	S1 C1	S1 C2	S1 C3	S1 C4	S2 C1	S2 C2	S2 C3	S2 C4	S3 C1	S3 C2	S3 C3	S3 C4	S4 C1	S4 C2	S4 C3	S4 C4	
Balcı	25.58 o	27.09 l-o	30.37 ıj	33.79 def	27.36 k-o	27.42 k-o	30.99 hı	31.51 ghı	26.96 l-o	27.02 l-o	34.36 cde	31.59 ghı	26.93 l-o	28.49 kl	35.85 abc	32.16 f-ı	29.84 B
Linas	27.74 k-n	26.94 l-o	31.43 ghı	33.92 def	27.49 k-o	27.42 k-o	32.77 e-h	33.24 efg	25.89 no	28.02 klm	37.08 a	32.17 f-ı	26.94 l-o	29.16 jk	35.39 a-d	31.08 hı	30.42 A
Remzibey	26.18 mno	28.35 kl	31.14 hı	32.81 e-h	26.26 mno	27.62 k-n	34.01 c-f	32.35 fgh	26.68 l-o	28.70 jkl	34.50 b-e	31.48 ghı	26.95 l-o	28.51 kl	36.27 ab	31.48 ghı	30.21 AB
Mean	**29.61 B**				**29.87 B**				**30.37 A**				**30.77 A***				

*Different letters in the same column indicate different groups

Table 9. Average values of the effect of chitosans at different concentrations applied to safflower cultivars under salt stress on CAT (U g^{-1} FW) activity.

Cultivars	Salt doses × Chitosan doses																Mean
	S1 C1	S1 C2	S1 C3	S1 C4	S2 C1	S2 C2	S2 C3	S2 C4	S3 C1	S3 C2	S3 C3	S3 C4	S4 C1	S4 C2	S4 C3	S4 C4	
Balcı	0.57 m	1.15 ıjk	1.96 ef	2.05 def	0.88 klm	1.77 e-h	1.91 ef	2.18 de	0.76 klm	1.71 fgh	2.40 cd	1.94 ef	0.91 klm	1.83 efg	2.67 bc	1.72 fgh	1.65 B
Linas	0.63 lm	1.39 hıj	1.92 ef	1.88 ef	0.99 kl	1.87 ef	1.93 ef	2.05 def	0.98 klm	1.44 ghı	2.73 bc	1.91 ef	0.69 lm	1.86 ef	3.79 a	1.70 fgh	1.73 A
Remzibey	0.83klm	2.00 def	1.93 ef	1.96 ef	0.83 klm	1.39 hıj	2.09 def	1.91 ef	1.05 jkl	1.72 fgh	2.63 bc	1.92 ef	1.04 jkl	1.86 ef	3.00 b	1.75 efgh	1.74 A
Mean	**1.52 D**				**1.65 C**				**1.77 B**				**1.90 A***				

*Different letters in the same column indicate different groups

Chlorophyll content: The amount of photosynthetic pigment consisting of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid differencies depending on species, type of stress, duration of stress, a period of the plant in the life cycle, and the intensity of stress in plants under stress (Turfan, 2017). Salt stress affects chlorophyll metabolism and caused chlorophyll production to decrease significantly (Qin *et al*., 2019; Gürsoy, 2020). The highest chlorophyll was determined using 0.4% chitosan. There was no significant difference between the other administered chitosan concentrations. Abu-Muriefah (2013) studied bean plants and reported that chitosan application on leaf had an effect on increasing chlorophyll content compared to the plants that were not treated with chitosan. Similarly, Dzung, (2005) has reported that chitosan increased chlorophyll content in soybean and peanuts

Carotenoid content: Stahl & Sies (2003) reported that carotenoids pigments played a very important role in the protection of plants against photooxidative processes in plants and as antioxidants that play an extremely active role in scavenging the harmful effects of free oxygen radicals. It was determined that there was an interaction among cultivars, salt, and chitosan concentrations. They showed increased carotenoid content in parallel to an increase in chitosan concentrations. The highest carotenoid content was determined in no salt treatment and 0.4% chitosan. Rahman *et al*., (2018) reported the application of chitosan to strawberry plants in the form of a spray caused an increase in the carotenoid content of the plant. They reported that the efficiency of the low dose of chitosan was increased by spraying and simultaneously increased more than one antioxidant content and their activities were high in the harvested fruits.

Lipid peroxidation (MDA): Cell membrane stability is affected by lipid peroxidation caused by active oxygen species under various stress conditions. Lipid peroxidation is known as MDA content. Accordingly, MDA concentration is an indicator of lipid peroxidation plant cells (Fu & Huang, 2001; Feng *et al*., 2009). Jabeen & Ahmad (2013) reported that MDA content of seeds at low chitosan concentrations decreased stress conditions in their study where they applied chitosan under saline conditions on safflower and sunflower plants. Chitosan was applied to aerial parts and their treatment with the seeds was more effective in terms of MDA content in this study. Taher *et al*., (2018) reported that MDA content sunflower cultivars was increased with increasing salt concentrations.

Superoxide dismutase (SOD) activity: Moghaddam *et al*., (2019) has reported many changes in antioxidant enzyme activities in plants under salinity stress. It is reported that antioxidants have a vital role in salt tolerance to clean reactive oxygen species (Ramesh *et al*., 2013). Beyaz & Kır (2019) has reported that CAT, SOD, APX, GR enzymatic activities in which plants show an antioxidative effect to survive in the production and accumulation of reactive oxygen species. Taher *et al*., (2018) displayed that sunflower cultivars induce morphological and biochemical changes under saline conditions (0, 50, 150, 250 mM), but their SOD activity is increased up to 150 mM salt concentration but their activity showed decreased activity using 250 mM salt concentration. Moghaddam *et al*., (2019) reported that SOD activity increased with increasing salt density in *Tagetes minuta* seedlings in a study in which they studied antioxidant enzyme activities under salt stress. The highest SOD activity was displayed at 100 mM salt concentration of 0.77 U g^{-1} FW and the 150 mM salt concentration). Similarly, the highest SOD activity was noted using a 0.4% chitosan application that played a role in improving SOD activity.

Catalase (CAT) activity: There is increase in antioxidant enzyme activities under stress conditions of plants. It is known that this increase is also a result of the plant's resistance mechanism. It was determined that the catalase enzyme was the highest at the 150 mM salt concentration. The highest CAT was determined using 100 mM salt concentration.79 U g^{-1} FW and 150 mM salt concentration together with 2nd chitosan concentration. In general, the amount of catalase enzyme increased as the concentrations of chitosan increased together with increasing salt concentrations. Chitosan application caused an increase in catalase enzyme under salt stress conditions. Therefore, the plant's tolerance mechanism enabled it to resist stress. Jabeen & Ahmad (2013) applied chitosan to safflower and sunflower seeds under saline conditions. They reported that the seeds treated with 0.25-0.50% chitosan had higher catalase activity compared to the control. Turfan (2017) reported that the spinach plant was quite high in $FeCl_3$, $NiCl_2$, $ZnCl_2$, 75 and 225mM NaCl applications in the study after evaluation of CAT activity under various stress conditions.

Conclusion

Besides the negative effects of salt concentrations in 0.4% mM, the positive effects of chitosan application on safflower cultivars were noted at different levels in this study. The most advantageous results in terms of morphological (seedling length, stem length, seedling fresh weight, stem fresh weight) and biochemical (chlorophyll, carotenoid, MDA, SOD, CAT) parameters were obtained from the cultivar Linas. Additionally, with the increase of salt concentrations, chlorophyll, carotenoid, SOD and CAT enzymes showed an increase in enzyme activities after chitosan treatments. The 2nd concentration of (0.4%) chitosan showed the most advantageous results. However, there was no positive effect of chitosan in decreasing MDA content. As a result of the study, it was concluded that chitosan can be evaluated as a natural material that can be effective in improving tolerance of plants under stress conditions.

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