EFFECT OF CHITOSAN PRETREATMENT ON SEEDLING GROWTH AND ANTIOXIDANT ENZYME ACTIVITY OF SAFFLOWER (*Carthamus tinctorius* L.) CULTIVARS UNDER SALINE CONDITIONS

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Abstract. Today, salinity has become a major problem in agricultural areas all over the world. However, plants develop a defense mechanism against stress by increasing various antioxidant enzyme activities in order to tolerate salt and other stress factors. In addition to this mechanism, the effects of stress are attempted to be reduced by various applications that increase enzyme activities. One of these applications is chitosan application. In this study, 4 different doses of chitosan pretreatment (0 (control) (Ch1), 0.2% (Ch2), 0.4% (Ch3), 0.6% (Ch4)) were applied to safflower cultivars (Balcı, Linas, Remzibey) for 4 hours under laboratory conditions. For each chitosan application, 50 seeds were germinated under saline conditions (0 (control) (S1), 50 mM (S2), 100 mM (S3), 150 mM (S4)) in petri dish. As a result of the study, it has been determined that chitosan applications provide increases in seedling length, root length, seedling wet weight, root wet weight, germination percentage, total chlorophyll, carotenoid, β-carotene and lycopene parameters. In the study, it was determined that the most effective chitosan application was Ch3 in terms of the properties examined in stress conditions. According to the research results, it was concluded that chitosan can be considered as a natural material that can positively affect in the defense mechanism of plants under stress conditions.
Keywords: *abiotic stress, environmental stress, damage, seed germination, seedling development*

Introduction

High soil salinity is an serious factor that limits agricultural production in many regions of the world (Yamaguchi and Blumwald, 2005). Salt soil conditions are also one of the most important environmental stress factors that damage plants significantly (Bulgari et al., 2019; Jafari and Garmdareh, 2019). In addition, stress factors that plants are exposed to divided into two types abiotic and biotic (Bulgari et al., 2019). Salinity, one of the abiotic stress factors, affects plants in two ways. Firstly it causes a decrease in soil water content through osmotic stress. In this way, the water intake of the plant is restricted. The second is that it causes excessive ion intake. In particular, it increases the uptake of Na^+ and Cl^- ions (Abogadallah, 2010). Various environmental stresses cause oxidative damage in plants, causing damage and even death of the plant's cells (Sharma et al., 2012). A variety of reactive oxygen species (ROS) are usually produced in plants under stress conditions. Plants develop molecular defense systems to avoid the effects of damage caused by ROS and limit ROS formation (Rejeb et al., 2014). To reduce oxidative damage in ROS cells, plants develop a defense system containing antioxidant enzymes as well as reduced glutathione, tocopherol, carotenoids and flavonoids such as non-enzymatic ascorbate (Nú˜nez et al., 2003; Azevedo-Neto et al., 2006).

Although germination of seed is one of the critical stages for seedling development and successful crop production, it is a complex process that is very sensitive to the negative effects of environmental conditions (Almansouri et al., 2001; Fan et al., 2013; Kataria et al., 2017). The negative effects of salt application on seed germination and

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seedling development occur as physiological and biochemical changes such as osmotic stress, ion toxicity and oxidative damage (Yu et al., 2013; Alsaeedi et al., 2017; Fang et al., 2017). In plants, salt tolerance can be increased with some environmental applications as well as genetic mechanisms (Razzaq et al., 2020). Therefore, pretreatment applications to seed stimulate the metabolic processes of germination and increase the performance of the seed against various environmental conditions (Jisha et al., 2013; Kataria et al., 2017).

Recently, with the use of biostimulants, the resistance of plants to abiotic stress conditions has been increased and thus agricultural production and quality increase has been provided (Boehme et al., 2008; Mahdavi et al., 2011; Safikhan et al., 2018).

Biostimulators are defined as substances that stimulate the development of the plant obtained from various organic and inorganic substances and also play an important role in reducing the effects of abiotic stress (Boehme et al., 2008; Mahdavi et al., 2011; Du Jardin, 2015). Biostimulant application is one of the approaches to reduce abiotic stress and increase the yield and quality of the product in most plants (Safikhan et al., 2018). Today, one of the biostimulant applications is chitosan application. It is a natural, non-toxic biopolymer obtained by deacetylation of chitosan chitin (Katiyar et al., 2015; Younes and Rinaudo, 2015). In addition, the Crustacea family of the chitin is an important ingredient in crustaceans (crab, shrimp, crayfish, etc.) (No et al., 2002; Gürsoy et al., 2018). It is also stated by the researchers that the chitin is a natural aminopolysaccharide that is abundant in nature (Ravi Kumar, 2000). In addition to being biologically renewable, chitosan is biodegradable, biocompatible, antigenic and non-toxic, and biofunctional structure, and this polymer and the materials obtained using this polymer have been used in biomedical applications such as wound dressing material and drug delivery systems (Kim et al., 2007; Hosseinnejad and Jafari, 2016; Muxika et al., 2017).

In plants, it has been reported that antimicrobial activity, stimulating plant growth and development, inducing chitinase activity and increasing seed yield in the seed coating (Tay, 1993; Tham, 2001; Vasyukova, 2001; Devlieghere et al., 2004). Plant growth, seed germination, chlorophyll content and ion uptake can be increased with chitosan application (Ahmed et al., 2020).

Safflower (*Carthamus tinctorius* L.) is an annual medicinal and aromatic oilseed crop (Kumar and Kumari, 2005; Moghadam and Mohammadi, 2014; Golkar and Taghizadeh, 2018). In addition, it is an oil plant with flowers in yellow, red, orange, colors, with and without thorns, resistant to drought, with an average oil rate of 30-50% (Gürsoy, 2019).

In this study, it was aimed to determine the effect of chitosan pretreatment on the development of safflower cultivars, photosynthetic activity and antioxidant enzyme activities in saline conditions.

Materials and methods

Research material and growth conditions

Safflower cultivars (Balci, Linas, Remzibey) were obtained from the Central Field Crops Research Institute, Ankara, Turkey. The research was carried out at the Aksaray University Scientific and Technological Research Laboratory (ASÜBTAM). Before commencing the experiment, seeds of cultivars were kept in 5% sodium hypochlorite solution for 5 minutes for surface sterilization. Then washed with pure water and subjected to 4 hours priming with different concentrations of chitosan (Ch) solutions. Chitosan (Sigma-Aldrich, medium molecular weight, at 85% -acetylated, viscosity 270 cP, CAS: 9012-76-4) after dissolving in 0.1% acetic acid of commercially available

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chitosan [0 (control) (Ch1), 0.2% (Ch2), 0.4% (Ch3), 0.6% (Ch4)]. For each Ch dose, 50 seeds were placed in sterile petri dishes on Whatman No:1 blotting papers and 10 mL of different doses of salt (0 (control) (S1), 50 mM (S2), 100mM (S3), 150mM (S4)) concentrations were added from solutions containing NaCl (Merck). Only water was added to the control petri dish. In order to prevent evaporation the petri dishes are wrapped with parafilm. The petri dishes were left to germinate at 24 ± 1^0C. The research randomized plots experimental desing were made with 3 replication according to the trial pattern. Measurements and observations were made on the 14th day of the study.

Germination percentage (%)

Germination percentage was calculated using the formula below.

$$\text{Germination\%} = (\text{number of germinated seeds} / \text{total number of seeds}) \times 100 \quad \text{(Eq.1)}$$

(Siddiqi et al., 2007)

Chlorophyll (mg/g)

The young leaf samples (0.25 g) from each safflower cultivar were filtered after homogenizing in 80% acetone (Merck) and the extracts were filtered with 25 ml with acetone. These samples were read at 663 nm and 645 nm wavelength (Beckman coulter DU 730 Life Sciences UV / VIS Spectrophotometer) followed by calculation of chlorophyll using the formula given below (Lichtenthaler, 1983; Amira and Qados, 2011; Kabay and Şensoy, 2016). Before each reading, the device was reset using blind reading.

$$\text{Chlorophyll a (mg/g)} = (12.7*663\ \text{nm})-(2.69*645\ \text{nm})*V/W*10000 \quad \text{(Eq.2)}$$

$$\text{Chlorophyll a (mg/g)} = (22.91*645\ \text{nm})-(4.68*663\ \text{nm})*V/W*10000 \quad \text{(Eq.3)}$$

$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b} \quad \text{(Eq.4)}$$

Carotenoid (mg/g)

The carotenoid amount was determined according to the Jaspars formula by reading the extract used in determining the chlorophyll amount at 450 nm wavelength (Beckman Coulter DU 730 Life Sciences UV / VIS Spectrophotometer) (Turfan, 2017).

$$\text{Carotenoid} = (4.07\times A450-(0.0435\times\text{Chlorophyll a}+0.367\times\text{Chlorophyll b}) \quad \text{(Eq.5)}$$

β-Carotene (mg/g)

100 mg sample was homogenized for 1 minute in a mixture of 10 ml acetone-hexane (92:3) and filtered. The absorbance of the filtrate at 453, 505 and 663 nm was recorded. It is preferred in the calculation of carotene amount. (Turfan, 2017).

$$\text{mg β-Carotene/100 mg} = 0.0458 \text{ x } A663+0.372 \text{ x } A505–0.0806 \text{ x } A453 \quad \text{(Eq.6)}$$

Lycopene (mg/g)

100 mg sample was homogenized for 1 minute in a mixture of 10 ml acetone-hexane (92:3) and filtered. The absorbance at 453, 505 and 663 nm was recorded in the filtered

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extract. The following formula was used to determine the amount of lycopene (Turfan, 2017).

$$\text{mg Lycopene/100 mg} = 0.216xA663 - 0.304xA505 + 0.452xA453 \quad \text{(Eq.7)}$$

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 means of applications.

Results

A statistically significant difference was determined at the level of $P < 0.01$ in Cultivars × Salt doses× Chitosan doses triple interaction (*Table 1*) in all the features studied.

Table 1. *Variance analysis results on the effect of chitosan pretreatment on seedling growth and antioxidant enzymes of safflower cultivars under saline conditions*

Variation sources	D f	Seedling Length		Root Length		Seedling Wet Weight		Root Wet Weight	
		Mean square	F	Mean square	F	Mean square	F	Mean square	F
Cultivar	2	0.185	1.85	0.02	1.64	0.002	5.78**	0.0005	3.66*
Salt	3	13.21	132.57**	0.547	44.69**	0.023	59.05**	0.003	28.00**
Cultivar×Salt	6	1.123	11.26**	0.038	3.104**	0.001	3.26**	0.002	13.42**
Chitosan	3	11.04	110.76**	1.702	139.15**	0.025	65.52**	0.015	122.46**
Cultivar×Chitosan	6	0.061	0.613	0.075	6.12**	0.001	1.38	0.001	6.98**
Salt×Chitosan	9	1.38	13.88**	0.239	19.52**	0.002	6.03**	0.001	9.70**
Cultivar×Salt×Chitosan	18	0.3	3.01**	0.068	5.546**	0.002	4.39**	0.001	8.25**
Error	96	0.1		0.012		0.0003		0.0001	
Total	143								
CV%		3.88		3.4		5.59		7.24	

Variation sources	D f	Germination percentage		Total chlorophyll		Carotenoid		β -Carotene		Lycopene	
		Mean square	F	Mean square	F	Mean square	F	Mean square	F	Mean square	F
Cultivar	2	1.646	4.74*	0.024	0.3922	0.25	6.18**	0.044	32.55**	0.008	9.18**
Salt	3	18.94	54.62**	7.145	115.54**	0.239	5.92**	0.134	98.147**	0.02	24.74**
Cultivar× Salt	6	1.741	5.02**	0.533	8.62**	0.251	6.22**	0.012	8.57**	0.003	3.58**
Chitosan	3	15.45	44.57**	8.061	130.35**	5.164	127.95**	0.313	229.61**	0.118	142.33**
Cultivar× Chitosan	6	0.137	0.395	0.124	2.008	0.343	8.48**	0.017	12.62**	0.002	2.378*
Salt×Chitosan	9	2.144	6.184**	0.634	10.24**	0.792	19.62**	0.065	47.69**	0.031	37.68**
Cultivar×Salt×Chitosan	18	0.882	2.543**	0.147	2.38**	0.325	8.051**	0.019	13.66**	0.003	4.03**
Error	96	0.347		0.062		0.04		0.001		0.001	
Total	143	----------		----------		----------		----------		----------	
CV%		0.60		10.02		6.16		5.96		4.74	

** $P<0.01$, *$P<0.05$

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When the seedling length feature is examined (*Table 2*), the longest seedling length was obtained from Balcı variety in S1 Ch3 application as 10.00 cm. The minimum seedling length was obtained in S4 Ch4 application and Balcı variety. Generally, the longest seedling length in all cultivars has been obtained in Ch3 application, and it is determined that chitosan is effective in eliminating the harmful effects of salt while increasing the salt doses. Chitosan application seems to inhibit the effects of salt stress, causing the seedling to grow higher. However, chitosan application had different effects on varieties. For example, in S1 and S4 applications in Balcı cultivar, it is seen that the seedling height is longest in Ch3. The same is true for the Linas variety. In Remzibey cultivar, Ch3 was found to be the longest seedling length only in S1 application, and in all other salt doses, the longest seedling length was detected in Ch1 application. In the Remzibey cultivar, a positive effect of chitosan applications against salt doses in terms of seedling length could not be determined. Cultivars × S doses × Chitosan doses interaction caused significant difference in seedling length feature at $p<0.01$ level. In this respect, when the study results were examined, it was determined that the harmful effects of salt doses were inhibited by interaction with chitosan. Similarly, salt doses interacted with varieties and showed different reactions in each variety.

Table 2. *Average values the effect of chitosan doses applied to safflower cultivars on saline length (cm) under saline conditions*

Cultivars	Seedling Length (cm)							
	S1				S2			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	9.53 ab	7.52 n-r	10.00 a	7.82 ı-q	9.35 a-c	8.47 d-l	8.63 c-ı	7.94 h-p
Linas	9.56 ab	8.54 d-j	9.89 a	8.22 d-n	8.82 b-f	7.77 j-q	8.21 d-n	7.90 h-q
Remzibey	8.90 b-e	8.68 c-h	9.74 a	7.96 g-p	8.75 c-g	7.62 m-r	7.63 m-r	7.52 n-r
	S3				S4			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	8.23 d-n	7.74 j-q	7.26 p-s	7.74 j-q	7.74 j-q	6.74 st	8.10 e-o	6.40 t
Linas	8.92 b-d	7.68 k-r	8.30 d-n	8.07 f-p	7.84 ı-q	7.12 q-t	7.86 ı-q	6.45 t
Remzibey	8.86 b-f	7.66 l-r	8.50 d-k	8.36 d-m	8.21 d-n	7.29 o-s	7.93 h-q	6.92 r-t
LSD%1	0.6786							

According to the Duncan test dissimilar letters in the column show different groups

In terms of root length (*Table 3*), the longest root length (3.80 cm) was detected in Remzibey variety and S1 Ch1 application. The shortest root length was determined as 2.59 cm in Remzibey cultivar and S4 Ch4 application. It is seen that root length increases with increasing Ch doses in all cultivars. However, this increase didn't occur only in the S1 dose, and it is the dose that does not apply salt since the S1 dose is a control application. In other words, with the application of salt, the effectiveness of chitosan has been revealed and it has been effective in lengthening the root length by inhibiting the negative effects of salt. Although this effect was determined in all varieties, the most effective chitosan application was determined as Ch3. Cultivars × S doses × Chitosan doses were determined statistically significant differences in root length characteristics in terms of $p<0.01$. With this interaction in all cultivars, root length feature was affected and increased due to salt doses and chitosan doses.

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Table 3. *Average values of the effect of chitosan doses applied to safflower varieties under saline conditions on root length (cm)*

Cultivars	Root Length (cm)							
	S1				S2			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	3.30 e-k	3.39 d-j	3.64 a-d	3.22 g-n	3.11 j-p	3.20 g-n	3.46 b-g	3.16 h-p
Linas	3.29 e-l	3.30 e-k	3.40 c-ı	3.23 f-n	3.00 l-q	3.20 g-n	3.60 a-d	3.17 h-o
Remzibey	3.80 a	3.42 c-h	3.50 b-f	3.14 h-p	3.11 j-p	3.13 ı-p	3.64 a-d	3.06 k-q
	S3				S4			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	2.98 n-q	3.04 k-q	3.72 ab	2.97 n-q	2.99 m-q	3.07 k-q	3.45 b-g	2.63 r
Linas	2.97 n-q	3.15 h-p	3.66 abc	2.99 m-q	2.88 pq	3.52 b-e	3.22 g-n	2.80 qr
Remzibey	3.04 k-q	3.15 h-p	3.27 e-m	3.06 k-q	2.90 o-q	3.65 a-d	3.48 b-g	2.59 r
LSD%1	0.2351							

*According to the Duncan test dissimilar letters in the column show different groups

Seedling wet weight (*Table 4*) was determined as the most seedling wet weight of 0.45 g in Linas cultivar in S1 Ch3 application. The minimum seedling wet weight was determined as 0.28 g in S4 Ch1 application, that is, when S doses are highest but Ch dose is control, ie 0%. In all other applications, seedling wet weight increased with Ch application. Cultivars × S doses × Chitosan doses were determined statistically significant differences in seedling wet weight in terms of interaction with $p<0.01$. All cultivars were significantly affected by S and Ch doses. While the application of Ch3 in S2, S3 and S4 in the Balcı cultivar causes the formation of seedling wet weight the most, Ch3 application in all salt doses in Linas and in S2 and S3 in Remzibey were effective in obtaining the seedling wet weight the most. In S1 and S4, the dose of Ch3 was more effective.

Table 4. *Average values the effect of chitosan doses applied to safflower varieties under saline conditions on seedling wet weight (g)*

Cultivars	Seedling Wet Weight (g)							
	S1				S2			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	0.41 a-c	0.34 g-o	0.38 c-g	0.34 g-o	0.33 h-p	0.40 b-e	0.44 ab	0.31 k-p
Linas	0.35 g-n	0.40 b-f	0.45 a	0.35 g-n	0.34 g-n	0.37 c-j	0.41 b-d	0.33 h-p
Remzibey	0.35 g-n	0.38 c-h	0.44 ab	0.35 f-m	0.31 k-p	0.37 c-j	0.36 d-k	0.33 g-o
	S3				S4			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	0.31l-p	0.35 f-m	0.38 c-g	0.35 g-n	0.30 m-p	0.36 e-l	0.35 g-n	0.32 j-p
Linas	0.32 j-p	0.35 g-n	0.37 c-ı	0.35 g-n	0.30 n-p	0.33 h-p	0.34 g-n	0.31 k-p
Remzibey	0.32 ı-p	0.36 e-l	0.35 g-n	0.35 g-n	0.28 p	0.32 j-p	0.33 h-p	0.29 op
LSD%1	0.04183							

* According to the Duncan test dissimilar letters in the column show different groups

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In the root wet weight feature (*Table 5*), the maximum root wet weight was determined in Remzibey cultivar in Ch3 application in S1. Although S1 was a control dose, Ch affected root caused an increase in wet weight. However, in other S doses, the root wet weight increased with the increase of Ch. In terms of root wet weight, the most effective Ch doses were Ch2 and Ch3. Cultivars × S doses × Chitosan doses interaction showed statistically significant difference at $p<0.01$ in terms of root wet weight.

Table 5. *Average values of the effect of chitosan doses applied to safflower varieties under saline conditions on root wet weight (g)*

Cultivars	Root Wet Weight (g)							
	S1				S2			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	0.14 g-m	0.12 l-n	0.15 f-l	0.16 d-ı	0.17 d-f	0.15 e-k	0.21b	0.14 g-m
Linas	0.16 d-h	0.14 f-l	0.18 cd	0.15 e-k	0.17 d-f	0.18 cd	0.18 c-e	0.14 g-m
Remzibey	0.15 e-k	0.15 d-j	0.24 a	0.16 d-h	0.13 j-n	0.17 d-f	0.16 d-h	0.14 g-m
	S3				S4			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	0.14 h-n	0.15 d-j	0.17 d-f	0.15 f-l	0.12 l-n	0.13 ı-n	0.18 cd	0.13 ı-n
Linas	0.13 ı-n	0.16 d-h	0.20 bc	0.15 e-k	0.11 n	0.15 e-k	0.17 d-g	0.12 l-n
Remzibey	0.11 mn	0.16 d-h	0.21 ab	0.12 k-n	0.15 f-l	0.15 d-j	0.15 d-j	0.11n
LSD%1	0.02351							

* According to the Duncan test dissimilar letters in the column show different groups

In germination percentage (*Table 6*), the highest germination rate was determined as 99.97% in Balcı cultivar in S2 Ch1 application. The least germination was detected in Linas cultivar and Ch4 application with 95.73%. Cultivars × S doses × Chitosan doses interaction showed statistically significant difference in $p<0.01$ level in terms of germination percentage. In all cultivars, the germination rate decreased with increasing salt doses. However, an increase in germination rate was determined in all cultivars and all S doses with Ch applications. For example, in the Remzibey cultivar, it is seen that the germination percentage is highest with the application of Ch3 at the dose of S4. Similarly, the most germination was determined in S3 and S4 in Ch3 in Balcı cultivar.

In terms of total chlorophyll (*Table 7*) Cultivars × S doses × Chitosan doses interaction showed statistically significant difference at $p<0.01$ level. Differences between varieties were determined with chitosan applications in applied salt doses. Although chlorophyll amount is expected to decrease with increasing salt doses, it has been determined that it increases with chitosan applications. Chitosan had a positive effect by eliminating the negative effect of salt by playing an encouraging role. The highest chlorophyll content was obtained in S1 and Ch1 and the lowest chlorophyll was determined in S4 Ch4 as 1.04 mg g^{-1}. However, the effects of S and Ch doses on varieties were in different ways. While S3, S3 and S4 increase Ch3 chlorophyll in the Balcı cultivar, it is determined that the application of Ch2 in S1 and S2 and Ch4 in S3 and S4 are more effective in linas cultivar. In Remzibey variety cultivar, Ch3 was determined as the most effective application dose in S2, S3 and S4.

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Table 6. *Average values of the germination percentage (%) of chitosan doses applied to safflower varieties under saline conditions*

Cultivars	Germination percentage (%)							
	S1				S2			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	99.60 a-c	98.73 a-ı	99.60 a-c	98.40 b-k	99.97 a	98.80 a-ı	98.40 b-k	98.57 a-j
Linas	99.57 a-d	99.10 a-f	99.80 ab	97.57 f-q	98.43 b-k	97.97 e-p	98.13c-m	98.07 d-o
Remzibey	99.43 a-e	98.80 a-ı	98.33 b-l	98.10 c-n	97.90 e-p	96.90 k-s	98.27 c-l	97.73 f-p
	S3				S4			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	97.43 h-q	97.43 h-q	98.97 a-h	96.60 n-s	97.53 g-q	96.57 o-s	98.40 b-k	96.50 p-s
Linas	98.07 d-o	96.83 l-s	98.40 b-k	97.03 j-s	98.00 e-p	96.73m-s	97.73 f-p	95.73 s
Remzibey	97.33 ı-r	96.20 q-s	99.03 a-g	96.93 k-s	98.07 d-o	98.07 d-o	98.87 a-ı	95.87 rs
LSD%1	1.264							

* According to the Duncan test dissimilar letters in the column show different groups

Table 7. *Average values the effect of chitosan doses applied to safflower varieties under saline conditions on total chlorophyll (mg g^{-1} FW)*

Cultivars	Total chlorophyll (mg g^{-1})							
	S1				S2			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	3.92 a	2.81 c-k	1.95 n-q	2.13 l-q	3.31 bc	2.41 g-p	3.23 b-d	2.37 ı-p
Linas	3.32 bc	3.04 b-g	2.93 c-j	3.02 c-h	3.00 c-ı	2.33 j-q	2.95 c-j	2.04 m-q
Remzibey	3.64 ab	3.18 b-e	3.08 b-f	3.00 c-ı	2.80 c-k	2.05 m-q	2.60 d-m	2.09 l-q
	S3				S4			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	3.31 bc	1.95 n-q	2.70 c-l	1.99 m-q	2.57 e-n	1.92 o-q	2.23 k-q	1.30 rs
Linas	3.26 bc	1.92 o-q	2.51 f-o	1.84 p-r	2.26 k-q	1.93 n-q	2.15 l-q	1.14 s
Remzibey	3.08 b-f	2.04 m-q	2.39 h-p	1.72 qr	2.55 e-o	1.84 p-r	2.26 k-q	1.04 s
LSD%1	0.5343							

* According to the Duncan test dissimilar letters in the column show different groups

When the amount of carotenoid is examined (*Table 8*), the most carotenoid amount is determined as S4 Ch3 as 4.09 mg g^{-1}. The minimum amount of carotenoids was determined as 1.59 mg g^{-1} in S1 Ch1. It was determined that as the S doses increased, the amount of carotenoids increased in all cultivars. However, it is seen that this increase is higher in Ch3. Cultivars × S doses × Chitosan doses interaction showed statistically significant difference at $p<0.01$ in terms of carotenoid amount. In all varieties, especially in S2, S3 and S4, the carotenoid decreased slightly in Ch2, but increased in Ch3.

When examined in terms of β-carotene (*Table 9*), the most β-carotene was found as S4 and Ch3 application in Balcı cultivar as 0.86 mg g^{-1}. It was determined as at least β-carotene 0.24 mg g^{-1} FW in S1 Ch1 application and Balcı variety. However, the

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interaction of Cultivars × S doses × Chitosan doses showed a statistically significant difference in $p<0.01$ level in terms of β-carotene. In terms of β-carotene, the effects of S and Ch doses on varieties were determined, and the highest β-carotene amount in Ch3 was determined in all S doses. Especially in S3, which has the highest salt dosage, the highest β-carotene was determined in all cultivars.

Table 8. *Average values the effect of chitosan doses applied to safflower varieties on saline conditions on the amount of carotenoid (mg g^{-1} FW)*

Cultivars	Carotenoid (mg g^{-1} FW)							
	S1				S2			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	1.59 r	2.67 n-q	3.77 a-e	3.66 a-h	2.96 k-p	2.66 o-q	3.68 a-f	3.66 a-g
Linas	2.21 q	2.84 m-p	3.65 a-h	3.59 a-ı	3.59 a-ı	2.85 m-p	3.58 a-j	3.27 e-m
Remzibey	3.68 a-f	2.72 n-p	3.77 a-e	3.60 a-ı	3.60 a-ı	3.09 ı-o	3.41 c-l	3.55 b-j
	S3				S4			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	2.96 k-p	3.62 a-h	3.45 c-k	3.33 d-m	2.91 l-p	3.31 d-m	3.81 a-d	3.24 f-m
Linas	3.14 h-o	3.07 j-o	3.81 a-d	3.14 h-o	2.56 pq	3.15 g-o	3.99 ab	3.26 e-m
Remzibey	2.94 k-p	3.27 e-m	3.88 a-c	3.17 f-n	2.65 o-q	3.01 k-p	4.09 a	3.03 k-p
LSD%1	0.4292							

* According to the Duncan test dissimilar letters in the column show different groups

Table 9. *Average values the effect of chitosan doses applied to safflower varieties on saline conditions on the amount of β-carotene (mg g^{-1} FW)*

Cultivars	β-Caroten (mg g^{-1} FW)							
	S1				S2			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	0.24 p	0.50 ı-l	0.69 bc	0.69 bc	0.39 no	0.37 o	0.65 b-f	0.65 b-e
Linas	0.69 b	0.42 m-o	0.69 b	0.64 b-f	0.51 ı-l	0.48 k-m	0.84 a	0.68 b-e
Remzibey	0.52 ı-l	0.36 o	0.66 b-e	0.60 e-h	0.55 h-k	0.48 j-m	0.71b	0.63 b-g
	S3				S4			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	0.68 b-d	0.67 b-e	0.66 b-e	0.65 b-e	0.61 c-h	0.68 b-e	0.86 a	0.54 h-k
Linas	0.69 bc	0.69 bc	0.82 a	0.65 b-f	0.57 f-ı	0.71 b	0.82 a	0.56 g-j
Remzibey	0.65 b-f	0.65 b-f	0.85 a	0.60 d-h	0.55 h-k	0.70 b	0.80 a	0.45 l-n
LSD%1	0.0678							

* According to the Duncan test dissimilar letters in the column show different groups

When the study results are examined in terms of lycopene (*Table 10*), the most lycopene was determined as 0.81 mg g^{-1} in Linas and Remzibey varieties in S4 and Ch3. The least lycopene was determined as 0.44 mg g^{-1} in S2 Ch1. Especially in S3 and S4 doses in which salt doses increased, the highest lycopene in Ch3 was determined. As in carotene, S4, which has a high salt dose in lycopene, has the highest lycopene content in all varieties in Ch3 application.

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Table 10. *Average values the effect of chitosan doses applied on safflower varieties on saline conditions on the amount of lycopene (mg g^{-1} FW)*

Cultivars	Lycopene (mg g^{-1} FW)							
	S1				S2			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	0.55 f-k	0.60 d-g	0.60 c-g	0.69 b	0.44 l	0.60 c-g	0.63 b-f	0.62 b-g
Linas	0.51 ı-l	0.57 d-ı	0.62 b-g	0.64 b-e	0.49 kl	0.64 b-e	0.60 c-g	0.63 b-f
Remzibey	0.52 h-k	0.64 n-d	0.60 d-g	0.64 b-d	0.54 g-k	0.61 b-g	0.59 d-ı	0.64 b-e
	S3				S4			
	Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4
Balcı	0.49 j-l	0.60 d-h	0.60 d-h	0.59 d-h	0.56 e-k	0.60 c-g	0.78 a	0.56 d-k
Linas	0.55 f-k	0.60 c-g	0.68 bc	0.57 d-ı	0.62 b-g	0.57 d-j	0.81a	0.56 d-k
Remzibey	0.54 g-k	0.60 c-g	0.78 a	0.59 d-h	0.63 b-f	0.58 d-ı	0.81a	0.59 d-h
LSD%1	0.06786							

* According to the Duncan test dissimilar letters in the column show different groups

Discussion

In this study, in which different doses of salt were applied with different doses of chitosan pretreatment to the safflower varieties, although the results were evaluated, there was a decrease in some morphological features due to the increase of salt doses, and with the application of chitosan, the characteristics of seedling length, root length, root wet weight, seedling wet weight and germination rate an increase was observed. However, there are increases in biochemical properties with the effect of chitosan application in stress conditions. The most effective chitosan dose was determined as Ch3 in all the properties studied. Ma et al. (2012) reported that application of 0.0625% oligochitosan to the nutrient solution in which wheat seeds were grown reduces the negative effect of salt stress. Jabeen and Ahmad (2013), applied chitosan to sunflower and safflower varieties under salty conditions. As a result of the study, they reported that low concentration of chitosan application caused increase in germination parameters of both cultivars. In the study conducted by Sheikha and Al-Malki (2011), they reported that chitosan applications had an effect on the plant's growth parameters, such as seedling and root height, wet and dry weight. Al-Tawaha et al. (2018), 81.94 cm in plant height control application, 84.06 cm in 30 mg/L chitosan application, 84.38 cm in 60 mg/L chitosan application, 84.81 in 60 mg/L chitosan application, they reported that they had determined in cm. Salt stress affects chlorophyll metabolism and causes a significant decrease in chlorophyll production (Qin et al., 2019). Chlorophyll content is widely used in plants as an indicator of abiotic stress tolerance. In addition, chlorophyll decreases in plants exposed to stresses such as salinity, as a result of which growth is delayed (Safikhan et al., 2018). In recent studies, it has been reported by researchers that chitosan increases chlorophyll content in soybean and peanuts (Dzung, 2005). Yahyaabadi et al. (2016), applied chitosan to fenugreek plants under salt stress. As a result of the study, they reported that the application was effective in promoting plant growth by reducing salt stress on the water content of the leaf and photosynthetic pigment parameters. Turfan (2017), applied various abiotic stresses (salt, heavy metal, drought and lime) to the spinach plant. As a result of the study, it was reported that chlorophyll a, b, total chlorophyll and carotenoid,

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β-carotene and lycopene content increased in drought and lime stress applications. Stahl and Sies (2003), reported that carotenoids are pigments that play a very important role in the protection of plants against photooxidative processes in plants and are antioxidants that play an extremely active role in eliminating the harmful effects of free oxygen radicals. In addition to having antioxidant capacity, carotenoids also play a very important role in reacting to various stress conditions (Boba et al., 2011). In plants under stress, the amount of photosynthetic pigment consisting of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid varies depending on factors such as species, type of stress, duration of stress, the period of the plant in the life cycle, and the intensity of stress (Turfan, 2017). Linic et al. (2019), it is suggested that carotenoids, which are specific metabolites, can play a positive role as natural substances in stress management in tolerant species. Rahman et al. (2018), reported that the application of chitosan to the strawberry plant in the form of a spray causes an increase in the carotenoid content of the plant. ß-carotene is an organic red-orange colored pigment that is abundant in plants (Pop et al., 2019). Falcinelli et al. (2017), reported that appropriate doses of salt cause increased phenolic compounds in rapeseed. He et al. (2020), reported that NaCl and $CaCl_2$ application can be used as a strategy that will increase the antioxidant activity through the accumulation of carotenoids as a result of their study in corn plant. In this study, it was determined that pre-application of chitosan to the seeds of safflower varieties against salt stress promotes the strengthening of the defense system by causing the enzyme activities of the varieties to increase.

Conclusion

In this study, besides the negative effects of salt doses, the positive effects of chitosan application, safflower varieties were affected at different levels. The most advantageous results were obtained from the Remzibey variety in terms of morphological (seedling length, root length, seedling wet weight, root wet weight, germination percentage) and biochemical (chlorophyll, carotenoid, β-carotene and lycopene) parameters. In addition, it has been determined that chitosan plays a role in promoting the increase of enzyme activities, which is the defense mechanism of plants under stress conditions. In this study, the third dose of chitosan (Ch3) caused the most advantageous results. As a result of the study, it was concluded that chitosan can be evaluated as a natural material that can be effective in the defense mechanism of plants under stress conditions. It has been concluded that with the pretreatment of chitosan in regions with salty soil conditions in the world, the germination and seedling development of plants can be increased accordingly it was concluded that yield and quality can be increased. However, applications should be made in other plants and under various stress conditions and their results should be evaluated.

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