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Original Article

Effects of Foliar Application of Chitosan on Some Vegetative Growth and Biochemical Parameters of Strawberry Under Salt Stress

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ABSTRACT

Aim/Background: This research, which was carried out as a pot experiment in a plastic greenhouse to examine the effects of three different salt (NaCl) doses (Control, 30, 60 mmol/L) and three different chitosan doses (Control, 1, 2 and 3 ppm) applied to Albion strawberry variety (*Fragaria ananassa* Duch.). **Materials and Methods:** In this study, plant characteristics such as leaf area, Fresh Weight (FW) and Dry Weight (DW) of root, crown and leaf were investigated. Biochemical parameters including chlorophyll analysis was determined by the method of Witham *et al*., carotenoid analysis by Krik and Allen, total phenolic compound content by Folin Ciocalteu colorimetric method and Malondialdehyde (MDA) content by Rao and Sresty in leaf tissues. **Results:** The effects of applied salt stress showed an initial increase in all studied parameters, followed by a decrease with increasing salt concentration (60 mmol/L). Plant weight, leaf area, chlorophyll a, chlorophyll b, total chlorophyll and carotenoid increased during the fruiting period. In the experiment, the effects of chitosan sprayed on strawberry leaves to reduce salt stress were found to be statistically significant some vegetative and biochemical parameters. Chitosan doses of 1 ppm (DW of leaf and leaf area) and 2 ppm (FW of root, DW of root and crown) increased plant growth. The effect of chitosan on total phenolic compounds and MDA was found to be statistically significant. The effect of chitosan on photosynthetic pigments was found to be statistically insignificant. **Conclusion:** Chitosan has been found to positively affect plant growth in strawberries improving yield and quality characteristics under salt stress.

Keywords: Strawberry (*Fragaria ananassa)*, Chitosan, Salt Stress, Phenolic Compound, Vegetative Growth.

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INTROCUCTION

Modern cultivated strawberries (*Fragaria x ananassa* Duch) belong to the *Rosaceae* family[1] and are a hybrid of two wild strawberry species, *Fragaria virginiana* and *Fragaria chiloensis*.[2] Strawberries are fragrant, fleshy and juicy false fruits. Strawberry flavor is the result of a complex mixture of numerous volatile and organoleptic compounds combined with properties such as aroma and taste.[3]

Plants encounter numerous biotic and abiotic environmental stress factors throughout their lives and must exhibit at least some tolerance to these adverse conditions. Biotic and abiotic environmental stress factors are major constraints that can limit plant growth and productivity, leading to significant losses in yield and biodiversity.[4,5] Biotic factors include infection-causing microorganisms (fungi, bacteria, viruses), pests (insects, nematodes) and competition with other plants. Abiotic (physicochemical) factors, on the other hand, encompass environmental changes such as extreme temperatures, drought, heavy metals, nutrient deficiencies and metal toxicity.[6,7]

Soil salinity is the primary environmental factor limiting crop yield in horticultural crops. Recent research on food security has highlighted the issue of soil salinity as a critical concern.[8] Soil salinity predominantly occurs in arid and semi-arid regions characterized by reduced rainfall. In such environments, irrigation can rapidly exacerbate salinization. During evaporation, salts from the lower soil layers are transported upwards via capillarity and accumulate in the root zone of plants. The causes of salinization include inadequate drainage, excessive salt content in irrigation water and improper irrigation practices.[9,10]

The effect of saline water on the osmotic potential of plants and soil results in a decrease in water availability due to restricted water uptake by plants. Additionally, excessive Na^+ and Cl^- uptake can lead to limited assimilation, transport and distribution of mineral nutrients, as well as nutrient imbalances within

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plants.[11,12] The exclusion or sequestration of toxic Na^+ and Cl^- ions in meristematic tissues and reproductive organs is a critical plant physiological mechanism for conferring salinity tolerance.[11-14]

Strawberries are among the most sensitive species to soil salinity.[15,16] Strawberry plants begin to experience salt stress in soils with an electrical conductivity exceeding 2 mmhos/cm or containing more than 960 ppm (960 mg/L) of soluble salts. This negatively impacts the vegetative and generative growth of strawberry plants, leading to reductions in yield levels.[17] Additionally, leaf number and leaf surface area rapidly decrease with increasing environmental salinity. The primary reason for this is the increase in solution osmotic pressure caused by the NaCl compound.[18] Strawberry cultivation is suitable for both greenhouse and open field production and has a high adaptability to different ecological conditions. However, one of the main problems in its cultivation is salinity, which restricts plant growth. Salinization poses a significant challenge in greenhouse cultivation, one of the most intensive forms of agricultural production, due to factors such as continuous and intensive use of a specific area and heavy fertilization.[19]

Chitin is the second most abundant polysaccharide on Earth after cellulose. Chitin is a long-chain homopolymer of (1-4)-linked 2-acetamido-2-deoxy-β-d-glucan, which is a derivative of glucose known as N-Acetyl-d-Glucosamine (GlcNAc).[20] Chitosan is a derivative obtained by partial or complete deacetylation (removal of the acetyl functional group from an organic compound) of chitin in an alkaline medium. Its basic structure is poly-[β-(1,4)-2-amino-2-deoxy-β-D-glucopyranose].[21] Chitosan, which can be obtained in abundance from many natural sources containing chitin such as mushrooms, crayfish, shrimp and the exoskeleton of crabs, is more advantageous than other biopolymers including chitin in terms of its non-toxicity to organisms, easy biodegradability and biocompatibility.[22] Consequently, chitosan is a natural, safe and inexpensive biopolymer with diverse applications across various industrial sectors, including food, medical, pharmaceutical, cosmetics, agriculture, wastewater treatment and textiles.[23,24] Chitosan, which has recently increased its use in agriculture, has antiviral, antibacterial and antifungal properties and is an effective agent in controlling and reducing the spread of diseases by activating the defense system of plants.[24-26]

Studies have shown that chitosan promotes plant growth, protects the safety of edible products and enhances plant defense responses against abiotic and biotic stresses in various horticultural crops.[27] In the first study in which the stimulatory effect of chitosan was determined in pea (*Pisum sativum* L.) and tomato (*Solanum lycopersicum* L.) plants, it was shown to increase defense responses against abiotic and abiotic stress.[28] An initial oxidative burst with Hydrogen Peroxide (H_2O_2) accumulation was observed in different plants and plant cell cultures upon chitosan application.[26,29,30] This is thought to lead to the synthesis of secondary metabolites, such as polyphenols, lignin, flavonoids and phytoalexins and the induction of plant defense enzymes.[26] Its use to protect plants against biotic stress is generally in two forms: the first is to coat the product/seed to protect it from pathogens, or the second is to apply it to plants before possible infection to activate the resistance mechanism. Due to its properties of helping to strengthen plant defense against pathogens and various stress factors and increasing crop yield, research has focused on these areas in recent years.[31,32]

Strawberry, which is an important fruit species with its high anthocyanin and antioxidant capacity both in our country and in the world, was preferred because its growing conditions are relatively faster and safer for scientific studies. The project covers the determination of the resistance of chitosan applications to salt stress and some changes in plant growth (biochemical changes and plant development parameters) in the strawberry plant, which has economic importance worldwide. In our study, it was aimed to improve the toxicity of strawberry roots caused by NaCl (Control, 30 and 60 mmol/L) by foliar application of chitosan at different concentrations (Control, 1, 2 and 3 ppm).

MATERIALS AND METHODS

Materials

This study was carried out in the greenhouse area of the Department of Crop and Animal Production in Amasya University Suluova Vocational School Campus (520m, 40°50'42.8' N and 35°38'0.08' E) during the production period of 2021. Neutral day strawberry variety "Albion" obtained from a commercial company was used as material. 2 L sterilized plastic pots were filled with a 1:1 mixture of peat: perlite and frigo strawberry seedlings were transplanted on 04/06/2021. Before transplanting, the seedlings were soaked in water for one hour. One seedling was planted in each pot.

Chitosan and Salt Applications

After transplanting, when the seedlings had 3-4 leaves, Control, 1, 2 and 3 ppm chitosan was applied to the seedlings by foliar spraying on 08/07/2021. Salt applications were started 24 hours after this application. A salt solution containing Control, 30 and 60 mmol/L NaCl was applied to the pots twice a week at 100 mL and the excess solution was drained. Salt applications continued for 6 weeks. The control groups were watered with irrigation water in the designed experiments. Throughout the study, the pots were watered when the soil surface became dry to maintain soil moisture. Before the flowering period of the plants, ammonium sulfate fertilizer was applied to the pots at a rate of 20 kg/da.

Plant Sampling and Vegetative Growth Analysis

Three plants were harvested from each treatment on 10/ 08/ 2021 (flowering; two months after transplanting) and on 10 /09/ 2021 (fruiting stage; three months after transplanting). The plants were extracted from their pots and immediately separated into their constituent parts: crown, root and leaf. Then Fresh Weight (FW)

Table 1: Effect of salt and chitosan treatments on Dry Weights (DW) of leaf, root and crown of strawberry during flowering and fruiting periods.

Flowering Period														
	DW of Leaf (g)					DW of Root (g)				DW of Crown (g)				
	CHT (ppm)					CHT (ppm)				CHT (ppm)				
Salt App. (mM)	0	1	2	3	Mean	0	1	2	3	Mean	0	1	2	3	Mean
S0	2.52±0.45	2.42±0.47	2.71±0.49	2.41 ±0.40	2.51±0.41	5.13±0.74	5.69±1.32	5.05±0.31	1.88±0.62	4.44±1.72	1.72±0.49	3.85±1.30	1.39±0.26	0.72±0.04	1.92±1.36
S30	2.57±0.33	2.54±0.23	2.73±0.23	2.21±0.12	2.51±0.28	3.81±0.24	3.75±0.46	5.77±0.33	3.68±0.80	4.25±1.01	1.63±0.40	0.32±0.15	4.89±0.27	2.19±0.82	2.26±1.78
S60	3.07±0.70	1.50±0.31	2.41±0.28	2.29±0.50	2.31±0.71	3.94±0.41	5.99±1.38	6.87±0.51	2.79±0.59	4.89±1.82	2.30±0.80	5.75±0.16	4.58±0.40	0.92±0.36	3.39±2.01
Mean	2.72±0.52	2.15±0.58	2.62±0.34	2.30±0.34	2.45±0.49	4.29±0.76	5.14±1.44	5.89±0.86	2.78±0.97	4.53±1.54	1.88±0.60	3.33±2.47	3.62±1.70	1.28±0.82	2.52±1.80
Fruiting Period															
S0	3.79±0.36	5.02±0.33	3.47±0.17	4.38±0.16	4.16±0.65	5.73±0.05	4.93±0.75	5.46±0.25	4.30±0.80	5.11±0.74	2.08±0.27	1.30±0.30	2.61±0.23	1.48±0.36	1.86±0.59
S30	2.36±0.52	4.99±0.41	3.37±0.39	3.06±0.55	3.44±1.08	4.74±0.27	3.26±0.68	4.60±0.60	6.76±1.27	4.84±1.46	1.31±0.22	0.60±0.16	0.57±0.04	0.52±0.05	0.75±0.35
S60	3.15±0.27	3.49±0.40	2.30±0.45	2.72±0.19	2.91±0.55	2.34±0.36	3.38±0.17	2.18±0.46	2.63±0.38	2.63±0.57	0.87±0.17	0.62±0.07	0.36±0.05	0.48±0.07	0.58±0.21
Mean	3.10±0.70	4.50±0.82	3.05±0.64	3.38±0.81	3.51±0.93	4.27±1.52	3.86±0.95	4.08±1.52	4.56±1.95	4.19±1.49	1.42±0.56	0.84±0.38	1.18±1.08	0.82±0.52	1.06±0.70
CHT Mean	2.91±0.63[b]	3.32± 1.39[a]	2.83±0.54[b]	2.84±0.82[b]		4.28±1.17 [b]	4.50±1.36[b]	4.99±1.52[a]	3.67±1.75[c]		1.65±0.61[bc]	2.07±2.13[ab]	2.40±1.86[a]	1.05±0.71[c]	

Means expressed with different letters in the same column were found to be significantly different at the 5% level according to the Duncan test. CHT: Chitosan, App: Application.

of each component (g) was determined using a 0.001 g precision balance. The plant parts were dried at 70°C to a constant weight and weighed to determine their Dry Weight (DW).[33] Leaf area (cm^2) according to Demirsoy[34] were determined.

Biochemical Analyzes

Chlorophyll analysis was determined by the method of Witham *et al.*,[35] carotenoid analysis by Krik and Allen,[36] total phenolic compound content by Folin Ciocalteu colorimetric method[37] and Malondialdehyde (MDA) content according to Rao and Sresty,[38] each analysis being repeated three times.

Statical Analyses

The experiment was set up using the Randomized Complete Block Design with three different salt concentrations (Control, 30 and 60 mmol/L) and four different chitosan doses (Control, 1, 2 and 3 ppm) applied to the Albion strawberry variety. There were three replications with 15 seedlings per replication. The comparison of averages of each treatment was analyzed by Analysis of Variance (ANOVA) using SPSS version 22 and Duncan's multiple range test to at significance level 5% ($p \leq 0.05$).

RESULTS AND DISCUSSION

Vegetative Growth Parameters

In this study, chitosan-treated strawberry plants successfully coped with prolonged exposure to salt stress (Control, 30 and 60 mmol/L NaCl) as evidenced by morphological and biochemical measurements.

Dry and Fresh Weight (g)

The physiological effect of different levels of salt application used in the study on strawberry plants was in the form of decreases in the biomass weight of the plants (Tables 1, 2). Dry Weight (DW) and Fresh Weight (FW) of leaf, crown and root of strawberry plants and leaf area, adversely affected by salt applications. When the averages of DW of leaf at flowering and fruiting periods in the experiment were examined, it was determined that the highest values were obtained from the control treatment in which salt was not applied and decreased by 7.96% and 30.48%, respectively, as the amount of salt increased. In the experiment, FW of root were most affected by salt stress treatments. As the salt application increased, 49.68% in the flowering period and 35.91% in the fruiting period decreased compared to the control. Similarly, FW of crown decreased by 19.10% during the flowering period and 48.73% during the fruit development period compared to the control and were affected by salt treatments. Salt stress also results in a considerable decrease in the FW of leaves, crowns and roots in strawberry[39-41] in parallel with our results. The reduction in growth parameters observed in our study under elevated salt stress conditions can be explained by the induction of osmotic stress in strawberry plants, as previously reported by Turhan[42]

Table 2: Effect of salt and chitosan treatments on Fresh Weights (FW) of leaf, root and crown of strawberry during flowering and fruiting periods.

Flowering Period															
	FW of Leaf (g)					FW of Root (g)					FW of Crown (g)				
	CHT (ppm)					CHT (ppm)					CHT (ppm)				
Salt App. (mM)	0	1	2	3	Mean	0	1	2	3	Mean	0	1	2	3	Mean
S0	7.01±0.40	6.71±0.64	6.70±0.85	5.08±1.12	6.37±1.04	4.33±0.15	3.26±0.33	19.33±0.41	5.28±0.74	8.05±6.85	3.77±0.17	3.82±0.10	3.38±0.35	4.30±0.96	3.82±0.56
S30	5.64±1.07	7.00±0.79	7.06±0.55	10.99±2.40	7.66±2.38	4.61±0.41	5.68±0.52	2.11±0.11	6.87±0.06	4.82±1.85	3.70±0.84	3.72±0.41	2.43±0.03	1.65±0.67	2.87±1.04
S60	6.23±0.35	8.23±0.41	9.90±1.50	4.60±0.34	7.24±2.20	3.49±0.43	4.90±0.47	4.42±0.07	3.38±0.20	4.05±0.72	3.19±0.21	2.54±0.09	3.06±0.29	3.57±0.24	3.09±0.43
Mean	6.29±0.84	7.31±0.89	7.88±1.76	6.87±3.33	7.09±1.98	4.14±0.59	4.62±1.13	8.62±8.09	5.17±1.56	5.64±4.37	3.55±0.52	3.36±0.65	2.96±0.48	3.17±1.32	3.26±0.81
Fruiting Peroid															
S0	13.28±3.64	15.95±4.49	12.91±2.93	11.38±1.38	13.38±3.31	21.93±3.88	24.30±9.56	24.78±9.73	16.86±2.15	21.97±6.94	3.73±1.05	4.19±0.66	5.14±2.54	4.36±0.47	4.35±1.33
S30	13.72±2.24	14.20±5.13	15.51±4.12	15.20±6.58	14.66±4.15	13.97±2.03	15.26±4.86	15.88±3.80	15.96±3.20	15.27±3.20	3.42±0.97	2.16±0.45	2.66±0.55	1.57±0.54	2.45±0.90
S60	14.32±0.92	15.90±1.21	11.80±3.73	11.43±3.63	13.36±3.00	12.64±2.17	17.10±3.91	14.61±4.35	11.96±4.10	14.08±3.80	2.32±0.56	2.13±0.90	2.53±0.90	1.94±0.67	2.23±0.69
Mean	13.77±2.23	15.35±3.57	13.41±3.55	12.67±4.26	13.80±3.47	16.18±4.99	18.89±7.05	18.42±7.41	14.93±3.61	17.10±5.94	3.15±1.00	2.83±1.18	3.44±1.87	2.62±1.40	3.01±1.38
CHT Mean	10.03±4.18	11.33±4.84	10.64±3.93	9.77±4.76		10.16±7.08^{b}	11.75±8.82^{ab}	13.52±9.06^{a}	10.05±5.69^{b}		3.35±0.80	3.09±0.96	3.20±1.35	2.90±1.35	

Means expressed with different letters in the same column were found to be significantly different at the 5% level according to the Duncan test. CHT: Chitosan, App: Application.

Table 3: Effect of salt and chitosan treatments on leaf area and MDA of strawberry leaves during flowering and fruiting periods.

Flowering Period										
	Leaf Area (cm^2)					MDA (nmol /g TA)				
	CHT (ppm)					CHT (ppm)				
Salt App. (mM)	0	1	2	3	Mean	0	1	2	3	Mean
S0	212.53±30.71	161.49±34.78	118.88±15.65	143.45±13.49	159.08±41.92	9.29±1.79	8.23±3.06	7.52±6.13	5.93±2.16	7.74±3.40
S30	159.30±4.94	118.88±13.18	191.97±4.55	271.78±28.18	171.48±40.36	9.35±1.95	8.23±1.73	6.45±4.86	7.63±0.86	7.91±2.62
S60	177.84±14.16	117.37±18.36	182.72±11.30	96.72±20.50	143.66±41.49	8.30±0.41	7.33±1.29	7.80±4.09	5.97±1.19	7.35±2.11
Mean	183.22±28.97	132.58±30.01	164.52±35.86	151.98±55.20	158.08±41.96	8.98±1.43	7.93±1.92	7.26±4.46	6.51±1.55	7.67±2.69
Fruiting Period										
S0	253.82±16.61	371.79±22.02	204.97±3.57	166.04±13.63	249.15±81.84	7.39±4.60	4.94±1.31	5.35±2.73	4.90±1.64	5.65±1.76
S30	189.43±36.56	377.81±45.49	369.39±40.57	279.05±8.08	303.92±85.62	7.26±1.65	5.91±2.07	3.89±0.43	5.14±0.92	5.55±1.76
S60	195.20±12.77	263.64±4.59	153.07±12.27	324.10±22.66	234.00±69.31	4.96±1.64	5.18±0.73	4.58±1.26	4.94±2.61	4.91±1.47
Mean	212.81±37.36	337.75±61.15	242.48±65.08	256.39±71.86	262.36±82.74	6.54±2.83	5.34±1.35	4.60±1.65	4.99±1.61	5.37±2.00
CHT Mean	198.02±35.82^{b}	235.16±115.44^{a}	203.50±83.23^{b}	204.19±82.16^{b}		7.76±2.51^{a}	6.64±2.09^{ab}	5.93±3.53^{b}	5.75±1.72^{b}	

Means expressed with different letters in the same column were found to be significantly different at the 5% level according to the Duncan test. CHT: Chitosan, App: Application.

Table 4: Effect of salt and chitosan treatments on chlorophyll a, chlorophyl b and total chlorophyl of strawberry leaves during flowering and fruiting periods.

Flowering Period															
	Chlorophyl a (mg/g FW)					Chlorophyl b (mg/g FW)					Total Chlorophyl (mg/g FW)				
	CHT (ppm)					CHT (ppm)					CHT (ppm)				
Salt App. (mM)	0	1	2	3	Mean	0	1	2	3	Mean	0	1	2	3	Mean
S0	0.23±0.06	0.21±0.04	0.20±0.07	0.27±0.04	0.23±0.05	0.28±0.03	0.28±0.06	0.22±0.02	0.23±0.003	0.25±0.04	0.51±0.06	0.49±0.10	0.42±0.09	0.50±0.05	0.48±0.07
S30	0.24±0.005	0.67±0.52	0.23±0.10	0.38±0.21	0.38±0.30	0.32±0.03	0.32±0.15	0.22±0.06	0.40±0.33	0.32±0.17	0.56±0.003	1.00±0.67	0.46±0.16	0.78±0.54	0.70±0.43
S60	0.17±0.02	0.18±0.05	0.50±0.16	0.33±0.18	0.29±0.17	0.23±0.05	0.20±0.02	0.32±0.06	0.26±0.11	0.25±0.07	0.40±0.07	0.38±0.08	0.83±0.17	0.60±0.26	0.55±0.23
Mean	0.21±0.04	0.35±0.35	0.31±0.17	0.32±0.14	0.30±0.21	0.27±0.05	0.27±0.10	0.25±0.06	0.29±0.19	0.27±0.11	0.49±0.08	0.62±0.44	0.57±0.23	0.62±0.32	0.58±0.29
Fruiting Period															
S0	0.37±0.11	0.25±0.08	0.27±0.04	0.34±0.08	0.31±0.08	0.30±0.09	0.26±0.06	0.26±0.03	0.28±0.04	0.28±0.05	0.67±0.20	0.52±0.14	0.54±0.05	0.63±0.11	0.59±0.13
S30	0.51±0.25	0.40±0.32	0.31±0.06	0.28±0.05	0.37±0.20	0.38±0.25	0.34±0.14	0.27±0.04	0.26±0.04	0.31±0.13	0.89±0.10	0.75±0.46	0.59±0.10	0.54±0.08	0.69±0.25
S60	0.27±0.06	0.28±0.09	0.25±0.09	0.18±0.05	0.24±0.07	0.26±0.04	0.41±0.17	0.25±0.06	0.24±0.04	0.29±0.11	0.53±0.08	0.70±0.27	0.50±0.15	0.42±0.08	0.54±0.17
Mean	0.38±0.17	0.31±0.18	0.28±0.06	0.26±0.09	0.31±0.14	0.31±0.14	0.34±0.13	0.26±0.04	0.26±0.04	0.29±0.10	0.70±0.20	0.65±0.29	0.54±0.10	0.53±0.12	0.61±0.20
CHT Mean	0.30±0.15	0.33±0.27	0.29±0.13	0.29±0.12		0.29±0.11	0.30±0.12	0.26±0.05	0.28±0.13		0.59±0.18	0.64±0.36	0.56±0.17	0.58±0.24	

Means expressed with different letters in the same column were found to be significantly different at the 5% level according to the Duncan test. **CHT:** Chitosan, **App:** Application.

Table 5: Effect of salt and chitosan treatments on total phenolic and carotenoid of strawberry leaves during flowering and fruiting periods.

Flowering Period										
	Total Phenolic Content (mg GAE/g DW)					Carotenoid (mg/g FW)				
	CHT (ppm)					CHT (ppm)				
Salt App. (mM)	0	1	2	3	Mean	0	1	2	3	Mean
S0	289.85±18.60	256.12±4.36	215.96±19.11	225.77±4.01	246.92±32.32	0.14±0.03	0.15±0.02	0.11±0.02	0.12±0.01	0.13±0.02
S30	190.58±10.75	272.76±11.56	285.05±3.25	287.30±4.90	258.92±42.22	0.26±0.02	0.20±0.10	0.11±0.03	0.21±0.15	0.19±0.10
S60	200.68±0.85	289.62±8.46	232.48±2.98	155.64±1.54	219.60±51.09	0.11±0.14	0.10±0.002	0.23±0.06	0.14±0.06	0.15±0.06
Mean	227.04±48.51	272.83±16.32	244.50±32.74	222.90±57.14	241.82±44.58	0.17±0.07	0.15±0.07	0.15±0.07	0.16±0.09	0.16±0.07
Fruiting Period										
S0	150.12±28.78	143.56±45.23	140.43±1.140	125.53±1.68	139.93±24.74	0.15±0.03	0.13±0.03	0.13±0.01	0.16±0.02	0.14±0.02
S30	180.13±16.33	130.71±25.06	149.49±27.23	125.51±12.22	146.46±28.70	0.25±0.07	0.20±0.13	0.14±0.02	0.13±0.01	0.18±0.08
S60	143.96±11.66	113.00±9.03	119.54±8.83	95.16±9.86	117.91±20.12	0.13±0.01	0.17±0.05	0.13±0.02	0.10±0.01	0.13±0.03
Mean	158.07±24.26	129.12±29.44	136.49±19.55	115.40±17.11	134.77±27.05	0.18±0.07	0.16±0.07	0.13±0.02	0.13±0.03	0.15±0.05
CHT Mean	192.55±51.41[a]	200.98±77.46[a]	190.49±61.42[a]	169.15±68.80[b]		0.17±0.06	0.16±0.07	0.14±0.05	0.14±0.06	

Means expressed with different letters in the same column were found to be significantly different at the 5% level according to the Duncan test. CHT: Chitosan, App: Application.

and Turhan and Eriş.[42] This situation was also determined in the study of Turhan and Eriş[42] with Camarosa and Tioga strawberry varieties. Turhan and Eriş (2005) found that DW of leaf decreased in both cultivars when the salt application dose was increased. In other studies of strawberry, it has been reported that salt excess suppresses plant growth, causing damage to different organs of plants and serious reductions in plant characteristics and drying of the leaves from the edges to the inside.[43] The effect of chitosan on DW of root, leaf and crown and FW of root was statistically significant. The 1 ppm dose of chitosan increased DW of leaf by 14.08% compared to the control. The 2 ppm dose of chitosan increased FW of root, DW of crown and root by 16.58, 45.45 and 33.07 % respectively. As in our study, foliar application of chitosan increased all growth parameters of maize and alleviated salt stress.[44]

Leaf Area (cm²)

Our findings indicate that salt stress induced noticeable symptoms in strawberry plants. Furthermore, analysis of plant growth parameters clearly showed that the vegetative parts were the most sensitive to salt damage. Table 3 shows that leaf area initially increased at a salt dose of 30 mmol/L compared to the control, but subsequently decreased as the salt concentration was raised. Salt stress in strawberries causes leaves to curl inward from the edges and dry out, inhibiting plant growth and causing damage to various plant organs.[43] In a study conducted by Üzal and Yıldız,[45] it was observed that leaf weight and leaf area were significantly reduced in various strawberry cultivars subjected to salinity stress. The extent of this reduction was found to be influenced by both the duration of salt exposure and the specific cultivar. Bag and Kocaman[41] found that increasing salt concentration led to a decrease in leaf area in the Monterey strawberry cultivar. In the experiment, strawberry plants harvested three months after planting during the fruiting period exhibited an increase in leaf area because of the growing of plants. In both periods, the 30 mmol/L salt dose had a positive effect on the leaf area, resulting in higher values compared to the control. The effect of chitosan on leaf area was found to be statistically significant. The healing effect of chitosan was also seen in the leaf area and it was found that applying 2 ppm increased the leaf area by 18.75%. The application of chitosan in agriculture is important because chitosan can activate plant defense systems against biotic and abiotic stress.[46] In our study, chitosan reduced the negative effects of salt on leaf area. We propose that chitosan mitigates salt stress by inducing stomatal closure, thereby reducing Na+ and Cl^- uptake by roots. Chitosan sustains plant development under saline conditions, safeguards the photosynthetic apparatus, enhances the production of osmolytes, activates antioxidant enzymes and induces stomatal closure to curtail the uptake of sodium and chloride ions from roots.[47]

Biochemical Parameters

MDA Content (nmol/g TA)

The changes in Malondialdehyde (MDA) content in strawberry leaves due to salt and chitosan applications are presented in Table 3. It was observed that MDA levels decreased significantly as both the duration and concentration of salt treatments increased, particularly during the fruit development phase. According to the statistical analysis, the application of increasing doses of chitosan to alleviate the negative impacts of salinity resulted in a significant reduction in MDA accumulation in strawberry leaves. At a concentration of 3 ppm of chitosan, there was a 25.90% reduction in MDA accumulation compared to the control group. This illustrates the healing effect of chitosan, as evidenced by the decrease in MDA accumulation. In another study where sunflower and safflower seeds were treated with chitosan and its derivatives, a significant decrease in MDA content was observed, resulting in the mitigation of membrane damage. This was suggested as the reason for the increased tolerance to salt stress.[48] MDA is a reliable biomarker for assessing the extent of oxidative damage in plants under salt stress. Therefore, the measurement of MDA content is a widely used method to evaluate the severity of salt stress and the efficacy of various stress mitigation strategies.[49] Yaşar *et al.*[50] found that watermelon genotypes tolerant to salt stress had lower total MDA accumulation compared to salt-sensitive genotypes when subjected to salt stress.

Chlorophyll and Carotenoid Contents (mg/g FW)

Another parameter investigated in the study was the photosynthetic pigment content and the results of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents of the compounds applied to the plant under salt stress are presented in Tables 4 and 5. Results showed that the chlorophyl a, chlorophyl b and total chlorophyl concentration as well as chlorophyl activity remained unchanged under salt stress with or without chitosan treatment. However, considering the contents of chlorophyll a (0.33±0.27 mg/g FW), chlorophyll b (0.30±0.12 mg/g FW) and total chlorophyll (0.64±0.36 mg/g FW), it was found that the highest values were obtained from the treatments in which chitosan was 1 ppm. Furthermore, salt concentration of 30 mmol/L effectively improved chlorophyl content under both salt-stress and chitosan. In the study, the negative effect of salt on chlorophyll content was observed more during the fruiting period. The significant decrease seen in the samples taken fruiting period (3 months after planting) can be interpreted as prolonged exposure to salt application. Tuna and Eroğlu[51] reported that chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents decreased due to salt stress (100 mM) in pepper. Ashraf and Harris[52] reported that increasing salinity in photosynthetic tissues resulted in the stacking of adjacent grana membranes, leading to the shrinkage of thylakoids and the degradation of chlorophylls. Yıldız *et al.*[53] reported that high salinity caused

a disruption of the molecular structure of chlorophylls and a decrease in their quantity.

Total Phenolic Content (mg GAE/g DW)

In the study in which the effects of salt and chitosan applications were investigated in strawberry, the amount of total phenolic matter was between 289.85 and 113.49 mg GAE/g DW in Table 5. It is seen in the table that the amount of total phenolic content decreased as the concentrations of salt applications increased in both periods. In addition, as shown in the table, the amount of phenolic content decreased with time, the lowest value (95.16 mg GAE/g DW) being found in the S60 salt treatment during the fruiting period. Contrary to our findings, Şahin[54] reported increased levels of phenolic compounds in the leaves of Sweet Charlie and Camarosa strawberry cultivars when subjected to higher concentrations of NaCl compared to the control. There was a statistical difference between the chitosan doses in the mean total phenolic content and the total phenolic content was the highest at 1 ppm chitosan dose (200.98 mg GAE/g DW). Chitosan treatments have been shown to significantly increase the antioxidant content, particularly phenolic compounds, in various plants such as tea (*Camellia sinensis*),[55] broccoli (*Brassica oleracea* L.),[56] sweet basil (*Ocimum basilicum* L.)[57] and tomato.[58]

CONCLUSION

In this research, the impact of treating Albion cv. strawberry leaves with chitosan under salt condition on various aspects, such as dry weight, fresh weight, leaf area, MDA, chlorophyll and carotenoid content was examined.

Salinity stress treatments damaged morphological and biochemical parameters of strawberry plants. Chitosan, an environmentally friendly natural polymer, is an alternative to chemical methods that can be used to reduce economic losses during cultivation. Chitosan treatments probably mitigated the adverse impacts of salinity by serving as an abiotic elicitor, thereby stimulating yield enhancement and the production of secondary metabolites.

The findings indicate that the application of chitosan has a pronounced effect on enhancing various parameters in strawberries subjected to salt stress. Specifically, chitosan treatments led to a notable decrease in MDA, a key indicator of plant defense mechanisms. Furthermore, chitosan significantly improved yield and quality attributes such as DW of all parts, FW (root), leaf area and the content of phenolic compounds.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

There is no Ethical Approval required for the study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MDA: Malondialdehyde; **GlcNAc:** N-acetyl-d-glucosamine; **FW:** Fresh weight; **DW:** Dry weight; **CHT:** Chitosan.

SUMMARY

• Salinity is one of the most important factors negatively affecting soil fertility and limiting crop yields in strawberry production.

•Chitosan is an effective agent with antiviral, antibacterial and antifungal properties that enhances the defense mechanisms of plants, thereby protecting them against biotic and abiotic stress.

• The samples investigated from different organs (root, crown and root) at two growth stages (flowering and fruiting) were used to monitor dry and fresh weight of strawberry.

•Phenolic content, chlorophyll and MDA were analyzed to see the effects of chitosan on salt stress in strawberry leaves.

• The effects of chitosan sprayed on strawberry leaves to reduce salt stress were found to be statistically significant some vegetative and biochemical parameters.

• Chitosan has been found to positively affect plant growth in strawberries improving yield and quality characteristics under salt stress.

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