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Research Article

Revealing the Influences of Exogenous Chitosan Supplements on Yield, Nutritional Values and Enzyme Activities in Taşköprü Garlic

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Abstract: Garlic (*Allium sativum* L.) Taşköprü garlic is a valuable source of antioxidative molecules, including phenolic compounds, flavonoids, phenolic acids, enzymes, and minerals. A two-year study was conducted in an open field to compare the potential influence of exogenous chitosan (CHT) supplements on garlic yield, ash content, secondary metabolite generations, antioxidant enzyme activity, and mineral status of Taşköprü garlic in comparison to the untreated groups. The applications were arranged as control (0), CHT (CHT-1:0.5 Mm, CHT-2: 1 mM, and CHT-3:2 mM), and NPK. The influences of the applications were measured by monitoring bulb and plot yield, total phenolic, flavonoid, phenolic acids, ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) activity, and K, P, S, Ca, Mn, Fe, Ni, Cu, Zn, and Se accumulations. Results revealed that CHT-1 application improved bulb yield; benzoic acid and rosmarinic acid; Cl, K, Ca, and Se accumulation in garlic samples, but CHT-2 application increased total phenol content, POD and SOD enzyme activity, and the Mn, Fe, Ni, Cu, and Zn concentrations. The CHT-3 application enhanced the flavonoid generation in the garlic tissues. In summary, exogenous chitosan supply improved bulb growth by inducing flavonoids, total phenolics, benzoic acid, K, Na, Cl, and Ca accumulation and by activating POD and SOD. Moderate levels of chitosan (CHT-1 and CHT-2) could be offered to garlic cultivation, and data obtained can also provide potential knowledge about pre-harvest traits of garlic bulbs for further investigation.

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1. Introduction

Garlic (*Allium sativum* L.), one of the oldest cultivated plant species, has been used for culinary and medicinal purposes for thousands of years. Beyond its cultural and health significance, it is a valuable crop with a significant economic impact. Many studies have demonstrated that this vegetable, which is consumed fresh, cooked, pickled, dried, powdered, or in garlic oil, is rich in minerals, vitamins, carotenoids, phenolic acids, flavonoids, carbohydrates, proteins, enzymes, and organosulfur compounds (Turfan et al., 2024; Ünal, 2024a). It has been used since ancient times to treat numerous illnesses (ailments), and its use continues in modern medicine today (Ünal, 2024b). Also, it is a significant source of income for producers, providing livelihoods and contributing to the development of regional economies in Türkiye (Turfan and Turan, 2023). The Taşköprü district in Kastamonu province is renowned for its high-quality garlic and has a crucial significance in the garlic industry, and also Taşköprü garlic is among the Geographical Indications of Türkiye. The higher dry matter content compared to other varieties is considered an essential factor in its superior quality and extended shelf life (Abdelaal et al., 2021; Tahmas Kahyaoğlu, 2021). However, heavy rain and hail experienced in the region during the spring months cause the suppression of vegetative growth and the emergence of various diseases, which will result in serious yield loss (Ünal, 2024a). In addition, the manufacturer could not sell its products at the expected price and suffered significant economic losses (Badal et al., 2019; Mohamed et al., 2023; Turfan et al., 2024).

In modern agriculture, it is important to select resistant plant species to improve yield and nutritional quality in plant production, but exogenous use of antioxidants that strengthen the resistance of the plant to abiotic and biotic stress factors can also bolster this expectation (Elshamly and Nassar, 2023; Turfan and Turan, 2023; Yoldas et al., 2024). Despite using inorganic fertilizers and pesticides to improve growth, inhibiting the loss of yield in crops or obtaining more products is very common; they often cause environmental degradation and threaten food safety (Turfan et al., 2024). Recently, the interest in sustainable and environmentally friendly agricultural practices has further evoked research into chitosan (CHT) applications (Jabber, 2021; Zhang et al., 2021; Gürsoy, 2022). CHT is a biopolymer derived from chitin, found in crustacean exoskeletons and fungal cell walls. (Hassan et al., 2021; Rameshjan et al., 2024). It may provide numerous advantages in agriculture due to its biodegradability, biocompatibility, non-allergenicity, and non-toxicity to human health (Moradkhani and Jabbari, 2023). Many researchers have shown that CBT has beneficial effects in improving yield and nutritional values in many species using different mechanisms and even alleviating stress-induced damages (Rahman et al., 2021; Moradkhani and Jabbari, 2023; Mohamed et al., 2023). CHT can enhance growth rate by inducing photosynthetic activity, promoting cell proliferation in meristematic tissues (El Amerany et al., 2022; Krupa-Malkiewicz et al., 2024), and facilitating mineral uptake from the soil (Geries et al., 2020). The mentioned positive effects of CHT applied during the growth stages were proven in many vegetables, including potatoes (Falcon et al., 2017), onions (Rameshjan et al., 2024), tomatoes (Adamuchio-Oliveira et al., 2020; Attia et al., 2021), eggplant (Sultana et al., 2017), and corn (Elshamly and Nassar, 2023). The ameliorative influences of chitosan supplementation on stress-related damage in plants subjected to salt (Golkar et al., 2019; Gürsoy, 2022), drought (Handayani and Dinoto, 2022), and heavy metal (Krupa-Malkiewicz and Ochmian, 2024) stress have been demonstrated in safflower, lettuce, and tomato. Researchers have claimed that the protective roles of CHT against stress may be associated with its strengthening of both enzymatic and non-enzymatic defence systems (Attia et al., 2021), stimulation of secondary metabolic pathways, and inhibition of the accumulation of toxic compounds such as MDA and ROS in cells. Abdelaal et al. (2021) revealed that foliar CHT alleviated cellular damages induced by drought in garlic, by enhancing chlorophyll content and activation of antioxidant enzymes, including ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD). Similarly, Golkar et al. (2019) in safflower and Abdelaal et al. (2021) in garlic found that these enzymes increased upon CHT supply even under drought stress conditions. Chitosan can alter the accumulation of phenolic acids (such as rosmarinic acid, gallic acid, ferulic acid, and chlorogenic acid) in plant cells by modulating the activation of some molecules involved in secondary metabolite pathways (Chakraborty et al., 2009; Pirbalouti et al., 2017a and 2017b). It has been reported by Fooladi et al. (2019), and Park et al. (2019) that CHT can regulate the amounts of phenols, flavones, and phenolic acids in plant organs by activating phenylalanine lyase, one of the enzymes responsible for the synthesis of phenolic acids. A study conducted by Gmaa (2016), which examined the effects of exogenous CHT, K, and salicylic acid supplements on garlic growth rate, bulb development, and nutritional quality, revealed that chitosan application yielded superior results compared to the other two supplements. Exogenous CHT provision can alter the mineral uptake from the soil by altering the activation of ion transporters of root cell membranes (Krupa-Małkiewicz and Ochmian, 2024). Geng et al. (2020) reported that CHT stimulates the secondary metabolite pathway, which leads to the release of organic acid from the root surfaces, and thus can control mineral absorption. The positive effects of exogenous chitosan provision during the growth season to improve yield, and agronomic and nutritional quality are well documented in different crops. However, to our knowledge, no study investigates the effects of exogenous CHT applications during the vegetation period on the nutritional quality of garlic bulbs after harvest, taking into account yield, secondary metabolite production, antioxidant enzyme activation, and mineral accumulation, especially in Türkiye. Therefore, more studies need to be conducted to explain how foliar applications during the vegetation period affect garlic's yield and post-harvest nutritional value. The main objective of this study was to reveal the effects of different chitosan concentrations on total phenolic compounds, flavonoids and phenolic acid accumulations, APX, CAT, POD, and SOD activities, and mineral contents in Taşköprü garlic. This could contribute to our understanding of how agricultural practices impact garlic's nutritional and medicinal properties. It will also provide clues for further research on the effects of chitosan on agronomic properties and the nutritional quality of garlic during storage.

2. Material and Methods

2.1. Garlic cultivation

In this investigation, the plant material studied was Taşköprü garlic obtained from the Taşköprü district of Kastamonu province. The study is a two-year study, 2021 and 2022, and was carried out in an open field in Uzunkavak village of Taşköprü district (41°19'16" North latitude, 33°59'44" E longitude and 633 m altitude). The climatic data of the experimental area are presented in Figure 1.

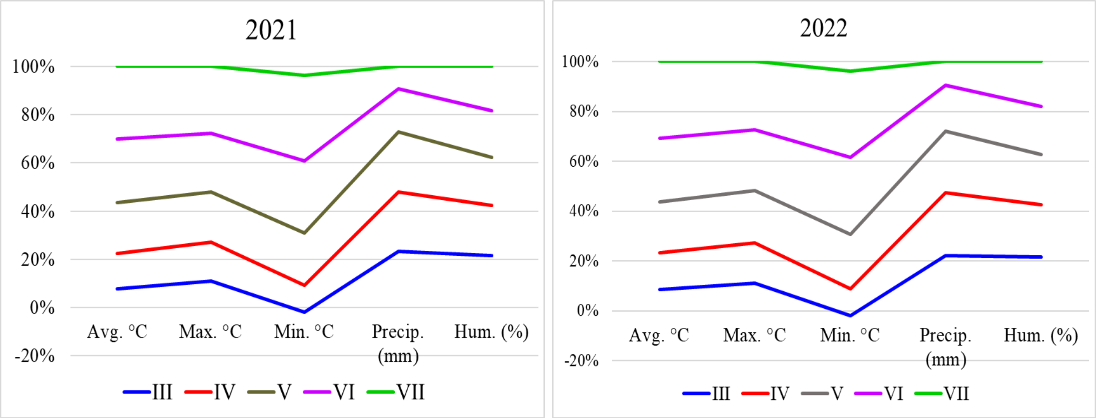

Figure 1. Climatic factors of the study area during the garlic cultivation.

The mean climate variations observed during the study are shown in Figure 1. It was determined that the monthly mean relative humidity in the district was approximately 58-68 (2021) and 56%-67% (2022). Whereas the mean temperature ranged from 5.6 to 21.5 in 2021 and from 6.2 to 22.4 °C in 2022; the lowest temperature ranged from -0.8 to 15.63 °C in 2021 and from -0.84 to 15.42 °C in 2022. The average level of precipitation varied between 27 to 72 mm.

Garlic cultivation was initiated on March 08 with the sowing of the cloves into the soil, and the season was completed with the bulbs harvesting on July 16 in both years. The experiment was arranged in a randomized block design (RBD) with three replications. Before the experiment was set up, some soil samples were collected from the different parts of the field (layer of 20 cm) and used for element analyses. The application pattern was arranged as control, NPK (nitrogen-phosphorus-potassium), and chitosan (CHT1, CHT-2, and CHT-3). While chitosan supplements were conducted from leaves, NPK fertilizer was performed from the soil. No fertilizer was added to the control application and CHT-applied groups. The experimental plots were prepared for 1 m^2 (1 m × 1 m). Larger-sized cloves were sown in rows in the soil with their tips visible (Vural et al., 2000). NPK treatment, diammonium phosphate (DAP), and potassium nitrate (KNO_3) fertilizers were applied as 16 kg N da^{-1}, 8 kg P_2O_5 $da^{-1,}$ and 8 kg K_2O da^{-1}. Inorganic fertilizers were used twice, before planting and when the plants were 30-35 cm. Irrigation was carried out regularly during the growing period. No plant protection product was used during the experiment. The mean nutrient components were: available Mg 25.35%, K 18.22%, Ca 8.66%, Na 8.12%, P 4.20%, S 2.54%, Mn 966 mg kg^{-1}, Fe 40240 mg kg^{-1}, Ni 144.6 mg kg^{-1}, Zn 1454.4 mg kg^{-1}, and Cu 42.6 kg^{-1}. pH value of the soil was found to be 6.38. The electrical (EC) conductivity was 6.63 dS m^{-1}. Mature bulbs were harvested when the leaves had turned completely yellow and dried. The harvested samples were then spread out on the field and left to dry for one week. After sufficiently drying the bulbs, all the samples were transported to the laboratory in paper bags.

Chitosan (Sigma Aldrich, CAS: 448877, 2-amino-2-deoxy-D-glucosamine: CHT) was applied as a stimulant for garlic seedlings. First, 3 g of CH was dissolved in 2% acetic acid and then sprayed onto the seedlings twice a week for four weeks to get the upper and lower surfaces of the leaves. Plants were sprayed (5 mL) with acetic acid as a control. The four application models were as follows: 1) Control (2% acetic acid), 2) CHT-1 (0.5 mM), 3) CHT-2 (1 mM), and 4) CHT-3 (2 mM), respectively. The solutions were prepared fresh for each application to the seedlings. The doses supplemented were adjusted according to a preliminary study with pot culture. Doses of CHT that visibly improve seedling development have been selected for 2021 and 2022.

2.2. Determination of biochemical properties, mineral status of samples

Estimation of the amount of total phenolic compounds in the ethanolic garlic extracts was performed, using the Folin-Ciocalteu method (Agbar et al., 2008). Waiting for all samples in the dark for 45 minutes, the absorbance was noted at 760 nm. Total phenols were calculated using a calibration curve prepared with Gallic acid as a standard and expressed as gallic acid (R^2: 0.9892, $y=0.0132x-0.0152$) equivalents (GAE) g^{-1} FW. The total flavonoid level in the ethanolic extracts was measured based on Kumaran and Kumaran's method (2006). A calibration curve was prepared using quercetin as a standard (0.03125-0.5 mg ml^{-1}). The absorbance was recorded at 415 nm after 20 minutes by the beginning of the reaction. Total flavonoids were expressed as quercetin (R^2: 0.9914, $y=0.0126x + 0.0026$) equivalents g^{-1} FW.

The modified HPLC procedure (Rodriguez-Delgado et al., 2001) was used to measure phenolic acids such as benzoic acid, caffeic acid, chicoric acid, chlorogenic acid, cinnamic acid, coumaric acid, ferulic acid, gallic acid, rosmarinic acid, tannic acid, and vanillic acid, as well as the flavonoid quercetin in garlic tissues. Phenolic acids standards for all mentioned above were prepared with 0.0312, 0.0625, 0.1250, 0.2500, 0.5000, and 1.0000 mg L^{-1} methanol using 10 mg L^{-1} stock solution. All standards were sourced from Sigma-Aldrich with a purity of 99% from Taufkirchen, Germany. For the analysis, 10 g of samples were initially chosen from each replicate and homogenized by a food-grade blender. Subsequently, these samples were centrifuged at 8050 g at 4 °C for 30 min. The resulting supernatants were then filtered first through coarse filter paper and then through a 0.45 µm membrane filter (Millipore Millex-HV Hydrophilic PVDF; Millipore, Massachusetts, USA) before being injected into an HPLC system from Agilent in California, USA. Chromatographic separation was employed using a 250 × 4.6 mm, four µm ODS (Octadecyl-silica) column from HiChrom in Leicestershire, UK. The mobile phase for separating phenolics and flavonoids from the garlic samples consisted of Solvent A (methanol: acetic acid: water, 10:2:28) and Solvent B (methanol: acetic acid: water, 90:2:8). UV spectral measurements were recorded at 254 and 280 nm, and the flow rate and injection volume were set at 1 mL min^{-1} and 20 µL, respectively (Taş et al., 2022).

Antioxidant enzyme activity measurements of garlic samples were carried out following the protocols of Zhang et al. (2006). 500 mg of fresh garlic tissue was extracted with 5 ml of sodium phosphate buffer (50 mM, pH 7.0), and then these samples were centrifuged at 10000 rpm and 4 °C for 15 min. APX, CAT, POD, and SOD activities were measured in the collected supernatant. CAT activity was determined by taking into account the decrease in H_2O_2 concentration at 240 nm for one minute. In the method based on the ability of catalase to catalyze the decomposition of hydrogen peroxide into water and oxygen, the decline in the H_2O_2 concentrations reflects the enzyme's catalytic activity. The activity was measured with a reaction mixture (3 mL) containing 100 µl of enzyme extract and 2.9 ml of buffer (50 mM, pH 6.0), including ten mM H_2O_2. POD activity was performed with 3 mL reaction mixtures by recording the absorbance at 470 nm. The activity of POD was described as the content of enzyme that caused an increase in absorbance at 470 nm of 0.001 per minute. SOD activity was conducted based on the reduction of NBT (nitroblue tetrazolium). The reaction solution consisted of 100 µl of enzyme extract, two µM riboflavin, 65 µM NBT, 13 µM methionine, and one µM EDTA in sodium phosphate buffer (50 mM, pH 7.8). This solution was initiated by illumination for 2 min at 25 °C, and the absorbance of blue formazan was noted with a spectrophotometer at 560 nm. One unit of SOD activity (EU) was expressed as the amount of enzyme that caused 50% inhibition of NBT reduction.

Dry matter (%) was measured by drying the garlic samples in a drying oven at 65 °C until reaching a stable weight. Dried samples (25 g) were put into a tared crucible and combusted in an ash furnace at 525±25 °C until becoming white ash. The %ash was calculated using the % ash formula (Cemeroğlu, 2007).

Some soil samples were collected before cultivation experiments to assay elemental analysis (Mg, P, S, K, Ca, Na, Cl, Mn, Fe, Ni, Zn, and Cu). Then, soil and garlic cloves were dried at 65 °C and powdered. Approximately 250 mg dried sample was burned with nitric acid and completed with water in a 10 ml volumetric flask (Sarker et al., 2022) and used in the analysis of elements at the Kastamonu University's Central Research Laboratory using inductively coupled plasma-optical emission spectrometry (ICP-OES, SpectroBlue II).

2.3. Statical analyses

All analyses were performed with three replications. All results were expressed as the mean values ± standard errors. The results were subjected to variance analyses (ANOVA) by using SPSS 23.0 statistical software. Tukey's Honestly Significant Difference (HSD) test evaluated the differences among means, and the significance level was accepted as $P<0.05$.

3. Results and Discussion

3.1.Influence of exogenous chitosan supplements on yield and ash content

The influence of exogenous CHT supplements on yield efficiency is present in Table 1. In 2021, the plot yield ranged from 622 (control) to 779 g (CHT-1), while in 2022, it ranged from 654 (control) to 832 g (CHT-1). The bulb weight was in the range of 25 and 31 g (control-CHT1)in 2021 and the range of 27 and 38 g (control-CHT1)in 2022. The clove numbers of the bulbs were between 12.50 and 13.98 (control-CHT-1) in the first year, while it was between 12.67 and 14.64 (control-CHT-1)in the second year. The results indicated that the CHT-1 dose exhibited better results than other concentrations, and the lowest levels of bulb and plot yields were achieved in the control plants. Despite observing an increase in the yield of NPK-applied samples, it was lower than that of the CHT-1 and CHT-3 applied groups (Table 1). Further, clove numbers were relatively higher in the CHT-1 and NPK-applied groups (Table 1). The yield results displayed that CHT concentrations significantly increased the bulb growth and plot yields compared to the control and NPK-applied garlic, but the highest values were achieved with the CHT-1. Studies conducted in Turkiye have shown that the yield of garlic bulbs can vary with the growing environment and the treatments applied during the growth stages (Badal et al., 2019). Reports displayed that the average fresh weight of garlic bulbs generally ranges between 16.00 and 28.50 g (Turfan, 2022) and 20.00 and 39.68 g (Badal et al., 2019). Also, the number of cloves per bulb typically varied between 9.00 - 18.00 (Turfan, 2022) and 17.15 - 27.63 (Badal et al., 2019). A similar finding was reported by Jabber (2021), who observed that the fresh weight of bulbs ranged from 20.60 to 24.16 g and clove number ranged from 20.04 to 23.52, with the highest weight observed in the CHT-treated group. In a study conducted by Mohamed et al. (2023), it was shown that bulb diameter (38.65-48.84 mm) and clove number were improved by CHT provision compared to the control, especially with 2 cm L^{-1}. In a study conducted with onions, it was shown that chitosan doses (0, 1000, and 2000 ppm) applied early growth rate promoted seedling growth and bulb yield, and the highest yield was achieved with 2000 ppm (Rameshjan et al., 2024). Similar results regarding the improvement effect of CHT were reported by Ramadan and El Mesairy (2015) in okra, Attia et al. (2021) in tomatoes, Falcon et al. (2017) in potatoes, Rahman et al. (2021) in carrots, and Geries et al. (2020) in onions.

An increase in yield may be due to the stimulation of photosynthetic metabolism by chitosan (Abdelaal et al., 2021) and the resistance of garlic to climatic adversities and diseases (El Amerany et al., 2022). However, according to Elshamly and Nassar (2023), consistent with this study, the interaction of CHT with plants is also closely related to its applied concentration. They expressed that appropriate levels promoted growth and yield in crops, whereas high levels resulted in a reduction by provoking metabolic perturbations. Differences between the values determined regarding the plot yield, bulb weight, and clove number compared to the literature were related to the examined garlic variety, growing conditions, and the concentration and diversity of the substances used in the applications.

Table 1. Influences of exogenous chitosan application on the plot and bulb yields of garlic samples

	Plot yield (g)		**Bulb yield (g)**		**Clove number (per plant)**		**Ash (%)**	
	2021	**2022**	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**
Cont.	622.77±0.18e*	671.43±0.20c	25.30±0.14c	27.93±0.17d	12.93±0.28ab	12.96±0.17b	88.15±0.15d	88.85±0.21d
NPK	722.15±0.20c	715.48±0.33bc	28.81±0.19b	30.52±0.16c	13.58±0.15a	13.73±0.18ab	92.91±0.09c	92.88±0.15c
CHT-1	779.42±0.22a	832.69±0.25a	31.78±0.14a	38.18±0.27a	13.98±0.19a	14.64±0.15a	98.25±0.21ab	99.30±0.36ab
CHT-2	638.80±0.37d	654.54±0.26d	25.82±0.10c	30.58±0.16c	12.64±0.18b	12.67±0.13b	100.99±0.08a	101.27±0.46a
CHT-3	728.56±0.45b	744.11±0.15b	28.56±0.15b	35.36±0.16b	12.50±0.19b	13.29±0.19b	95.79±0.14b	97.88±0.15b
F	157482	140378	327.3	496.3	10.13	21.84	1254.6	307.1
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*: Means (±: n= 15) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $P \leq 0.05$. Cont: Control, NPK: nitrogen-phosphorus-potassium, CHT-1: 0.5 mM Chitosan; CHT-2: 1 mM Chitosan; CHT-3: 2 mM Chitosan.

Ash amount, which is an essential indicator of biomass, is one of the quality elements of garlic (Gmaa, 2016). In the present study, the ash content of samples ranged from 88.15% to 100.99% in the first year and from 88.85% to 101.27% in the second year. The highest amount of ash was obtained from CHT-2 application, followed by CHT-1. However, the lowest ash content was seen in the control group, followed by the NPK application (Table 1). The result elucidated that in CHT concentrations, especially CHT-2, a significant increase occurred in the ash content of garlic in comparison with the control and NPK applications for both years (Table 1). The positive influence of CHT supplements on the ash content may be attributed to the higher levels of secondary metabolites, POD and SOD activities, and K content in the bulbs, as cited by Khaled et al. (2021), and Turfan and Turan (2023). High levels of these compounds can increase biomass by increasing the cellular water content, promoting the accumulation of storage substances in the cloves, and strengthening resistance to diseases and stress conditions; hence, ash levels may increase (El Sagan and El Dsouky, 2015; Falcon et al., 2017).

3.2. Influences of exogenous chitosan supplements on the secondary metabolite variations

In this current study, the exogenous CHT supplements had a notable effect on the levels of total phenolic and flavonoids in garlic samples across two years (Figure 2), but there were no significant differences across the years. The total phenolic compound and flavonoid variations of cloves of garlic are presented in Figure 2. The results were similar between the years; however, the CHT-2 applied group was the richest in total phenolic compound (14.33 and 14.15 mg), which was significantly higher than control (9.06 mg and 8.97 mg) and NPK-supplied samples (10.57 mg, 9.24 mg). Regarding the flavonoid content, the CHT-3-supplied garlic had the highest total flavonoid content in comparison to the control (53.48-55.69 mg), followed by the CHT-2 applied group (52.14-52.53 mg) (Figure 2). Total phenolic and flavonoid were influenced positively by CHT supplements in comparison to the control. For both years, the highest total phenolic was recorded with CHT-2 supplements and the highest flavonoid with CHT-3 (Figure 2). Consistent with this study, research exhibited that the total phenolic compounds in garlic typically range from 14.55 to 55.00 mg, while the total flavonoid content varies from 0.07 mg to 37.56 mg (Tahmas Kahyaoğlu, 2021; Turfan, 2022). Incremental values of total phenolic and flavonoid with CHT supplement were reported by Pirbalouti et al. (2017a) in basil, Golkar et al. (2019) in safflower, and Fooladi vanda et al. (2019) in melissa. In their study examining the effects of chitosan doses on the secondary metabolite profile in strawberry fruits, Rahman et al. (2021) found that chitosan stimulated total phenolic (310.4-370.9 µg; control-CHT) and flavonoid (532.7- 947.8 µg; control-CHT) synthesis in the fruit compared to the control, and the highest value was achieved with 1000 ppm chitosan. Similar results in garlic were reported by Khaled et al. (2021), who observed that CHT supplements positively influenced the accumulation of total phenols and flavonoids in garlic by activating secondary metabolite pathways. Likewise, Chakraborty et al. (2009), and Park et al. (2019) showed that CHT applications promoted polyphenol and flavonoid generation in Coconut and Buckwheat.

Bulb-yielding efficiency is considered a quality marker in garlic, but an equally important trait in garlic is the content of secondary productions, e.g. total flavonoid, phenolic compounds, and phenolic acids (Zeroual et al., 2024). They are involved in vegetative growth, bulb development, and tolerance to adverse environmental conditions (Turfan and Turan, 2023); they also offer various health benefits to people who consume them (Alide et al., 2020).

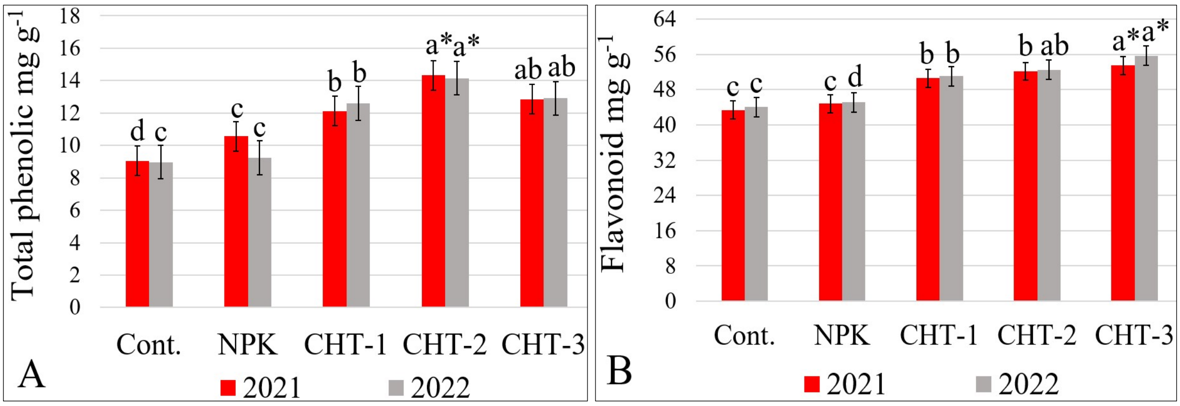

Figure 2. Variation of total phenolic compound (2A) and flavonoid contents (2B) in garlic samples. *: Means (±:n= 3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at P≤0.05. Cont: Control, NPK:nitrogen-phosphorus-potassium; CHT-1: 0.5 mM Chitosan; CHT-2: 1 mM Chitosan; CHT-3: 2 mM Chitosan.

Phenolic acids are a significant class of secondary metabolites that can be present at various rates in flowers, leaves, stems, and roots, which contribute to nutritional quality, coloration, and flavour (Arista et al., 2023; Nurcolis et al., 2023). The variations of phenolic acid concentrations in the garlic samples are presented in Table 2 and Table 3. Despite CHT and NPK supplements showing remarkable effects on phenolic acid accumulations in garlic samples, there was no significant change across the years.

Among the phenolic acids, the highest amount was benzoic acid. For both years, the highest benzoic acid was achieved with the CHT -1 application, while the lowest value was seen in the control plants. However, a second peak was observed in the NPK fertilizer (Table 2). Moreover, while chicoric acid and vanillic acid decreased in CHT and NPK-supplied groups compared to the control, benzoic acid, rosmarinic acid, gallic acid, and chlorogenic acid increased with CHT and NPK applications (Table 2). As a flavonoid compound, quercetin was negatively affected by CHT and NPK applications, but the decrease in NPK fertilization was statistically insignificant (P<0.05) (Table 2). The lowest quercetin concentration was recorded with a CBT-1 dose, followed by a CHT-3 supply (Table 2). According to the obtained data, the most abundant phenolic acids in garlic samples were benzoic acid, rosmarinic acid, caffeic acid, chicory acid, vanillic acid, gallic acid, and chlorogenic, while the abundant flavonoid compound was quercetin (Table 2, Table 3).

Table 2. Influence of CHT supplements on the variation of phenolic acids in garlic (2021)

2021	**Control**	**NPK**	**CHT-1**	**CHT-2**	**CHT-3**	**F**	**P**
GA	0.758±0.002^{b*}	0.890±0.009^{a}	0.850±0.002^{ab}	0.78±0.031^{b}	0.760±0.002^{b}	16.96	<0.001
Chl	0^{d}	0.382±0.013b	0.238±0.002c	0.56±0.001a	0.391±0.003b	1250	<0.001
Van	3.774±0.07a	2.493±0.01c	2.493±0.01c	3.12±0.02b	3.145±0.01b	267	<0.001
Chic	2.689±0.002a	2.165±0.014e	2.319±0.005d	2.37±0.002c	2.485±0.004b	836	<0.001
Ben	119.01±0.24e	150.55±0.84d	219.74±0.11a	187.19±0.48b	169.5±0.45c	5982	<0.001
Ros	7.84±0.09e	16.396±0.44b	19.842±0.11a	13.66±0.04c	11.999±0.25d	752.5	<0.001
Qu	5.877±0.02a	5.853±0.01a	5.605±0.02d	5.68±0.01c	5.753±0.01b	97.4	<0.001

*: Means (±: n= 10) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at P≤0.05. Control, NPK: nitrogen-phosphorus-potassium; CHT-1: 0.5 mM Chitosan; CHT-2: 1 mM Chitosan; CHT-3: 2 mM Chitosan; GA: gallic acid, Chl: chlorogenic acid, Van: vanillic acid, Chic: chichoric acid, Ben: benzoic acid, Ros: rosmarinic acid, Qu: quercetin.

Table 3. Influence of CHT supplements on the variation of phenolic acids in garlic (2022)

2022	Control	NPK	CHT-1	CHT-2	CHT-3	F	P
GA	0.747±0.002d	0.886±0.004a	0.864±0.004ab	0.820±0.003b	0.774±0.003c	430	<0.001
Chl	0	0.353±0.004c	0.230±0.002d	0.578±0.003a	0.409±0.003b	8599	<0.001
Van	3.884±0.007a	2.459±0.003c	2.515±0.004c	3.152±0.002ab	2.873±0.505b	6.6	<0.003
Chic	2.57±0.003a	2.22±0.002e	2.31±0.004d	2.35±0.003c	2.50±0.004b	1827	<0.001
Ben	115.9±0.22e	148.03±0.46d	214.66±0.13a	185.6±0.24b	172.41±0.18c	19241	<0.001
Ros	7.74±0.06d	17.15±0.16b	20.83±0.29a	13.48±0.03c	11.77±0.10cd	1955	<0.001
Qu	5.829±0.007a	5.810±0.004a	5.564±0.005c	5.791±0.013b	5.772±0.003b	243	<0.001

*: Means (±: n= 10) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at P≤0.05. Control, NPK: nitrogen-phosphorus-potassium; CHT-1: 0.5 mM Chitosan; CHT-2: 1 mM Chitosan; CHT-3: 2 mM Chitosan; GA: gallic acid, Chl: chlorogenic acid, Van: vanillic acid, Chic: chichoric acid, Ben: benzoic acid, Ros: rosmarinic acid, Qu: quercetin.

The results are in harmony with the literature, which indicated that the predominant phenolic acids found in garlic are gallic acid, benzoic acid, catechin, chlorogenic acid, rosmarinic acid, syringic acid, vanillin, coumaric acid, ferulic acid, cinnamic acid, tannic acid, kaempferol, and quercetin (Beato et al., 2011; Nagella et al., 2014; Shang et al., 2019). Also, studies conducted by Fooladivanda et al. (2019), Golkar et al. (2019), Park et al. (2019), and Hassan et al. (2021) elucidated that chitosan affects phenolic acid levels in lemongrass, safflower, buckwheat, and *Catharanthus roseus.* Furthermore, Chakraborty et al. (2009), and Geng et al. (2020), reported that types and concentrations of phenolic acids in several fruits and vegetables were enhanced by the CHT application, and this increase was closely related to the CHT-induced PAL activity. However, research claimed that high doses of CHT can suppress the concentration of phenolic acids in plant tissue. Park et al. (2019) examined the amount of benzoic acid, caffeic acid, catechin, chlorogenic acid, and gallic acid in buckwheat plants, and in the control plants, the recorded values were (in μg g^{-1} dry weight) as follows: 74.48, 77.99, 56.18, 58.92, and 6.09, respectively. Also, they determined that benzoic acid (71.34, 58.17, and 68.56 μg), caffeic acid (82.52, 81.25, and 70.27 μg), catechin (64.32, 96.59, and 66.34 μg), chlorogenic acid (81.62, 99.66, and 66.56 μg), gallic acid (6.27, 9.19, and 5.61 μg) contents changed depended on CHT levels (0.01%, 0.1%, and 0.5%). The result displayed that benzoic acid was reduced by the CHT, but others were increased. In another study, Geng et al. (2020) and Elshamly and Nassar (2023) observed incremental values of phenolic acids in corn, and creeping bentgrass upon CHT application.

While general benzoic acid and rosmarinic acid were relatively higher in garlic samples, gallic, vanillic, and chlorogenic acid showed lower values. Similarly, quercetin is highest in the control group of garlic (Table 2). These results are consistent with the findings of Park et al. (2019). In addition, the decrease in the amount of quercetin, vanillic acid, chichoric acid, and quercetin in the CHT and NPK applied group could be attributed to the difference in the effects of CHT and NPK application on secondary pathways (Chakraborty et al., 2009; Diretto et al., 2017). Pirbalouti et al. (2017b), and Hassan et al. (2021) suggested that secondary metabolite pathways can be induced by some stimulants but inhibited by other substances.

3.3. Influences of exogenous chitosan supplements on the activity of antioxidant enzymes

The influence of CHT supplements on the APX, CAT, POD, and SOD activities is shown in Figure 3. Data revealed that for both years, exogenous CHT supplements elevated POD and SOD activity, especially with CHT-2 application. In contrast, APX and CAT activity were negatively influenced by CHT supply. Further, CHT-1 inactivated APX activity by 24.52% for the first year and 20.20% for the second year, compared to the control. Regarding the control, the lowest CAT activity was achieved with CHT-3 (17.59% for the first year and 20.20% for the second year) (Figure 3). On the other hand, the highest POD activity was recorded with CHT-2 (30.1%, 30.2%), followed by CHT-3 (16.2%, 16.9%), in comparison to control. Similarly, SOD activity was promoted the most by CHT-2 among CHT concentrations (by 10.62% and 10.66%) in both years (Figure 2). Additionally, NPK application led to a slight decrease in the activity of the four enzymes examined in both years (Figure 3).

In agreement with this study, Abdelaal et al. (2021) observed that CHT provision activated SOD and POD enzymes in garlic even under dry conditions. Increased values in CAT and GPX activities in lemon grass upon CHT supplementation at 100 and 150 mg CHT doses were reported by Fooladi vanda et al. (2019). A study conducted on safflower under salt stress revealed that SOD and CAT activity were elevated in seedlings treated with exogenous CHT, and the increase in SOD activity was 0.4%. Attia et al. (2021) revealed that spraying CHT solutions on tomatoes improved the activities of SOD (10.07%-24.84%), CAT (10.07%-24.45%), and POD (36.175-46.335) compared to the control. The increased levels of POD and SOD activity have been attributed to the stimulating effect of CHT supplements on cell division and expansion (El Amerany et al., 2022), photosynthetic metabolism (Zhang et al., 2021), and secondary metabolite accumulation (Pirbalouti et al., 2017b), which promotes vegetative growth and bulb development in garlic. On the other hand, the differences in antioxidant enzymes detected in garlic applied with CHT were consistent with the studies of Geng et al. (2020) and Attia et al. (2021), who reported that a higher CHT (300 mM) induced activity of these enzymes, but higher or lower levels inactivated the enzymes. Researchers have attributed these differences to the properties of the chemicals in which chitosan is dissolved. Similarly, Hasan et al. (2021), Khaled et al. (2021), and Moradkhani and Jabbari (2023) highlighted that exogenous chitosan applications stimulated enzyme activities in *Catharanthus roseus*, *Allium sativum*, and *Cuscuta campestris* plants.

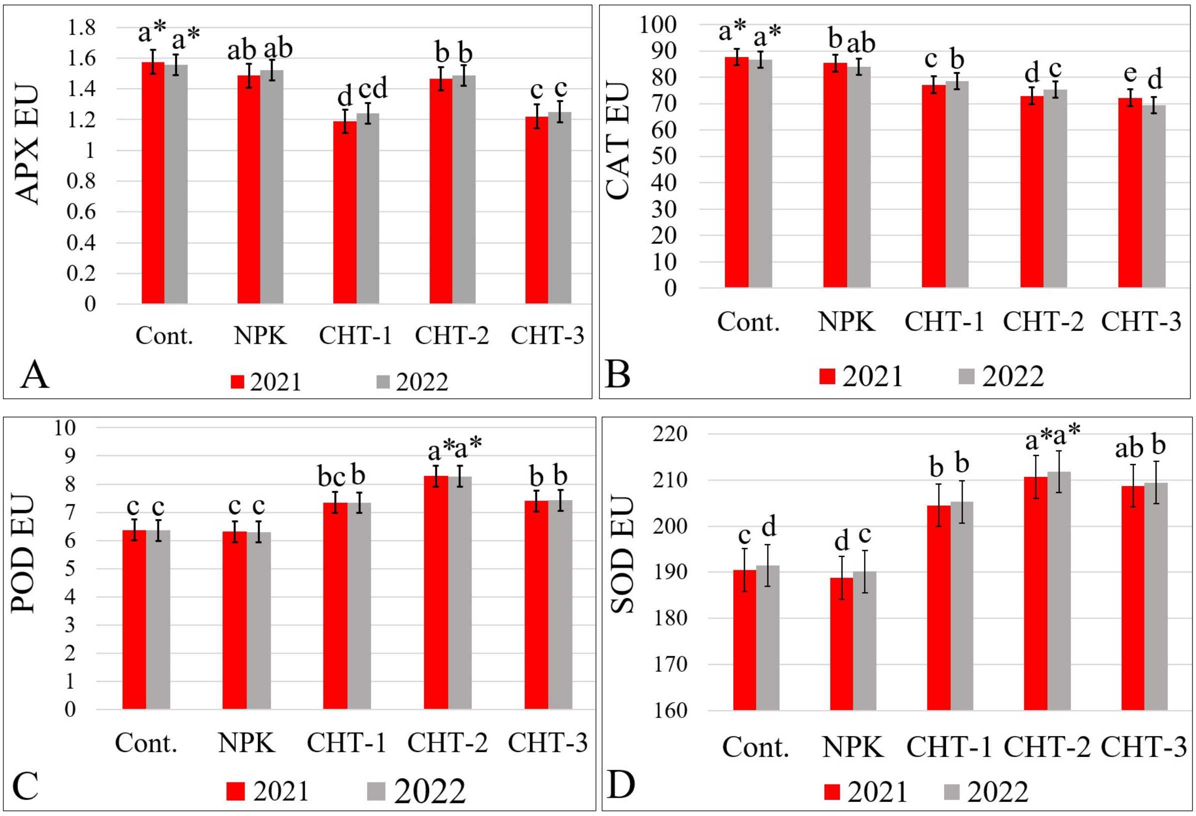

Figure 3. Variation of APX (3A), CAT (3B), POD (3C), and SOD (3D) activity in the garlic samples. *: Means (±:= 3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at P≤0.05. 0: Control, NPK: nitrogen-phosphorus-potassium; CHT-1: 0.5 mM Chitosan; CHT-2: 1 mM Chitosan; CHT-3: 2 mM Chitosan.

3.4. Influences of exogenous chitosan supplements on mineral status of garlic samples

Identifying the elemental fluctuation of CHT-supplied garlic may provide vital information for monitoring how pre-harvest agricultural practices can regulate mineral accumulation. The mineral fluctuations of garlic samples as macro and trace elements are shown in Table 4 and Table 5. The data indicated that the element accumulations of garlic samples differed significantly between groups but did not significantly differ across the years. Considering both years, the lowest and highest values recorded for the elements Na, Cl, Mg, P, S, K, and Ca were (in mg g^{-1}) in the range of 133-960.8, 558-960.8, 23.6-37, 5432-10730, 10460 -22990, 18500-23500 and 3537-9460, respectively (Table 4).

Regarding trace elements (Mn, Fe, Ni, Cu, Zn, and Se), the highest levels were recorded as 77.7 mg, 35.44 mg, 40.7 mg, 52.3 mg, and 0.74 mg, respectively, while the lowest values of the elements were 25.58 mg, 40.5 mg, 30.20 mg, 15.34 mg, 44.64 mg, and 0.42 (Table 5). The obtained data presented in Table 4 indicates that regarding essential elements, CHT supplements improved Na (CHT-2), Cl (CHT-2), K (CHT-1), and Ca (CHT-1) contents, but NPK caused an increase in Mg and P contents ($P<0.05$). Further, sulfur content was over in the control group for both years. Regarding trace elements, the samples supplemented with CHT-2 had the highest trace element contents in both years. In contrast, the lowest Mn was detected in the CHT-applied samples, but the lowest Fe was detected in the CHT-1-applied group. Moreover, a decrease in Ni was recorded with NPK, whereas reductions in Cu and Se were noted in the control group (Table 4, Table 5).

Table 4. Influences of exogenous chitosan application on the major elements (Na, Cl, K, Mg, P, S, and Ca) and trace elements (Mn, Fe, Ni, Cu, and Zn) content (in mg g^{-1}) in garlic samples (2021)

2021	Control	NPK	CHT-1	CHT-2	CHT-3	F	P
Na	134b*	133c	134b	136a	135b	1583	<0.001
Cl	558.5	561.4	924.6	960.8	693	1123570	<0.001
Mg	33b	37a	24.6d	25.7cd	26.5c	271777	<0.001
P	8 609b	10 730a	5 755d	5 845c	5432e	14637571	<0.001
S	22 990a	22 220b	10 460e	10 822d	1 1455c	69662315	<0.001
K	18 500e	22 630c	23 500a	22 688b	18 766d	6124583	<0.001
Ca	3537e	6 276d	9 460a	6 855c	8 255b	6587795	<0.001
Mn	28.8c	32b	31.96bc	36.22a	27.66d	140543	<0.001
Fe	48.2d	61.1b	42.16e	75.77a	55.68c	18467694	<0.001
Ni	30.6cd	30.2d	33.46b	35.44a	30.88c	176198	<0.001
Cu	16.6d	17.7c	21.8b	38.8a	16.8cd	753807	<0.001
Zn	50.2b	48.4c	50.5b	52.3a	45.7d	112873	<0.001
Se	0.42e	0.46d	0.66a	0.53c	0.64b	2991	<0.001

*: Means (±: n= 10) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $P\leq0.05$. NPK:nitrogen-phosphorus-potassium, CHT-1: 0.5 mM Chitosan; CHT-2: 1 mM Chitosan; CHT-3: 2 mM Chitosan.

Table 5. Influences of exogenous chitosan application on the major elements (Na, Cl, K, Mg, P, S, and Ca) and trace elements (Mn, Fe, Ni, Cu, and Zn) content (in mg g^{-1}) in garlic samples (2022)

2022	Control	NPK	CHT-1	CHT-2	CHT-3	F	P
Na	132d*	134b	133c	138a	134b	15142	<0.001
Cl	563.4d	563.6d	922.6b	955.5a	684.7c	237919068	<0.001
Mg	24c	38a	26.6b	23.6cd	24.7c	546.4	<0.001
P	8544b	10 544a	5 644d	5 755c	5 566e	10521652	<0.001
S	23 455a	22 566b	11 233e	11 566d	13 455c	54946702	<0.001
K	19 566d	22 456b	21 455c	24 355a	17 444e	13047559	<0.001
Ca	3422e	6422d	9345a	6654c	8544b	1165000	<0.001
Mn	30.5bc	31.6b	34.6a	34.2a	25.5c	1845854	<0.001
Fe	53.3c	60.6b	40.5d	77.7a	53.5c	4376128	<0.001
Ni	28.8d	32.5b	31.2c	33.5a	27.6d	149246	<0.001
Cu	17.6c	17.4c	24.6b	40.7a	15.5d	2446651	<0.001
Zn	54.4a	47.8d	49.7c	50.4b	44.4e	137804	<0.001
Se	0.66c	0.47e	0.72b	0.57d	0.76a	2945.2	<0.001

*: Means (±: n= 10) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $P\leq0.05$. NPK:nitrogen-phosphorus-potassium, CHT-1: 0.5 mM Chitosan; CHT-2: 1 mM Chitosan; CHT-3: 2 mM Chitosan.

Also, the contents of Ca and Mn reached their maximum values with CHT-1, while the contents of Cl, K, Ni, and Cu peaked with CHT-2. In contrast, the highest Mg and P were achieved with the NPK-applied groups. Zhang et al. (2021) noted that exogenous CHT induced the K content in lettuce, but it did not Na level did not significantly affect Na accumulation. Similarly, Ramdan and El Mesairy (2015) found that 200 ppm CHT increased the leaf K percentages compared to the control. Higher Na, Cl, K, and Ca levels with CHT applications may be attributed to the increased uptake of these elements from the root rhizosphere due to chitosan elevating the microbial population, facilitating the conversion of organic substances into inorganic nutrients, and boosting the intracellular osmotic potential, which all collectively improve mineral absorption by roots (Geng et al., 2020;

Rahman et al., 2021). Diretto et al. (2017), Elshamly and Nassar (2023), and Yoldas et al. (2024) suggested that the rates of essential elements in vegetative tissue are much higher due to their critical roles in the optimal growth of vegetative and reproductive organs, the uninterrupted continuation of physiological processes, the maintenance of turgor/osmosis in cells, and also the activation of many enzymes. Similarly, trace elements Mn, Fe, Zn, Cu, and Ni are essential components of essential enzymes in photosynthesis, respiration, protein and carbohydrate metabolism; therefore, they may have accumulated more in garlic tissue (Adamuchio-Oliveira et al., 2020; Macit et al., 2023). The high amounts of essential and trace elements matched with the high bulb fresh weight detected in CHT-supplied samples confirmed the positive effect of chitosan on mineral uptake from the soil and accumulation in garlic tissue (Table 1 and Table 3).

Conclusion

CHT is a natural polysaccharide with extensive application areas, especially in agriculture, as a safe and inexpensive exogenous stimulator antioxidant. In this study, the effects of exogenous CHT provision during the early growth stages on the yield, ash content, biochemicals, enzyme activities, and mineral status of Taşköprü garlic. The results revealed that the effects of chitosan on the examined traits changed according to concentrations, but no significant change was detected in the measured parameters depending on the years. While total phenolic compound production in the samples was promoted by CHT-2, total flavonoid synthesis was stimulated by CHT-3. While vanillic acid, chlorogenic acid, and quercetin acid decreased with CHT doses, gallic acid, benzoic acid, rosmarinic acid, and chichoric acid accumulation were improved by CHT supplementation. Further, while the amount of rosmarinic acid and benzoic acid peaked with CHT-1, chlorogenic acid reached the highest value with CHT-2. The highest gallic acid was achieved by the NPK. Also, the enzymes POD and SOD were activated by CHT-2. In terms of nutrients, all CHT doses promoted macro elements (Na, Cl, K, and Ca) and trace elements Mn, Fe, Cu, Zn, and Ni accumulation in garlic samples. However, the CHT-2 samples were found to be rich in Cl, Mn, Fe, Ni, Cu, and Zn, while the CHT-1 group had a greater concentration of calcium, potassium, and selenium. Also, the samples provided solely with NPK were the richest in the P, S, and Mg elements.

Considering all examined parameters, CHT-1 proved beneficial in improving bulb yield, phenolic acids (especially benzoic and rosmarinic acid), as well as the levels of potassium, calcium, and selenium. In addition, CHT-2 was useful in enhancing total phenolic compound, POD, and SOD activity, as well as Mn, Fe, Ni, Cu and Zn accumulation. The results of the study may offer insights for further exploration of chitosan applications in garlic production, aimed at enhancing quality yield and keeping quality during storage.

Ethical Statement

Ethics approval is not required for the study because there is no need for a permit for the investigation of the experimental plant variety.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Author Contributions

NT arranged the research topic, wrote and, checked the text. AÇA carried out planting, chemical application and chemical analyses.

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References

Abdelaal K., Attia K. A., Niedbała G., Wojciechowski T., Hafez Y., Alamery S., Alateeq T. K., & Arafa S. A. (2021). Mitigation of drought damages by exogenous chitosan and yeast extract with modulating the photosynthetic pigments, antioxidant defense system and improving the productivity of garlic plants. *Horticulturae*, *7*(11), 510. https://doi.org/10.3390/horticulturae7110510.

Adamuchio-Oliveira, L. G., Mazaro, S. M., Mógor, G., Santanna-Santos, B. F., & Mógor, Á. F. (2020). Chitosan associated with chelated copper applied on tomatoes: Enzymatic and anatomical changes related to plant defense responses. *Scientific Horticulture*, *271*, 109431. https://doi.org/10.1016/j.scienta.2020.109431.

Agbar, Z. A., Shakya, A. K., Khalaf, N., & Haroon, M. (2008). Comparative antioxidant activity of some edible plants. *Turkish Journal of Biology, 32,* 193-196.

Alide, T., Wangila, P., & Kiprop, A. (2020). Effect of cooking temperature and time on total phenolic content, total flavonoid content and total in vitro antioxidant activity of garlic. *BMC Research Notes, 13*, 564.

Arista, R. A., Priosoeryanto, B. P., & Nurcholis, W. (2023). Total phenolic, flavonoids contents, and antioxidant activities in the stems and rhizomes of java cardamom as affected by shading and N fertilizer dosages. *Yuzuncu Yıl University Journal of Agricultural Sciences, 33*(1), 29-39. https://doi.org/10.29133/yyutbd.1193221.

Attia, M. S., Osman, M. S., Mohamed, A. S., Mahgoub, H. A., Garada, M. O., Abdelmouty, E. S., & Abdel Latef, A. A. H. (2021). Impact of foliar application of chitosan dissolved in different organic acids on isozymes, protein patterns and physio-biochemical characteristics of tomato grown under salinity stress. *Plants, 10*, 388. https://doi.org/10.3390/plants10020388.

Badal, D. S., Dwivedi, A. K., Kumar, V., Singh, S., Prakash, A., Verma, S., & Kumar, J. E. (2019). Effect of organic manures and inorganic fertilizers on growth, yield and its attributing traits in garlic (*Allium sativum L*). *Journal of Pharmacognosy and Phytochemistry*, *8*, 587-590.

Beato, V, Orgaz, F, Mansilla, F, & Montano, A. (2011). Changes in phenolic compounds in garlic (*Allium sativum* l.) owing to the cultivar and location of growth. *Plant Foods for Human Nutrition, 66*(3), 218-223.

Cemeroğlu, B. (2007). Food analyses. *Gida Teknolojisi Derneği Yayinlari, 34*, 168-171.

Chakraborty, M., Karun, A., & Mitra, A. (2009). Accumulation of phenylpropanoid derivatives in chitosan-induced cell suspension culture of *Cocos nucifera*. *Journal of Plant Physiology*, *166*, 63-71.

Diretto, G., Rubio-Moraga, A., Argandoña, J., Castillo, P., Gomez-Gomez, L., & Ahrazem, O. (2017). Tissue-specific accumulation of sulfur compounds and saponins in different parts of garlic cloves from purple and white ecotypes. *Molecules, 22*, 1359.

El Amerany, F., Rhazi, M., Balcke, G., Wahbi, S., Meddich, A., Taourirte, M., & Hause, B. (2022). The effect of chitosan on plant physiology, wound response, and fruit quality of tomato. *Polymers, 14*, 5006.

El Sagan, M. A. M., & El Dsouky, G. A. (2015). Effect of ırrigation systems and chitosan on productivity of garlic under siwa conditions. *Zagazig Journal o*f *Agricultural Research, 42* (6), 427- 439.

Elshamly, A. M., & Nassar, S. M. (2023). The impacts of applying cobalt and chitosan with various water irrigation schemes with different growth stages of corn on macronutrient uptake, yield, and water use efficiency. *Journal of Soil Science and Plant Nutrition, 23*, 2770-2785. https://doi.org/10.1007/ s42729-023-01233-3.

Falcon, A., Costales, D., Gonzalez, D., Morales, D., Mederos., Y, Jerez ,E., & Cabrera, J. (2017). Chitosans of different molecular weights enhance potato (*Solanum tuberosum* L.) yield in a field trial. *Spanish Journal of Agricultural Research, 15*(1), e0902. https://doi.org/10.5424/sjar/2017151-9288.

Fooladi vanda, G., Shabani, L. & Razavizadeh, R. (2019). Chitosan enhances rosmarinic acid production in shoot cultures of *Melissa officinalis* L. through the induction of methyl jasmonate. *Botanical Studies, 60*, 26. https://doi.org/10.1186/s40529-019-0274-x.

Geng, W., Li, Z., Hassan, M. J., & Peng, Y. (2020). Chitosan regulates metabolic balance, polyamine accumulation, and na+ transport contributing to salt tolerance in creeping bentgrass. *BMC Plant Biology, 20*(1), 1-15.

Geries, L. S. M., Omnia, H. S., & Marey, R. A. (2020). Soaking and foliar application with chitosan and nano chitosan to enhancing growth, productivity and quality of onion crop. *Plant Archives, 2*(2), 3584-3591.

Gmaa, S.S. (2016). Improving yield, quality and storageability of garlic *(Allium Sativum L.)* by pre-harvest foliar application. *Journal of Plant Production, 7*(1),7-12.

Golkar, P., Taghizadeh, M., & Yousefian, Z. (2019). The effects of chitosan and salicylic acid on elicitation of secondary metabolites and antioxidant activity of safflower under in vitro salinity stress. *Plant Cell Tissue and Organ Culture, 137*(3), 575-585. https://doi.org/10.1007/s11240-019-01592-9.

Gürsoy, M. (2022). Effect of foliar aminopolysaccharide chitosan applications under saline conditions on seedling growth characteristics antioxidant enzyme activity, chlorophyll and carotenoid contents of safflower (*Carthamus tinctorius* L.), *Pakistan Journal of Botany*, *54*(5), 1605-1612. hhttp://Dx.Doi.org/10.30848/PJB2022-5(15).

Handayani, R., & Dinoto, A.(2022). Chitosan application for maintainingt the growth of lettuce (*Lactuca sativa*) under drought condition. *Earth and Environmental Science, 980*, 012013. https://doi:10.1088/1755-1315/980/1/012013.

Hassan, F. A. S., Ali, E., Gaber, A., Fetouh, M. I., & Mazrou, R. (2021). Chitosan nanoparticles effectively combat salinity stress by enhancing antioxidant activity and alkaloid biosynthesis *in Catharanthus roseus* (L.) G. Don. *Plant Physiology and Biochemistry, 162*, 291-300. https://doi.org.10.1016/j.plaphy.2021.03.004.

Jabber, M. D. (2021). *Effect of Nitrogen and Chitosan Raw Material Powder on the Performance on Yield of Garlic* (*Allium sativum* L.). (Master Thesis). Sher-E-Bangla Agricultural University, Department Of Soil Science, Dhaka, Bangladesh.

Khaled, A., Kotb, A. A., Gniewko, N., Tomasz, W., Yaser, H., Salman, A., Talal, K. A., & Arafa, S. A. (2021). Mitigation of drought damages by exogenous chitosan and yeast extract with modulating the photosynthetic pigments, antioxidant defense system and ımproving the productivity of garlic plants. *Horticulturae, 7*, 510.

Krupa-Małkiewicz, M., & Ochmian, I. (2024). Sustainable approaches to alleviate heavy metal stress in tomatoes: exploring the role of chitosan and nanosilver. *Agronomy*, *14*, 2477. https://doi.org/10.3390/agronomy14112477.

Kumaran, A., & Karunakaran, R.J. (2006). Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. *Food Chemistry, 97*, 109-114. https://doi.org/10.1016/j.foodchem.2005.03.032.

Macit, M., Aras, A., Capanoglu Guven, E., & Bakir, S. (2023). Investigating the content and bioaccessibility of phenolic compounds in roots of *Rosa canina* L. and *Rosa pimpinellifolia* L. *Yuzuncu Yil University Journal of Agricultural Sciences*, *33*(2), 163-173. https://doi.org/10.29133/yyutbd.1231881.

Marshall, M. R. (2010). Ash Analysis. Food Analysis. Boston, MA, Springer US: 105-115.

Mohamed, I. H., Abdelrasheed, K. G., Abbas, H. A., & Shehata, M. N.(2023). Effect of foliar application by chitosan on five garlic genotypes in aswan the Southern Egypt. *Aswan University Journal of Science and Technology,3*(3), 68-90. https://doi.org/10.21608/AUJST.2023.325643.

Moradkhani, S., & Jabbari, H. (2023). The effects of foliar spray of chitosan nanoparticles on tomato resistance against of *Cuscuta campestris* yunck. *The Open Medicinal Chemistry Journal, 17*(1). https://doi.org/10.2174/18741045-v17-230223-2022-7.

Nagella, P., Thiruvengadam, M., Ahmad, A., Yoon, J. Y., & Chung, I. M. (2014). Composition of polyphenols and antioxidant activity of garlic bulbs collected from different locations of Korea. *Asian Journal of Chemistry, 26*, 97-902. https://doi: 10.14233/ajchem.2014.16143A.

Nurcolis, W., Iqbal, T. M., Sulistiyani, S., & Liwanda, N. (2023). Profile of secondary metabolites in different parts of the butterfly pea (*Clitoria ternatea*) plant with antioxidant activity. *Yuzuncu Yil University Journal of Agricultural Sciences*, *33*(2), 231-247. https://doi.org/10.29133/yyutbd.1251495.

Park, C. H., Yeo, H. J., Park, Y. E., Chun, S. W., Chung, Y. S., Lee, S. Y., & Park, S. U. (2019). Influence of chitosan, salicylic acid and jasmonic acid on phenylpropanoid accumulation in germinated buckwheat (*Fagopyrum esculentum* Moench). *Foods*, *8(5),* 153. https://doi.org/10.3390/foods8050153

Pirbalouti, A. G., Malekpoor, F., Salimi, A., & Golparvar, A. (2017a). Exogenous application of chitosan on biochemical and physiological characteristics, phenolic content and antioxidant activity of two species of basil (*Ocimum ciliatum* and *Ocimum basilicum*) under reduced irrigation. *Scientia Horticulturae, 217*, 114-22. https://doi.org/10.1016/j.scienta.2017.01.031

Pirbalouti, A. G., Malekpoor, F., Salimi, A., Golparvar, A., & Hamedi, B. (2017b). Effects of foliar of the application chitosan and reduced ırrigation on essential oil yield, total phenol content and antioxidant activity of extracts from green and purple basil. *Acta Sci. Pol. Hortorum Cultus*, *16*(6), 177-186. 10.24326/asphc.2017.6.16

Rahman, M. A., Jannat, R., Akanda, A. M., Khan, M. A. R., & Rubayet, M. T. (2021). Role of chitosan in disease suppression, growth and yield of carrot. *European Journal of Agriculture and Food Sciences, 3*(3), 34-40.

Ramadan, M. E. & El Mesairy M. M. A. (2015). Effect of humic acid and chitosan on growth and yield of okra (*Abelmoschus Esculentus* L.) under saline conditions. *Egyptian Journal of Desert Research, 65*(1), 47-60.

Rameshjan, Y., Asgari, A., & Abdollahi, F. (2024). Evaluation the Effects of ırrigation periods and chitosan as a biological elicitor on onion (*Allium cepa* L.) growth and yield. *Iranian Journal of Field Crops Research, 22*(3), 291-309. https://doi.org/10.22067/jcesc.2024.86155.1293

Rodriguez-Delgado, M. A., Malovana, S., Perez, J. P., Borges, T., & Garcia-Montelong, F. J. (2001). Separation phenolic compounds by high-performance liquid chromatography with absorbance and fluorimetric detection. *Journal of Chromatography A, 912*, 249-257. https://doi.org/10.1016/S0021-9673(01)00598–2.

Sarker, U., Oba, S., Alsanie, W. F., & Gaber, A. (2022). Characterization of phytochemicals, nutrients, and antiradical potential in slim amaranth. *Antioxidants*, *11*, 1089.

Shang, A., Cao, S. Y., Xu, X. Y., Gan, R. Y., Tang, G. Y., Corke, H., Mavumengwana, V., Li, H. B. (2019). Bioactive compounds and biological functions of garlic (*Allium sativum* L.). *Foods*, *8*, 246.

Sultana, S., Islam, M., Khatun, M. A., Hassain, M. A., & Huque, R. (2017). Effect of foliar application of oligo-chitosan on growth, yield and quality of tomato and eggplant. *Asian Journal of Agricultural Research, 11*(2), 36-42. https://doi.org/ 10.3923/ajar.2017.36.42

Tahmas Kahyaoğlu, D. (2021). Comparison of the antioxidant activity of garlic cloves with garlic husk and stem: Determination of utilization potential of garlic agricultural wastes. *Turkish Journal of Agricultural and Natural Sciences*, *8*(2), 463-469.

Takim, K., Koyuncu, M. & Sayaslan, A. (2020). Comparison of amount of phenolic compounds by using LC-MS / MS in Tunceli mountain garlic (*Allium tuncelianum*) different extracts. *Harran Journal of Agricultural and Food Science*, *24*(1), 44-52. https://doi.org/10.29050/harranziraat.591171.

Taş, A., Kuru Berk, S., Kibar, H., & Gündoğdu, M. (2022). An in-depth study on post-harvest storage conditions depending on putrescine treatments of kiwifruit. *Journal of Food Composition and Analysis*, *111*, 104605. https:// doi. org/ 10. 1016/j. jfca. 2022. 104605

Turfan, N. (2022). Comparison of bulb yield, some bioactive compounds and elemental profile of Taşköprü garlic (*Allium sativum* L.) grown in greenhouse and open field conditions. *Journal of Tekirdag Agricultural Faculty, 19*(2), 248-261.

Turfan, N., & Turan, B. (2023). Effects of glutamic acid applications on the yield and growth parameters in garlic (*Allium sativum* L.) cultivation. *Harran Journal of Agricultural and Food Science, 27*(1), 1-14. https://doi.org/10.29050/harranziraat.1176239.

Turfan, N., Kibar, B., & Kibar, H. (2024). Role of storage on quality, nutritional and biochemical properties of garlic bulbs grown depending on different fertilizer applications. *Vegetos*, *37*(5), 2095-2121. https://doi.org/10.1007/s42535-023-00668-4.

Ünal, H. G. (2024a). Kastamonu garlic processing facilities situation analysis, problems and expectations. *Turkish Journal of Agriculture-Food Science and Technology*, *12*(9), 1600-1607.

Ünal, H. G. (2024b). Production habits and problems in Taşköprü garlic. *Turkish Journal of Agriculture-Food Science and Technology*, *12*(7), 1156-1161.

Vural, H., Eşiyok, D., & Duman, I. (2000). Culture Vegetables, 440 p. ISBN: 975-90790-0-2.

Yoldas, F., Ceylan, Ş., & Saatçi Mordogan, N. (2024). Assessment of residual impacts of poultry manure on nutrient, sucrose, fructose and glucose content of second crop onion (*Allium cepa* L.). *Yuzuncu Yil University Journal of Agricultural Sciences*, *34*(2), 235-245. https://doi.org/10.29133/yyutbd.1394395.

Zeroual, S., Gaouaoui, R., & Deghima, A. (2024). Polyphenolic content and *in vitro* antioxidant activity of three algerian date (*Phoenix dactylifera*) varieties. *Yuzuncu Yil University Journal of Agricultural Sciences*, *34*(2), 211-223. https://doi.org/10.29133/yyutbd.1392549

Zhang, L. L., Han, S.C., Li, Z. G., Liu, N., & Li, L. Y. (2006). Effects of the ınfestation by actinote thalia pyrrha (fabricius) on the physiological ındexes of mikania micrantha leaves. *Acta Ecologica Sinica, 26*(5), 1330-1336.

Zhang, G., Wang, Y., Wu, K., Zhang, Q., Feng, Y., Miao, Y. (2021). Exogenous application of chitosan alleviate salinity stress in lettuce (*Lactuca sativa* L.). *Horticulturae, 7*(10), 342. https://doi.org/10.3390/horticulturae7100342.