







Modulation of Biochemical Properties in Wheat by Silver Nanoparticles Under Salt Stress Conditions

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Abstract

Salt stress is a major abiotic factor that adversely affects wheat growth, leading to biochemical imbalances and reduced crop productivity. In recent years, nanotechnology has emerged as a promising approach to mitigate the detrimental effects of salinity in plants. The objective of the research is to investigate the role of silver nanoparticles (AgNPs) in modulating the biomechanical properties of wheat (*Triticum aestivum*) under salt-stress conditions. The effect of seed priming AgNPs at concentrations of 0, 1, 2, 5, 10, and 15 mM was followed by exposure to 175 mM NaCl to induce salt stress. Taurine, starch content, catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) activity are used to evaluate the biochemical characteristics. Compare the growth and biochemical parameters under different conditions (control, salt stress, and AgNP treatments). Analyze the effect of various concentrations of AgNPs on wheat growth and stress-related biochemical responses. Results showed that the 175 mM NaCl salt stress significantly reduced shoot fresh and dry weights and taurine and starch content while increasing CAT, POD activities, and SOD activity. Seed priming with AgNPs improved wheat growth under normal and salt-stressed conditions, with higher concentrations (10 mM and 15 mM) showing enhanced shoot fresh and dry weight. Salt stress and combined AgNPs increase the levels of taurine and starch content while decreasing CAT activity and raising POD activity. The research highlights the potential of nanotechnology in sustainable agriculture and provides insights into the use of AgNPs for improving crop tolerance to adverse environmental conditions.

Keywords:

Biochemical properties, silver nanoparticles (AgNPs), wheat (triticum aestivum), salt stress conditions, shoot fresh and dry weight.

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Introduction

An evolving issue that influences the environment and socioeconomic connections is the salinity of soils. The quantity and quality of food produced are also directly affected by saltwater intrusion, which endangers farming systems (Nafiza Begum & Vijaya, 2024; Ondrasek et al., 2022). Specifically, wheat has been used as an everyday food by the globe's population due to its nutritious importance (Bosco et al., 2018). Salt stress represents one of the most prominent abiotic environmental factors that limits the production of wheat (Shahzadi et al., 2024). One of the noteworthy biochemical parameters is taurine, which helps the plants to grow regardless of the different abiotic stresses (Ranjith et al., 2023). Other factors, like superoxide dismutase (SOD), ascorbate peroxidase (APX), and peroxidase (POD), are crucial for increasing the effectiveness of the photosynthesis processes and improving the yield of wheat (Ravindra et al., 2025). The advancement of nanotechnology provides a reliable and sustainable approach to salinity reduction and reduces negative effects on the environment due to harmful chemicals while increasing productivity in agriculture (Soni et al., 2024). Silver nanoparticles (AgNP) are vital for managing environmental stressors, especially salt stress, as an improvement of photosynthesis productivity, activation of oxidative mechanisms defense, and regulation of water distribution (Monica Nandini, 2024; Kamal et al., 2024). The application of AgNPs strengthened the plants' capacity to change the function of the antioxidant enzymes, decreased damage from oxidation, and increased the plant's capability to oppose salt stress (Awaly et al., 2023).

The simultaneous impact of glyphosate (Gly) and silver nanoparticles (AgNP) on the rhizosphere microbes, and the transcription of genes, in the physiologic structure of wheat was analyzed by (Bitu et al., 2021; Feng et al., 2021). The necessary information was gathered from the dealings of the wheat grains with Gly, AgNPs, and their combinations. Then the growth, expression of genes, and microbiological diversification of the wheat were examined using the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. The combined AgNPs and Gly treatment substantially increased wheat growth, changed the expression of genes, and reduced the quantity of positive rhizosphere micro-organisms. Komatsu et al., (2022) explored the potassium nitrate (KNO₃), nicotinate, and AgNPs interactions to influence the growth of wheat and sterilizing. Ninety proteins were shown to be impacted by the combination by proteomics analysis, with 25 exhibiting an increase and 65 showing a decrease in activity. Proteins linked to biotic stress and oxidants have been demonstrated to undergo significant alterations. Lower concentrations of pathogen-related proteins and glutathione reductase or peroxiredoxin were found by immunoblot analysis, but the starch synthase and liver lip were lowered and improved, respectively.

The influence of bio-synthesized silver nanoparticles (B-AgNPs) on the initial development of seeds, roots, coleoptile, and cytoplasm metabolic rates, and wheat fertilization was analyzed by (Daabool, 2022; Lahuta et al., 2022). In solutions comprising 10–40 mg/L of B-AgNPs, grains were fertilized successfully; however, root development slowed and coleoptile growth decreased by 25%. The primary metabolism, store material mobilization, and sugar/amino acid translocation were all affected by the stimulation of Reactive Oxygen Species (ROS) and the modification of metabolic profiles. The effect of silver ions and AgNPs on wheat springtime plant development and its yield examination was carried out by (Pociecha et al., 2021). Two varieties of negatively-charged AgNPs and silver ions were administered to use hydroponically grown wheat

for 14 days before the crop was transplanted into the soil. The results revealed that AgNP application increased yield, accelerated blossoming, and stimulated development, all of which were associated with modifications to the balance of Phytohormones particularly in gibberellin A6 (GA6).

Elshazly et al., (2022) recommended generating AgNPs with extracts of seeds from *Nigella Sativa* and its influence in the wheat. At different AgNP concentrations ($12.5\text{--}100\text{ }\mu\text{g}\cdot\text{L}^{-1}$), toxicity and gene toxicity were evaluated by examining molecular indicators, root/coleoptile dimension, and sprouting of the seed. The outcomes proved that AgNPs enhanced the seedling biomass but lowered root and coleoptile length, with no apparent impact on germination. Khan et al., (2023) evaluated the pearl millet's transcriptomic responses to salinity stress caused by AgNP. The pharmacological and transcriptome investigations were conducted when seedlings were given treatments with the sodium chloride (NaCl) (250 mM), AgNPs (20 mg/L), and NaCl + AgNPs. The function of antioxidant enzymes, whereas salt stress raised oxidative stress indicators, was strengthened by AgNP. The two techniques of KEGG and Differentially Expressed Transcripts (DET) offered effective mechanisms linked to stress.

The influence of AgNPs in contrast with Nitrogen, Phosphorus, and Potassium (NPP) fertilizers on the proline information, POD, and CAT enzymes in two wheat cultivars named Ibaa 99 and AI-Rasheed was analyzed by (Atia & Oraibi, 2024). A graveolens extraction was used to create AgNPs, which were then investigated using X-ray scattering, and ultraviolet (UV) spectroscopy. While peroxidase and catalase activities increased significantly in both cultivars, findings demonstrated that proline dropped in AI-Rasheed but increased in Ibaa 99. In the millets of wheat and maize, the function of AgNPs in decreasing the harmful effects of lead (Pb) was performed by (Danish et al., 2024). A pot experiment with Pb-contaminated (400 mg kg^{-1}) and non-contaminated soil, with AgNPs administered at 0 and 100 mg kg^{-1} , was conducted to collect information. The assessment was done on nutrition absorption, growth, and photosynthetic pigments. The findings indicated that 100 mg kg^{-1} AgNPs enhanced the physiological features, shoot length, and chlorophyll content, indicating AgNPs' capacity to reduce Pb cytotoxicity.

Kannaujia et al., (2022) proposed an approach to evaluate the B-AgNP's moderate ozone-induced phytotoxicity in wheat varieties of HD-2967 and DBW-17 when compared to ethylenediure (EDU) within ozone exposure; the growth, physiological in nature antioxidant protection, and yield characteristics were examined. Wheat volume, index of harvesting, and yield were all considerably enhanced by B-AgNPs (25 mg/L), with DBW-17 reacting faster. As shown by the results, B-AgNPs are an alternative for EDU in wheat protection, as it successfully increase ozone obstruction. The correlations among wheat development measurements and enzyme functions when administered silver (Ag) and gold (Au) and the nanoparticles were carried out by (Manaf et al., 2021). The antioxidant enzymes of Ascorbate Catalase (CAT), Peroxidase (POD), and guaiacol POD and the developmental variables of biomass production, area of leaves, and chlorophyll content were employed as information statistics. All three nanoparticles dramatically increased the activity of enzymes, which increased wheat growth, as demonstrated by variance (ANOVA).

The purpose of the investigation is to explore the impact of the AgNPs seed priming on wheat's growth and modulation in key biochemical responses, including taurine and starch content, along with enzyme activities under salt-stress environments (Akgün & Ergün, 2023).

The remaining parts of the investigation are structured as follows: Part 2 covers the brief process of seeding with AgNP with the plant sources and the experimental design. Part 3 focuses on the achieved outcomes of the suggested approach and Part 4 briefly discusses the overall outcomes. Finally, the conclusion is provided in Part 5.

Methods and Materials

The characteristics of the wheat plant (*Triticum Aestivum*) and its growth process, the applied technique for the AgNP seeding, the important biochemical factors with the measuring parameters, and the utilized analysis technique are explained comprehensively below.

Plant Material

Triticum aestivum, or common wheat, is a frequently grown wheat type that enables the measurement of the stress reduction potential of AgNPs under salt stress. Before receiving exposure to different experimental circumstances of salt stress up to 175 mM NaCl, the seeds were sprouted and surface-sterilized on wet filter paper in Petri plates under restricted culture ambient circumstances (temperature: $23 \pm 2^\circ\text{C}$, humidity: 45–50%) to guarantee the uniformity of the size and age of the seeds.

Design of Experiment

The impacts of AgNPs on wheat exposed to salt stress (175 mM NaCl) have been assessed using a randomized technique. Wheat seeds were primed with AgNP solution at different concentrations of 0, 1, 2, 5, 10, and 15 mM, where 0 mM served as a control that did not receive any AgNPs, while the other quantities represented different AgNP treatment levels. After the appearance of four leaves from the plant growth above the ground, the different concentrations of AgNP salt were applied to the sprouted wheat seedlings. Following priming, the seeds were transferred to white sand that had been cleaned and dried (with particles ranging from 0.5 to 1 mm), and it received watering twice a week using distilled water for irrigation. For 15 days, the experiment was carried out in a growth environment with regulated conditions (25°C). To determine AgNPs' contribution to reducing salt stress, the biochemical characteristics were examined.

Seed priming with AgNPs

Seed priming combined with AgNPs has evolved into a potent wheat seed preparation technique that helps seeds manage multiple stressful environments. By immersing the wheat seeds in a solution of AgNP at various concentrations of 0, 1, 2, 5, 10, and 15 mM, the seeds were soaked with AgNPs in deionized water. At room temperature, the seeds were allowed to grow in these solutions. Before being planted, the seeds were dried to take out any remaining moisture after the soaking time.

Following the priming, the seedlings were placed in white sand (0.5–1 mm particle size) that was already dehydrated and sterilized with distilled water. Inside each container, 70 g of sand was combined with 2.5 ml of the corresponding AgNP solution. The plants were exposed to 175 mM NaCl with the intent to resemble salt stress. For 15 days, seedlings were kept in a monitored growing environment and given 10 mm of distilled water two times per week. To avoid associations between nanoparticles and nutritional components, no additional supplements were offered. By using the effective priming strategy, wheat's early development was improved, also its resilience to stress mechanisms was strengthened, and important biochemical characteristics, such as taurine levels, starch content, and antioxidant enzyme activities (SOD, CAT, and POD), were manipulated. For further biochemical analysis, control and treated plant samples were gathered and preserved.

Biochemical Assessment

The following tests were carried out to assess the biochemical characteristics of wheat under the various concentrations of AgNP.

Peroxidase (POD) – The enzyme POD is crucial for wheat growth and its activity was determined by the guaiacol method that contributed to the decomposition of (H_2O_2) (hydrogen peroxide), resulting in a brown-colored product that was quantified by measuring absorbance at 470 nm. POD enhances overall growth, improving defences against abiotic challenges, including drought and salt.

Starch content - A significant indicator of plant development and storage of energy is the amount of starch in wheat. Under ideal conditions, starch builds up in the connective tissue of wheat, giving it nutrients necessary for growth, and the progress is calculated by the anthrone method. Here, the starch in the plant tissue was hydrolyzed and combined with anthrone to form a green color.

Superoxide Dismutase (SOD) – SOD is essential for protecting wheat from damage from oxidation, particularly when it is exposed to salt stress. The reduction of NBT (Nitro Blue Tetrazolium) at the wavelength of 560 nm is suitable for assessing the SOD. Wheat growth, antioxidant defence, and stress tolerance are all improved by increased SOD activity.

Taurine - In wheat development, taurine promotes improved tolerance to stress, membrane stabilization, cellular framework protection, increased activity of antioxidants, and osmotic regulation that promotes better growth and development in different stress circumstances. A spectrophotometric method is employed to measure the taurine content by the absorbing capacity of a colored complex created in a chemical process.

Catalase (CAT) - Through the separation of H_2O_2 , into water and oxygen at 240 nm wavelength, the CAT activity is measured and offers a significant role in wheat progress. The oxidative stress is mitigated and encourages growth in salty conditions.

Measurements of Growth

To evaluate the growth response of wheat under different treatments of AgNP, the two significant parameters were measured below.

Shoot Fresh Weight: The biomass of a plant's above-ground parts, like its stem and leaves, just after harvesting is referred to as shoot fresh weight and provides the analytical balance. Under different experimental conditions, fresh weight is monitored. In the context of wheat under salt stress, shoot fresh weight can be substantially decreased due to the adverse effects of salinity on plant physiology. **Shoot dry weight:** The total weight of a plant after it has been entirely drained at a temperature of 65°C for 48 hours to exclude any wet matter is known as shoot dry weight. By analysing the dry weight, the wellness of AgNP can modulate the biochemical properties of wheat plants, and the potential to maintain growth in the circumstances of salt exposure is kept tracked.

For recording the impacts of salinity conditions and AgNP priming on wheat growth over time, these growth measures were taken at periodic times, usually every seven days, during the time of experimentation which is generally measured in terms of g (gram).

Statistical Analysis

To evaluate the overall effects of AgNPs priming on wheat growth and stressing-related physiological reactions an analysis of variance (ANOVA) test with the Duncan's multiple range test was conducted. The variables of SS (sum of squares) indicate the total fluctuations in the statistics of the plants that received the different treatments. An ensemble quantity of autonomous factors that can alter the data is mentioned by DF (degree of

freedom). The proportion of the SS to DF is known as MS (mean square), which compares the variations between the groups. The changes in the MS in the two groups are calculated by F-statistics. A higher F-value indicates a significant difference between groups. In ideal circumstances, the probability of the observed information is denoted as the P-value. Typically, the value decreased by 0.05 and the symbol * indicates the significant effects.

Result

The examination of how the biochemical properties, like the enzyme activities and the taurine and starch, are analyzed and the findings were validated through ANOVA with Duncan's multiple range test analysis. The effects of various NaCl concentrations (0 mM and 175 mM) and AgNP concentrations (0–15 mM) on plant shoot development are visualized in Figure 1 (a and b).

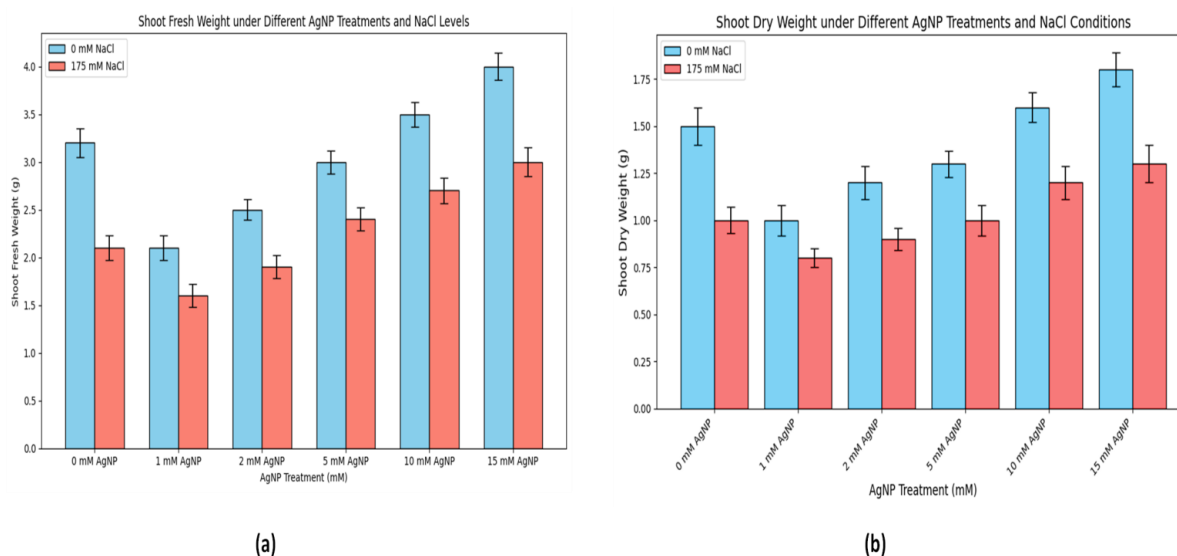


Figure 1. Different concentrations of AgNP and NaCl in shoot fresh (a) and shoot dry (b) weights

The AgNP treatment significantly influences plant growth, with higher AgNP concentrations (10-15 mM) enhancing shoot fresh and dry weight under both normal and salt stress conditions. While 175 mM NaCl limits the growth, AgNP mitigates the effect, and improving biomass proved the AgNP's potential in promoting plant resilience against salt stress. The shoot fresh weight demonstrated the initial effect of AgNP on plant water retention and the shoot dry confirmed the AgNP's role in enhancing actual biomass production, specifically under salt stress. Examining these two parameters helped to determine the AgNP impacts on the plant's structural growth. The various NaCl and AgNP concentrations on the biochemical parameters of SOD, starch, and CAT are visualized in Figure 2 (a - d), and the POD activity is represented in Figure 3.

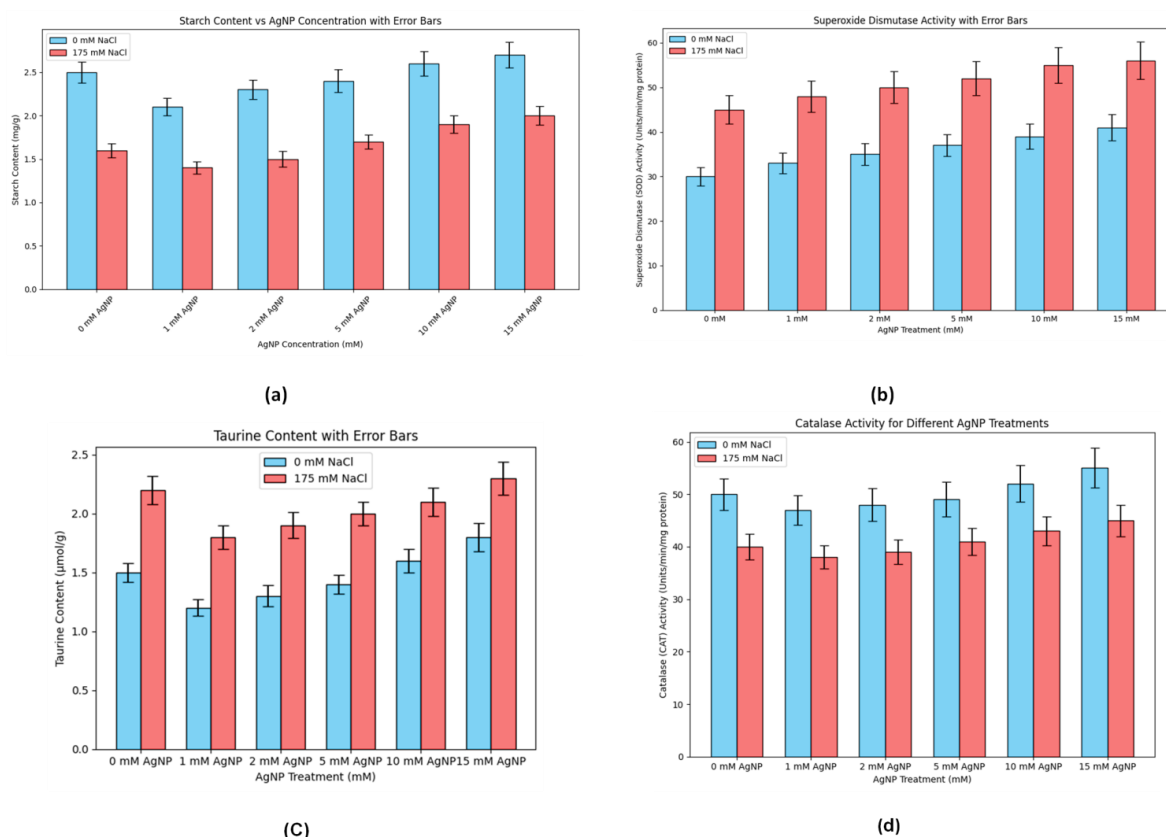


Figure 2. The effect of AgNP and NaCl on (a) starch (b) SOD (c) taurine (d) CAT parameters

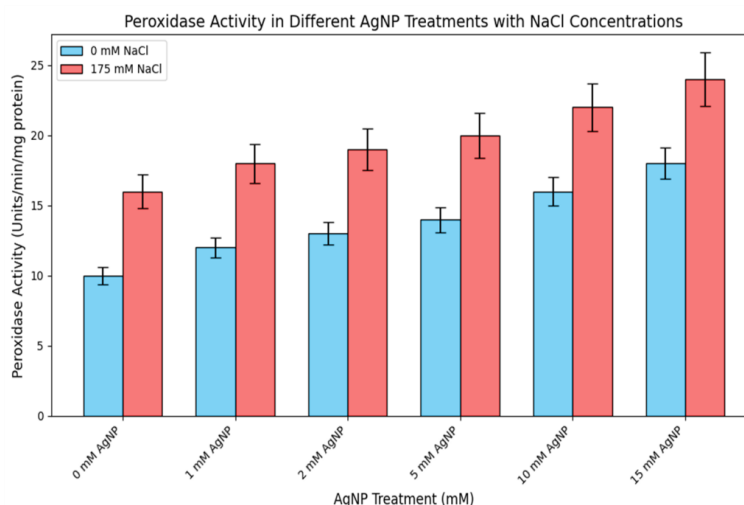


Figure 3. Influence of AgNP and NaCl on POD activity

Under 175 mM NaCl, the three biochemical parameters of SOD activity strengthened the antioxidant defenses, salt stress tolerance, and effective wheat growth. The POD activity elevates to enhance wheat's resistance to damage from oxidation, promoting enhanced development and resistance to salt stress. The taurine promotes cellular integrity enhancing antioxidant activity and adaptability to salinity.

In contrast to the mentioned three parameters the CAT activity at high concentration of salt stress (175 mM NaCl), the plants could not be able to adequately maintain the required enzyme activity, and the structure could change its metabolic procedures in response to the stress. As a result, CAT activity decreases under

higher salinity. Similarly in the starch content at 175 mM NaCl, high salinity disrupts photosynthetic efficiency, reducing carbohydrate production and leading to lower starch content. Table 1 demonstrates the impacts of various concentrations and treatment levels on five biochemical factors.

Table 1. ANOVA outcomes for biochemical properties with AgNP and NaCl treatments

Source of Variation	SS	df	MS	F-statistics	p-value
Starch Content					
AgNP Treatment	0.12	5	0.024	4.29	0.008*
NaCl Level	0.15	1	0.15	9.29	0.004*
Interaction (AgNP \times NaCl)	0.03	5	0.006	0.93	0.47
Error	0.05	18	0.0028	-	-
Total	0.35	29	-	-	-
Taurine Content					
AgNP Treatment	0.08	5	0.016	6.43	0.002*
NaCl Level	0.10	1	0.10	5.23	0.03*
Interaction (AgNP \times NaCl)	0.02	5	0.004	0.83	0.56
Error	0.04	18	0.0022	-	-
Total	0.24	29	-	-	-
SOD Activity					
AgNP Treatment	100.24	5	20.048	8.17	0.001*
NaCl Level	120.37	1	120.37	18.02	0.0001*
Interaction (AgNP \times NaCl)	20.46	5	4.092	0.69	0.65
Error	25.89	18	1.44	-	-
Total	266.96	29	-	-	-
CAT Activity					
AgNP Treatment	150.56	5	30.11	12.05	0.0002*
NaCl Level	180.23	1	180.23	35.71	0.00001*
Interaction (AgNP \times NaCl)	12.56	5	2.512	0.73	0.60
Error	70.01	18	3.89	-	-
Total	413.36	29	-	-	-
POD Activity					
AgNP Treatment	85.16	5	17.032	7.01	0.003*
NaCl Level	112.45	1	112.45	20.13	0.0001*
Interaction (AgNP \times NaCl)	16.98	5	3.396	1.02	0.43
Error	59.64	18	3.31	-	-
Total	273.23	29	-	-	-

An MS (0.016), F-statistics (6.43), and a p-value (0.002 *) and SS (0.08) reveal a robust influence of AgNP treatment on taurine concentration. For starch content, an effective influence is obtained by the AgNP treatment illustrated by SS (0.12), MS (0.024), F-statistics (4.29), and a p-value (0.008 *). The AgNP treatment had a substantial effect on SOD activity, as evidenced by 20.048 (MS), 8.17 (F-statistics), 0.001 (p-value), and 100.24 (SS). With SS (150.56), MS (30.11), F-statistics (12.05), and a p-value (0.0002*) in CAT activity, an important difference is achieved, indicating the efficacy of AgNP treatment. The SS (85.16), F-statistics (7.01), p-value (0.003), and MS (17.032) all confirm that AgNP treatment has a substantial effect on POD activity. Similarly, the NaCl level has an enormous effect as proved by SS (112.45), MS (112.45), F-statistics (20.13), and p-value (0.0001).

Discussion

The results showed significant effects of AgNP treatment and NaCl levels on various biochemical parameters, including taurine, starch, and enzyme activities and shoot measurements. The analysis delivered the AgNP

treatment considerably positively affected the three biochemical parameters of SOD, POD activities and the taurine content with higher F-statistics and low p-values proved the plant's growth and robust treatment effect. In contrast to these parameters, the AgNP treatment CAT activity and starch content denoted the adversely affect in the high concentration of salinity. However, p-values greater than 0.05 demonstrated that there was no apparent interaction between AgNP and NaCl. Furthermore, the presence of NaCl identified the detrimental effects of AgNP on plant growth, which is reduced in the shoot fresh and dry weight at higher concentrations, highlighting a cooperative stress effect between AgNP and NaCl.

Conclusion

The investigation demonstrated the capability of AgNP as an efficient method to minimize the negative effects of salt stress on wheat (*Triticum aestivum*). The selection and preparation of wheat seeds is firstly performed and the experimental design was employed to assess the effects of AgNPs on wheat under salt stress. The wheat's resilience against the salinity is enhanced by AgNP seed priming. The AgNP seed priming influenced the various biochemical properties comprising starch and taurine as well as the function of the antioxidant enzymes like SOD, CAT, and POD. Under salt stress, the wheat performance is decreased at higher AgNP concentrations (10 mM and 15 mM), as offered by lower shoot fresh and dry weights. The ANOVA results highlighted that AgNPs could enhance wheat's resistance to salt stress by positively modulating the biochemical parameters of taurine, POD, and SOD activities. Although the starch and CAT activities did not have a significant effect in higher concentrations of NaCl, the findings revealed that AgNP could deliver a promising technique for sustainable farming domains to enhance the productivity of crops under challenging environmental circumstances like salinity and proved a valuable tool for improving wheat growth and resilience in saline-prone areas. While the technique is demonstrated as a suitable solution for improving wheat growth some limitations exist. The functions of antioxidant enzymes were determined, but the molecular mechanisms by which AgNPs influence stress tolerance were not considered. Further research could explore the genetic and molecular mechanisms that explain AgNPs' improved ability to resist stress.

Author Contributions

All Authors contributed equally.

Conflict of Interest

The authors declared that no conflict of interest.

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