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Abstract

Oats (Avena sativa L.) are the 6th-ranked crop (salt-sensitive) in the world, and common salt stress occurs in Pakistan. Salinity stress poses a significant threat to agricultural productivity worldwide, with its adverse effects on crop growth and yield being increasingly pronounced. The parameters used here are directed towards the efficiency of chitosan application as growth regulators to improve the negative effects of salinity stress on the growth of oats, such as plant height, chlorophyll content, relative water content, water potential, and yield. The experiment was carried out at Islamia University of Bahawalpur, Pakistan, to investigate the impact of chitosan on salt stress on oat plants. It was laid out in a completely randomized design with a factorial arrangement. Six treatments were provided by the two factors: salinity and chitosan application, and a positive relationship between chitosan foliar application and enhancing the performance of oat plants under salt stress conditions was found. The plant height (17.65%), grain weight (27.78%), grain yield (58.16%), biological yield (40.69%), harvest index (13.04%), RWC (20.90%), High WPP (10.19%), and Improved Chlorophyll content (43.72%) are consistently found superior in Chitosan treated oat crop than normal and only salt treated oat plants. The use of chitosan to improve the resilience of oats (Avena sativa L.) to salinity stress by evaluating the effects of different chitosan concentrations on plant growth.

Key Words:

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INTRODUCTION

Oats (*Avena sativa* L.), a versatile cereal crop from the family Poaceae, have been a critical component of human and livestock nutrition since ancient times. (Giraldo et al., 2019). Oats are an important health promoting foods due to their nutritional value, that presents the presence of beta-glucans, a soluble fiber that has been correlated with lowering cholesterol and controlling blood sugar (Paudel et al., 2021). Moreover, oats are rich in antioxidants like phenolic acids and avenanthramides, known for reducing the risk of cardiovascular diseases and type 2 diabetes. (Martínez-Villaluenga & Peñas, 2017).

In Pakistan, oats are mainly grown as a winter forage crop, both as a rain-fed and irrigated crop. They are appreciated for their feeding value and palatability, and have made considerable inputs in the rations of ruminants, and are also used for silage and hay production to overcome the shortage of fodder. Oats may be suggested as an important crop for Pothohar regions (Attock, Chakwal, Rawalpindi, Islamabad, and Jhelum), which receive 400 mm of rainfall during the growing season with a temperature range of 16–32°C. With the help of improved varieties of oats, a greater volume of these products, meat, milk, and butter, can be produced in the dairy industry, which may lead to the production of green fodder in quantities ranging between 148.1 and 197.7 tons/ha. Oats were the sixth most cultivated cereal in the world in 2017, with a total of 158.5 million hectares cultivated and 478 million tons harvested. Nevertheless, the nature of the climate, varieties, and other factors like irrigation continue to affect the yield, especially in Pakistan, where rainfed varieties yield approximately 20 tons/ha, while varieties like introduced cultivars, Scott, and Cuscade yield up to 69 tons/ha. (Niazi et al., 2023). But due to the more difficult hurdles of poor return and yields, low investment, etc., oats are classified as a “minor crop” (Gorash et al., 2017).

Oats offer superior nutritional quality compared to other cereals, containing 4% protein, 9% fat, 0.27% phosphorus, and 0.82% calcium. (Alemayehu et al., 2023). Furthermore, the grains and leaves of this plant are high in carotene and carbohydrates, and their fat content is 6% in some cultivars, which include omega-3 fatty acids, to have energy-rich and nutritious. (Kouřimská et al., 2018). I.e., its superior protein profile to that of common mealworms is due to essential amino acids like lysine and threonine, while minerals such as magnesium, phosphorus, thiamine, iron, and zinc are supportive of energy production, immune function, and bone health (Sterna et al., 2016). Land degradation and salinity are huge challenges to oat cultivation. It is estimated that land degradation currently affects 43 percent of South Asia’s agricultural area and has an annual financial loss in the range of USD 10 billion. With the numbers for Pakistan, 20,644 km² of land is degraded, with the highest impacts noticed in the high mountains, Sindh, and Baluchistan (Ullah et al., 2018). The problem is further compounded by the effect of salinity, which leads to 20 percent of cultivated and 33 percent of irrigated lands being globally affected, including 22–30 million hectares in Pakistan (Qadir et al., 2019). In agriculture, chitosan can be administered to plants using foliar application, dealing with seeds by nutrition through soil, or directly as a soil fertilizer (Kocięcka & Liberacki, 2021). However, oats have qualities such as salt and alkali tolerance that should enable them to be used for rehabilitating saline-alkali soils despite these challenges. Breeding salt-tolerant oat varieties, seed priming, and the application of biostimulants have been demonstrated to have the potential to increase resilience and yield under saline conditions. (Adem et al., 2014) (Sarkar & Roy, 2020). In addition, biopolymers such as chitosan have been

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successfully used to counteract salt stress by increasing the activity of antioxidant enzymes and keeping ions balanced, which improves crop performance in the areas under salt stress (Elkarmout et al., 2022; Safikhan et al., 2018). While chitosan is already widely used, this study specifically explores its potential to enhance salinity stress tolerance in oats (*Avena sativa* L.). Additionally, it evaluates how varying concentrations of chitosan influence overall plant growth.

Material and Methods

The experiment was conducted at the Islamia University of Bahawalpur, Pakistan. Sandy loam soil was collected from the Agronomy Research Field of the university, manually cleaned of clods, stubbles, and stones, and used as the growth medium. The experiment was designed as a Complete Randomized Design (CRD Factorial) with three replications. Treatments consisted of two salinity levels (0 mM NaCl as control and 150 mM NaCl to induce salinity stress) and three chitosan concentrations (0 µM as control, 15 µM, and 30 µM), making a total of six treatment combinations. Before sowing, soil samples were collected from depths of 0–15 cm and 15–30 cm using a soil auger, labeled, and sent to the Soil and Water Laboratory of the Regional Agricultural Research Institute (RARI), Bahawalpur, for physicochemical analysis.

Table 2.1. Physiochemical analysis of the experimental soil

Characteristics		Status	Values
Texture	Sand (%)	Sandy loam	54
	Silt (%)		32
	Clay (%)		14
PH		Medium alkaline	8.42
EC (dSm^{-1})		Non-saline	0.68
N (%)		Low	0.044
P_2O_5 (ppm)		Low	6
K_2O (ppm)		Medium	149
Organic Matter		Low	0.46
$CaCO_3$		Low	2.12

The oat variety *Sgd.Oats-2011* were sown manually on December 2, 2023. The plots were irrigated twice weekly. Manual hoeing was used for weed control. Temperatures during the experimental period (December 2023–April 2024) ranged from 18°C to 31°C. The crop was harvested on April 27, 2024.

For the salinity treatment, the required 150 mM NaCl solution was prepared by dissolving 8.775 g of NaCl per liter of water, calculated based on the molecular weight of NaCl (58.5 g/mol). For 500 mL of solution, 4.38 g of NaCl was used. Chitosan solutions were prepared for concentrations of 15 µM and 30 µM. Using the molecular weight of chitosan (501 g/mol), 0.003 g and 0.006 g of chitosan were dissolved in 400 mL of water to prepare the respective solutions. These treatments were applied as foliar sprays according to the experimental layout.

Procedure to Record the Observations

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Agronomic Parameters

Plant Height at Maturity (cm)

The stem length of the Oat plant was measured using the meter scale from the base of the stem to the top of the leaf. The centimeter scale or the rod was used from the soil surface to the tip of the topmost plant part. Measured averages were calculated.

Yield parameters

100 Grains weight

From every pot, 100 grains were picked up and weighed with the help of an electric balance. The weight was measured in grams.

Grain Yield (g/pot)

A plant sample from each pot was chosen; the pods were removed and weighed after the grain was extracted from the pod.

Biological Yield (g/pot)

A weighed sample from each container was picked, and the grams of biological yield were found after sun drying to finalize the biological yield as g/pot.

Harvest Index (%)

The harvest index was calculated by using the following formula

$$Harvest\ index = \frac{Grain\ yield}{Biological\ yield} \times 100$$

Quality Parameters

Relative Water Content

The relative water content (RWC) was calculated via the method given by Barrs and Weatherley (1962) by obtaining the fresh weight (Wf) of leaves and then subsequently soaking them for 4 hours before the saturated weight (Ws) was measured. Leaves that were over-saturated and dried in the oven afterward got a constant dry weight (Wd), and RWC was calculated from the equation.:

$$RWC = \frac{Wf - Wd}{Ws - Wd} \times 100$$

Leaf Water Potential (ψw)

Water content and stomatal conductance were examined from the leaves of the plant. The leaf-answering exercises for these qualities were done early in the morning. Leaf water potential (ψw) was measured using a pressure chamber (Model 1000; Planeete et Humaine, Albany, OR, USA.

Chlorophyll Content

A chlorophyll meter is a device that is used to measure the chlorophyll level in the leaves of a plant without damaging the plant. It works by turning on the light on a leaf and determining the amount of light absorbed by the chlorophyll. By this, we can see the plant's health and nutrient status. A chlorophyll meter was utilized to check the chlorophyll levels of various plant groups that were subjected to different environmental conditions; thus, data were collected to analyze the effects of these conditions on plant health and development.

Percentage formula

The general formula to calculate a percentage is:

$$= \frac{Final\ value - Intial\ Value}{Intial\ Value} \times 100$$

The initial Value is the starting amount.

The final Value is the ending amount.

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Multiply by **100** to express the result as a percentage.

Results

Results showed that there was a significant effect of different chitosan treatments and salinity stress on the physiology of oats and plant growth. Salinity showed a significant effect on plant height, showing 15.00% reduction under saline stress without chitosan as compared with non-saline conditions. But, chitosan application had positive results on plant height in both control and saline conditions. Non-saline conditions showed only a slight decrease in height with 15 µM chitosan, while a significant increase of 17.23% was seen with 30 µM chitosan. A comparison of the plant height of untreated plants and plants treated with 30 µM chitosan under saline conditions indicated that chitosan treatments helped counteract the stress conditions by increasing the height of Pinus spp. plants by 17.65%. Grain weight was similar. Grain weight (mg/grain) was adversely affected by salinity, giving a 12.62 % decrease in grain weight for untreated plants with salinity as compared to non-saline plants. But, both 15 and 30 µM chitosan groups enabled 16.50% and 24.27% enhancements, respectively, to the weight of the grains compared with the non-saline growing conditions. The result presented in Fig. 3.1 shows that under saline stress conditions, both 15µM and 30µM chitosan significantly increased the grain weight by 11.11% and 27.78%, respectively, when compared with the salinity-stressed control plants, indicating the efficacy of chitosan on grain development.

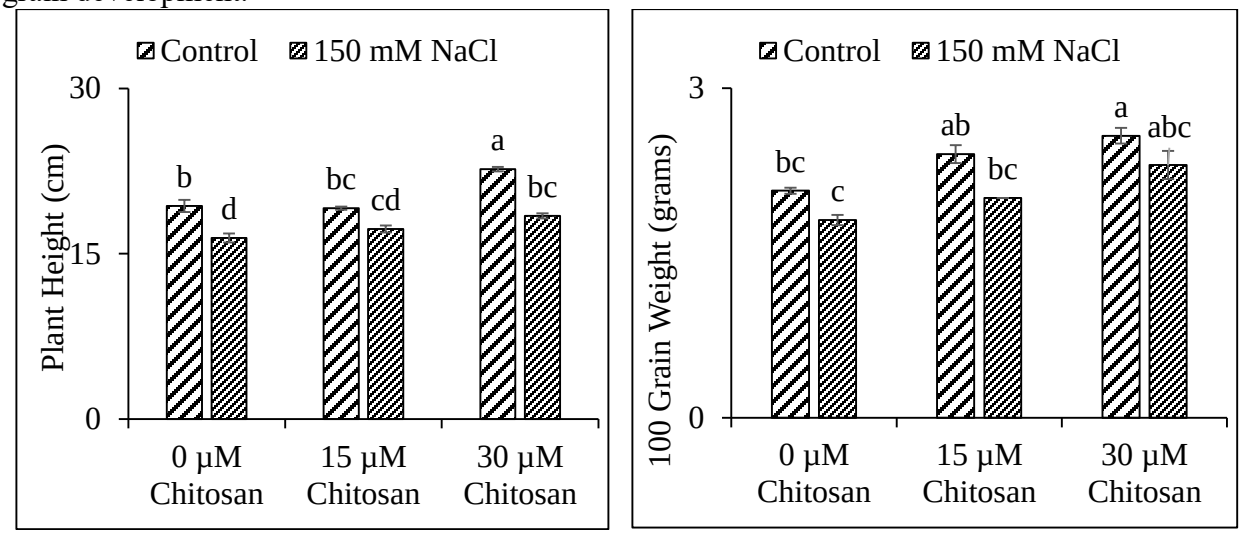


Fig. 3.1 Effect of chitosan application on plant height and 100-grain weight of oat under salinity stress.

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Grain yield was significantly impacted, with a 20.60% reduction observed under saline stress without chitosan. But, in the absence of salinity, treatment with 15 µM and 30 µM of chitosan resulted in a yield increase of 35.55% and 47.18%, respectively. Grain yield also improved in the same trend with 51.05% and 58.16% increase under saline conditions, 15 µM and 30 µM of chitosan, respectively, as compared to stressed untreated plants, showing that chitosan helps in yield enhancement under stress.

The biological yield was also affected, declining by 14.9% under saline conditions without chitosan. In non-saline conditions, the treatments of 15µ M and 30µ M of chitosan resulted in 26% and 33.09% increase in biological yield, respectively. The results in Figure 3.2 showed that, under salinity stress, 15µM and 30µM chitosan treatment resulted in 37.5% and 40.69% higher biological yield than untreated salinity-stressed plants, indicating its salinity stress alleviation capacity.

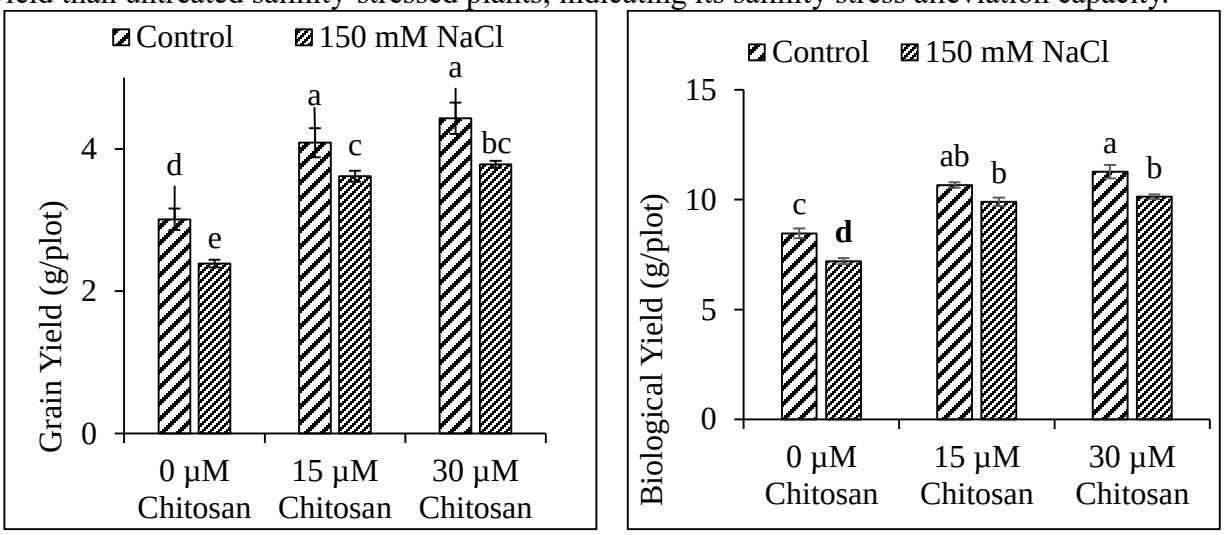


Fig. 3.2 Effect of chitosan application on Grain yield and Biological yield of oat under salinity stress.

The harvest index (HI%) was also highly variable. Saline stress resulted in a decrease in HI% values by 6.59%, while using chitosan at 15 µM and 30 µM increased HI% by 8.84% and 11.70%, respectively, in a non-saline environment. The beneficial effect of HI% in a saline environment can be seen by comparing with the saline environment (control), where HI% increased by 10.89% and 13.04%, respectively, using 15 µM and 30 µM chitosan. Saline stress resulted in a decrease in RWC by 18.88% in the saline control, while chitosan application resulted in an increase in RWC. In the non-saline treatments, 15 and 30 µM chitosan increased RWC by 3.74 and 12.57%, respectively. It has been observed that under saline conditions, RWC upon chitosan application at 15 µM and 30 µM had increased by 10.76% and 20.90%, respectively, indicating the capacity of chitosan in water holding property under stress situations (Figure 3.3).

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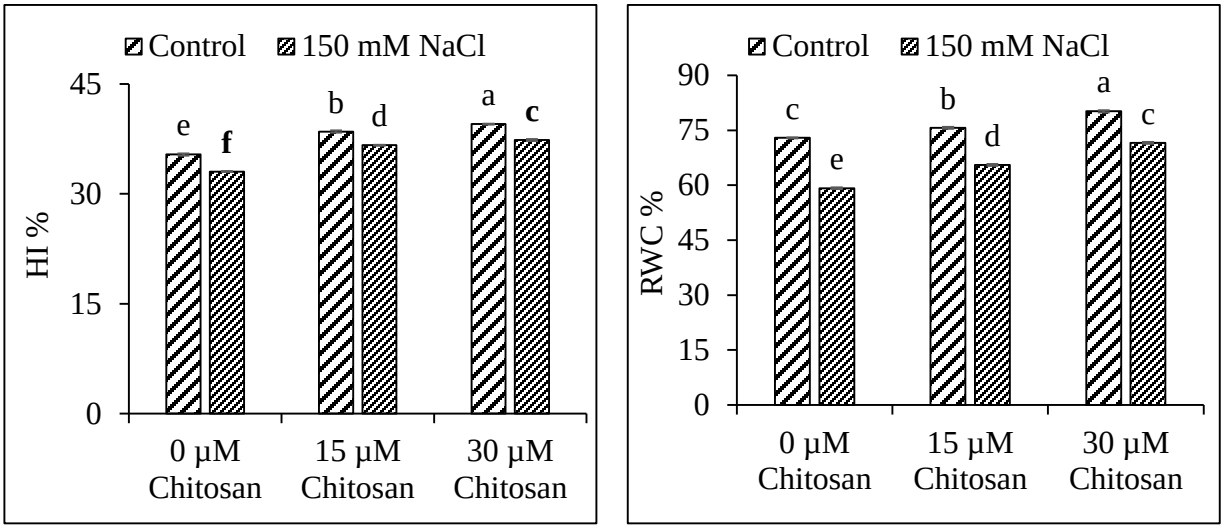
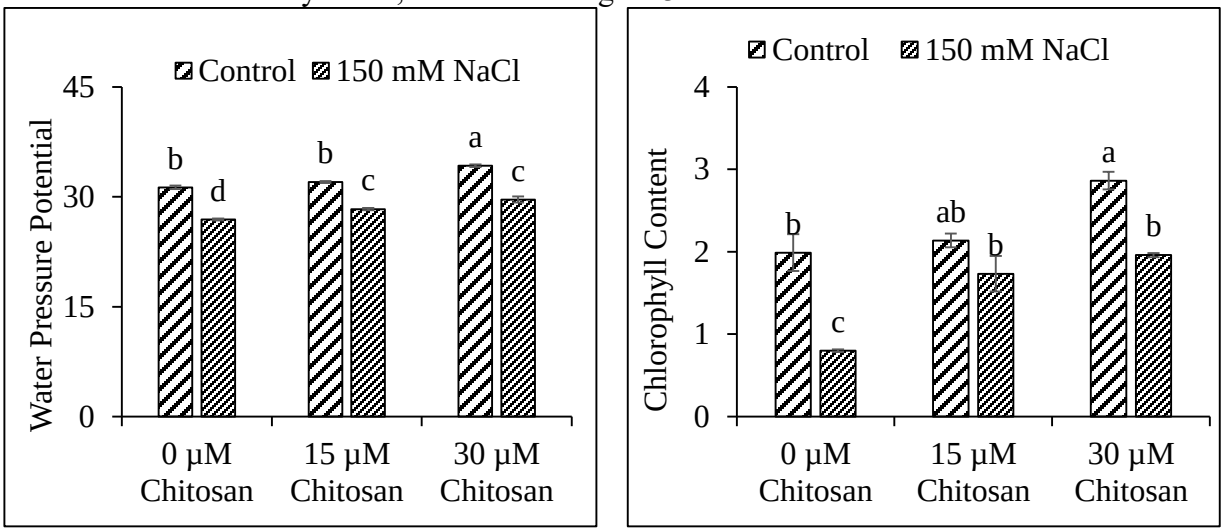


Fig. 3.3 Effect of chitosan application on Harvest Index and Relative Water Content of oat under salinity stress.

Water pressure potential (WPP) also exhibited significant variations. Saline stress caused a 14.06% reduction in WPP without chitosan, but chitosan mitigated this effect. Under non-saline conditions, 15 µM and 30 µM chitosan increased WPP by 2.33% and 9.42%, respectively. Under saline conditions, 15 µM and 30 µM chitosan improved WPP by 5.31% and 10.19%, respectively, underscoring its effectiveness in alleviating stress-induced reductions. Finally, **chlorophyll content** was greatly affected by treatments. Salinity caused a 59.80% reduction in chlorophyll content without chitosan, but chitosan application significantly improved levels. Under non-saline conditions, 15 µM and 30 µM chitosan increased chlorophyll content by 7.04% and 43.72%, respectively. Under saline stress, 15 µM and 30 µM chitosan improved chlorophyll content significantly, highlighting its potential to mitigate stress effects on chlorophyll levels. Overall, the application of 30 µM chitosan was most effective in improving growth and physiological parameters under non-saline conditions while also providing substantial mitigation against the adverse effects of salinity stress, as shown in Figure 3.4.



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Fig. 3.4 Effect of chitosan application on Water Pressure Potential and Chlorophyll Content of oat under salinity stress.

Discussion

The present study shows that chitosan significantly improves plant growth and physiological performance in control and saline stress conditions of oats. (Gul et al., 2022). Today, Salinity is one of the major constraints to crop production in the world. The growth, biomass yield, RWC, and plants were observed to be reduced by salt stress. (Ali et al., 2023). The application of chitosan at 30 µM was highly effective and decreased these effects to a large extent, especially under control and saline conditions. The results of these findings are in agreement with previous findings, implying that the chitosan was assisting in abiotic stress-enhanced tolerance due to its effects on water retention, ionic balance, and photochemical efficiency (Mukhtar Ahmed et al., 2020). The improved RWC and WPP enhancement under saline stress indicates the critical role of chitosan in osmotic adjustment, which is an essential factor for stress tolerance. (Fang et al., 2024). It also supports vital and physiological enzymatic processes by maintaining cellular hydration. These results are in agreement with previous studies demonstrating chitosan's capacity to modify osmotic balance, enhance the antioxidant activity of stressed plants, and stabilize membranes, thus reducing the oxidative damage caused by stressed plants. (Mukarram et al., 2023). The application of chitosan in this study resulted in one of the most commonly found effects being an increase in chlorophyll content, especially under saline conditions. Both chlorophyll and its converse degradation under stress are major contributors to reduced plant productivity. (Hameed et al., 2021). This increase in chlorophyll content with 30 µM chitosan is due to its protective effect on the photosynthetic apparatus, which may be due to the attenuation of oxidative damage and the stabilization of chloroplast membranes. The results are consistent with the findings from the previous studies, where it is established that applying chitosan increased photosynthetic efficiency and biomass production in stressed plants. (Singh et al., 2020). Under controlled and saline conditions, chitosan significantly increased the grain yield by enhancing reproductive success, nutrient uptake, photosynthetic activities, and antioxidant defense, resulting in better resource allocation and a higher harvest index. (Shobana et al., 2021). From an agricultural point of view, these observations offer the opportunity to use chitosan as an eco-friendly and sustainable replacement for synthetic agrochemicals to improve crop yield. The unique properties of biodegradability, non-toxicity, and compatibility with a variety of crops make it a useful agricultural tool that aids in solving the challenges of global agricultural losses due to salinity and other abiotic stresses. (Asif et al., 2023). The chitosan abrogates stress-induced perturbation in the manner in which phytohormones like salicylic acid (SA) and abscisic acid (ABA) function. Besides that, it can enhance soil properties and enable nutrient delivery and support of various microbial communities, thus becoming a valuable sustainable crop management tool (Shahrajabian et al., 2021).

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Conclusion

The most effective concentration, as measured by increased oat crop growth and yield under salt stress conditions, is chitosan at 30 µM. The improvement to plant height, chlorophyll, harvest index, and stress-related physiological functions all contribute to this improvement. As a bio-stimulant, Chitosan mitigates the adverse effects of salinity and supports the sustained agricultural practices by enhancing the plant's resilience. Its function of acting as a growth regulator makes it a good tool to enhance crop performance under stressful environments and facilitates agricultural progress in the future.

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