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The Effect Of Salicylic Acid On The Soluble Protein Content In Azolla (Azolla Spp.) And Duckweed (Lemna Minor) Plants Under Salinity Stress

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Abstract: This study examines the issue of increasing the stress resistance of high-productivity aquatic plants, Azolla (Azolla spp.) and Duckweed (Lemna minor), under conditions of water resource salinization. The research was conducted using natural water samples with varying degrees of salinity from the "BUXOROBALIQ" water basin. To mitigate the negative effects of the stress factor, the plants were treated with salicylic acid (SA) at concentrations of 10-4M, 10-5M, and 10-6M. The total soluble protein content in the plants was analyzed as the primary biochemical indicator. The results showed that the effect of salicylic acid varied depending on the plant species and concentration.

Keywords: Salinity stress, salicylic acid, Azolla (Azolla spp.) , Duckweed (Lemna minor), soluble protein, abiotic stress.

Introduction: The salinization of soil and water resources remains one of the most pressing global challenges in agriculture. This abiotic stress factor negatively affects all stages of plant growth and development, from seed germination, thereby severely limiting productivity. Climate change and shortcomings in irrigation practices are leading to the expansion of salinized land areas. This situation necessitates the development of innovative approaches aimed at increasing plant resistance to stress [1].

Higher aquatic plants such as Azolla (Azolla spp.) and Duckweed (Lemna minor)are distinguished by their agronomic and ecological significance. Due to their high protein content and nitrogen-fixing ability, they are a valuable feed source for fish, livestock, and poultry. They can also be used as phytoremediators to clean water bodies of pollutants like heavy metals. However, the productivity of these plants is highly sensitive to salinity, a factor that severely restricts their large-scale cultivation.

Salinity stress induces several negative processes in cells. An increase in the amount of salt in the water leads to the formation of reactive oxygen species (ROS). ROS damage proteins, including antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT).

This weakens the plant's defense system, leading to more severe cellular damage. Soluble protein content is a critical indicator for assessing the overall condition of plant cells under stress. The stabilization or increase in protein content indicates that the plant is successfully adapting to stress. It has been noted in scientific literature that SA, as a signaling molecule in plants, activates the synthesis of protective proteins and enzymes against stress [2, 3].

In the Bukhara region, a number of studies have been conducted on the bioecological distribution of plants and microalgae in drainage waters and their role in reducing water mineralization. According to the results obtained, among the plants found in water bodies, duckweed (Lemna minor) and azolla (Azolla spp.) were noted to have a relatively higher ability to absorb salts and heavy metals from the water compared to other studied species. [8]

The purpose of this study is to select the optimal concentration of salicylic acid to enhance the abiotic stress resistance of Azolla (Azolla spp.) and Duckweed (Lemna minor) plants and to biochemically evaluate its effect on their defense mechanisms in water conditions with varying degrees of salinity.

METHODS

The research was conducted at the "Buxorobaliq" water basin. Hydrochemical analyses of the water were performed at the "Biotechnology and Ichthyology" laboratory of Bukhara State University and the laboratory of the State Committee for Ecology and Environmental Protection, based on the methodologies of Yu.Yu. Lure and N.S. Stroganov.

The higher aquatic plants Azolla (Azolla spp.) and Duckweed (Lemna minor)were cultivated in fresh water (control) and in environments with varying levels of salinity and mineral content from the "Buxorobaliq" water basin, including incoming drainage water, outgoing drainage water, and pond water. The plants in each water sample were treated with solutions of salicylic acid (SA) at concentrations of 10-4M, 10-5M, and 10-6M. To evaluate the plants' response to salinity stress and the effectiveness of SA, the total soluble protein content in them was measured.

RESULTS AND DISCUSSION

The main hydrochemical indicators of water from different sources in the "Buxorobaliq" basin (incoming canal water, incoming drainage water, outgoing drainage water, and pond water) were determined, revealing significant differences between them. The results are presented in Table 1.

Table 1.
Analysis of hydrochemical indicators of water composition in the “Buxorobaliq” basin (2023)

№	Name of the detected indicators in water	Amount of indicators in various water samples				
		Unit of measurement	Result obtained			
			Incoming ditch water	Incoming drainage water	Outgoing drainage water	Pond water
1	pH		6,5±0,2	7,0±0,2	6,8±0,2	6,0±0,2
2	Dry residue	*mg/l*	1350±35	4100±60	5350±60	3250±45
3	Chlorides	*mg/l*	260,7±5	1008,2±7	1201,3±8	617,8±6
4	Sulfates	*mg/l*	216,1±5	1152,7±8	1308,6±8	733,6±6
5	Ammonium ion	*mg/l*	0,17±0,02	0,78±0,02	0,97±0,02	0,65±0,02
6	Nitrites	*mg/l*	0,2±0,001	0,6±0,002	0,8±0,002	0,60,002
7	Nitrates	*mg/l*	6,5±0,2	5,1±0,2	4,7±0,2	4,8±0,2
8	Suspended solids	*mg/l*	24±0,8	46±1,4	57±1,8	52±1,8

As can be seen from the results presented in Table 1, significant differences were observed among the hydrochemical indicators of the various water sources in the “Buxorobaliq” basin. The acidity level of the water (pH) ranged from 6.0 to 7.0, which is considered optimal for fish farming. While the lowest pH was found in the pond water (6.0), it was 6.5 in the canal water, and 6.8-7.0 was recorded in the drainage waters. Thus, the incoming drainage water has a relatively more alkaline environment, and as a result of biological processes in the pond, the pH has slightly decreased. The amount of dry residue, which represents the total mineralization level in the water, was 1350 mg/l in the canal water, while it increased to 4100 mg/l in the incoming drainage water. In the outgoing drainage water, 5350 mg/l was recorded, which indicates that salt accumulation and water salinization are occurring at a high level in the basin. The dry residue of the pond water was 3250 mg/l, and this is a variable quantity that decreases when a large amount of canal water is supplied to the pond and, conversely, increases when the ponds are filled with drainage water. The chloride content was found to be relatively low in the canal water at 260.7 mg/l, while in the incoming and outgoing drainage waters, it was 1008.2 and 1201.3 mg/l, respectively. This result indicates that salinization processes in the area are intensive. The presence of nitrogen compounds reflects the decomposition of organic matter in the water and the activity of nitrogen cycle processes. The high amount of suspended solids in the drainage waters (up to 57 mg/l) and in the pond water (52 mg/l) indicates an increase in water turbidity and that filtration processes in the ecosystem are not occurring completely.

The higher aquatic plants Azolla (Azolla spp.) and Duckweed (Lemna minor), selected as the research objects, are considered a valuable natural feed supplement not only for fish but also for livestock and poultry due to their high productivity, role in nitrogen fixation, and high protein content. Furthermore, they also have great potential as a means of phytoremediation for cleaning water bodies of heavy metals and other pollutants. However, their productivity is sensitive to the water's salinity level, and this factor severely restricts their cultivation in saline or salinized waters.

Salinity stress is a complex process that damages plant cells in several ways simultaneously. An increase in the amount of dissolved salts in the water triggers a chain of biochemical processes in the plant. To mitigate the negative consequences of stress caused by water salinity, an external application of salicylic acid solution, known as a plant hormone and signaling molecule, was performed. In the experiment, the total soluble protein content was selected to measure the response to stress. The experimental results are presented in Figures 1 and 2.

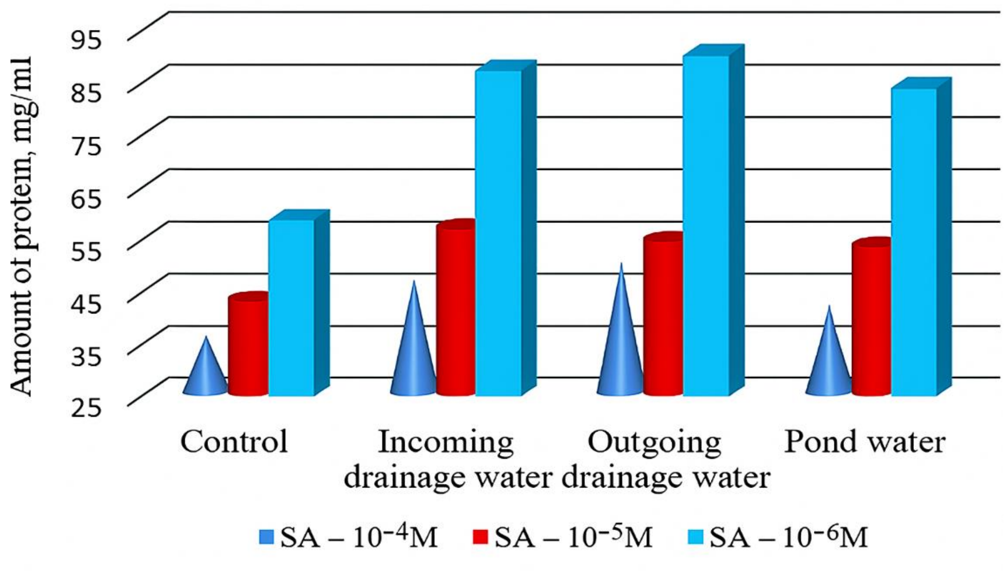

Figure 1. The effect of different concentrations of salicylic acid on the soluble protein content of the Azolla (*Azolla* spp.) plant under saline water conditions

The experimental results in Figure 1 show that the soluble protein content in the control group was relatively low, which represents the metabolic state of the plants under normal conditions. However, under saline water conditions, a significant increase in protein synthesis was observed. These results indicate that low concentrations of salicylic acid stimulate anti-stress defense mechanisms. It has been noted in scientific literature that salicylic acid participates as a signaling molecule in plants, activating the synthesis of protective proteins and antioxidant enzymes [4-5].

However, under the influence of a high concentration (10-4M), the protein content decreased, and values close to the control were recorded. This situation is explained by the phytotoxic effect of SA in high doses. Previous studies have also reported that concentrations of SA outside the optimal range can suppress protein biosynthesis [6].

Analysis by salinity levels shows that the soluble protein content was highest in Azolla (Azolla spp.) samples grown in the outgoing drainage water. This indicates an activation of protein synthesis as a response of the plants to a stronger stress factor. In the pond water, average values were recorded. These results prove the adaptability of Azolla (Azolla spp.) to different salinity levels. It is known that under stress conditions, proteins, especially protective enzymes and stress-induced proteins, function as key mechanisms for protecting plant cells [7][9][10]

The same pattern was observed in all four water samples, meaning the soluble protein content showed an inverse proportionality to the SA concentration. The highest protein content was recorded at the lowest concentration of 10-6M SA, and the lowest value was recorded at the highest concentration of 10-4M SA. For example, while treatment with 10-6M SA in the most saline outgoing drainage water increased the protein content to 93 mg/ml, this indicator was only 50 mg/ml at 10-4M SA. This clearly indicates that the 10-6M concentration of SA is optimal for stimulating the defense mechanisms of Azolla (Azolla spp.) under these experimental conditions, and that higher concentrations may exert a phytotoxic effect or suppress the defense reaction.

The results of the experiment conducted on the duckweed (Lemna minor) plant with different concentrations of SA solution in water samples of varying salinity levels are presented in Figure 2.

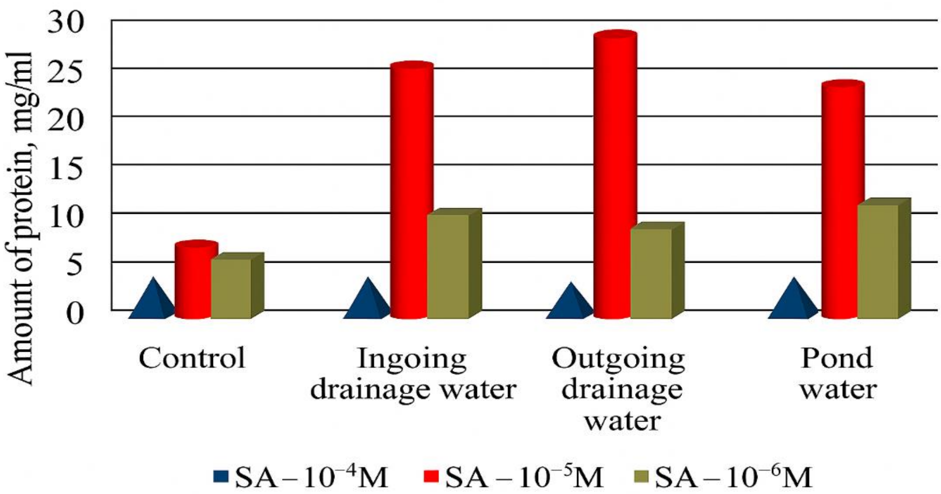

Figure 2. The effect of different concentrations of salicylic acid on the soluble protein content of the Duckweed (*Lemna minor*) plant under saline water conditions

In the duckweed (Lemna minor) plants of the control group, the soluble protein content recorded the lowest value compared to other samples, which indicates the plant's basal metabolic activity under normal, stress-free conditions. However, in plants grown in saline water samples (incoming and outgoing drainage waters, pond water), a significant increase in protein content was observed. This indicates the activation of defense mechanisms in the plant in response to the abiotic stress caused by salinity.

In the experiment, the effect of SA varied depending on the concentration. The highest protein synthesis was observed in the samples treated with 10-5M SA. This suggests that this concentration of SA acted as a signaling molecule that increases stress resistance in the duckweed (Lemna minor), enhancing the synthesis of protective proteins (e.g., stress proteins, antioxidant enzymes). Studies show that low doses of salicylic acid improve the adaptation of plants to adverse conditions such as salinity. However, when the highest concentration of salicylic acid, 10-4M solution, was applied, the soluble protein content sharply decreased, and results close to those of the control group were recorded. This phenomenon is explained by the fact that a high amount of SA has a phytotoxic effect on the plant, meaning it disrupts normal metabolic processes in the cells, including protein biosynthesis.

CONCLUSION

1. The hydrochemical analysis of the “Buxorobaliq” water basin, especially the high levels of salts and chlorides in the drainage waters, indicates that intensive salinization processes are occurring in the area.

2. Salicylic acid can be an effective tool for increasing

the resistance of Azolla (Azolla spp.) and duckweed (Lemna minor) aquatic plants to salinity stress, but its effect depends on the plant species and concentration. It was found that the most effective concentration for activating the anti-stress defense mechanisms in Azolla (Azolla spp.) was 10-6M, while for duckweed, the optimal concentration to increase stress resistance was 10-5M.

3. High concentrations of SA were found to have a phytotoxic effect, reducing protein synthesis. Therefore, using its low concentrations could be a promising biotechnological method for increasing the productivity of valuable aquatic feed plants like Azolla (Azolla spp.) and duckweed (Lemna minor) in saline water basins.

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