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Effect of Biosolvent on Salt Contents in Saline Soils and Antioxidant Enzymes of Cotton in the Bukhara region of Uzbekistan

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Abstract

Among abiotic stresses, soil salinization is a very serious environmental stress for plants, reducing yields by up to 20% on irrigated lands worldwide. The study of antioxidant enzymes in the activation mechanisms for salt resistance and the scientific analysis of the salt influence in the biochemical processes of plants leads to the activation of the immune system which serve in quantitative and qualitative increase of plant productivity. Different biochemical processes which are specifically activated under salt stress in the early stages of plant germination. The activities of peroxidase, polyphenol oxidase and phenylalanine ammonia-lease were significantly recorded lower value in roots and leaves of salt-affected crops. The review considers the activity of antioxidant enzymes under salt stress. After washing the saline soil with the biosolvent preparation, the activity of peroxidase, polyphenol oxidase and phenylalanine ammonia-lease increased in plants exposed to salt stress, which mitigated the negative impact of salt stress. Enzymatic activity was calculated based on Microsoft Excel at the rate of 1 mg g^{-1} in relation to the protein content. The activity of plant oxidoreductase enzymes in saline soil was studied using simple and experimental methods. For simple variants, water was used and the experiment was washed with biosolvent.

Key words: Biosolvent, Antioxidant enzymes, Soil salinity, Cotton, Peroxidase, Polyphenol oxidase, Phenylalanine Ammonia-lease

Introduction

The increase in salinized areas has a detrimental effect on crop yields. This negatively affects the country's economy. Such problems require scientists to create environmentally friendly and effective salt detergents when washing soil salinity, which will be useful not only for the structure of the soil but also for the plant organism. Salinization, which is one of the abiotic stresses, is characterized by an increase for salts in the soil, such as NaCl, $CaCl_2$, $MgCl_2$, Na_2SO_4, Mg_2SO_4, Na_2CO_3 and other carbonates, bicarbonates and nitrites. The presence of these salts in the soil limits the productivity of plants. Salinity is a measure of the number of dissolved salts in the water. It is usually expressed in parts per thousand (ppt), or the number of grams of dissolved salts present in 1,000 grams of water. Cotton agricultural crop is classified as moderately tolerant to salt stress with a salinity limit of 7.7 dS m^{-1} (Munns and Tester, 2008). Salinity sensitivity depends on the stage of plant growth and the type of salt and features of soil and properties in the soil. One of the main problems in the cultivation of agricultural crops is salinity, which is characterized by a direct effect on seed germination, growth, biochemical mechanisms of the plant and, consequently, on its productivity (Munns and Tester, 2008). Three types of salinization are distinguished in the literature: the first is the presence of salts in groundwater, the second is the presence of salts in irrigation water, and the third is transitional salinity (Jabbarov *et al*., 2023). Accumulation of salts in dry areas is faster because of rapid rate of evaporation as compared to humid areas (Stavi *et al*., 2021) and water used for agriculture purpose have more amount of salt (Singh, 2022). It is known, from the literature that salinity directly affects the root shoot of the plant. Saline soils are usually poor in nitrogen and organic matter and rich in salts (Green *et al.*, 2008). Salt concentration depends on the chemical properties, physical properties and soil structure. For example, in 1974 Szabolcs divided saline soils into two types viz., (i) saline soils, the soils that contain more quantity of salts affect the growth of plant and sodium chloride and sodium sulfate are found predominantly in this type of soil and soil electric conductivity is < 8. 2 (cited by James *et al.*, 2011); (ii) sodic soils containing high amount of sodium salts and other name of this soil are known as alkali soil. Initially salt stress inhibits growth and development in the form of osmotic stress, and then this stress continues with ion toxicity (Rahnama *et al.*, 2010). Under the osmotic effect of increased salt accumulation in soils and plants, salinity decreases, and the loss of water by leaves accelerates; therefore, salt stress is also called hyperosmotic stress (Liu and Han, 2021). The osmotic process in the early stages of salt stress causes salt swelling of the root system, which causes various physiological changes such as membrane rupture, imbalance of nutrient uptake through the root, and reactive oxygen species (ROS) detoxification capacity. (Farooq *et al.*, 2015) Most importantly, differences in the activity of antioxidant enzymes, as well as a decrease in photosynthetic activity are because of reduced leaf opening (Munns and Tester, 2008). It is clear that, depending on the level and duration of salt stress, various changes occur in the physiological and morphological processes of the plant, leading to the inhibition of productivity (Gupta and Huang, 2014). Researches have shown that groundwater and irrigation water contain many types of salts, such as calcium chloride, sodium chloride and carbonates, but sodium chloride has a more detrimental effect on cotton yields than other salts (Rengasamy, 2006). It is important to note that salt stress is also considered hyperactive ionic stress. One of the most detrimental effects of salinity is the accumulation of Na^+ and Cl^- ions in plant tissues in soils with high NaCl content. The entry of Na^+ and Cl^- into cells causes a pronounced imbalance of ions, and an increase in the level of Na^+ and Cl^- ions (through tissues) can lead to physiological disorders. Soil salinization is one of the most urgent problems in the modern world. In the world, 25% of arable land is saline to one degree or another, 60% of the lands of Central Asia, and in Uzbekistan, this figure is 60-65%. Existing systems of agro-technological land reclamation measures (traditional salt leaching) require a significant amount of water to wash away water-soluble salts in the surface layers of the soil. In soils with high salinity, these methods do not give the expected effect. Accordingly, in order to improve the agro-reclamation state of irrigated arable lands in agriculture it is important to improve chemical methods of reclamation and create effective preparations. Today, many studies are being carried out in the world on the synthesis of ion-exchange polymers with ion-exchange properties, the study of their chemical structure, molecular size and biological activity, and the creation of biodegradable compositions based on them to improve the agro-reclamation state of irrigated lands and agricultural land. Polycarboxylic acids containing carboxyl groups occupy a special place in terms of high ion exchange properties between ionic polymers. In this regard, the synthesis of water-soluble polycarboxylic acids with a cis-structure and the creation of compositions based on them with effective leaching properties of soil salts of various salinity, as well as chemical soil treatment, i.e., chemical amelioration.

Antioxidant regulation of salinity tolerance

Abiotic and biotic stress in plants can cause overflow, dysregulation, or even disruption of electronic transport chains (ETCs) in chloroplasts and mitochondria. Under these conditions, molecular oxygen ($1O_2$) acts as an electron acceptor, causing the accumulation of ROS. Singlet oxygen (1O), hydroxyl radical (OH), superoxide radical (O_2^-), and hydrogen peroxide (H_2O_2) are highly oxidizing compounds and therefore potentially detrimental to cell integrity (Sachdev *et al.*, 2021). Antioxidant metabolism, including antioxidant enzymes and non-enzymatic compounds, plays an important role in the detoxification of ROS caused by salt stress. Plants grow under the influence of environmental stresses such as salinity, waterlogging and drought, temperature, radiation, mineral deficiency, which qualitatively and quantitatively affect plant productivity. Although assessment of the effects of all various stresses on plants is significant, in view of the running literature, the researchers concentrate their interests on salinity than on other stresses. Salt tolerance positively correlates with the activity of antioxidant enzymes such as peroxidase, polyphenol oxidase, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPC), ascorbate, APX) and glutathione reductase (GR), as well as with the accumulation of non-enzymatic antioxidant compounds (Agarwal and Pandey, 2004). Antioxidant enzyme responses to NaCl stress in cotton. Plant peroxidases are widely distributed in all higher plants, and these enzymes are involved in various plant physiological processes: cross-linking of cell wall proteins and hydroxyproline-rich polysaccharides, both oxidation and polymerization of soluble phenolic compounds, H_2O_2 formation, and chlorophyll degradation and aging. One of the main functions is connected with the role as a part of defense enzyme complex in cells, ensuring detoxification of activated O_2 forms. This function is very important in the formation of metabolic response of plants to different stress factors (Kamran *et al*., 2019). Peroxidase (POX) plays a special role in cell wall strengthening and lignification processes, which are highly resistant to bigot age (Johjima *et al*., 1999).

Many scientists have studied that polyphenol oxidase plays an important role in lignification reactions as peroxidase (Chen *et al*., 2021) and produces quinones using oxygen, which increases plant resistance to insects and salt stress (Duffey and Stout, 1996). Polyphenol oxidases (PPO) are a group of Cu-containing enzymes that catalyze the oxidation of several phenols to o-quinones. In turn, o-quinones are highly reactive molecules that can undergo non-enzymatic secondary reactions to form brown complex polymers known as melanin has and cross-linked polymers with protein functional groups (Rolff *et al*., 2011). An increase in the amount of POX and PPO under the influence of salt stress and their participation increases the resistance of the plant to the action of abiotic and biotic stress.

Phenylalanine ammonia-lease is activated by the phenylpropanoid pathway and is a key enzyme in increasing plant stress resistance and its prolongation. In many literatures, it can be observed that the FAL enzyme is activated under the influence of stress, and phenolic compounds or secondary metabolites such as flavonoids are activated in the plant. This is because the involvement of the FAL enzyme in the duration of plant resistance to stressors has been repeatedly studied. FAL as phenolic compounds have strong antioxidant properties, a role in the protection against ROS during salt-water stress adaptation has been long suggested (Akula and Ravishankar, 2011). Previously it was reported that the activity of antioxidant enzymes in plants subjected to salt stress increased (Caverzan *et al*., 2016).

In order to improve the performance of crops growing under salt stress, it is important to understand how plants cope under such conditions. Salt tolerance of plants is a complex phenomenon that involves physiological, biochemical, and molecular processes as well as morphological. Furthermore, salinity tolerance is unlikely to be determined by a single gene or gene product (Cai *et al*., 2021). Keeping above facts in mind the present studies were conceptualized.

Materials and Methods

Saline soils (irrigated meadow soil) of the Bukhara district of the Bukhara region of Uzbekistan were selected for this study. Biosolvent increases the efficiency of leaching saline soils (Hudoynazorv, 2018). Cabinet of Ministers of the Republic of Uzbekistan with the requirements of the technological regulations PR-2008 intended for use of biosolvent in all types of irrigation systems as a plant protection and chemicals and have shown an improvement in soil composition. Sample variants were analysed by washing with water. In these two types of soil studies, physicochemical and biochemical analysis of soil composition was carried out using standard methods (Fan *et al*., 2022; Kaur *et al*., 2022). At the same time, in order to study the effect of soil salts on the immune system of cotton variety Bukhara-8 which was grown in experimental soils and the activity of antioxidants such as peroxidase (POX), polyphenol oxidase (PPO) and phenylalanine ammonia lysis (PAL) in control variants was determined. Correlations between sample and experimental variants of the roots, stems and leaves of seedlings are compared and analysed.

Analysis of the influence of biosolvent on the process of leaching of salts from the soil in the field was done**.** In field studies, the effectiveness of the use of the preparation "Biosolvent" in saline conditions was analysed in terms of parameters of physicochemical and colloidal properties. In experiments to evaluate the effectiveness of the technology for using a 0.5-10% solution of the Biosolvent preparation, 3 l/he and 5 l/he was tested under conditions of high salinity. In experiments in the Bukhara district of the Bukhara region, the preparation Biosolvent was used by washing the soil.

Methods for studying the physicochemical properties of soils under saline conditions

The magnitude of the electrical conductivity (dS/m) of the soil-water suspension was determined in the ratio EC 1:1 - 1:1 as an objective indicator of the degree of soil salinity - an instrument in an electro conductometer (Russia). The soil pH was determined using a standard pH meter (Germany). Determination of dry residue, HCO^{3-} Cl-, $SO_4{}^{2-}$, Ca^{2+}, Mg^{2+}, K^+ and Na^+ ions in soil samples was carried out using standard indicators and a set of chemical reagents, a photometer, and an electro conductometer. The studied soil sample was weighed on a laboratory scale, mechanically crushed in a mortar and passed through a sieve with a mesh size of 0.25–1 mm.

Determination of carbonates in soil: For the determination of carbonates ($CaCO_3$; $MgCO_3$ $Na_2CO_3 \times 10H_2O$) an approximate method was initially used. In this method, HCl solution (10%) was dropped into a soil sample (irrigation regime of cotton using sprinkler and drip irrigation in the lower Volga region, 2019). In the experiments, salt leaching was determined by the concentration and mechanical composition of salts in the soil at the end of the growing season under normal field conditions. In this case, the removal of salts was calculated according to the following formula, developed by V. R. Volobuev for 1 m of the soil layer, taking into account the water-physical properties of saline soils and the number of salts (Khojiyev *et al.*, 2020).

Here is the coefficient of free salt transfer, $S_{i,}$ S_{adm} are the number of salts in the soil before and after salt leaching (in percentage of the dry mass of the soil).

$N = 10000 \times lg[Si/Sadm]^a\ m^3/ha$

Plant material collection and preparation of extract

As a source of peroxidase, cotton of C-6524 variety were collected from local agricultural field of district Bukhara and carried at 4°C to the laboratory and stored at -20°C until used. In fresh roots, stems and leaves of 12-day-old seedlings of cotton, the activity of peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase enzymes was determined. Cotton was sown on a field treated with Biosolvent at 3 l/he and 5 l/he, for the experiment, and not in a control variant. Each object of the sample was analysed based on the results of three surveys.

Extraction of total soluble protein

One gram of cotton root, stem, leaf tissue per sample was powdered using liquid nitrogen, homogenized in 3 ml extraction buffer (20 mM HEPES, 10% glycerol, 1mM EDTA 100μM PMSF, 5 mM DTT, and 1 mM benzamidine) and centrifuged (16,000 × g, 10 min, 4 °C). Supernatant was collected in fresh tube, dialyzed against 20 mM Tris-Cl and stored at 4 °C for further experiments. The amount of protein was determined by the Lori method. The enzymatic activity was calculated using the Microsoft Excel program at the rate of 1 mg/g in relation to the protein content.

Peroxidase assay

Assay of peroxidase was carried out according to the method of Malik and Singh with certain modifications. In the 2 ml of phosphate buffer (pH 6.0/ pH 7.0), 100 μl of plant extract and 1ml of o-dianisidine solution were added. The reaction was initiated by adding 100 µl of 0.2 × 10> ³M H_2O_2 and the absorbance was read at (460 nm) every 30-second interval up to 5 minutes. The peroxidase activity was calculated using extinction co-efficient of o-dianisidine and the enzyme activity was expressed as unit per mg of protein.

Polyphenol oxidase assay

Polyphenol oxidase activity was determined by the method described previously (Galeazzi and Sgarbieri, 1981). Activity was determined using pyrogallol substrates in absorbance at 494 nm. For enzyme activity, 1 mL of the extract was placed in a cuvette containing pyrogallol substrate and 50 µL enzyme extract was added. The blank sample contained the same concentration of solution, except for 50 µL enzyme extract in 1 ml. One unit of PPO activity was defined as the amount of enzyme causing an increase in absorbance of 0.001 per minute in 1 mL reaction mixture.

Phenylalanine ammonia-lease assay

Assay of PAL was performed using the method (Gómez Vásquez et al., 2004) by using the optimal pH (8 for PAL) of these enzymes. The reaction mixture consisting of 0.5 ml of enzyme extract and 150 mM of L-phenylalanine or L-tyrosine was adjusted to 3 ml with the extraction buffer. Incubation was done at 40 °C for PAL for 30 min. PAL activity was determined at 290 nm, following the formation of E-cinnamic acid. Specific activity of enzymes was expressed acid formed per minute per milligram of protein (mmol. min^{-1} mg^{-1} of protein).

Results and Discussion

Looking at the process of washing with Cl ions, there was a significant difference in the experimental variants compared to the control, but very significant changes were observed in the experimental variant in soil samples taken at a depth of 0-30 cm (Table 1).

Table 1. Dynamics of changes in the number of ions in the soil under the influence of the biosolvent

Ions	Experiment parameters (Indicators)	Control (Average of 3 points)			Experiment 3l/ha (Average of 3 points)			Experiment 5l/ha (Average of 3 points)		
	Horizons (cm)	0-30	30-70	70-100	0-30	30-70	70-100	0-30	30-70	70-100
HCO_3^-	Before washing mg/eq	0.010	0.009	0.009	0.010	0.009	0.010	0.000	0.000	-0.019
	After washing mg/eq	0.012	0.011	0.011	0.009	0.010	0.010	-0.002	-0.001	-0.021
	Change (%)	13.7	20.4	19.1	-11.5	4.3	4.2	-25.3	-16.2	-23.3
Cl^-	Before washing mg/eq	0.031	0.033	0.029	0.040	0.037	0.035	0.009	0.005	-0.064
	After washing mg/eq	0.020	0.025	0.025	0.012	0.014	0.018	-0.008	-0.011	-0.043
	Change (%)	-22.2	-17.7	-5.5	-67.8	-59.6	-42.3	-45.7	-41.9	47.8
SO_4^{2-}	Before washing mg/eq	0.466	0.538	0.474	0.451	0.481	0.438	-0.015	-0.056	-0.912
	After washing mg/eq	0.352	0.382	0.355	0.259	0.301	0.305	-0.093	-0.082	-0.659
	Change (%)	-11.6	-21.8	-16.6	-39.2	-35.2	-26.5	-27.7	-13.4	43.2
Ca^{2+}	Before washing mg/eq	0.086	0.105	0.093	0.130	0.142	0.119	0.044	0.037	-0.212
	After washing mg/eq	0.066	0.064	0.059	0.051	0.054	0.050	-0.015	-0.010	-0.109
	Change (%)	-15.7	-32.5	-24.4	-59.5	-60.5	-54.0	-43.8	-28.0	78.5
Mg^{2+}	Before washing mg/eq	0.052	0.057	0.049	0.022	0.024	0.023	-0.029	-0.034	-0.072
	After washing mg/eq	0.041	0.048	0.042	0.034	0.037	0.036	-0.008	-0.011	-0.078
	Change (%)	-4.4	-2.9	5.7	55.5	74.7	72.8	59.9	77.6	-78.5
Na^+	Before washing mg/eq	0.068	0.071	0.069	0.069	0.071	0.067	0.001	0.000	-0.136
	After washing mg/eq	0.031	0.045	0.049	0.020	0.031	0.041	-0.011	-0.014	-0.091
	Change (%)	-48.5	-34.9	-25.8	-69.6	-56.3	-34.2	-21.1	-21.3	60.0
K^+	Before washing mg/eq	0.018	0.016	0.013	0.021	0.017	0.013	0.003	0.001	-0.027
	After washing mg/eq	0.015	0.014	0.012	0.012	0.010	0.009	-0.003	-0.003	-0.021
	Change (%)	-1.5	-5.5	-0.2	-44.1	-33.9	-21.1	-42.5	-28.4	21.4

The data presented in Table 1 show the positive effect of the Biosolvent on the chemical composition of the soil. Biosolvent studies in saline soils convincingly showed the significant changes in the composition of Cl-, SO_4^{2-} Ca^{2+}, Mg^{2+}, Na^+, and K^+ in the soil for control and experimental variants. Particularly high differentiation was observed in soils obtained at a depth of 30–70 cm. Therefore, in the experiments it was noted that under the conditions of washing (control) of saline soils with ordinary water, the amount of HCO^- in the soil increased slightly, and when washing with the Biosolvent preparation, it did not change or went up steadily. These results indicate that the alkalinity in the composition of the soil does not increase significantly and is a positive condition. Cl^- ions also increased in the control group from 17.7 to 28% after washing in the soil horizon of 30-70 cm and from 59.6 to 63.2% after washing with a Biosolvent. It has been established that the value of this indicator in the soil horizon of 70-100 cm changes from 5.5 to 9.6% and 42.3 to 43.7%, respectively, in the control and Biosolvent group. After washing the saline soil with ordinary water (control), the SO_4^{2-} content in the soil horizon 30-70 cm changed from 21.8 to 29.3% and after washing with a Biosolvent from 35.2 to 45%. After washing with ordinary water (control), the content of Ca^{2+} in the soil horizon 30-70 cm ranges from 32.5 to 39.8%, and when washed with Biosolvent from -60.5 to 60.7%. Experiments have shown that after washing with ordinary water (control), the content of Mg^{2+} in the soil horizon of 30-70 cm increases from 2.9 to 9.8%, and when washed with the Biosolvent preparation, from 52.8 to 74.7%. In the control group, the content of Na^+ in the soil horizon of 30-70 cm increased from 34.9 to 41.1%, and in the washes with the preparation Biosolvent from 56.3 to 64.3%. In the experiments it was found that in the control group, the content of K^+ in the soil horizon of 30-70 cm ranges from 5.5 to 16.1%, and in the flush with the preparation "Biosolvent" from 33.9 to 40.4%. Washing with the Biosolvent significantly increases the rate of leaching of ions including Cl^- ions from 35 to 42%, SO_4^{2-} from 13 to 16%, Ca^{2+} from 21 to 28%, Na^+ increase from 21 to 23%, Mg^{2+} from 63 to 68% and K^+ from 24 to 28%, which are harmful to plants in saline soils. Biosolvent with water in the ration of 1:10 at the rate of 3l/ha and 5l/ha showed the effectiveness in washing off the salts in saline soils and it saves 2000 m^3/ha of pure water. The Biosolvent had a positive effect on the productive reduction of harmful cations and anions in saline soil, leads to increase of the activity on the biochemical processes of cotton crops.

Figures 1, 2, 3 depict the activity of enzymes which were shifted upwards in the variants of the experiment. The results showed that there was a rise slightly in the activity of peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase when applying 3 liters of biosolvent per hectare. In experiments with a flow rate of 5 liters per hectare, it was found that the amount of all enzymes increased significantly compared to control samples.

Figure 1 presents that three types of enzymes were determined from root, stem and leaf of cotton. All enzymes were studied in a control

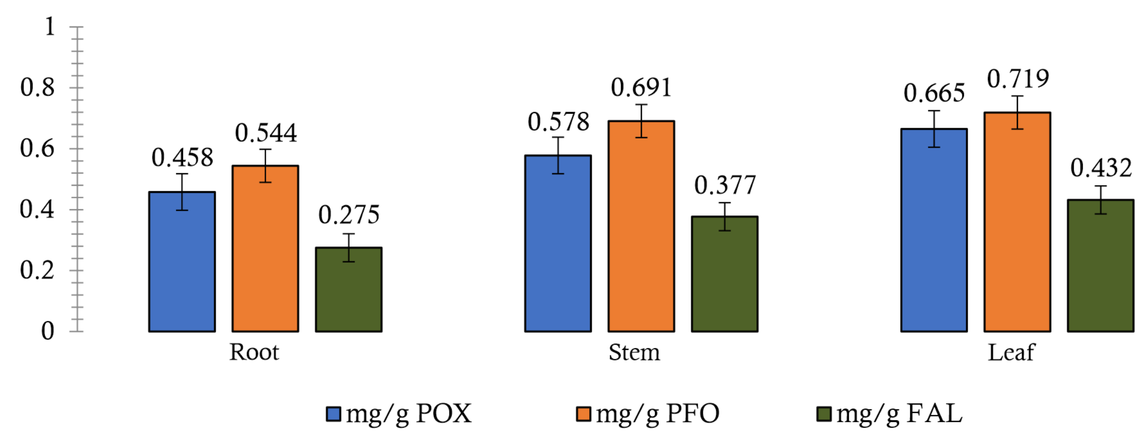

Fig. 1 Activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase (control)

variant, for example, control, control 3 l/ha and control 5 l/ha. The focus was to compare the enzyme activities of cotton after leaching with biosolvent. Comparison of the three types of experimental soils can provide a clear scientific conclusion of this study. It is clear from the fig.1 POX activity (blue colour) was 0.458 at root and 0.578 at the stem as well as 0.665 in the leaf. High level of POX was found at the leaf. PFO activity was high at the stem and leaf no major differences was found between two plant parts. In addition, FAl activity were lower from all parts of cotton. However, its activity was slightly rise stem and leaf to compare root system 0.377 and 0.432 respectively.

Figure 2 showed a slight increase in all enzymes compared to the control variant. The soil is washed with biosolvent 3 l/ha and the effect of this preparation on the enzyme activity of cotton variety Bukhara-8 was studied. There was focused on the fact that biosolvent had a positive effect on the soil, reducing the amount of toxic salts. After this, we planted cotton and studied the biochemical process of plants after receiving a reduced titter of salts from the soil. Because if soil properties improve, this can have a positive effect on plant tissue. In this case, biochemical processes were activated first, especially enzyme activity. POX activity was low, but compared to the control variant, there was a difference in the root system. PPO activity increased in the experimental variant by 0.094 and 0.157 at the stem and leaf, respectively. PAL activity was high on the leaf 0.494 and was 0.432 in the control.

The greatest difference was in peroxidase activity can be found from figure 3. At the same time, the enzyme activity in the plant stem increased by 32% compared to the control. It was studied that the activity of this enzyme in the leaf and root of the plant was 32 and 43% higher than in the control, respectively. Another oxido-reductase enzyme, polyphenol oxidase activity, was found to be significantly increased in plant leaf and root by 25%, respectively. However, no significant changes was observed in PFO activity in the plant stem. These results are consistent with the data of other authors on oxidoreductase enzymes in plants under salt stress. Zhang *et al.* (2013) found that *Broussonetia papyrifera* showed changes in the concentration of peroxidase activity under NaCl stress. It is reported that POD activity decreases under conditions of salt stress at a concentration of 100 mM.

Studies of *Glycine max L.* showed that the stress effect of NaCl on stress leads to an increase in the activity of peroxidase and polyphenol oxidase and a decrease in the content of hydrogen peroxide. These results indicate that the number of salts in the soil is not in all cases directly proportional to the activity of oxidoreductase enzymes in plants. These results indicate that the number of salts in the soil is not in all cases directly proportional to the activity of oxidoreductase enzymes in plants. In experiments on different varieties of tomato plants, a non-stationary increase and decrease in the activity of ascorbate peroxidase in leaves under the influence of NaCl stress was revealed in comparison with the activity of the pre-stress

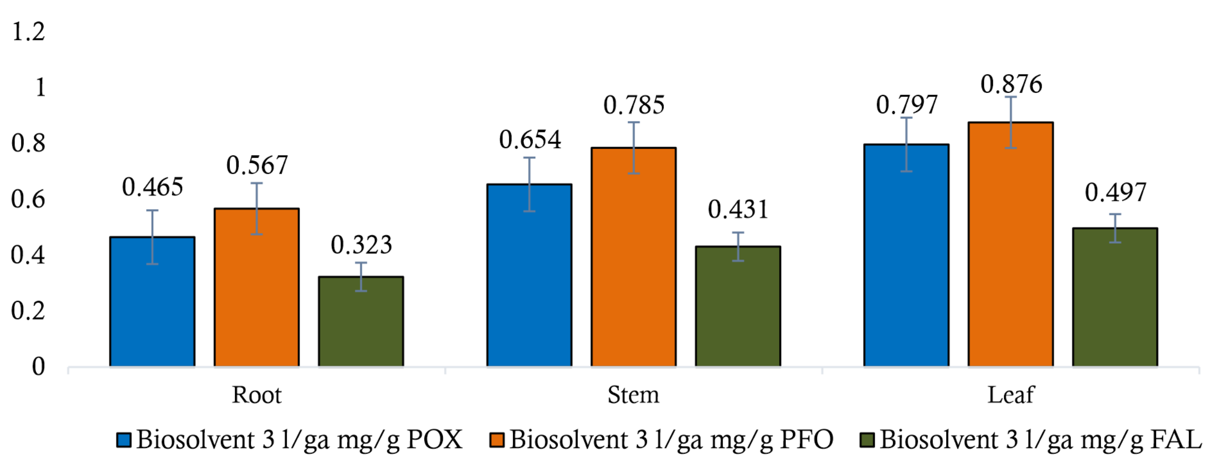

Fig. 2 Activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase (Experiment 3l/ha Biosolvent)

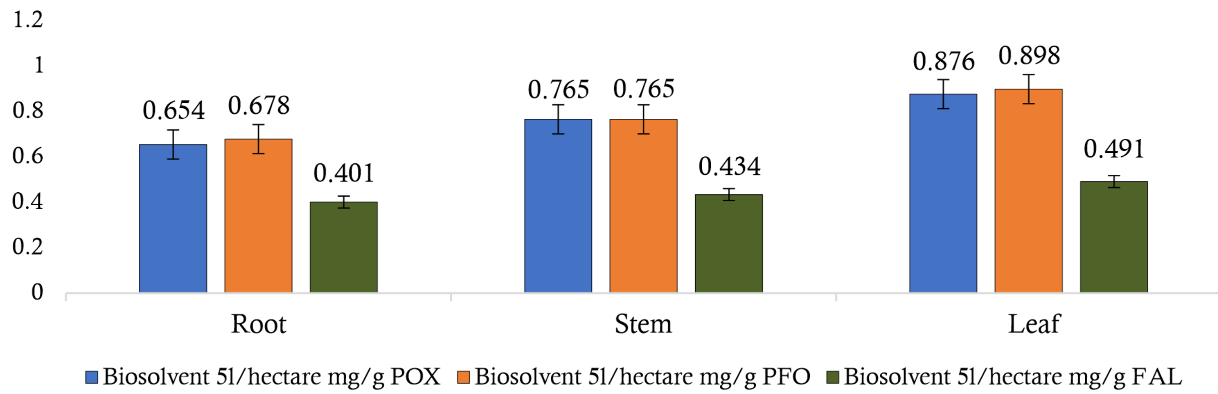

Fig. 3 Activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase (Experiment 5l/ha biosolvent)

enzyme. It has been established that the activity of enzymes in the root part of the plant periodically decreases in two of the three varieties studied and increases in one. In our study, it was found that the activity of PAL did not change significantly when using a biosolvent at a concentration of 3 l/ha. At a rate of 5 l/ha, no concentration-related changes in stem and leaf results were observed. Only in the root part of the plant, the activity of the enzyme was 46% higher than in the control samples. In contrast to oxidoreductase enzymes, no significant changes in PAL activity were observed in plant leaves and stems. These results are consistent with the following literature data. The expression of the DY523333 PAL gene of the tomato plant was found to be statistically unchanged in plants of salt-tolerant cultivars Ouyang et al. In a study by a decrease in PAL activity was observed under the influence of a single stress in the presence of 150 mM NaCl. However, under the influence of 3-fold stress, a significant increase in PAL activity relative to the control was observed. These results explain that the change in PAL activity in plant tissues under salt stress is concentration dependent. The difference between PAL activity and its m RN AA level is explained by a decrease or inhibition of PAL activity under the influence of high concentrations of trans-cinnamic acid, which is formed under the influence of FAL activity. The reason for the difference between changes in PAL and peroxidase/polyphenol oxidase activity revealed in our study may be related to the above phenomenon.

Soil salinity is a massive abiotic stress, which can decline water potential and induce nutrient imbalances in plants, and these adversely affect plant growth (Mansour and Salama, 2004; Chinnusamy *et al.*, 2005; Genc *et al.*, 2007). The inclusive results of the current study showed that cotton is a stress tolerant crop due to salinity. Full maturation of sensitive variety plants under severe salt stress (in soil containing sodium chloride, and sulfate) made it possible to compare and contrast the responses of tolerant and sensitive varieties. It was tested and selected tolerant and susceptible varieties based on physiological and morphological traits to salt stress in order to explain the underlying physiological, anatomical and antioxidant mechanisms of the defense systems involved. Mechanisms have played a significant and differential role in susceptible and tolerant varieties. Biochemical changes that occur in plants at high concentrations of harmful salts in the soil occur when plants are exposed to salt stress - these include superoxide, hydrogen peroxide and reactive oxygen species . Hydroxyl radicals (Van Breusegem *et al.*, 2001). The activity of peroxidase polyphenol oxidase and phenylalanine ammonia lyase of cotton seeds, formed as a result of salt stress was determined by washing with a bioreactor of 3 l/ha and 5 l/ha. These results are consistent with those of Ben Amor and others. (2006) and Sekmen et al. (2007), where they correlated an increase in PO and PPO activity with tolerance. The activation of the three types of enzymes studied in cotton under the influence of salt stress, especially after washing the soil with biosolvent, showed good results. These data did show that cotton under salt stress provides salt tolerance or adaptive function.

Conclusions

The preparation of "biosolvent" has a high effect on the leaching of salts in the 0-30 cm soil horizon, a good effect on the 30-70 cm horizon and a decrease in relative efficiency on the next 70-100 cm horizon. The results of studying the individual impact of the composition of biosolvent and its components on soil salts and the size of the soil structure allow to draw conclusions about the mechanism of action of the composition. Studies have shown that the introduction of the preparation "biosolvent" in the form of a solution into the saline soil layer and subsequent irrigation significantly reduces the number of salts in the soil rhizosphere of plants. Laboratory studies have shown that under the influence of the "biosolvent" preparation, the content of sulfates in the soil under saline conditions decreased by 23% compared to the control group. It was also established that the salinity of saline soils under the influence of 2-10% of the drug "biosolvent" decreased by 2.4-12 times compared with the control, and the number of salts of general toxic action - by 1.7-12 times. 2.5 times. Magnesium sulfate decreased by 1.9-2.9 times, sodium chloride by 1.1-1.3 times and magnesium chloride by 1.5 times compared with the control.

The biosolvent acts as a conditioner when washing out salts in saline soils, because of which the negative impact of salts on the soil is reduced. In the studies carried out, an inorganic polymer (polymetharylic acid) of a given structure was synthesized and the ratios of its functional groups were determined. In addition, on the basis of an inorganic polymer, a composition has been created that acts at the phase boundary, which ensures the transfer of sparingly soluble salts in the soil into a soluble form, and its physicochemical characteristics have been studied. The effectiveness of the developed polymer composition "biosolvent" for washing soil salts was confirmed based on laboratory and field tests.

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