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Participation of Antioxidant Enzymes in Defense Reactions of Wheat to the Stressing Environments

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ABSTRACT

Activity of antioxidant enzymes of catalase (CAT) and peroxydase (PO) was analysed in the sprouts and roots of 7-day-old germs of spring wheat *Triticum aestivum L.,* variety Aray. To determine the activity of the said enzymes the infection background with the fungus strain *Fusarium graminearum 0142* was created. Before seeding, the wheat seeds were disease-treated by various treaters. The peculiarities of functioning of antioxidant enzymes in defense response to pathogene and treaters are shown. The change in the activity of antioxidant enzymes when fusarium and different treaters are active and combined action of treaters and pathogene show that these stressors have impact on the defense reaction of plants. When these stressors are active the change in the activity of antioxidant enzymes in spring wheat had different directionality.
Keywords: catalase (CAT), peroxydase (PO), reactive oxygen species (ROS), spring wheat, activity of antioxidant enzymes, stressing environment, plant body, oxidative burst, disease-treatment.

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INTRODUCTION

Living bodies are subjected to the influence of unfavorable factors of environment. Due to their attached mode of life, plants are dependent significantly on the impact of stressors. One of the most wide-spread unfavorable factors is hypooxygenation, as well as oxidative stress arising after returning to the normal conditions of aeration. Latest experiments revealed that in the conditions of reaeration and hypoxia the reactive oxygen species (ROS) are accumulated in the cells of plants, in the area of cell walls and in anoplast. Plants growing in the conditions of hypoxia accumulate the reactive oxygen species (ROS) in the cells weakly. Apparently, such effect is related to the work of antioxidant system, in particular, to the enzyme catalase, performing the dissipation of molecules H_2O_2 [1].

An important role in the forming of plant resistance to phytopathogenes belongs to the biochemical defense mechanisms. Biochemical defense mechanisms play a critical role in the formation of plant resistance to pathogens and other stressing impacts of environment. The earliest response of plant body to the stressing factors is a generation of reactive oxygen species (ROS), oxidative burst launching the chain of the following defense reactions [2].

Oxidative burst is launched by elicitors, biological inducers and defense reactions of plants. Many metabolites of phytopathogenic microorganisms, in particular, fungi, have this property. Because of the high reaction capacity, the reactive oxygen species (ROS) can harm any macromolecules (lipids, DNA and proteins). In normal conditions the availability of antioxidant protection allows the cells to support the intracellular concentration of oxidants at the safe level. Reactive oxygen species (ROS) are an integral part of the pathological states [3].

Antioxidant systems protect the plant body from extreme oxidative stress. For pathogenic microorganisms it is extremely important because they are subjected to the impact of their "own" ROS and reactive oxygen species (ROS), produced by the host. If a parasite uses destructive force of reactive oxygen species (ROS) to destruct the tissues of the host, it shall possess a sufficiently powerful antioxidant apparatus for its own defense.

Special danger for grain crops is an affection of plants by fungi: *F. acuminatum, F. avenaceum, F. culmorum, F. equiseti, F. graminearum, F. semitectum,* etc. Damage caused by the disease is revealed not only in significant crop losses and decrease of the quality of seed material but also in the decrease of nutritive value of grain. This disease is wide spread in the areas where wheat is grown, especially in the humid environments [4].

The role of antioxidant enzymes in the formation of fungus pathogenity *F. graminearum,* and the regulating mechanism of these processes are insufficiently studied. Due to this fact, the aim of this project was to analyse the activity of antioxidant (CAT, PO) in spring wheat in the conditions of disease treatment and creation of infection background by the exciter of fungus *Fusarium graminearum 0142.*

OBJECTS AND RESEARCH METHODS

Preliminarily treated and dried seeds were put into a Petri dish on damp absorbent paper. Seeds were treated by suspension of conidiums of fungus *Fusarium graminearum,* concentration $18x10^6$**.**

Fungus strain *Fusarium graminearum* 0142 (pathogenic) was received from the Republican collection of microorganisms. Fungus was cultivated on solid modified Czapeck's medium. Cultures were grown at room temperature in tubes. All tests were conducted in three biological and in three analytical replications. To determine the oxidation-reduction enzymes we took seven-day-old sprout germs and roots of wheat.

CAT activity was determined by means of spectrophotometrical method according to breaking of H_2O_2 at 240 nm in Na+-phosphate buffer (pH 6.5). The reaction mixture contained 2ml of 0.1M Na+- phosphate buffer (pH 6.5), 100 mcl of H_2O_2 (final concentration 12.5 mM), 50 mcl of plant extract [5]. PO activity was determined according to the initial speed of oxidation of o-dianisidine at room temperature at 460 nm. Reaction rate was determined according to the slope of initial areas of kinetic straight lines of optical density change temporally [6]. Protein was determined by microbiuretic method [7]. The results were processed statistically by means of master program of statistic processing application Microsoft Excel 2006 [8].

RESULTS AND DISCUSSION

Treaters of seeds are chemicals (fungicides, bactericides) of defense and disinfectant action that penetrate into seed germs and protect them from smut infections and root rots, fungi, bacteria and pests living in the soil. Nowadays, the chemicals of single-purpose and complex impact can be used for seed treatment.

The first ones protect the plants from one of types of threat. The second ones render complex impact and protect the plant from pests and fungi, rot, mould, bacteria. After treatment of a plant as far as it grows the components of treaters are distributed equally along the sprout body continuing to protect it from internal and surface seed infections. In Figure1 there are seeds of spring wheat on the fifth day of germination.

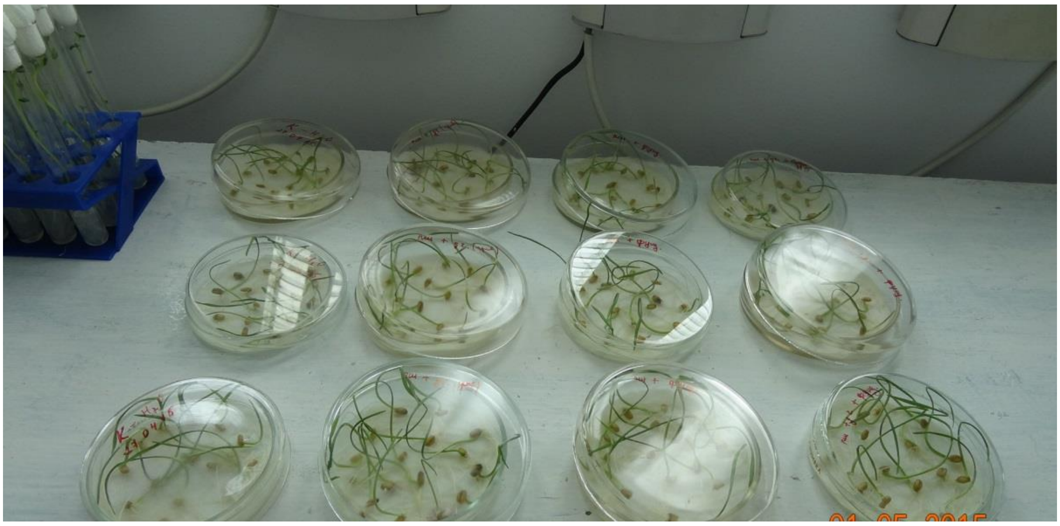

Figure 1. Planted wheat seeds according to the test variants (Institute of Molecular Biology and Biochemistry named after M.A. Aitkhozhin, 2015)

Contamination of (disease-treated) seeds by different bacterial and saprophytic fungi is shown in the Table 1.

Table 1 – Contamination of (disease-treated) seeds by different bacterial and saprophytic fungi (Institute of Molecular Biology and Biochemistry named after M.A. Aitkhozhin, 2015)

Revealed microorganisms						
Name of samples	*Mucor*	*Fusarium. graminearum*	*Bacterial exudate*	*Alternaria*	*Penicillium*	*Efficiency of treaters, %*
Control sample	+	-	-	+	+	97%
F.graminearum		+++++++	-	-	-	93%
Maxim Star, suspension concentrate (fludioxonil, 19 g/l + cyprokonazole, 6.3 g/l)		-	-	+	-	99%
Maxim Star, suspension concentrate (fludioxonil, 19 g/l + cyprokonazole, 6.3 g/l) + gr.	-	+++++	-	-	-	95%

Vincite, suspension concentrate (flutriafol, 25 g/l + tiabendazol, 25 g/l)	-	-	-	-	-	100%
Vincite, suspension concentrate (flutriafol, 25 g/l + tiabendazol, 25 g/l) + gr.			++			98%
Tenazole ultra, suspension concentrate (tebuconazole, 120 g/l)	-	-	-	-	-	100%
Tenazole ultra, suspension concentrate (tebuconazole, 120 g/l) + gr.	-	++	-	-	-	98%
Bastion, 34% water-suspension concentrate (carboxin 170 g/l + thiram 170 g/l)	+	-	-	-	-	99%
Bastion, 34% water-suspension concentrate (carboxin 170 g/l + thiram 170 g/l) + gr.	-	++++	-	-	-	96%
Vitavax 200 FF, 34% water-suspension concentrate (carboxin 170 g/l + thiram 170 g/l)	-	-	-	+++	-	97%
Vitavax 200 FF, 34% water-suspension concentrate (carboxin 170 g/l + thiram 170 g/l) + gr.	-	+++	-	++	-	95%
Note: -not revealed; +amount of contaminated seeds.						

During the test, the contamination of seeds by funfus diseases when treating by means of different treaters was studied. The results showed that the seeds were contaminated more when using the treaters Maxim star, Tenazole super, Bastion and Vitavax. Other treaters depress the contamination and molding of seeds by different bacterial and saprophytic fungi very well. According to the table, it can be seen that seed treaters depress significantly the formation of fungus diseases.

Formation of reactive oxygen species (ROS) and activation of antioxidant enzymes is the result of fast signal reactions of plants to the stressing environment factors. Catalase activity was investigated in sprouts and roots of spring wheat. In Figure 2 there is an activity of catalase (CAT) in the sprouts of spring wheat.

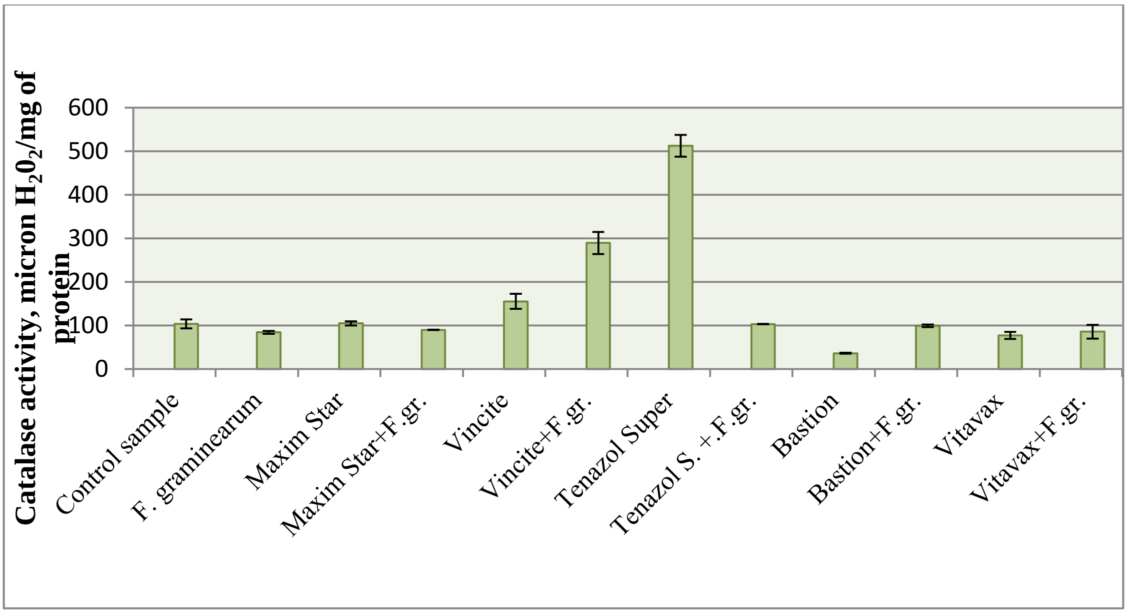

Figure 2. Change of CAT activity in the sprouts of spring wheat, variety "Aray" at treatment by treaters and fungus infection (Institute of Molecular Biology and Biochemistry named after M.A. Aitkhozhin, 2015)

CAT activity in the sprouts during infection was 22.8% below the control variant. In the wheat sprouts with treater Maxim Star the enzyme activity was 1.06% above the control variant. In the sprouts with treater Vincite it was 49.8% above the control variant. Vincite together with pathogene was 178.9% above the control variant; it was 86.2% above the control variant with Vincite. At treatment by Tenazole super, the activity of enzyme in sprouts was 394.0% above the control variant. CAT activity was also studied in the roots of spring wheat. The results of the study are shown in the Figure 3.

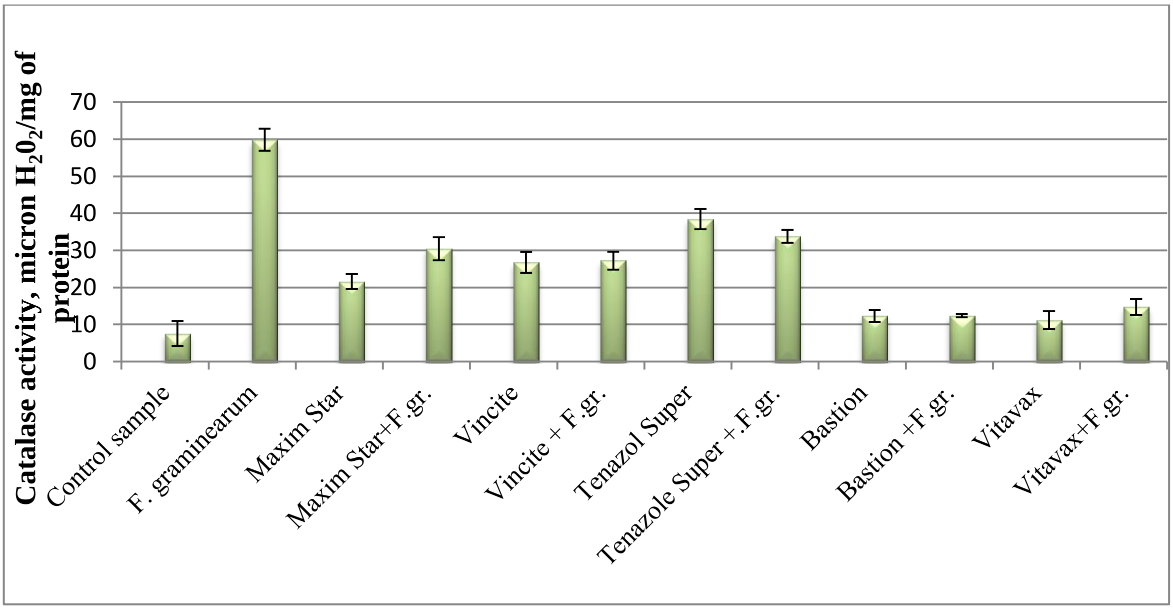

Figure 3. Change of CAT activity in the roots of spring wheat, variety "Aray" at treatment by treaters and fungus infection (Institute of Molecular Biology and Biochemistry named after M.A. Aitkhozhin, 2015)

Catalase activity in the roots at infection was 693.4% above the control variant. At infection and treatment by Maxim Star, the activity of enzyme was 303.9% above the control variant. In the variant with treater Tenazole super, in the roots the catalase activity was 409.9% above the control variant. Change of activity of soluble PO in the wheat sprouts is shown in the Figure 4.

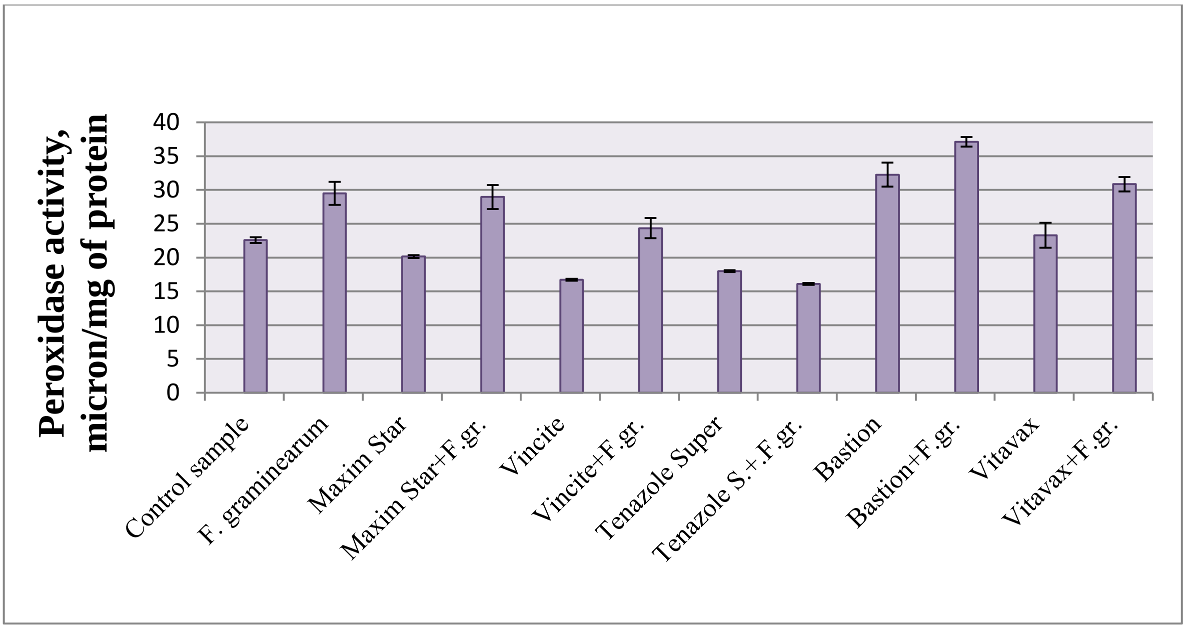

Figure 4. Change of soluble PO activity in the sprouts of spring wheat, variety "Aray" at treatment by treaters and fungus infection (Institute of Molecular Biology and Biochemistry named after M.A. Aitkhozhin, 2015)

In a soluble form the PO activity of enzyme at infection without disease treater was 31% above the control variant. In the combined variant of the treater Maxim Star and at infection the enzyme activity in the sprouts was 29% above the control variant and 44% above the treater itself. When treating by Vincite and at infection the enzyme activity was 8% above the control variant. Bastion showed an increased activity together with pathogene. Enzyme activity in this variant was 64% above the control variant and 15% above the variant where only treater was used, and 26% above the variant where the pathogene without treater was used. Vitavax together with fungus was 37% above the control variant and 5% above the infected variant without treater. Enzyme activity in the variant with Tenazole super decreased by 25% in comparison with the control variant. Activity of soluble PO in the roots is shown in the Figure 5.

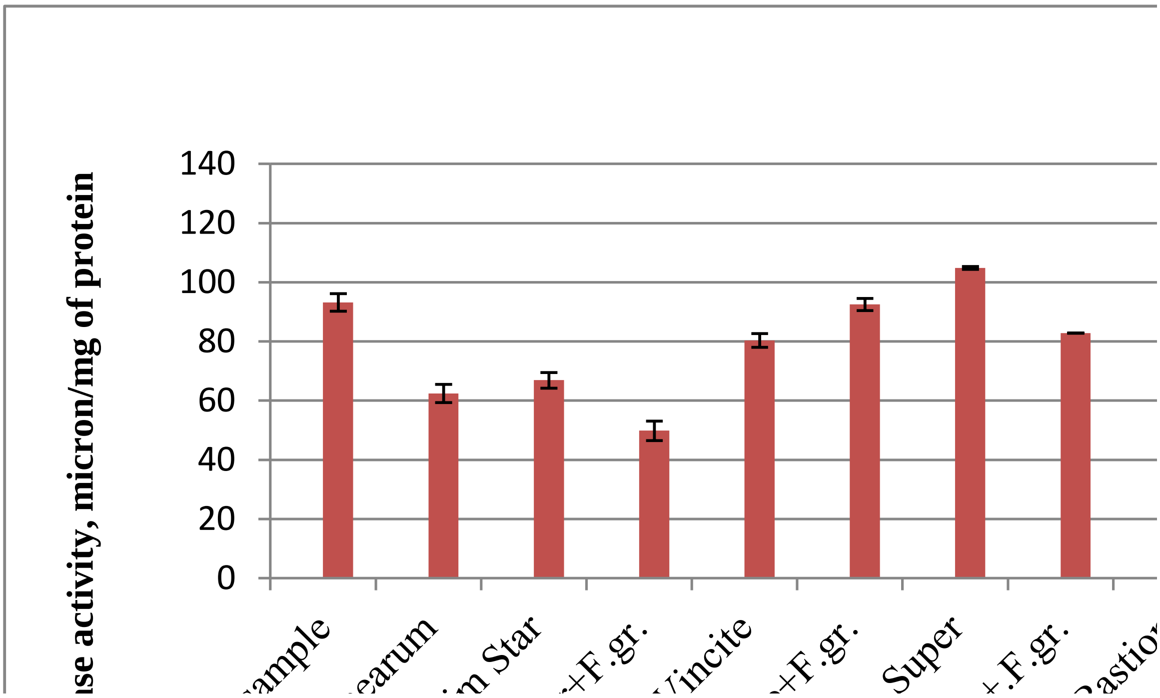

Figure 5. Change of soluble PO activity in the roots of spring wheat, variety "Aray" at treatment by treaters and fungus infection (Institute of Molecular Biology and Biochemistry named after M.A. Aitkhozhin, 2015)

In the PO soluble form in the roots the activity at infection was 49% below the control variant. In the variant with Tenazole super, the enzyme activity was 12.6% above the control variant and 68.0% above the variant with pathogene. In the variant with Bastion, in the roots the activity of soluble PO was 24.0% above the control variant ad 84.0% above the variant with pathogene. Vitavax showed 28.0% above the control variant. These treaters had the most significant impact for PO in the roots of spring wheat.

In the bound form PO activity of enzyme was similar to the soluble form. Enzyme activity in the sprouts varied from 19.0% up to 150.0% in comparison with the control variant. In the roots the enzyme activity in the bound form was from 36.0% up to 131.0%.

CONCLUSION

As a result of the study it can be concluded that treaters of seed material make a significant impact on biometrical values of wheat. During observation we mentioned that treater Tenazole super depressed the growth of wheat germs. But the treaters Bastion and Vitavax, on the contrary, increased the growth of wheat. Growth level in these variants was equal to the control variant.

Also the contamination of seeds by the fungus diseases was studied. It was revealed that the plants were contaminated more by fungus diseases after treatment of seeds by treaters Maxim Star, Tenazole super, Bastion, Vitavax. Other treaters depressed well the contamination and molding of seeds by different bacterial and saprophytic fungi. The results show that the treaters of seeds depress significantly the contamination of fungus diseases of the wheat plants.

Activity of antioxidant enzymes in spring wheat varied in the sprouts and roots. In contaminated seven-day-old sprouts and roots the ROS activity was above the control variant and infected variant. The treater together with the pathogene had the most significant impact on the ROS activity in the sprouts and roots. In the sprouts, the PO enzyme reacted more actively to the pathogenic impact. Bastion together with the pathogene showed the increased activity. Enzyme activity in this variant was 64% above the control variant and 15% above the treated variant and 26% above the infected variant.

In the PO soluble form in the roots the activity was mentioned in the variants with treaters. In the variant with Bastion in the roots the activity of soluble PO was 24% above the control variant and 84.0% above the infected variant. Vitavax showed the activity of 28% above the control variant.

Thus, in the wheat roots the activity of soluble PO was impacted more by the treatments of treaters than contamination by pathogene. It can be said that these treaters made a significant impact on antioxidant status of plants. In PO bound form in the sprouts and roots the PO activity was noticeably higher in the variant with the treater used during infection.

The key moment of adaptation and survival of plants is activation of the system of antioxidant enzymes because “oxidative burst”, that is, excessive accumulation of ROS, is related to the universal stress reaction at infection [9]. ROS generation is a particular stress reaction of plants to the infection by pathogenes. ROS plays the protective function of interaction of plant and pathogene.

In conclusion it can be said that treaters and pathogens can activate the activity of CAT and PO enzymes. The change in the activity of antioxidant enzymes at the impact of fungus exciter and different treaters, and the combined action of treaters and pathogene show that these stressors have an impact on protective reaction in plant body. At the impact of these stressors the change in the activity of antioxidant enzymes in spring wheat had different directionality. Following the studies performed, it can be concluded that treatment by treaters resulted in oxidative stress; the changes in the antioxidant system of plants at the impact of treaters and pathogens depend upon the type of plants, time and mode of impact of a stressing agent. These changes were expressed in reversible increase and decrease of enzyme content. Studied enzymes participate in the formation of lignin, in providing of plant cells with energy, in formation of reactive oxygen species that lead to the death of pathogens. Activation of oxidant enzymes does not only increase the immunity of plants to different unfavorable environments but also prevents the development of pathogenic fungi. This, in its turn, is very important for good crops in agriculture [10].

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