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## TYRE-TREAD ROLE OF TRICHODERMA HARZIANUM 203 IN SPROUTS INFESTATION OF WHEAT BY FUSARIUM OXYSPORUM

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### Abstract:

It is shown that the fungi-antagonist *Trichoderma harzianum* 203, performs protective function influencing the pathogenic fungi *Fusarium oxysporum*, causing root decay. During the conducted research it was established: positive influence of this fungi on sowing seed quality and accumulation by sprouts of dry and crude biomass an underground part both in monitoring, and in case of infestation with a pathogenic fungi. It is revealed that the fungi-antagonist *Trichoderma harzianum* 203, reduces peroxide oxidation of lipids. Decrease in peroxide oxidation of lipids under the influence of *Trichoderma harzianum* 203 takes place at the expense of antioxidants of the nonferment nature.

**Keywords:** mycology, phytopathology, plant protection, plant stability

### Introduction

Currently in agricultural practice the tendency of greening the production and using the least toxiferous medicines for plant protection is observed. A new milestone in developing ecologically safe complex protection of crops against different diseases is using inductors of systemic stability, such as nonpathogenic or opportunistic microorganisms, falling into to the separate ISR type (induced systemic resistance) interinfluences, the most perspective from the practical point of view. Till 1998 the *Trichoderma* sort fungi spp. were not considered as a potential plant stability inductor. The first data confirming an opportunity to use them as stability inductor were published by De Meiller [1]. The author showed that at colonization of bean roots of *T. harzianum* in root system ISR [2] is started. As Trigerrami induced systemic plant stability due to the influence of the *Trichoderma* sort fungi jasmonic, salicylic and ethylene acids act, which was confirmed by many authors [3; 4; 5; 6; 7]. Fungi of this sort synthesize antibiotics, toxins on the basis of which enzymes receive medicines for biological monitoring of diseases and stimulation of plant growth [8]. Being adapted by a saprophyte *Trichoderma* to live and prosper in various ecological conditions external environments, at the same time synthesizing a wide range of enzymes, such as hitinaza, cellulase. Due to these

At the heart of the protective effect mechanism of these synthesizable enzymes, their activity concerning degradation of polysaccharide of the chitine which is a part of a cell-like wall of fungi – pathogens lies [9]. The Fissile Forms of Oxygen (FFO) play an important role in plant protection as they are capable of alarming molecules. Under monitoring of AFK there are reinfluences of supersensitivity and apoptosis. In pathogenesis due to reinfluence of supersensitivity the pathogen zone is formed around of the dead plant cells containing the antimicrobial connections in great numbers. [10] It is shown that the fungi *Trichoderma harzianum* induces resistance to root rotting in many crop species. In this regard the purpose of our research was to assess the tire-tread influence of fungi-antagonist *Trichoderma harzianum* 203 in case of sprouts infestation of wheat with a pathogenic fungi *Fusarium oxysporum*. The studied parameters were showing signs of seed quality – germination energy and viability; growth indexes - length of elevated and underground part of sprouts and accumulation of dry and crude biomass of the wheat sprouts. For definition of the antioxidatic status the analysis of malonic dialdehyde maintenance was carried out and activity of the main antioxidatic enzymes - catalases, peroxidases, ascorbateperoxidases investigated.

## **Methods**

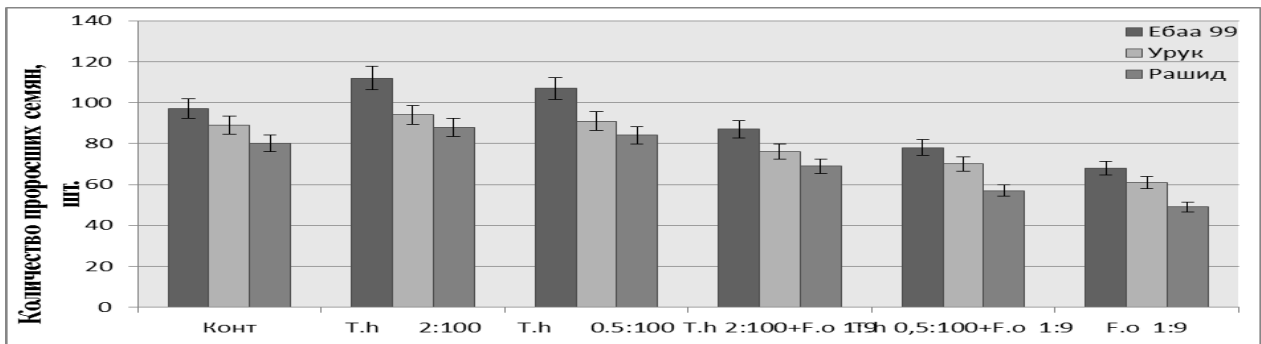
Research objects were sprouts of three sorts of the weak spring-sown field of the Iraqi selection (*Triticum aestivum* L.), differing on pathogens resistance: a sort of Ebaa 99 - high-resistant, Uruk – medium-resistant, Rasheed – unresistant. As a substratum for cultivation of wheat sprouts sterile river sand was used. Sterilization of sand was carried out in a furnace at 160° for 7 hours. For the research the sort *Trichoderma harzianum* T 203 was selected for territories of ancient burials of the Republic of Tatarstan which showed earlier high performance on resistance to many activators of plants was used. The used concentration of sporous-micellar suspension of a micromycete made  $1 \times 10^6$  colonies/ml which was added to the cultivation environment in several cultivation. The used titre *Fusarium oxysporum* made  $1 \times 10^6$  colonies/ml. Activity of enzyme in catalase (KF 1.11.1.6.) calculated according to a technique in a homogenate of fabrics or suspension of mitochondrions [11]. Calculation was made on UNICO 2800 spectrophotometer; measured activity falling of density at 240 nanometers in 4 min. 30 sec. (quartz).

Activity of ascorbateperoxidase was calculated by a technique of Y. Nakano, K. Asada. We used a colorimetric method of calculating activity of peroxidase according to Boyarkin [12]. The level of peroxide oxidation of lipids (POL) judged on accumulation of product – malonic dialdehyde (MD). Maintenance of MD was estimated on extent of accumulation of a product of its reinfluence with thiobarbituric acid (TBK) [13].

**Results and Discussion**

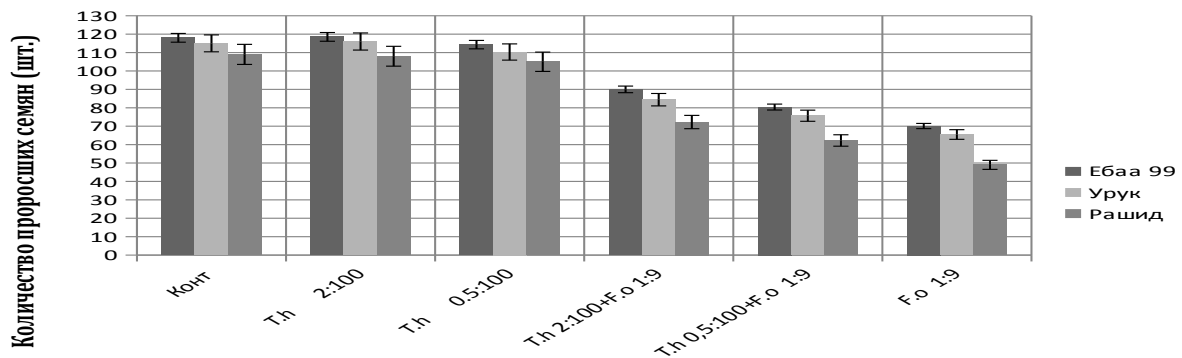
**Germination and viability of seeds**

Currently in literature rather huge number of data devoted to influence of soil microorganisms on sowing seed quality is saved up. Soil fungi-antagonists of the Trichoderma sort are capable to synthesize and allocate various factors of growth - auxins, cytokinin, ethylene, organic acids, amino acids, vitamins and other substances joining in metabolism of the fissile plants. It is shown that fungi sorts of the sort Trichoderma depending on patrimonial accessory, can render the effect both stimulating, and inhibiting on germination energy and viability of cereal seeds [14]. Some sorts of the Trichoderma have no reliable effect [15].



Конт	Cont. (тут и далее)
Количество проросших семян, шт.	Quantity of the sprouted seeds, piece.
Ебаа	Ebaa
Урук	Uruk
Рашид	Rasheed

**Figure 1 - Influence of T.harzianum and F. oxysporum on germination energy (quantity of the sprouted wheat seeds on the 5th day taken from 120 seeds of various stability sorts).**



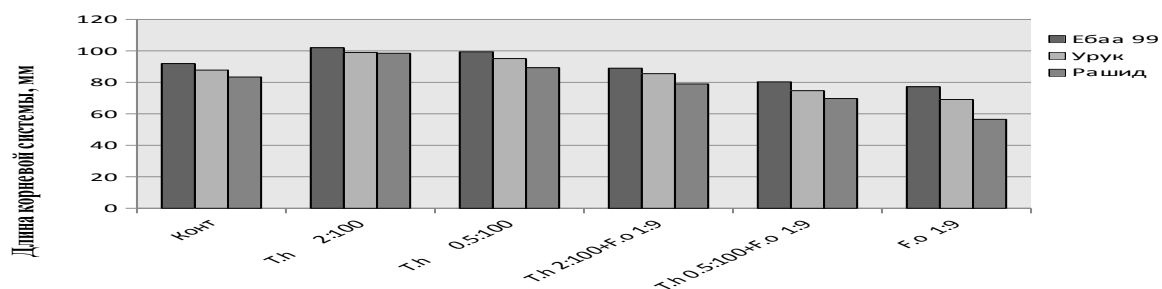
Количество проросших семян, шт.	Quantity of the sprouted seeds, piece.
Ебаа	Ebaa
Урук	Uruk
Рашид	Rasheed

**Figure 2 - Influence of T.harzianum and F. oxysporum on quantity of the sprouted wheat seeds on the 8th days (viability of seeds) taken from 120 seeds of various stability sorts.**

In research for influence of fungi *T.harzianum* on sowing seed quality, in particular, germination energy in sorts of wheat, various on stability, was calculated; it was established that the sort *T.harzianum*, the one we used, possesses stimulating properties. The greatest stimulating influence was established in concentration 2:100 cultural liquids. The greatest stimulating effect was found in steady against pathogens sort of Ebaa 99. The stimulating effect of *T.harzianum* concerning germination energy and viability of seeds can be explained by possibility of synthesis physiologically of the active materials with the studied sort of *T.harzianum*. Influencing plants of a pathogenic fungi of *F.oxysporum* energy of seed germination decreased by 24%, and viability of seeds by 30%, which corresponds to data on its phytotoxicity. Various sorts had an identical intensity of germination energy, and seed viability decreased equally to phytotoxic impact of *F.oxysporum* on sowing seed quality and did not depend on sort stability. In collateral impact of fungi-antagonist *T. harzianum* and a pathogenic fungi of *F. oxysporum* on sorts of wheat, various on stability, observed partial decrease in negative-phytoxic influence of pathogen on plants. Partial removal of phytotoxiferous influence of pathogen fungi on germination energy and viability are bound to aborting its development.

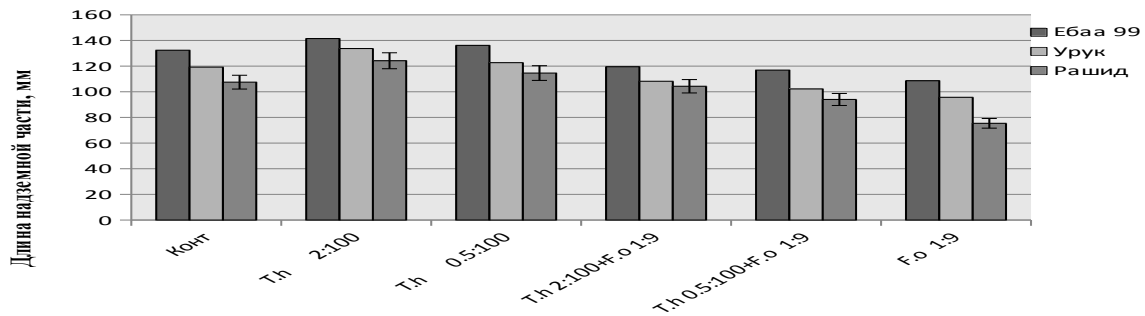
**Morphological indexes of wheat sprouts influencing the cultural liquid of *T.harzianum* and *F. Oxysporum* fungi**

The *Trichoderma* sort fungi spp., aside from antagonistic properties, can have the stimulating effect on growth and developing of a plant in general. Proceeding from literary data, they can allocate auxins, gibberellina, cytokinin, abscisic acid and ethylenes which are plant hormones and be in charge of growths and development of plants, maturing of fruits. The phytostimulating influence of different sorts and species of the *Trichoderma* sort fungi is not studied in a due measure. Inducing growth and development of plants can happen due to change in rizosphere and synthesis of the metabolites possessing the stimulating influence. In research of morphological indexes for the wheat sprouts differing in phytopathogens resistance it was established that the unstable sort had height of elevated and underground parts less than steady (figure 5,6). When influencing sprouts of cultural *T.harzianum* with liquid in concentration 2:100 the growth-stimulating effect in all sorts grew, and underground parts of sprouts in comparison with monitoring was observed.



Длина корневой системы, мм	Length of root system, mm
Ебаа	Ebaa
Урук	Uruk
	Rasheed

**Figure 3 - Influence of T.harzianum and F. oxysporum on length of underground part after 8 days of influencing the various stability sorts**



Длина надземной части, мм	Length of an elevated part, mm
Ебаа	Ebaa
Урук	Uruk
Рашид	Rasheed

**Figure 4 T.harzianum Influence and F. oxysporum on length of an elevated part after 8 days of influencing the wheat sprouts of various stability sorts.**

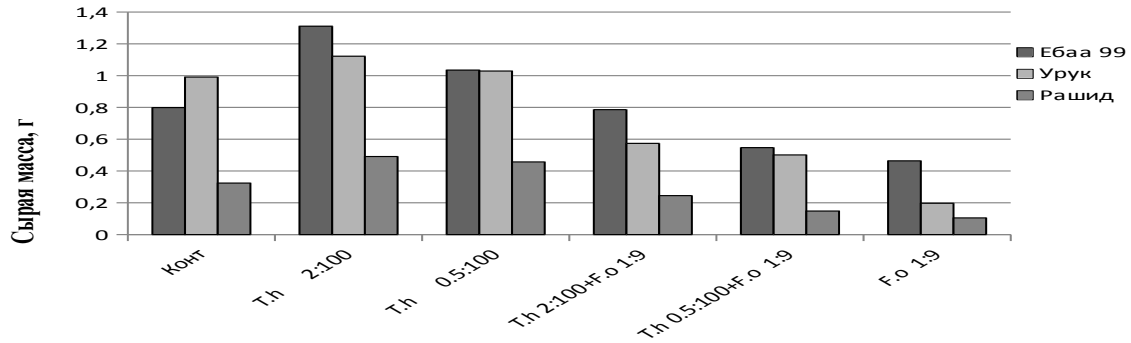
Apparently, the sort we researched synthesizes the substances possessing growth - the stimulating influence what the data obtained by us confirm. It is shown that in a bench and numerous field test of the sort of T-22 there is a developing root system in plants of corn and in other cultures. This effect remained during all life of an annual plant. Colonization of roots the sort of T-22 led to developing secondary roots already at a depth of 25-75 cm from the surface of the soil. The quantity of secondary roots in experience exceeded control indicators twice. As a result of a plant were steadier against a drought, and, perhaps, gained resistance to consolidation of the soil [16]. in case of sprouts infestation with a pathogenic fungi of F.oxysporum we revealed inhibition of length of an elevated and underground part. The most strong inhibiting effect was found in an unstable sort Rasheed. At the plants colonized by the Trichoderma sort fungi and pathogenic fungi partial removal of the effect inhibiting growth is observed.

**Accumulation of crude and dry weight wheat sprouts influencing the T.harzianum and F. oxysporum**

The integral indicator characterizing plant growth and its bodies in the conditions of a pathogenesis is its biomass. At a research of level of accumulation of crude and dry biomass at wheat sprouts, various on stability, reliable increase in crude mass of an underground part was established, influencing the fungi-antagonist of T.harzianum. It is established that using the studied sort at cultivation of cultural liquid in the ratio 2:100 leads to the greatest

accumulation of crude and dry weight of sprouts an underground part, irrespective of sort stability. influencing the this fungi of the antagonist influence of this fungi on accumulation of a dry and crude weight an elevated part at the sprouts possessing the strong and average pathogens resistance is not revealed.

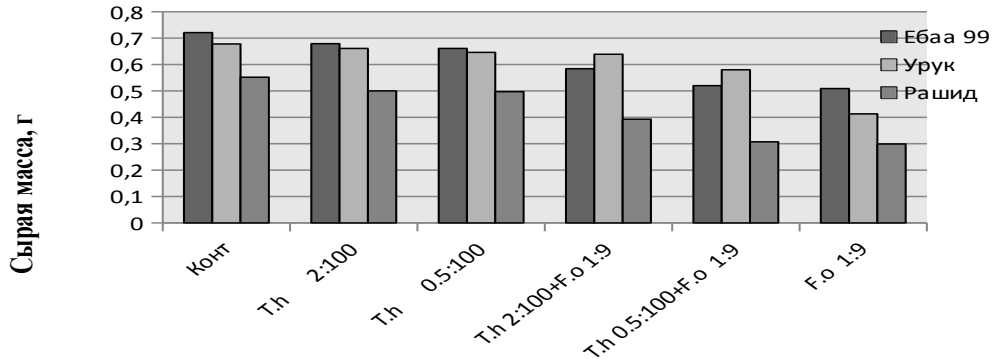
At influence on sprouts of a pathogenic fungi of *F. oxysporum* its toxic influence was noted. Decrease in accumulation of crude and dry biomass irrespective of wheat sort was so recorded.



Сырая масса, г.  
Ебаа  
Урук  
Рашид

Crude weight. g  
Ebaa  
Uruk  
Rasheed

**Figure 5 - Influence of *T.harzianum* and *F. oxysporum* on accumulation of crude weight of the underground part wheat sprouts of various stability sorts.**

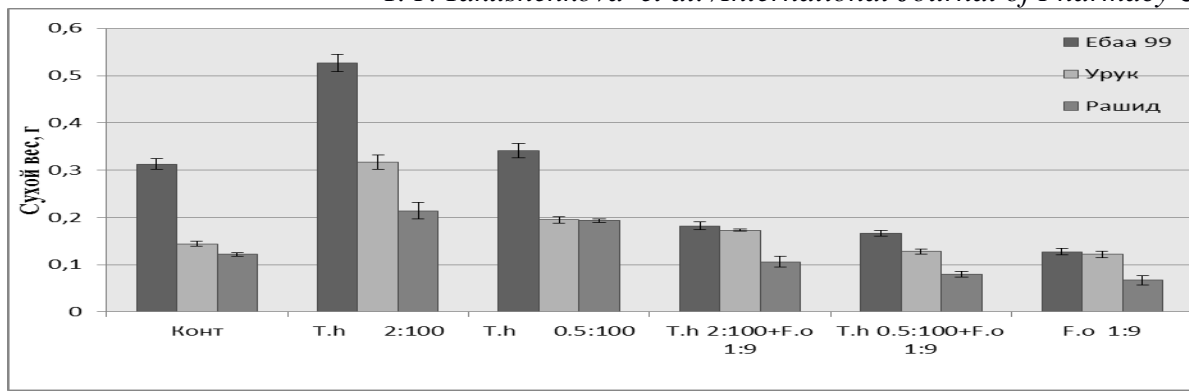


Сырая масса, г.  
Ебаа  
Урук  
Рашид

Crude weight.  
Ebaa  
Uruk  
Rasheed

**Figure 6 - Influence of *T.harzianum* and *F. oxysporum* on accumulation of crude weight (g) elevated part of the wheat sprouts of various stability sorts.**

Collateral influence of fungi-antagonist and fungi of pathogen either did not influence these indicators, or reduced them in relation to monitoring (figures 7-10). I the unstable sort Rasheed we observed some decrease in accumulation of both dry, and crude weight an elevated part under the influence of *T.harzianum*.

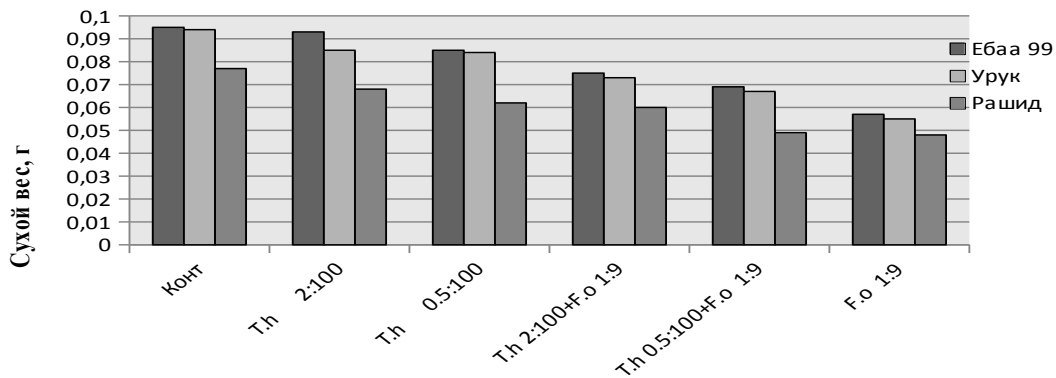


Сухой вес, г.  
Ебаа  
Урук  
Рашид

Dry weight. g  
Ebaa  
Uruk  
Rasheed

Figure 7 - Influence of T.harzianum and F. oxysporum on accumulation of dry weight (g)

underground part wheat sprouts of various stability sorts.



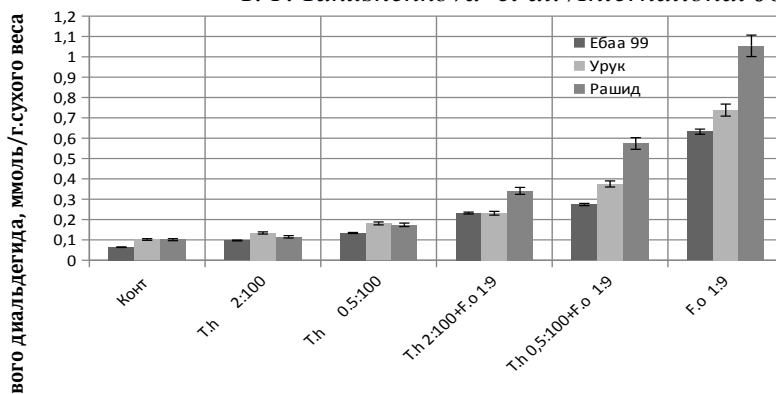
Сухой вес, г.  
Ебаа  
Урук  
Рашид

Dry weight. g  
Ebaa  
Uruk  
Rasheed

Figure 8 T.harzianum and F. oxysporum influence on accumulation of dry weight (g) in elevated part wheat sprouts of various stability sorts

MD contents in roots of wheat sprouts various on stability influencing the T.harzianum and F. oxysporum

It is known that toxic effect of pathogens on plants is shown in developing an oxidizing stress and forming the fissile forms of oxygen. The fissile forms of oxygen are capable to initiate peroxide oxidation of lipids therefore there is a damage of membranous structures. It is considered that such product of peroxide oxidation of lipids as a malonic dialdehyde can be used as the biological indicator of developing an oxidizing stress of plants at a pathogenesis. At impact on wheat sprouts of fungi of T.harzianum essential changes in the maintenance of a malonic dialdehyde at sprouts are not observed. At impact on sprouts of a pathogenic fungi of F. oxysporum ten multiple increase in this connection at an unstable sort Rasheed are observed that demonstrates activation of peroxide oxidation of lipids, and, therefore developing infectious process in a plant.



Содержание малокового диальдегида, г.  
Ебаа  
Урук  
Рашид

Maintenance of galactagogue dialdehyde. g  
Ebaa  
Uruk  
Rasheed

**Figure 9 - Influence of T.harzianum and F. oxysporum on the maintenance of amalonic dialdehyde (WELL) in roots after 8 days of influencing the wheat sprouts of various stability sorts**

In resistant and medium-resistant sort the increase in the maintenance of a malonic dialdehyde was observed, but to a lesser extent 7-8 times. Collateral processing of sprouts fungi-antagonist and a pathogenic fungi considerably reduced the maintenance of a malonic dialdehyde at a steady sort of Ebaa99 and an average and steady sort Uruk. The unstable sort Rasheed positive effect – had the least accumulation of a malonic dialdehyde at cultivation of cultural T.harzianum 2:100 liquid. Thus, we established the beneficial effect of fungi antagonists of T.harzianum on pathogenesis – the developing of root decay. Thus, we have discovered, that the effect can be realized due to increase in both synthesis of antioxidatic enzymes, and their activity.

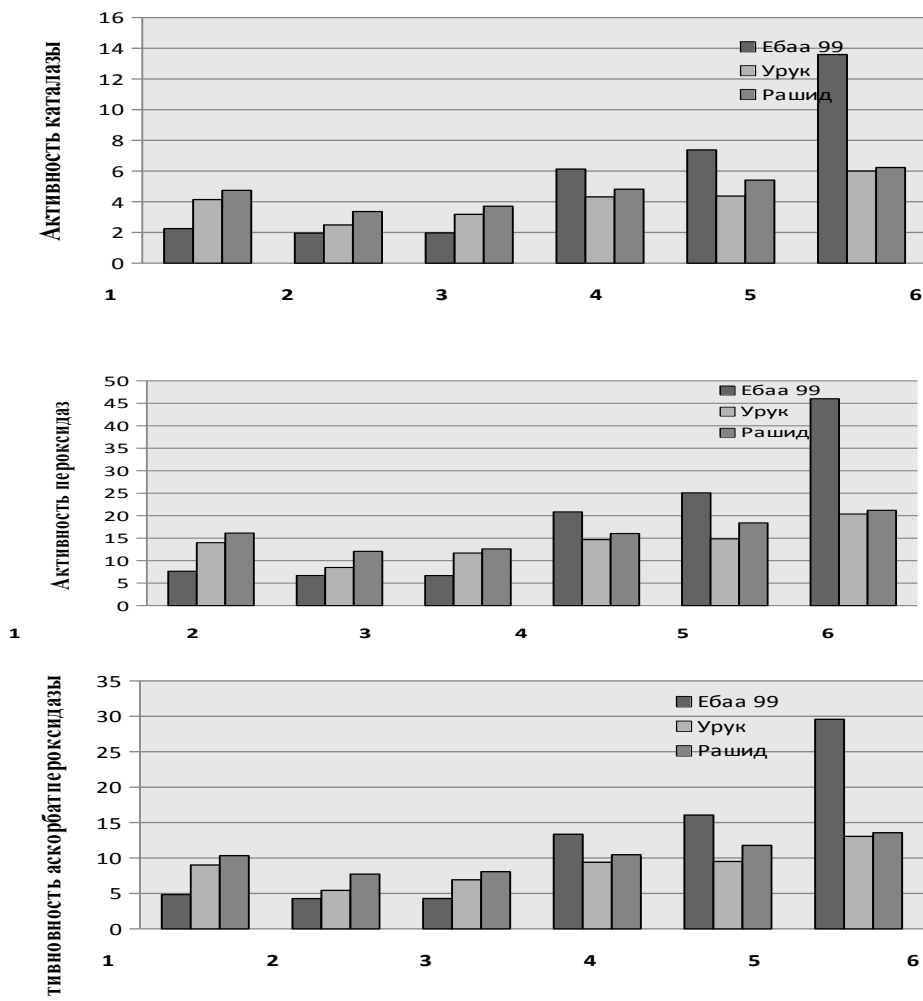
**Activity of antioxidatic enzymes influencing sprouts of T.harzianum and F. oxysporum wheat**

At the initial stage of pathogenesis in plants there is a reinfluence of oxidizing explosion in the place of introduction of a microorganism which leads then to supersensitivity reinfluence. Recently Make (2010) [17] showed that processing of seeds of tomatoes of T.harzianum accelerates seed germination, increases viability, improves water exchange, increases resistance to salinization, an osmotic stress, - hypo and hyperthermias, due to activation of antioxidatic systems. At plants of control option the greatest activity of antioxidatic enzymes was observed at an unstable sort Rasheed. It is possibly bound to infection of seeds of this sort with pathogenic micromycetes - Fusarium oxysporum and Puccini graminis tritici. When determining phytocontamination at a steady and average and steady sort defeat was not revealed by a phytopathogenic microbiota. At plants of control option the greatest activity of antioxidatic enzymes was observed at an unstable sort Rasheed. It is possibly bound to infection of seeds of this sort with pathogenic micromycetes - Fusarium oxysporum and Puccini graminis tritici. When determining



phytocontamination at a steady and average and steady sort defeat was not revealed by a phytopathogenic microbiota.

In research of influence of T.harzianum on activity of the antioxidant enzymes it was established that the used sort of a fungi antagonist of T.harzianum does not cause changes in activity of antioxidatic enzymes in a steady sort of Ebaa 99, and causes some decrease of the activity of antioxidatic enzymes in average and unstable sorts. Perhaps the fungi antagonist of T.harzianum produces a number of connections antioxidants - Acidum ascorbinicum, the restored glutathione, flavonoids. All these connections are capable to neutralize the fissile forms of oxygen with no enzymes taking part. In case of sprouts infestation with a fungi pathogen of F. oxysporum reliable increase of activity of all studied enzymes at a steady sort is observed. The obtained data on activity of antioxidatic enzymes correspond to the maintenance of a malonic dialdegad in these wheat sprouts.



- |                                  |  |
|----------------------------------|--|
| 1. активность катализы           | 1. activity catalyses                      |
| 2. активность пероксидов         | 2. activity of peroxide compounds          |
| 3. активность аскорбатпероксидов | 3. activity of ascorbateperoxide compounds |
| Ебаа                             | Ebaa                                       |
| Урук                             | Uruk                                       |
| Рашид                            | Rasheed                                    |

Figure 10 - Influence of T.harzianum and F. oxysporum on activity of antioxidant enzymes in roots after 8 days of impacting the wheat sprouts of various stability sorts:

### **The maintenance of micromycetes (the number of colonies, piece) in wheat sprouts allocated from roots**

When determining phytocontamination in resistant and medium resistant sort decrease was not revealed by a phytopathogenic microbiota. Seeds of an unstable sort were inflicted with *Fusarium oxysporum* and *Puccini graminis tritici*. After the cultivation of wheat sprouts, various sorts distinguishing on pathogens resistance in the environment of *T.harzianum* fungi antagonist the greatest colonization by this fungi was observed in resistant and medium resistant sorts. After cultivation of wheat sprouts in the environment with fungi-antagonist and a pathogenic fungi *Fusarium oxysporum* the number of colonies of a fungi-antagonist decreases, at the same time there is a colonization of roots by pathogenic micromycete. Colonization level of a fungi pathogen is in direct dependence on stability sort. So, the least number of pathogen fungi colonies is observed in resistant sort of Ebaa 99 (Table 1).

### **Conclusion**

As a result of the conducted research the stimulating effect of the studied sort *Trichoderma harzianum* T 203 on an incipient state of developing plants expressed in increase in germination energy and viability of seeds was found. This effect is undoubtedly caused by penetration of a fungi antagonist into seeds of wheat and development by it of the physiologically active materials influencing positively the sowing seed quality of wheat. Increase in accumulation of crude and dry biomass in the underground part is caused by wheat sprouts under the influence of *Trichoderma harzianum* T 203 first of all with their colonization [18] and, possibly, with the changes happening in morphology and anatomy of roots [19]. The fungi antagonist did not increase activity of antioxidatic enzymes, but at the same time the level of peroxide oxidation of lipids did not increase as well.

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