***fusarium* extracts influence on some biochemical indexes of seed germinating**

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# **Introduction**

*Work importance.* The inoculum can be highly affected by pathogenic microflora. The rapid desease progress was found out with the help of barley phytoseed analysis. Of course all this demand special attention.

The application of chemical seed protectants has been popular until nowadays, but at the moment it demands revision for the next reasons:

 Negative influence on people;

 The remains of chemical drugs collect in soil, water, food stuffs;

 Chemical substances lead to appearance of stable disease inducers ;

 Chemical substances break biological balance between the rhizosphere of natural organisms.

Modern scientific research and experiments show that it's better to applicate various biological agents along with environmental and health control and this will provide high technical and economic efficiency.

***The work purpose*** is to reveal the extract of mushroom *Fusarium sambucinum* influence on biochemical indexes of the seed germination. To reach this purpose the following problems were concidered:

 To investigate the Fusarium influence on metrical indexes of the culture growth;

 To study the influence of *Fusarium sambucinum* on catalase activity in barley plantlets

\* to study some indexes of peroxide oxidation of lipids (TBA-REACTING compounds);

 To define the proportion of mushroom *Fusarium* extract which render the most expressed effect on the seed germination indexes .

 **The influence of the nonpathogenic extract of the Fusarium Sambucinum on the seed germination has been explored for the first time.**

# Fusarium mushrooms

Fusarium is really widespread in nature. Some Fusariums are causative agents of the deseases of more than 200 species of cultivated plants. But separate kinds of Fusarium can synthesise various biologically active agents (for example, vitamins and antibiotics).

# Pathological effect of toxines produced by Fusarium

Grains affected by Fusarium usually have low weight, they lose lifeability or they can have some rot of plantlets. The growth of mushrooms cause accumulation of the toxic metabolites which are hazardous to health of people and animals

# System of plant protection

Chemical protection of plants is spread as a measure of fighting against plant desease, especially in systems of intensive technologies of crop cultivation. However it is not environmentally friendly and it should be combined with biological protection.

# Methods of this research

This experiment is carried out on seeds of gramineous [grəˈmınıəs] culture of spring barley. The seeds were parted into 4 groups: the 1st group (n=3)was the control group (seeds were steeped in water), the 2d group (n=3) the seeds were steeped in an extract of *Fusarium sambucinum* in prorortion 1:1000 (Р1), in the 3d groop (n=3) the seeds were steeped in an extract of mushroom *Fusarium sambucinum* in proportion 1:10000 (Р2), in the 4th group (n=3) the seeds were steeped in an extract of mushroom *Fusarium sambucinum* in proportion 1:100000 (Р3).

#  Equipment and reagents

We used the following reagents during our experiment: 70 % ethanol, the Folin's-Chiokalto's reagent, 10 % solution of Na2CO3, 2 % solution FeNH4 (SO4) 2, 1n HCL, N-BUTANOL, 0,25 % solution of TBA, 10 % solution of CCl3COOH .

To carry out this experiment we used the following equipment: a spectrophotometer, analytical balance, a whizzer, a water bath, a thermostat.

# The seed germinating

Petri dishes were wiped with alcohol, warmed up with the help of a drying chamber. The seeds were put in Petri dishes on 2 beds of filter paper. Petri dishes were put in a thermostat at temperature 250С for 4 days. After this we chose 30 seeds from each group with root length of 0,5-1,2 sm. Then we measured root length, weighed seeds in one Petri dish. The measured seeds were put in another Petri dish on new filter paper with probed solutions of the mushroom extract 1:1000 (Р1), 1:10000 (Р2), 1:100000 (Р3) in amount of 10 ml. In control probe we flowed 10 ml of distilled water. Then we put this Petri dish in a thermostat for 24 hours. After this we measured the plantlets length and the weight of plantlets and shifted seeds in Petri dishes on distilled water to grow under natural lighting. After 8 days we measured the root length, the offspring length and the weight in one Petri dish.

# The catalase activity definition

To define catalase activity we used the method based on definition of Нydrogenium peroxide amount which was not decomposed after its incubation with catalase. This amount was found with spectrophotometric registration of the coloured resultant of peroxide interaction with ammonium molybdate .

In experimental pattern we added 0.1 ml of filtered citosole to 1 ml of hydrogenium peroxide solution. We were centrifuging this probe for 30 minutes.

In the control pattern we used distilled water instead of Hydrogenium peroxide. In "empty" pattern we added to Н2О2  0,1 ml of Н2О instead of biological stuff . We incubated the reaction mixture within 10 minutes at ambient temperature and then we poured 0,5 ml of ammonium molybdate to it. Optical density was measured by a spectrophotometer at wave length of 412 nanometers.

The catalase activity was counted with the help of the following formula:



 E"empty", Eoptical - optical density of "empty" and control hallmark;

 V - final volume of a reaction mixture, ml;

 *ε* - quotient of a molar extinction, 22200 sm-1 × М-1;

 t - an incubation period of hallmark, mines;

 *l* - length of a dish, sm;

 mtissue - tissue mass in a reaction mixture, mg;

Ferment strength is expressed in mkmol/ g of tissue.

# 2.5 Definition of TBA-REACTING bonds

Various toxins, including heavy metals, can cause oxidative stress in plants. They stimulate formation of active forms of oxygen in cells. They possess very high aggressiveness and they are capable of damaging almost all cell components . Active radicals, mainly HO, interact with organic matters and form hydroperoxide compounds of DNA, protein, lipids (ROOH). Hydroperoxide compounds turn into alcohol, aldehydes, epoxides and other acidifyed compounds during the meta[ae]bolism. Formation of these compounds is called peroxide oxidation. Peroxide oxidation of lipids is a display reaction of cellular membranes damage. As a result of peroxide oxidation final meta[ae]bolites are formed.These final products react with the thiobarbituric acid, so they are called TBA-REACTING products.

# The TBA-reacting compound determination

The reaction mixture consisted of 100 ml of distilled water dissolved in 10 g of trichloroacetic acid and 250 mg of thiobarbituric acid.

 We mixed the plant material (300 mg of plantlets) in a stamp with a small amount of the reaction mixture consisting of the solution of the thiobarbituric acid in the solution of trichloroacetic acid. This homogenate [hə'mäjəˌnət]was put into the measuring tube. Then the volume was led up to 4 ml. The patterns were put into a water bath for 30 minutes. Then the hallmarks were quickly cooled. Their contents was centrifuged. Optical density was measured on a spectrophotometer against the control pattern which consisted of 0,25 % solution ТBA in 10 % solution in threechloracetic acid. [of the reaction mixture]

# Results of the research

# 3.1 Parameters of grain germination. Germinating ability

The germination value of barley seeds growing in the environment of *Fusarium sambucinum* extract and taken for analysis practically does not differ from germinating ability of seeds in control bunch (see appendix 1). Neither the root quantity, nor their average length did not variated. At the mass change assessment it was found out that the mass of control grains enlarged for 0,38 g. When we used the extract in proportion 1^1000 the mass of grains enlarged for 0.43g, and when the proportion was 1: 10000, the mass has enlarged for 0.47g.(The appendix 1).

Speaking about the germinative energy of barley seeds we can say that they germinated in different ways. Some samples which were growing in extract *Fusarium samb.* prepared in proportion 1:10000, had healthy tops. Other had fusarium desease and rot.(They were dissolved in *Fusarium samb.* Extract in proportion 1:1000 and 1:100000) As a result some amount of samples had been lost. Maybe, nonpathogenic Fusarium can be harmful at some conditions.

# The Fusarium Sambucinum extract influence on catalase activity and concentration of TBA-reacting compounds.

 *Fusarium sambucinum* extract prepared in proportion 1:1000 significantly [sɪg'nɪfɪkəntlɪ] enlarges catalase activity in 1,8 times in comparison with catalase activity in plantlets in control hallmark. The increase of catalase activity was noticed in groups where the mushroom extract was prepared in proportion 1:10000 and 1:100000. Catalase activity became higher in 1,3 and 1,4 times accordingly.

 The received results let us to say that catalase participate in formation of protective functions in vegetable organism against pathogenic doses of strain. The increased formation of peroxide oxidation products is capable to make toxic effect displayed in cell membrane damage.

# The conclusion

Mycorhize mushrooms play positive role in supply of higher plants.

 Some kinds of fusarium grow in the root zone of plants and they not always possess parasitic properties.

# The received results allow to make the following conclusions:

1. The action of *Fusarium sambucinum extract* dissolved in proportion 1:1000 - 1:10000 cause statistically significant [sɪg'nɪfɪkənt] weight enlargement on 0,4 - 0,5g.

2. The extract of mushroom *Fusarium sambucinum* prepared in proportion 1:1000 enlarges catalase activity on 81 % and reduces the content of peroxide oxidation products on 13 %.

3. The optimal proportion of *Fusarium sambucinum extract* is 1:1000.

# Business offers

To enrich the sowing qualities of seeds and to increase yield [jɪ͟əld] and quality of grain crops we recommend to use the extract of BKMF 3051 strain of Fusarium Sambucinum in industrial amounts. It should be cultivated in 1000 times to stimulate seed germination.