Project name: AP19676907 "Development of mushroom's extracts and spent substrates efficient use technology as means potato protection against phytopathogens and feed additives manufacturing."

Relevance:

Until now, in a number of foreign countries, as in the Republic of Kazakhstan, antifungal and antiviral effects of medicinal fungi in the field of potato protection have not been studied. The production of mycelium, fruit bodies and extracts of edible and medicinal xylotrophic fungi with high biological value opens up wide prospects for the creation of new environmentally friendly productions and their application in food industry, agriculture and medicine. In addition, used substrates for growing mushrooms need bioconversion, which involves the production of feed bioadditives containing a large amount of protein, lignin and other nutrients. The success of the accelerated production of environmentally friendly products of basidial macromycetes largely depends on the correct choice of lignocellulosic substrate and can be enhanced with the help of modern safe growth-stimulating and nutritional supplements.

Purpose:

The purpose of the project is to develop a technology for the effective use of mushroom extracts and spent substrates of edible and medicinal xylotrophic mushrooms as a means of protecting potato from viral and fungal pathogens with the manufacture of feed additives.

Expected and achieved results 2023:

Within the framework of the project on the basis of NJSC " S. Seifullin KATU" (KATU) plans to: study the antimicrobial and antioxidant effects of medicinal mushrooms; identify BAS producing strains, carry out genetic certification and create a collection of promising species of edible and medicinal basidiomycetes, develop an effective automated technology for their cultivation, study the possibility of using processed mushroom waste as effective feed additives for animals and secondary bioadditives for mushroom cultivation.

As a result of the project, 3 recommendations will be prepared: on obtaining and using antiviral and antifungal preparations from xylotrophic fungi; on the intensive technology of cultivation of edible and medicinal basidial macromycetes' promising species based on plant waste available in Kazakhstan; production and use of a highly nutritious mushroom feed additive for farm animals and a secondary additive for the cultivation of mushrooms. The developed recommendations will serve as a methodological basis for obtaining additional domestic environmentally friendly products for food and agricultural purposes. The project has a high interdisciplinarity, as work will be carried out in several directions and specialists in the field of plant protection and immunity, mycology, biotechnology, biochemistry, veterinary medicine, and information technology will be involved.

Based on the results of the research, at least 2 (two) articles and (or) reviews will be published in peer-reviewed scientific journals indexed in the Science Citation Index Expanded of the Web of Science database and (or) having a CiteScore percentile in the Scopus database of at least 50 (fifty); as well as at least 1 (one) article or review in a peer-reviewed foreign or domestic publication recommended by the CQASHE.

In addition, based on the results of research, master's and doctoral dissertations (PhD) will be defended, materials of international scientific and practical conferences will be published, an application for a patent of the Republic of Kazakhstan for an invention will be submitted to RSE "NIIP".

1. Development of optimal parameters for edible and medicinal mushrooms intensive cultivation technology using plant organic waste and bioadditives.

The mother culture of *Hericium erinaceus* and oyster mushroom (*Pleurotus ostreatus*) was obtained and propagated. Optimization of nutrient media for the cultivation of mother culture of the studied xylotrophic fungi was carried out. The best growth characteristics of mother cultures of fungal strains *Pleurotus ostreatus* and *Hericium erinaceus* on the 7th day of cultivation were demonstrated by variants with wheat-sucrose agar, wheat-glucose agar, barley-glucose agar and wheat agar, exceeding the performance of potato-glucose agar (control) by 1.3-

1.5 times. The seed mycelium of *Hericium erinaceus* and oyster mushroom (*Pleurotus ostreatus*) was obtained and maintained in a sterile culture. When studying the effect of a secondary sunflower husk (SH) based additive on the growth of mother culture *Pleurotus ostreatus*, the maximum rate of culture development was revealed when adding 6% of the additive to the nutrient medium, which exceeded the control by 13.2%. Mycelium growth on a medium with an additive from wheat straw at a dose of 3, 6 and 12% was at the standard level (PGA), and the best indicator was noted when a secondary additive was added to the medium at a dose of 9%, where the mycelium growth rate exceeded the control version by 11%. When studying the effect of a secondary additive based on SH in the range of 3-12% on the growth of the mother culture of *Hericium erinaceus*, it was found that the most intensive development of mother culture occurs in an agarized medium with the addition of 12% SH additive with an excess of control (PGA) by 13.4%. In addition, the formation of primordiums was noted in this version of the experience. Mycelium growth on medium with wheat straw additive at a dose of 9-12% was at the standard level (PGA). On secondary additives based on sunflower husk and wheat straw in doses of 6-12%, the formation of primordia was observed directly from the mother culture of this type of fungus, and a dose of 12% leads to earlier fruiting.

The composition of the nutrient substrate for the cultivation of seed mycelium of the studied basidiomycetes has been optimized. In an experiment to study the growth of the seed mycelium of the fungi *Pleurotus ostreatus* on various substrates (grain and straw) with the addition of secondary additives from processed mushroom blocks based on wheat straw and sunflower husks, the following data were obtained: - the growth of the seed mycelium is significantly faster on a substrate with straw by 70 %, compared to the colonization of grain substrate; - a secondary additive based on sunflower husks did not have a significant effect on the growth of seed mycelium compared to controls (barley grain, straw); - a secondary additive based on wheat straw improves the growth rate of mycelium on a straw substrate by 17.8% compared to the control experimental variant (straw), on a grain substrate - by 18.2% compared to the control (grain).

The introduction of a secondary additive based on sunflower husk into the substrate of beech sawdust improves the growth rate of the seed mycelium *Hericium erinaceus* by 13.5% compared with the control (sawdust).

When studying the effect of organic cellulose-containing crop waste on the fruiting of the studied fungal species, it was found that strains K-80 and NK-35 of *Pleurotus ostreatus*, like *Hericium erinaceus*, had the maximum weight of fruiting bodies on the variant of the substrate with sawdust. The average productivity of fungal fruiting bodies in relation to the mass of the substrate on day 29 was: for *Hericium erinaceus* based on sawdust - 3.1%, based on straw - 2%; for *Pleurotus ostreatus*: on the sawdust variant, strain K-80 - 29.1%, on a substrate with straw - 14.2%; strain NK-35 on the version with sawdust - 28.1% and on the basis of straw - 14.4%.

2. Automation of climate-control based on microprocessors on a development board for basidiomycetes cultivation.

For the cultivation of basidiomycetes, a system for automating climate control and regulating the main climate parameters in the growth chamber was designed. Adjustable parameters: carbon dioxide content, humidity and temperature, as well as the frequency of turning on the light (to create the effect of daylight) and turning on the internal fan to average the air in the chamber. A controller was manufactured based on the "ESP 8266 development board NodeMCU" processor. The controller, using contact relays, controls the light in the chamber, the internal fan, the supply ventilation, and the air humidifier. Temperature, humidity and carbon dioxide sensors transmit a signal to the ESP 01 microprocessor which has a WiFi transmitter. Using WiFi, the ESP 8266 processor receives data from sensors, analyzes and controls the corresponding relay. The controller board was installed on the instrument panel in room 5017. The operation of the relay board was adjusted and the interaction of all controller components was tested. An internal and supply ventilation fan, an air humidifier are installed and connected

to the light switch in the chamber. Software and a prototype of a demo version of climate control equipment based on ARDUINO were developed and tested.

3. Screening and creation of mushroom's productive strains and BAS-producing strains collection.

Screening and creation of a collection of productive fungal strains and BAS-producing strains were carried out. To carry out PCR identification of the macromycetes *Pleurotus ostreatus* and *Hericium erinaceus* and genetic certification of fungi, primers are designed. It has been established that the composition of oyster mushroom caps includes: Proteins 3.3%, Fats 0.04%, Chitosan 8.4%, Ash 1%, moisture 86.9%. The composition of *Hericium erinaceus* mushroom caps includes: Proteins 13.5%, Fats 2.4%, Chitosan 6.6%, Ash 0.85%, moisture 76.25%. Research is currently being conducted on the “legs” of the fruiting body of the oyster mushroom.

The genomic DNA of fungal strains was isolated, and the quality and quantity of genomic DNA were analyzed. Fragmentation and amplification of ITS target regions for genetic identification were carried out.

Extraction of fat and protein from the mushrooms *Pleurotus ostreatus* and *Hericium erinaceus* was carried out, including the following stages: preparation of raw materials - drying and grinding to a powder state; fat extraction using a Soxhlet apparatus; aqueous extraction of defatted mushrooms; alkaline extraction.

4. Finding and obtaining extracts of active against potato pathogens mushrooms.

Work has been carried out to research and obtain extracts of cultivated fungi that are active against pathogens of potato diseases. Various extraction methods have been tested to obtain extracts of *Pleurotus ostreatus* and *Hericium erinaceus* mushrooms with optimal extract composition. A literature search was conducted on methods for obtaining extracts from fruiting bodies (frozen, dried, fresh). Extraction of fats and proteins from the mushrooms *Pleurotus ostreatus* and *Hericium erinaceus* was carried out. Fat extraction was carried out using a Soxhlet apparatus using chloroform as an extractant. After extraction, chloroform was evaporated to obtain a pure fat fraction. Protein extraction was carried out in 2 stages - the first with water, the second with an alkaline solution. During aqueous extraction, proteins and active substances pass into the extract. During alkaline extraction, mainly proteins pass into the solution.

5. Determination of regulations for use of mushroom extracts in potato protection against pathogens.

Pure cultures of *Fusarium* and *Alternaria* pathogens have been isolated and maintained *in vitro,* and their identification has been confirmed. As a result of testing potato plantings using enzyme immunoassay, varieties were identified that were monoinfected with viruses: PVY, PVX, PVM. The antiviral and antifungal activity of *Pleurotus ostreatus* and *Hericium erinaceus* extracts against the main potato phytopathogens was assessed. Extracts of the fungi *Pleurotus ostreatus* and *Hericium erinaceus* were tested on pure cultures of *Fusarium* and *Alternaria* pathogens, and on potato plants infected with viruses (PVY, PVX, PVM). Testing of mushroom extracts on a pure culture of *Fusarium oxysporum* was carried out by diffusion into agar with disks. Aqueous extracts and proteins of *Pleurotus ostreatus* and additionally the water-soluble fungus *Lentinula edodes* were tested. In each variant, an experiment was carried out with different doses of mushroom extracts: 1:1, 1:10, 1:100. A comparison was also made with a positive control (Falcon drug, active ingredient 167 g/l tebuconazole, 43 g/l triadimenol, 250 g/l spiroxamine) and a negative control (distilled water). Filter paper discs treated with mushroom extracts, pesticide and water were placed on a PGA with a pure culture of *Fusarium oxysporum*. Around the disks treated with oyster mushroom extracts, partial zones of growth inhibition of colonies of pure *Fusarium oxysporum* culture were observed. A similar picture was observed in all variants of the experiment with mushroom extracts of *Pleurotus ostreatus*. The positive control zone showed little contamination, while the negative control zone showed complete contamination. Using the enzyme immunoassay (ELISA) method, samples monoinfected with viruses: PVY, PVX, PVM were identified in potato field plantings, which were transferred to a

sterile culture (culture of isolated plant organs, in vitro). The antiviral activity of oyster mushroom extracts was studied by introducing the extract through a membrane filter into the Murashige-Skoog nutrient medium in which infected plants grew. As a result, no antiviral effect of oyster mushroom extracts was noted against PVY, PVX, PVM by adding the extract to the nutrient medium.

6. Development of technology for obtaining of highly nutritious and easily digestible feed additives for farm animals and secondary bioadditives for cultivation of basidiomycetes based on spent mushroom substrates.

The development of a technology for recycling waste from mushroom production has begun by obtaining a highly nutritious and easily digestible feed additive for farm animals and a secondary bioadditive for the cultivation of basidiomycetes based on waste mushroom substrate. Technological equipment has been prepared for the subsequent processing of waste from the production of *Pleurotus ostreatus* mushrooms: extruder PE-350, mixer SG-800, pneumatic crusher PD-700, cooler, hay-straw chopper, storage hopper with unloading auger, etc. Raw materials were prepared from mushroom blocks (MB) with a particle fraction from 1.0 to 2.0 mm. Chopping of mushroom blocks was carried out using a hay-straw chopper SI-200, with a productivity of 400 kg/h. A technology has been developed for the production of a feed additive based on the use of mushroom blocks. Grain components (barley, wheat, oats) were mixed with mushroom blocks in a ratio of 1:3. In this ratio, the volume of the grain part was the same, while the number of mushroom blocks was varied at 20%, 25% and 30%. The feed additive includes the following components: extruded wheat, extruded barley, extruded oats, tricalcium phosphate, flax, amino acids, premix, BioFeed-P, table salt, water, mushroom blocks. Determination of the chemical composition and nutritional value of feed additive samples was carried out using an infrared feed analyzer NIRS DS 2500 (FOSS). An analysis was carried out to change the component composition of feed additives with the inclusion of 20 and 30% mushroom blocks before and after extrusion. The protein content in the feed additive with the addition of 20% MB before extrusion was 17.3%, after extrusion the figure decreases slightly and amounts to 16.3%, while the proteins pass into a more digestible form. On the contrary, the fat content increased after extrusion, so in the feed additive with 20% MB before treatment it was 5.5%, and after it was 6.5%. It should be noted that the moisture content decreased after extrusion from 13.1% to 10.1%. The mineral content is reflected by the ash content, which after extrusion remains practically at the same level - 5.1% - as before processing. The protein content in the feed additive with the addition of 30% MB after extrusion was 17.5%. The fat content after extrusion also increased from 5.5% to 6.5%. Humidity has decreased to 13.9%, which allows to extend the shelf life of the finished product. Microbiological analysis of feed additives was carried out on ready-made Compact Dry nutrient media with the study of the total microbial number, group of *E. coli*, fungi, yeast and salmonella. According to the research results, it was revealed that barothermal treatment made it possible to destroy *Escherichia coli* microbes. Thus, before extrusion of a sample with 30% MB, 209 CFU were detected, while after extrusion, analysis of this sample showed the absence of *E. coli*. It was also revealed that the sample with 30% MB was contaminated with yeast, but after extrusion they were not detected. In the sample with 20% MB, multiple colonies of mold fungi were found before processing, but these were not detected after extrusion. Salmonella was not detected in all tested samples. To assess the productivity of animals based on the principle of pairs of analogues, 2 groups of rabbits were formed (taking into account age, live weight and breed). Microclimate parameters were the same in all groups. The animals are clinically healthy: breathing is rhythmic, visible mucous membranes are pale pink, the animals willingly eat food, the posture is natural. To control weight indicators, individual weighing was carried out; the average live weight in the experimental group was 1.6 ± 0.008 kg, in the control group 1.59 ± 0.15 kg. The experimental group was given extruded feed additives containing 30% MB. The rate of introduction of the feed additive is 100 g/head per day, 80 g/head of hay per day. On the 7th day of the experiment, control weighing of the animals was carried out, the absolute gain was 160 g in the MG, 140 g in the control group, thus the

average daily gain in the MG was 22.8 g and 20 g, respectively. Before the experiment, blood samples were taken for biochemical analysis and assessment of the body's homeostasis. Blood analysis was performed on a veterinary biochemical analyzer model SMT-120V (Chengdu Seamaty Technology Co., China, Sichuan). The results of blood taken before the experiment indicate potential changes in liver function and lipid metabolism in animals in the experimental group compared to the control group. The average value of total protein in the experimental group was 63.87±2.47 g/l, while in the control group it was 60.10±2.99 g/l. The alanine aminotransferase level in the experimental group was 107±16.54 U/L, while in the control group it was 81.00±23.46 U/L. Elevated ALT values may indicate liver damage or stress in animals. The average value of total cholesterol in the experimental group was 2.52±0.17 mmol/l, while in the control group the figure was 1.99±0.36 mmol/l. This indicates that the level of total cholesterol in animals in the experimental group is higher than in the control group. Elevated cholesterol levels may be associated with changes in lipid metabolism or metabolic processes in animals in the experimental group. Glucose (GLU) was slightly reduced in the experimental group and amounted to 1.51±0.30 mmol/L, in the control group 3.39±1.40 mmol/L. The remaining parameters studied were within the physiological norm; no significant differences were observed between the groups. On the 7th day of the experiment, the ALT level in the blood of animals in the experimental group returned to normal. A decrease in cholesterol level in the blood of rabbits of the experimental group was revealed: 2.26±0.22 mmol/L, which is also close to the upper limit of normal. The concentration of glucose in the blood of rabbits in the EG was 1.62±0.15 mmol/L, in the CG it was 3.03±0.74 mmol/L. Based on these data, we can conclude that the studied feed additives based on mushroom blocks do not have a negative effect on the animal’s body, but, on the contrary, bring indicators such as ALT and total cholesterol levels to normal.

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List of publications and patents published within the framework of this project: (with links to them):

Two articles published in an international research conference:

1. Vadim Khassanov, Celal Bal, Mustafa Sevindik. Lion's mane mushroom in terms of biological activity. II. International KORKUT ATA Scientific researches conference, October 7-8, 2023 / Ankara, Turkey, 1041-1048.

2. Vadim Khassanov, Celal Bal, Mustafa Sevindik. Fairy ring mushroom and biological activities. II. International KORKUT ATA Scientific researches conference, October 7-8, 2023 / Ankara, Turkey, 1064-1069.