

**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:**

To date, in a number of foreign countries, as well as in the Republic of Kazakhstan, the antifungal and antiviral effects of medicinal mushrooms in the field of potato protection have not been studied. Obtaining mycelium, fruiting bodies, as well as extracts of edible and medicinal xylotrophic mushrooms with high biological value opens up broad prospects for the creation of new environmentally friendly industries and their use in the food industry, agriculture and medicine.

In addition, spent substrates for mushroom cultivation require bioconversion, which involves the production of feed bioadditives containing a large amount of protein, lignin and other nutrients. The success of accelerated production of environmentally friendly products of basidiomycetes largely depends on the correct choice of lignocellulose substrate and can be enhanced with the help of modern safe growth-stimulating and nutritional additives.

**Objective of the project:** 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.

**Results obtained for 2024:**

The mother mycelium of shiitake (*Lentinula edodes*) was obtained and propagated. Nutrient media for culturing the mother mycelium of the studied xylotrophic fungus were optimized. On the 12th day of cultivation, the highest growth characteristics were demonstrated by the variants prepared with the addition of sunflower husk and hericium mushroom blocks (HMB), surpassing the indicators of potato-glucose agar (control) by 0.9 times. The seed mycelium of the shiitake mushroom strains *Lentinula edodes* was obtained and maintained in sterile culture.

PCR identification of the studied macromycetes was carried out. Based on the results of DNA barcoding for samples based on comparison with reference sequences, the following species were identified: *Ganoderma sanduense*, *Pleurotus ostreatus*, *Trichoderma rifaii*, *Lentinula detonsa*, *Herichium rajendrae*, *Auricularia australiana*.

Pure cultures of fusarium and alternariosis pathogens with confirmed identification have been isolated and maintained *in vitro*. Potato plantings have been tested by enzyme immunoassay, which has revealed cultivar samples mono-infected with PVY, PVX, and PVM viruses. Antiviral and antifungal activity of *Lentinula edodes* extracts against the main potato phytopathogens has been assessed. These extracts have been tested on pure cultures of fusarium and alternariosis pathogens, as well as on potato plants infected with PVY, PVX, and PVM viruses. Fungal extracts have been tested on a pure culture of fusarium

(*Fusarium oxysporum*) by agar diffusion with disks. Aqueous extracts and water-soluble proteins of *Lentinula edodes* have been tested. In each variant of the experiment, different doses of mushroom extracts were used: 1:1, 1:10, 1:100. In addition, a comparison was made with a positive control (Falcon preparation containing 167 g/l tebuconazole, 43 g/l triadimenol and 250 g/l spiroxamine) and a negative control (distilled water). Wells were made on potato glucose agar with a *Fusarium oxysporum* culture, into which extracts were added in different concentrations, as well as positive and negative controls. On the 7th day, the aqueous extract of shiitake showed the same high efficiency in suppressing fusarium as the fungicide Falcon. On the 14th day, some contamination was observed in dishes with aqueous and alkaline extracts of shiitake. There was no contamination in dishes with Falcon. Thus, the water extract of shiitake showed comparable effectiveness with Falcon in the early stages, and at later stages, shiitake extracts were more effective than hedgehog's mushroom. The same results were noted in the experiment with alternaria.

The effect of aqueous and alkaline extracts of shiitake on the lower fungi *Phytophthora infestans* and *Alternaria solani* was examined. A pure culture of the pathogen was used as a positive control (K+), and the fungicide Falcon (Spiroxamine 250 g/l, Tebuconazole 167 g/l, Triadimenol 43 g/l) was used as a negative control (K-).

A recipe and technology for the production of extruded feed with the inclusion of 10 and 15% of spent mushroom blocks (oyster mushroom substrate) as a feed additive have been developed. Experiments have been conducted to analyze the effect of feed enriched with mushroom substrate on the physicochemical parameters of milk and milk productivity of Kamori goats.

The chemical composition of the feed was determined using the developed method using the FOSS infrared analyzer. The analysis revealed the following content in the finished feed: protein - 14.5%, fat - 5.6%, fiber - 7.79%, ash - 5.01%, starch - 48.48%.

The safety parameters of the feed according to the developed recipe were studied; when determining the general toxicity on guppy fish, it was found that the feed was non-toxic; no death of biotests was detected within 24 hours.

The following mycotoxin content was determined in the feed samples using the ICA method: Fumonisin – 0.3 mg/kg, Aflatoxin – 0.002 mg/kg, which corresponds to the established standards. The results of the enzyme immunoassay showed that the content of Aflatoxin Total is 1750 ng/kg (equivalent to 0.00175 mg/kg), which is below the established maximum level.

The introduction of the feed according to the developed recipe into the diet resulted in an increase in milk yield by 0.5 liters, as well as an improvement in the physicochemical parameters of milk. Thus, the fat content in milk by the end of the experiment reached 5.93%, which exceeds the initial level by 95.7%. In addition, the protein content was 3.29%, and lactose - 4.89%. No deviations were found when studying the biochemical and hematological parameters of the goats' blood.

Based on the results of research for 2024, a scientific article was published in a journal with a CiteScore percentile in the Scopus database of 88 (eighty-eight)

Q1 in the direction of Plant Science: Sevindik Mustafa, Ayşenur Gürgen, Vadim Tagirovich Khassanov, Celal Bal. Biological Activities of Ethanol Extracts of *Hericium erinaceus* Obtained as a Result of Optimization Analysis //Foods. - 2024. - Vol. 13. - No. 10. - P. 1560.<https://www.mdpi.com/2304-8158/13/10/1560>. A scientific article was published in the edition recommended by Committee for Quality Assurance in the Sphere of Science and Higher Education: Kanapina M.M., Vologin S.G., Khassanov V.T. The influence of natural viral infection on potato productivity in the conditions of Akmola region // Bulletin of Science of the Kazakh Agrotechnical Research University named after Saken Seifullin (interdisciplinary). - Astana, 2024. - No. 1 (120). - P.160-171.<https://bulletinofscience.kazatu.edu.kz/index.php/bulletinofscience/article/view/1633/1137>. In addition, the results of the research work were published in the materials of the international scientific and practical conference: Khassanov V.T., Beisembina B., Kanapina M.M., Borodin E.V. (2024). Evaluation of the effect of organic additives on the growth of the mother mycelium of *Auricularia auricula-juda* during *in vitro* cultivation. Modern Scientific Technology, (7). P.131-136 <https://ojs.publisher.agency/index.php/MSA/article/view/3998>

An application for the invention “Method for preparing a feed additive from spent mushroom substrate” was submitted to the RSE “NIIP” of the Republic of Kazakhstan, registration number – No. 2024/0773.1.

#### **Research group members:**

**project manager**– Khassanov Vadim Tagirovich, professor, candidate of biological sciences, h-index – 3 (Web of Science), 3 (Scopus), ORCID: 0000-0002-9054-5551, Scopus Author ID: 57188854211 <https://www.scopus.com/authid/detail.uri?authorId=57188854211> Web of Science Researcher ID: O-7172-2017 <https://www.webofscience.com/wos/author/record/1199319>

#### **research group:**

Sevindik Mustafa – foreign consultant, associate professor, PhD, h-index Scopus-19, Web of Science-25, ORCID: 0000-0001-7223-2220, Scopus Author ID: 57195056820 <https://www.scopus.com/authid/detail.uri?authorId=57195056820> Web of Science Researcher ID: J-1060-2019 <https://www.webofscience.com/wos/author/record/1734137>

Weixing Shan - scientific consultant. Professor, PhD, h-index Scopus –24, Web of Science- 23, Web of Science Researcher ID: GDY-7223-2022 ORCID: 0000-0001-7286-4041, Scopus Author ID: 35895917700.

Beisembina Bibigul – leading researcher, PhD, h-index Scopus – 1, Web of Science – 1, ORCID: 0000-0001-6667-8541, Scopus Author ID 57188854892 <https://www.scopus.com/authid/detail.uri?authorId=57188854892> Web of Science Researcher ID: O-7166-2017 <https://www.webofscience.com/wos/author/record/41156>

Baldzhi Yuriy Aleksandrovich – senior researcher, candidate of veterinary sciences, h-index Scopus - 2, Web of Science - 1, Web of Science Researcher ID: AAF-2915-2020 (C-6504-2017)

<https://www.webofscience.com/wos/author/record/1950386>  
<https://www.webofscience.com/wos/author/record/1601092>, ORCID: 0000-0002-5006-3224, Scopus Author ID: 57204942823  
<https://www.scopus.com/authid/detail.uri?authorId=57204942823>

Zhatkanbaeva Zhanna Kalanbekovna – researcher, candidate of chemical sciences, h-index Scopus - 0, Web of Science – 3, Web of Science Researcher ID: O-8229-2014 <https://www.webofscience.com/wos/author/record/1809394> ORCID: 0000-0001-6584-2565, Scopus Author ID - 57202887991  
<https://www.scopus.com/authid/detail.uri?authorId=55858254400>

Zharmakin Bolatkhan Kaikenovich – researcher, master of technical sciences, h-index Scopus - 0, Web of Science – 0, ORCID: 0000-0002-5323-3460.

Suleiman Madina Akbaralykyzy – senior researcher, master of agricultural sciences, h-index - 0, ORCID: 0000-0002-7670-5352, Web of Science Researcher ID: ACQ-0840-2022  
<https://www.webofscience.com/wos/author/record/2959581>

Kanapina Meruert Maratovna – senior researcher, master of agricultural sciences, h-index - 0 ORCID: 0000-0002-7091-1163

Daulet Daniyar – researcher, h-index – 0, ORCID:0009-0000-5923-3616

Borodin Evgeny Valerievich – technologist, h-index – 0

Akhmetzhanov Marat Talgatovich - laboratory assistant, h-index - 0 ORCID: 0000-0002-6489-5814

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

Sevindik Mustafa, Ayşenur Gürgen, Vadim Tagirovich Khassanov, Celal Bal. Biological Activities of Ethanol Extracts of *Hericium erinaceus* Obtained as a Result of Optimization Analysis //Foods. – 2024. – T. 13. – No. 10. – P. 1560.  
<https://www.mdpi.com/2304-8158/13/10/1560>

Kanapina M.M., Vologin S.G., Khassanov V.T. Influence of natural viral infection on potato productivity in the Akmolra region // Science Bulletin of the Kazakh Agrotechnical Research University named after Saken Seifullin (interdisciplinary). - Astana, 2024. - No. 1 (120). - P.160-171.  
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Khassanov V.T., Beisembina B., Kanapina M.M., Borodin E. V. (2024). Evaluation of the effect of organic additives on the growth of the mother mycelium of *Auricularia auricula-juda* during *in vitro* cultivation. Modern Scientific Technology, (7). P.131-136  
<https://ojs.publisher.agency/index.php/MSA/article/view/3998>

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