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# IDENTIFICATION, PATHOGENICITY AND ANTIFUNGAL SUSCEPTIBILITY OF SEA BUCKTHORN FUNGAL PATHOGENS

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## ABSTRACT

This study presents a comprehensive identification and characterization of fungal pathogens that affect sea buckthorn (*Hippophae rhamnoides*). Utilizing microbiological methods, biochemical analyses, molecular genetic diagnostics including PCR and DNA sequencing and pathogenicity testing, three species of phytopathogenic fungi were identified: *Aureobasidium pullulans*, *Didymella glomerata*, and *Epicoccum nigrum*. The pathogenicity of these fungi towards sea buckthorn was experimentally confirmed. An evaluation of the isolates' sensitivity to six antifungal agents (terbinafine, nystatin, ketoconazole, clotrimazole, itraconazole, and fluconazole) indicated notable species-specific differences in susceptibility to fungicidal effects. The findings enhance our understanding of the etiology of fungal diseases affecting sea buckthorn and can inform the development of effective strategies for phytosanitary control and plant protection.

**Key words**: *Hippophae rhamnoides, Aureobasidium pullulans*, *Didymella glomerata*, *Epicoccum nigrum,* identification, pathogenicity, antifungal sensitivity.

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## 1 INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides*) is a promising agricultural crop from the *Elaeagnaceae* family, distinguished by its high nutritional and pharmacological value. Sea buckthorn fruits are widely used in the food and pharmaceutical industries for the production of juices, wines, sea buckthorn oil, vitamin supplements and drugs that help strengthen the immune system and improve overall health [1]. Due to its unique chemical composition, sea buckthorn demonstrates a wide range of biological activity, including antioxidant, anti-inflammatory, adaptogenic, antimicrobial, antiviral, hepatoprotective and cardioprotective properties [2, 3]. The fruits, leaves and seeds of the plant contain more than 200 bioactive compounds, including carotenoids, flavonoids, riboflavonoids, pectins, organic and fatty acids, tocopherols, folic acid, as well as macro- and microelements (magnesium, iron, boron, sulfur, silicon) [4, 5]. Of particular importance in the formation of the immunostimulating properties of sea buckthorn is the high concentration of phytoncides and vitamin C, as well as the significant content of vitamins A, B, P and K [6].

However, despite the value of this crop, the cultivation of sea buckthorn is accompanied by a number of biotic and abiotic stresses, among which fungal diseases pose a serious threat. Phytopathogenic fungi can significantly reduce crop yields, deteriorate the quality of fruits and cause plant death, which leads to significant economic losses in agriculture [7].

Fungal infections are widespread among sea buckthorn plantations and cause various pathological changes, including leaf wilting, necrotic bark lesions, leaf and fruit spotting, and root rot [8]. The most significant phytopathogenic fungi affecting sea buckthorn are representatives of the genera *Verticillium, Fusarium, Phoma*, and *Alternaria* [9]. These microorganisms are highly adaptable to various environmental conditions and are capable of causing chronic or acute infections, leading to a decrease in plant productivity and their mass death.

Some of the most common symptoms of fungal diseases of sea buckthorn include *Verticillium* wilt (*Verticillium* spp.) – characterized by sudden yellowing and wilting of leaves, development of vascular necrosis, which ultimately leads to the death of the plant; *Fusarium* wilt (*Fusarium* spp.) – characterized by damage to the vascular system, necrotic changes in the root system, leading to the death of young seedlings and weakening of adult plants; *Phoma* wilt (*Phoma* spp.) – causes the appearance of black spots on leaves and fruits, cracking of the bark and the formation of cankers; *Alternaria* leaf spot (*Alternaria* spp.) – manifests itself as dark spots on leaves, brown lesions on fruits and weakening of the general condi-

tion of plants [10]. The development of these diseases leads to significant economic losses, as infected plants lose productivity, and affected fruits become unsuitable for processing and consumption.

Factors contributing to the development of fungal infections. The increase in the incidence of sea buckthorn fungal pathogens is due to several factors, including: 1. changing climatic conditions - increased temperature and humidity create a favourable environment for the spread of fungal infections; 2. intensive farming - the use of monoculture plantings without crop rotation contributes to the accumulation of pathogens in the soil; 3. decreased genetic diversity – growing a limited number of varieties with a low resistance to phytopathogens increases the likelihood of disease outbreaks; 4. insufficient use of agrotechnical protection methods – the lack of preventive measures, such as regular sanitary pruning, a fungicide treatment and soil moisture control, contributes to the spread of infection [11].

In the context of climate change and increasing agricultural intensity, the risk of phytopathogenic infections is increasing, which requires the development of more effective measures for diagnostics, prevention and control of fungal diseases of sea buckthorn. Research aimed at studying the aetiology and pathogenesis of fungal infections is important for preserving the harvest and ensuring the sustainable development of agriculture. Thus, an integrated approach to studying fungal diseases of sea buckthorn, including molecular genetic methods for identifying pathogens, assessing their pathogenicity and sensitivity to antifungal drugs, as well as developing effective methods for plant protection, is an urgent task in modern phytopathology [12]. The results obtained can be used to improve phytosanitary control, develop new methods of prevention and create more resistant varieties of sea buckthorn, which in the future will increase the productivity and profitability of its cultivation.

Biotechnological methods are used to preserve and accelerate the propagation of valuable medicinal plant species, including obtaining a callus culture of plants. The technology involves using healthy plants and their cultivation on nutrient media. This is achieved by sterilizing plants with various chemicals, such as sodium hypochlorite (NaClO), hydrogen peroxide ($H_2O_2$), and corrosive sublimate ($HgCl_2$) [13]. However, it is not always possible to achieve the desired result due to the contamination of plants with pathogens that grow deep into plant tissue and productive buds [14]. This study aimed to study the main phytopathogens of sea buckthorn, their microbiological and genetic characteristics, and determining sensitivity to the main antifungal drugs.

## 2 MATERIALS AND METHODS

### *2.1 Isolation and cultivation of pathogens*

The study of pathogenic fungi affecting sea buckthorn began with isolating pure cultures of microorganisms from infected kidney tissue. For this purpose, a fragment of the affected tissue was taken from each sample and then placed on a nutrient medium. Potato dextrose agar (PDA) was used as a selective medium. The samples were incubated in a thermostat at an optimal temperature for 7-14 days.

The colonies of interest were reseeded to obtain a pure culture necessary for further research. Macroscopic examination of the isolated cultures was performed on the 5th, 7th and 10th days of incubation, while the morphological characteristics of the colonies, such as shape, colour and texture, were recorded. Microscopic analysis was carried out using a ZEISS Axio Scope A1 microscope, which made it possible to study the morphological features of sporulation and the cellular structure of the studied fungi.

### *2.2 Biochemical analysis of cultures*

Four different nutrient media were prepared for the biochemical analysis: corn agar, honey agar, potato dextrose agar and Czapek-Dox medium. Four-section Petri dishes were used as an experimental method. Inoculation was carried out by spot application of spores of each of the isolated strains onto the appropriate medium. Cultivation was carried out for 7 days at the optimal temperature, with daily measurement of the colony diameter.

### *2.3 Genetic analysis of crops*

For molecular genetic analysis, DNA was isolated from the pure culture. For this, a small fragment of the culture was transferred to a 1.5 ml microcentrifuge tube, 500 µl of lysis buffer and 5 µl of proteinase K were added. The resulting mixture was homogenized using a sterile pestle, which was then incubated in a thermal block at 65°C for 2 hours with stirring every 20 minutes.

After incubation, 100 µl of 3M NaCl was added and mixed on a vortex. Then 65 µl of a 10% CTAB solution with NaCl, preheated to 65°C, were added and incubated for 10 minutes at the same temperature. Next, 500 µl of a mixture of chloroform and isoamyl alcohol (in a ratio of 24:1) were added, mixed thoroughly and kept at 4°C for 20 minutes. Centrifugation was carried out at 13,000 rpm for 5 minutes. The upper aqueous layer was transferred to a clean tube, and 380 µl of cold isopropanol was added and left to precipitate DNA at -20°C for 20 minutes. The precipitated DNA was centrifuged at 13,000 rpm for 5 minutes, then washed with 70% ethanol, dried and dissolved in 50 µl of TE buffer. The samples were kept at 50°C for 5 minutes to improve solubility.

Quantitative and qualitative assessment of the isolated DNA was performed using a Nanodrop spectrophotometer.

For PCR analysis, a 25 µl reaction mixture was prepared, including buffers (1×), dNTPs, forward primer *ITS* 4F (5`-TCCTCCGCTTATTGATATGC-3`), reverse primer *ITS* 5R (5`- GGAAGTAAAAGTCGTAACAAGG -3`) [15], Taq polymerase and degassed water ($ddH_2O$). Seven samples were used in the study, including positive and negative controls. Amplification was performed on an Eppendorf Mastercycler thermal cycler using the following temperature regime: initial denaturation at 95°C for 5 minutes, followed by 35 cycles consisting of denaturation at 95°C (30 seconds), primer annealing at 55°C (30 seconds), and extension at 72°C (1 minute). A final extension was performed at 72°C for 7 minutes, after which the samples were stored at 4°C until further analysis.

Qualitative analysis of amplified fragments was performed by electrophoresis in 1% agarose gel using 1× TAE buffer. The PCR product was purified by exonuclease and alkaline phosphatase (SAP) treatment by dephosphorylation, incubating samples at 37°C for 30 minutes. Samples were prepared

for sequencing using alcohol acetate precipitation.

Sequencing was performed using the BigDye® Terminator kit, and the resulting nucleotide sequences were uploaded to the GenBank database and analyzed by comparison with available reference sequences.

*2.4 Pathological test of isolated strains*

To study the pathogenicity of the isolated fungal strains, they were cultivated on potato dextrose agar (PDA) slants. Healthy sea buckthorn sprouts were used as test objects. Spore suspension washes were prepared using sterile saline. 200 µl of saline was added to each test tube and incubated for 2-3 minutes, then 10 µl of the suspension was taken and applied to the leaves and root system of the test plants. The infected plants were kept under natural light at room temperature for 5 days. At the end of the incubation period, a macroscopic analysis was conducted to assess the manifestation of infection symptoms.

*2.5 Analysis of susceptibility of strains to antifungal drugs*

To study the sensitivity of the isolated fungal strains to antifungal agents, they were cultivated on a Sabouraud medium. Each strain was preliminarily inoculated in three test tubes on slopes and incubated for 7 days at the optimal temperature.

To assess sensitivity, six antifungal drugs (ketoconazole, intraconazole, clotrimazole, terbinafine, fluconazole and nystatin) were used in various concentrations (0.5; 1.0; 1.5; 2.0; 3.0; 5.0; 10 µg/ml). Concentrations were calculated based on the amount of active substance in the drugs.

To set up the test, a suspension of fungal spores was prepared in Sabouraud broth. 50 µl of an antibiotic solution of a given concentration and 50 µl of the fungal suspension were added to a 96-well plate. The plate was covered with a lid and incubated in a thermostat. Optical density measurements were performed on a BioRad iMark spectrophotometer at a wavelength of 490 nm over time: on the 1st, 2nd, 3rd, 6th and 7th days. After the last measurement, the strains were reseeded onto Petri dishes with potato dextrose agar (PDA), divided into 8 sectors by the number of antifungal concentrations. Sowing was performed with a sterile bacteriological loop, starting from the control zone and moving on to sections with antibiotics (0.5-10 µg/ml). Colony growth was assessed daily for 3 days.

*2.6 Statistical methods*

To process the data we obtained, we used standard classical methods of analysis for testing statistical hypotheses. Calculations were performed using the «Function Wizard» in the «Statistical» category of Microsoft Excel. Differences at $P \leq 0.05$ were considered reliable values.

## 3 RESULTS

As a result of the study, fungal colonies were successfully isolated and identified from five samples (No. 1, No. 3, No. 4, No. 5 and No. 6) (Figure 1). However, isolating a pure culture from sample No. 2 was impossible due to the high degree of contamination, which prevented reliable results from being obtained.

Sea buckthorn branch fragments incubated on a nutrient medium. Active development of fungal microflora is observed in the bud locations, manifested as seeding with fungal colonies. In all vials, turbidity of the nutrient medium is observed, indicating the growth of microorganisms and their metabolic activity.

Pure cultures were isolated by inoculating samples onto an agar nutrient medium of potato dextrose agar (PDA). Microscopic analysis of the morphological characteristics of fungal structures was carried out using a light microscope at a magnification of ×40–100 (Figure 2).

Colonies are rounded with smooth edges and beige with a dark center. The surface is smooth and shiny. The microscopic structure of the yeast-like culture isolated from samples No. A, D, E. Polymorphic structures characteristic of yeast-like fungi are observed. In the early stages of growth, oval or elongated blastoconidia (yeast cells) predominate, forming by

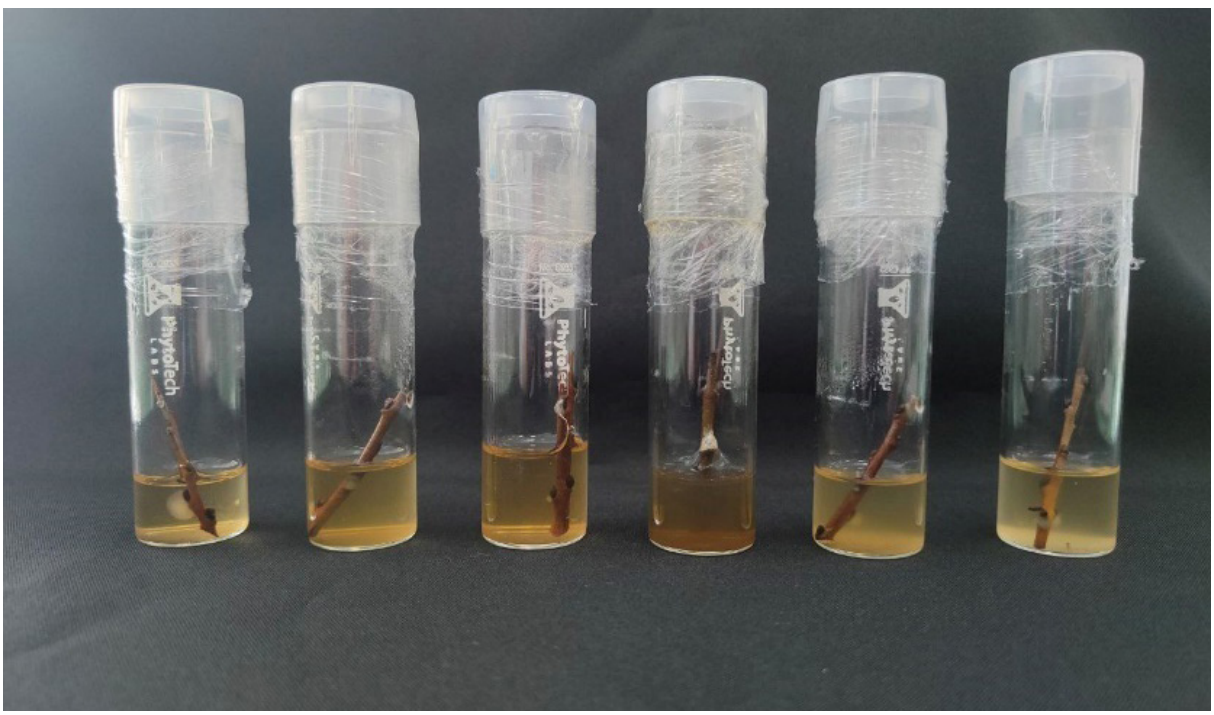

**Figure 1** – Fragments of sea buckthorn branches.

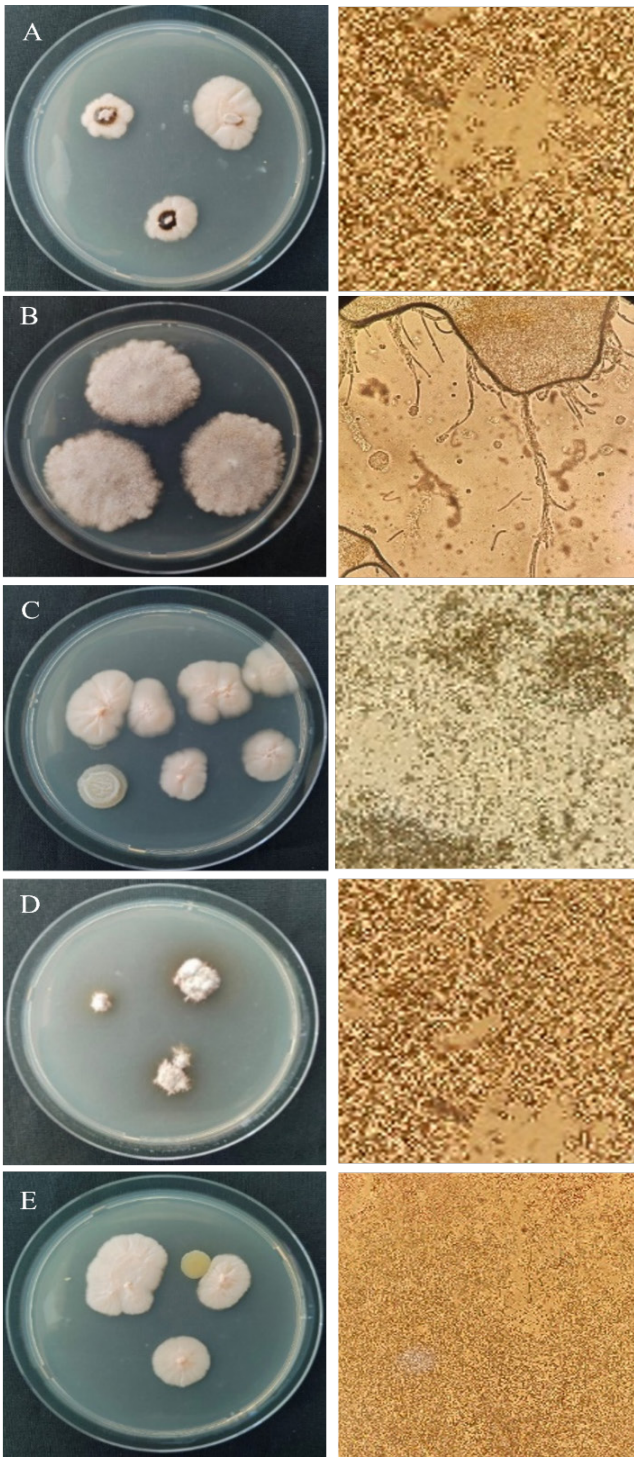


**Figure 2** – Colonies and microscopy of pure cultures.

budding. During the development of the fungus, a transition from yeast-like cells to a mycelial form is observed, which is expressed in the formation of thin septate hyphae.

Macro- and micromorphology of colonies of fungal culture isolated from sample №B Colonies are round, light brown, with pronounced aerial mycelium raised above the surface of the medium. A denser structure is noted in the central part. Microscopic analysis revealed a branched system of hyphae with a septate structure. In sample №C, colonies vary in size from small to medium, round with uneven edges, and light grey and white. The developed fluffy aerial mycelium is noted and raised above the surface of the medium. Microscopy revealed the presence of filiform hyphae with septa, as well as spore-bearing cells of a rounded shape, consisting of clusters of cells.

*3.1 Biochemical analysis of culture*

During the study of the biochemical properties of isolated cultures, the colonies' diameter was measured to assess their growth depending on the composition of the nutrient medium, as well as to study the effect of various carbon sources. Four agar nutrient media with different carbohydrate compositions were used as substrates: corn agar containing molasses; honey agar enriched with fructose; potato agar, in which the main source of carbohydrates is starch; and Czapek-Dox medium containing sucrose. The data obtained made it possible to identify the features of the metabolic activity of the studied strains depending on the carbon substrate used (Figure 3).

The growth of colonies was measured over a period of three days, since all the studied strains showed pronounced growth activity during this period (Table 1).

Analysis of the experimental data showed that the ability to grow on different nutrient media varied significantly between the strains. Strain №3 was the most active, especially on honey agar (average growth 3.76 cm, ± = 1.43), with pronounced growth also on potato agar and Czapek medium. Strain №5 showed good results on honey agar (average growth 1.85 cm, ± = 1.43) and potato agar (average growth 1.19 cm) but was accompanied by high variability. Strain №1 demonstrated moderate and stable growth (maximum on corn agar – 0.95 cm, ± = 0.17), indicating its preference for media with complex carbohydrates but with limited adaptability. Strain №6 also showed moderate growth, especially on potato agar (1.05 cm), with a low deviation, indicating its ability to grow stably. Strain №4 was the least active, with minimal growth on all media.

To improve the clarity and ease of interpretation of the results, the growth dynamics of fungal strains on various nutrient media were presented in the form of graphs (Figure 4).

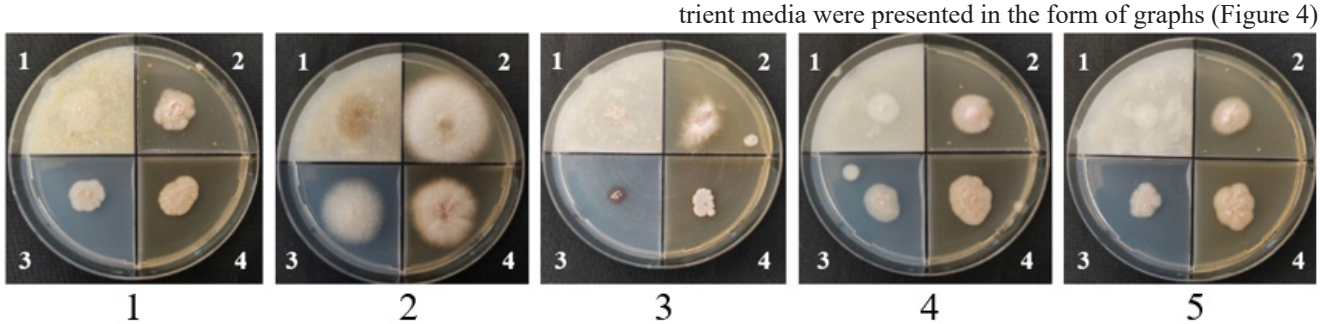


**Figure 3** – Results of strain growth on day 3 on various nutrient media: 1 – corn agar; 2 – honey agar; 3 – Czapek-Dox medium; 4 – potato agar.

**Table 1** – Measurement of strain growth on different media

| strain №1 | | | | | |
|---|---|---|---|---|---|
| **Nutrient medium** | **day 1** | **day 2** | **day 3** | mean value | SD (±) |
| Corn agar | 0,8 | 0,84 | 1,2 | 0,95 | 0,17 |
| Honey agar | 0,36 | 0,84 | 1,36 | 0,85 | 0,34 |
| Potato agar | 0,36 | 0,75 | 0,9 | 0,67 | 0,21 |
| Czapek | 0,315 | 0,55 | 0,84 | 0,57 | 0,18 |
| strain №3 | | | | | |
| Corn agar | 0,35 | 1,53 | 1,7 | 1,19 | 0,56 |
| Honey agar | 1,62 | 4,06 | 5,61 | 3,76 | 1,43 |
| Potato agar | 0,55 | 2,2 | 3 | 1,92 | 0,91 |
| Czapek | 0,315 | 1,62 | 3 | 1,65 | 0,90 |
| strain №4 | | | | | |
| Corn agar | 0,1 | 0,27 | 0,27 | 0,21 | 0,08 |
| Honey agar | 0,36 | 0,85 | 1,235 | 0,82 | 0,30 |
| Potato agar | 0,4 | 0,48 | 0,65 | 0,51 | 0,09 |
| Czapek | 0,06 | 0,06 | 0,06 | 0,06 | 0 |
| strain №5 | | | | | |
| Corn agar | 0,28 | 0,825 | 1,105 | 0,74 | 0,30 |
| Honey agar | 4 | 0,78 | 0,78 | 1,85 | 1,43 |
| Potato agar | 0,84 | 1,2 | 1,53 | 1,19 | 0,23 |
| Czapek | 0,35 | 1,08 | 1,425 | 0,95 | 0,40 |
| strain №6 | | | | | |
| Corn agar | 0,1 | 0,28 | 0,405 | 0,26 | 0,11 |
| Honey agar | 0,315 | 0,77 | 1,12 | 0,74 | 0,28 |
| Potato agar | 0,66 | 1,05 | 1,44 | 1,05 | 0,26 |
| Czapek | 0,4 | 0,55 | 0,78 | 0,58 | 0,14 |

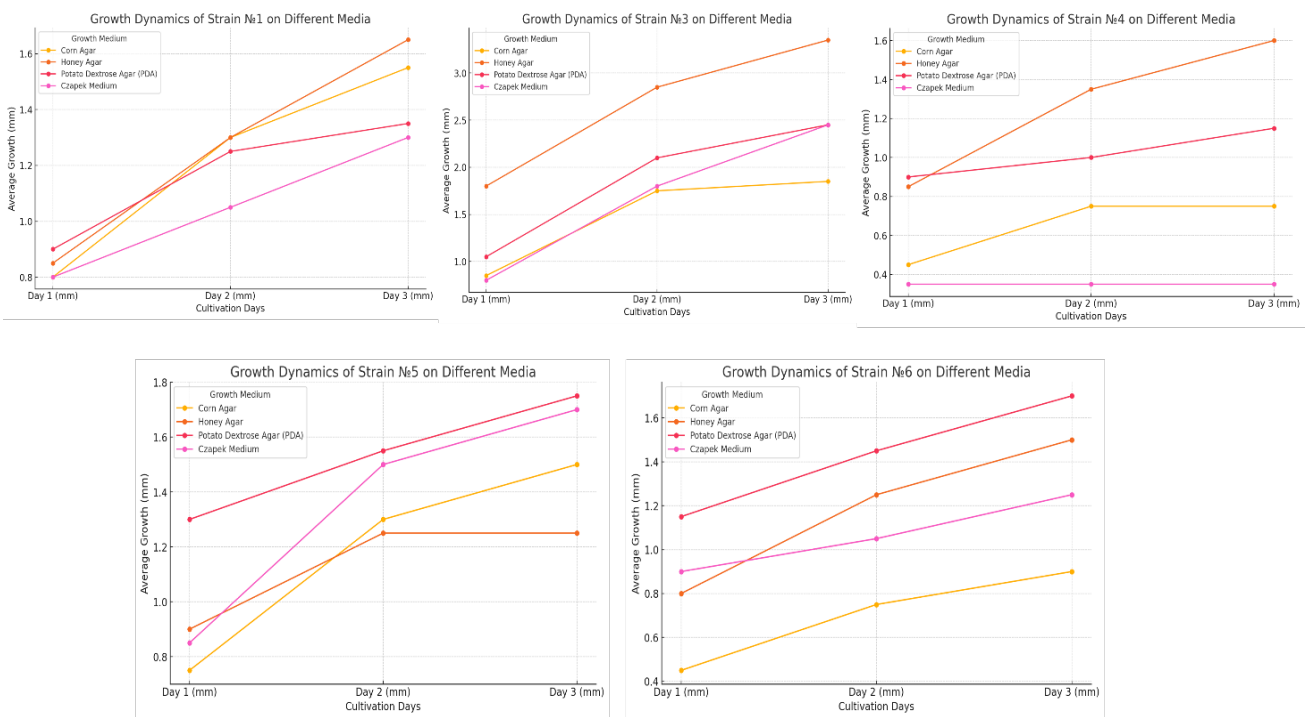


**Figure 4** – Growth dynamics of fungal strains on various nutrient media.

The most active growth is observed in strain №3, especially on honey agar, where the highest values are achieved by the third day. Strain №4 demonstrates weak growth on the Czapek medium, which indicates a low ability to assimilate sucrose as a carbon source. Strains №1, №5 and №6 show better growth on potato agar and honey agar, which indicates their ability to use starch and fructose as nutrients. Czapek medium is generally the least favorable for most strains, except for №5, which demonstrates moderate growth.

In general, honey agar was the most productive for most strains, while the Czapek medium showed the least efficiency. Thus, the composition of the nutrient medium has a decisive effect on the growth of fungi, and taking into account both the average growth rate and its stability allows an objective assessment of the physiological potential of each strain.

*3.2 Genetic analysis of culture*

DNA extraction was performed for further study and precise identification of the isolated fungal cultures, followed by molecular genetic analysis. During the study, amplification of the isolated DNA was carried out using species-specific primers, which made it possible to obtain clear amplicons of about 600-650 nucleotide pairs in size (Figure 5).

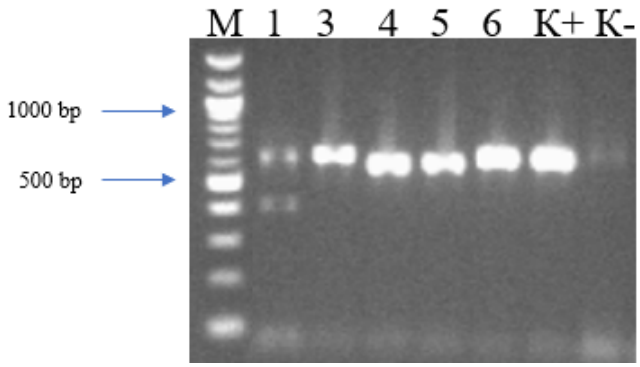


**Figure 5** – Electropherogram results: M – DNA ladder (100 bp); K- – negative control; K+ – positive control; 1,3,4,5,6 – DNA.

The results indicate the high specificity of the applied primers and the efficiency of the conducted amplification, which provides a reliable basis for further sequencing.

As a result of sequencing, nucleotide sequences were obtained, which were then subjected to comparative analysis with reference sequences presented in the international NCBI GenBank database. According to the results of BLAST analysis, identification of the isolated strains showed a high degree of homology with known fungal species. In particular, samples №1, №5 and №6 were identified as *Aureobasidium pullulans*, sample №3 as *Didymella glomerata*, and sample №4 as *Epicoccum nigrum*. These results confirm the correctness of the molecular genetic approach and allow us to classify the isolated cultures as pathogens of phytopathogenic fungi.

*3.3 Pathological examination of strains*

A pathological test confirmed the pathogenicity of the identified phytopathogenic fungi. Spore suspensions of each isolated strain were applied to healthy sea buckthorn seedlings to assess their infectious potential. Spore suspensions prepared from pure cultures of *Aureobasidium pullulans, Didymella glomerata* and *Epicoccum nigrum* were used as inoculum. The treated plants were incubated under controlled conditions at optimal temperature and humidity for 5 days (Figure 6).

At the end of the incubation period, a macroscopic analysis of the plants was carried out, assessing the development of disease symptoms, such as tissue discoloration, the appearance of necrotic spots, wilting and deformation of leaves. Repeated isolation of the pathogen and identification based on morphological features and sequencing confirmed the presence of pathogens *A. pullulans, D. glomerata* and *E. nigrum*. The results obtained confirmed the pathogenicity of the studied strains to sea buckthorn, which indicates their potential role in the development of fungal diseases of this crop.

*3.4 Testing strains for resistance to antifungal agents*

The test for resistance to antifungal drugs was performed

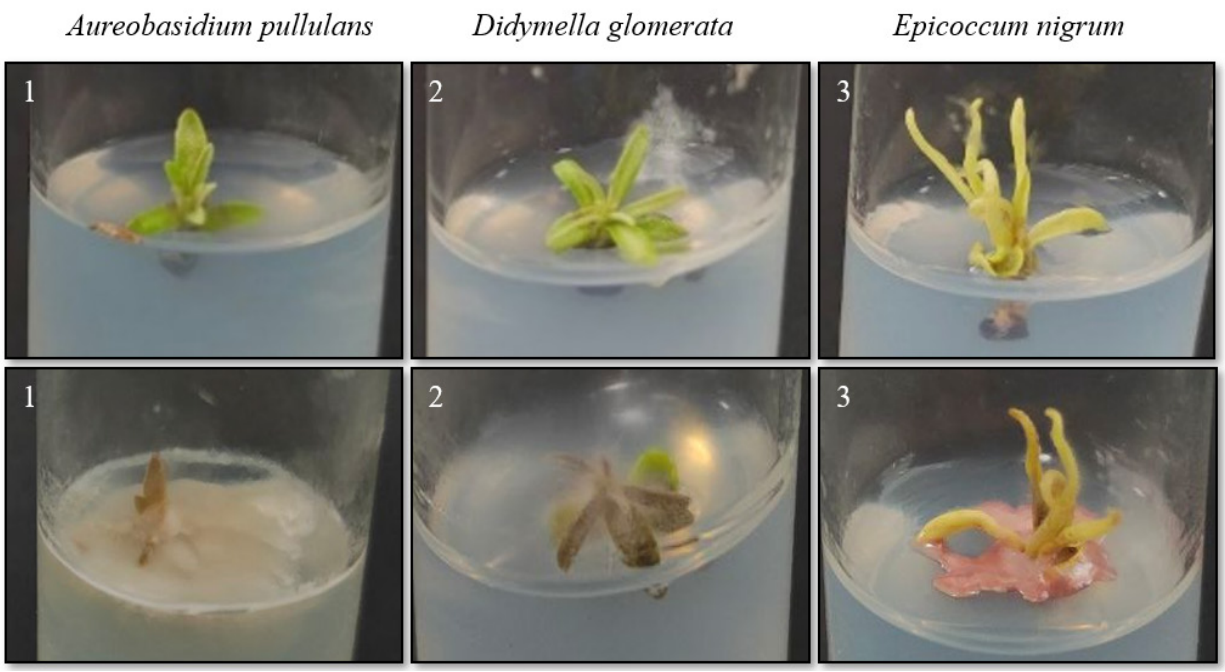


**Figure 6** – Pathological test results: top – healthy plants; bottom – 5 days after infection.

on three isolated fungal strains (*A. pullulans, D. glomerata* and *E. nigrum*). Six antifungal drugs were used to assess susceptibility: itraconazole, clotrimazole, ketoconazole, terbinafine, fluconazole and nystatin in various concentrations (Figure 7).

The experiment results demonstrate different degrees of resistance of the studied fungal strains to antifungal drugs. The *Aureobasidium pullulans* strain showed complete resistance to ketoconazole and fluconazole. The use of nystatin and intraconazole had a restraining effect on the growth of the culture, but significant suppression was observed only at high concentrations of the drugs. At the same time, terbinafine and clotrimazole completely inhibited the growth of this strain.

The *Didymella glomerata* strain showed sensitivity to all the studied antifungals, except intraconazole and fluconazole, where insignificant growth of the culture was observed at a concentration of 0.5 μg/ml.

The *Epicoccum nigrum* strain was susceptible to intraconazole, ketoconazole, nystatin, fluconazole and terbinafine. However, when exposed to clotrimazole at concentrations of 0.5 and 2.0 μg/ml, the growth of the culture was preserved, indicating its partial resistance to this drug.

## 4 DISCUSSION

The study's results confirm that *Aureobasidium pullulans, Didymella glomerata* and *Epicoccum nigrum* are significant

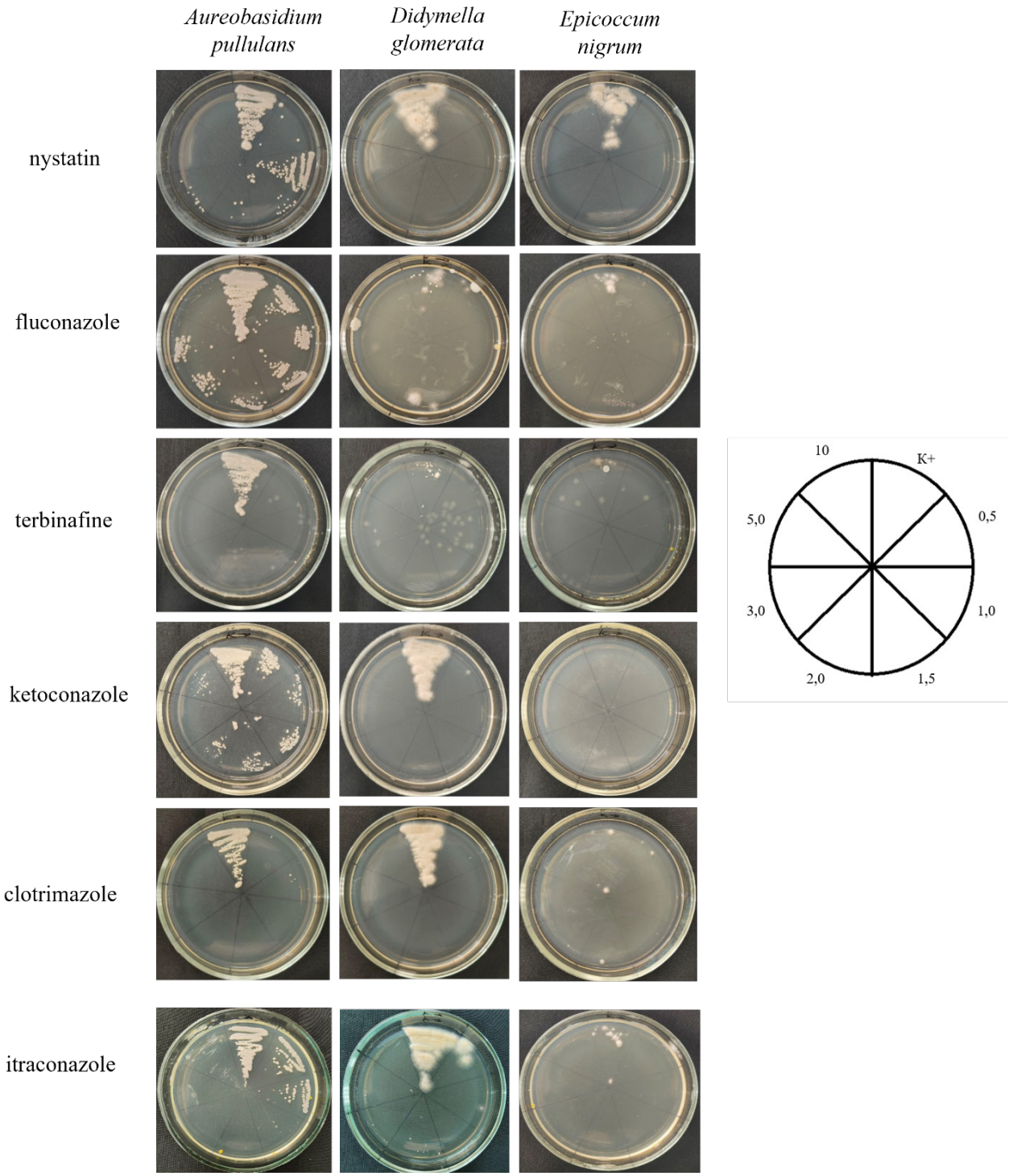


**Figure 7** – Results of resistance to antifungal agents.

sea buckthorn phytopathogens capable of causing diseases of this crop. Isolation and identification of these fungi and experimental confirmation of their pathogenicity indicate the need for further study of their biology, pathogenicity mechanisms and interactions with the host plant. Establishing their role in developing sea buckthorn diseases is especially important in searching for effective measures to protect and control infections [16].

*Aureobasidium pullulans* is an opportunistic fungus that is widespread in various ecological niches. In the literature, it is known as a saprotroph, a biological agent for controlling phytopathogens, but its role as a causative agent of plant diseases remains poorly understood [17]. The discovery of *A. pullulans* as a sea buckthorn pathogen indicates its ability to cause infections under certain conditions, which may be associated with changes in environmental factors or stress conditions of plants. This fungus probably contributes to developing complex infections together with other phytopathogens, which requires additional research.

*Didymella glomerata* represents the genus *Didymella*, which includes many phytopathogenic species that affect woody and herbaceous plants. This study confirmed its participation in the pathogenesis of sea buckthorn, with the manifestation of characteristic symptoms such as leaf spotting and wilting [18]. This pathogen is likely to cause chronic infections and gradually weakening plants, which can significantly reduce the productivity of sea buckthorn plantations.

*Epicoccum nigrum* is a ubiquitous fungus, mostly known as a saprotroph, but its ability to cause plant diseases has been confirmed by several studies [19]. The present study demonstrated pathogenic properties when artificially infected with sea buckthorn seedlings. This may indicate its potential ability to switch from a saprotrophic to a parasitic lifestyle under certain conditions. It is known that some *Epicoccum* representatives can produce phytotoxic metabolites, which may play a key role in the development of infection.

The results of the analysis of the sensitivity of the isolated strains to antifungal drugs revealed varying degrees of susceptibility of fungi to different classes of fungicides, which emphasizes the complexity of choosing effective means of control.

The *Aureobasidium pullulans* strain was completely resistant to ketoconazole and fluconazole, indicating the presence of defense mechanisms that prevent the effects of these drugs. Nystatin and intraconazole demonstrated only partial inhibition of culture growth, with a pronounced effect observed only at high concentrations. At the same time, terbinafine and clotrimazole showed complete suppression of this strain's growth, making them promising for use in the fight against this pathogen.

*Didymella glomerata* showed sensitivity to all antimycotics studied, except intraconazole and ketoconazole at low concentrations (0.5 μg/ml), where insignificant growth was observed. This indicates the possibility of using these drugs in the fight against this pathogen, but additional studies are needed to determine the optimal dosages and application regimens.

The *Epicoccum nigrum* strain was susceptible to itraconazole, ketoconazole, nystatin, fluconazole and terbinafine. However, in the presence of clotrimazole at concentrations of 0.5 and 2.0 μg/ml, the culture grew, which may indicate the presence of partial resistance mechanisms.

The obtained data emphasize the importance of an individual approach when choosing antifungal agents to control each specific type of pathogen. Differences in the sensitivity of strains to fungicides may be due to specific features of cell walls, as well as the ability of fungi to produce enzymes that destroy antifungal compounds.

The results of this study are of practical importance for the development of strategies for protecting sea buckthorn from fungal pathogens. The detection of *Aureobasidium pullulans, Didymella glomerata* and *Epicoccum nigrum* as pathogens of this crop emphasizes the need to monitor the phytosanitary condition of sea buckthorn plantings. It is important to take into account the potential participation of these fungi in mixed infections, which can complicate the diagnosis and control of diseases.

The obtained data on the sensitivity of the isolated pathogens to antifungals can be used to justify the use of fungicides in protecting sea buckthorn plantations. For example, terbinafine and clotrimazole can be recommended to control *Aureobasidium pullulans*. At the same time, a wider range of drugs, including ketoconazole, intraconazole and fluconazole, can effectively suppress *Didymella glomerata* and *Epicoccum nigrum*.

Thus, the study not only expands knowledge about sea buckthorn phytopathogens but also lays the foundation for the development of effective methods for combating fungal diseases of this crop, which can contribute to an increase in the yield and quality of sea buckthorn fruits, as well as a decrease in economic losses in agriculture.

## CONCLUSION

The study identified three strains of phytopathogenic fungi affecting sea buckthorn: *Aureobasidium pullulans, Didymella glomerata* and *Epicoccum nigrum*. For their identification, pure cultures were isolated on a nutrient medium, biochemical analysis and microscopy were carried out, and molecular genetic methods were used, including DNA extraction, amplification (PCR) and sequencing. The pathogenicity of the strains was confirmed through a pathological test conducted on young sea buckthorn seedlings by artificial infection.

Analysis of resistance to antifungal drugs revealed a differentiated reaction of the strains to various groups of antifungal agents. The data obtained can be used to develop effective strategies for protecting sea buckthorn from fungal infections, including recommendations for using fungicides and selecting the most effective drugs for pathogen control.

## AUTHOR CONTRIBUTIONS

VK, BD, AS and KhO: conceptualization, study design, data validation, and writing–original draft preparation. KhO, BD, and AS: collection of biological material BD, and AS: data curation and laboratory experiments. VK, BD, AS and KhO: data analysis. VK: funding acquisition. VK, BD, AS and KhO: revising and final approval of the manuscript. All authors contributed to the article and approved the submitted version.

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## CONFLICT OF INTEREST

There are no conflicts of interest to declare.



## LITERATURE

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# ИДЕНТИФИКАЦИЯ, ПАТОГЕННОСТЬ И АНТИМИКОТИЧЕСКАЯ ЧУВСТВИТЕЛЬНОСТЬ ГРИБКОВЫХ ПАТОГЕНОВ ОБЛЕПИХИ

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**АБСТРАКТ**

В рамках данного исследования проведена комплексная идентификация и характеристика грибковых патогенов, поражающих облепиху (*Hippophae rhamnoides*). С использованием методов микробиологии, биохимического анализа, молекулярно-генетической диагностики (ПЦР и секвенирование ДНК) и патогенетического тестирования были выявлены три вида фитопатогенных грибов: *Aureobasidium pullulans*, *Didymella glomerata* и *Epicoccum nigrum*. Экспериментально подтверждена их патогенность в отношении облепихи. Оценка чувствительности изолятов к шести антимикотическим препаратам (тербинафин, нистатин, кетоконазол, клотримазол, интраконазол и флуконазол) выявила видоспецифические различия в восприимчивости к фунгицидному воздействию. Полученные данные расширяют представления об этиологии грибковых заболеваний облепихи и могут быть использованы для разработки эффективных стратегий фитосанитарного контроля и защиты растений.

**Ключевые слова:** *Hippophae rhamnoides, Aureobasidium pullulans, Didymella glomerata, Epicoccum nigrum, идентификация, патогенность, антимикотическая чувствительность.*

# ШЫРҒАНАҚТАР ҚОЗДЫРҒЫШТАРЫН ТҮРІН, ПАТОГЕНДІЛІГІН ЖӘНЕ САҢЫРАУҚҰЛАҚҚА ҚАРСЫ СЕЗІМТАЛДЫҒЫН АНЫҚТАУ

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**АҢДАТПАСЫ**

Бұл зерттеу теңіз шырғанағына (Hippophae rhamnoides) әсер ететін саңырауқұлақ патогендерін жан-жақты анықтауға және сипаттауға бағытталған. Микробиологиялық әдістерді, биохимиялық талдауды, молекулалық-генетикалық диагностиканы (ПТР және ДНҚ секвенциясы) және патогенетикалық тестілеуді қолдана отырып, фитопатогенді саңырауқұлақтардың үш түрі анықталды: Aureobasidium pullulans, Didymella glomerata және Epicoccum nigrum. Олардың теңіз шырғанағына патогенділігі тәжірибе жүзінде расталды. Алты зеңге қарсы препараттарға (тербинафин, нистатин, кетоконазол, клотримазол, итраконазол және флуконазол) изоляттардың сезімталдығын бағалау фунгицидтік әсерге сезімталдықта түрге тән айырмашылықтарды анықтады. Алынған деректер теңіз шырғанақтарының саңырауқұлақ ауруларының этиологиясы туралы түсінігімізді кеңейтеді және фитосанитарлық бақылау мен өсімдіктерді қорғаудың тиімді стратегияларын әзірлеу үшін пайдаланылуы мүмкін.

**Түйін сөздер**: *Hippophae rhamnoides, Aureobasidium pullulans, Didymella glomerata, Epicoccum nigrum, идентификациясы, патогенділігі, саңырауқұлаққа қарсы сезімталдығы.*