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REVIEW ARTICLE

## Detection Of The Microbiome And Mycotoxin-Producing Micromycetes In Dried Grapes (Raisins) And Vineyards

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### ABSTRACT

This study investigates the microbiome of dried grapes (White Kishmish raisins) and vineyards in Uzbekistan, focusing on mycotoxin-producing micromycetes. Microbiological analyses identified *Aspergillus*, *Fusarium*, and *Uncinula* as dominant genera. These micromycetes synthesize secondary metabolites, including hazardous mycotoxins such as aflatoxins, ochratoxins, and fumonisins, which pose serious health risks to humans and animals. The research involved microbiological and genetic analyses, including MALDI-TOF and 16S rRNA sequencing, to accurately identify fungal strains. The study confirmed the presence of *Aspergillus carbonarius* UZB-1, *Alternaria tenuissima* UZB-5, *Fusarium equiseti* UZB-6, and *Uncinula necator* UZB-3, all of which were registered in the NCBI database. The results highlight the significant contamination of stored raisins and vineyard soils, emphasizing the necessity of developing biological control strategies. Chemical treatments are not recommended for organic grape production. Instead, biopreparations based on antagonistic microorganisms should be applied to prevent fungal proliferation. The findings contribute to the development of eco-friendly solutions for grape and raisin production, ensuring food safety and reducing economic losses due to fungal contamination.

**Keywords:** White raisins”, *Aspergillus*, *Fusarium*, *Uncinula*, micromycetes, microbiome.

### INTRODUCTION

Mycotoxins are secondary metabolites of micromycetes that are classified as carcinogenic, genotoxic, teratogenic, dermatotoxic, nephrotoxic, and hepatotoxic. Currently, due to the negative consequences of mycotoxins in industry, veterinary medicine, feed production, and fruit drying, economic losses are being incurred. Therefore, preventing the formation of mycotoxins in food and feed and eliminating the effects of existing mycotoxin contamination require new scientific research.

Mycotoxins are primarily toxic secondary metabolites produced by micromycetes belonging to the genera *Fusarium*, *Aspergillus*, and *Penicillium* (Heidler and

Schatzmayr, 2003; Aslam *et al.*, 2015). Studies have identified that 300 metabolites produced by these genera are potentially toxic to animals and humans. Aflatoxins, ochratoxins, fumonisins, deoxynivalenol, zearalenone, trichothecenes, and patulin have been proven to exhibit immunotoxic, carcinogenic, nephrotoxic, hepatotoxic, neurotoxic, and teratogenic activity in humans and animals (Mis’hra and Das, 2003). Among the most hazardous mycotoxins for crops are aflatoxins and ochratoxins (OTA). The impact of these mycotoxin classes on human and animal health has been extensively studied worldwide.

Due to the harmful effects of mycotoxins, it is necessary to develop strategies to prevent the proliferation of toxic micromycetes and detoxify food and feed. These strategies include preventing mycotoxin contamination, neutralizing existing mycotoxins in food and feed, and more. Many physical and chemical detoxification methods have been tested to eliminate mycotoxins; however, their effectiveness is low, and they do not ensure complete safety (Aurangzeb *et al.*, 2014; Turaeva *et al.*, 2021).

Before harvest, grape bunches can rot and deteriorate due to various microfungi species such as *Alternaria* spp., *Aspergillus* spp., *Botrytis cinerea*, *Cladosporium* spp., *Eurotium* spp., *Penicillium* spp., and *Rhizopus* spp. These species are considered the primary natural contaminants of grapes (Turaeva *et al.*, 2020; Turaeva *et al.*, 2023a). Studies conducted in Argentina have demonstrated that *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* are the dominant microfungi in harvested grapes. In Spain, the most frequently detected micromycetes in cultivated grapes belong to the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Rhizopus*, and *Penicillium* (Turaeva *et al.*, 2023b; Usman *et al.*, 2024).

Micromycetes can appear at various stages of plant development in vineyards. As a result of frequent contamination of grapes with microfungi, mycotoxins are formed (Bakhora *et al.*, 2024). These mycotoxins are secondary metabolites produced by only a few species of micromycetes. Mycotoxins can cause various harmful effects in humans, ranging from allergic reactions to immunosuppression and cancer. Different micromycete species synthesize mycotoxins that degrade the quality of food products (Valero *et al.*, 2005).

Mycotoxin contamination poses a significant threat to human health. The primary micromycetes responsible for mycotoxin production belong to the genera *Aspergillus*, *Fusarium*, and *Penicillium*. These micromycetes synthesize mycotoxins such as aflatoxins, ochratoxins, fumonisins, and patulin. Aflatoxin poisoning often occurs through food consumption, but the most toxic aflatoxin compound, B1, can also be absorbed through the skin (Aydogdu and Gucer, 2009).

*Aspergillus* species are commonly found in soil, decaying plant residues, and unharvested crops affected by microbial spoilage. However, when favorable conditions arise, they can colonize all types of organic substrates. The optimal conditions for aflatoxin production by *Aspergillus* species include high humidity (at least 7%) and temperatures ranging from 55°F to 104°F (13°C to

40°C). The ideal temperature range is 27°C to 30°C (81°F to 86°F). Aflatoxins can contaminate crops both before and after harvest.

Four primary aflatoxin groups pose a risk to human health: G2, G1, B2, and B1. Unlike the micromycetes that produce them, aflatoxins are difficult to detect because they are colorless and odorless. Chemically, aflatoxins are highly stable and cannot be easily removed from food products, even when exposed to high temperatures during food processing. Aflatoxins are genotoxic carcinogens. According to the World Health Organization (WHO), aflatoxin B1 is classified as a Group 1 carcinogen. Based on this classification, there is sufficient evidence to conclude that long-term consumption of aflatoxins can lead to cancer in humans. Aflatoxin B1 is the most potent genotoxic carcinogen, followed by G1, B2, and G2. Even small doses of aflatoxins can cause chronic aflatoxicosis (Boonen *et al.*, 2012).

Ochratoxins are a group of mycotoxins synthesized by certain species of micromycetes belonging to the genera *Aspergillus* and *Penicillium*. Among *Penicillium* species, the primary producers of ochratoxins are *Penicillium verrucosum*, *Aspergillus ochraceus*, and some other species, including *A. carbonarius* and *A. niger*. All ochratoxins exhibit strong nephrotoxicity and rapidly bind to proteins in the bloodstream. The most widespread and toxic ochratoxin is ochratoxin A (OTA) (Voth-Gaeddert *et al.*, 2018).

Ochratoxin A has been detected in grapes and dried grape products. It has also been identified in grape juice, must, and wine. The contamination of grapes with ochratoxin A begins in vineyards, and its presence in grape-derived products has raised significant concern in many countries (Yelinov, 1989; Bragulat *et al.*, 2008). The contamination of grapes and grape products with mycotoxins (particularly ochratoxins) is primarily caused by micromycetes belonging to the *Aspergillus* genus.

To minimize the risk of ochratoxin A contamination in vineyards, it is necessary to reduce the presence of phytopathogenic micromycetes in the soil and develop biological control measures to prevent grape damage (Aydogdu and Gucer, 2009).

Among all food products, dried fruits are widely consumed worldwide due to their taste and beneficial properties. Dried fruits are a source of vitamins (A, B1, B2, B3, B5, B6) and minerals (iron, magnesium, potassium, calcium, phosphorus). The caloric content of dried fruits is approximately 250 kcal per 100 g. However, during

storage and sale, food products undergo various microbiological, biochemical, and enzymatic changes that lead to spoilage (Hocking *et al.*, 2007).

The microbiological spoilage of dried fruits results in the loss of their beneficial properties. Many food products serve as nutrient-rich environments that promote microbial growth. Under certain conditions, some microorganisms can alter the organoleptic properties of food products, leading to their spoilage.

It is crucial to develop cost-effective methods to prevent food contamination by mycotoxins and ensure food safety while minimizing potential losses. To achieve this, the microbiological characteristics of products should be examined, and the degree of contamination with micromycetes should be accurately determined.

## MATERIALS AND METHODS

### Sampling and Microbiological Analysis of Raisins and Vineyards:

Samples were taken from raisins made from White Kishmish grapes stored for export. The collected samples were cleaned with 3% hydrogen peroxide, alcohol, and distilled water. A 1-gram portion of plant samples was weighed and ground. The prepared samples were placed into sterile flasks containing 50 mL of 0.85% physiological saline solution and shaken for 30 minutes. A 100  $\mu$ L suspension prepared from the samples was diluted within a range of  $10^3$ - $10^8$  and then plated onto MRS, Czapek, and PDA agar media with the addition of 0.0025% crystal violet indicator. The incubation was conducted in a thermostat at 37°C for 48-72 hours. During the study, microorganism colonies grown on agar media were purified by repeated streaking.

To identify the types of micromycetes, XSP-136 B and OLYMPUS BX 41 light microscopes (with 400x magnification) were used. Additionally, vineyards infected with phytopathogens were selected, and samples were taken from diseased plants and soil. Standard methods accepted in soil microbiology were used to analyze soil samples microbiologically. To determine the microorganism groups in vineyard soils, soil samples were collected from a depth of 0-10 cm.

For microbiological analysis, 1 gram of each soil sample was weighed and mixed with 9 mL of sterile water, followed by shaking in a shaker (IKA KS 130 BASIC) for 20 minutes. A 1 mL aliquot was taken from the prepared suspension and added to a sterile test tube containing 9 mL of sterile water. This dilution process was carried

out sequentially. From the final suspension, 1 mL was taken and inoculated onto specialized agar media in Petri dishes in triplicate. Samples were also collected from infected plant parts using sterile laboratory equipment.

In the Samarkand region, samples were taken from the stem and root rhizosphere of the local White Kishmish grape variety. Stem samples were cleaned 10 times with 3% hydrogen peroxide, alcohol, and distilled water. The samples were then ground in the laboratory using glass instruments to rupture the cell walls and were inoculated under sterile conditions onto nutrient media. Additionally, samples were taken using the classical microbiological smear method, for which sterile cotton swabs were prepared and soaked in 0.9% physiological saline solution.

From the root rhizosphere, 1-gram samples were weighed and diluted in 5 mL of sterile water across 1-10 test tubes, with 0.5 mL transferred per step. Diluted samples (3rd-4th and 5th-6th dilutions) were inoculated onto nutrient media. Samples prepared for microbiological analysis were inoculated onto meat-peptone agar (MPA), potato-dextrose agar (PDA), oat agar (OA), Czapek agar, and Sabouraud agar, and then incubated in thermostats at temperatures ranging from 20°C to 38°C. Phytopathogenic micromycetes were isolated in pure culture by repeated subculturing onto nutrient media.

## RESULTS AND DISCUSSION

In our research, the microbiome of stored raisins and grape plants was studied. According to the research results, it was impossible to count the CFU (Colony Forming Units) of microorganisms in the collected samples. The level of contamination was determined to be complete and 100%. In each experimental variant, it was found that micromycetes completely covered the surface of the Petri dishes (Figure 1). The micromycetes belonging to the genera *Aspergillus*, *Fusarium*, and *Uncinula* were identified as dominant.

For environmentally friendly grape cultivation, it is recommended to:

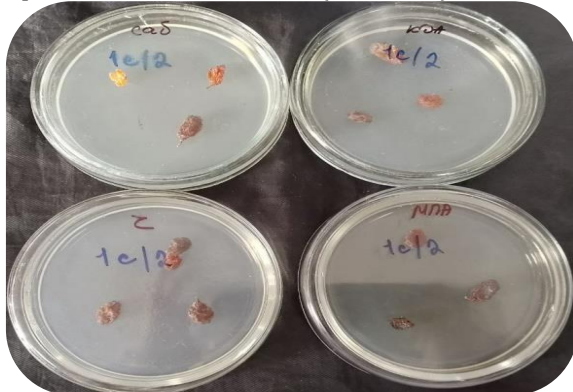
- Initially apply microbiological treatment to the soil.
- Treat both the plant and the soil with microbial preparations that have a high antifungal activity against *Aspergillus*, *Fusarium*, and *Uncinula* species, ensuring a multifunctional impact mechanism.



Samples taken from the initial (untreated) stored raisins



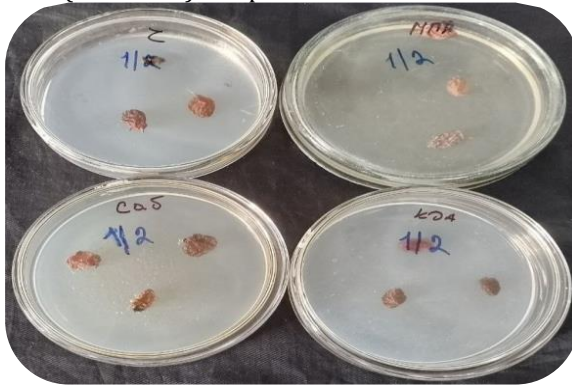
Experimental variant (3 days)



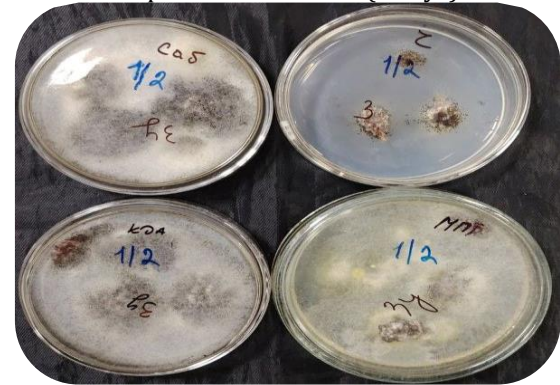
Initial (untreated) samples taken from stored raisins



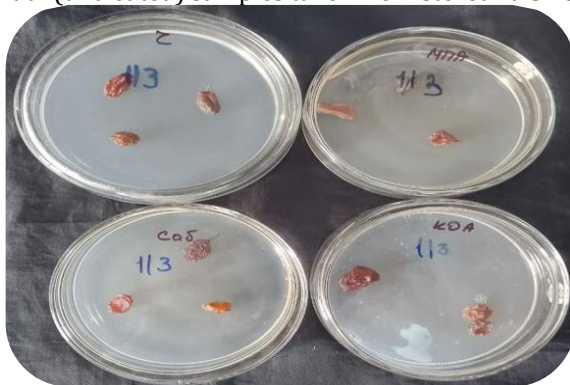
Experimental variant (3 days)



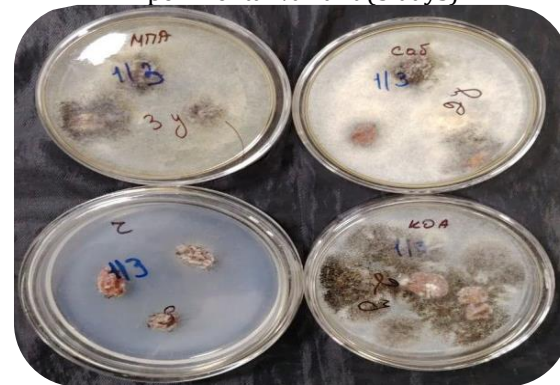
Initial (untreated) samples taken from stored raisins



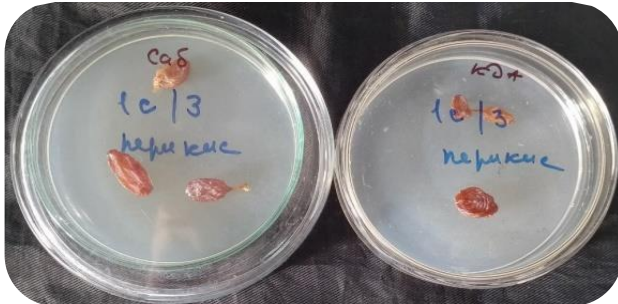
Experimental variant (3 days)



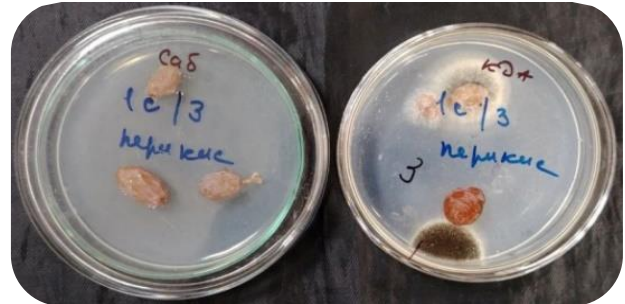
Initial (untreated) samples taken from stored raisins



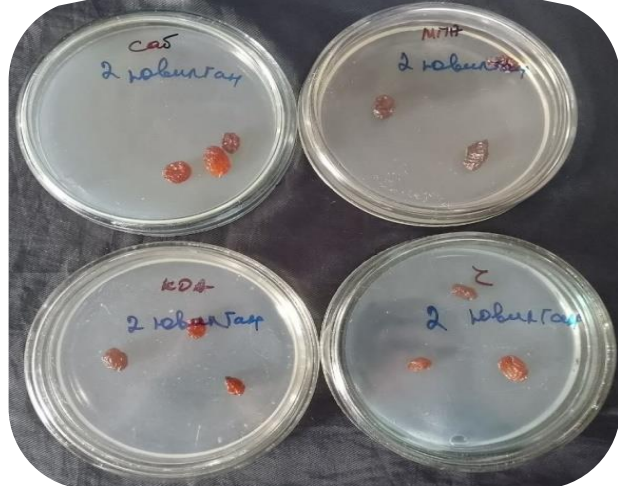
Experimental variant (3 days)



Initial (untreated) stored raisins treated with H<sub>2</sub>O<sub>2</sub>



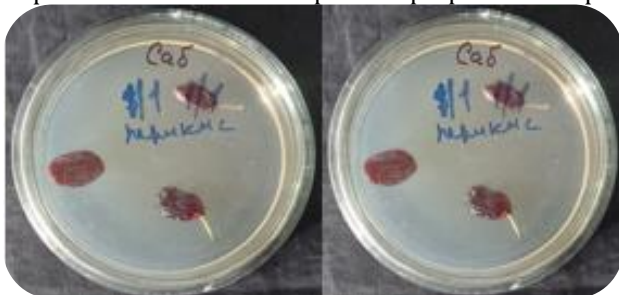
Experimental variant (3 days)



Samples taken from the final product prepared for export



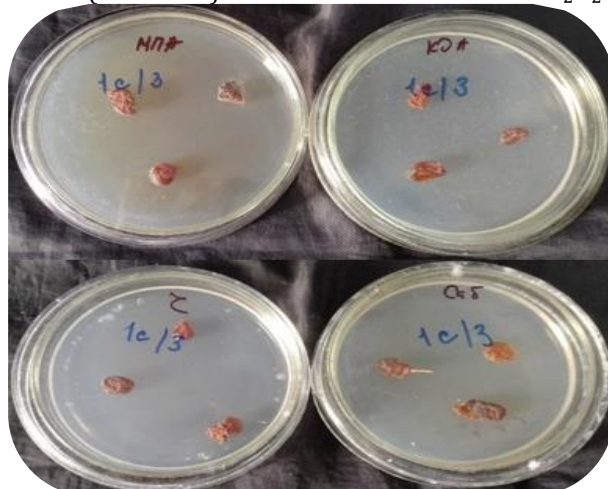
Experimental variant (3 days)



Initial (untreated) reserve raisins treated with H<sub>2</sub>O<sub>2</sub>



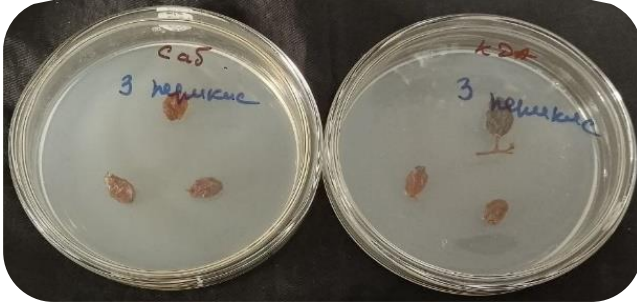
Experimental variant (3 days)



Initial (untreated) samples taken from stored raisins



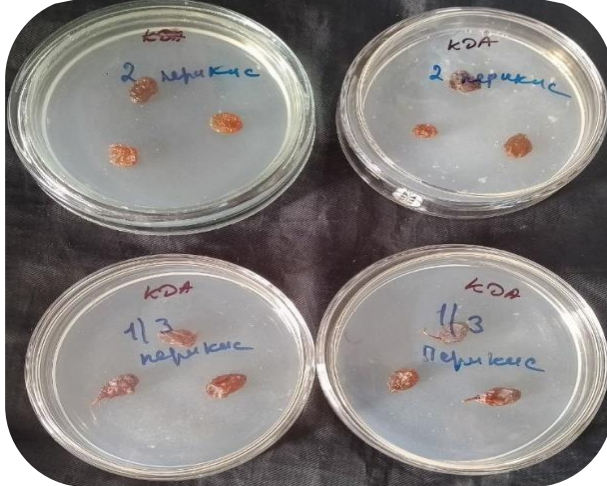
Experimental variant (3 days)



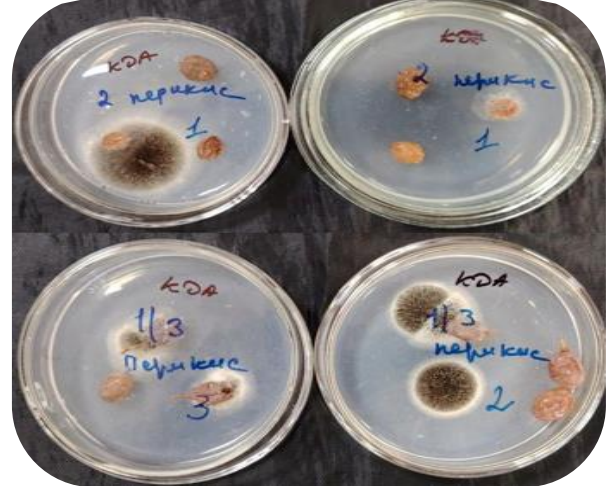
Samples taken from fumigated raisins



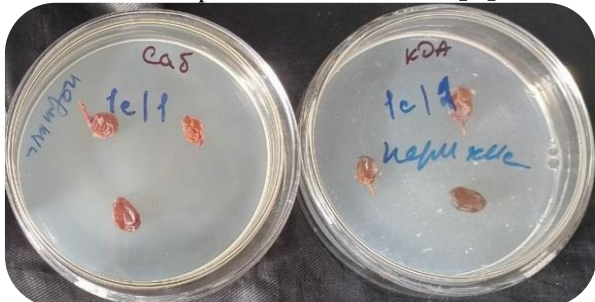
Experimental variant (3 days)



Initial (untreated) reserve raisins and samples from the finished product treated with H<sub>2</sub>O<sub>2</sub>.



Experimental variant (3 days).



Initial (untreated) stored raisins treated with H<sub>2</sub>O<sub>2</sub>.



Experimental variant (3 days).



Initial (untreated) stored raisins treated with H<sub>2</sub>O<sub>2</sub>.



Experimental variant (3 days).



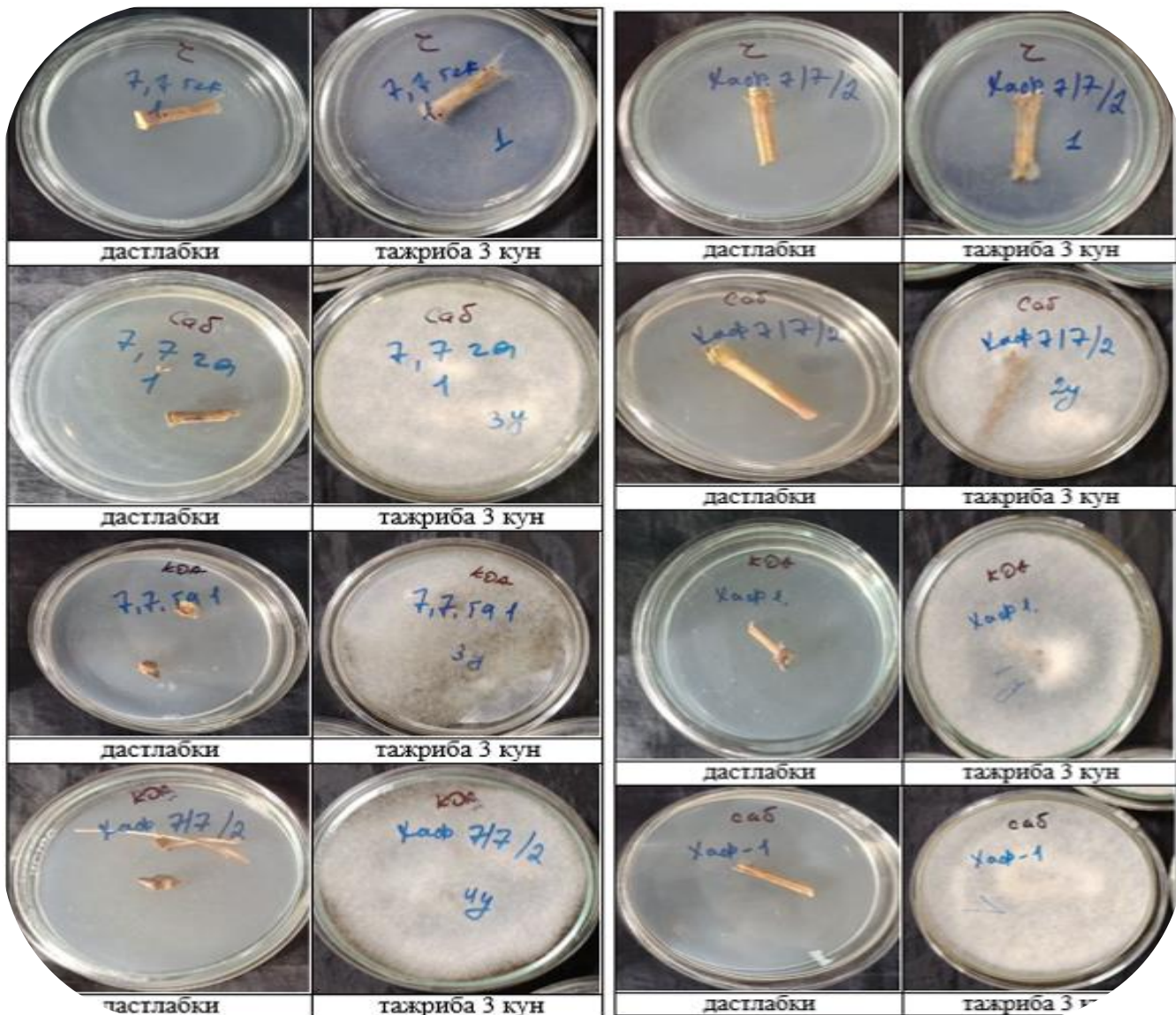
Initial (untreated) sample taken from stored raisins.

Experimental variant (3 days).

Figure 1. Microbiological analysis results of stored raisin samples intended for export.

During the research, grapevine cuttings grown in a symbiotic state were examined, and samples were taken from 25 plants in each of the 8 land plots. The samples were cultured on microbiological nutrient media using standard methods. The experimental

samples were placed in incubators at temperatures of 20°C and 37°C for three days, and the results were recorded after five days. Based on the obtained results, the plant infection coefficient was determined to be 95%.



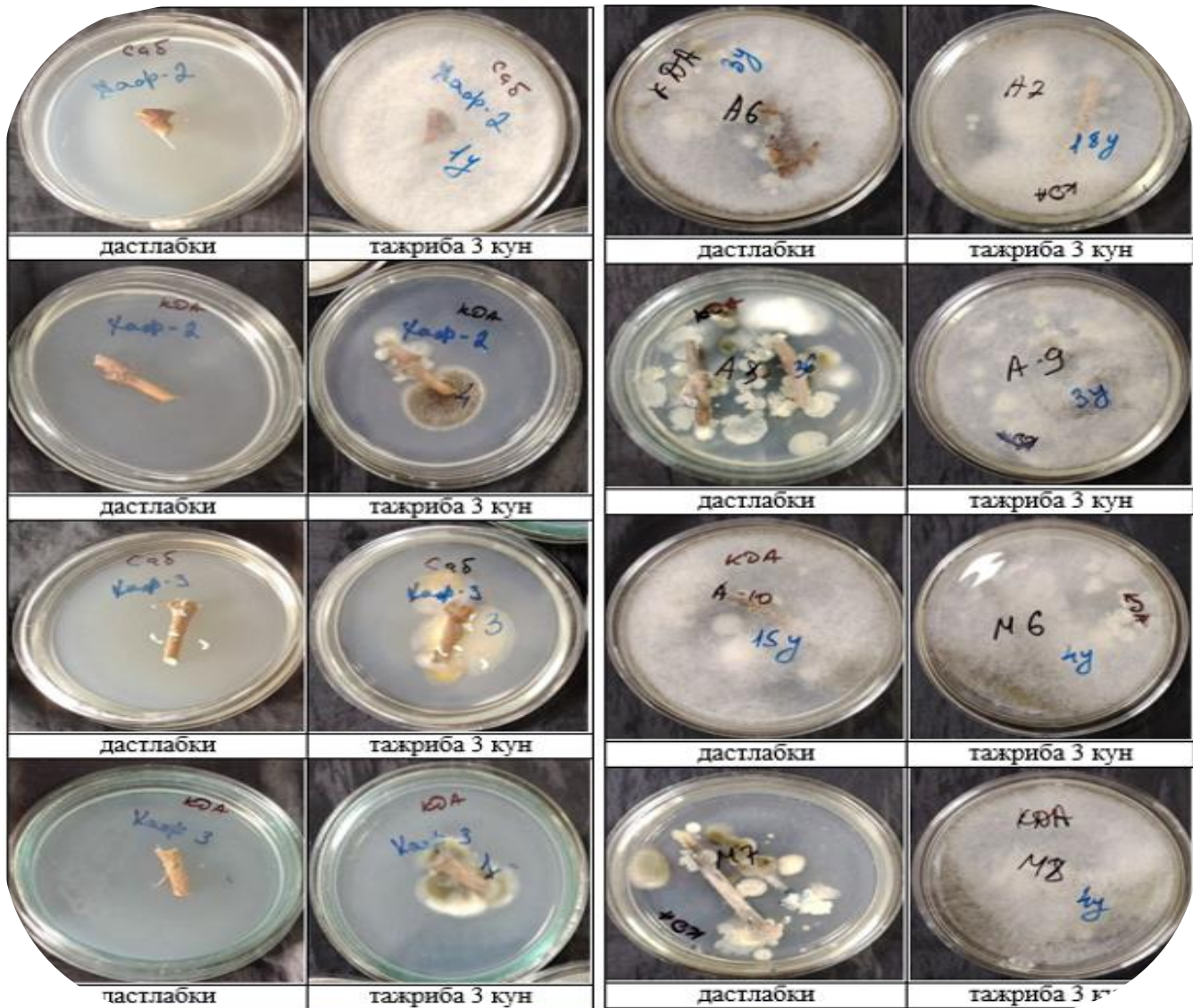


Figure 2. Microbiological analysis results of samples taken from grapevine cuttings in vineyards of the Samarkand region. Colonies of micromycetes causing grapevine diseases were found to completely cover the surface of the Petri dish.

- **Anthraco**se: A disease caused by micromycetes that affects grapevine shoots, leaves, inflorescences, and grape berries.
- **Powdery rot**: A disease resulting from grapevine infection by micromycetes, affecting its green organs throughout the entire vegetation period.
- **Oidium**: A fungal disease caused by microfungi that affects grape clusters, buds, and shoots throughout the entire vegetation period.
- **Grapevine necrosis**: A highly dangerous disease caused by various infections and unfavorable environmental factors, leading to the death of certain

tissues or plant organelles.

- **Chlorosis**: A grapevine disease that disrupts the process of chlorophyll formation in leaves, manifested by a color change from green to pale green, then yellow-green, and eventually deep yellow. According to this state standard, all the aforementioned diseases are caused by phytopathogenic microorganisms that spread from the soil to the air, the surrounding environment, and vineyard plantations. Our research revealed that when samples taken from grapevine shoots were cultured on nutrient media, phytopathogenic fungi were detected. These pathogens can be transmitted through water, contaminate soil and crops, and may also spread when untreated or non-disinfected organic fertilizers are

applied. Additionally, vineyard soil may already be contaminated with phytopathogenic microorganisms. During our research, the *Actinomucor sp.*

phytopathogenic fungus, responsible for causing powdery rot disease, was identified.

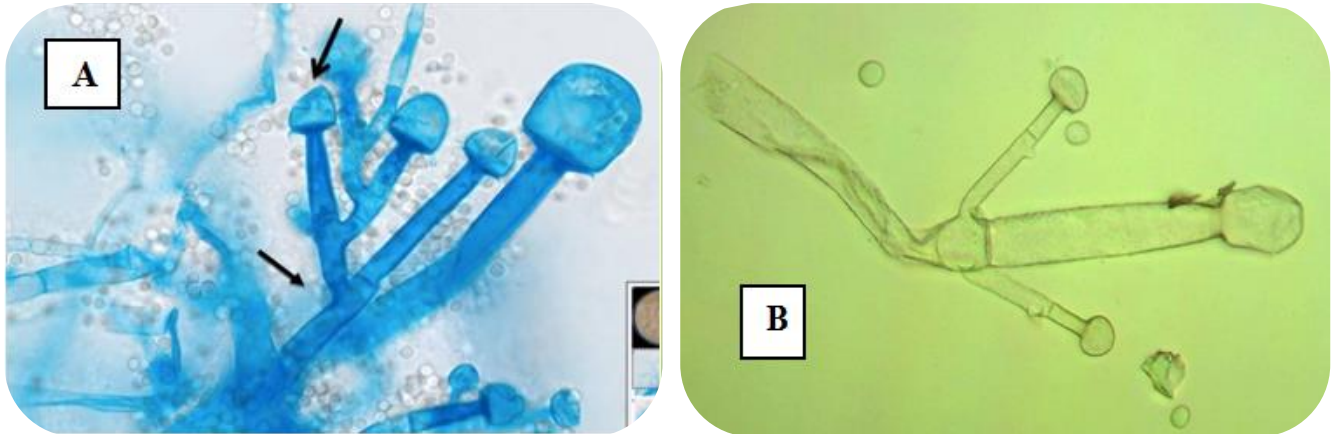


Figure 4. Microscopic appearance of *Actinomucor sp.* micromycete (A – data from scientific literature, B – isolated from a grapevine sample).

*Uncinula sp.* micromycete is a pathogenic microorganism primarily affecting soft berry-producing crops. It develops on the leaves, stems, and roots of plants, causing damage. According to the research results, *Uncinula sp.* micromycete was identified in the experimental variant (Figure 5).

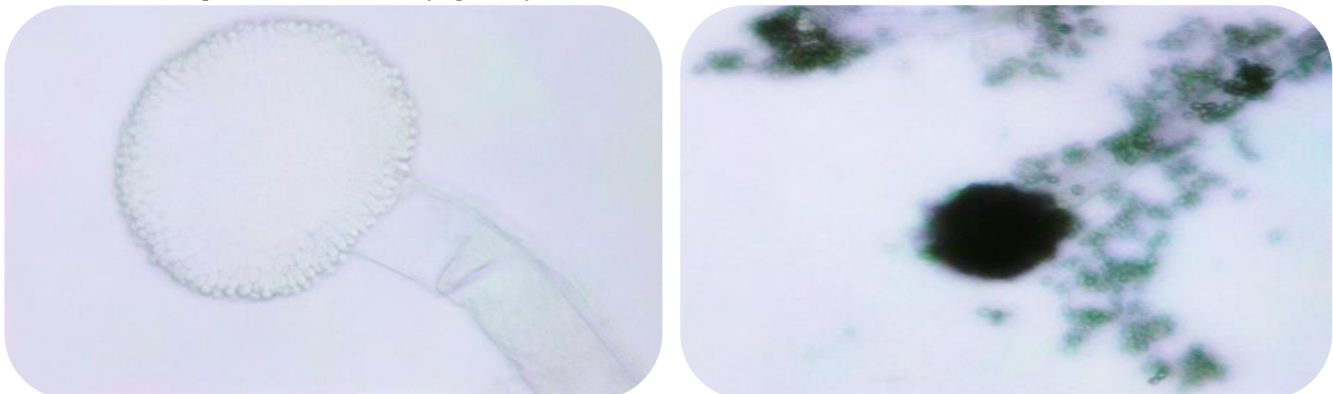


Figure 5. Microscopic view of the phytopathogenic fungus *Uncinula sp.* identified in samples taken from grapevine shoots. *Alternaria* fungus is considered a highly dangerous phytopathogen for grape plantations. It develops on the leaves of the plant, affecting the leaf blade and stem, leading to the wilting of growing plants and causing *Alternaria* disease. In the experimental variants, the phytopathogenic *Alternaria sp.* fungus was identified (Figure 5).

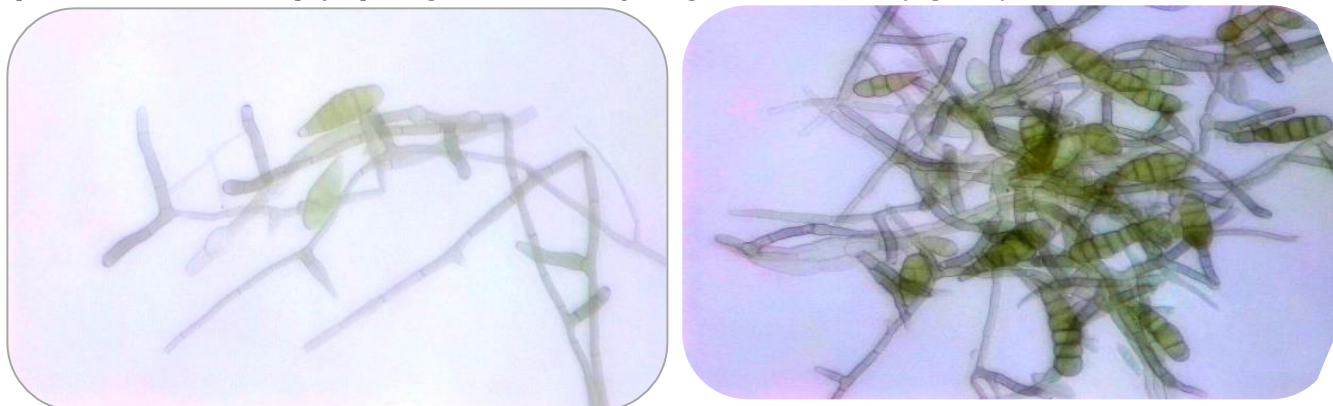


Figure 6. Microscopic view of *Alternaria sp.* phytopathogenic fungal spores identified in experiments conducted on grape samples.

This phytopathogenic microorganism is considered highly dangerous due to its ability to produce potent toxic mycotoxins, such as Aflatoxin and Fumonisin, which can lead to plant destruction. Scientific studies conducted by researchers worldwide have provided data on the mycotoxin-producing properties of phytopathogenic fungi, their isolation, and their hazardous characteristics. Research conducted at the Institute of Microbiology of the Academy of Sciences of Uzbekistan has shown that the

*Aspergillus sp.* micromycete found in grapevine and stored raisin samples synthesizes the dangerous mycotoxin aflatoxin. This fungus develops in soil, various plant residues, and untreated organic fertilizers. It can enter the human body through contaminated plant products and cause serious health issues, including allergies. In our research, a *Aspergillus sp.* phytopathogenic fungal strain was identified in the analyzed grape samples (Figure 7).

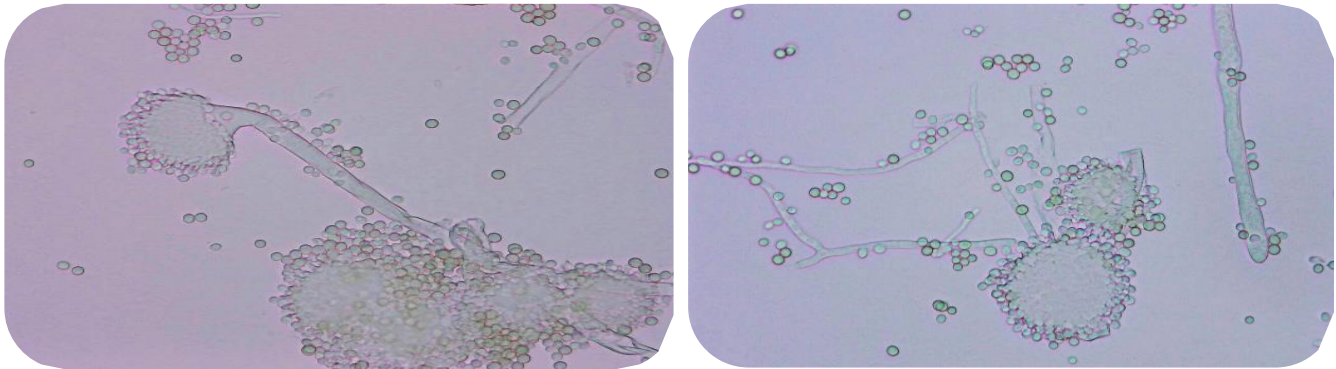


Figure 7. Microscopic appearance of the *Aspergillus sp.* phytopathogenic fungus identified in the experimental samples.

A wide variety of *Fusarium* fungi species are distributed worldwide and are recognized as a major group of phytopathogens posing a threat to all types of crops. In Uzbekistan, infections caused by *Fusarium* species are frequently observed across all regions. During our

research, *Fusarium sp.* phytopathogenic fungal strains were identified in samples collected from symbiotic grapevine plantations. These phytopathogenic fungi infect the plant's root system, stem, and leaves, causing gray rot, white rot, and fusariosis diseases.

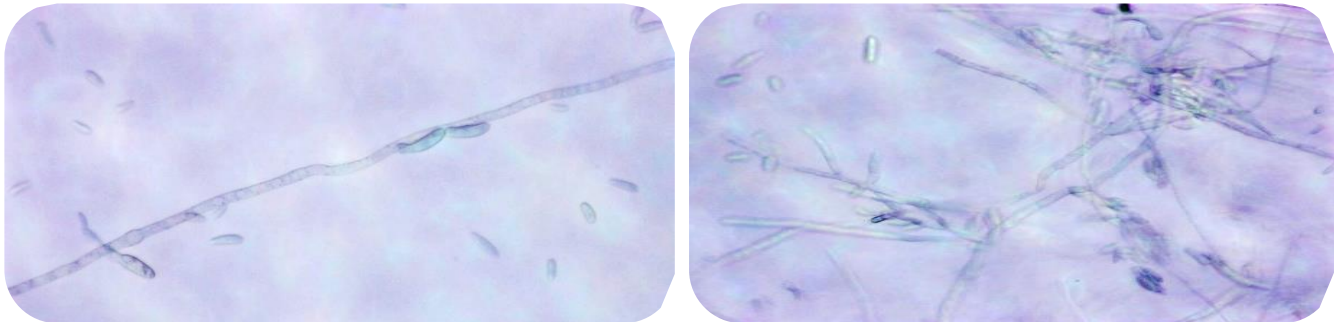


Figure 8. Microscopic appearance of *Fusarium sp.* phytopathogenic fungi.

This passage discusses the identification of fungal strains isolated from dried raisins and grapevine cuttings using the MALDI-TOF method and genetic identification based on 16S rRNA sequencing.

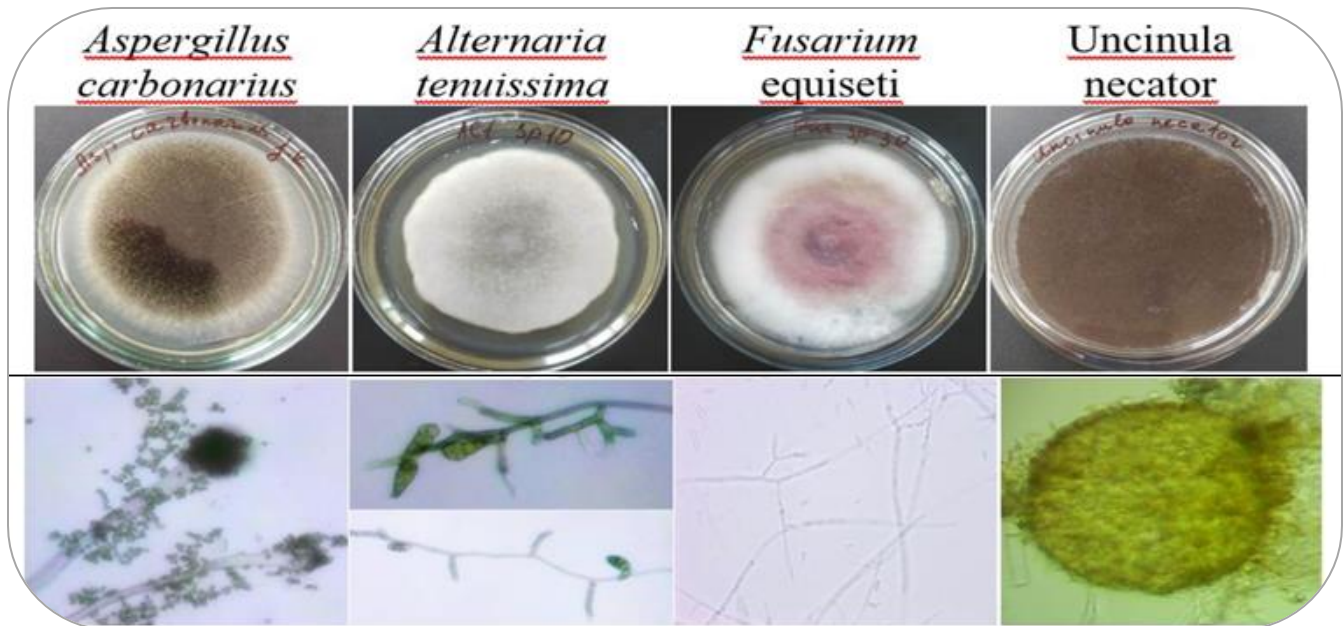
The *Aspergillus sp.* strain was identified as *Aspergillus carbonarius* UZB-1 based on its 16S rRNA sequence: TTCCGTAGGGTAACCTGCGGAAGGATCATTACCGAGTCCGGGTCTTTGGGCCAACCTCCACCCGTGTCTATTGTACCTGTTGCTTCGGCGGGCCC

This strain was registered in the NCBI (National Center for Biotechnology Information) database under accession number OQ632520. Phylogenetic analysis

revealed that its closest relative is *Aspergillus carbonarius* 081 (MF072667.1) with 99.82% similarity.

The *Alternaria sp.* strain was identified as *Alternaria tenuissima* UZB-5 based on its 16S rRNA sequence: TCGGTAGGTGAACCTGCGGAAGGATCATTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGTTACAGCCTTGCTGAATT

This strain was registered in the NCBI database under accession number OQ632521. Phylogenetic analysis determined that its closest relative is *Alternaria tenuissima* AT2 (AF314584.1) with 99.81% similarity.



9 Figure. Microscopic and giant colony morphology of *Aspergillus carbonarius*, *Alternaria tenuissima*, *Fusarium equiseti*, and *Uncinula necator* Fungal Strains (Magnified 400x).

This research identified and genetically characterized *Fusarium equiseti* UZB-6 and *Uncinula necator* UZB-3 fungal strains using 16S rRNA sequencing.

- *Fusarium equiseti* UZB-6 was identified based on its 16S rRNA sequence:

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TGGAAGTAAAAGTCGTAACAACGCTCCGTTGGTGAACCA
GCGGAGGGATCATTACCGAGTTTACAACCTCCAAACCCCT
GTGAACATACCTATCGT
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The strain was deposited in the NCBI database under accession number OQ632597. The closest genetic relative in GenBank is *Fusarium equiseti* ODF52 (EU016677.1), with 99.83% similarity.

- *Uncinula necator* UZB-3 was identified based on its 16S rRNA sequence:

```
ATTACAGAGCGAGAGGCTCAGCCATGACGGTAGTCGTGTG
CTGGGTCGACCCTCCACCCGTGCCGATATGTATTTGTTG
CTTTGGCGGGCCGGGT
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The strain was deposited in the NCBI database under accession number OQ632523. The closest genetic relative in GenBank is *Uncinula necator* (AF073346.1), with 99.83% similarity.

During the study, the microbiome of white Kishmish raisins and grape samples cultivated and stored in Uzbekistan was thoroughly analyzed. Microbiological tests identified *Aspergillus*, *Fusarium*, and *Uncinula* genera as dominant micromycetes. These fungi are known to synthesize aflatoxins, ochratoxins, and fumonisins—highly hazardous mycotoxins that pose

serious risks to human and animal health (Heidler and Schatzmayr, 2003; Mis'hra and Das, 2003).

The identification of mycotoxin-producing fungi such as *Aspergillus carbonarius*, *Alternaria tenuissima*, *Fusarium equiseti*, and *Uncinula necator* using MALDI-TOF and 16S rRNA gene sequencing confirms the real threat posed by these pathogens. Among them, aflatoxin B1 is the most potent genotoxic and carcinogenic mycotoxin, classified by the World Health Organization (WHO) as a Group 1 carcinogen (Valero *et al.*, 2005).

The raisins and vineyard samples collected in this study showed 100% contamination, as observed by the full coverage of Petri dish surfaces with fungal growth. This high contamination level reflects the widespread presence of micromycetes. Similar findings have been reported by Valero *et al.* (2005), who documented the prevalence of ochratoxin A-producing *Aspergillus* spp. in sun-dried grapes.

Treating soil and plants with biopreparations containing antifungal antagonistic microorganisms is considered an ecologically sound strategy (Turaeva *et al.*, 2020). Chemical treatments, by contrast, are discouraged in organic agriculture due to their negative impact on soil microbiota. This aligns with the findings of Turaeva *et al.* (2023), who reported the isolation of phytopathogens such as *Fusarium*, *Oidium*, and *Alternaria* from vineyard soils and plants.

The detection of aflatoxin- and fumonisin-producing

fungi in this study emphasizes the importance of ensuring both food safety and compliance with international export standards for grape products. Mycotoxins such as OTA and aflatoxins are strictly regulated in international trade, and their presence can result in rejected shipments (Boonen *et al.*, 2012).

Moreover, the high level of contamination highlights the need for systematic monitoring of vineyard microbiomes. Micromycetes can persist not only in plant tissues but also in soil for extended periods and may become active again under favorable environmental conditions (Turaeva *et al.*, 2020; Bragulat *et al.*, 2008; Usman *et al.*, 2025).

### CONCLUSION

The study confirms that grape cultivation and raisin storage in Uzbekistan are highly susceptible to contamination by mycotoxin-producing micromycetes. Six genera of phytopathogenic fungi were identified, with *Aspergillus*, *Fusarium*, and *Ucinula* being dominant. The presence of toxic mycotoxins such as aflatoxins and fumonisins highlights the significant risks associated with fungal contamination.

To mitigate these risks, microbiological treatments for soil and plants are recommended. The use of biopreparations containing antagonistic microorganisms can effectively suppress fungal growth while maintaining organic production standards. Chemical treatments should be avoided, as they compromise environmental sustainability and food safety.

Additionally, long-term monitoring of vineyard microbiomes is essential since phytopathogenic fungi persist in soil and plant residues for extended periods. Future research should focus on refining biological control strategies and improving grapevine resistance to fungal infections. Implementing these measures will contribute to the sustainable production of high-quality, contamination-free raisins and grapes while ensuring human health and minimizing economic losses.

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### ETHICAL APPROVALS

As this is a review article based on previously published

literature, no ethical approval or informed consent was required.

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#### Contribution of Authors:

Baxora Turaeva	: Conducted trials and wrote a manuscript
Guzal Qutlieva	: Helped in manuscript write-up and research trials
Boburbek Koziyev	: Contributed to manuscript preparation and supported experimental research
Hulkar Kamolova	: Prepared all data tables, figures, and graphical representations
Amir Maxkamov	: Developed and formatted all tables, figures, and graphical data representations