Research Article

Phytopathogenic Micromycetes of *Vitis vinifera* Grown in Uzbekistan

Turaeva Bakhora Ismoilovna[1], Kutlieva Guzal Zhumaniyazovna[1], Zukhritdinova Nigora Yuldashevna[1], Soliev A'zam Bohodirovich[2], Mahkamov Amirjon Muxammad o'g'li[3]

[1]Institute of the Microbiology, Academy of Sciences of the Republic of Uzbekistan, Tashkent, UZBEKISTAN.
[2]Republican Scientific and Practical Center of Sports Medicine, Tashkent, UZBEKISTAN.
[3]Samarkand State University (Named after Sharof Rashidov), Samarkand, UZBEKISTAN.

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ABSTRACT

In the cultivation of grapes in Uzbekistan, phytopathogenic micromycetes cause dangerous diseases and cause the death of most of the crop. Therefore, it is important to identify the micromycetes that spread the disease from grapes. The article covers research aimed at studying micromycetes that spread disease in grapes grown in Uzbekistan. This makes it possible to grow ecologically clean products in viticulture and identify dangerous diseases in advance. The aim of our study was to study the microbiome of the soil of the grape growing area, infected young vines, leaves and fruits of grapes and to identify the phytopathogenic micromycetes that spread the disease. As part of our research, samples of infected branches, leaves, and fruits of "Kishmish" and "Rizamat" grape varieties grown in Urgut and Altyarik districts of Uzbekistan were brought. Phytopathogenic micromycetes were isolated from the samples. Cleaned 10 times with 3% hydrogen peroxide, alcohol and distilled water was grown meat-peptone agar (MPA), potato-dextrose agar (PDA), Oat agar (OA), Chapek nutrient media. Micromycete isolates belonging to the genera Fusarium, Alternaria, Penicillium and Aspergillus were detected in the soils of grape growing and in samples taken from infected grapes.

Keywords: *Vitis vinifera* L., *Fusarium, Penicillium, Alternaria alternata, Aspergillus niger,* Phytopathogenic micromycetes, Vine diseases.

Correspondence:
Dr. Turaeva Bakhora Ismoilovna
Republican Scientific and Practical Center of Sports Medicine, Tashkent, UZBEKISTAN.

Email id: jaloliddinshav-kiev1992@gmail.com

INTRODUCTION

At present, increasing the export potential of Uzbekistan and meeting the needs of the population in environmentally friendly products is one of the pressing issues. The study of the prospects for the development of environmentally friendly products has shown that, based on the requirements of the world market for harvests in farms specializing in viticulture, the competitiveness of products, the reduction of export opportunities and production volumes is the result of insufficient attention to the development of intensive technologies. Grape is one of the important agricultural crops of the Mediterranean. Major grape-growing countries include Italy, France, Spain, Turkey, Greece and Portugal. Turkey and Greece are the most important exporters of dried grapes. Many biotic and abiotic factors cause quantitative and qualitative losses of grape products every year. In particular, mycotoxins synthesized by phytopathogenic micromycetes as secondary metabolites, being extremely harmful to the grapes, cause serious diseases in it, which leads to the loss of crop.[1] Micromycetes have been isolated from *Alternaria alternata, Alternaria padwickii, Aspergillus flavus, Bipolaris hawaiiensis, Bipolaris oryzae, Curvilaria lunata, Curvilaria oryzae, Fusarium culmorum, Fusarium oxysporium, Melanospora zamiae, Rhizoctonia solani, and Verticillium sp* and reported to cause crop failure.[2] Bekale Be Ndong *et al.* determined the pathogenicity of the micromycete Alternaria towards plants and studied the morphological features of different conidiophores; conidias' difference or their chain formations.[3] "Rizamat" is an early and medium ripening grape variety. It is a medium-sized

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plant, with large fruits, weighing about 350-400 g per bunch. Fruits have cylindrical shapes, with a pink-coloured, firm and crispy taste. Fruits ripen in 52 days at the end of July. Productivity is 200-250×102 kg per hectare.

"Black kishmish" is a local variety of grape. A high vine, large branches, and fast-growing characterize it. It is not resistant to diseases and pests, easily affects by cercosporiosis powdery mildew. The grape bunches are large with an average weight of 172 g. The shape of the cylinder is conical, porous, sometimes dense, and black in color, the flesh is smooth, soft, and the seed inconspicuously small. The productivity is around 281×102 kg per hectare, with the highest harvest reaching up to 358.5 kg per hectare. The grapes contain many beneficial substances for human health and are the second most consumed fruit in the world after bananas. Even in the wine industry, when preparing high-quality, expensive wines that meet international standards, environmentally friendly biological products are used. The vine is a type of perennial plant that annually absorbs large amounts of nutrients from the soil and is infected with about 700 diseases. The most common dangerous diseases of this variety are alternations, gray rot, oidium and bacterial cancer. They severely damage the vine, spoil the quality of the product and reduce the yield by 25-70%.

The diseases such as oidium or powdery mildew (*Uncinula necator*), anthracnose (*Gloeosporium ampelophagum*), cercosporosis (*Cercospora vitis*), gray rot (*Botrytis cinerea*), black rot (*Phoma lenticularis*), cypress necrosis (*Rhacodiella vitis*), bacterial root collar cancer (*Bacterium tumefaciens*) is common in the vine.[4,5] Since the vine is a perennial plant, the plant-growing soil and its stem are the main sources of the disease[6] and the damage by phytopathogenic microorganisms is observed every year.[7] Phytopathogens that cause damage to plants include bacteria, viruses, mycromycetes and nematodes.[8] Pathogenic microorganisms can be classified as necrotrophs, biotrophs, and hemibiotrophic based on their growth cycle and distribution. Necrotrophic pathogens synthesize lytic enzymes and toxins that destroy living plant cells and feed on dead tissues. Biotrophic pathogens destroy the structure of living tissues, while hemibiotrophic pathogens start from the stage of biotrophic infection and proceed to the final necrotrophic stage, killing the host organism.[9] The most dangerous group of disease-spreading microorganisms in vine plantations are micromycetes, on the basis of which many grape diseases have been identified.[10] The fungus Alternaria alternata is a phytopathogen that parasitizes many agricultural crops. It may damage citrus fruits,[11] the surface of the leaf plate, the stem, develop on the leaves of plants and cause the plant to dry up, spreading the disease of Alternaria.[12] Based on the analysis of soil microflora, phytopathogenic micromycetes such as *Ascochyta fabae*, *Alternaria tenuissima*, *Cladosporium herbarum, Fusarium culmorum*, *F.oxysporum, F.solani*, *Ulocladium botrytis* have been identified.[13] In Mexico, up to 70% of plants are infected with phytopathogenic micromycetes. Up to 100 kg of toxic chemical pesticides per one ha are used annually to protect vineyards from phytopathogenic microorganisms that cause a number of harmful ecological inconveniences to the environment and human health. High humidity and temperature are favorable conditions for the development of phytopathogenic fungi belonging to *Alternariya* and *Aspergillus* species.[14] The regular use of chemicals against pathogens leads to an increase in the adaptability and viability of phytopathogens, as well as to a high level of damage to vineyards and complete loss of the crop.[9] Therefore, it is of scientific importance to identify phytopathogenic organisms that negatively affect the development of the vine and the preservation of grapes.

MATERIALS AND METHODS

The samples of local varieties of "Kishmish" and "Rizamat" grown in the highlands of Urgut District of the Samarkand Region and Altyarik District of the Fergana Region of Uzbekistan were brought. The samples were taken from stems, leaves, fruits and root rhizosphere of infected grape varieties. The stem, fruit and leaf samples were cleaned 10 times with 3% solution of hydrogen peroxide, alcohol and distilled water. In Mexico, up to 70% of plants are infected with phytopathogenic micromycetes. The infected plants developed 5 mm round, dark brown spots. Small pieces (5 mm 2) were cut from infected plant leaves, washed with, 70% ethanol for 30 sec in purified and sterilized water, planted on a potato dextrose medium with agar and incubated in a thermostat at 25°C. Morphological characteristics were studied and 3 isolates were isolated according to our research.[15]

In laboratory conditions, cell walls were disrupted using crushed glass and cultured into nutrient media under sterile conditions. The samples were taken using the classic microbiological (smearing) method, as well, for which sterile cotton swabs were prepared and placed in 0.9% physiological solution. 1g sample was taken from the root rhizosphere and diluted in 5 ml of sterile water by adding 0.5 ml to 1-10 test tubes.[16] The samples of 3-4 and 5-6 dilutions were planted in nutrient media.

The samples prepared for microbiological analyses were planted on meat-peptone agar (MPA), potato-dextrose agar (PDA), Oat agar (OA), Chapek nutrient media with agar and placed in a thermostat with temperature ranges from 20°C to 38°C[17] XSP-136 B and NLCD-307B (that can make X400 magnification) light microscopes were used to identify the types of microorganisms. In determining the types of microscopic fungi[18-21] identifiers were used.[22,23] Phytopathogenic fungi were also identified using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF) method in the sanitary-hygiene laboratory of the Ministry of Health of the Republic of Uzbekistan.[24] Studies are still being conducted to identify antagonists of isolated phytopathogenic micromycetes in order to develop biological control measures against phytopathogenic fungi.

RESULTS

The samples of 10-year "Kishmish" and "Rizamat" varieties grown in Urgut District of Samarkand Region and Altyarik District of Fergana Region were brought for the microbiological analyses (Figure 1). The obtained samples were cleaned with 3% solution of hydrogen peroxide, alcohol, distilled water, and planted in nutrient media under sterile conditions.

Figure 1: Samples of "Kishmish" and "Rizamat" grape varieties taken from infected 10-year vine plantation.

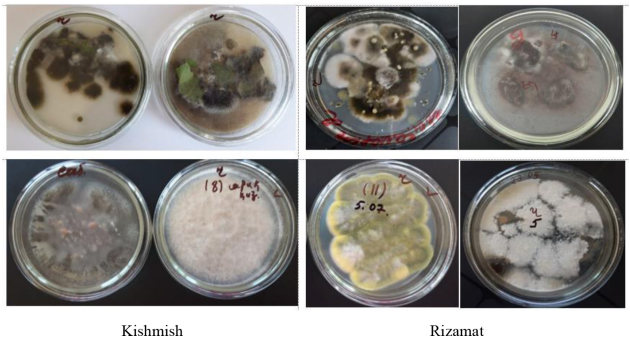

Figure 2: Microbiome of diseased «Kishmish» and «Rizamat» grape varieties grown in culture media

For microbiological analysis, according to the moist chamber method, phytopathogenic microorganisms were cultured on meat-peptone agar, starch-ammonium agar, potato dextrose agar, Mandal's agar and Chapek's agar (Figure 2). Then, the samples were put on the thermostats with a temperature range from 20°C to 38°C.

Clean micromycete colonies were obtained by replanting from the samples incubated for 4 days in agar Chapek nutrient medium at a temperature of 20°C. Morphological characteristics of pure colonies were studied. Based on the research, the conidia were found to be arranged in a chain at the top of the conidiophores with a spherical or spherical swollen shape, and an average diameter of 3.12 ± 2.24 μм, color ranging from dark brown to black, with uneven cell walls. Conidiophores with smooth walls and flat spherical spores were found to belong to *Aspergillus* Micheli ex Fries.

In our study, the morphological features of the micromycetes were found to have long chains, and conidiophores produce different types of spores from the ends of hyphae. The conidiophores were found to produce short-beaked brown-smooth, flat-surfaced conidia with an average diameter of 10 ± 2.10 μм and exhibit characteristics typical to the genus of *Alternariya* further identified as *Alternaria alternata* (Figure 3 B). The micromycete that forms fluffy white colonies on the 1st- 2nd day of the development, and turns to black on the 4th and 5th days, having the light purple color of the upper part has also been studied. The micromycete did not produce microconidia but produced large amounts of macroconidia. Macroconidia were thick having 2.5 to 5.0 μм and along with the size of 20 to 30 μм, and divided into 3 or 5 parts. They were also able to grow separately from phialides in isolation. Chlomydospores ranging from 9 to 14 μм in diameter,

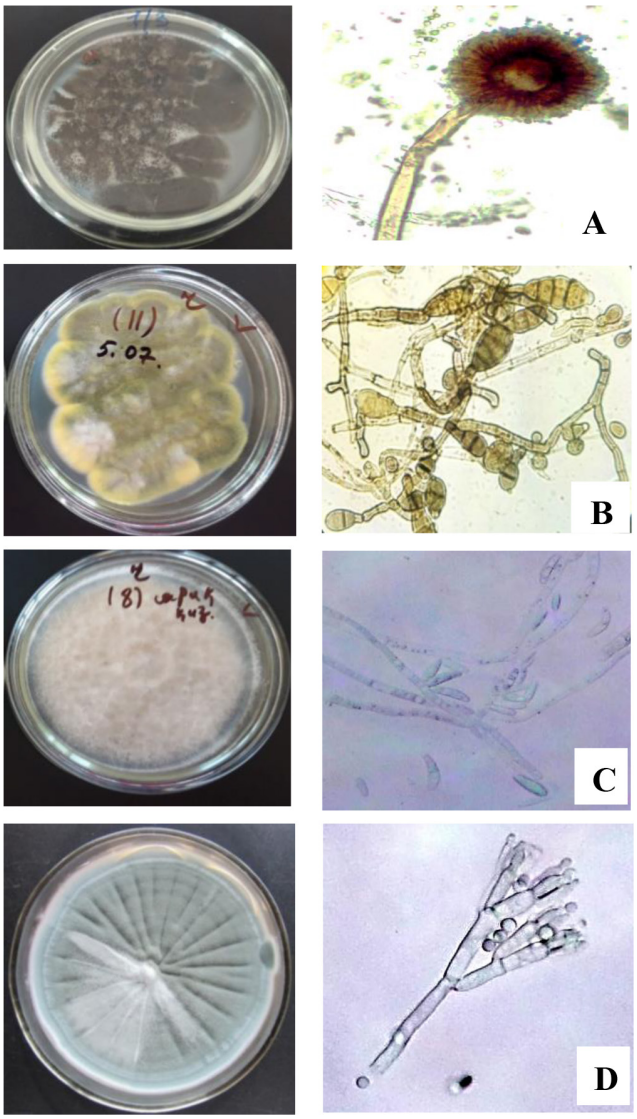

Figure 3: Colonies and microscopic appearance of micromycetes identified in experimental samples.

thick-walled, globose, singly, together or in chains were found in both hyphae and macroconidia. The viability of chlamydospores in macroconidia lasts longer. These morphological characteristics belong to the genus of *Fusarium* sp. fungi (Figure 3 C). In our research, the morphology of the purified micromycete colony in an agar medium was studied. White and gray, ranging from blue-green to dark green colonies have been formed. The spores with spherical, homogeneous, 200-500 μм long, more often 2-3, and four-layered, thin-walled, smooth-walled conidiophores have been formed. 7-14 μм in size, cylindrical, smooth, individual globular conidia have also been formed. These characteristics are features of micromycetes belonging to the genus of *Penicillium,* and therefore the isolated micromycete was identified as *Penicillium* sp. (Figure 3 D). Grapes are affected by a variety of biotic stress agents, including pathogenic fungi that cause severe disease symptoms in various plant organs. In all grape-growing regions, the most dangerous vine diseases are diseases affecting woody tissues, vine diseases, which cause significant yield loss and vine mortality and economic losses. In research, phytopathogenic micromycetes were isolated from samples taken from the soil of the grape growing area, infected young vine branches, leaves and grape fruits. It has chain-shaped conidia, the tip of the conidiophores is spherical or spherical, the average diameter is 3.12 ± 2.24 μm, from dark brown to black, the cell walls are uneven, the walls of the conidiophores are smooth, and the spores are spherical. In our studies, conidia with a straight elongated chain-like or flexible appearance, light or dark brown conidiophores, and different types of spores from conidiophore hyphal ends, conidiophores with a short beak, brown smooth, flat surface with an average diameter of 10 ± 2.10 μm, respectively. The causative Alternaria alternata fungal strain was isolated from infected grape leaves that produced the spot. Biosampling and microbiome studies of infected plants in grape plantations may allow early detection of diseases spread by phytopathogenic micromycetes and identification of biological control agents.

DISCUSSION

As stated in the above statement, the microbiome of plant organoids and fruits of 10-year grape (*Vitis vinifera* L.) plantations in the territory of Uzbekistan was studied. The studied grape varieties «Kishmish» and «Rizamat» are also used for consumption. Since grapes are a perennial plant, phytopathogenic microorganisms live in the soil and damage the next year's crop. In accordance with our research, phytopathogenic micromycetes (Alternaria alternata, Penicillium expansum, Botrytis cinerea, Verticillium dahliae and Fusarium oxysporum) were identified in the soil after harvest. Phytopathogenic Fusarium, Penicillium, Alternaria alternata and Aspergillus niger micromycetes that spread disease to grapes were isolated. These characteristics of a micromycete have been previously described for the mycelial fungus Aspergillus niger by other researchers.[18,25] According to our research results, the phytopathogenic micromycete *Aspergillus niger* has been developed in the experimental variants (Figure 3 A). According to a study done by Pakistani researchers, micromycete *Aspergillus niger* spreads powdery mildew disease (powdery mildew) in 12-17% of the grape (*Vitis vinifera*) in 5 regions of Pakistan. Those infected fruits of grapes covered with black or ink-colored spores were

incubated in a potato dextrose agar nutrient medium for 3 days at a temperature of 25°C. Phytopathogenic micromycetes spread to vineyards through water and air. They hibernate in perennial host plants and the remains of infected plants of previous years may damage new plants, and thus, completely destroy the whole crop [26]. Fungi belonging to the genera *Aspergillus, Penicillium* and *Alternaria* have been found to produce toxic substances such as ochratoxin A, fumonisins and patulin.

These complex diseases are caused by many taxonomically unrelated phytopathogenic fungi.[27] Due to the colonization of pathogenic micromycetes in grape leaf plate, stem and young branches, general withering and complete death of the plant is observed.[28] Phytopathogenic micromycete Aspergillus niger was identified. spreads powdery mildew (muchnistaya rosa) disease in farm crops.

In the study of Jihane Kenfaui *et al*., infected black spored fruits of grape in Pakistan were incubated in potato dextrose agar medium at 25 0C for 3 days and phytopathogenic micromycetes were isolated.[29] Won Ki Kim *et al*. isolated 12 Penicillium isolates from grapes infected with blue mold in Korea and identified them as *P. bialowiezense*, *P. citrinum*, *P. echinulatum*, *P. expansum*, *P. solitum* and Penicillium species based on their morphological characteristics.[30] In accordance with our research, W. Habib *et al*.'s research shows that representative isolates of the main toxigenic genera that affect the grapes for consumption are Aspergillus, Penicillium, and Alternaria, and that they produce toxic substances such as ochratoxin A, fumonisins, and patulin.[31] Micromycetes belonging to the genus *Alternaria* have a negative effect on the cultivation of agricultural crops and the growth of the economy. A fungal strain of *Alternaria alternata* has been found to cause leaf joint damage in spinach.[32] Phytopathogenic micromycetes *A. alternata and A. tenuissima* cause *alternariosis*.[33-35] Biospecimens were taken from the affected part of grape plantations where leaf spots appeared, and pure cultures of *A. alternata* 203-7 and *A. tenuissima* 198-1-1 strains were taken as research objects.[36] Micromycetes belonging to the genera *Aspergillus* and *Penicillium* synthesize toxic metabolites and damage grapefruit. Fungi belonging to the genera Fusarium, *Penicillium*, and *Alternaria* are recorded as dominant species found in grapes.[37] *Fusarium* and *Penicillium* genera isolates were also identified from grape plantations grown in Uzbekistan according to our research.

CONCLUSION

The microflora of *Vitis vinifera* grown in Uzbekistan was studied. Phytopathogenic micromycete strains of *Aspergillus niger and Alternaria alternata* were isolated and identified. *Fusarium* sp. and *Penicillium* sp. isolates belonging to the genus have also been identified. Since the vine is a perennial plant, taking samples from the plantation soil, and microbiological analysis of the plant microbiome allows for early detection of diseases. To ensure the continuity of the research, to combat phytopathogenic micromycetes, to isolate a new generation of antagonistic strains from the vine, to study the effect on the physiological properties of the plant and use them as a biological control agent are being aimed for our further research.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MALDI-TOF: Matrix-assisted laser desorption/ionization mass spectrometry; **MPA:** Meat-peptone agar; **PDA:** Potato-dextrose agar; OA: Oat agar.

SUMMARY

In the cultivation of grapes in Uzbekistan, phytopathogenic micromycetes cause dangerous diseases and cause the death of most of the crop. This article describes new research aimed at the first study of micromycetes that spread disease in grapes grown in Uzbekistan. Micromycete isolates belonging to the genera Fusarium, Alternaria, Penicillium and Aspergillus have been identified in the soil where grapes are grown and in samples taken from infected grapes.

Authors' Contribution

The contributions of the study were divided among the authors. A supervised the research and wrote the article. B helped to write the article. C identified the type of micromycete. D recorded the results. E brought samples and participated in the research.

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