

# Determination of Fungal Pathogens in the Species of Genus of *Pyrus L.* as Biotic Factor in Greater Caucasus, Azerbaijan

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Our findings highlight the significant biotic factors affecting *Pyrus L.* and provide insight into the health challenges faced by these species in their natural habitat. This research underscores the importance of monitoring fungal pathogens to develop effective management strategies for the conservation of *Pyrus L.* biodiversity in the region. The stationary stations were the North-Eastern regions of the Greater Caucasus (Guba, Khizi in Azerbaijan area) along with the Central Botanical Garden with in ex situ conditions. Various microscopic pathogenic fungi have been investigated in this research. Morphological (colony morphology, spore form) and different determinants were used to determine their characteristics. It was found that the various pathogens were observed in *Pyrus L.* in species of the genus *Pyrus* microscopic fungi they cause disease such as *Phyllosticta pinna* Sacc., *Fusicladium pirinum* Fuck., *Botrytis cinerea* Pers. Different infection sources are thoroughly described in relation to both harvesting and post-harvesting handling and intervention practices. The diseases are influenced by various environmental factors, with specific temperature ranges outlined for disease development and causal agents identified. Previous studies have focused on diagnostic indicators, such as optimal environmental conditions for fungal growth in *Pyrus L.*, including temperature, pressure, pH, water activity, and oxygen levels, summarized under both in situ and ex situ conditions. The research included five species of the genus *Pyrus L.*: *Pyrus caucasica* Fed., *Pyrus communis L.*, *Pyrus georgica* Kuth., *Pyrus vsevolodi* Heideman, and *Pyrus salicifolia* Pall.

**Keywords:** Fungi; Microorganism; *Pyrus L.*; Pathogen; Spoilage.

One of the most important matters facing modern biological science is reducing the number of harmful microorganisms and pathogens related to plant protection, preventing their mass formation, and creating plants that are resistant against such microorganisms. Studies have shown that pests and pathogens reduce crop productivity by 20–40%<sup>3-4, 6, 9, 19</sup>.

In plants that are susceptible to disease, the interactions between the host, pathogen, and environment are not spatially defined, ranging from complete disease facilitation to no disease

at all. Environmental variables, alongside biotic and abiotic stress responses, significantly impact the host plant's condition, including its growth, resistance mechanisms, and pathogen survival<sup>2, 7</sup>. Research indicates that plant extracts have traditionally been used to treat various fungal infections<sup>16</sup>.

Fungal pathogens in plants spread through both insect vectors and host plants in response to environmental changes. Diseases transmitted by vectors are more likely to occur locally and regionally, depending on the environmental

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needs of the vectors. The host's phenology is also influenced by environmental changes, which affects its susceptibility to pathogens. Due to global warming, pathogens can spread within a host and horizontally to new hosts<sup>20</sup>.

Plant disease outbreaks significantly threaten global food security and environmental sustainability, leading to losses in primary productivity and biodiversity that adversely affect the environmental and socio-economic conditions of the impacted regions. Climate change exacerbates these risks by altering pathogen evolution, host-pathogen interactions, and enabling the emergence of new pathogenic strains. Pathogen ranges can shift, increasing the spread of plant diseases to new areas<sup>8,18</sup>

Biologically control agents (BCAs) such as *Trichoderma*, *Beauveria* and *Metarhizium* species have been suggested as potential medicinal agents (IPDM) incorporated into several natural products such as tea and others. However, the agricultural industry has been able to achieve this goal, although there is little evidence to support the use of beneficial fungi as microbial pesticides<sup>12-13</sup>.

The growth of filamentous fungi in water is destroyed, and pears are not very important in their production, which can damage the economy. At least 13 fungal diseases of pear from different cultivars have been reported worldwide<sup>17</sup>.

The spread of the disease for the growing year and the disease begins in winter, its infected in pear trees and falls to the ground at the end of the past season. Microscopic spores are usually found in tree sap in winter and are released from the leaves into the winter ball. Ascospores are released over a period of 3–4 months, and the number of spores increases the most during flowering<sup>21</sup>.

The initial indication of infection is the appearance of small red spots, which result from lesions, particularly those inflicted by birds. Under warm conditions, itchy white blisters can rupture the skin, often leading to the formation of infection circles. In the culture medium, there is a high concentration of yellow-brown spores (conidia). The fungus has the potential to spread to adjacent healthy fruits and can remain robust and persistent even in its later stages<sup>19</sup>.

Being a perennial, it creates favorable climatic conditions for the development of various fungal diseases, which collectively result in

significant yield losses both in quantity and quality. These chemicals are employed to manage animal-related issues<sup>7</sup>.

The protection of plants and trees from diseases and pathogens under local- *in situ* conditions is one of the most important challenges faced by scientists working in this field. There is no doubt that the socio-economic importance of plant biomass is high worldwide<sup>1</sup>.

Firstly, it is essential to adapt fungal species to changing environmental conditions. The compactness and structure of soil significantly influence its biological activity. Soil pH affects the mobility of ions in soil solution and their absorption by plants and soil microflora. Excess water can impact mold growth, as these microorganisms are sensitive to reduced oxygen levels. Mechanical soil cultivation has a minimal effect on fungal spores. However, soil movement can alter the population of arbuscular mycorrhizal fungi within the soil profile. The geographical location, topographical variation of cultivated land, and fluctuations in climatic factors all influence the population and symbiotic activity of arbuscular mycorrhizal fungi in the soil<sup>6</sup>.

## METHODS

Between 2016 and 2020, species from the genus *Pyrus* L. were collected from both natural (*in situ*) and cultivated (*ex situ*) environments. Fungal presence was detected in various organs of these pear species. Pathogens were isolated from the collected plant samples using agarized malt juice and Sabouraud agar as nutrient media. The cultivation process occurred in a thermostat set to 28°C, with colonies developing over 4-5 days before being identified based on their microscopic characteristics<sup>10,23</sup>.

The conditions for conidia infection are similar to those for ascospores, but conidia require approximately 2 hours more wetness than ascospores at temperatures below 53°F. In areas with overhead irrigation, daytime irrigation and nighttime dew can create additional infection cycles beyond those caused by rainy days.

The initial identification of fungal infections on pear organs and adults included assessing colony morphology, spore size, and spore shape of the fungal isolates. Spore sizes

were measured using a microscope. The methods for initial identification varied<sup>11, 15, 23</sup>. The fungal cultures were archived in the Biological Active Compounds (BAC) laboratory at the Microbiology Institute in Baku, Azerbaijan.

## RESULTS AND DISCUSSION

### *Phyllosticta pinna* Sacc

The fungus that causes pear disease is often found on pear leaves. In the species *P. salicifolia*, *P. vsevolodii* and *P. communis*, confluent brown, round or irregular spots appear on diseased leaves. Symptoms of leaf damage with brown spot during the period of spot growth are similar to leaf burn. Over time, small pointed brown fruits appear in diseased leaf tissue. The leaves turn yellow and fall prematurely, which affects the development of shoots and the proper development of trees. Additionally, it reduces the plant's frost resistance.

### *Fusicladium pyrinum* Fuck

Cold and rainy weather promotes fungal development. Symptoms of this disease include olive-colored spots on the leaves of *P. salicifolia*, *P. georgica*, and *P. caucasica*. The disease progressively spreads to the pear fruit, creating velvety spots on the surface. This condition decreases the fruit yield and can also affect apple plants, with *Fusicladium dendriticum* (Wallr.) being a fungus responsible for apple disease. It mainly targets young shoots, negatively impacting the flowering and fruiting processes.

The disease's impact is influenced by nutrient status and specific solutes. Carbohydrate-rich substrates are more conducive to fungal

growth compared to protein-rich foods, which are preferred by bacteria. Most molds can use nitrate, ammonium ions, or organic nitrogen sources with equal efficiency and are generally indifferent to the type of nitrogen source

### *Botrytis cinerea* Pers.

The disease manifests in the form of large brown spots with no borders on pear fruits and leaves. On rainy days, many gray spores of the fungus develop on infected tissues. *P. caucasica* and *P. communis* occur in pear varieties. After some time the resulting mycelium dries out and small round black sclerotia multiply inside. In dry, hot weather, spots on plant leaves crack and fall off. The disease adversely affects the proper development and productivity of the plant.

*Botrytis cinerea* can penetrate the stems and reach the flesh after a period of cold storage, leading to spoilage. The presence of gray mold has been linked to the concentration of spores on fruit surfaces. Reports indicate that *B. cinerea* attacks the flowering parts of pear fruits during bloom and causes calyx tip rot during storage<sup>23</sup>.

Cooling fruits post-harvest helps to extend their storage life by slowing down physiological changes and limiting fungal growth that leads to spoilage. Fruits should be kept out of direct sunlight, which is an inexpensive cooling method. Additionally, harvesting fruits early in the morning ensures they remain cool during transportation. For long-term storage, fruits are kept in the refrigerator or freezer. However, *Botrytis cinerea* can still infect pears under cold, wet conditions, which are common in freezers. Infected pears develop tiny lesions in the field before harvest, and these lesions



Fig. 1. *Phyllosticta pinna* Sacc. in different *Pyrus* L. species



**Fig. 2.** *Fusicladium pyrinum* in different *Pyrus* L. species



**Fig. 3.** *Botrytis cinerea* Pers. in different *Pyrus* L. species

can easily spread to healthy pears during storage<sup>14</sup>.

#### **Optimal conditions for fungal growth in *Pyrus* L. Spp.**

The colonization of fruit products by fungi and the production of mycotoxins are influenced by a variety of factors, both intrinsic and extrinsic, as explored in this research. Intrinsic factors include initial contamination of fruits before storage and the type of contaminants. These factors encompass water activity, fruit pH, biological structure, and nutritional content of the fruit. For instance, *Pyrus* L. species have low pH, high water activity, and provide abundant nutrients, making them ideal for fungal growth<sup>4,21</sup>.

Extrinsic factors include storage temperature, relative humidity, and the atmospheric composition during packing and storage. Fruits are expected to be nutritious and offer a favorable environment for microorganisms. Since fruits consist of living tissues, fungal pathogens must overcome natural barriers the plant has developed to avoid infection-induced death.

Thus, there is a specificity between fruits and various mold species, with fungal pathogens often bypassing the fruit's defense mechanisms, leading to spoilage. Berries, being acidic, mainly restrict damage to fungi, which is why fungal infections in fruits are a significant area of study<sup>19</sup>.

<sup>22</sup>. *Pyrus L.* species are now frequently sold far from their origin. The choice of post-harvest storage conditions for pears affects the extent of fungal damage and fruit loss. Approximately seven key physical and chemical factors contribute to this damage

### CONCLUSION

Abiotic factors influence the growth and germination of fungi affecting fruit. General characteristics cannot be uniformly applied to *Pyrus L.* species across different geographical regions. Although these species belong to the same genus, they may still be vulnerable to similar or identical pathogens. The impact of abiotic factors is not isolated but rather involves synergistic effects. Future research may need to include in vivo evaluations of fungal growth under varying environmental conditions.

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### Conflict of Interest

The authors do not have any conflict of interest.

### Data Availability Statement

This statement does not apply to this article.

### Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

### Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

### Authors' Contribution

Prof. Elman Iskender : conceptualized the research study and designed the methodology. Dr. Sabina Jafarzadeh : conducted the data collection and performed the statistical analysis and supervised the article and provided critical insights throughout the research process.

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