CONTRIBUTION TO THE STUDY OF FUNGI CAUSING STORAGE ROT OF SOME TREE FRUITS

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Abstract

Pathogens can cause considerable quantitatively or qualitatively losses during storage both by degrading appearance, taste also can be a major food safety problem for the consumer by producing mycotoxins. Thus, the knowledge and study of the biology and morphology of the micromycetes present on fruits during storage is extremely important. Studied biological material was taken from the natural ventilated warehouse of the horticultural farm „Vasile Adamachi" from "Ion Ionescu de la Brad" Iasi University of Life Sciences (IULS), during December 2022 and March 2023 and it is represented by fruit trees as apple, pear and quince. The sampled fruits were brought to the laboratory, sorted according to the species and the present pathological manifestations, and were prepared for the pathogens identification. From the total of stored and analyzed fruits, was found that *Monilinia fructigena* fungus showed the highest percentage of infection, by 45% followed by *Penicillium* spp. fungus which showed a percentage of 43%.

Key words: fungi, storage rot, fruits

In 2019, the total production of apples in Europe was 10,626,790 t and Romania took the 7th place presenting a production of - 26% compared to 2018 (FAO). Fruit production can be diminished and at the same time depreciated primarily by some pathogens in orchards during the period of formation, growth and ripening, as well as after harvest during storage (Hatman M. *et al*, 1998).

Fruit deterioration is a normal process during storage, but the degradation stage depends on the present conditions. Strict compliance with the rules that are required to prevent fruit contamination lead to a high production in terms of quality and quantity. Among the more common storage pathogens on fruit, we can mention: *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. and that in addition to crop losses, these pathogens are also responsible for the mycotoxinsn production (Zain M.E., 2011). Mycotoxins are considered natural substances, secondary metabolites that appear during the development of parasitic fungi on plants in the field or in storage, plants that can later be used in human and animal food, having a negative effect on people and the health of domestic animals (Lelde G., 2015). Cause of alterations is represented by bacteria, viruses, molds and phytopathogenic fungi that are widespread both in the natural environment and in fruit storage and processing areas (Nybom H. *et al*, 2020). They can cause infections in all phases of the production and utilization process, starting from the field phase until the product is sold. In the field phase, the alterations caused by the action of the numerous pathogenic microflora, well-represented and more aggressive field manifest themselves immediately after infection and the completion of the incubation period or can be realized later during storage when favorable conditions occur (Neri F. *et al*, 2019). This category includes: moniliosis known as rown rot or mummified (*Monilinia fructigena*) fruit trees as pear, quince and apple; gray rot *(Botrytis cinerea*) on pear, apple or strawberry fruits; blight (*Alternaria alternata*) on quince fruits, apple fruits, pear fruits etc. At the end of the growth phase and the beginning of ripening, the saprophytic microflora of the field attacks the fruits with serious consequences at storage in the warehouses. Most fruits are invaded by molds that destroy the pericarp allowing surface germs to penetrate inside the pulp. *Alternaria*, *Cladosporium*, *Fusarium*, *Rhizopus*, *Trichothecium*, *Phoma* etc. are the most common fungi that present a high frequency in all species.During harvesting, transport and conditioning, the intermediate saprophytic microflora infects the products via spores (resistant forms) spread through the means of harvesting, packaging or transport (Kumari N. *et al* 2018).

Warehouse microflora manifests itself in most cases only in warehouses throughout the storage period and until utilization. The most famous molds in this category are: black mold

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(*Aspergillus niger*), blue mold (*Penicillium expansum*), green mold (*Penicillium digitatum*).
Many of the species involved in post-harvest fruit decay (*Rhizopus*, *Penicillium*) are opportunistic pathogens unable to penetrate healthy tissues or attack healthy plants during their growth period (Watkins C.B., Mattheis J.P., 2019).

MATERIAL AND METHOD

Studied biological material was taken from the warehouse with natural ventilation of the horticultural farm ,,Vasile Adamachi" from "Ion Ionescu de la Brad" Iasi University of Life Sciences (IULS).

The fruits of the studied trees were represented by: *Malus domestica* (apple tree), *Pyrus communis* (pear tree) and *Cydonia oblonga* (quince tree). The varieties of apple tree fruits stored and used in the determination of micromycetes causing damage in storage are Idared, Florina and Generos variety. The pear and quince tree plantation were established in the fall of 2010, the pear tree varieties sampled are red Williams, Clapp's Favorite and Curé and for quince tree the Aromate and Beretzki varieties. For the study of the biology and morphology of micromycetes, fruits were taken from seed fruit trees (apple, pear and quince) from the warehouse of the horticultural farm ,,Vasile Adamachi", from "Ion Ionescu de la Brad" Iasi University of Life Sciences (IULS) during December 2022 and March 2023.

The evaluation of the attack on the fruits was carried out periodically throughout the storage period and the determination of the pathogens was carried out according to the appearance of the fruits, but especially according to the morphological characters of the pathogens, using the specialized literature. The samples were collected aseptically, sterile, dry, clean utensils were used to avoid external contamination during transport to the laboratory. In the results section is showed the percentage of attacked fruits referring to the most important pathogens from an economic point of view and highlighted their morphological characters.

In the laboratory, the sampled fruits were sorted according to the species and the pathological present manifestations and were prepared for the pathogens identification. The identification of the pathogenic fungi from the sampled fruits was carried out by direct microscopic examination.

For the fruits that showed minor pathological changes and was not precisely identified the pathogen, the wet room method for a fungal growth stimulation was used. The wet room method consists of wallpapering Petri dishes or various transparent plastic dishes with rounds of filter paper of appropriate sizes on which fragments of the material to be analyzed are placed. Incubation lasted 7 days at a temperature of 25℃, after which the developed colonies were analyzed by microscopic preparation. Accurate identification of the micromycetes genera from the stored fruits was made on the basis of the microscopic preparations and the specialized determinants within the disciplines of Microbiology and Phytopathology.

RESULTS AND DISCUSSIONS

During the storage period from December to January, 4 types of micromycetes were identified in the *Pyrus communis* species, on the red Williams, Clapp's Favorite and Curé varieties which produce, more or less important, economically and qualitatively damages. The micromycetes identified during storage on the *Pyrus communis* species are *Alternaria* spp., *Fumago vagans*, *Monilinia fructigena* and *Penicillium* spp. (*figure 1*).

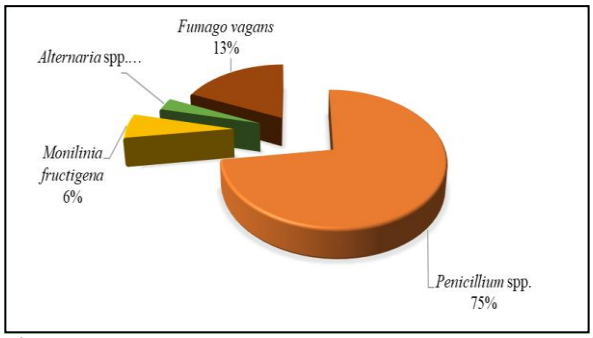

Figure 1 **Mcromycetes spectrum on *Pyrus communis* stored fruits.**

According to figure no. 2, between December and January, 5 genera of micromycetes were identified fruits of *Cydonia oblonga* species, on the Aromate and Bereczki varieties, whose action was carried out differently (*figure 2*). Compared to the *Pyrus communis* fruit tree species, *Cydonia oblonga* fruits showed a much higher resistance to storage conditions.

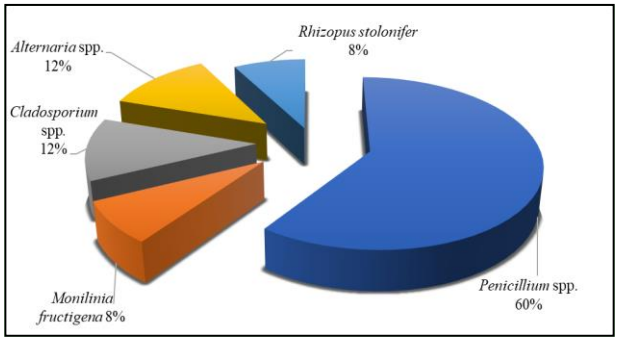

Figure 2. **Mcromycetes spectrum on *Cydonia oblonga* stored fruits.**

On the fruits of *Cydonia oblonga* species, *Penicillium* spp. was identified as the predominant pathogen, in a percentage of 60% (*figure 3*).

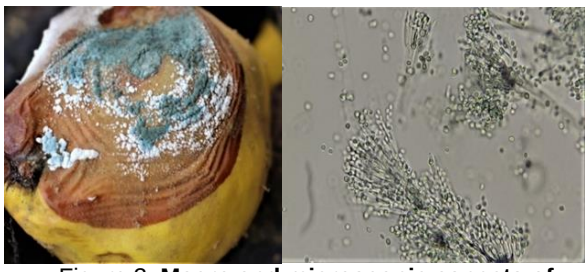

Figure 3. **Macro and microscopic aspects of *Monilinia fructigena* fungus on quince strored fruits**

For the stored fruits of *Malus domestica* species with the varieties Idared, Florina and Generos, due to the much higher production, the sampling and identification of micromycetes was carried out over a period of 3 months (3 times) and on a larger number of fruits (*figure 5*). Identified micromycetes on the analized stored fruits of this species are: *Botrytis cinerea* (*figure 4*), *Fumago vagans, Monilinia fructigena* and *Penicillium* spp. (*figure 6*).

Figure 4. **Macro and microscopic aspects of *Botrytis cinerea* fungus on apple strored fruits**

The degree of depreciation of the stored apple fruits varies depending on the pre-harvest treatments, temperature and humidity conditions, but also due to the fruit sorting process.
Between January and February period, the spectrum of micromycetes found on apple stored fruits was reduced to *Penicillium* spp. and *Monilinia fructigena* fungus.

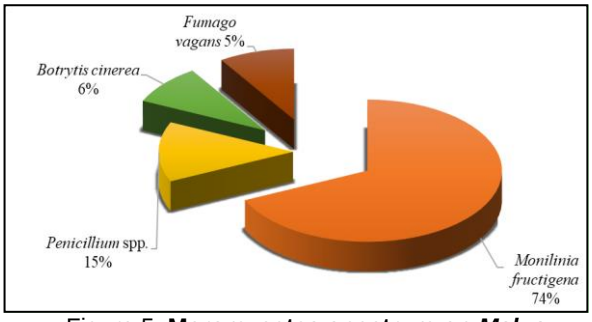

Figure 5. **Mcromycetes spectrum on *Malus domestica,* during December and January months**

On stored apple fruits, *Penicillium spp.* fungus showed an infection rate of 55% and *Monilinia fructigena fungus* of 45%, the difference between the two pathogens being only 10%.

Identified micromycetes spectrum on apple stored fruits during February-March period was reduced to *Penicillium* spp. in percentage of 57% and *Monilinia fructigena* 43% (*figure 6*).

Figure 6. **Macro and microscopic aspects of *Monilinia fructigena* fungus on apple strored fruits**

The stored fruit species on which the study was carried out behaved differently depending on the period of storage. Following the observations that were done we noticed that *Monilinia fructigena* and *Penicillium* spp. fungus were predominant pathogens in all fruit tree species analyzed, *Penicillium* spp. fungus registered the highest presence percentage by 75% and the lowest 15%. *Monilinia fructigena* fungus presented the highest percentage of his presence on stored fruits of *Malus domestica* species (by 75%) and the lowest on stored fruits of *Pyrus communis* species, only of 6% (*figure 7*).

From the total of stored and analyzed fruits, was found that *Monilinia fructigena* fungus showed the highest percentage of infection, by 45% followed by *Penicillium* spp. fungus which showed a percentage of 43%, the difference between the two pathogens being extremely small, by 2%.

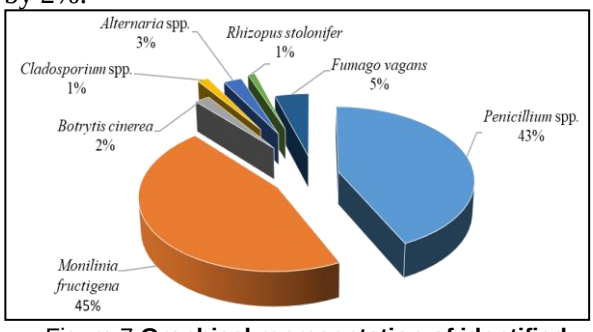

Figure 7 **Graphical representation of identified micromycetes on stored fruits**

Proportion of stored fruit attacked by *Alternaria* spp., *Fumago vagans* and *Botrytis cinerea* was extremely reduced, only of 2-5%. The presence of *Rhizopus stolonifer* and *Cladosporium* spp. fungi was less common, of 1% of all attacked stored fruits. Stored fruits play an important role for the human population both from an economic and alimentary point of view.

Obtained data in the work outline the role of micromycetes on the stored fruits as well as on the phytosanitary status of the fruit tree. With the help of these results and the knowledge of the fungi that are causing strorage rot to the stored fruits we realize the influence of cultural hygiene measures and compliance with harvesting and storage conditions.

CONCLUSIONS

The change in the rate of infected fruits by various species of micromycetes at certain evaluation time periods provides important information regarding climatic conditions, storage conditions and if the vegetation treatments were properly or not applied.

Using of storage method with natural ventilation the temperature and humidity are able to fluctuate producing in this way major changes on fungal presence that can damage stored fruits. Also, an improper sorting can cause quite a lot of damage in fruits warehouses. Both *Monilinia fructigena* and *Penicillium* spp. fungi are pathogens in which the infection can by carried out from one fruit to another, which can justify such a high percentage of occurrence.

The damage produced by storage fungi on fruits are considerable because it affects both the quality and quantity of production and the health of the consumer, as some micromycetes can produce mycotoxins that are toxic to the human body.

A strict compliance with the rules of cultural hygiene, carrying out pre-harvest treatments, appropriate harvesting, warehouses disinfection, fruits sorting during storage and permanent checking of temperature and humidity are some basic measures that could prevent the infection of fruits by the identified pathogens.

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