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DAMAGES ON HAZELNUT KERNELS CAUSED BY *PENICILLIUM* SPP.

Desislava Vladimirova Boneva

Agricultural University – Plovdiv, Bulgaria
E-mail*:* desiboneva_93@abv.bg

Abstract

The presence of *Penicillium* spp. was observed during a laboratory examination of nuts (*Corylus avellana* L.) collected from April 2019 to April 2023 from hazelnut trees, grown in different regions of Bulgaria. The obtained isolates were identified following the Koch's postulates and their cultural and morphological characteristics were studied. Three-hundred ninety seven (397) samples, with suspected presence of pathogen, were taken from nuts. *Penicillium* spp. strains were identified as a cause for damages in approximately 2% of all examined kernels.

Keywords: *Penicillium* spp., hazelnut, pathogenicity

INTRODUCTION

The hazelnut (*Corylus avellana* L.) belongs to the genus *Corylus* (family *Betulaceae*) which includes European (common) hazelnut (*C. avellana* L.), Constantinople (large-fruited) hazel (*C. maxima* Mill), Turkish (tree-like) hazel (*C. colurna*) and Pontic hazel (*C. pontica* C Koch.), (Mihaylov, 2018). The hazelnut is a monoecious, dioecious, anemophilic plant. Many of the cultivars are self-sterile, which requires planting of several different cultivars. The hazelnut is the earliest flowering fruit species.

The common hazelnut holds considerable significance for the food industry in several countries (FAO, 2020). The main producer of hazelnut in the world is Turkey followed by Italy. The filbert is found in its natural state throughout Europe. The nut contains significant amounts of fat, proteins, essential amino acids (lysine, arginine, leucine, etc.), carbohydrates and phospholipids. The nutrients in the hazelnuts are favorable for development of many saprophytic and pathogenic fungi that produce secondary metabolites (mycotoxins). Mycotoxins cause various toxic effects in animals and humans - carcinogenic, mutagenic, cytotoxic and neurotoxic (Ukwuru et al., 2021). According to studies, ochratoxins are often found in nut samples (Skrbic et al., 2014) and they are secondary metabolites produced by some *Penicillium* and *Aspergillus* species (Heussner et al., 2015). Ochratoxin A is considered as the most toxic type of ochratoxin with immunotoxic, neurotoxic, teratogenic, and hepatotoxic effect on some animals, and with carcinogenic effect on humans (Tao et al., 2018). Another mycotoxin identified in hazelnuts – patulin (polyketide lactone), produced by *Penicillium* spp. and others, also poses a serious threat to health (Mahato et al., 2021). A kernel contamination with mycotoxins (secondary metabolites) is due to fungal infection either during fruit development (before harvesting), or during fruit storage (postharvest). The symptoms of fruit rot can be only on the kernel, hidden by the shell, or can cover the entire fruit and to be visible (Salvatore et al., 2023). The hazelnuts can be eaten raw or as roasted after heat treatment. Despite that roasting prevents the development of pathogens and microorganisms it is unable to eliminate the heat-resistant mycotoxins (Siciliano et al., 2017).

According to studies, a several different fungal species can attack the nuts: *Penicillium*, *Aspergillus*, *Trichothecium*, *Mucor*, *Ulocladium*, *Cladosporium*, *Alternaria*, *Scopulariopsis*, and *Drechselera* (Saffari et. Al, 2021, Botondi, 2019, Wiman et. Al, 2019). A comprehensive analysis on 134 isolates from hazelnut kernels revealed that the most widespread *Penicillium* species were *P. commune* (69 isolates), *P. solitum* (36 isolates) and *P. expansum* (29 isolates) (Lombardi et. al, 2022).

In Bulgaria, the hazelnut is among the less common fruit species, despite the favorable conditions for its cultivation. In the Southern Bulgaria, where are the most favorable growing conditions the hazelnut trees are often found only in private yards.

The objective of this study, conducted as part of an extensive research initiative, was to gather specific information concerning the presence of *Penicillium* species on hazelnut kernels.

MATERIALS AND METHODS

Hazelnuts fruits sampling

The plant samples, consisted of hazelnut fruits, were taken during the harvest period from hazelnut plantations and private yards from different regions of Bulgaria from April 2019 to April 2023 (Table 1).

Table 1. Description and locations of surveyed hazelnut plots

№	Object of visual assessment	Location
1.	Hazelnut - private yard	Belgun / Dobrich
2.	Hazelnut plantation	Vidno / Dobrich
3.	Hazelnut plantation	Kavarna / Dobrich
4.	Hazelnut - private yard	Kavarna / Dobrich
5.	Hazelnut plantation	Balgarevo / Dobrich
6.	Hazelnut plantation	Sveti Nikola / Dobrich
7.	Hazelnut plantation	Rakovski / Dobrich
8.	Hazelnut plantation	Shabla / Dobrich
9.	Hazelnut plantation	Kardam / Dobrich
10.	Hazelnut plantation	Kalina / Dobrich
11.	Hazelnut plantation	Plenimir / Dobrich
12.	Hazelnut plantation	Sredina / Dobrich
13.	Hazelnut - private yard	Kolartzi / Dobrich
14.	Hazelnut plantation	Primorci / Dobrich
15.	Hazelnut plantation	Pobeda / Dobrich
16.	Hazelnut plantation	Kalnovo / Shumen
17.	Hazelnut plantation	Smyadovo / Shumen
18.	Hazelnut plantation	Diankovo / Razgrad
19.	Hazelnut plantation	Osenec / Razgrad
20.	Hazelnut plantation	Yunatsite / Pazardjik
21.	Hazelnut - private yard	Ihtiman / Sofia
22.	Hazelnut plantation	Trud / Plovdiv

Isolation

The whole nuts were cracked open and estimated for mold presence. After visual evaluation, 397 samples, with suspected presence of pathogens in the tissues, were collected. The Koch's postulates were applied to establish the etiology of causative agent in the nuts. To prevent the air contamination kernels

were placed briefly (about 10 seconds) in ethanol 70%, rinsed with sterile distilled water and allowed to air-dry. Small pieces were cut from the rotted internal parts, and the sample was taken from the border between infected and healthy tissue. The pieces were placed onto Petri plates containing potato dextrose agar (PDA) and incubated in the dark at 24 to 25°C for 5 to 7 days. Small pieces were plated also on slanted agar (PDA). All isolates received collection numbers and were stored at 4°C for further laboratory tests.

Pathogenicity test

The obtained isolates were tested for pathogenicity. Visibly healthy hazelnut kernels from plantation (Kavarna, private yard, no pesticides used) were collected. After removing the shells, mycelial discs (5 mm in diameter) of 10 days old cultures were cut with a sterile borer and were placed in the heart of the kernels. In order to facilitate the penetration of the pathogen in the kernel, with a sterile needle, in the tissues were made small holes. The kernels were wrapped with parafilm and incubated at room temperature for seven days. A control samples was also prepared but the inserted in the kernels disks (5 mm in diameter) were cut from the agar medium (PDA) (3 replicates).

Cultural and morphological characterization of isolates

The cultural and morphological characteristics of the *Penicillium* spp. isolates were determined on PDA. Disks of 5 mm were cut with a sterile borer from the edge of the studied colonies. The disks were placed on the Petri dishes, with 20 ml PDA (3 replicates) and were incubated in dark at 25°C. Colony growth dynamics were observed on the fifth, seventh and tenth day. The development of the colonies, their color and morphological characteristics such as size, shape and color of spores were defined by direct observation and microscopically. The calculation of the mean spore size was based on the measurement of one hundred spores from each isolate.

RESULTS AND DISCUSSION

The symptoms of nuts consist of dark necrotic sunken spots covered with mold (Fig. 1). They covered only the nuts, remaining covered by the shell, or cover the shell as well.

It was found that from all analized nut samples, around 2% of the isolates belong to *Penicillium* spp. Most of the infected nuts were collected from the North-Eastern Black Sea coast of Bulgaria - Kavarna town (hazelnut – private yard), where the annual humidity is relative higher. The other infected nuts came from the city of Ihtiman (hazelnut – private yard), and from the village of Trud, Plovdiv district (hazelnut plantation). According to studies in Spain, in areas with higher rainfall, the number of cases of nuts with mold symptoms tend to increase (Tous et. al, 2001). An important measure against phytopathogens spread is conducting the harvesting in a very short period after the fruit reach full maturity (when the natural fruit drops occur). During harvest, the fruits with visibly damaged shells must be removed. The moisture content in the nuts after harvesting is high - around 30%, which requires further drying either under direct sunlight or in dryers; after that fruits should be stored in a dry, cool and well ventilated place.

The pathogenicity tests proved that the isolates can infect the healthy kernels (Fig. 2). The results were reported 7 days after inoculation. From eight isolates with a presence of *Penicillium* spp., only two gave a positive reaction in the pathogenicity test. The re-isolations revealed isolates, identical to the initial ones. In the control samples the nuts remained healthy.

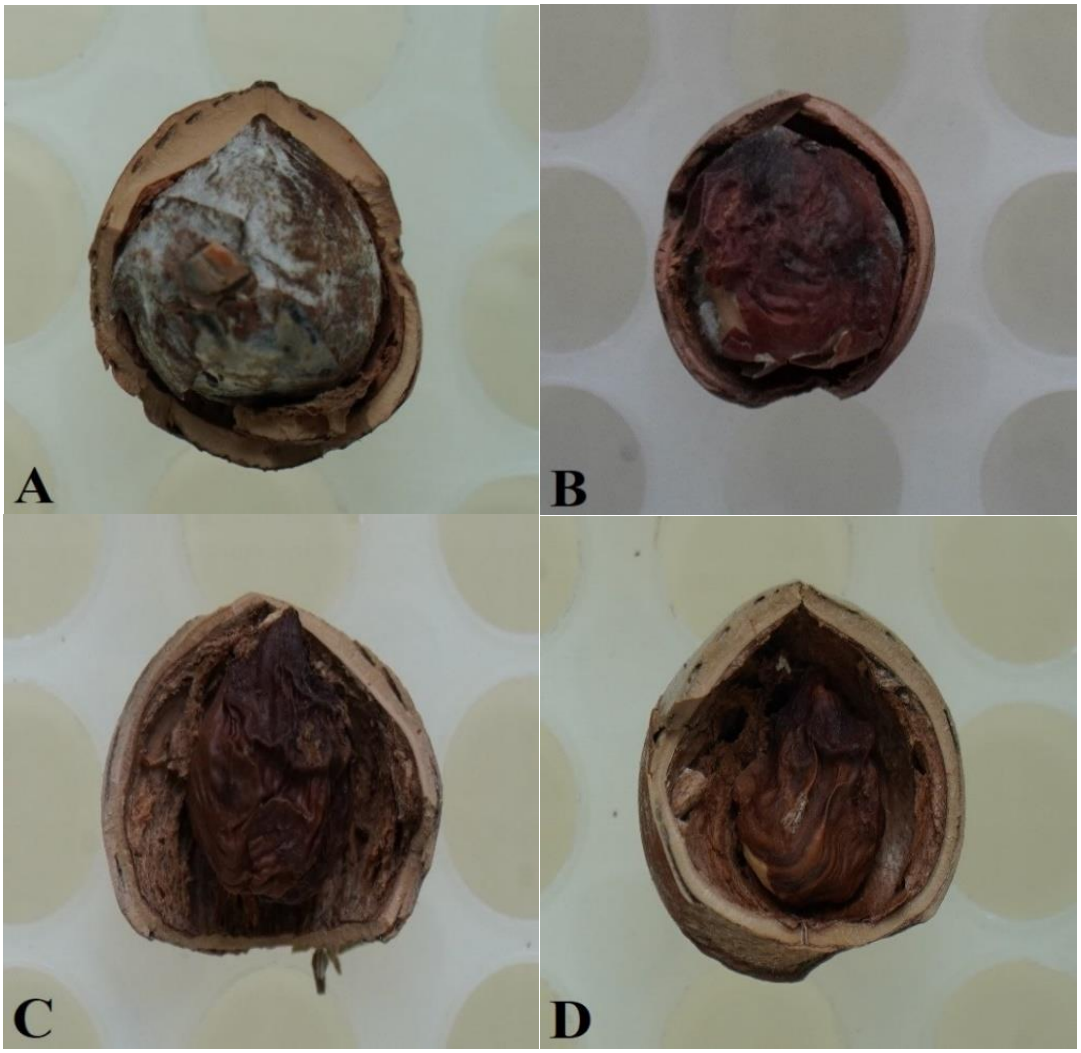

Figure 1. Necrosis and fungal structures on analyzed nuts.
Above – mold (A – sample 393; B – sample 348). Below – complete necrosis and mummification (C – sample 296; D – sample 281)

Figure 2. Pathogenicity test on hazelnut kernels

On PDA *Penicillium* spp. strains form flat, scattered, velvety colonies, with a slow growth rate. The color of the colonies is grayish-green, with a lighter periphery. On the bottom of the Petri dish, the colony was yellowish (Fig. 3). The isolate 281 forms the colony with a diameter of 27 mm on the fifth day, 40 mm on the seventh day, and 48 mm on the tenth day.

A light microscope Leica DM500 was used for microscopic analysis in the laboratory of Agricultural University Plovdiv. In agreement with the description of Lombardi (Lombardi et al., 2022) the septate conidiophores, broom-like branched at the upper end were observed under the microscope. A chain of conidia forms on the branches. The conidia were small in size (2-3.5 μm), unicellular, oval to ovoid in shape, colorless (Fig. 4).

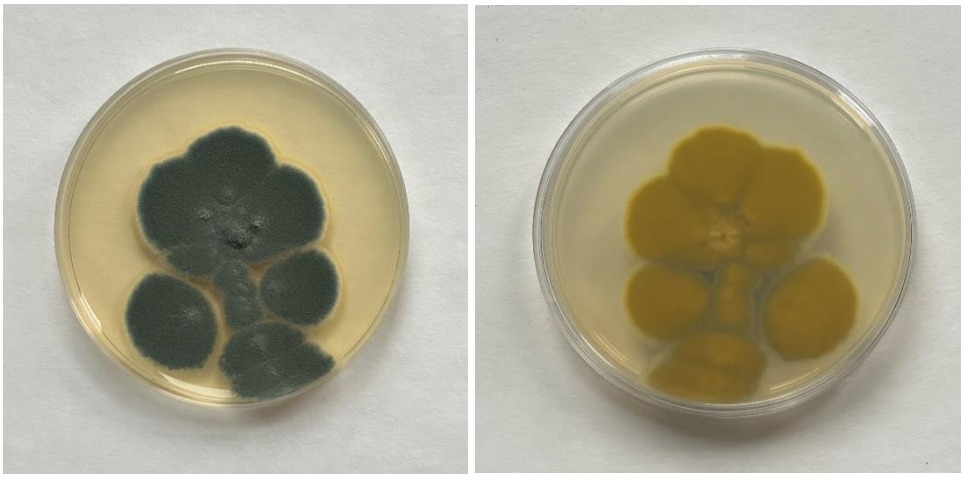

Figure 3. Fungal colony of isolate 281 on PDA – views from the surface and bottom sides

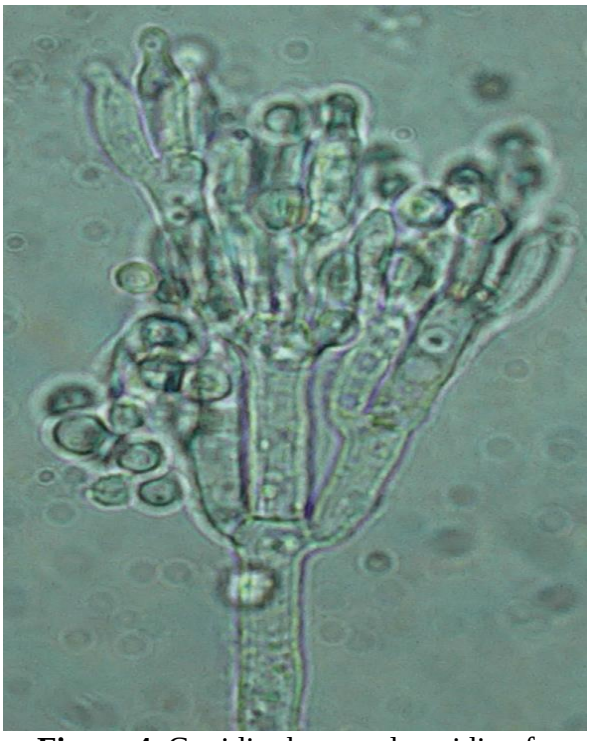

Figure 4. Conidiophore and conidia of *Penicillium* spp. (magnification 400x)

CONCLUSION

It was found that from all 397 nut samples, with suspected presence of pathogens in the tissues, *Penicillium* was identified as a causative agent in approximately 2% of infected kernels. Two of the isolates (nuts with both symptoms - necrosis and mold presence) showed a positive reaction in the pathogenicity test. The isolates were taken from samples collected from the North-Eastern Black Sea coast of Bulgaria (Kavarna), where the annual humidity is relatively higher.

Generally, the diseases caused by *Penicillium* spp. arisen as a problem in rainy years and during the storage. In such cases, the mixed fungal infection may also be observed which increases the threat to production, and could facilitate toxin synthesis. To prevent the development and spread of pathogens on the nuts, it is important to harvest them in time and keep them properly.

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