PHENOTYPIC AND PATHOGENIC CHARACTERIZATION OF *COLLETOTRICHUM* SPP. ASSOCIATED WITH BITTER ROT ON APPLE FRUITS IN POST-HARVEST STORAGE

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Abstract

Postharvest diseases are a major problem in long storage of apple (*Malus pumila* Mill.) worldwide. Among them bitter rot caused by *Colletotrichum* spp. complex (*C. gleosporioides* and *C. accutatum*) is one of the prevalent. The pathogen affects the fruit pre-harvest in orchards and/or post-harvest in storage, resulting in considerable economic losses. Advanced symptoms of bitter rot and anthracnose canker, with dark brown to black fruiting bodies called acervuli, were visible on stored apple fruits grown in the region of Berovo, Republic of Macedonia. Acervuli bear abundant one-celled fusiform macroconidia. Two groups of fungal isolates were obtained using standard laboratory procedure on PDA medium. The first group formed white mycelium that become smoke-grey with the time forming black aggregated conidiomata. The second group formed white areal mycelium with orange-pink colour on the reverse side with orange-pink conidiomta that appear black on the reverse side. One-celled elliptic to fusiform macroconidia are observed on PDA medium. Microconidia were not observed. The pathogenicity of the isolates was tested by wound inoculation of healthy 'Idared' apple fruit. After ten days, post-inoculation lesions up to 2-3 cm in diameter developed on inoculated fruit while the control remained healthy. Acervuli also developed in the rotted areas of inoculated fruits. Biological and morphological characterization of isolates was performed *in vitro*. The isolates were preliminary identified based on the morphology, temperature of growth and sensitivity of pesticides. Due to the overlapping morphological characters, species delimitation based on morphology alone is hardly possible in the genus of *Colletotrichum.* Multilocus sequence analyses combined with a polyphasic approach is generally suggested for species differentiation.

Keywords: post harvest disease, apple, anthracnose, acervuli

Introduction

Post harvest diseases mainly occurs on post harvest fruits and causes severe loses during storage. Major pathogens are fungi like *Colletotrichum* spp. and *Neofabraea* spp. but losses can be caused from some others like *Nectria* spp., *Botrytis* spp., *Fusarium* spp., and *Monilinia* spp. The study deals with morphological and pathogenic characteristics of *Colletotrichum* spp. causing bitter rot and antrachnose on apple fruit grown in the region of Berovo, Republic of Macedonia. The genus was recently voted the eighth most important group of plant pathogenic fungi in the world, based on perceived scientific and economic importance. *Colletotrichum accutatum* and *Colletotrichum gleosporioides* are major species from the genus *Colletotrichum* isolated from symptomatic fruits. Both species are cosmopolitan, causing losses worldwide attacking many different plant hosts. Occurs on a wide range of plant families like pome fruit (apple, pear, pomegranate), berries (strawberry, blueberry), nuts (almonds) citrus fruit (orange), cucurbites (cucumbers, watermelon, pumpkin), micelaneus fruit (kiwi, avocado, banana, mango), legumes (bean, soybean, peas), leafy vegetable (spinach), cereals (wheat), bulb vegetables (garlic) and fruiting vegetables from the family Solanaceae (tomato, aubergin, peppers) (Waller, 1992). It is considered that the teleomorphic stage of the fungus belong to the genus Glomerella spp.

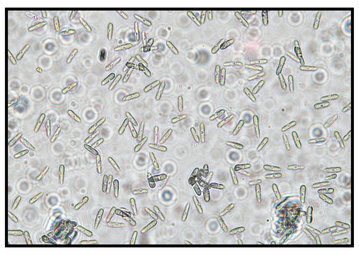

Fig.7 Conidia from diseased tissue

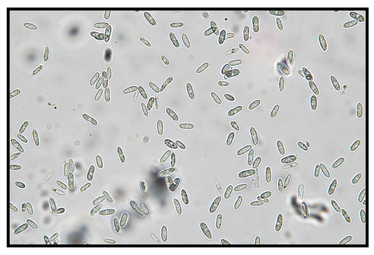

Fig.8 Conidia from conidiomata developed on PDA medium after 10 days.

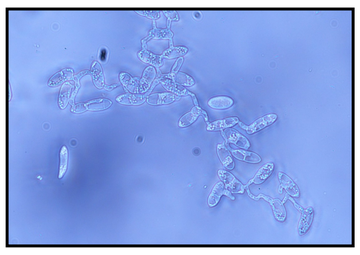

Fig. 9 Anastomosis formed between conidia submerged in water for 24 h

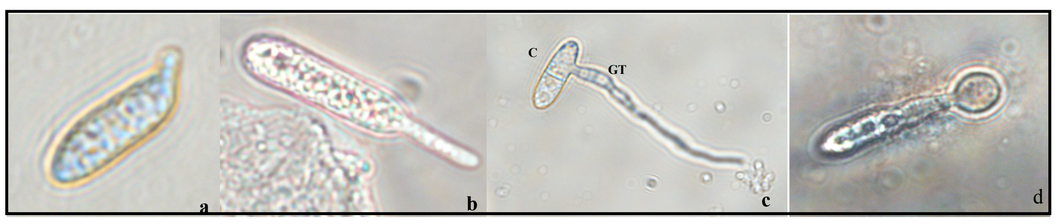

Fig. 10 Light micrographs of the early development stages of Colletotrichum. (a, b) Beginning of conidia germination; (c) Septated conidia (C) with germ tube (GT); (d) Appressorium formation by conidia and early stage of melanization.

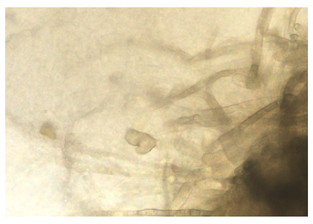

Fig. 11 Melanized appresoria formed by mycelia

Fig. 12 Septate mycelia with conidiophores

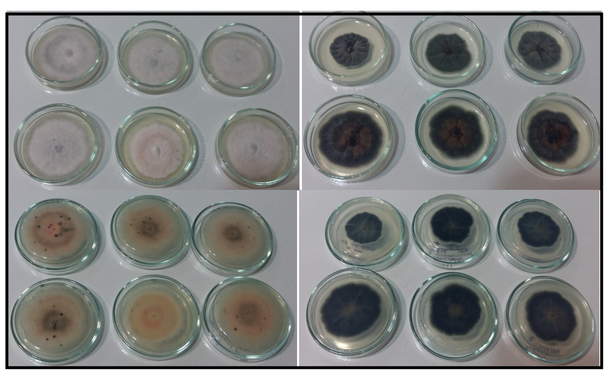

Fig. 13 Two groups of isolates obtained by the single spore isolation

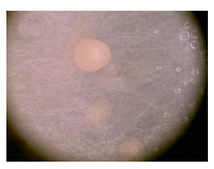

Fig. 14 Orange conidiomata

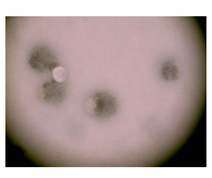

Fig. 15 Black conidiomata

Materials and methods

Isolation of the fungus was made on PDA from the symptomatic apple fruit grown in the region of Berovo, Republic of North Macedonia and stored for two months. Isolates were kept in the incubation chamber on 25 ^{0}C until the development of the mycelium and the conidia, then single spore isolation was made according to Choi et al., (1999). Morphological characteristics of the obtained isolates were investigated by microscopic observations on microscope type Olympus BX 41, equipped with Mini VID camera, model MVC-U6MP-USB3.

Koch's postulates were performed in order to confirmed the pathogenicity of the isolates. Pathogenicity was performed on ripe fruits of apple, pear, avocado and pepper by wood inoculation and stored on room temperature. Disease severity was evaluated 3th, 5th, 7th and the 10th day of inoculation after which a re-solation of the pathogen was performed on PDA and obtained isolates were compared to the initial ones. Culture characteristics such as colony color and formation of conidial masses were observed and recorded after 7days of incubation. To induce appresoria formation, conidia were transferred to a cavity slide, mounted with fresh apple juice and incubated at 27° ± 1°C for 24-48 h.

Fig. 1 Symptom of bitter rot on apple fruit

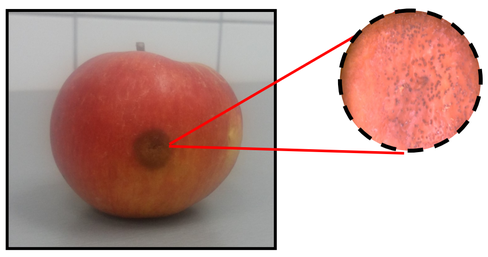

Fig 3 Occurrence of a symptom on apple 3 days after artificial inoculation with visible appearence of numerous acervuli.

Fig.2. Occurrence of a symptom on apple 10 days after artificial inoculation.

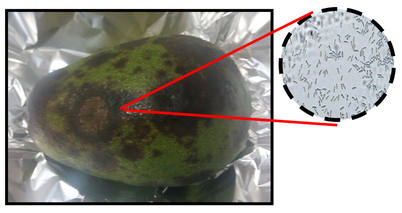

Fig. 4 Occurrence of a symptom on avocado 6 days after artificial inoculation

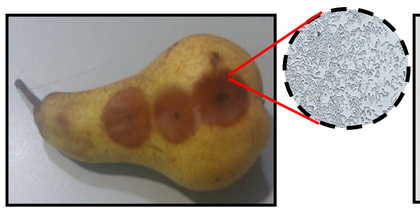

Fig. 5 Occurrence of a symptom on pear, 6 days after artifial inoculation

Fig. 6 Absence of symptoms in pepper, 6 days after artificial inoculation

Results and discussion

In November 2019 symptoms of bull's eye were observed on apple fruits grown in the region of Berovo and stored for two months. Symptoms appeared as advanced fruit antracnose with numerous acervuli. Under optimal conditions in storage, a bright orange spore masses occurred (Fig 1). The primary production of conidia is in acervuli. Conidia usually have both end rounded (Fig. 7, Fig. 8). Seta were not observed. Strains exhibited high variability in colony color and spore formation. Two groups of isolates were observed after the single spore isolation. The first group look like typical *Colletotrichum* isolates, appearing white at the beginning ant than turned to pink and gray on the upper side and orange-pink on the reverse side. The formation of conidiomata was observed very early 5th day of incubation on 25 ^{0}C. Conidiomata are single or aggregate (Fig 13, left). Second group doesn't produce mycelium only grayish-black formation of conidia which occasionally produce pink mases in the center (Fig 13, right) Although the two groups of isolates appeared very differently it was observed that conidia which originate from the orange masses (Fig ?) produce isolates on PDA as in the first group. When conidia which originate from young black conidiomata without orange masses are grown on PDA, isolates as the second group developed. Germination of conidia appear very fast on the one or on the both sides of the conidia after several hours. Conidia and mycelium formed melanized appressorium (Fig. 10(d) and Fig.11). When conidia germinate a septa developed and the young non septed conidia become one septed (Fig 10 (c)). Asexual recombination was observed between conidia by forming anastomosis tubes (Fig 9). Teleomorphic stage was not observed.

Conclusion

Studies on apple anthracnose and *Colletotrichum spp.* have shown that due to the overlapping morphological characters, species delimitation based on morphology alone is hardly possible in *Colletotrichum*. However, the precise determination of the etiology of Colletotrichum diseases requires more than just the isolation and identification of a Colletotrichum species from plant tissue.Multilocus sequence analyses combined with a polyphasic approach including the analysis of geographical, ecological and morphological data is generaly suggested for species differentiation within the genus.