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SHORT COMMUNICATION

First Report of Olive Anthracnose, Caused by *Colletotrichum gloeosporioides*, in Morocco

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Abstract

Ripe and overripe olive fruits (Picholine marocaine) showing circular spots of 5 to 20 mm in diameter, slightly depressed and reddish-brown in color, were collected from two orchards located in the regions of Ouazzane in Morocco. *Colletotrichum gloeosporioides* was isolated from symptomatic fruits and Koch's postulate was fulfilled. According to the literature, this is the first report of *Colletotrichum gloeosporioides* causing anthracnose in olives in Morocco.

Key words: *Olea europea*, Picholine marocaine, *Colletotrichum gloeosporioides*, Morocco.

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Introduction

Olive (*Olea europaea* L.) is subjected to be attacked with a variety of fungal pathogens, which affect its health, yield and its oil quality (Sanei et al., 2011). Anthracnose caused by *Colletotrichum acutatum* J.H. Simmonds and *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (syn. *Gloeosporium olivarum* Alm.), widespread disease of olives in most olive-growing regions in the world, causing pre-and post-harvest problems (Sergeeva et al., 2008).

The disease was first reported in Portugal. Subsequently, it was reported in the Mediterranean countries such as Italy (Ciccarone, 1950; Agosteo et al., 2002; Moral et al., 2008), Spain (Martín et al., 2002), and Serbia and Montenegro (Latinovic and Vucinic, 2002), and Tunisia recently (Rhouma et al., 2010). The disease has also been reported in other countries such as Japan, Uruguay, Argentina, Brazil, South Africa, California, China, India, Australia and New Zealand (Margarita et al., 1986; Bompeix et al., 1988; Mugnai et al., 1993; Latinovic and Vucinic, 2002; Sergeeva et al., 2008; Duarte et al., 2010;).

Anthracnose symptom was, in most cases found on immature or mature olive fruits (between 50 and 80%)), while in some cases the leaves were also affected in two orchards (10 to 15%) located in the region of Ouazzane (North-East of Morocco).

The aim of the present work was to investigate the etiology of the anthracnose observed in December 2012 on fruit olives.

Materials and Methods

Samples of infected olive fruits were collected from two olive orchards in Ouazzane region (North-East of Morocco), placed in a cooler and taken to the laboratory. (refrigerated at 4 °C). These olives showed a soft circular rot on their surface consisting of slightly depressed reddish brown spots. Such spots expanded to up to 20 mm in diameter and coalesced to form the characteristic circular sunken lesions (Fig. 1).

At the laboratory, portions (5 mm2) of the infected fruits, were removed at the point of progression of disease symptom; cut into small pieces and then soaked into in 10% sodium hypochlorite (NaOCl) for 3 min, rinsed three times with sterile water, dropped after on sterile paper towels before plating them onto Potato Dextrose Agar (PDA) and incubated at 25°C for 10 days. Isolated colonies were, sub-cultured into fresh plates until pure cultures were obtained. Pure cultures obtained were identified by visual examinations and viewing under stereo microscope. They were then described and classified based on conidia and colony morphology as described by Barnett et Hunter Barry (1999).

Figure 1. Olive Fruits showing brown spot symptoms of anthracnose.

Figure 2. Macroscopic aspect of *Colletotrichum gloeosporioides* after ten days on PDA medium.

Pathogenicity Tests

Preparation of Spore Suspension

In order to verify the pathogenicity of the isolated pathogen, ten olives (cv. Picholine marocaine) were surface-sterilized as above and the disinfected fruits were, then rinsed in three changes of sterile distilled water and air before inoculation. Suspension of conidia was prepared by suspending mycelia scraped from 10 days old cultures of pathogens fungi in PDA. The resulting suspension was filtered through 2-layer cheesecloth. The concentration of spore suspension was adjusted to 106 conidia.milliliter-1 using haemacytometer.

Inoculations

The fruits were each, pierced with sterilized needle in one place; each fruit was injected with 25 µl of spore suspension of pathogen, then sealed in moist plastic bags, and incubated for 5 days in a moist chamber. Control fruits were, inoculated with sterile distilled water. Typical anthracnose symptoms were evaluated after 5 days (100%).

Re-isolation of isolated fungal pathogens The causative organism in the diseased parts was re-isolated on potato dextrose agar as described in isolation of pathogen. The characters of the re-isolated pathogens were compared with their original isolates.

Results and Discussion

Obtained isolates on PDA from the affected fruit of olive trees, consistently produced one type of colonies. The cultures contained dense, white mycelium with a few orange conidial masses near the inoculum point (Fig. 2), simple conidiophores with an hyaline ovoid conidia, conidia size is 9-16.65 µm in lengh × 3,33- 5 µm in width (Fig. 3). The waxy acervuli (Fig. 4),

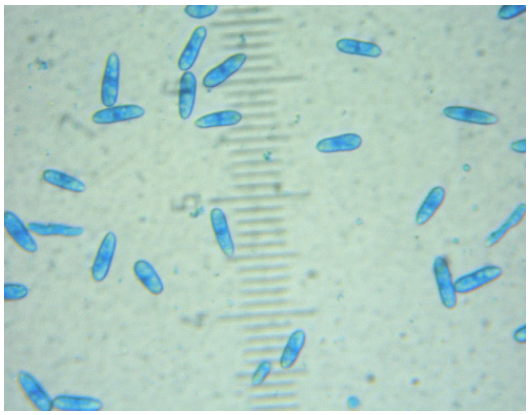

Figure 3. *Colletotrichum gloeosporioides* spores colored with cotton blue × 400.

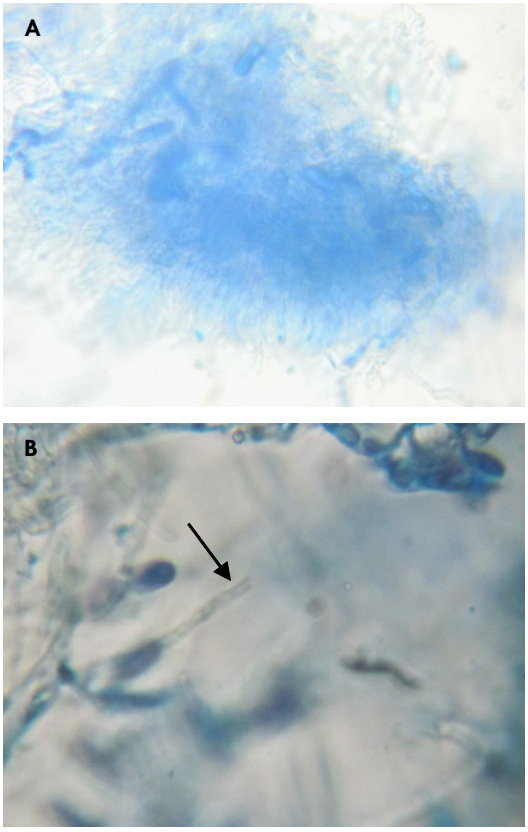

Figure 4. *Colletotrichum gloeosporioides* acervuli (A) with setae (B) colored with cotton blue × 400.

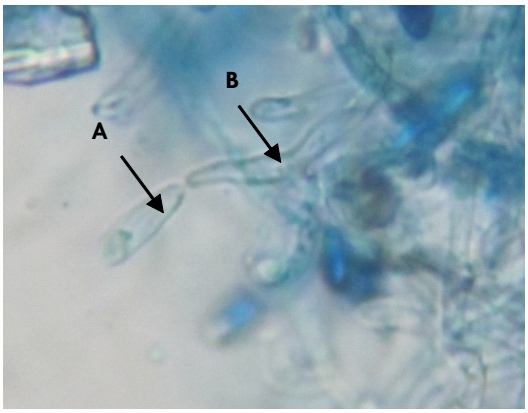

Figure 5. *Colletotrichum gloeosporioides* conidia (A) with a short erect conidiophore (B) colored with cotton blue × 400.

typically with setae, and simple, short, erect conidiophores (Fig. 5). According to these characters and to Barnett key, this species could be Colletotrichum gloeosporioides. The colonization process of olive fruits by Colletotrichum such as spores adhesion and germination on cuticle to form an appressoria (Gomes et al., 2012). C. *gloeosporioides* usually attacks ripe or overripe fruits, and only rarely the leaves, peduncles and shoots. On the fruits, the disease causes soft circular rotted spots, on which slimy orange-colored masses of spores are produced under high humidity.

Figure 6. Typical anthracnose symptoms were observed after 5 days on the olive fruits after their inoculation with C. *gloeosporioides* (b) and No symptoms in olive fruits sprayed with sterile distilled water (a).

The pathogenicity of C. *gloeosporioides* was confirmed by artificial inoculation using a spore suspension (106 conidia.milliliter-1). Symptoms began to appear after 5 days of inoculation (Fig. 6). C. *gloeosporioides* was re-isolated from these lesions fulfilling Koch's postulate. No symptoms were produced in olive fruit sprayed with sterile distilled water.

Although C. *gloeosporioides* was reported on many hosts in different countries of the world (Farr et Rossman, 2011), to our knowledge, this is the first report of C. *gloeosporioides* affecting particularly the olive fruit in Morocco.

Anthracnose caused by C. *gloeosporioides* is one of the most important fungal foliar diseases affecting olive trees and the major disease of olive fruits. Fruit rot and mummification are the most important disease symptoms which result in high acidity and a reduced organoleptic quality of olive oil.

Besides C. *gloeosporioides*, C. *acutatum* also sporadically causes olive anthracnose. The co-occurrence of these two fungi was reported in Spain on a very limited scale (Martín and García-Figueres, 1999). However, Talhinhas et al. (2005) stated that these two species were not seen together on the same plant. With molecular and phenotypic assays, these authors reported that the occurrence of C. *acutatum* in Portuguese olive orchards was higher (>97%) than C. *gloeosporioides* (<3%). *Colletotrichum acutatum* produced orange to pink coloured colonies with whitish aerial mycelium on potato dextrose agar (PDA). Colletotrichum gloeosporioides produced grey colonies with whitish aerial mycelium on PDA (Sergeeva et al., 2008). Also, conidia of C. *gloeosporioides* isolates are cylindrical with obtuse ends and measured 13 to 24 µm in length, whereas conidia of the C. *acutatum* isolates are elliptical-fusiform, tapered and acute at both ends, and measured 13 to 20 µm in length (Gunnell et al., 1992).

In olive-growing areas where anthracnose was endemic, disease control was primarily based on early harvesting in order to escape secondary infections of the very susceptible mature and overripe drupes. Direct control measures involving regular fungicide sprays were used both to prevent defoliation and to avoid yield losses. Aerial spraying of Bordeaux mixture or copper oxychloride had been successfully attempted; two or three

preventive treatments from late September to the end of December proved effective against fruit anthracnose (Martelli and Piglionica, 1961; Graniti et al., 1993; Pennisi et al., 1993).

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