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Short notes

FIRST REPORT OF THE PHYTOPATHOGENIC FUNGUS *ALTERNARIA TENUISSIMA* IN CEDARWOOD (*CEDRUS ATLANTICA* M.) IN MOROCCO

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ABSTRACT

Our research focuses on identifying lignivorous fungus from decayed cedarwood. A sample was taken from Azrou forest's cedar grove, which is a part of Morocco's Ifrane National Park. On a water agar medium first, and subsequently a PDA medium, the isolated fungus was cultured and purified. After the fungus was purified, an optical microscope morphological analysis allowed us to identify the pathogen *Alternaria tenuissima*. These findings were confirmed by a molecular characterisation, which had a coverage rate of 94% and an identity of 94,88%. This is the first report of *A. tenuissima* in decomposing cedarwood that we are knowledge of.

KEYWORDS: *Alternaria tenuissima*, cedar wood, *Cedrus atlantica* M., cubic brown rot (Saboune), red ring rot (M'jej).

INTRODUCTION

Cedar is a highly sought-after tree on a global scale due to the economic value of its wood and the use of its extracts and oils in perfumery and the pharmaceutical industry. There are four species of cedar: the Atlas cedar (*Cedrus atlantica* Manetti) represents the Moroccan and Algerian cedar forests, the Cyprus cedar (*Cedrus brevifolia* Henry), the Lebanon cedar (*Cedrus libani* London), and the Himalayan cedar (*Cedrus deodora* London), which ranges from India to Afghanistan. Unfortunately, many problems, including pollarding, deforestation, over-exploitation, and pest and fungus infestations, have caused a sharp decline in cedar forests

worldwide, which has caused the environment to deteriorate. In addition, the lack of plant and soil cover makes it difficult to mobilize water supplies and silting causes dams to fail. The main fungi that attack cedar wood include *Ungulina officinalis, Fomitopsis pinicola, Phellinus chrysoloma,* and *Trametes pini. T*his fungus species cause two cedar diseases: the red ring rot, known in Morocco as ‘M’jej disease’ and the cubic brown rot, known among Moroccan sawyers as "Saboune disease" (Aberchane et al. 2003, Chauiyakh et al. 2022, Isikov 2020). This affected wood is a crucial source of essential oils used in perfumery and synthesising some bioactive compounds (Adams 1991, Jaouadi et al. 2021, Ninich et al. 2022).

The objective of this study is the morphological and molecular identification of other parasitic fungi of cedar in the cedar forest of Azrou in the National Park of Ifrane-Morocco.

MATERIALS AND METHODS

Raw material

Samples in the form of small pieces of 1 to 2 cm of decomposed wood were taken from the trunks of cedar trees (*C. atlantica*) visually infected by the two diseases at the forest of Azrou in the Ifrane national park in Morocco (Fig. 1). This fungus was isolated from a dead tree trunk infected with red ring rot (M'jej) at 33°25'14"N,5°04'22"W.

Fig. 1: Trunk of Cedrus atlantica visually infected in the core (Azrou Forest 33°25'14"N, 5°04'22"W).

Culture, purification morphological and molecular identification

The isolation, purification, morphological and molecular identification of this fungus was done according to the protocol described by (Chauiyakh et al. 2022) in another article in order to identify another lignivorous fungus of cedar.

Phylogenetic analysis

The sequences derived from ITS were concatenated into combinatorial sequences using DNA baser assembler, multiple sequences were compared using MUSCLE algorithm of MEGA 11. The phylogenetic tree was constructed using the maximum likelihood method (1,000 replicates).

Pathogenicity test

Koch's postulates were complemented by inoculation and subsequent re-isolation of the fungus from infected cedar wood. Ten pieces of the live stem were inoculated into a wound made in the wood with a scalpel, and mycelial plugs (5-6 mm in diameter) from 10-day-old PDA plates of the isolated and identified fungus were inserted into the wound. Another set of ten pieces of healthy wood was used as a negative control.

RESULTS AND DISCUSSIONS

After five days of incubation at a temperature of 22–26°C, the colonies at a temperature of 22–26°C appear white-grey (60–70 mm in diameter) with a cottony mycelium. Short conidial chains consisting of 4–7 or more conidia were found, often branched, ovoid or ellipsoidal, rarely with a short pale brown, cylindrical or conical beak with smooth or warty walls. The conidiophores were short or elongated, straight, branched, and sometimes superficial. Morphological and cultural characters were consistent with *Alternaria tenuissima*, represented systematically in Tab. 1. The study of (Aktaruzzaman et al. 2015) on the morphology of this fungus confirms our results. It shows that the conidiophores are branched, straight, measuring 23.7–40.2 um long and 3.6-5.0 µm thick, and that the conidia are short chains composed of 3–7 or more conidia, occasionally branched, dark brown, and size of conidia varied from 12.5-48.0 µm in length and 8.1-14.4 µm in width with a short-tapered beak (1.6-3.2 µm) or no beak (Fig. 2).

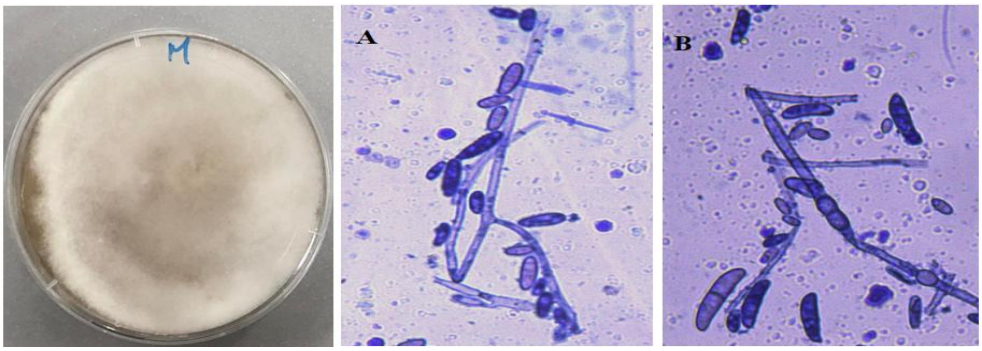

Fig. 2: Alternaria tenuissima: a) colony on PDA medium after 5 days of incubation at 22-25°C, b) mycelium of the fungus with different forms of phialides and conidia, c) conidias. (Microscopic observation ×40).

The blast of the DNA sequence obtained after the assembly of the sequencing results allowed us to identify *A. tenuissima*, with a coverage percentage of 94% and a similarity of 94.88%. The phylogenetic tree (Fig. 3) showed that, based on the nucleotide sequence of the ITS regions, *A. tenuissima* identified in our study clustered in the same clades with other sequences of *A. tenuissima* downloaded from the NCBI database and identified by their accession code, with percentages of 99% and 100%. However, other species are classified in other clades such as *A. alternata, A. ventricosa, A. conjuncta.* After inoculation, eight pieces inoculated with mycelium plugs, showed necrosis with reddish spots. *A. tenuissima* was

successfully reisolated from the necrotic areas of wood inoculated with mycelium plugs (Fig. 4).

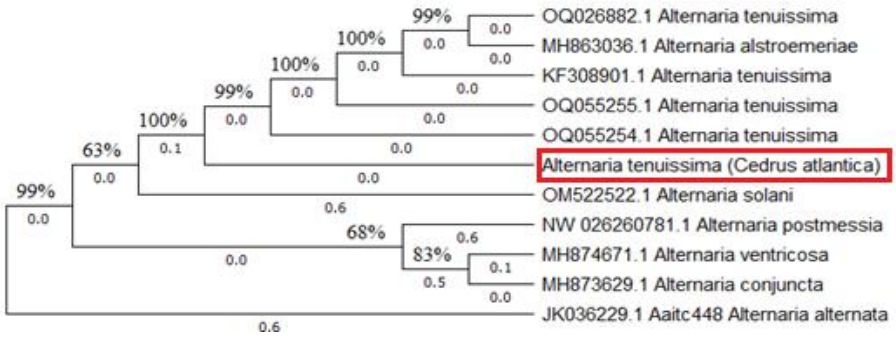

Fig. 3: Phylogenetic tree based on ITS fragment from Alternaria strains and constructed using the maximum likelihood method.

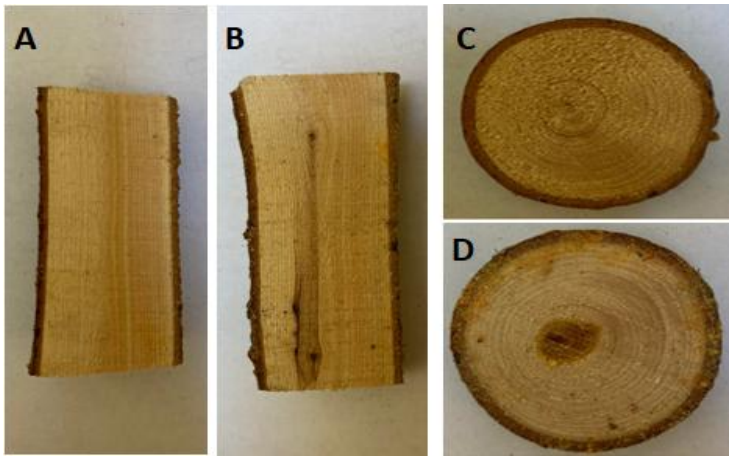

Fig. 4: Results of the pathogenicity test; a) and c) controls; b) and d) necrotic lesions affecting the heartwood of the stems.

Alternaria genus is known for its pathogenicity and the degradation of different plants; this fungus produces metabolites like mycotoxins capable of degrading the different parts of plants. Its species and mycotoxins have been isolated from a wide range of foods, such as cereals, fruits, vegetables, and their derived products (Del Mondo et al. 2022, Patriarca 2016). In South Africa, *A. tenuissima* and other species of *Alternaria* were reported in the core rot of apples by producing secondary metabolites that degrade apple's core (Pavicich et al. 2020, Serdani et al. 2002). In China, it causes tuber rot in stored potato, leaf spot on *Dioscorea zingiberensis*, the blight disease on Chinese yam (*Dioscorea polystachya* Turczaninow), and the leaf black spot on pecan (*Carya illinoinensis*), which represents an essential fruit in the China markets with significant economic effect. Disease incidence was approximately 40% in 2019 and 50% in 2020. Small, dark-brown to black spots on leaves were observed initially, and spots expanded quickly into circular or irregular patches when spots coalesced. In severe cases, the disease can obviously weaken tree vigor, ultimately leading to losses in yield. Disease symptoms were not observed on the fruit (Liu et al. 2019, Qian et al. 2022, Yan et al. 2022). This fungus was also reported as leaf spot in *Datura metel* and Black chokeberry (*Aronia melanocarpa*) in Korea in 2015, which causes circular to irregular brown or light

brown spots ranging from 4 to 11 mm on the leaf (Aktaruzzaman et al. 2015, Wee et al. 2016). The degradation of the leaves is among the leading causes of the plant's decline since these leaves are the primary unit of photosynthesis. This fungus isolated from a trunk of cedar causes severe damage, such as the deperissement of the plant, by degrading the cellulose, which represents the primary compound of trunk wood. Cellulose is a polysaccharide composed of monomers β-glucose present in plant cells; it provides rigidity and structure to the cell.

According to other previous studies, the fungal pests have also affected the cedar groves around the world, such as *Pleurostoma richardsiae* and *Paecilomyces maximus* in a first report by (Chauiyakh et al. 2022, Chauiyakh et al. 2023), *Phellinus chrysoloma*, *Ungulina officinalis*, *Trametes pini*, *Coniophora puteana* and *Porodaedalea pini* (Aberchane et al. 2003, Zaremski et al. 2007). The first signs of infection are the appearance of a pale yellow to brownish discoloration of the wood. In the advanced stage of the attack, the wood takes on a brown and cubic appearance and crumbles over time into cubic pieces. This brown rot is typical; only the cellulose is degraded. Fungi can cause heart rot in living trees, but their prominent role is to decompose the wood of trees killed by other pathogens. Infection usually begins via a wound already present on the tree. Since the fungi mostly colonize dead material, there can be many sites of infection. When the disease is well established, small spindle-shaped cells of white tissue, characteristic of the disease, become visible (Chauiyakh et al. 2022, Sarkhad et al. 2022, Wang et al. 2021).

To biocontrol *A. tenuissima*, (Liu et al. 2019) used chitosan. It has been suggested as an environmentally acceptable substitute for synthetic fungicides for treating postharvest diseases on agricultural commodities. It is a naturally occurring polysaccharide called poly-β-(1→4) N-acetyl-D-glucosamine. The essential oils can also be an ecological solution to limit or stop the development of pathogenic fungi like lignivorous fungus (Chauiyakh et al. 2022).

CONCLUSIONS

Alternaria tenuissima is a phytopathogenic fungus that causes wood and leaves dieback. Several studies have reported it as an aggressive and pathogenic agent of apple trees in South Africa, as well as a pathogenic agent of potato, pecan in China, leaf spot in *Datura metel* and Black chokeberry (*Aronia melanocarpa*) in Korea. This fungus produces metabolites like mycotoxins capable of degrading the different parts of plants. Our study identified this fungus in a sample isolated from a dead and depleted cedar trunk, which shows symptoms of severe infection by a disease known as M'jej in Morocco. This disease causes the decay of the cedar wood, which leads to losses of 40% of the wood. To our knowledge, this is the first report of *A. tenuissima* in cedarwood.

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