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Short notes

FIRST REPORT OF THE LIGNIVOROUS FUNGUS *PAECILOMYCES MAXIMUS IN CEDRUS ATLANTICA* M. IN MOROCCO

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ABSTRACT

This study is interested in isolating fungi from the wood parts of cedar trunks withered and identifying these lignivorous fungi. A sample was isolated from the cedar grove of Tazekka National Park located south of the city of Taza in Morocco. The culture and purification of the isolated fungus were done on a water agar medium and then on a PDA medium. After the purification of the fungus, a morphological study by scanning electron microscope allowed us to identify the pathogen *Paecilomyces*. A molecular characterization identified *Paecilomyces maximus* with a coverage percentage of 99% and an identity of 98.77%. To our knowledge, this is the first report of ***P. maximus*** in decayed cedar wood.

KEYWORDS: *Cedrus atlantica* M., cedar wood, *Paecilomyces maximus*, red ring rot, M'jej, cubic brown rot, Saboune.

INTRODUCTION

Cedar is among the most sought-after plants in the world due to its wood quality, socio-economic interest, and medicinal and cosmetic uses through its oils and extracts in phytotherapy and perfumery. There are four species of cedar: *Cedrus atlantica* Manetti, known as the Atlas cedar and which represents the Moroccan and Algerian cedar forests, the Lebanon

cedar (*Cedrus libani* London), the Cyprus cedar (*Cedrus brevifolia* Henry), and the Himalayan cedar (*Cedrus deodora* London). The systematic position of this plant is represented in Tab. 1.

Tab.1: Taxonomical classification of Cedrus (Chaudhary et al. 2011).

Kingdom	*Plantae*
Subkingdom	*Tracheobionta*
Division	*Conoferophyta*
Class	*Dinopsida*
Ordrer	*Pinales*
Family	*Pinaceae*
Genus	*Cedrus*
Species	*C. atlantica Manetti, C. deodara London, C. libani London, C. brevifolia Henry*

Cedar groves worldwide suffer from attacks by several pests that cause damage to the roots, trunk, branches, and fruit. These attacks sometimes cause the death of the plant, as well as the decay of the wood. Parasitic pests of cedar were documented as follow: Leaf and needle processionaries, such as *Thaumetopoea bonjeani* (Linares et al. 2013), *Thaumetopoea pityocampa* (Bouzar-Essaidi et al. 2021, Sbabdji and Kadik 2011), and *Thaumetopoea* (Alptekin et al. 1997). Needle leafrollers caused by *Acleris undulana* (Erler et al. 2010), *Epinotia cedricida* (Fabre et al. 2001). Aphids on young twigs and needles caused by *Cedrobium laportei* (Fabre et al. 1989), *Cinara cedri Mimeur* (Ülgentürk et al. 2020). The scolytes *Phloeosinus cedri* (Beghami et al. 2020) attack the branches and the trunk, and *Phloeosinus acatayi Scheldl, Orthotomicus tridentatus Egg, Orthotomicus erosus (Wollaston), Xyloterus lineatus Olivier,* that attack the Xylem of the plant (Avci and Sarikaya, 2009, Fabre et al. 2001, Schvester 1986, Skrzecz et al. 2015), the beetles *Phaenops (Melanophila) marmottani* (Mouna, 2009) and the Cerambycide *Ergates faber* (Gales et al. 2018) that attack the trunk of the tree. The chalcidien *Megastigmus schimitscheki Novitzky* (Alptekin et al. 1997) attack the seeds.

Two primary diseases of cedar are related to attacks by fungi that cause wood decay and tree dieback. Red rot caused by *Trametes pini* or *Phellinus chrysoloma* (Hakam et al. 2017) and cubic brown rot caused by *Fomitopsis pinicola* or *Ungulina officinalis* (Aberchane et al. 2003). In Morocco, these damages cause losses estimated by more than 30% of the national production of cedar wood (Chauiyakh et al. 2022, El Yousfi 1994, Lanier 1994).

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

MATERIALS AND METHODS

Study area and biological material

The National Park of Tazekka is located in the municipality of Taza in Morocco, bounded by the Middle Atlas in the South, the Rif in the North, the plains of Gharb in the West, and

Guercif in the East. It was created in 1950 on an area of 680 ha, and now it covers an area of 13737 ha. The cedar grove is located on the summit of Jbel Tazekka and occupies an area of over 800 ha. 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 (Fig. 1). This fungus was isolated from a dead tree trunk infected with red ring rot (M'jej) at 34°05'41"N,4°10'37"W.

Culture and purification

The samples were deposited after their collection in Petrie dishes containing only agar water (20 $g \cdot L^{-1}$ of agar-agar); the latter is a minimum medium to inhibit bacterial growth. After the mycelium's appearance, the fungal strains' purification was done by subculturing in malt-agar medium (30 $g \cdot L^{-1}$ of malt + 20 $g \cdot L^{-1}$ of agar-agar + 50 $mg \cdot L^{-1}$ of the antibiotic chloramphenicol). The antibiotic is used for the inhibition of the bacterial growth. In 100 mL of a liquid medium containing 5 $g \cdot L^{-1}$ of yeast extract + 5 $g \cdot L^{-1}$ of malt extract + 5 $g \cdot L^{-1}$ of sucrose + 50 $mg \cdot L^{-1}$ of chloramphenicol, the obtained strains were seeded to ensure an excellent mycelial growth. The mycelium obtained after one week was lyophilized in Eppendorf tubes for preservation and DNA extraction.

Fig. 1: Decayed cedar tree (Tazekka National Park), a) decayed tree; b) white mycelium visible on the trunk.

Morphological characterization

From a Petri dish containing the purified fungus, a fragment of the mycelium was taken and dried in the oven at a temperature of 60°C for 2 to 3 hours. After drying, the piece of the fungus was observed by scanning electron microscope.

DNA extraction and PCR

From 20 to 30 g of the mycelium, DNA was extracted by the Bioline ISOLATE II Plant DNA Kit, BIO-52070. A NanoDrop measured the purity of the obtained DNA. The ITS sequences of the rDNA genes were amplified by the two universal primers ITS1 (5' -TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The reaction mixture volume of 25 µL consists of 1 µL of DNA, 0,5 µL of dNTP, 0.5 µL of Taq DNA polymerase, 5 µL of Buffer, 1.25 µL of each ITS primer, and 15.5 µL of ultra-purified water. The amplification was under the following conditions: a denaturation phase at 95°C for 2 min, a 35-fold cycle (denaturation phase at 95°C for 30 s, hybridization phase at 57°C for

30 s, and elongation phase at 72°C for 30 s), with a final extension at 72°C for 1 min. After amplification, a 1% agarose gel electrophoresis was performed to check the quality of the PCR product obtained.

PCR product purification

The Purification Kit (ExoSAP-IT™ PCR Product Cleanup Reagent) was used to purify the PCR product by adding to 5uL of DNA (PCR product) 1 uL of EXO-SAP-IT, the thermocycler program is 37°C/15 min to activate the enzymes, and 80°C/15 min to inactivate these enzymes.

Sequence reaction

BigDye™ Terminator v3.1 cycle sequencing kit was used in the sequence reaction by adding to 1 uL of purified PCR product the following reagents: 2 uL of BIG DYE Seq 3.1, 2 uL of 5X Buffer, 1 uL of Primer (10μM), and 4 uL of ultrapure H_2O. The conditions for the sequence reaction were as follows: 96°C/1 min, 25-fold cycle (96°C/10S, 50°C/5 s, 60°C/4 min). The purification of the sequence reaction was done following the manual of the Xterminator SAM solution kit.

Sequencing and bioinformatics analysis

Sequencing was done using an Applied Biosystems 3130xl Genetic Analyzer Sanger sequencer. The DNA Baser Assembler software did the assembly of the results. The ITS sequences obtained were blasted with the NCBI nucleotide library collection.

RESULTS AND DISCUSSION

Colonies multiply on PDA medium after 3 to 5 days of incubation at 24 ± 2°C in the dark. They are fast growing, tufted or funiculose, powdery and yellow-white or sand coloured. They appear with a white-yellowish coloration with a light green center. At the bottom of the colony, the mycelium appears white and has a yellowish coloration under the green colony; its diameter after five days of incubation was 6 cm. Condiophores bearing dense, vertillately arranged branches bearing phialides, that are cylindrical or ellipsoidal, tapering abruptly into a long and cylindrical neck. Conidia are subspherical, ellipsoidal to fusiform, smooth-walled, 4 × 2 μm, and are produced in long divergent chains (Fig. 2).

The morphological characteristics of this colony are the same as the genus *paecilomyces,* this genus is an anamorphic form of *Byssochlamys* which is characterized to have branching on the conidiophores, widening phialids on the lower front and narrow elongated ends, as well as producing ellipsoidal or cylindrical chained conidia with olive brown color (Houbraken et al. 2020). A similar finding was reported by Dong et al. (2012) who identified fungi with phylogenetic results closer to the genus *Paecilomyces* (Nandika et al. 2021).

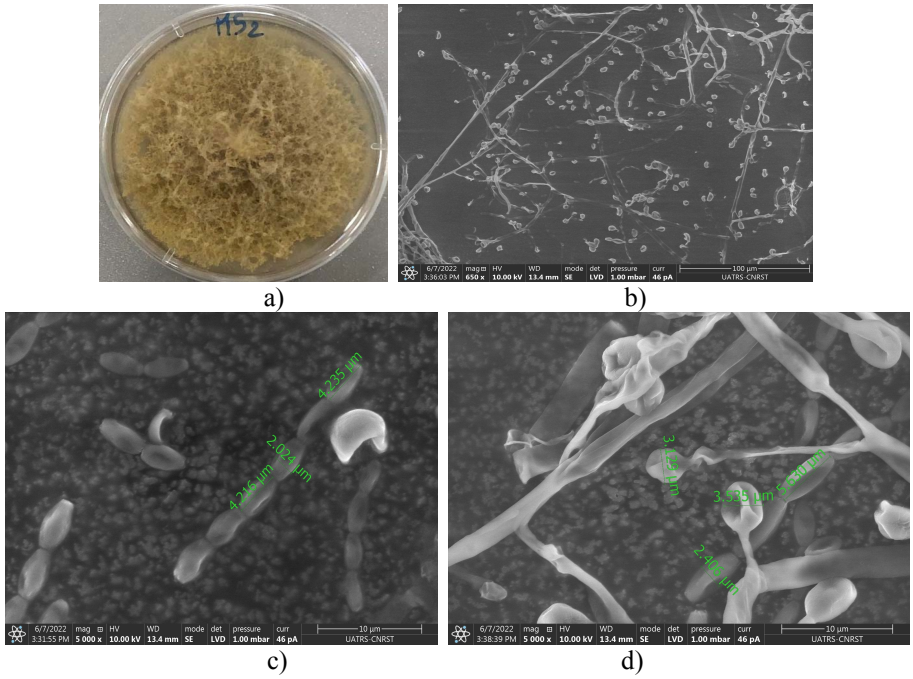

Fig. 2: Scanning electron microscope observation of Paecilomyces maximus, a) macromorphology on PDA after five days of growth; b) mycelium structure and conidiophores; c) conidia in chains; d) phialides and conidia.

The blast of the DNA sequence obtained after the assembly of the sequencing results allowed us to identify *Paecilomyces maximus*, with a coverage percentage of 99% and a similarity of 98.77%. Tab. 2 shows the systematic classification of this fungus.

Tab. 2: Taxonomical classification of Paecilomyces maximus.

Kingdom	*Fungi*
Subkingdom	*Dikarya*
Division	*Ascomycota*
Subdivision	*Pezizomycotina*
Class	*Eurotiomycetes*
Subclass	*Eurotiomycetidae*
Order	*Eurotiales*
Family	*Thermoascaceae*
Genus	*Paecilomyces*
Species	*maximus*

Paecilomyces maximus is known by *Paecilomyces formosus* (Nandika et al. 2021). This species is known for its pathogenicity and its effect on animals and plants' health. Humans are rarely exposed to this infection (Batarseh et al. 2020). For instance, in 2015, the first case of a child infection was confirmed. Symptoms like having brownish-yellow patches on his back's skin extending to the buttocks shown just after six days of his birth. Fine nodules in another patient with chronic granulomatous disease (CGD) and fibrosis of the lungs was reported at that

time (Kuboi et al. 2016, Heshmatnia et al. 2017). Moreover, other species such as *Paecilomyces variotii* and *Purpureocillium lilacinus* are said to have a significant effect on immunocompromised humans who suffer from dermatitis, ocular infections, pneumonia, disseminated mycosis and peritonitis (Foley et al. 2002, Houbraken et al. 2010).

Additionally, *P. maximus* was reported in 2021 as the causal agent of rubberwood (*Hevea brasiliensis*) in Palembang, South Sumatra Province, Indonesia (Nandika et al. 2021), as well as in Turkey and Iran as the causal agent of Pistachio dieback in more than 400 ths hectares of Iranian pistachio orchards. This disease is characterized by dieback of twigs and branches, necrosis and darkening of bark and wood tissues, gummosis and canker lesions of *Pistacia vera L.* (Heidarian et al. 2018, Ozan et al. 2022). In this context, after the evaluation of the antagonistic effect of two strains of *Streptomyces misionensis* on *Paecilomyces formosus*. Torabi et al. (2019) has shown that these two strains of *S. misionensis* known for their biocontrol powers, inhibited the growth of the fungus *P. formosus*. This latter is declared to be the major causal agent of pistachio Dieback.

According to other previous studies, the fungal pests have also affected the cedar groves around the world, such as *Pleurostoma richardsiae* in a first report by Chauiyakh et al. (2022), *Phellinus chrysoloma*, *Ungulina officinalis*, *Trametes pini*, *Coniophora puteana* and *Porodaedalea pini* (Aberchane et al. 2003, Zaremski et al. 2007, Chauiyakh et al. 2022).

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 (Wang et al. 2021, Chauiyakh et al. 2022, Sarkhad et al. 2022).

CONCLUSION

Paecilomyces maximus is a lignivorous fungus that causes wood dieback on trunks and twigs. Several studies have reported it as an pathogenic and aggressive agent of *Pistacia vera L.* in Iran and Turkey, as well as an agent of rubberwood (*Hevea brasiliensis*) in Indonesia. 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 or Saboune 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 *P. maximus* in cedarwood.

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