First report of *Pythium schmitthenneri* on olive trees and in Morocco

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Received: 22 October 2021 / Accepted: 26 January 2022 / Published online: 4 February 2022
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Abstract

In a grove in north-eastern Morocco in 2020, severe root rot and crown rot symptoms were noticed on olive trees (*Olea europaea*). *Pythium schmitthenneri* was identified as the pathogen based on conidia morphology and cytochrome oxidase subunit II (COXII) gene sequence analysis. Koch's postulates were verified by re-isolation of the fungus *P. schmitthenneri* from artificially inoculated olive seedlings after a pathogenicity test. This is the first time *P. schmitthenneri* has been identified as the cause of olive tree root and crown rot in Morocco.

Keyword *Olea europaea* · *Pythium schmitthenneri* · Sequence analysis · Pathogenicity

The olive tree (*Olea europaea*) is one of the Mediterranean Basin's oldest and most traditional crops, and it was one of the first plants to adapt to the Mediterranean environment (Fraga et al. 2021). Olive cultivation supports the economies of Mediterranean countries (Caselli and Petacchi 2021), including Morocco, which is among the top countries in terms of olive output and area (Anonyme 2019; Harbouze et al. 2019, 2021).

Olive trees, like all crop plants, are susceptible to a variety of pest and disease problems that can result in major consequences and financial losses by causing tree death (Chliyah et al. 2017). Severe root rot and crown rot symptoms (Fig. 1) were noticed on olive trees inside a grove in Ain Blouz, Fes Prefecture (North-eastern, Morocco), in February 2020. The disease's causative agent was effectively identified from affected roots using cornmeal agar (CMA). Small sections of root tissues

were disinfested in 2% sodium hypochlorite for 2 min, then rinsed three times in distilled sterile water (DSW) for 30 s, plated on CMA, and incubated for four days at 25 °C. Single spore cultures produced from the isolates were sub-cultured on potato dextrose agar (PDA) to identify the pathogen. White colonies with non-septate hyphae grew from these cultures (Fig. 1). Sporangia were globular or sub-globular with/without papilla and measured $15.13 \times 33.44 \mu\text{m}$ on V8 medium after 7 days of incubation (Fig. 1). The shape of the hyphae and conidia, as well as the size of the spores, were similar to *Pythium* sp. (Ellis et al. 2012).

Two isolated fungus was identified at the species level using sequencing analysis of the cytochrome oxidase subunit II (COXII) gene. Total DNA was isolated from each fungal isolate's mycelium using the Doyle and Doyle (1990) protocol. Total DNA was used in the polymerase chain reaction (PCR), which amplifies 563 bp of the highly conserved COXII gene using the primer pair FM66/FM58 (Martin 2000). Sanger sequencing was used to determine the nucleotide sequences of two PCR amplicons. MZ466379 and MZ466380 are the accession numbers for these amplicons in GenBank. Both isolates clustered with *Pythium schmitthenneri* (GenBank Accession Number JM895530) according to phylogenetic analysis, with a 100% bootstrap value, they were also clustered with *Pythium hypogynum* and *Pythium acrogynum*, as these species are closely related to each other (Ellis et al. 2012; Senda et al. 2009) (Fig. 2), moreover, temperature growth experiment have also

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Fig. 1 *Pythium schmitteneri* causing root rot on olive trees (*Olea europaea* L.). **A.** Decline symptom observed on an olive tree in a grove in north-eastern Morocco, **B.** Morphology of 6-days-old colonies of *P. schmitteneri* grown on potato dextrose agar at 25 ± 1 °C in the dark, **C, D.** sporangia and hyphal structure, Bars = 10 μ m

confirmed the identity of our isolates as *P. schmitteneri*, which is the only one species able to grow at temperature below 4 °C as previously reported by Ellis et al. (2012). Both PH1 and PH2 isolates have been deposited in the PhytoENA-Meknès-Microorganisms Collection (CMENA) under the accession number CMENA01 and CMENA02 respectively. As a result of morphological traits, temperature experiment and sequence analysis of one gene, the COXII, the pathogen was identified as *P. schmitteneri*.

The above two isolates were utilized individually to artificially inoculate healthy one-year-old olive seedlings for pathogenicity assays (Santilli et al. 2020). One-year-old olive seedlings were transplanted into free-draining pots with sterile soil and inoculum. The latter involved a 21-day culture of the isolates in darkness at 25 °C in pots containing a sterilized medium composed of 50 g wheat seeds and 50 mL V8 juice. Plants in the healthy controls were transplanted into pots with sterile soil but no inoculum. After transplantation, all of the plants were kept in wet soil for 48 h before getting moved to the greenhouse room and kept at 25 °C. This experiment was carried out twice using 5 olive saplings per treatment.

All inoculated plants exhibited significant root rot, leaf chlorosis, defoliation, wilting, and eventual mortality six weeks following inoculation (Fig. 3), whereas controls remained symptomless. The pathogen was re-isolated and morphologically characterized from infected roots. Koch's postulates were met by the technique outlined above. *Pythium* sp. is a fungus that affects a wide range of plants all over the world. Several *Pythium* species have been associated with olive tree root rot in previous research (El-Morsi et al. 2009; Hernández et al. 1998; Hiar et al. 2017; Shaima and Elkanzi 2021). Chliyah et al. (2014) described the symptoms of root rot and wilt diseases on olive plants and concluded that they are caused by *Fusarium* species. This is the first report of *P. schmitteneri* as the cause of root rot and crown rot on olive trees in Morocco that we are aware of its impact. To ensure proper management of fungal diseases widespread in Moroccan olive orchards, greater efforts are undoubtedly required to study species diversity, community composition, and disease incidence caused by *P. schmitteneri* and other similar species.

Fig. 2 Phylogenetic analysis of 8 *Pythium* (two of this study GenBank under the Accession Numbers MZ466379 and MZ466380), 7 *Phytophthora*, and 3 *Phytophthora* isolates based on cytochrome oxidase subunit II sequences using MEGA X. The tree was constructed using the maximum likelihood method. Numbers on the branches represent bootstrap values

Fig. 3 Wilt of a rooted olive seedling in a pot (on the left) artificially inoculated with *Pythium schmitthenneri* Ph1/ Ph2 through the soil, 6 weeks after transplanting into infested soil (Ph1 and Ph2), and an uninoculated olive seedling (on the right)

Acknowledgements The authors wish to acknowledge the olive farmers of Meknes-Fes for providing them with diseased plants.

Funding This study was supported by the Phytopathology Unit of the Department of Plant Pathology- Ecole Nationale d'Agriculture de Meknès. This Research was financially supported by MESRSI under PRIMA Project Section 2, SIRAM.

Data availability statement The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors of this work declare that no conflict of interest.

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