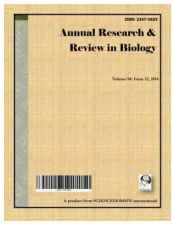

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New Appearance of *Phytophthora palmivora* as a Pathogen of the Olive Trees in Sidi Kacem Region (Morocco)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study aims to study and evaluate the presence and geographical distribution of *Phytophthora palmivora,* the causal agent of the olive trees wilting in Morocco. In 2015 surveys, carried out in Sidi Kacem region, made it possible to note the presence of olive trees showing symptoms of dieback. The diseased samples were taken from these trees in order to determine the causative agent of the disease. *P. palmivora* was isolated and the Koch's postulate was verified. The pathogenicity of one isolate, selected among other isolates was studied. After five months of inoculation, this isolate proved able to induce different types of symptoms in olive plants: growth reduction in the vegetative and the root parts and the number of Leaves and buds. Sometimes the newly formed buds dry up. The values of the growth parameters are very low in the inoculated seedlings, the number of leaves, branches and buds are respectively 101, 12 and 13.5. On the other hand, these values are respectively 338.66, 29.83 and 11.83 in the control plants, the root mass of the Inoculated

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seedlings is also very low (13.1 g) versus 75.1 g for the control seedlings. The fungus was found in different parts of the inoculated olive plants: roots, stems and dried out buds.

Keywords: Olive; Phytophthora palmivora symptoms; Koch's postulates; pathogenicity; Sidi Kacem.

1. INTRODUCTION

In Morocco, the olive tree is susceptible to different types of fungal attacks which can cause serious economic losses. There are pathogens that attack the vegetative part of the olive and fruit [1]. *Cycloconium oleaginum*, the causative agent of the disease 'peacock eye', is responsible for the early fall of leaves of the olive tree [2,3]. *Colletotrichum gloeosporioides*, the causative agent of olive anthracnose, was reported in the olive groves of the Ouazzane region [4]. *Glocosporium olivarum* [2] and *Septoria oleagina* [5] can also attack the leaves. *Pestalotia fici* is considered an agent of fruit rot [1].

The olive tree roots are also subjected to attack by soil fungi: *Verticillium dahliae*, the causative agent of verticillium wilt [6,7] and *Phytophthora palmivora* is the causative agent of rotten roots [8]. These two pathogens are recognized as the responsible agents of the olive groves withering in several Moroccan regions. *P. palmivora*, for example, has been reported in different regions of Morocco: Souk Larbaa, nursery Sidi Taibi Kenitra, Sraghna, Marrakech (Aattaouia, Jaidate and Tassaout) [1].

Surveys, conducted in Sidi Kacem's region, helped locate trees with dieback. Sometimes these types of symptoms are observed in trees of the city. A diagnosis was performed to confirm the causative agent of these dieback and Koch's postulates was applied to verify the pathogenicity of the isolated species.

2. MATERIALS AND METHODS

Surveys at the olive groves of Sidi Kacem's region helped to note the presence of some trees showing dieback (Fig. 1). Samples of dry branches were brought back to the laboratory to isolate the causative agent of decay and fungal species that accompany it. They were cut into small pieces, disinfected with 95% alcohol for five minutes, washed with sterile distilled water, dried on sterile filter paper and then placed in Petri dishes containing the PSA (200 g potato 20 g sucrose, 15 g agar, 1000 mL distilled water) and incubated at 28℃. After seven days, mycelial colonies started to grow. Among the isolated fungal species, there are *Phytophthora* sp. and other fungi. All these species were planted on a planting environment (PSA) for their identification. Determining keys were used to determine the names of fungal species isolated [9,10].

Fig. 1. Wilting symptoms on olive trees in the Sidi Kacem region

The isolation percentage (Pi) of all the isolated fungal species was calculated using the following formula:

$$Pi = NsX/ NT \times 100$$

NsX: Number of segments containing the fungal species X.

NT: Total number of used segments.

The production of the inoculum was performed by planting *Phytophthora* sp. on an Oatmeal Agar plates (30 g of oats, 15 g agar, 1000 ml distilled water) in the dark at 28℃ for 14-21 days. The mycelium was transferred to sterile Petri dishes containing 20 ml of sterile distilled water, incubated overnight at 28℃ in the light. Then, the plates were cooled for 5 min at - 20℃ to induce the release of zoospores. The inoculum concentration was adjusted to 10^5 zoospores / ml. Other fungus was planted on PSA environment.

For *Phytophthora* sp. Koch's postulate has been verified by the inoculation of olive seedlings of the "Moroccan Picholine kind" with an isolate chosen at random from the other isolated. Other olive plants of the same variety were used as control.

The 18 months old olive plants originated from a nursery in Meknes region, are dug up from their substrate. The root portion of the plants is washed under running water to remove the traces of soil, and then it is soaked for 6 hours in a fungal suspension of 10^5 conidia / ml. The same operation was performed to control plants, but the spore suspension was replaced by distilled water. The inoculated plants are replanted in pots containing disinfected soil (2 hours at 200℃) and placed in a greenhouse.

The symptoms induced by *Phytophthora* sp. were assessed four months after inoculation: number of leaves, twigs and buds formed. The effect of the inoculation was also observed on the weight of roots and vegetative masses of plants, evaluated by their weighing in comparison with those of control plants. The Re-isolation of *P. palmivora* is made from plants inoculated four months after inoculation. To do this, root fragments, stem and branches of 2 cm in length are recovered, the sample is taken after every 10 cm over a length of 70 cm from the collar. These fragments are disinfected in a sodium hypochlorite solution at 5% for 2 minutes, transferred in 70% ethanol for 30 seconds and rinsed with sterile distilled water. Each fragment is cut into 10 small fragments which are planted in the PSA environment and amended with streptomycin (100 mg / ml). The Plates are incubated at 28℃. After 10 to 14 days of planting, colonies are identified.

3. RESULTS

Collected isolates of *Phytophthora* sp. were identified according to morphological criteria [11, 12]. They form chlamydospores and sporocysts from ellipsoid to egg shaped. The sporocysts are papillate, and can be distinguished from those of other species of *Phytophthora*. Indeed, they are obsolete and have a short pedicel. The chlamydospores are intermediate and terminal (Fig. 2). The organs of sexual reproduction were not observed (the species is heterothallic). These characters are those of *Phytophthora palmivora* [8,10,13].

On PSA medium, colonies appeared four to six days around the stem segments and roots from diseased olive trees (Fig. 3). Microscopic observations have allowed identifying several fungal species. The most encountered are *Phytophthora* sp. (P = 64%), *Alternaria alternata* (P = 20.6%), *Aspergillus niger* (P = 7.3%) and *Fuzarium solani* (P = 4.5%) and other species (Pi = 3.6).

P. palmivora isolate used as the inoculums proved able to induce different types of symptoms in olive plants of the variety 'Picholine Marocaine': necrosis, buds withering, defoliation, rotten roots, vascular browning, wilting, degradation, and reduced root system (Figs. 4 and 5).

The percentage of branches showing wilting is 47%. *P. palmivora* could also disrupt the emission of leaves and twigs in inoculated plants, respectively about 101 and 12. In control plants, the number of leaves and twigs are respectively 338.6 and 29.8. The average root mass in inoculated plants is 13.1 g and that of the control plants 75, 1 g (Fig. 6).

Similarly, *P. palmivora* was re-isolated from the roots and from other higher parts of inoculated olive plants. Re-isolation percentages were 55 and 68 respectively from the roots and buds. The fungus was also re-isolated from the bark of the inoculated plants.

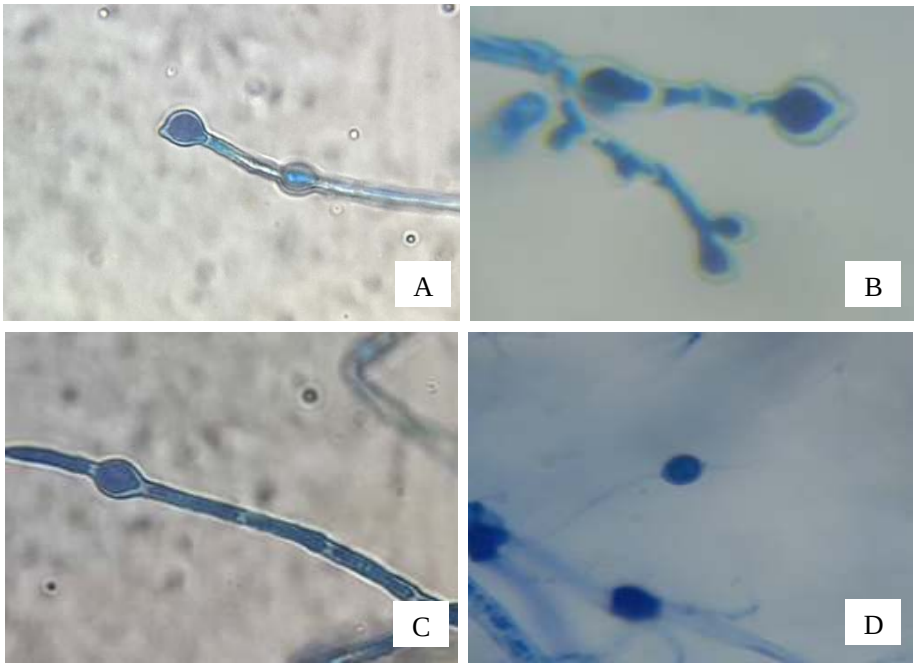

Fig. 2. Morphological characterization of *Phytophthora palmivora*. Papillate sporangia (A and B), Intermediate chlamydospore (C), Terminal chlamydospore (D) on cotton blue (×400)

Fig. 3. *Phytophthora palmivora* colony isolated from leaf petioles of the inoculated olive plants

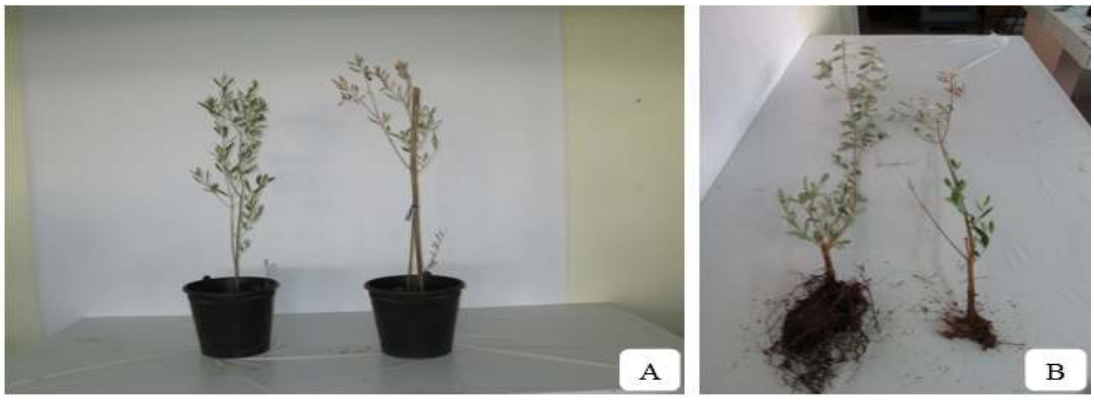

Fig. 4. Symptoms of wilting, yellowing and defoliation of the aerial part (A), root parts of the witness plant and the inoculated plant (B)

Fig. 5. Browning of the root vessels

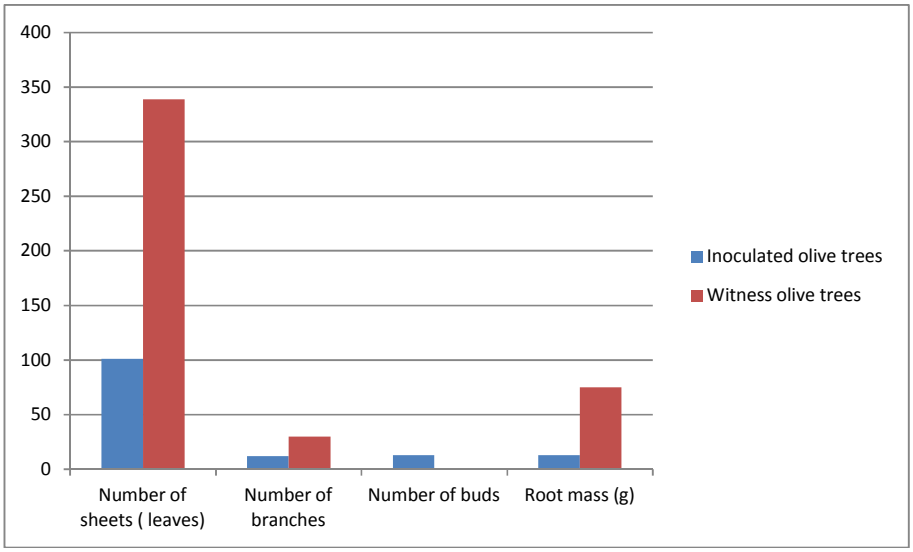

Fig. 6. Comparison between the trees inoculated with *Phytophthora palmivora* and control olive trees on the level of the aerial and root parts

4. DISCUSSION

Phytophthora palmivora was reported for the first time on the olive tree in Morocco in 2013. After observing the symptoms of the withering crown, rotten roots, and the defoliation of the two-year old olive trees in commercial plantations nurseries in Sidi Taibi. These symptoms were also observed in trees aged from twenty to fifty years in Souk El Arbaa's olive groves (Gharb region). A simple isolation enabled to note the presence of *P. palmivora* verified by the application of Koch's postulate [8].

Other surveys were done in several regions have identified a geographical distribution of *P. palmivora* in Morocco. The fungus was reported in six regions with different isolation percentages: Souk Larbaa (85%), Sidi Taibi (73.6%), Aattaouia (64%), Jaidate (47%), Sraghna (40%) and Tassaout (32%). This study has allowed distinguishing the olive trees attacked by *P. palmivora* by less dense foliage, small leaves, and a yellow coloration [1].

P. palmivora was reported as a pathogenic agent in olive trees in several countries. In Argentina, the identification of this fungus was made by Lucero and his coworkers [14] who considered this species as one of the responsible agents of olive trees "dry branch" that mainly attack young trees managed by modern technologies [15]. The pathogenicity of collected isolates was tested on one year trees and the developed symptoms were varied: leaves necrosis and defoliation, reduced root mass by 40% [14].

In the summer of 1999, the same symptoms of withering associated with rotten roots in young commercial plantations were reported in Calabria (southern Italy) and the presence of *Phytophthora* was verified by the application of Koch's postulate on young olive plants [16]. This pathogen has recently been described as the causative agent of the rotting of the olive trees roots in Italy [17]. Similarly, *P. palmivora* was considered as the causative agent of "dry syndrome" in the olive tree. *Phytophthora megasperma* is also responsible for the symptoms and the pathogenicity of two *Phytophthora* species depends on the expense of the water content in the soil [18].

In Iran, Gorgan region, *P. palmivora* was reported after a series of inspections in irrigated olive trees fields between 2009 and 2010 [19]. This pathogen is probably native from South Asia, but is now pantropical. It leads to significant losses in tropical fruits and vegetable crops. It is a ubiquitous pathogen agent tackling a wide range of host plants [13,20,21]. It was reported on cocoa in Nigeria [22], in Papua New Guinea [21], Solomon Islands [23] and betel [24]. The disease was also observed on papaya in the Philippines, Sri Lanka, Santo Domingo, India, Indonesia, Malaysia, Hawaii, Mauritius, Mexico, Australia, Brazil, Spain, Taiwan, and perhaps elsewhere [25].

Phytophthora spp. species are responsible for one of the most devastating group diseases. They are virtually present in all the cultivated lands and attack the root system, especially in hot and humid conditions. Almost all plants are sensitive to rotten roots; this disease is difficult to control after the installation [26].

Another feature of *Phytophthora* species, *P. palmivora* in particular, is their capacity to cause multiple diseases in the same host [20]. The disease symptoms most often encountered are: rotten roots, rotten crown, canker trees, damage, rotten buds, burned leaves, rotten fruit, and rotten corm [27].

The pathogenicity of *P. palmivora* is due to several factors: ability to produce different types of spores: sporocyst, zoospores, chlamydospores and oospores, fast sporulation (3-5 days) and the ability to survive in or out of the host tissues [27].

5. CONCLUSION

This study made it possible to note that the symptoms of dieback observed in the olive trees of Sidi Kacem region are due to *Phytophthora palmivora*. This pathogenic fungus, isolated from different levels of trees that show total or partial desiccation, was able to induce these same symptoms in olive plants. It was able to reduce root and vegetative growth, disrupt the emission of branches and leaves, and cause dry root rot in all the inoculated plants. The branches of the inoculated plants also showed drying symptoms and the pathogen was re-isolated from all levels of the inoculated plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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