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Characterization of *Phytophthora* and *Pythium* Species Associated with Root Rot of Olive Trees in Morocco

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Abstract: The olive tree is one of the most important fruit crops grown in Morocco, yet extensive decline associated with the root rot of this crop has been observed in many regions. This study aimed to identify and characterize the oomycetes associated with root rot disease in olive trees. During the 2021 and 2022 growing seasons, symptomatic root tissues and soil samples were collected for isolation. Based on morphological traits and the sequencing of the internal transcribed spacer (*ITS*) region of rDNA, 10 oomycete species were identified, belonging to the *Phytophthora* and *Pythium* sensu lato (s.l.) genera. Seven species were assigned to *Phytophthora*, namely, *P. palmivora*, *P. plurivora*, *P. acerina*, *P. oleae*, *P. cactorum*, *P. gonapodyides*, and *P. megasperma*. The *Pythium* s.l. genus was represented by three species, including *P. schmitthenneri*, *P. aphanidermatum*, and *P. irregulare*. A pathogenicity assay was conducted by soil infestation to evaluate the effect of these pathogens on one-year-old olive saplings (var. *Picholine Marocaine*). Results revealed that all 10 species were pathogenic to olive saplings. Inoculated saplings exhibited symptoms, such as root rot, vascular discoloration, and wilting. The pathogens were successfully re-isolated from necrotic roots, thereby fulfilling Koch's postulates. These findings highlight the complex etiology of root rot disease in olive trees, as multiple species can induce similar symptoms. This study represents the first detailed report of *Phytophthora* and *Pythium* s.l. species associated with olive root rot disease in Morocco.

Keywords: *Olea europaea*; oomycetes; root rot; *Phytophthora* spp.; *Pythium* s.l. spp.; *ITS* sequencing; pathogenicity

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1. Introduction

The olive tree (*Olea europaea* L.) is one of the oldest domesticated fruit crops and a key component of Mediterranean agroecosystems [1]. With origins tracing back over 7000 years, current cultivated varieties are believed to have been derived from the wild Mediterranean olive, or oleaster [2]. Over centuries, human activities have facilitated the expansion of olive cultivation beyond its native range, introducing the crop to regions such as Australia, California, Chile, China, South Africa, and New Zealand [3–8]. Today, olive oil and table olives represent vital agricultural products worldwide, with annual production exceeding

20 million tons, largely concentrated in Mediterranean countries, which account for over 95% of this output [9].

In Morocco, the olive tree occupies a central role in the agricultural economy, covering an estimated growing area of over 1.2 million hectares [10]. The sector contributes significantly to rural livelihoods, generating approximately 51 million working days annually and significantly contributing to national exports, which positions Morocco among the leading olive-producing countries globally [11]. The olive industry also supports socioeconomic development by ensuring food security and generating foreign exchange through exports [12]. Despite these substantial contributions, olive production in Morocco is increasingly threatened by various biotic and abiotic stressors. Among the biotic factors, pests and diseases pose a substantial risk, reducing productivity and threatening the long-term sustainability of olive orchards [13].

Globally, olive trees are affected by several diseases, including Verticillium wilt, caused by the soilborne pathogen *Verticillium dahlia* [14]; olive leaf spot, incited by *Fusicladium oleagineum* (syn. *Venturia oleaginea*) [15,16]; and anthracnose, caused by *Colletotrichum* spp. [17]. Recently, root rot diseases caused by oomycetes, particularly species of *Phytophthora* and *Pythium*, have emerged as significant threats to olive production [18]. These soilborne pathogens survive in organic debris and soil as oospores, hyphae, and sporangia, enabling them to persist for many years under unfavorable conditions [19]. When conditions, such as waterlogged and poorly drained soils, occur, these pathogens become highly destructive, leading to root necrosis, reduced root vigor, wilting, chlorosis, and tree decline [20]. Reports of root and collar rot caused by *Phytophthora* spp. and *Pythium* s.l. spp. are increasing worldwide, with notable species, such as *P. plurivora*, *P. megasperma*, *P. palmivora*, *P. heteropora, P. drechsleri*, and *P. aphanidermatum*, linked to root rot [21–25]. Symptoms include chlorosis, wilting, defoliation, twig dieback, and, in severe cases, tree mortality, all of which result in significant economic losses [26].

In recent years, root rot disease has been reported in olive orchards in Morocco, posing a new challenge to olive growers. In 2013, Chliyeh et al. [27] identified *P. palmivora* as a pathogen causing stem lesions as well as root and collar rot in young olive trees. More recently, *Pythium schmitthenneri* was reported to cause root rot in northeastern Morocco [28]. Despite their growing importance, little information is available on the prevalence, diversity, and pathogenicity of these oomycetes in Moroccan olive orchards [13]. This lack of knowledge complicates the development and implementation of effective management strategies to mitigate the impact of these diseases on olive production.

Therefore, given the emergence of root rot disease in various Moroccan olive orchards and the still limited information available about its etiology, this study was conducted in different Moroccan regions to isolate and identify the main species involved, as well as to evaluate their pathogenicity.

2. Materials and Methods

2.1. Sampling

During the 2021 and 2022 growing seasons, olive orchards in various locations across Morocco were surveyed to identify key symptoms associated with root rot disease, such as wilting, yellowing of the leaves, and root rot. Trees exhibiting these symptoms were carefully selected for further analysis. Root samples displaying characteristic symptoms of rot, such as dark brown to black discoloration and the presence of decayed tissues, were collected from the affected olive trees in six provinces, including Benimellal (4 orchards), Berkane (5), Errachidia (4), Fes (3), Meknes (5), and Khenifera (3) (Figure 1). Both root and soil samples were collected from each symptomatic tree. The samples were placed in

separate sterile plastic bags, transported to the laboratory, and stored at 4 °C until further processing for pathogen isolation and identification was carried out.

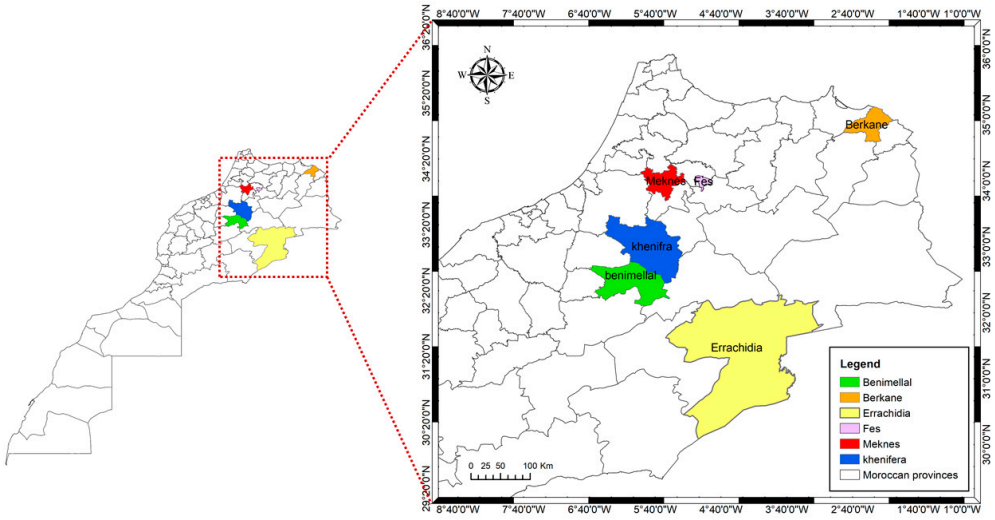

Figure 1. Map of Moroccan provinces where symptomatic olive trees were samples, created using ArcGIS software (version 10.8).

2.2. Isolation of Pathogens

Pathogen isolation was conducted following the method described by Drenth and Sendall [29]. Specifically, soil samples were placed separately in sterile plastic boxes and flooded with sterile distilled water (SDW). Apple cotyledons, which were surface-sterilized with 70% ethanol followed by rinsing with sterile distilled water to reduce the risk of fungal contamination from the apple tissue itself, were used as bait and were placed on the surface of the moistened soil. Plastic boxes were maintained at 22 °C for five days. After the incubation period, the cotyledons were carefully removed and examined under a light microscope for the presence of sporangia. Subsequently, the cotyledons were air-dried in a laminar flow hood and placed in Petri dishes containing potato dextrose agar (PDA) supplemented with streptomycin (100 µg/mL).

Isolation was also performed directly from symptomatic root tissues. The roots were rinsed under tap water to remove soil particles, cut into small pieces (approximately 4 cm), surface-sterilized in a 2% sodium hypochlorite solution for 30 s, dipped in 70% ethanol for 30 s, and rinsed three times with SDW. The disinfested root fragments were placed on sterile filter paper, and air-dried under sterile conditions, and then, fragments were placed onto Petri dishes containing the same PDA–streptomycin medium (three fragments per dish).

For both methods, all the Petri dishes were incubated in the dark at 22 °C for three days. The growing colonies were subjected to a series of subcultures until pure cultures were obtained for further characterization.

2.3. Identification of the Isolates

Since our study focused on oomycetes, all fungus-like isolates were initially grouped into morphotypes based on their colony growth characteristics, including both surface and reverse colony appearances, after 7 days of incubation on PDA and 22 °C in the dark. Morphological features of sporangia, oogonia, antheridia, chlamydospores, and hyphal swellings were also checked and compared with species already described in the literature [30,31].

In addition, DNA sequence data analysis was employed to confirm the species identification of all isolates. Genomic DNA was extracted from the mycelium of 7-day-old pure culture, following the method described by Doyle and Doyle [32]. The complete internal

transcribed spacer (*ITS*) region of rDNA was amplified and sequenced for all isolates using the primers *ITS*1 (5′-TCCGTAGGTGAACCTGCGG-3′) and *ITS*4 (5′TCCTCCGCTTA TTGATATGC-3′) [33]. Polymerase chain reactions (PCR) were carried out in 25 µL reaction volumes, containing 2.5 µL of extracted DNA, 17.4 ultrapure water (PCR grade; Thermo Scientific, Carlsbad, CA, USA), 0.1 µL DreamTaq Hot Start Green Taq Polymerase (Thermo Scientific, Carlsbad, CA, USA), 2.5 µL green buffer (Thermo Scientific, Carlsbad, CA, USA), 0.5 µL dNTP mix, and 1 µL of each primer. The PCR conditions were as follows: an initial denaturation at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 25 s, annealing at 54 °C for 35 s, and extension at 72 °C for 45 s, with a final elongation step of 8 min at 72 °C in a thermal cycler.

The PCR products were visualized through migration in 1.5% agarose gel electrophoresis. Sequencing was then performed bi-directionally using the Sanger method. The resulting sequences were edited utilizing BioEdit software v7.0.5.3 (Raleigh, NC, USA) and compared with sequences in the National Center for Biotechnology Information (NCBI) database using the BLAST search tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 1 August 2024). The final sequences were submitted to GenBank.

Phylogenetic analysis was performed using MEGA software v11.0.8 (Philadelphia, PA, USA). The *ITS* sequences obtained were utilized to construct a phylogenetic tree, with major clusters determined based on sequence similarity. The phylogenetic relationships were inferred using the maximum likelihood (ML) method, and branch support was assessed through 1000 bootstrap replications to ensure robust confidence in the resulting relationships.

2.4. Pathogenicity Assay

The pathogenicity assay was conducted in a greenhouse at the National School of Agriculture in Meknes, to fulfill Koch's postulates. The experiment utilized one-year-old asymptomatic olive trees (var. *Picholine Marocaine*), and pure cultures of an isolated representative of the main species were identified. Four saplings were inoculated per isolate, with additional four serving as controls.

To initiate the trial, olive saplings were carefully removed from their substrate and cleaned with tap water to eliminate any adhering soil particles. The roots were then wounded using a sterile scalpel to facilitate pathogen entry. For inoculation, mycelial plugs of the isolate, cultured on V8 agar medium at 25 °C for 14 days, were distributed around the root system of each olive seedling and then covered with sterile soil [34]. The inoculated olive saplings were grown in a greenhouse environment maintained at 25 °C. To facilitate proper interaction between the isolates and the saplings, the plants were maintained in flooded soil conditions for 48 h, with flooding initiated immediately after soil infestation. Control plants were treated similarly, using sterile V8 agar plugs, as described above. Throughout the experimental period, the plants were irrigated regularly, to ensure optimal growth conditions, for three months. Three months after inoculation, the saplings were examined for the presence of disease symptoms and carefully removed from the soil for the assessment of disease severity. The severity of disease symptoms on plant roots was visually assessed utilizing a 1–5 scale [35]:

- 1: Healthy white roots with no observed disease (0%);
- 2: Slightly affected roots, 25% root rot or initial symptoms of disease;
- 3: Moderately affected roots, 50% root rot with early browning;
- 4: Severely affected roots, 75% root rot with advanced browning;
- 5: Dead roots, 100% disease severity.

Symptomatic roots were sampled for the re-isolation of the pathogen to confirm its identity and ensure the validation of Koch's postulates.

2.5. Statistical Analysis

The pathogenicity assay was replicated twice across time, employing a completely randomized design (CRD). Data were analyzed via analysis of variance (ANOVA) using IBM SPSS Statistics version 26 (New York, NY, USA) to evaluate the effects of isolate inoculation. When significant effects were detected, mean separation was performed using Tukey's test at a significance level of $p < 0.05$.

3. Results

3.1. Symptoms of Root Rot in Surveyed Olive Orchards

Infected olive trees displayed distinct symptoms of root rot, characterized by a sudden decline and a greenish-grey discoloration of the stems, branches, and collar. These symptoms often progressed to complete tree decline and eventual death. In the orchard, various dieback symptoms were observed (Figure 2), including leaf chlorosis, defoliation, wilting, and root rot, which ultimately resulted in the mortality of affected trees.

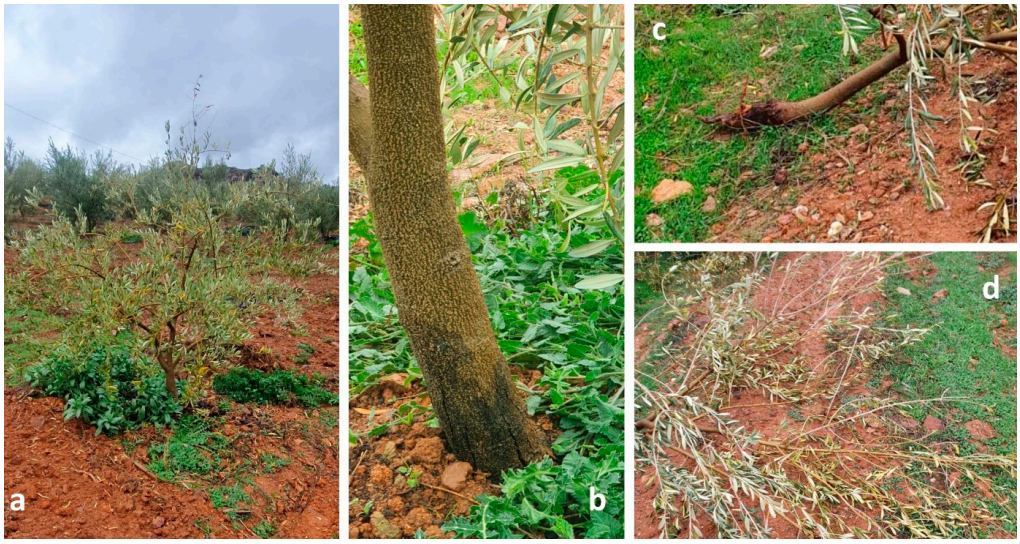

Figure 2. Infected olive trees exhibited several characteristic symptoms of root rot in the field, including (**a**) leaf yellowing and wilting, (**b**) greenish-grey discoloration of stems and branches, and collar, (**c**) root rot with the loss of fine roots in a 5-year-old plant, and (**d**) increased canopy transparency, with foliage becoming sparse, ultimately resulting in plant death.

3.2. Identification of Fungi-like Isolates Associated with Symptomatic Samples

A total of 21 fungus-like isolates were obtained from the samples (Table 1). Of these, 9 were isolated from roots, while 12 were recovered from apple cotyledons used as baits. Based on morphological characteristics, including the colony morphology (Figure 3), the shape and size of reproductive structures, such as sporangia and gametangia, and the *ITS* rDNA sequence phylogenetic analysis (Figure 4), the isolates were identified as oomycetes belonging to the *Phytophthora* and *Pythium* genera (Figures 3 and 4). Fifteen of the isolates were assigned to the genus *Phytophthora* and classified into seven species, namely, *P. palmivora* (six), *P. plurivora* (three), *P. acerina* (two), *P. oleae* (two), *P. cactorum* (one), *P. gonapodyides* (one), and *P. megasperma* (one). The six remaining isolates belonged to the genus *Pythium* s.l. and represented three species, as follows: *P. schmitthenneri* (syn. *Globisporangium schmitthenneri)* (three), *P. aphanidermatum* (two), and *P. irregulare* (syn. *Globisporangium irregulare*) (one).

Table 1. Origin, sampling year, and GenBank accession numbers of *Phytophthora* and *Pythium* s.l. isolates recovered from olive plantations with root rot symptoms.

Code	Species	Origin	Accession Number	Sampling Year
BK3	*Phytophthora acerina*	Berkane	PQ198696	2021
MK-O14	*Phytophthora oleae*	Meknes	PQ202240	2021
ER-C7	*Phytophthora cactorum*	Errachidia	PQ202267	2021
MK-PP2	*Phytophthora palmivora*	Meknes	PQ203303	2021
F-PM1	*Phytophthora palmivora*	Fes	PQ615098	2021
ER-P9	*Phytophthora plurivora*	Errachidia	PQ203317	2021
ER-P10	*Phytophthora plurivora*	Errachidia	PQ203383	2021
BK-1	*Pythium irregulare*	Berkane	PQ216304	2021
MK-PH4	*Pythium schmitthenneri*	Meknes	PQ615100	2021
BM7	*Phytophthora acerina*	Beni Mellal	PQ201093	2022
BK-O6	*Phytophthora oleae*	Berkane	PQ202136	2022
BK-G1	*Phytophthora gonapodyides*	Berkane	PQ202351	2022
MK-P18	*Phytophthora palmivora*	Meknes	PQ203306	2022
BM11	*Phytophthora palmivora*	Beni Mellal	PQ206311	2022
BM-22	*Phytophthora palmivora*	Beni Mellal	PQ614906	2022
KH-1	*Phytophthora plurivora*	Khenifra	PQ216019	2022
KH-2	*Phytophthora megasperma*	Khenifra	PQ216286	2022
MK-A01	*Pythium aphanidermatum*	Meknes	PQ203392	2022
BM-23	*Pythium aphanidermatum*	Beni Mellal	PQ203459	2022
F-PH3	*Pythium schmitthenneri*	Fes	PQ611592	2022
F-PH5	*Pythium schmitthenneri*	Fes	PQ611756	2022

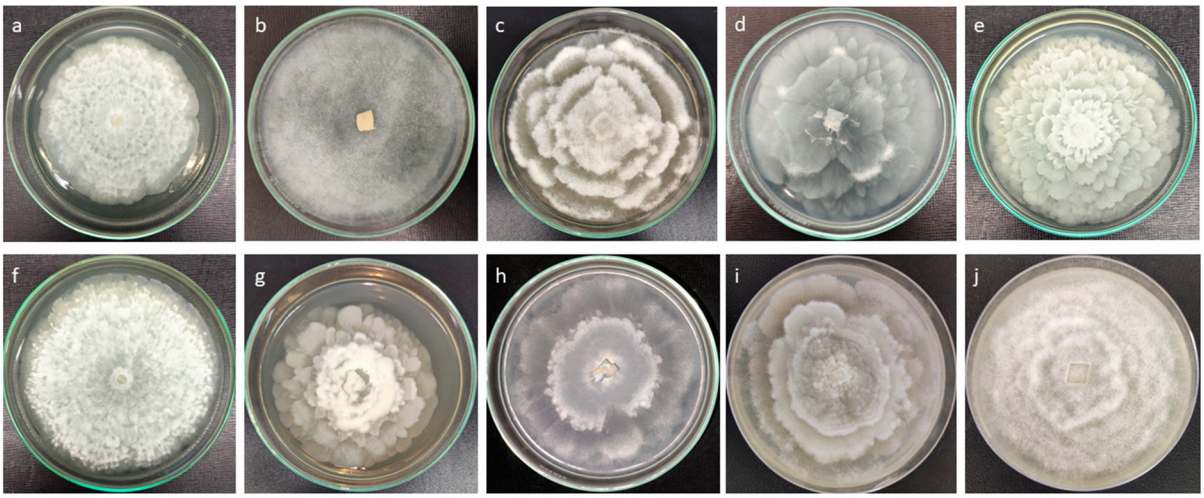

Figure 3. Colony morphology of *Phytophthora* and *Pythium* s.l. isolates on PDA medium incubated for 7 days in the dark at 25 °C. *P. acerina* (**a**), *P. cactorum* (**b**), *P. gonapodyides* (**c**), *P. megasperma* (**d**), *P. palmivora* (**e**), *P. plurivora* (**f**), *P. oleae* (**g**), *P. aphanidermatum* (**h**), *P. irregulare* (**i**), and *P. schmitthenneri* (**j**).

Phylogenetic analysis of *ITS* sequences revealed that three *Phytophthora* species clustered within *ITS* clade 2, making it the most represented clade. Clade 6 included two species, while clades 1 and 4 were represented by one species each. Similarly, the *Pythium* s.l. species were distributed across three distinct clades (A, F, and E), highlighting the genetic diversity of the oomycete populations associated with olive root rot.

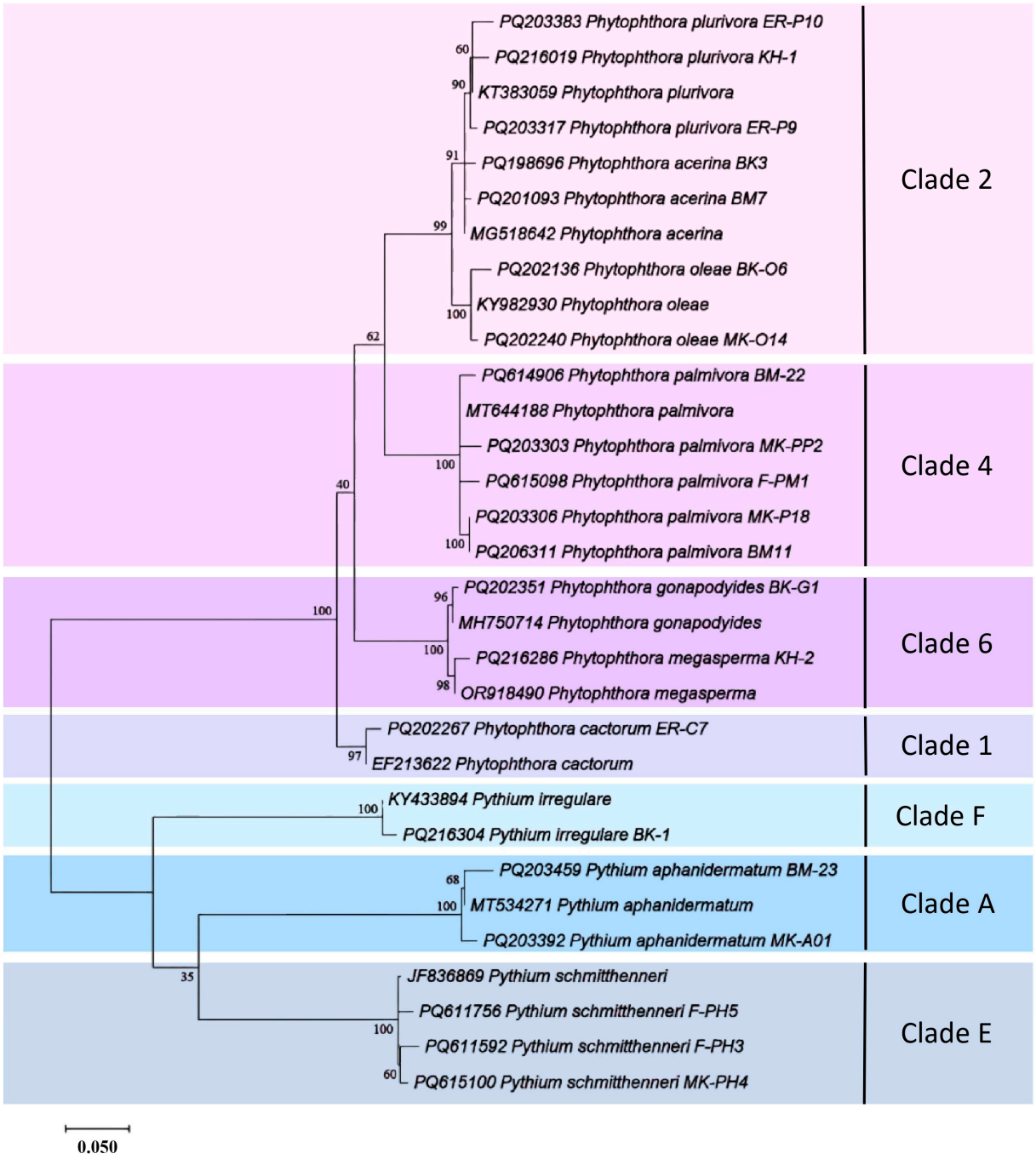

Figure 4. Phylogenetic tree of *Phytophthora* and *Pythium* s.l. isolates associated with olive root rot. The tree was constructed using maximum-likelihood analysis of rDNA *ITS* gene sequences from the 21 isolates and reference sequences from GenBank. The Kimura two-parameter model was performed in MEGA 11 software (v11.0 13), with 1000 bootstrap replications to evaluate the tree.

3.3. Pathogenicity

The pathogenicity of all tested species was evaluated on olive saplings (var. *Picholine Marocaine*). Results demonstrated that both *Phytophthora* and *Pythium* s.l. species were pathogenic when inoculated into wounded plants (Figure 5). After three months, significant differences in disease severity were observed among the tested species (Table 2). *P. schmitthenneri* was the most aggressive, causing 100% disease severity, followed by *P. palmivora* and *P. plurivora*, each with 92%. The remaining species exhibited variable disease severities, ranging between 50% and 75%.

Figure 5. Root rot symptoms in olive saplings at the end of the pathogenicity test (three months post-inoculation) under greenhouse conditions: (**a**) aerial part of a healthy plant (uninoculated control); (**b**–**c**) wilted leaves and stem desiccation in plants inoculated with *P. schmitthenneri* and *P. palmivora*, respectively; (**d**) leaf wilting and discoloration in a plant inoculated with *P. irregulare*; (**e**) healthy roots (control); (**f**,**g**) black discoloration, reduction in size and length of roots, and degradation of fine feeder roots in plants inoculated with *P. schmitthenneri* and *P. palmivora*, respectively; (**h**) formation of necrotic lesions on roots of a plant inoculated with *P. irregulare*; (**i**–**k**) brown discoloration, desquamation, and necrotic spots on the collar; (**l**,**m**) detachment of outer root tissues, leaving only the central thin white fibers.

Inoculated saplings displayed a range of symptoms, including root rot, vascular discoloration, and wilting. Specifically, affected plants exhibited wilted leaves and stem desiccation. Their root systems were significantly reduced in size, with shorter root hairs compared with non-inoculated control plants. Saplings that survived infection showed necrotic lesions on their secondary roots. However, the final stage of infection, characterized by complete leaf desiccation, was not consistently observed.

Table 2. Results of pathogenicity test showing disease severity (%) on olive tree roots inoculated with pathogenic isolates after three months of incubation at 25 °C within glasshouse conditions.

Species	Isolate	Disease Severity (%)	Re-Isolation (%)
Phytophthora palmivora	BM11	92 ^{c,d}	100
Phytophthora plurivora	ER-P10	92 ^{c,d}	100
Phytophthora acerina	BK3	75 ^{b,c}	100
Phytophthora oleae	MK-O14	67 ^{b,c}	100
Phytophthora cactorum	ER-C7	50 ^{b}	100
Phytophthora gonapodyides	BK-G1	50 ^{b}	100
Phytophthora megasperma	KH-2	58 ^{b}	100
Pythium schmitthenneri	F-PH3	100 ^{d}	100
Pythium aphanidermatum	BM-23	58 ^{b}	100
Pythium irregulare	BK-1	50 ^{b}	100
Uninoculated control	_	0 ^{a}	_

Values with the same letter do not differ significantly at $p < 0.05$, according to Tukey's test.

All ten tested species were successfully re-isolated from necrotic roots, thereby fulfilling Koch's postulates. In contrast, no fungal isolates were recovered from control plants, which remained healthy throughout the trial.

4. Discussion

This study is the most comprehensive investigation to date on *Phytophthora* and *Pythium* s.l. species associated with root rot disease in olive plantations in Morocco. The findings have allowed us to clarify both the symptomology and etiology of this emerging disease affecting olive trees in various provinces, including Beni Mellal, Berkane, Errachidia, Fes, Meknes, and Khenifra.

In all surveyed trees, the most frequent disease symptoms consisted of leaf chlorosis, defoliation, wilting, the greenish-grey discoloration of stem, branches, and collars, as well as root rot. In severe cases, these symptoms resulted in the dieback of affected trees. The symptoms observed in this study are the same as those previously reported by many authors [9,18,27].

Isolates were successfully identified based on morphological traits and sequencing of the *ITS* region of the DNA. Seven *Phytophthora* species, representing four of the twelve major *Phytophthora* clades, were identified. These include *P. palmivora*, *P. plurivora*, *P. acerina*, *P. oleae*, *P. cactorum*, *P. gonapodyides*, and *P. megasperma*. In regard to the genus *Pythium* s.l., three species were identified, including *P. schmitthenneri*, *P. aphanidermatum*, and *P. irregulare,* each belonging to distinct *ITS* clades. The detection of 10 distinct species across two genera of the family *Phytiaceae* in Moroccan olive-growing regions highlights the complexity of root rot disease etiology. These findings strongly suggest that different *Phytophthora* and *Pythium* s.l. species can produce similar symptoms in olive trees.

Among these pathogens, *P. palmivora,* a member of clade 4, has been identified as the causative agent of root rot disease in several major olive-producing regions worldwide, first reported in Spain by Sánchez-Hernández et al. [36]. Since then, it has been documented in other olive-producing regions, including Italy [37–40], Tunisia [41], Argentina [42], and Australia [43]. In Morocco, *P. palmivora* was first reported by Chliyeh et al. in 2013 [44] as a causative agent of root rot in the northwestern region of the country.

In our study, three species within clade 2, *P. plurivora*, *P. acerina*, and *P. oleae*, were identified. *P. plurivora* is a polyphagous pathogen with a broad host range, including members of the *Betulaceae, Fagaceae*, and *Oleaceae* families, in both natural ecosystems and agricultural settings [45,46]. This species was first reported in olive trees in Northern Italy

(Veneto) in 2018 [47]. A few years later, this polyphagous species was found on olive in other regions of the country [20].

Similarly, *P. acerina* was described in 2018 as an agent causing sudden death to olive trees in Italy [47]. The high virulence displayed by *P. acerina* in inoculation tests on olive saplings highlights its potential as an extremely aggressive pathogen [20]. However, its geographic distribution and host range remain poorly understood.

Phytophthora oleae, originally identified as a causal agent of rot on mature olive fruits in Southern Italy [48], has also been associated with root rot in wild olive trees in Spain [49] and the dieback of wild olive trees in Sardinia (Italy) [50]. Moreover, this species was recovered from the soil of a typical Mediterranean plant community, also encompassing olive (Oleo-Quercetum virgilianae) in a nature reserve in Sicily (southern Italy) [51]. As reported by Linaldeddu et al. [20], *P. oleae* is one of the most prevalent pathogens involved in root rot symptoms. Its ability to infect olive roots, stems, and also, fruits highlights the risk posed by this species on olive trees.

From clade 1, *P. cactorum* was also reported to cause root rot in olive plantations. This species is a highly invasive and polyphagous pathogen known to thrive in diverse climates, from tropical to temperate, where it causes severe diseases in various agricultural and forestry crops [52,53].

Moreover, two species from clade 6, *P. megasperma* and *P. gonapodyides*, were detected. *P. megasperma* was first isolated from young olive trees (less than 10 years old) in Spain in 1997 [54] and has since been linked to olive disease in Spain [22,54,55], Italy, Tunisia, and Iran [40,41,56,57]. Its widespread distribution and persistence in olive-growing regions emphasize its relevance as a root rot pathogen.

There have been no previous reports regarding an association between *P. gonapodyides* and olive trees. However, this study revealed for the first time that the species is present in olive plantations in Morocco. This finding indicates that the species may be involved in root rot disease progression. Nevertheless, this oomycete has been reported to cause root rot and stem cankers in various forest trees and crops, including holm oak in Spain [58], beech in Sweden and Italy [59,60], apple in China [61], and raspberry in Canada [62]. *P. gonapodyides* has a prevalently aquatic lifestyle and is frequently recovered from streams and riparian ecosystems as an opportunistic pathogen [63].

In contrast, few studies have documented the role of *Pythium* s.l. species in causing root rot in olive trees worldwide. In this study, we have identified three species associated with the disease, including *P. aphanidermatum*, *P. irregulare,* and *P. schmitthenneri*. Among these, *P. aphanidermatum* has previously been associated with the branch wilting and dieback of olive trees in Egypt, Jordan, and the north of Iran [24,57,64]. Similarly, *P. irregulare* has been reported on olive in Spain, where it was associated with root necrosis, root rot, wilting, and the rapid death of the trees in waterlogging conditions [36,54]. These two *Pythium* s.l. species were widely known to cause root rot in a range of crops, including tomato [65], stevia [66], onion [67], bell pepper [68], as well as apple and citrus trees [69]. Notably, our study also identified *P. schmitthenneri* as a causative agent of root rot in olive plantations. This finding confirms our earlier work, in which *P. schmitthenneri* was first reported as a pathogen of olive trees in Morocco [28].

Following Koch's postulates, the pathogenicity of the 10 oomycete species isolated from olive samples was tested on olive saplings under greenhouse conditions. The results demonstrated that all tested *Phytophthora* and *Pythium* s.l. isolates were capable of inducing a range of disease symptoms, which, in severe cases, led to plant death. However, significant variation in disease severity was observed among the isolates. Specifically, *P. schmitthenneri*, *P. palmivora*, *P. plurivora*, and *P. acerina* exhibited the highest virulence, with disease severity ranging from 75% to 100%. In contrast, the remaining species were

less aggressive, inducing moderate disease symptoms with severity levels between 50% and 68%. These findings are consistent with those of Linaldeddu et al. [20], who reported that the degree of aggressiveness can vary significantly among different species.

Much evidence suggests that the primary cause of olive decline is excessive irrigation, as such conditions provide the high moisture required by these pathogens for their proliferation. Moist environments promote the production of sporangia, as well as the dissemination of zoospores [26,70]. Consistently with this hypothesis, Moein et al. [71] demonstrated that higher irrigation regimes significantly increased disease severity in apple saplings inoculated with *P. ultimum*, *P. irregulare*, or *P. cactorum*. Consequently, it can be concluded that irrigation practices may have facilitated infections caused by oomycetes in olive orchards, also acting as the inoculum vehicle of these oomycetes [72].

The management of root rot diseases caused by pathogenic oomycetes remains a significant challenge for farmers. Thus, enhancing our knowledge of the *Phytophthora* and *Pythium* s.l. species responsible for root rot and the environmental factors influencing disease development is crucial for effective control strategies. Currently, no curative methods exist to eliminate this devastating disease once it has been established in an orchard. Therefore, an integrated management approach that combines sanitation, the regulation of irrigation practices, the use of biological control agents, chemical treatments, and resistant varieties has been reported as the most effective method for combating root rot disease [73–77].

5. Conclusions

The findings of our study enhance our understanding of the biodiversity and pathogenicity of *Phytophthora* and *Pythium* s.l. species associated with root rot disease in Moroccan olive groves. Ten species were identified, including seven *Phytophthora* and three *Pythium* s.l. species, demonstrating significant variation in their virulence. These findings highlight the complexity of the disease etiology, as multiple species can induce similar symptoms, complicating diagnosis and management.

The discovery of several *Phytophthora* and *Pythium* s.l. species in Moroccan olive groves is particularly concerning, as it underscores the potential emergence and spread of these invasive pathogens. This warrants further research to explore the full diversity of oomycete communities in olive-growing systems, evaluate their host specificity, geographic distribution, and survival strategies, and investigate the environmental factors driving their emergence and dissemination.

Such efforts are critical for designing sustainable disease management strategies. Addressing these challenges will ensure the long-term productivity and resilience of olive plantations in Morocco and other regions affected by root rot disease.

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