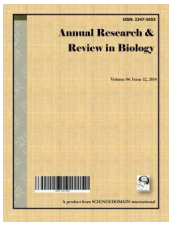

Annual Research & Review in Biology

13(5): 1-7, 2017; Article no.ARRB.33744
ISSN: 2347-565X, NLM ID: 101632869

Detection of *Fusarium solani* as a Pathogen Causing Root Rot and Wilt Diseases of Young Olive Trees in 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.

Article Information

DOI: 10.9734/ARRB/2017/33744
Editor(s):
(1) Viduranga Y. Waisundara, Faculty of Applied Sciences, Rajarata University of Sri Lanka, Mihintale, Sri Lanka.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Clint Magill, Department of Plant Pathology & Microbiology, Haryana Agric. University, India.
(2) Rafael Fernandez Da Silva, Department of Biology, FACYT-University of Carabobo, Venezuela.
Complete Peer review History: http://www.sciencedomain.org/review-history/19586

Original Research Article

Received 27th April 2017
Accepted 16th May 2017
Published 17th June 2017

ABSTRACT

The cultivation of the olive tree has several problems related to pests and diseases that can cause heavy economic losses by causing the death of trees. *Fusarium solani* was detected in the olive trees of Sidi Taibi's nurseries located in the national road between Kénitra and Rabat in spring 2012 and 2013 (1 to 2% of diseased plants). Koch's postulate was verified in the olive trees variety 'Picholine Moraine', inoculated with *F. solani*. Three months after inoculation of the plants, the extremities of the young buds began to dry out, then the drying became generalized and the roots of the inoculated plants showed rot and detached from the base of the plants. The pathogen was re-isolated from the roots, dried buds, bark and petioles of the leaves of the inoculated plants; the percentage of isolation was 100%.

Keywords: Olive; Fusarium solani; symptoms; Koch's postulates; pathogenicity; Morocco.

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1. INTRODUCTION

In Morocco, nurseries located in the Sidi Taïbi site, between Kénitra and Rabat, sell different types of plants, including olive trees. These, sometimes, show symptoms of decline. A diverse fungal complex containing *Verticillium dahliae*, *Phytophthora palmivora*, *Fusarium oxysporum* and *Fusarium solani* was isolated from these plants [1]. This fungal complex, which also contains *F. graminearum, F. eupionnates* and *Rhizoctonia solani*, has also been isolated from the dried roots and branches of olive trees growing in different olive regions of Morocco [1]. Pathogenicity of *Verticillium dahliae* has been studied on different olive varieties [2] as well as its distribution in the olive-growing areas of Morocco [3,4,5]. *Phytophthora palmivora* was encountered for the first time in Morocco [6]. Its distribution has also been confirmed in different olive-growing regions of Morocco [7,8]. Similarly, the pathogenicity of *Phytophthora palmivora* was compared with that of *Verticillium dahliae* [1]. Both species were able to induce diebacks of the branches of inoculated olive plants. On the other hand, the pathogenicity of *Fusarium oxysporum*, *Fusarium solani, F. graminearum, F. eupionnates, Rhizoctonia solani* against to the olive tree has never been studied in Morocco.

In this work, the pathogenicity of *Fusarium solani* was studied on the variety 'Picholine Marocaine'.

2. MATERIALS AND METHODS

Visits to the nurseries of Sidi Taibi; national road between Kénitra and Rabat, in the spring of 2012 and 2013, some olive trees with symptoms of drying and dieback (1-2%) were recorded (Fig. 1A). These plants were brought back to the laboratory and isolations were made from the roots, bark and dried branches. After cross section the fragments of a secondary stem, under the stereoscopic microscope, we observed browning inside these stems (Fig. 1B).

The samples were cut into 1 cm fragments, disinfected with alcohol, deposited on PSA medium (Potato Sucrose Agar: 200 g potato, 20 g sucrose, 15 g agar, 1000 mL of distilled water) and the cultures are incubated at 25℃. The identification of the fungal species was carried out using microscopic observations of the cultures, after purification on different culture media.

Isolations were also made from leaves; fragments of 1 to 2 cm were washed, disinfected with alcohol and placed in sterile Petri dishes containing three disks of filter paper moistened with sterile distilled water. The dishes were then incubated under continuous light.

The microscopic observations made it possible to identify the fungal species calculating their percentages:

$$Pi = N_S X / N_T \times 100$$

$N_S X$: X Number of colonies of a fungal species.

N_T: Total number of colonies of all isolated species.

Among the isolated fungal species, an isolate of *Fusarium solani* was randomly selected to inoculate olive plants. The inoculum was prepared after 10 days of culturing the parasite on PSA medium at a temperature of 25℃. Spore suspension was prepared by scraping the cultures in sterile distilled water and then adjusted using a hemocytometer 10^5 spores mL^{-1}.

Four-month-old olive trees grown from woody cuttings were first removed; the roots were washed under a stream of water and then rinsed with sterile distilled water. The plants were subsequently soaked for one hour in a prepared conidia suspension. Afterwards, the plants were planted in their pots and watered with 250 ml of the conidia suspension. The same operation was carried out for the control plants, except that the sportive suspension was replaced by sterile distilled water.

All plants are placed in a greenhouse and irrigated each week with tap water. The development of symptoms was monitored for four months in inoculated olive plants. The re-isolation of *Fusarium solani* from the inoculated olive plants was also carried out four months after root inoculation. The inoculated plants were removed from their soil and stripped of their substrate and washed extensively with running water. Cross-sections of the roots, bark, dried buds and leaf petioles were separately deposited in the alcohol at 90° for 1 to 2 minutes, rinsed thoroughly with sterile water, dried on a sterile filter paper. The fragments of the plants were thus deposited in Petri dishes containing PSA medium. The observations of the cuts were done after one week.

Fig. 1. Olive tree plant showing symptoms of dieback and wilting from Sidi Taibi nurseries (A); Cross section of a secondary stem of an olive plant (B)

3. RESULTS AND DISCUSSION

Isolations from the roots of olive plants with symptoms of drying and dieback have recorded the presence of *Fusarium solani* (23%), *Alternaria alternata* (17%), *Drechslera australiensis* (13%), *Rhizoctonia solani* (7%), *Fusarium oxysporum* (6%) and *Verticilium dahliae* (4%) (Table 1).

Table 1. Isolation percentage of fungal species from olive plants showing symptoms of drying and dieback

Fungal species	Percentage (%)
Fusarium solani	23 [a]
Alternaria alternata	17 [a]
Drechslera australiensis	13 [ab]
Rhizoctonia solani	7 [b]
Fusarium oxysporum	6 [b]
Verticillium dahliae	4 [b]

Two results read on the same column followed by two different letters, differ significantly at the 5% threshold.

On PSA medium, *F. solani* forms fluffy or cottony colonies of white to cream color with a pale reverse (Fig. 2A), this description is consistent with that of Chermette and Bussieras (1993). In older crops, the white cottony mycelium is purple or pink white. Under the microscope, *F. solani* is characterized by a septate mycelium and the presence of microconidia (11.8 µm) (Fig. 2B), united or bicellular of various shapes (ovoids, fusiform, cylindrical, piriformes) and multicellular macroconidia (18.1 µm), fusiform and septate (Fig. 2C). The resistance spores are chlamydospores in the terminal or intercalary position (Fig. 2D).

The isolate of *F. solani* tested was able to induce, three months after inoculation, symptoms of drying in olive trees of the 'Picholine Marocaine' variety (Fig. 3). The drying occurs from top to bottom and generalizes. Dried leaves remain attached to young branches for a long time.

F. solani was also able to cause dry root rot of inoculated plants, The roots break and detach from the base of the plants. *F. solani* was re-isolated (100%) From the roots, dried buds and leaf petioles (Fig. 4).

Several telluric fungi, *Verticillium dahliae* [9,10,11,12], *Rhizoctonia* sp. And *Fusarium* sp. are responsible for the symptoms of wilting and partial or total dieback of the olive tree [13,14,15,16]. These soil fungi also cause significant damage in several countries of the Mediterranean basin [17,18,19,10]. The Fusarium genus includes 44 species according to Booth [20], phytopathogens capable of attacking a large number of plants, and causes great economic losses. Fusarium solani is one of the most important species of the Fusarium genus from a phytosanitary point of view. This fungus, the agent of root rot and collars of young olive trees, most common in nurseries and open fields [21,22,15,14,23].

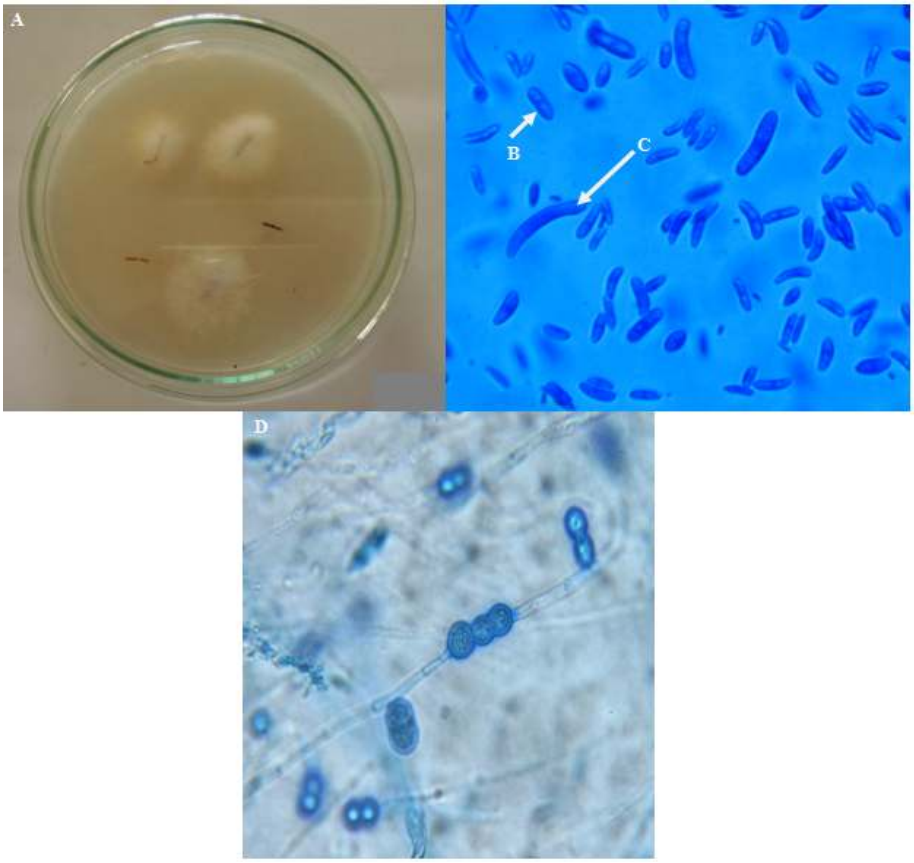

Fig. 2. Colonies of *F. solani* isolated from the roots of olive plants (A); microconidia (B); macroconidia (C); intercalary chlamydospores (D). (X 400)

Fig. 3. Control olive plant (not inoculated) (A); Inoculated plant with *F. solani* (A)

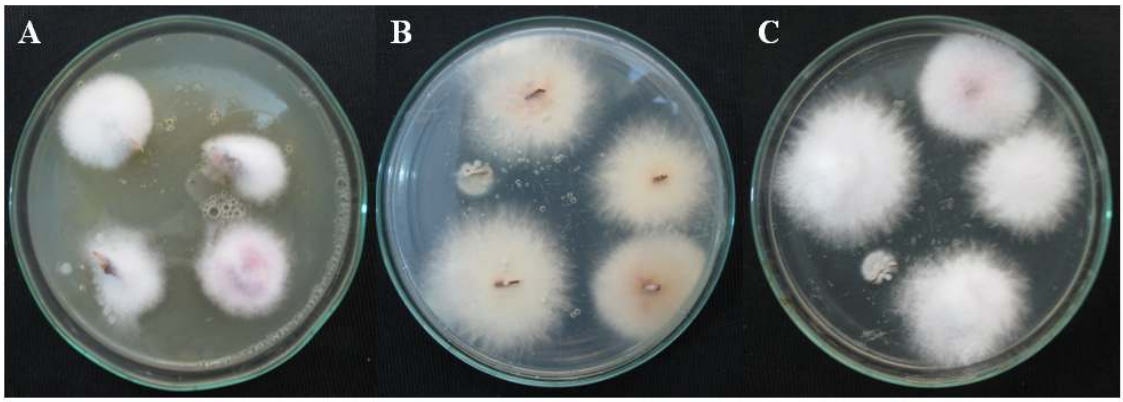

Fig. 4. Re-isolation of *F. solani* from leaf petioles (A), dried buds (B) and roots (C)

F. solani was reported for the first time in India in 1982 as a pathogen on olive tree [24]. The described symptoms were falling leaves, drying branches and root rots. Plants may die 3 to 4 months after infection. The fungus was reported in Iran [25], in Tunisia [26,23,27,28], in Egypt, regions of Giza and Fayoum [28,29], in Nepal [30], as one of the decaying agents and the root rots of the olive tree.

The damage caused by *F. solani* on olive trees in nurseries is sometimes very important [31,32,33,34]. The isolate of *F. solani*, originating from olive trees in the nurseries, was able to induce desiccation and root rot in the olive plants of the variety 'Moroccan Picholine' is widely cultivated in Morocco. Indeed, F. solani has been found in diseased plants in the presence of *Rhizoctonia solani* and *Verticillium dahliae*, soil fungi responsible for general or partial decline of olive trees in all olive-growing countries. In other works [1] on the olive-related fungi in Morocco, different species of the Fusarium genus has been isolated from the olive trees in different zones of Morocco. The pathogenicity of these species to the varieties of olive trees grown in Morocco is still unknown.

4. CONCLUSION

This is the first report of this pathogen in Moroccan olive trees. So, due to the severe symptoms and the increasing incidence recorded; *Fusarium solani* should be considered a potential threat to olive cultivation in Morocco. The commercialization of infected plants and their transfer to different olive-growing areas may contribute to the spread and development of the disease, which will constitute, in time, a real danger for the cultivation of the olive tree in Morocco. The risk is great when diseased plants at the nursery contain three pathogens.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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