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Bibliography. Figures. Tables.

I. *Verticillium dahliae* — Lebanon (South). II. Stone fruit — Diseases and pests — Lebanon (South). III. Olive — Lebanon (South).

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EVALUATION OF MORPHOLOGICAL CHARACTERISTICS AND PATHOGENICITY OF DIFFERENT ISOLATES OF *VERTICILLIUM* WILTS FROM DISEASED STONE FRUITS AND OLIVES

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Abstract

The study was undertaken in order to evaluate the economic importance of *Verticillium* wilt on olive trees in South Lebanon and stone fruits in North of Bekaa and Kesrwan. Only two samples from a total of 120 olive trees showed symptoms of *Verticillium dahlia*.

Identified isolates were then incubated under controlled conditions in order to study their morphological characteristics. Colonies from olives isolates were either orange or white. Independently of isolates origin, the colony growth, measured at 2 days interval and up to 28 days, was parabolic starting from the beginning of experiment and up to day 22 and then it became linear.

Studies on 9 isolates pathogenicity were carried out under growth chamber conditions. Results showed difference in isolates pathogenicity where olive twigs inoculated with isolates Am9 and M31 died 38 days after inoculation. In parallel, tomato seedlings inoculated with Am7 isolate died after 18 days of inoculation.

Key words: *Verticillium dahlia*, stone fruits, olive trees, pathogenicity, isolates.

Résumé

Une étude sur la présence du champignon *Verticillium dahliae* dans les fruits à noyaux et les oliviers a été réalisée dans les régions du nord de la Békaa, du sud du Liban et de Kesrwan. Les symptômes de 2 des 120 échantillons des oliviers ont été reliés à la présence de *V.*

dahliae. Les caractéristiques morphologiques des isolats obtenus ont été étudiées. Les colonies des 2 isolats d'oliviers avaient comme couleur orange ou blanche. La croissance des colonies mesurée sur 28 jours à un intervalle de 2 jours était parabolique au début de l'expérience, puis linéaire à partir du 22^{ème} jour.

Des études sur la pathogénicité des 9 isolats ont été faites sous conditions contrôlées. Elles ont montré une différence de pathogénicité entre les isolats testés. Les plantules d'oliviers inoculées par les isolats Am9 et M31 sont mortes 38 jours après inoculation, tandis que les plantules de tomates sont mortes 18 jours après inoculation lorsqu'elles étaient inoculées avec l'isolat Am7.

Mot clés : *Verticillium dahlia*, fruits à noyaux, oliviers, pathogénicité, isolats.

INTRODUCTION

The production of stone fruits and olives is constrained by many biotic and abiotic factors. One of these factors is *Verticillium* wilt caused by a soil-born fungus *Verticillium dahlia*. The disease is of increasing significance in Lebanese agriculture especially in fruit plantations (olive and stone fruits), where serious losses may occur, with incidence levels reaching 10% in the case of olives (Shams Eddine, 1998; Atallah, 2000). The main goal of this study can be summarized as follows:

- Evaluate the frequency of occurrence of *V. dahlia*;
- Isolate and identify the causal agent in the laboratory;
- Study the morphological characteristics (mycelial appearance, colony color and growth) of the different obtained isolates;
- Undertake studies for evaluating pathogenicity.

MATERIALS AND METHODS

Collection of samples and laboratory isolation

Two hundred samples were collected from almonds, cherries, peaches and olives trees located in North of Bekaa, Kesrwan and South of Lebanon. The orchards presented a low incidence of the disease concerned by the study. They were afterwards isolated in the laboratory in order to determine the fungus causing symptoms.

Morphological characteristics

Five parameters were taken into consideration: colony, color, texture growth, sclerotia appearance and arrangement. Two plates of each isolate were prepared. Petri dishes were incubated at a temperature of 23⁰ C with a 24 hours light period for a total period of 28 days. Measurements were taken within an interval of two days.

Pathogenicity test

The pathogenicity of some selected fungal isolates obtained from different samples and showing variability in their morphological characteristics was tested. Pathogenicity tests were undertaken on olives and tomatoes seedlings.

The method used in pathogenicity test was that of the root dip method. Nine isolates from peaches and olives were chosen for the study (Tab. 1).

Inoculum preparation

For olive and tomato seedlings, the spore concentration for inoculation was adjusted with sterile distilled water to 4×10^6 spores/ml.

Test in growth chamber Plant material

Olive twigs variety Soury (young, soft wood, 20–25 cm long,) from symptomless trees were collected at a commercial grove. Healthy tomato variety Baladi seedlings produced by standard procedures were supplied by a commercial nursery.

Inoculation of olive and tomato twigs

Four olive twigs and four tomato twigs were placed in test tubes containing the conidia suspension; one olive and one tomato twig were placed in test tubes containing sterile water tested used as control material (modified from Tsor and Erlich, 1996). The tubes were placed in a growth chamber under constant temperature (22–25°C).

Table 1: Isolates used in pathogenicity test

Location	Host	Sample number
Dar Beechtar	Olive	D2
Sariine	Almond	S5
Dar Beechtar	Olive	D6
Amioun		Am7
		Am9
		S24
Sariine		Peach
	S26	
Majdelzoun	Olive	M31
Abra		A102

Disease assessment

Wilt symptoms on olive twigs were recorded 3 weeks after inoculation, on a scale of 0-4, where 0 = no symptoms, 1 = up 50% of the leaves roll in, 2 = more than 50% of the leaves roll in and some are necrotic, 3 = up 95% of the leaves are necrotic, and 4 = leaves completely dry or defoliated (Tsor *et al.*, 2001).

Severity of chlorosis and wilt on tomato seedlings were scored 14 days after inoculation on a scale of 0-5, where 0 = no symptoms, 1 = chlorosis of lower leaves, 2 = moderate (30–50% of leaves) wilt with severe chlorosis, 3 = moderate wilt and necrosis, 4 = severe (more than 50% of leaves) wilt and necrosis, and 5 = dead plant.

RESULTS AND DISCUSSION

Severity and incidence

One olive sample collected from Majdelzoun showed moderate attack of *Verticillium dahlia* with 50% of incidence. Whereas one sample from Aabra showed severe attack of *Verticillium dahlia* with lower incidence (15%).

Morphological characteristics

Colonies morphology

One colony from olive tree A (102) isolate had white color and puffy growth. The other one from olive tree M (31) isolate had orange color and thick growth.

In various colonies, *V. dahliae* produced prostate, hyaline mycelium, whitish to cream in underside after one week, then gradually darkens with the formation of black, thick-walled microsclerotia (Pegg and Brady, 2002).

This difference in colony color could be related to different types of isolates. In fact, temperatures between 20-23°C can lead to the highest frequency of morphological mutants of *Verticillium dahliae* in cultures (Pegg and Brady, 2002). In addition, a higher frequency and range (9.1%) of morphological variants from microsclerotia compared with 0.5% from conidia has been found.

Growth rate

Results showed that colony growth was parabolic from day 2 and up to

day 18. From day 18 to the end of the experiment, the growth measured was linear as shown in figure 1.

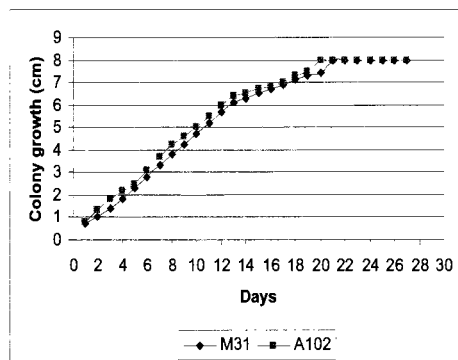


Figure 1: Colony size of olive tree isolates from Majdelzoun and Aabra, grown on PDA up to 28 days after isolate transfer. each value represents the mean of two replicates.

Pathogenicity tests on olive varieties

Almost all olive twigs used for pathogenicity tests wilted and died 36 – 42 days after inoculation.

All control twigs put in tube with sterile water did not show any symptoms, indicating that, the death of all inoculated twigs is related to the presence of the pathogen.

Variability in the pathogenicity of the *Verticillium dahlia* isolates tested was shown.

Isolates Am9 and M31 were the most severe, as symptoms appeared earlier than on twigs inoculated with other isolates. In addition, both isolates led to the death of twigs 38 days after inoculation.

Development of symptoms was the lowest on twigs inoculated with

isolate A102. Disease growth was similar on olive twigs inoculated in S24, S25 and S26 isolates; whereas symptoms development was similar on olive twigs inoculated with S5, Am7 and D6.

Pathogenicity tests on tomato varieties

Almost all tomato twigs used for pathogenicity tests wilted and died 25 – 31 days after inoculation.

All control twigs put in tube with sterile water did not show any symptoms, indicating that, the death of all inoculated twigs is related to the presence of the pathogen.

It was noticed that isolates S5, S25 and A102 were the most severe ones as symptoms appeared earlier than twigs inoculated with other isolates. In addition, both isolates led to the death of twigs 28 days after inoculation.

Development of symptoms was the lowest on twigs inoculated with isolate M31; disease growth was similar on tomato twigs inoculated in D2, Am9 and S24 isolates; whereas symptoms development was similar on tomato twigs inoculated with D6, Am7 and S26.

CONCLUSION

Olive trees were collected from Majdelzoun and Aabra in the South of Lebanon were the most susceptible to *Verticillium* attack.

Two colonies obtained from olive trees were white and orange. Colonies growth was parabolic at beginning and then linear.

Based on results of this experiment, *Verticillium dahliae* attack could not be neglected and the presence of isolates with different pathogenicity should lead to more detailed experiment using PCR and DNA studies.

Verticillium wilt has become an important problem in a number of fruit trees in Lebanon. Since curative control methods for this disease are not effective, especially in trees, preventive actions become critical. The most important preventive measure is the use of clean *Verticillium*-free nursery seedlings, since diseased seedling are often major sources of primary infection (Thanassouloupoulos, 1993).

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