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# PREVALENCE AND SURVEY OF *VERTICILLIUM DAHLIAE* ON STONE FRUITS AND OLIVE TREES IN TWO REGIONS OF LEBANON

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## ABSTRACT

*Field experiments were carried out and 96 samples were collected (46 from stone fruits trees and 50 from olive trees). Most of samples collected from stone fruits showed evident wilt symptoms whereas in olive samples, symptoms were not evident. Almond trees were found to be the most susceptible to Verticillium attack among stone fruits species and the Sariine village located in Central Bekaa was the village with the highest incidence of Verticillium dahliae. Concerning olive, Amioun and Dar Beechtar samples were found to be the most infected with the fungus.*

*Laboratory experiments permitted to isolate and identify the fungus from the collected samples. Results showed that symptoms in 32 collected samples out of 46 from stone fruits and in 13 collected samples from 50 olives were related to the presence of Verticillium dahliae. In fact, verticillate conidiophores shape and appearance of microslerotia were observed in these colonies. Identified isolates were then incubated under controlled conditions in order to study their morphological characteristics (color and growth of colony as well as appearance date of microsclerotia). Colonies from almond isolates were yellow whereas those from peaches and olives were 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 12 and then*

*it became linear. In parallel, microsclerotia appeared 7 to 10 days after incubation in all colonies.*

*Results revealed that the frequency of Verticillium dahliae was 69,5 % in stone fruits samples and 26 % in olive samples. Therefore, Verticillium dahliae attack could not be neglected and should be considered as a severe disease of the stone fruit trees in the Bekaa region and of olive trees in North Lebanon.*

***Keywords:*** *Verticillium dahliae, stone fruits, olive trees, frequency, morphological characteristics.*

## RÉSUMÉ

*Une enquête sur la présence du champignon Verticillium dahliae sur les fruits à noyaux et les oliviers a été réalisée dans la région du centre de la Bekaa et dans le nord du Liban. Au cours de cette enquête, des échantillons des branches et rameaux ont été collectés : 46 des fruits à noyaux et 50 des oliviers. La sévérité et l'incidence ont été évaluées dans les vergers visités. Il a été démontré que les amandiers sont les plus attaqués par la maladie et que l'incidence la plus élevée est rencontrée dans la localité de Sariine. Comme les échantillons malades collectés présentaient plusieurs types de symptômes, l'isolement et l'identification du champignon, agent causal de la maladie, ont été entrepris au laboratoire. Les symptômes de 32 échantillons des 46 collectés des fruits à noyaux ainsi que 13 des 50 échantillons des oliviers sont dus au V. dahliae identifié grâce à l'apparition des microsclérotes et à la forme verticillée des conidiophores. L'étude des caractéristiques morphologiques des isolats obtenus a été faite en les incubant sous conditions contrôlées et en notant la couleur et la croissance des colonies ainsi que la date d'apparition des microsclérotes. Les colonies des isolats d'amandiers sont jaunes tandis que celles provenant des isolats des pêchers et des oliviers sont blanches. Indépendamment de l'origine des isolats, 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 14$^{ième}$ jour. Quant aux microsclérotes, elles étaient apparues 7 à 10 jours après incubation.*

*Cette étude a montré que la fréquence de V. dahliae était de 69,5 % dans les échantillons collectés à partir des arbres fruitiers à noyaux et de 26 % dans ceux des oliviers. Ainsi, l'attaque de Verticillium dahliae ne doit pas être négligée.*

***Mot clés :*** *Verticillium dahliae, fruits à noyaux, oliviers, fréquence, caractéristiques morphologiques.*

## INTRODUCTION

Lebanese agricultural sector consists of many fruit crops. Among these fruit crops, olive trees are planted in Lebanon over a surface of 55,640 ha with a production of 189,5 thousand tones in 2000 and 85,8 thousand tones in 2001 as appeared in the statistics of the FAO (2002). As for stone fruits, they are cultured over a surface of 25,179 ha with a production of about 147,6 thousand tones in 2001 as mentioned also by the FAO (2002).

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-borne fungus *Verticillium dahliae*. The disease is of increasing significance in Lebanese agriculture especially in fruit plantations (olive and stone fruits) where serious losses may occur and has been reported or suspected in Lebanon on stone fruits and olives before. Attalah (2000) and while surveying and diagnosing diseases associated with almond has detected the presence of *Verticillium* wilt. Choueiri (2003) has suspected the presence of *Verticillium* wilt in some orchards as he was surveying stone fruits for viral diseases. It was also reported to occur in the most important olive producing areas at incidence levels of around 10 % (Shams Eddine, 1998).

However, at the present time, no structural complete survey of the presence and the impact of the vascular wilting in the orchards of stone fruits and the olive groves have been accomplished.

Our study is a part of a broader project entitled "Economic Importance and Integrated Management of *Verticillium* Wilts of Fruit Trees" financed by the National Council of Scientific research (CNRS) Lebanon. This project will contribute further to the knowledge gained due to the many research studies undertaken on this serious disease in surrounding countries of the Mediterranean region. It will help to identify the susceptibility of species and varieties of fruit trees grown in Lebanon (especially in nurseries) and to determine the most effective integrated control measures to manage this increasing problem in Lebanon and other regions.

The main goals of our study are:

- to lead an investigation in the Bekaa regions of stone fruits and in North Lebanon of olive groves;
- to evaluate the frequency of *V. dahliae*;
- to isolate and identify the causal agent in the laboratory;
- to study the morphological characteristics (mycelial appearance, colony color and growth) of the different obtained isolates.

## MATERIAL AND METHODS

Our study was undertaken in order to evaluate the economic importance of *Verticillium* wilt on stone fruits and olives trees. Therefore, field and laboratory experiments were carried out.

### 1. Field visits

Field experiments consisted on visits undertaken to Lebanese growing areas of stone fruits and olives. Visited regions were chosen based on the presence of the crops studied and the potential occurrence of this disease. Data from reported literature on areas infected by *Verticillium* fungus was used. In fact and while surveying viral diseases on stone fruits during 2000, 2001 and 2002 and in all Lebanese growing regions, orchard showing wilt symptoms were recorded (Choueiri, 2003). As for olive trees, preliminary studies (Shams Eddine, 1998) revealed the presence of *Verticillium* wilt in olive groves in North and South Lebanon.

Most visited villages were those of the Central Bekaa region where orchards of almonds and peaches presented a high incidence of the disease concerned by the study.

Thirteen orchards of stone fruits visited were located in six different localities of the Bekaa, two orchards in North Lebanon and one orchard in Laqlouq. Forty samples of twigs and branches were taken randomly from already tagged orchards in the Bekaa, five randomly from the North Lebanon and one from Laqlouq region. Twenty two samples of almond trees were collected from Sariine, Kafar Zabad, Laqlouq and Danieh-Markabta versus 19 of peach trees taken from Sariine, Khorbet Anafar and Kab Elias. Three samples of apricots where two of them from Bakhoun and one from Bar Elias were collected and two samples from plum trees from Bakhoun.

Concerning olives, only the North of Lebanon was visited. Out of the 50 samples collected from olive trees, 40 samples were collected from: Zghorta-Koura road, Amioun, Kafar Hazir, Kfar Akka, Dar Beechtar, Bterram and Bechmezine. Those did not show neither obvious exterior symptoms of wilting nor vascular discoloration, whereas ten samples of affected olive trees showing discolored tissues were collected from Amioun.

#### 1.1. Disease scale

In parallel to collecting samples, disease incidence and severity were evaluated in stone fruit orchards. The severity of the disease was evaluated accor-

ding to the scale described by Saad (2003) with mild attack (Md): one to two twigs from ten infected equivalent to ten percent of the tree; moderate attack (Mt): one third of the tree attacked equivalent to 10-60 % and severe attack (Sr): over 60 % of the tree infected.

Incidence was based on an estimation of trees number infected per orchard.

The ninety six samples (46 samples from stone fruits and 50 samples from olive trees) collected from new shoots, old shoots, small twigs and branches were kept in the refrigerator at $6^0$ C for laboratory experiments.

## 2. Laboratory experiments

Laboratory experiments were undertaken in order to isolate and identify *Verticillium* as well as to determine morphological characteristics of different isolates.

### 2.1. Isolation of fungi on agar media

Twigs showing typical *Verticillium* wilt symptoms, or branches of diseased stone fruits and olive trees, were collected from fields at various sites. Four to 5 cm long segments were cut into four pieces, placed on WA or HPDA and incubated at room temperature ($23^0$ C). Two replicates from each sample were done. Several days later and when the mycelium of the fungus was developed, a small part was transplanted onto two Petri dishes with PDA medium and then transported to the incubating room for the study of growth characteristics.

### 2.2. Pathogen identification

Fungal identification was done using a microscope and where pathogen fructification was mounted in lactophenol cotton blue (Surechem products LTD).

### 2.3. Morphological characteristics of the fungus

During the isolation and determination phase, some differences were noticed among *Verticillium* isolates. Therefore, a more detailed study was initiated to characterize the isolates according to their morphological differences. Two parameters were taken in consideration: colony color, texture and growth as well as sclerotia appearance and arrangement.

Two plates of each isolate were prepared. Petri dishes were incubated at a temperature of 23 °C with a 24 hours light period and that for 28 days. Measurements were taken within an interval of two days.

## RESULTS AND DISCUSSION

The vascular wilt caused by *Verticillium dahliae* was identified on most almonds, apricots and peach trees in the Bekaa region and on some olive trees in the North of Lebanon.

### 1. Description of visual symptoms

Collection of samples was based on visual symptoms for stone fruit trees in the Bekaa and randomly for olive trees and stone fruits in the north as they did not show any typical symptom.

#### 1.1. Stone fruits

Orchards of apricots, almonds and peaches were visited. Almonds and peaches were found to be the most attacked by *Verticillium* in Central Bekaa region whereas apricots were infected at lesser degree (one infected sample was collected from one orchard) and no orchards of cherries were found infected with the disease. Forty six samples of stone fruits twigs were collected from trees showing symptoms of wilting whereas one sample from Laqlouq was collected from trees without symptoms.

Where symptoms were evident, unilateral defoliation as well as unilateral wilting were the most symptoms observed. The exterior wilt symptom was confirmed by tissue discoloration of the vascular system in a cross and longitudinal sections of twigs and branches.

In some cases, on almonds, collected samples from Sariine and Kafar Zabad showed also dead leaves remaining attached to branches with die back of shoots.

*Verticillium* wilt has been reported or suspected in Lebanon on stone fruits before. Attalah (2000) and while surveying and diagnosing diseases associated with almond has detected the presence of *Verticillium* wilt. E. Choueiri (2003) has suspected the presence of *Verticillium* wilt in some orchards as he was surveying stone fruits for viral diseases.

Out of the 22 almonds collected samples, four samples from almond trees were taken from orchards in Sariine close to potato infected field. This closeness is dangerous as *Verticillium dahliae* can spread to orchard trees on residues from neighboring potato fields (Hiemstra, 1997). Six samples were collected from orchards intercropped with vegetables as tomatoes, cucumbers, cabbages

and others. But this intercropping with vegetables should be avoided since it increases the level of inoculum in the soil, soil cultivation often injures the roots thus providing a major source for *Verticillium dahliae* entry in the roots and irrigation increases the pathogen dissemination (Tjamos, 1993).

Peach and almond trees attacked by the disease were young (around 7-10 years and 10-15 years respectively). These results are in accordance with those of Guillaumin (1989) and López-Escudero and Blanco-López (1997). Guillaumin (1989) and while studying disease infecting almond roots identified *Verticillium* wilt on young trees. López-Escudero and Blanco-López (1997) reported that 25 % of olive groves less then 20 years old were affected by *Verticillium* wilt.

### 1.2. Olives

Olive groves, visited in North Lebanon, were chosen randomly and the choice was based on the relative importance of this area in terms of olive cultivation. Samples collected were from trees with or without symptoms. Symptoms were often associated with bad application of cultural practices as pruning and pesticides, with attack of insects as zeuzera and occurrence of physiological conditions. As a result, no clear diagnostic of *Verticillium* wilt symptoms was observed in the field. This observation was reported also by Shams Eddine (1998) while surveying Koura region for collection of olive samples.

In parallel, it is important to mention the high incidence of peacock eye disease on leaves in all visited localities.

Out of the 50 samples collected from olive trees, only ten samples showed discolored tissues. According to Johnson *et al.* (1997), olive show less coloration than other species and sometimes discoloration is not present.

Although young olive trees are attacked by *Verticillium* (Hernandez *et al.*, 1998), but olive trees of groves visited and showing *Verticillium* symptoms were old ones (mostly 100 years and above).

These attacks may result from microsclerotia in infected leaf petioles which can carry *Verticillium dahliae* (Tjamos and Tsougriani, 1990), infected trees being thus neglected source of inoculum (Hiemstra, 1997).

The majority of the samples, especially those of olive trees, did not show the symptoms of the fungus. Therefore, all the samples were cultured on different media in order to identify the cause of the diseased samples.

### 1.3 Severity and incidence of the disease

Severity and incidence of *Verticillium* wilt were evaluated while collecting samples from only stone fruits orchards with evident wilt symptoms.

Regarding host type, almond trees were the most susceptible to *Verticillium* attack as 14 samples out of 20 had a severe attack. Five samples showed moderate severity whereas one sample showed a mild attack. Concerning peaches, seven samples from Kab Elias were attacked severely by *Verticillium* whereas eight samples were moderately attacked and four samples presented a mild severity. Only one apricot sample had a severe attack.

The higher incidence of *Verticillium dahliae* was found in Sariine where eight of the collected samples were taken from fields with about 60 % incidence of the disease. Two fields showed an incidence of 2 %, five samples collected from fields with 6 %, four samples from fields with 30 %. The other villages located in the center of the Bekaa showed fewer incidences in the fields in comparison with the fields located in Sariine. Five samples from different fields of three villages had 1 % incidence. Only three samples were taken from fields with 5 % incidence.

Concerning olive diagnostic and as mentioned above was difficult to undertake. However, in one grove located in Amioun, incidence was around 30 % with highly severe attack.

## 2. Laboratory experiments

Microscopic observation, where a tiny amount of mycelium was mounted on slides, confirmed, in most cases, the visual wilt symptoms, thus relating them to *Verticillium dahliae*.

All samples, independently of host type and locality, which showed wilt symptoms in the field, were infected with *Verticillium* wilt. Samples which did not show evident symptoms of *Verticillium* were infected by a saprophytic disease. Three indicators for *Verticillium dahliae* identification were used: development of white mycelium, presence of microsclerotia and the verticillate shape of conidiophores (Pegg and Brady, 2002).

Sewell (1959) has reported that when mycelium grows from infected debris into the soil, the first-formed conidiophores are verticillate. In parallel, a comprehensive description of the pathogenic isolates of *Verticillium* described all the details of all the resting structures of the *Verticillium* species. In *V. dahliae*,

septation and swelling occur in contagious hyphae which continue to bud until globular, almost spherical cell masses form. These latters become melanized as the typical microsclerotia (Isaac, 1949, cited by Pegg and Brady, 2002).

Thirty two samples from stone fruit trees that showed symptoms when collected had shown also microsclerotia during the microscopic observation. Microsclerotia appeared also in thirteen olive isolates (Tab. 1).

The remaining samples showed either a contamination or did not show any presence of pathogens when cultured.

**Table 1:** Isolated samples of stone fruits and olives where Verticillium dahliae was identified according to microsclerotia and verticillate conidiophores.

| Sample number | Host | Locality | Isolate number |
|---|---|---|---|
| 1 | Almond | Sariine | S1 |
| 2 | | | S2 |
| 4 | | | S4 |
| 5 | | | S5 |
| 6 | | | S6 |
| 7 | | | S7 |
| 8 | | | S8 |
| 9 | | | S9 |
| 10 | | | S10 |
| 11 | | | S11 |
| 13 | | | S13 |
| 15 | | | S15 |
| 16 | Peach | Khorbet Anafar | KHP16 |
| 17 | | | KHP17 |
| 18 | Apricot | Bar Elias | B18 |
| 19 | Almond | Kafar Zabad | KZ19 |
| 20 | | | KZ20 |
| 21 | | | KZ21 |
| 22 | | | KZ22 |
| 23 | | | KZ23 |
| 24 | Peach | Sariine | SP24 |
| 25 | | | SP25 |
| 26 | | | SP26 |
| 27 | | | SP27 |
| 30 | | Kab Elias | KEP30 |
| 31 | | | KEP31 |
| 32 | | | KEP32 |
| 33 | | | KEP33 |
| 34 | | | KEP34 |
| 36 | | | KEP36 |
| 37 | | | KEP37 |
| 38 | | | KEP38 |

**Table 1** (cont'd):

| Sample number | Host | Locality | Isolate number |
|---|---|---|---|
| 72 | Olive | Dar Beechtar | DB72 |
| 75 | | | DB75 |
| 76 | | | DB76 |
| 77 | | | DB77 |
| 79 | | | DB79 |
| 80 | | | DB80 |
| 82 | | Amioun | A82 |
| 83 | | | A83 |
| 84 | | | A84 |
| 85 | | | A85 |
| 86 | | | A86 |
| 91 | | | A91 |
| 92 | | Bechmezine | B92 |

### 2.1. Morphological characteristics

After microscopic observation, mycelial growth, from plates where *V. dahliae* was identified, was then transferred on PDA media in order to study the morphological characteristics. As mentioned by Mol and Scholte (1995a, b), microsclerotial production is important for identification of *Verticillium dahliae*. For this reason, all samples were incubated at room humidity under temperature between 23 and 25 °C, this temperature being ideal for the *Verticillium* fungus for 28 days.

#### 2.1.1. Colonies morphology

Most of the cultures from stone fruit samples had a puffy growth in the incubating room; just one sample showed a thickness in growth. When incubated, the colonies did not have the same color.

The obtained results showed that most isolates having white colonies were from peaches, whereas the majority of those having yellow colonies were from almond trees.

In all isolates and after 7 to 10 days, few microsclerotia appeared at the beginning next to the center of the plate then continue their growth thus forming

concentric rings. This appearance of microsclerotia was also described by Ogawa *et al.* (1995) who reported that colonies turn black from the center after almost a week resulting from the production of microsclerotia.

This difference in colony color between isolates of *V. dahliae* was also reported by Shams Eddine (1998) and by Kawtharany (1998). But Ogawa *et al.* (1995) described colonies of *V. dahliae* as being white at first with moderate aerial mycelium and a regular margin then.

In all colonies, independently of trees type, *V. dahliae* produced prostate, hyaline mycelium, whitish to cream colonies seen from the underside of the plate after one week, then gradually darkens with the formation of black, thick-walled microsclerotia. This phenomena was also reported by Pegg and Brady (2002) when studying mycelial growth of *V. dahliae* on artificial media.

This phenomenon was also reported by Pegg and Brady (2002) when studying mycelial growth of *V. dahliae* on artificial media.

This difference in colony color could be related to different types of isolates. In fact, temperatures over 28 °C can lead to the highest frequency of morphological mutants of *Verticillium dahliae* in cultures (Roth and Brandt, 1964; cited by Pegg and Brady, 2002). Also a higher frequency and range (9,1 %) of morphological variants from microsclerotia compared with 0,5 % from conidia has been found (Tolmsoff, 1972). On the other hand, herbicides and insecticides have also been shown to act as mutagens on *Verticillium* mutants and resistant to toxic substances have also been studied (Galperina, 1990).

### 2.1.2. Growth rate

In parallel with studying colony morphology, the growth rate of each colony was also considered. At an interval of two days, measurements were done for colony size and that up to 28 days. The growth was similar in stone fruits and olive colonies during all the period of incubation and under the same conditions of temperature and light. An example of the obtained results is presented in figure 1. Results showed that colony growth was parabolic from day 2 and up to day 12. From day 14 to the end of the experiment, the growth measured was linear.

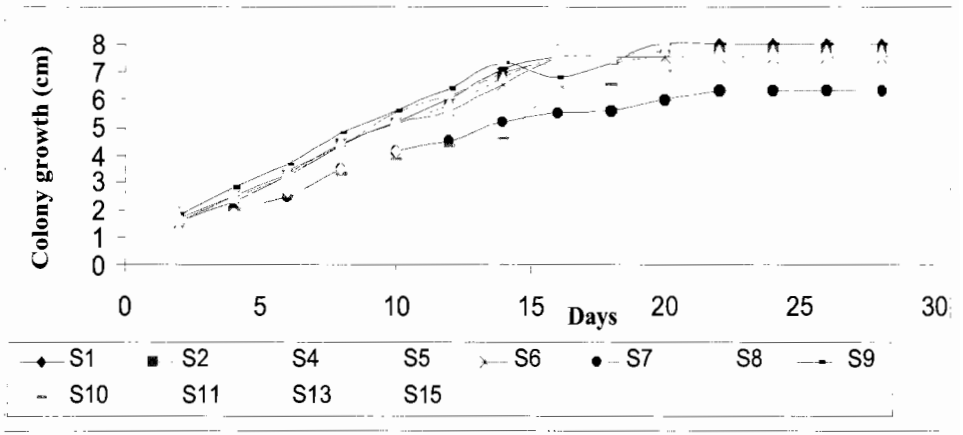


**Figure 1.** Colony size (in cm) of different almonds isolates from Sariine, grown on PDA up to 28 days after isolate transfer. Each value represents the mean of two replicates.

### 3. Frequency of Verticillium dahliae

Combining both visual and microscopic observations, it can be concluded that stone fruits, mainly almonds and peaches were severely attacked in Bekaa where the disease frequency was of 69,5 %. Frequency of the disease in olive samples was 26 %.

## CONCLUSION

Many visits had been done to the different regions cultivated with stone fruit and olives trees with more than one passage in stone fruit orchards. Severity and incidence of the disease were evaluated in stone fruit orchards. During field experiments, 96 samples were collected (46 from stone fruits trees and 50 from olive trees). Almond trees were found to be the most susceptible to *Verticillium* attack and the Sariine village located in Bekaa center was the village with the highest incidence of *Verticillium dahliae.*

In olive groves and because the symptoms were not evident, severity and incidence were not evaluated. Amioun and Dar Beechtar samples were found to be the most infested with the fungus.

Visited Orchards were intercropped with vegetables and olive groves were

neglected. But it is necessary not to intercrop vegetables in olive and stone fruit orchards because intercropping of vegetables crops increases the level of inoculum in the soil, soil cultivation often injures the roots thus providing a major source for pathogen entry in the roots and irrigation increases the pathogen dissemination (Tjamos, 1993).

Neglected infected trees are also source of inoculum (Hiemstra, 1997), therefore, cultural practices should be undertaken in order to reduce inoculum as microsclerotia are present in infected leaf petioles (Tjamos and Tsougriani, 1990).

After visiting and collecting samples from trees that showed the disease symptoms and others showing similar symptoms especially in olive groves, laboratory experiments were done.

The laboratory experiments allowed us to study the morphological characteristics of the disease. Laboratory experiments permitted to isolate and identify 45 isolates revealing the causal agent responsible of the wilting of trees from collected samples. Results showed that symptoms in 32 collected samples out of 46 from stone fruits and in 13 collected samples from 50 olives were related to the presence of *Verticillium dahliae*. In fact, verticillate conidiophores shape and appearance of microslerotia were observed in these colonies. These isolates were kept in slants and conserved in the refrigerator.

Combining both visual and microscopic observations, it can be concluded that stone fruits, mainly almonds and peaches were severely attacked in Bekaa where the disease frequency was of 69,5 %. Frequency of the disease in olive samples was 26 %.

The morphological characteristics of the isolates of *Verticillium dahliae* were studied by incubation of plates at $23^0$ C for 28 days.

Eleven out of the 14 colonies obtained from peach trees samples were white and 14 out of 17 colonies obtained from almond trees were yellow. Whereas for colonies obtained from olive samples, they were all white.

Colony growth was similar in all isolates independently from host types except for two olive isolates. In fact, colonies started to grow fast starting two days after incubation and up to 12 days then they growed slowly up to 22 days after incubation to reach a size of 7 to 8 cm and keep this size up to 28 days. The obtention of different isolates inclined more detailed studies in order to see whether these isolates entail different symptoms *in vivo* tests.

Since *Verticillium* wilt had become a severe problem, it is necessary to prevent it first of all by the use of clean *Verticillium*- free nursery seedlings, since diseased seedlings are often major sources of primerary infection (Thanassoulopoulos, 1993).

Based on results of this experiment, *Verticillium dahliae* attack could not be neglected and should be considered as a severe disease of the stone fruit trees in the Bekaa region and of olive trees in North Lebanon.

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