Effect of Arbuscular Mycorrhizal Fungi on Verticillium wilt development of olive trees caused by Verticillium dahliae

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Abstract
This study reports the effect of Arbuscular Mycorrhizal Fungi (AMF) on olive plants to enhance its tolerance against Verticillium dahliae under greenhouse conditions. Inoculated plants with native mycorrhizal consortium (Rhizolive) or with Glomus irregulare pure strain were treated three months after mycorrhization with V. dahliae. It was found that root colonization by Rhizolive or G. irregulare was similar. Results showed that disease severity and percentage of dead plants were significantly reduced in G. irregulare + V. dahliae (1.36 and 20% respectively) compared to Rhizolive + V. dahliae treatments (2.20 and 28.30% respectively).

Moreover, compared to controls, lower dwarfing index and leaf alteration index were shown in inoculated plants with Rhizolive + V. dahliae (-9.73% and 0.143 in 21th week); also, in inoculated plants with G. irregulare + V. dahliae (-2.96% and 0.200 in 21th week) treatments. Mycorrhization with Rhizolive or G. irregulare seemed to help olive plants to reduce the disease symptoms caused by V. dahliae.

Keywords: Olive plants, Arbuscular Mycorrhizal Fungi, Verticillium wilt, Tolerance.

Introduction
Olive (Olea europeae L.) is one of the most important fruit trees of the Mediterranean regions. In Morocco, the area given over to olive trees is approximately 1,000,000 ha. 1,500,000 tons of olives are produced per year and occupied the 4th place behind Spain, Italy and Greece.1,2 The predominant cultivar is “Picholine Marocaine” represented by 96% of orchards, with two clones Haouzia and Menara.3 This local cultivar is particularly adapted to the multiplicity of the climatic contexts. Nowadays, Picholine Marocaine suffers from several problems that affect both its production and workforce, the most important of which include fungal diseases.4 Verticillium wilt of olive trees, incited by the soil born Verticillium dahliae Kleb, caused several symptoms such as wilting, dieback, defoliation and death of susceptible trees.5,6 The impact of the disease is particularly severe in intercropped orchards during first year after establishment of the groves, especially during spring and summer causing serious damage to new plants, but tends to become rather mild with aging.7,8

Much research on olives has been focused on selecting resistant cultivars by using a highly virulent isolate of V. dahliae.9,10 Other studies have utilized continuous lighting to reduce the symptoms of the disease.11

Although, under field conditions, Mercado-Blanco12 has used bacterial PGPR like Pseudomonas fluorescens and Pseudomonas putida to control infections caused by V. dahliae, verticillium wilt of olive trees is a limitations factor for the cultivation of high yielding and excellent quality cultivars in Morocco and in Mediterranean basin.13,15

The benefit of arbuscular mycorrhizal fungi (AMF) in development of olive plants is well known.16-18 Establishing a symbiotic association between AMF and the plant root system can influence the diseases caused by soil-born fungi.19-22 Moreover, AMF can enhance efficiency of plant roots to absorb water, macro and microelements from the soil. In addition, AMF can protect the olive plants against environmental stresses such as soil salinity,23 drought,24 and pathogens like Verticillium wilt.22 AMF improves plant health, vigor and minimize stress.25 With less stress, there is greater plant resistance to pathogens and pests and a reduction in pesticide usage.26 Other benefits include enhanced seedling growth as well as increased adventitious root formation of cutting.27-31 Currently, the interaction between AMF and V. dahliae in olive trees is rarely mentioned in literature.

The results offered by Karajeh and Al-Raddad32 demonstrated that uses of Glomus mosseae improve the tolerance of olive seedlings to Verticillium wilt which corroborate with other research for other species.33-35 Nevertheless, other studies showed that the uses of AMF did not provide any benefits to overcome the symptoms in olive trees caused by V. dahliae.14,36 The present study aimed to evaluate the effectiveness of mycorrhizal consortium (Rhizolive) and a reference pure strain (G. irregulare) in controlling Verticillium wilt of olive cultivar.
Material and Methods

Plant material and transplantation: Five-month-old healthy olive seedlings cv. Hauzia were grown in plastic pots in nursery (semi-controlled conditions). Plants were removed from plastic bags surrounding their roots into pots of 1.5 kg capacity containing soil and peat mix (1:1); they were placed in a greenhouse at an average temperature of 25 ± 3 °C, 60% of relative humidity, 330 m² s⁻¹ of light and sprayed by tap water regularly. The substrate containing rhizosphere soil of olives, which is poor in available phosphorus (7.72 ppm) and peat were sterilized at 180°C at the interval of 3 hours and 1 hour respectively to avoid any microorganisms that could affect the function of AMF or the pathogenic fungus.

Inoculum mycorrhization and multiplication: Mycorrhizal consortium inoculum (Rhizolive) used in this study was collected by Kachkouch et al. from the soil and the root samples of the olive trees rhizosphere in different Moroccan olive groves. It is composed of 25 species of Glomus, Acaulospora, Gigaspora, Entrophospora and Scutellospora. In addition, the reference pure strain, Glomus irregulare (isolate of DAOM 197198) was provided by Laboratory of Research Institute of Plant Biology, University of Quebec in Canada. The AMF cannot be grown by Glomus irregulare acaulospora, Glomus et acaulospora, Entrophospora and Scutellospora. The highly virulent OMV isolate of V. dahliae was obtained from the collection of the Laboratory of Biotechnology and Molecular Bioengineering, Biology department, Cadi Ayyad University, Marrakesh, Morocco. It was prepared by taking mycelial disks of the pathogen that punched out with a manual disk puncher with a diameter of 5 mm. These disks were transferred on potato sucrose agar (PSA) medium. The pathogen was incubated at 25 °C for 15 days in the dark; thereafter, 10 ml of sterilized distilled water was added to each plate twice. The inoculation density was adjusted to 10² conidia/mL.

From each block, 58 plants were inoculated with V. dahliae by stem puncture using a hole which was pierced by using drill at 5 cm depth in the upper part of their main stem. Thereafter, 10 ml of inoculum were injected with test tube inside the hole. Control plants were inoculated with 10 ml of sterile distilled water.

Plant culture and experimental treatments: Four treatments were applied (inoculation with V. dahliae, with both Rhizolive and V. dahliae, with both G. irregulare and V. dahliae and controls) and arranged in a complete randomized design. Afterward, plants were incubated in greenhouse and sprayed twice per week.

Disease evaluation: Symptoms of V. dahliae were estimated by calculating per each plant, the disease severity, disease incidence, percentage of dead plants. They were assessed each three days after inoculation with OMV isolate of V. dahliae during 5 months. The symptoms were evaluated on a score ranging of 0 to 4, following a scale as described in table 1. The disease severity was determined according to the modified formula of El Said et al.:

\[ \text{Disease severity} = \frac{\text{nx(Ax0)} + \text{nx(Bx1)} + \text{nx(Cx2)} + \text{nx(Dx3)} + \text{nx(Ex4)}}{\text{Total numbers of plants}} \]

where n: number of plants in categories of A-E. The disease incidence and percentage of dead plants (PDP) were calculated according to the following formula:

\[ \text{Disease incidence} = \frac{(\text{Number of infected plants})}{(\text{Total number of plants})} \times 100 \text{ and PDP} = \frac{(\text{Number of dead plants})}{(\text{Total number of plants})} \times 100, \text{ respectively.} \]

Moreover, dwarfing index (DI) and leaf alteration index (LAI) were measured weekly per each plant during 5 months. The DI was determined according to the following formula:

\[ \text{D}. \text{I.} = \frac{(\text{M} - \text{X})}{\text{M}} \times 100 \]

where X: Stem height of the inoculated plants, M: Average size of the control plants. The LAI was determined according to the rating score of 0 to 5 used by Douira et al. following a scale as described in table 1:

\[ \text{L}. \text{A}. \text{I.} = \frac{(\Sigma (i \times x_i))}{(6 \times \text{Nt F})} \]

where i: Leaves appearance notes 0-5, x: Number of leaves with the note i, Nt F: Total number of leaves.

Maize seeds were disinfected and placed in germinating condition within the vermiculite (previously sterilized at 200 °C for 3 h) watered with sterile distilled water. After a week of germination, the maize plants were planted in plastic pots (13 x 09 cm) containing soils with AMF to be tested. These plants were watered regularly with distilled water with 30 ml weekly intake of modified nutrient solution of Long Ashton. After three months of culture, the mycorrhized roots of maize were disinfected for 10 min, rinsed three times for 10 min with sterile distilled water and cut into fragments of 1–2 mm long. The frequency of infection (F) of maize root was determined by the technique described by Trouvelot et al. (F = 100%).

Infection of the host plant by V. dahliae: Two blocks of 116 plants each were inoculated with 50g of Rhizolive or with G. irregulare. A similar number of non-inoculated plants were included as a control block. After three months of mycorrhization, the percentage of mycorrhizal colonization was estimated by using the technique of Phillips and Hayman, the frequency and intensity were calculated by assigning an index from 0 to 5 to root segments under a research microscope for every treatment.

The highly virulent OMV isolate of V. dahliae was obtained from the collection of the Laboratory of Biotechnology and Molecular Bioengineering, Biology department, Cadi Ayyad University, Marrakesh, Morocco. It was prepared by taking mycelial disks of the pathogen that punched out with a
The total score of leaves constituted the foliar index. An average was calculated for each lot of six plants. Furthermore, the area under the disease progress curve (AUDPC) was calculated for each treated block with reference to the maximum value potentially reached in period of assessment according to the formula of Campbell and Madden:

$$\text{AUDPC} = \frac{\sum t/n (S2 + 2 x S3 + 2 x S4 + ... + Si)/4 x n}{100}$$

where t: interval in days between observations, Si: final mean severity, 4: maximum disease rating and n: number of observations from the first reading of the symptoms. AUDPC and the final mean severity (FMS) were calculated in the end of the experience.

**Verticillium dahliae Re-isolation:** In the end of the experiment, plant infection was confirmed by isolating the V. dahliae from stems or roots in affected plants and controls. Samples were washed in running tap water, the bark was removed and tissue surface was disinfected in 0.5% sodium hypochlorite for 1 min. Roots and stems segments were transferred on PSA plates and incubated in the dark at 24 °C for a week.

**Statistical analysis:** Data were subjected to an analysis of variance for a randomized block design, using the SPSS 20.0 (software package). The mean values were examined by one-way ANOVA. Differences between treatments were determined using least significant difference (LSD) test at $P < 0.05$. Before data analysis, percentage values of mycorrhizal frequency and mycorrhizal intensity were arc-sin transformed.

**Results**

**Mycorrhization parameters:** Root colonization percentages, frequency (F) and intensity (I) were not significantly different between inoculated plants with a single strain (F = 98.66% and I = 79.23%) of AMF and plants inoculated with the mixture of several strains (F = 98.88% and I = 68.46%) of AMF (G. irregulare and Rhizolive, respectively). On the other hand, no root of control olive plants was mycorrhized (Figure 1).

**Disease symptoms:** Non-inoculated plants did not show any symptoms of Verticillium wilt (Figure 2), they grew normally and produced new twigs and leaves. The symptoms of inoculated plants were shown for a week in the V. dahliae inoculated plants and on the 10th day in mycorriza-treated plants (Figure 3). The most obvious symptoms observed were chlorosis and defoliation of green leaves, which started in the first week after inoculation and increased over time. However, wilt and dieback were slower and temporary in the G. irregulare + V. dahliae treatment. Chlorosis and dieback were extensive and progressive in both V. dahliae inoculated plants and Rhizolive + V. dahliae treatments. Defoliation started at low or moderate levels, became intense and increased over time.

In some cases, defoliation was partial in higher branches, especially in G. irregulare + V. dahliae treatment. The intensive defoliation was shown on the 13th day in V. dahliae inoculated plants and on the 17th in the other treatments. In some plants, yellowing was restricted to the basal leaves above the inoculation hole and wilting to leaves at the extremities. Disease incidence was showing extensive wilting or dieback affecting the whole plants in all treatments (Figure 3; Figure 4). After 43 days, the incidence was stable (57.99 %) in G. irregulare + V. dahliae treatment. However, it increased to attain 72.80% in the Rhizolive + V. dahliae treatment and 80% in the Verticillium inoculated plants (Figure 3; Table 4).

Important differences in the AUDPC and FMS values were found; they were significantly higher ($p < 0.05$) in V. dahliae inoculated plants (51.89% and 2.18) than in G. irregulare + V. dahliae treatment (27.68% and 1.17). Moreover, dual inoculation with G. irregulare + V. dahliae showed lower AUDPC and FMS values of 27.68% and 1.17 respectively (Table 4). The first dead plant was detected a week after inoculation in V. dahliae inoculated plants (Figure 4). Moreover, after 16th day this percentage increased in both treatments: V. dahliae and Rhizolive + V. dahliae. Meanwhile, G. irregulare + V. dahliae treatment showed the low death rate. Results indicated a significant gap between V. dahliae inoculated plants and G. irregulare + V. dahliae treatments.

Thus, at the end of the experiment, the pathogen had killed 32.65%, 27.19% and 20% of V. dahliae inoculated plants, Rhizolive + V. dahliae and G. irregulare + V. dahliae treatments respectively (Figure 3; Table 4). After 4 months of inoculation with V. dahliae, the disease symptoms were stable and affected plants exhibited yellowing, chlorosis and wilt leaves. Disease recovery as characterized by reducing and decreasing of the disease symptoms was accompanied by the production of new shoots and leaves; it was shown in mycorriza-treated plants. Furthermore, the high disease severity was observed in V. dahliae inoculated plants (2.49) and the lowest in G. irregulare + V. dahliae treatment (1.36) (Figure 2).

**Effect of AMF on dwarfing index and leaf alteration index:** Dwarfing index symptom appeared first in V. dahliae inoculated plants, then in G. irregulare + V. dahliae and Rhizolive + V. dahliae treatments after 6th, 9th and 12th weeks respectively (Table 2). The highest dwarfing index was found; they were significantly higher ($p < 0.05$) in V. dahliae inoculated plants (51.89% and 2.18) than in G. irregulare + V. dahliae treatment (27.68% and 1.17). Moreover, dual inoculation with G. irregulare + V. dahliae showed lower AUDPC and FMS values of 27.68% and 1.17 respectively (Table 4). The first dead plant was detected a week after inoculation in V. dahliae inoculated plants (Figure 4). Moreover, after 16th day this percentage increased in both treatments: V. dahliae and Rhizolive + V. dahliae. Meanwhile, G. irregulare + V. dahliae treatment showed the low death rate. Results indicated a significant gap between V. dahliae inoculated plants and G. irregulare + V. dahliae treatments.

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G. irregulare + V. dahliae showing the lower index compared to the control, the DI values were -2.96 % in the 21st week (Table 2). The leaf alteration index (LAI) of olive plants was lower in mycorrhiza-treated plants than in the control (Table 3). Thus, the higher LAI was observed in V. dahliae inoculated plants, it was 0.302 in 18th week. For first three weeks LAI was slower and abruptly increased thereafter in all treatments except control. LAI decreased after 15th week in G. irregular + V. dahliae, Rhizolive + V. dahliae and the control with values of 0.200, 0.143 and 0.062 in 21st week respectively (Table 3).

Figure 1: Mycorrhizal frequency and intensity of olive plants cv. Haouzia mycorrhized three months either with Rhizolive or with G. irregular

— V. dahliae — Rhizolive + V. dahliae — G. irregular + V. dahliae

Figure 2: Disease progress based on symptoms severity of olive plants cv. Haouzia after been mycorrhized three months either with Rhizolive or with G. irregular, then inoculated with V. dahliae

Table 1
Scale of wilt symptoms in olive plants

<table>
<thead>
<tr>
<th>Note</th>
<th>Categories / Description of symptoms</th>
<th>Disease severity</th>
<th>Leaf alteration index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>A : Healthy plant or no symptoms</td>
<td>i0 : Healthy leaf</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>B : Onset of dieback, no defoliation</td>
<td>i1 : Yellowing</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>C : Average dieback, average wilting</td>
<td>i2 : Chlorosis</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>D : Severe dieback, severe wilting</td>
<td>i3 : Necrosis</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>E : Dead plant</td>
<td>i4 : Wilting</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>i5 : Fallen</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3: Progress of incidence disease based on infected plants cv. Haouzia after been mycorrhized three months either with Rhizolive or with *G. irregular*, then inoculated with *V. dahliae*

![Graph showing disease incidence over time](image)

Figure 4: Plants death rate progress based on dead plants cv. Haouzia after been mycorrhized three months either with Rhizolive or with *G. irregular*, then inoculated with *V. dahliae*

![Graph showing death rate over time](image)

**Table 2**

Dwarfing index (%) of olive plants cv. Haouzia after been mycorrhized three months either with Rhizolive or with *G. irregular* then inoculated with *V. dahliae* during five-months

<table>
<thead>
<tr>
<th>Weeks after inoculation</th>
<th>Control</th>
<th><em>V. dahliae</em></th>
<th>Rhizolive + <em>V. dahliae</em></th>
<th><em>G. irregular</em> + <em>V. dahliae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 ± a</td>
<td>-11.89 ± 2.81 ab</td>
<td>-17.16 ± 2.55 b</td>
<td>-12.70 ± 2.76 ab</td>
</tr>
<tr>
<td>3</td>
<td>0 ± a</td>
<td>-10.71 ± 3.23 b</td>
<td>-23.17 ± 3.97 c</td>
<td>-12.59 ± 2.50 b</td>
</tr>
<tr>
<td>6</td>
<td>0 ± a</td>
<td>-8.92 ± 2.61 ab</td>
<td>-19.62 ± 2.87 c</td>
<td>-10.29 ± 2.85 bc</td>
</tr>
<tr>
<td>9</td>
<td>0 ± a</td>
<td>-1.37 ± 1.68 a</td>
<td>-15.15 ± 3.78 b</td>
<td>-5.61 ± 2.20 ab</td>
</tr>
<tr>
<td>12</td>
<td>0 ± a</td>
<td>3.94 ± 1.60 a</td>
<td>-11.93 ± 2.05 b</td>
<td>-0.53 ± 2.09 a</td>
</tr>
<tr>
<td>15</td>
<td>0 ± a</td>
<td>3.54 ± 1.25 a</td>
<td>-11.20 ± 2.11 b</td>
<td>-0.42 ± 2.04 a</td>
</tr>
<tr>
<td>18</td>
<td>0 ± a</td>
<td>4.77 ± 1.17 a</td>
<td>-10.47 ± 2.33 b</td>
<td>-1.13 ± 1.97 a</td>
</tr>
<tr>
<td>21</td>
<td>0 ± ab</td>
<td>5.13 ± 1.26 a</td>
<td>-9.73 ± 2.35 c</td>
<td>-2.96 ± 1.77 bc</td>
</tr>
</tbody>
</table>

Means ± SE within line followed by the same letter are not significantly different at *p* < 0.05
Verticillium dahliae re-isolation: Re-isolation of the pathogen from the root and stem tissues was higher in V. dahliae inoculated plants than in Rhizolive + V. dahliae and G. irregulare + V. dahliae treatments with values of 100%, 86% and 72% in stems respectively and 100% in roots. On the other hand, V. dahliae did not appear in plates concerning non-inoculated plants.

Discussion

This work proved beneficial for AMF to overcome the symptoms caused by V. dahliae in olive plants cv. Haouzia. The inoculation of olive plants either with mycorrhizal consortium (Rhizolive) or with G. irregulare pure strain showed a similar and higher mycorrhizal frequency and intensity, which has substrate with a low available phosphorus content. These results corroborate with those reported by. The application of AMF seemed to help the olive plants withstand the stress produced by the transplantion to the pressed pots soil and peat mix

We inoculated olive plants with Verticillium once AMF was established because pre-inoculation can nullify or reduce the detrimental effects of root pathogens on plants growth and increases of symptoms.

Various authors have reported that the interaction of pathogenic or non-pathogenic soil fungi with AMF increases tolerance to the disease e.g. AMF Glomus mosseae with the saprophytic fungi Trichoderma konizii and Fusarium solani with the phosphate producing fungus Aspergillus fumigatus with the shoot pathogen Oidium lini and the wilt pathogen Fusarium oxysporum.

The interaction between AMF and V. dahliae in olive plants has not been much studied. However, the study carried out by Karajeh and Al-Ruddah demonstrated the beneficial effect of using Glomus mosseae to reduce the disease severity of Verticillium wilt on olive plants cv. Nabali. Furthermore, under greenhouse conditions, Porras-Soriano et al. proved that application of different AMF (G. intraradices, G. mosseae or G. claroideum) did not seem to improve the tolerance of cv. Cornicabra olive seedlings to Verticillium wilt, which agrees with Kapulnik et al. None of the AMF (G. intraradices and G. mosseae) did not provide any benefits to susceptible cv. Picual used.

In the present study, plants were inoculated using stem injection which is an effective tool that allows to conidial suspension to impregnate the root system, rather than dipping the root. Also, young olive plants cv. Haouzia were mycorrhized either with Rhizolive or with G. irregulare for three months, then inoculated with V. dahliae. With these experimental conditions, symptoms appeared after a week and caused consistent infections in V. dahliae inoculated plants. In addition, all treated plants exhibited a high

### Table 3

Leaf alteration index of olive plants cv. Haouzia after been mycorrhized three months either with Rhizolive or with G. irregulare, then inoculated with V. dahliae during 5 months

<table>
<thead>
<tr>
<th>Weeks after inoculation</th>
<th>Leaf alteration index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>0.057 ± 0.008 a</td>
</tr>
<tr>
<td>1</td>
<td>0.102 ± 0.011 a</td>
</tr>
<tr>
<td>3</td>
<td>0.111 ± 0.016 a</td>
</tr>
<tr>
<td>6</td>
<td>0.103 ± 0.012 a</td>
</tr>
<tr>
<td>9</td>
<td>0.068 ± 0.005 b</td>
</tr>
<tr>
<td>12</td>
<td>0.065 ± 0.005 c</td>
</tr>
<tr>
<td>15</td>
<td>0.065 ± 0.005 c</td>
</tr>
<tr>
<td>18</td>
<td>0.062 ± 0.005 c</td>
</tr>
</tbody>
</table>

Means ± SE within line followed by the same letter are not significantly different at p < 0.05

### Table 4

Incidence, death, FMS and AUDPCP of Verticillium wilt of olive plants calculated after five months of inoculation with V. dahliae

<table>
<thead>
<tr>
<th>Disease incidence (%)</th>
<th>PDP (%)</th>
<th>FMS (%)</th>
<th>AUDPC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. dahliae</td>
<td>80.00 a</td>
<td>32.65 a</td>
<td>2.18 a</td>
</tr>
<tr>
<td>Rhizolive + V. dahliae</td>
<td>72.80 a</td>
<td>27.19 ab</td>
<td>1.72 b</td>
</tr>
<tr>
<td>G. irregulare + V. dahliae</td>
<td>57.99 b</td>
<td>20.00 b</td>
<td>1.17 c</td>
</tr>
</tbody>
</table>

Means ± SE within column followed by the same letter are not significantly different at p < 0.05

- **PDP** = percentage of dead plants
- **FMS** = final mean severity of symptoms 5 months after inoculation.
- **AUDPCP** = area under the disease progress curve with reference to the maximum value potentially reached over the assessment period.
frequency of positive isolations of *V. dahliae* from the root and stem tissues after 5 months of the experiment. Therefore, plants have become infected irrespective of their resistance as already found by some author\(^{56,57}\).

Disease severity was higher in *V. dahliae* inoculated plants whereas, *G. irregulare + V. dahliae* treatment showed the lowest disease severity, coinciding with findings reported by Karajeh and Al-Raddad\(^{32}\). Inoculation of olive plants with *V. dahliae* developed the same kind of symptoms than those reported in several studies\(^{8,43,57}\). After 5 months of mycorrhization with *G. irregulare*, olive plants significantly reduced their disease severity, disease incidence and PDP in comparison to *V. dahliae* treatment. Substantially lower values were recorded for mean AUDPC, FMS and PDP in *V. dahliae + G. irregulare* treatment. Verticillium wilt disease onset was delayed and its development was slower in mycorrhiza plants with pure strain ones related to the reduction in the deleterious effect of *Verticillium* on plant growth and on hormonal balance, which could differ between non-mycorrhizal and mycorrhalzal plants\(^{34}\).

In fact, the highest levels of cytokinins have been measured in mycorrhizal roots\(^{58}\). Previous studies showed that *G. irregulare* newly identified had a positive effect on young Jacaranda growth plants cultivated under nursery conditions and reduced strongly disease severity on potato plants inoculated with *Fusarium sambucinum*, which suggested that the biocontrol effect was due to stimulation of plants defense\(^{59,60}\).

Differences were significant between non-treated and mycorrhiza-treated plants and they appeared in all measured parameters. Moreover, induction of systemic resistance is suggested as the mechanism of disease suppression\(^{61}\). According to Morandi\(^{62}\), this resistance is due to the beneficial effect of AMF, which causes an accumulation of phenolics and associated isoflavonoids and flavonoids in the roots of their host plants. Similar results on shoot height, fresh and dry weight of tomato and eggplant seedlings have been reported by Karagiannidis et al\(^{34}\) where it was indicated that the use of *Glomus mosseae* reduces the detrimental effect of *V. dahliae*.

Dwarfing index and leaf alteration index were decreased in mycorrhiza-treated plants compared to the treated plants only with the pathogen (Tables 2, 3) which can be explained by producing new leaves in mycorrhized plants. According to Chliyeh et al\(^{13}\) and Sghir et al\(^{63}\), the combination of *V. dahliae* or *F. oxysporum f. sp* and AMF Rhizolive significantly increased DI and LAI in tomato seedlings and date palm respectively. Results in this work indicated production of new twigs and leaves in mycorrhiza-treated olive plants after three months of inoculation with *V. dahliae*; the pathogen is not able to kill all the olive plants in which it has caused severe disease symptoms. However, using the *V. dahliae* alone did not allow olive plants to recover from infection when symptoms were extreme. This fact demonstrates that inoculation with Rhizolive or *G. irregulare* improves the tolerance of susceptible olive plants cv. Haouzia to Verticillium wilt.

**Conclusion**

The olive plants inoculated at an early stage with AMF during the nursery could reduce the deleterious effect of *Verticillium* on olive plants and produce more vigorous plants that can resist the pathogen. However, bioprotection effect against *V. dahliae* was conditioned by plant stage at the moment of pathogen attack. The effectiveness of the inoculation method induced the appearance of symptoms after a week of inoculation. Disease severity, disease incidence and PDP were higher in plants inoculated only with *Verticillium* than in mycorrhiza-treated plants.

Moreover, AMF allowed DI and LAI to be reduced after 9\(^{th}\) and 15\(^{th}\) weeks respectively after inoculation with the pathogen and delayed the appearance of disease symptoms after 12 weeks in Verticillium inoculated plants. Although, root colonization with AMF seems to enhance the tolerance of olive plants to attack by *V. dahliae*.

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