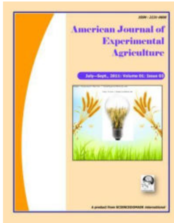

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Reaction of Some Olive Cultivars to *Verticillium dahliae* Isolates Agent of Vascular Wilt: A Comparative Study

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Research Article

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ABSTRACT

Verticillium dahliae Kleb. the causal agent of vascular wilt is an important pathogen of olive trees in growing areas of the world. To evaluate the reaction of the susceptibility of different commercial olive cultivars to the pathogen, six months old of eight commercial olive cultivars including Bladi, Kalamon, Koroneiki, Konservalea, Manzanilla, Mission, Rooghany, Sevillana and Zard cultivars and wild olive were root-dip inoculated. Nine-month-old nursery olive plants were inoculated with a non-defoliating (VCG4B, ND) or a defoliating (VCG1, D) isolate of *V. dahliae*. Resistance was evaluated by assessing symptom severity using a 0-4 rating scale and estimating the area under disease progress curves. Interaction between isolates of *V. dahliae* with different pathotypes and olive cultivars show that the defoliate pathotype cause higher disease severity index and stem colonization ($p< 0.01$). The percentage of plants killed and of those which recovered from the disease and stem colonization Index were used as additional parameters for including a particular cultivar into a defined category. One cultivar, 'Bladi', were susceptible or extremely susceptible to both pathotypes of *V. dahliae*. A second group showed differences of resistance depending on the pathotype used. They were susceptible or extremely susceptible to the D pathotype but resistant or moderately susceptible to the ND one. Finally, 'Kalamon' and 'Koroneiki' were resistant to both pathotypes of the pathogen. The resistance of these cultivars was evident by the plant ability to recover from infection with either isolates.

Keywords: Olive; Verticillium dahliae; defoliating and non-defoliating pathotypes;

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1. INTRODUCTION

Olive (*Olea europaea* L.), belonging to the Oleaceae botanical family, is a long-living and legendary tree with more than economical importance. Olive is cultivated in a wide variety of soils and can tolerate a broad range of physicochemical conditions (Lopez-Escudero and Blanco-Lopez, 2007). During the last two decades, Olive cultivation has expanded in Iran especially in Golestan province, the northern. In this province nearly 10,000 hectare of olive orchards are present, which represents about 20% of total national olive area (Anonymous, 2009). Commercial and native cultivars of olive are planted in Iran. Of course wild olive are the important genetical sources of olive, that residue of them can be seen in the East of Golestan province (Sanei et al., 2005b).

One of the major constraints for olive cultivation is Verticillium wilt, a vascular disease caused by the soil-borne fungus *Verticillium dahliae* Kleb. resulting in substantially reduced fruit yield and tree death (Al-Ahmad and Mosli, 1993; Blanco-Lopez et al., 1984; Cirulli and Montemurro, 1976; Dervis, et al., 2007; Hiemstra and Harris, 1998; Lopez-Escudero and Blanco-Lopez, 2004; Rodriguez-Jurado et al., 1993; Sanei et al., 2004; Serrhini and Zeroual, 1995; Thanassolopoulos et al., 1979), is a major disease affecting olive orchards in Iran (Sanei et al., 2004). The disease was first reported in Gorgan, Golestan province (Sanei et al., 1996) on cultivars Rooghany, and subsequently in many other olive districts in Iran (Taheri *et al*., 2006). *V. dahliae* infections were also observed on container-grown 2-year-old rooted cuttings of olive cv. Mission, Zard, Rooghany and Mary in many nursery in the province (Sanei et al., 2004). Disease incidence and severity are usually highest in young trees (Al-Ahmad and Mosli, 1993; Blanco-Lopez et al., 1984; Serrhini and Zeroual, 1995; Wilhelm and Taylor, 1965).

The symptomatology of verticillium wilt on olive is typical of vascular diseases. Usually, on young plants, the first symptoms appear in early spring, when leaves assume a colour varying from pale green to brown, roll up and fall off, leaving parts of the branches defoliated. The disease can extend to large portions of the foliage and 1–3 years after the appearance of symptoms, plants that are 5–15 years old may die (Cirulli, 1981; Sanei et al., 2004). On older plants, including those over 20 years old, the disease does not normally cause death, but does cause partial defoliation of some branches. Affected trees may recover, giving rise to a progressive reduction in disease incidence over several years (Sanei et al., 2010).

The new olive orchards established in Iran during the last 10-15 years have increased the importance of Verticillium wilt of olive (Sanei et al., 2004). Spread of the disease is linked to planting olive trees in fields previously used for growing susceptible hosts to the pathogen (Blanco-Lopez et al., 1984), or in those close to cotton fields infested by *V. dahliae* (Bejarano *et al*., 1996; Lopez-Escudero and Blanco-Lopez, 2001; Wilhelm and Taylor, 1965). The main factor for the increase in disease incidence and severity has been the establishment of orchards in fields previously cropped with *V. dahliae* host susceptible host especially cotton and vegetables for Golestan region (Sanei et al., 2000, 2004). Isolates of *V. dahliae* infecting olive can be classified as defoliating and non-defoliating pathotypes (Schnathorst and Mathre, 1966; Rodriguez Jurado et al., 1993; Sanei et al., 2004, 2005a, 2008) possessing, respectively, high and low aggressiveness. The defoliating strain was first recognized in cotton (Schnathorst and Mathré, 1966) and causes symptoms more severe on this species, also in olive than other strains (Lopez-Escudero and Blanco-Lopez, 2001; Rodriguez Jurado et al., 1993; Sanei et al., 2005a). In Iran, both pathotypes of *V. dahliae* were found infecting cotton and olive especially in the north (Sanei et al., 2008).

Several characteristics of *V. dahliae* make Verticillium wilt control difficult (Blanco-Lopez and Jimenez-Diaz, 1995). The pathogen can survive in the soil for long periods of time (Wilhelm, 1955), attack many dicotyledonous cultivated plants and weeds (Heale, 1988; Sanei et al., 2010; Vargas-Machuca, 1987), and chemical compounds are not effective, the use of cultivars resistant to wilt is, as with other wilt diseases (Blanco-Lopez et al., 1998), likely to be effective for olive. Research in Italy and other countries indicated that cultivars Coratina, Frantoio, Oblonga and Kalamon have useful resistance characteristics to the non-defoliating *Verticillium* pathotype (Barranco et al, 2000; Cirulli and Montemurro, 1976; Cirulli et al., 2009; Hartmann et al., 1971; Lopez-Escudero et al., 2004; Rodriguez Jurado et al., 1993; Tjamos et al., 1991; Schnathorst and Sibbett, 1971; Wilhelm and Taylor, 1965), while Ascolana, Cellino, Leccino, Manzanillo, Chemlalie, Konservolia, Mission and Picual are susceptible to the disease (Wilhelm and Taylor, 1965; Cirulli and Montemurro, 1976; Rodriguez Jurado et al., 1993; Tjamos, 1993; Lopez-Escudero et al., 2004). One clone of Arbequina named Allegra was also found to be highly resistant to the non-defoliating pathotype of *V. dahliae* (Tjamos, 1993). The use of resistant rootstocks could effectively contribute to the control of *V. dahliae*, even if the scion is susceptible (Cirulli et al., 2009; Porras Soriano et al., 2003; Sanei et al., 2005b).

The spread of the defoliating pathotype of *V. dahliae* in Iran (Sanei *et al*., 2005a, 2008) and its presence in commercial olive orchards (Sanei et al., 2004, 2008) make it necessary to determine which olive cultivars have higher resistance to *V. dahliae*. The objective of this work was to evaluate the resistance of olive cultivars to defoliating and non-defoliating pathotypes of *V. dahliae*, so that resistant cultivars can be identified and thus used for replanting, as rootstocks or as sources for resistance in future breeding programmes. The degree of susceptibility of this olive cultivar to verticillium wilt is not, however, known, also the present study aimed at evaluating the susceptibility to *V. dahliae* infections of some Golestan wild olives in comparison with other commercial olive cultivars.

2. MATERIALS AND METHODS

Two isolates of *V. dahliae*, were used in this study, obtained from diseased olive plants, in Golestan province and demonstrating different aggressiveness and symptomatology on cotton 'sahel cultivar', olive 'Zard cultivar' as differential plants (Sanei et al., 2008). Isolate D was a defoliating type (VCG1) and ND was a non-defoliating type (VCG4B) (Sanei *et al*., 2005a, 2008). The defoliating pathotype causes severe and rapid defoliation commonly extending to the entire plant. The non-defoliating (ND) pathotype causes typical leaf yellowing, necrosis, but infrequent defoliation (Sanei et al., 2004, 2008).

Inoculum was prepared from single-spore cultures of isolates, maintained on potato dextrose agar (PDA) slants at 4℃. Inoculations were made with a conidial suspension prepared from 7-day-old cultures grown on potato-dextrose-agar (PDA) slants in Petri dishes at 25±0.5℃ in the dark. The cultures were flooded with sterile distilled water and their surface was gently scraped with a sterile scalpel. The resulting suspension was filtered through cheesecloth to remove mycelial fragments. After filtration, the inoculum concentration was adjusted to 4×10^6 conidia per ml. Nine-month-old olive cultivars (Bladi, Kalamon, Koroneiki, Konservalea, Manzanilla, Mission, Rooghany, Sevillana and Zard), and wild olive stem-cutting collected from Minoodasht,Golestan province, propagated as rooted cuttings, were used to test the susceptibility to *V. dahliae* infection. Plants were inoculated by dipping their root systems in a conidial suspension for 30 min (Colella et al., 2008) and they were transplanted to sterile soil with 10 replications per experiment and placed in a glasshouse at 20-26℃ and 16-h photoperiod. Control trees were dipped into tap water.

The symptoms were rated 7 months after inoculation on a 0 to 4 scale according to Tjamos et al. (1991). To evaluate wilt resistance, disease severity was assessed weekly for 10 weeks, starting 2 weeks after inoculation. A scale 0–4 was used according to the percentage of plant tissue affected by chlorosis, leaf and shoot necrosis or defoliation (0=absence of symptoms, 1=light foliar symptoms in 1–9% of plant, 2=moderate foliar symptoms and light defoliation (10–25%), 3=severe foliar symptoms and moderate defoliation (26–50%) and 4=total defoliation or plant death). The percentage of dead plants (PDP), recovery from the disease (Lopez-Escudero and Blanco-Lopez, 2001; Hiemstra and Harris, 1998; Wilhelm and Taylor, 1965) and other symptoms such as marginal spots of leaves and irregular growth of twigs were also considered to estimate the severity of reactions. The area under the disease progress curve (AUDPC) was estimated for each cultivar considering its percentage with regard to the maximum possible value that could be reached in the 10 weeks period of assessment based on Lopez-Escudero et al. (2004): AUDPC= $[(t/2 * (S2 + 2 * S3 + \cdots + 2Si{-}1 + Si)/4 * n] * 100$ (t = interval in days between observations; Si = final mean severity; 4 = maximum disease rating; n = number of observations). Plant infection was verified by the isolation of the fungus from affected shoots or leaf petioles of affected plants during the experiments. Plants were arranged according to a split-plot completely randomised block design. The mainplot was the *V. dahliae* pathotype, and cultivars were assigned to sub-plots. The analysis of variance (ANOVA) of AUDPC of reference-cultivars in each experiment were performed to determine the variability among experiments. In experiments where reactions of cultivars were statistically different, values of AUDPC of cultivars included in these experiments were corrected regarding the percentage of the difference between the values of AUDPC for reference-cultivars in significant and non-significant experiments. Statistical analysis was performed by SAS program. Mean values were compared by the by the Fisher's protected LSD at $P = 0.05$.

To determine the colonization index, all leaves of inoculated and non-inoculated (control) cuttings were removed and the stem was disinfected by 1% NaOCl for 10 min, washed, dried and its epidermis peeled. Four segments (5 mm length) from each of two different parts (see below) were cut and placed on C'zapeck Dox Agar (supplemented with 100 ppm streptomycin) for 14 days at 25°C. Once *V. dahliae* was detected the segment was considered as infected. Colonization index (CI) was calculated as follows: CI= $(2 \times Nb + 6 \times Nt) / N$, where Nb, is the number of infected segments at the base of the stem- cutting, and Nt is the number of infected segments at the top (8 cm above the base), and N is the total number of tested segments. The number of infected segments from the base and upper parts was multiplied by the coefficient factors of 2, and 6, respectively, resulting in a calculated colonization index in range between 0 and 8. As detection of *V. dahliae* in the upper parts of the stem is more rare than in the base, detection in the upper parts may reflect a higher level of plant colonization (Tsror et al., 2001).

3. RESULTS AND DISCUSSION

Isolates from olive tested in this experiment were pathogenic to all olive cultuvars. The first symptoms developed 2 weeks after inoculation. Chlorosis was the most common symptom observed when the ND isolate was used. In plants inoculated with the D isolate, chlorosis was associated with cultivars showing certain level of resistance. Defoliation was also very frequent. It occurred, in the absence of chlorosis, in all susceptible cultivars inoculated with the D isolate, starting at 3–4 weeks after inoculation and intensifying from the seventh week after inoculation. Severity of vascular browning was the least useful index in differentiating the tested olive germplasm, because cross section of diseased plants with non-defoliating pathotype sometimes does not show any discoloration and low variation of vascular browning values on most tested cultivars did not allow differentation of the reactions of the accessions to the pathogen. Also, the non-defoliating pathotype induced a degree of vascular browning that was not much lower than that induced by the defoliating one, although the latter caused much higher external symptom severities and values of AUDPC%.

Defoliation was intensive in susceptible cultivars such as 'Mission', 'Manzanilla' and 'Sevillana', and slight and restricted to the middle of the main shoots of the plants in moderately resistant cultivars. Sudden wilt or apoplexy, characterised by the progressive rolling inward and chlorosis of leaves, was also observed in plants inoculated with the D or the ND *V. dahliae* isolate. Leaves became necrotic and remained attached to the twigs. The D isolate induced higher incidence of disease and symptom severity than the ND one, and earlier death of plants. Therefore, the D isolate caused between 70% and 100% of dead plants in 5 out of the 9 cultivars inoculated, whereas mortality (> 30%) was not observed when the ND isolate was used (Table 1). The pathogen was also isolated from petioles of green leaves collected immediately after defoliation.

Table 1. AUDPC, PDP and CI of olive cultivars inoculated with defoliating and non-defoliating isolates of *V. dahliae**

	Defoliating			Non-defoliating		
Cultivars	**AUDPC**	**PDP**	**CI**	**AUDPC**	**PDP**	**CI**
Bladi	85.4a**	100	2.5a	68.9a	20	2a
Kalamon	25.2d	30	1.5b	11.1d	0	1b
Koroneiki	22.8d	20	1.5b	12.8d	0	1b
Konservalea	81.4a	90	2.5a	57.2a	30	2a
Manzanilla	66.2b	60	2ab	36.7b	10	1.5ab
Mission	68.6b	50	2ab	34.3b	0	1.5ab
Rooghany	77.8b	100	2ab	54.9a	30	2a
Sevillana	57c	70	2ab	23.7c	0	1a
Wild olive	8.2 e	0	1c	3.1 e	0	1 a
Zard	80.3a	100	2.5a	53.1a	10	2a

**Nine-month-old olive plants were inoculated with defoliating or non-defoliating isolates of V. dahliae. Symptom severity was assessed weekly from 2 to 12 weeks after inoculation.*
AUDPC= area under the disease progress curve; PDP= percentage of dead plants; CI= colonization index
***Within columns, values followed by a common letter do not differ significantly at P=0.05 according to Fisher's protected least significant difference test*

Disease symptoms progress were always more severe in inoculations with the defoliate pathotype (Figure 1, Table 1). From the seventh week after inoculation, some cultivars showed recovery from the disease, expressed as a reduction in disease severity (Figure 1). This phenomenon mainly occurred in several cultivars inoculated with the ND isolate (Figure 1) and was associated with a certain level of resistance (Kalamon).

Cultivars were classified into susceptible and resistance categories as shown in Table 2. Most of the evaluated cultivars were susceptible to defoliate and non-defoliate isolate of *V. dahliae*.

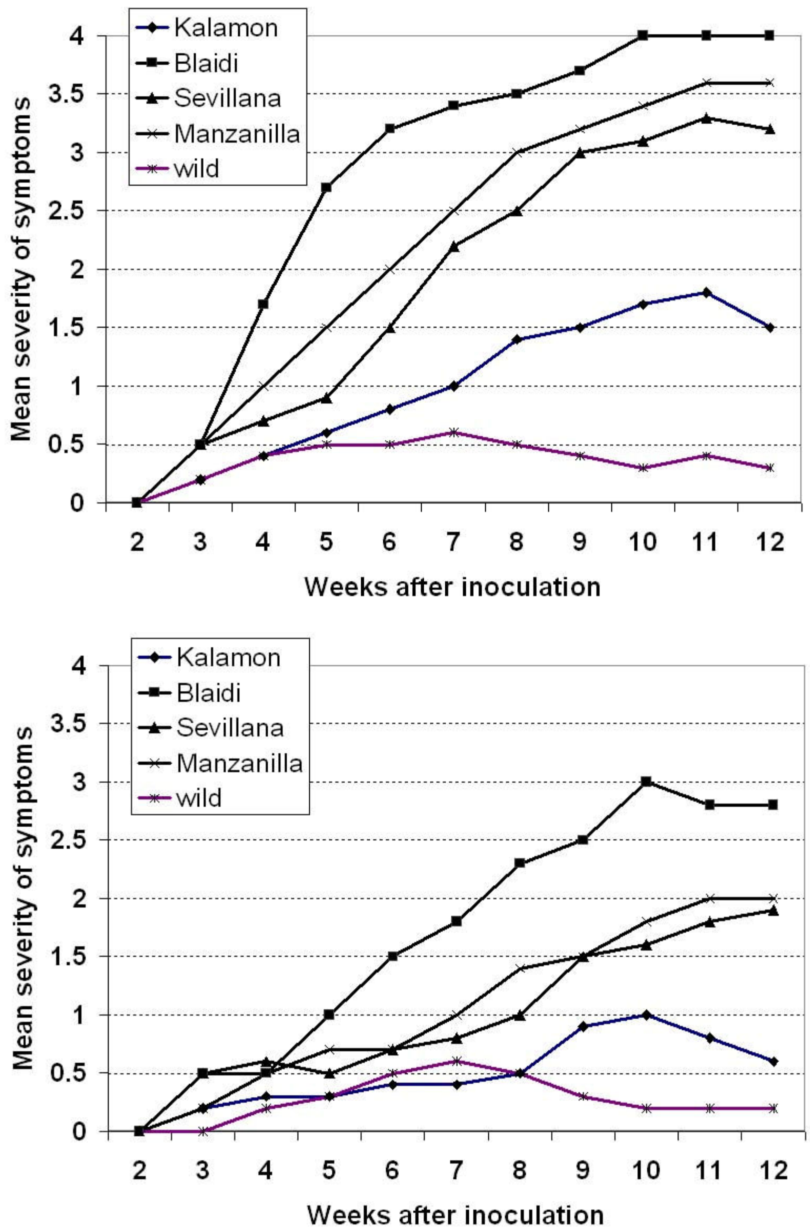

Fig. 1. Progress of the severity of symptoms in several widespread olive cultivars inoculated with the D (up) and ND (down) isolates of *V. dahliae.*

Severity of plant symptoms was weekly assessed for 12 weeks, starting at 2 weeks after inoculations, on a 0–4 rating scale according to percentage of plant tissue affected by chlorosis, leave and shoot necrosis, or defoliation (0 = healthy plant or plant without symptoms; 1 = affected plant in 1–33%; 2 = 34–66%; 3 = 67–99%; 4 = dead plant).

Moreover, all cultivars were more susceptible to the D pathotype than to the ND one. Bladi was extremely susceptible to both pathotypes of *V. dahliae* (Table 2) and Kalamon and Koroneiki were resistant to both pathotypes (Table 2). The extremely susceptible group to both pathotypes did not allow differentation of the reactions of the accessions to the pathogen.

Table 2. Resistance of olive cultivars to Verticillium wilt caused by defoliating (D) and non-defoliating (ND) isolates of *V. dahliae**

Susceptibility		**Cultivars**
D	ND	
E	E	Bladi
E	S	Konservalea, Rooghany, Zard
S	MS	Mission, Manzanilla
S	R	Sevillana
R	R	Kalamon, Koroneiki, Wild

** Resistance categories correspond to following interval of values of AUDPC for the D/ND isolates of V. dahliae: HR = 0–10%/0–10%; R = 11–30%/11–30%; MS = 31–50%/31–40%; S = 51–70%/41–60%; E = 71–100%/61–100%.*

Several authors have screened olive germplasm for response to *V. dahliae* in the quest for resistant cultivars or rootstocks and a wide range of susceptibility to Verticillium wilt was observed among the different cultivars (Cherrab et al., 2000; Lopez-Escudero et al., 2004). Cultivars such as Frantoio, Arbequina, Coratina, Empeltre and Frangivento were described as resistant while cultivars. Ascolana, Cellino and Leccino were described as susceptible (Cirulli and Montemurro, 1976). Tjamos (1993) also reported Arbequina as a cultivar resistant to *V. dahliae* but Lopez-Escudero et al. (2004) reported cv. Arbequina as susceptible.

Olive cultivars show a broad range of genetic variability for a large number of agronomic traits (Owen et al., 2005), including resistance to Verticillium wilt. To date, screening for resistance has been mainly carried out by examining the susceptibility, tolerance or resistance of different olive cultivars in artificial inoculation bioassays under greenhouse or semi-controlled conditions (Lopez-Escudero et al., 2004; Colella et al., 2008). Positive isolations of the fungus from affected plants during experiments demonstrated that olive plants were consistently infected, irrespective of the cultivar, pathotype and resistance level, which were similar to those reported for other cultivars by Rodrıguez-Jurado (1993). This research showed that main olive cultivars in Golestan province were very variable in resistance/susceptibility to defoliating and non-defoliating pathotypes of *V. dahliae*. Some cultivars were found to be highly resistant to verticillium wilt, especially Kalamon and Koroneiki cultivars since they proved to be highly resistant to both pathotypes (Table 2).

Almost all the evaluated cultivars have been categorized as susceptible or extremely susceptible to both pathogenic variants of *V. dahliae*, including the most important Iranian cultivars, ‘Rooghany’, ‘Zard’ and ‘Mission’ (Table 2). Moreover, all the cultivars were more susceptible and showed higher frequency of positive isolations of the pathogen from affected plant tissues when they were inoculated with the D pathotype than with the ND one. These results agree with studies of Hartman et al. (1971), Schnathorst and Sibbett (1971) and Rodrıguez-Jurado (1993). We have also demonstrated that ‘Zard’ and ‘Rooghany’, cultivars widely used nowadays in high-density orchards, shows more susceptibility compare to

'Mission', which already showed as susceptible (Tables 1 and 2, Sanei et al., 1998). Also, 'Konservalea' had similar susceptibility as 'Rooghany' and 'Rooghany' to the ND isolate (Tables 1 and 2), and tissues from affected plants yielded consistently cultures of the pathogen. This fact contrasts with the resistance recognised by Sanei et al. (2003) of this cultivar that was based on the low positive isolations of the pathogen from mature (> 8 years old) plants. Wild olive was resistant to the D and ND, Probably, this differential reaction makes them appropriate candidates to be tried as rootstocks in soil infested with inoculum densities of ND and D pathotypes of *V. dahliae* which reports for Golestan soils (Sanei et al., 2004; Sanei and Nasrollahnejad, 1995).

Our results indicate that 'Kalamon and 'Koroneiki' wild olive are resistant to *V. dahliae* especially to defoliating pathotype, so that they could be used for replanting or tried as rootstocks for other susceptible cultivars. although, the productivity, oil quality, early fruit maturation and easy mechanical harvesting and some disadvantages for commercial use of the cultivars, especially problems of fruit set and susceptibility to frost injury and other diseases or pests must be studied in this region. The main convenience for using the wild ollive as a rootstock consists of its rooting ability (Collela et al., 2008). Wild olive, in this experiment was the only cultivar in which neither of the isolates caused death of plants. All these reasons might justify the inclusion of this cultivar in breeding programmes for resistance to *Verticillium* wilt of olive.

The higher value of disease symptoms with defoliating pathotype confirmed the aggressiveness of the pathotype (Rodriguez Jurado et al., 1993; Bejarano Alcazar et al., 1996), which can be seen in other hosts especially susceptible cotton cultivars (Sanei et al., 2008). The host colonization of defoliating pathotype is more rapidly than the non-defoliating one and would be produced more population in the plant (Sanei and Nasrollahnejad, 1995). Although, The internal symptoms which do not show any discoloration in some cases, were similar to those reported by others (Blanco-Lopez et al., 1984; Thanassoulopoulos et al., 1979; Wilhelm and Taylor, 1965), The defoliating pathotype colonized the olive plant more rapidly than the non-defoliating one (Rodriguez Jurado et al., 1993) lower value of vascular browning should correspond to a weaker elicitation of the defence reaction by the plant opposing the advance of the pathogen (Rodriguez Jurado et al., 1993).

The results of the pathogenicity tests are consistent with previous reports indicating that heterologous *V. dahliae* isolates may infect olive (Schnathorst and Sibbett, 1971). The present report of an isolate from cotton and tomato capable of infecting olive extends the list of cultivated host species which could be a potential source of *V. dahliae* inoculum when they are cultivated near or together with olive trees. Cultivars Mission, Manzanilla proved to be only susceptible to moderately susceptible to *V. dahliae* (based on pathogen pathotype) infections compared with other olive cultivars commonly grown in Golestan region, which in contrast proved to be very susceptible in field servey (Sanei et al., 2003). This result and the previous result for field susceptibility by survey of resistance to Verticillium wilts in orchards with *V. dahliae* infection (Sanei et al., 2003) show that the age of the plant can influence susceptibility or resistance to V. dahliae infections.

4. CONCLUSION

This research identified 'Kalamon and 'Koroneiki' cuktivars and wild olive germplasm with high levels of resistance to verticillium wilt. Moreover, this is the first characterization of Golestan wild olive germplasm which highly resistant to the defoliating pathotype of *V.*

dahliae. The germplasm selected could be tested as rootstocks for susceptible olive cultivars and could also be very valuable in breeding programmes for resistance to verticillium wilt.

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