

## ORIGINAL ARTICLES

### Resistance of Some Olive Cultivars to Verticillium Wilt

El Said S. Hegazi, Ayman A. Hegazi and Abdou M. Abd Allatif

Pomology Department, Faculty of Agriculture, Cairo University, Giza, Egypt

---

#### ABSTRACT

The current study was conducted on twelve olive cvs. to investigate the resistance to Verticillium wilt a disease caused by *Verticillium dahliae*. One-year-old olive transplants were inoculated at green house with NET-5 isolate of *V. dahliae* during the period of October 2011 to March 2012. Resistance was evaluated by assessing symptom severity using (0 – 5) rating scale and the percentage of dead plants. Cultivars were classified into a defined category. Chlorosis was the most common disease symptoms appeared on the infected olive transplants, the chlorosis severity was determined in all the studied cultivars. Dolce cv. recorded the highest percentage of defoliation, while Picual cv. recorded the lowest percentage. The percentage of disease incidence recorded the highest percentage (83.33%) in Dolce cv, while Cairo 7 recorded the lowest percentage (27.78). Disease severity ranged from (2.50 to 0.48). Dolce cv. recorded the highest disease severity (2.50) followed by Eggizi and Coratina cvs. (1.43 and 1.07, respectively), while Frantoio and Cairo 7 cvs. recorded the lowest values (0.48 and 0.56, respectively). The percentage of dead plants ranged from (0 to 12.50%). Dolce cv. recorded the highest percentage of dead plants (12.50%) followed by Eggizi cv. (10.00%), while Frantoio, Arbequina, Verdal and Cairo 7 recorded the lowest.

**Key words:** Olive- Verticillium wilt - *V. dahliae* – Disease resistance.

---

#### Introduction

Incidence of verticillium wilt disease in olive trees is caused by *Verticillium dahliae* Kleb. is a widespread disease, wherever this crop is grown (Sesli *et al.*, 2010). Verticillium wilt disease considered as a serious problem in the majority of olive growing areas (Pagg and Brady, 2002). At present, control is based essentially on preventive methods such as the use of pathogen-free plants and soil when planting new olive orchards. Chemicals are not effective (Biris and Thanassouloupoulos, 1980).

Early in (1971) Hartmann *et al.*, in field studies to evaluate the degree of Verticillium wilt resistance in some olive rootstocks, they reported that trees grafting on Oblonga cv. remained free of symptoms, while 20 to 100 % of trees grafted on other rootstocks had the highest percentage of dead plants recorded for plants grafted on *Olea ferruginea*, while the lowest percentage was recorded for Oblonga (0%) and Sevillano (20%).

Studies showed that the olive cvs. Frantoio, Coratina, Frangivento, Oblonga and Kalamon have interesting resistance properties (Wilhelm and Taylor, 1965; Hartmann *et al.*, 1971; Schnathorst and Sibbett, 1971; Cirulli and Montemur-ro, 1976; Tjamos *et al.*, 1991; Lopez-Escudero *et al.*, 2004), while Ascolana, Cellina, Leccino, Manzanillo, Chemlali, Konservolia, Mission and Picual cvs. are susceptible (Wilhelm and Taylor, 1965; Cirulli and Montemurro, 1976; Tjamos., 1981; Wilhelm, 1981; Lopez-Escudero *et al.*, 2004).

According to Lopez-Escudero *et al.*, (2004) disease symptoms depending on the degree of resistance, chlorosis was associated with the cultivars showing certain level of resistance, while defoliation occurred in the susceptible cultivars such as Picual and Cobrancosa cvs.

Also, it has been determined susceptibility of some economically important olive cvs. and clones to *V. dahliae*. The severity of the disease has varied between 35.40 and 100% in the tested cultivars. The best results have been obtained from Gemlik cv. with the values of 35.40 - 36.25% (Erten and Yildiz, 2011).

According to Cirulli *et al.*, (2008) Frantoio cv. showed the least external symptoms (0.4). whlie, Coratina and Leccino cvs. showed the highest values of external symptoms

Sesli *et al.*, (2010) reported that a sudden wilt was observed in the susceptible cultivars, the percentage of dead plants ranged from 100% in Manzanillo, Edremit and Domat cvs. to 20% in Gernlik 2.

According to Lopez-Escudero *et al.*, (2004) percentage of dead plants differed according to cultivars and the pathogen isolates. The defoliating isolate of *V. dahliae* caused 70-100% dead plants in 13 of the 23 examined cultivars; the percentage of dead plants (PDP) reached 100% in olive cvs. Hendeno, Picudo, Manzanillo Sevilla and Arbequina, while Oblonga recorded 14.4%. On the other hand, the non defoliating

isolate (mild virulent) caused mortality in 7 cultivars with PDP ranged from 12.5 Arbequina to 50% in Picudo cv.

The re-isolation of the pathogen is very strong evidence on the occurrence of infection. Leopez -Escudero *et al.*, (2004) reported that the fungus of *V. dahliae* was recovered from 60% of the affected plants during the experiments, and recovered from 80% of the dead plants at the end of the experiments.

Sesli *et al.*, (2010) found that the pathogen was re-isolated from 70% of affected shoots, in the living plants and from 80% of the dead plant shoots.

On the other hand Cirulli *et al.*, (2008) found that, the differences in susceptibility of the tested cultivars are not related to re-isolation percentage of the pathogen, they added that, failure to detect the fungus in stem of inoculated plants also not exclude the presence of the fungus in the root system.

Sanei and Razavi (2011) stated that positive isolations of the fungus from affected plants during experiments of olive plants were consistently infected, in respective of the cultivar, pathotype and resistance level, which were similar to those reported for other cultivars by Rodriguez-Jurado *et al.*, (1993).

Mercado-Blanco *et al.*, (2003) found that isolations from roots of Acebuche-L plants indicated that 11% of V138I-inoculated plants and 44% of V4I-inoculated ones were infected with *V. dahliae*. Also, *V. dahliae* was isolated from Picual roots of 18.5% of V138I-inoculated plants and 25.9% of V4I-inoculated ones.

The present aims to study to determine the resistance of twelve olive cultivars to Verticillium wilt which included (severity of external symptoms, disease incidence %, disease severity, disease severity index % and percentage of dead plants).

## Materials and Methods

### Plant materials:

Twelve olive cultivars namely (Koroneiki, Cairo 7, Picual, Manzanillo, Verdal, Teffahi, Dolci, Frantoio, Arbequina, Chemlali, Eggizi and Coratina) were investigated according to their resistance to *V. dahliae* during the period of October 2011 to March 2012. Thirty healthy uniformed, one year old transplants of each cultivar were selected.

### Artificial production of *V. dahliae*:

The source of *V. dahliae* isolate (NET-5) was obtained from the collection of Plant Pathology Department, Faculty of Agriculture, Ain Shams University.

### Inoculum preparation:

Production of conidial suspension based on method of (Lopez -Escudero and Blanco-Lópe, 2007). The pure isolates of *V. dahliae* were used to inoculate a potato agar dextrose media (PAD) in 10 mm Petri dishes and incubated at 24 °C in dark. Conidial suspension, were prepared by adding sterile distilled water to the plates and left to rest for 20 min, then gently shaken, conidial suspension were collected .

### Inoculation technique:

The technique of plant inoculation was based on Porrás-Soriano *et al.*, (2003). Olive transplants were removed from the soil and the roots washed carefully to eliminate the soil adhering to roots. The roots were cut at 1 cm from the end to allow maximum penetration of the pathogen; the olive bare roots were dipped for 30 min in the conidial suspension then transplanted into polyethylene bags.

### Disease severity:

Verticillium wilt disease degrees of every plant were assessed from 0 to 5 using the arbitrary scale (Cirulli *et al.*, 2008) as in Table (1).

**Table 1:** Severity of external symptoms.

Category	Value	Symptoms
A	0	Healthy plants
B	1	Light foliar symptoms in 1-9% of the plants
C	2	Severe foliar symptoms and moderate defoliation
D	3	Defoliation (26- 50)
E	4	Total defoliation
F	5	Dead plant
M	-----	Total number of plants

Disease Severity (DS) and disease severity index (DSI) were determined four months after cultivation with the following formula, Booth (1970).

$$DS = \left[ \frac{(A \times 0) + (B \times 1) + (C \times 2) + (D \times 3) + (E \times 4) + (F \times 5)}{M} \right]$$

$$DSI = \left[ \frac{\sum[(\text{Rating no.} \times \text{no. of plants in rating}) \times 100]}{(\text{Total no. of plants} \times \text{highest disease rating})} \right]$$

The obtained results were used for the following resistance categories corresponding to the following DSI ranges: highly resistant (HR) =0–10%, Resistant (R) =11–30%, Moderately Susceptible (MS) =31–50%, Susceptible (S) =51–70%, Highly Susceptible (HS) =71–100% (a modified scale from López-Escudero *et al.*, 2004).

#### *Disease incidence:*

Disease incidence was calculated as percentage of infected plants to the total numbers of investigated plants.

$$\text{Disease incidence} = \left[ \frac{\text{Number of infested plants}}{\text{Total number of plants}} \right] \times 100$$

**Percentage of dead plants (PDP)** was estimated as percentage of dead transplants to the total number of used transplants in each cultivar.

#### *Resistance categories:*

The tested cultivars were arranged on the base of evaluation scales under the following categories

HR= Highly Resistance

R= Resistance

MS= Moderately Susceptible

S = Susceptible

HS= Highly Susceptible.

#### *Positive re-isolation of the pathogen:*

At the end of experiment re-isolation of *V. dahliae* were performed according to (Lopez- Escudero and Blanco-Lópe, 2007).

Samples were taken from dead and effected plants, washed in running tap water. The bark was removed and woody tissues were surface sterilized in 0.5% sodium hypochlorite for 1 min. Wood chips were placed into PAD media and incubated at 24°C in the dark for 10 days Figs (5 and 6).

#### *Experimental Design and Data Analysis:*

This study followed the complete randomized block design and data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1980) using Mstat C program (1989) software, and means of the treatments were compared by Least Significant Difference (L.S.D) according to Waller and Duncan (1969) at significance level of 0.05.

## **Results and Discussion**

#### *Severity of external symptoms:*

Chlorosis was the most common symptoms of disease appeared on the infected olive plants (Table, 2). The obtained results revealed that Frantoio and Arbequina cvs. showed some level of resistance.

**Table 2:** Percentage of olive transplants in the different categories of external symptoms.

Cultivars	Severity of external symptoms					
	0	1	2	3	4	5
Koroneiki	56.67	33.33	3.33	3.33	0.00	3.33
Cairo 7	72.22	16.67	5.56	0.00	0.00	5.56
Picual	64.29	25.00	3.57	0.00	0.00	7.14
Manzanillo	59.09	18.18	13.64	0.00	4.55	4.55
Verdal	36.36	54.55	9.09	0.00	0.00	0.00
Teffahi	65.63	21.88	3.13	3.13	0.00	6.25
Dolce	15.38	19.23	3.35	23.08	19.23	19.23
Frantoio	58.62	34.48	6.90	0.00	0.00	0.00
Arbequina	44.44	33.33	11.11	5.56	5.56	0.00
Chemlali	60.00	26.67	3.33	0.00	3.33	6.67
Eggizi	36.67	33.33	10.00	0.00	10.00	10.00
Coratina	40.00	33.33	13.33	3.33	3.33	6.67
LSD at p<0.05	1.007	0.975	0.871	0.005	0.929	0.722

On the other hand, the percentage of leaves defoliation was more evident in the susceptible cultivars compared with the other cultivars. Dolce cv. recorded the highest percentage of defoliation, while Cairo 7, Frantoio and Verdali cvs. recorded the lowest percentage.

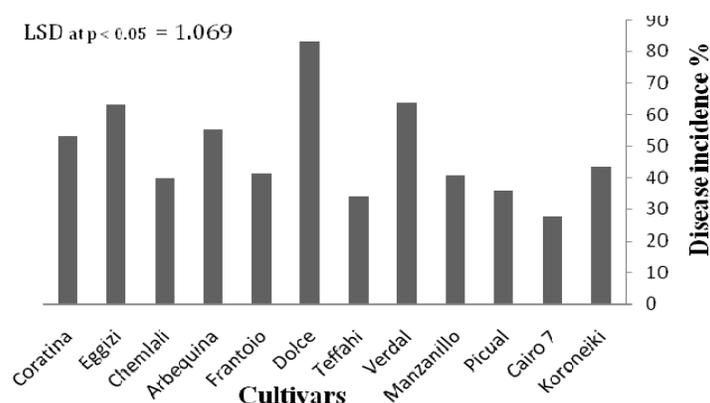
The obtained results indicated that the infected plant showed typical symptoms of disease infection.

Verticillium wilt symptoms were characterized by die back of twigs, usually sudden collapse of one or few branches followed by collapse of other branches, the small trees often wilt and die (Ciccarese *et al.*, 2002). Also, the branches become defoliated but may retain some brownish colored leaves (Bellahcene *et al.*, 2000).

Sesli *et al.*, (2010) reported that, defoliation was the most common symptoms observed, it occurred in all the susceptible cultivars. Defoliation was intensive in plants from extremely susceptible cultivars like Manzanillo, Edremit, Memecik, Uslu, Gemlik 5, and Domat and the symptoms were slight in moderately resistant cultivars like Gemlik 2 cv.

#### Disease incidence:

Results in Fig. (1) showed that the percentage of total plants with disease symptoms (disease incidence) was higher in all the studied cultivars. The percentage of disease incidence ranged from 83.33% in Dolce cv. while Cairo 7 recorded the lowest percentage of disease incidence (27.78%).

**Fig. 1:** Percentage of disease incidence % of the studied olive cvs.

#### Disease severity:

Disease severity is usually expressed as the percentage or proportion of plant area destroyed by a pathogen (Agrios, 2005). It is used in the case of a disease that caused varying degrees of damage to plants throughout the crop, disease severity ranged from (2.50 to 0.48), Dolce cv. (Fig. 2) recorded the highest disease severity (2.50) followed by Eggizi and Coratina (1.43 and 1.07, respectively), while Frantoio and Cairo 7 cvs. recorded the lowest value (0.48 and 0.56 respectively).

#### Disease severity index:

Disease severity index recorded the highest value in the susceptible cultivars Dolce (50.00) followed by Eggizi and Coratina cvs. (28.67 and 21.33, respectively), while Frantoio and Cairo 7 were the lowest (Fig.3).

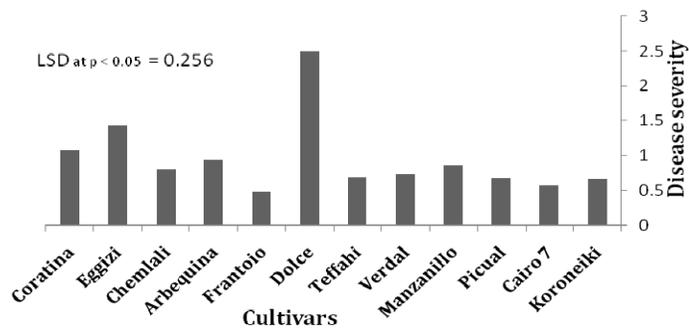


Fig. 2: Percentage of disease Severity of the studied olive cvs.

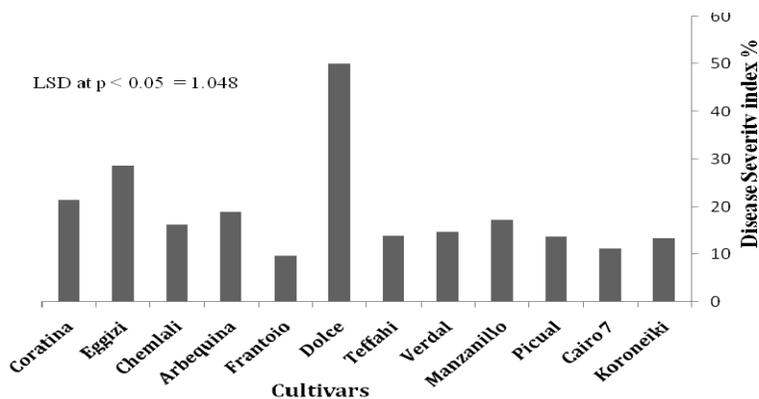


Fig. 3: Percentage of disease Severity index % of the studied olive cvs.

Cirulli *et al.*, (2008) reported that, Frantoio cv. was slightly affected by the disease, the value of external symptoms observed in this cv. were the lowest (0.4), while in the susceptible cv. Leccino, the value of disease severity in Leccino recorded (7.4) and the pathogen caused leaf yellowing, necrosis and defoliation. Porras-Soriano *et al.*, (2003) showed that plants of Cornicabra cv. were highly susceptible to *V. dahliae* with severity value of (4.2); the other cultivars showed some degree of resistance with severity value ranged from 1.2 in Empletre to (2.3) in Arbequina. cv. They also observed that grafting Cornicabra cv, on Frantoio cv. reduced its susceptibility from 4.2 to 1.8.

Also, it has been determined the susceptibility of some economically important olive cultivars and clones to *V. dahliae*, the severity of the disease has varied between (35.40 and 100%) in the tested cultivars. The best results had been obtained from Gemlik cultivar with the values of (35.40 - 36.25%) (Erten and Yildiz, 2011)

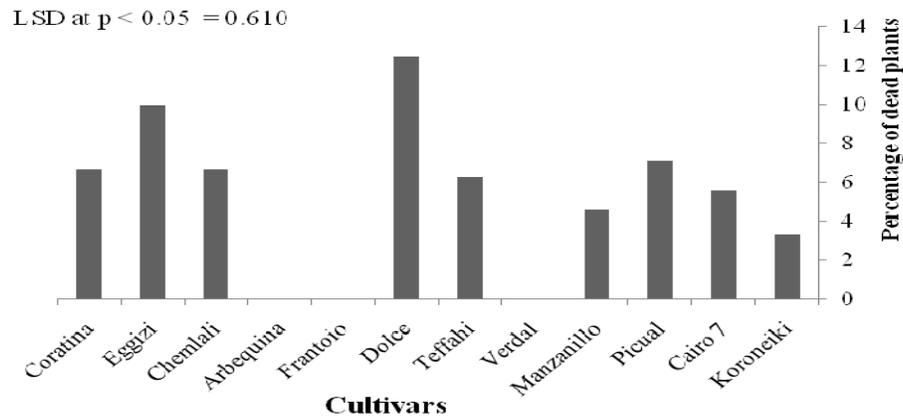
Mercado-Blanco *et al.*, (2003) found that very low disease incidence and severity, and consequently low disease intensity index (DII), occurred in Acebuche-L olives indicating that this genotype was resistant both to D and ND *V. dahliae*. development of Verticillium wilt disease in inoculated Arbequina plants occurred (V138I-inoculated plants, 5% disease incidence) and (V4I-inoculated plants, 30% disease incidence).

**Percentage of dead plants (PDP):**

As shown in Fig. (4) there was an obvious variation between the studied cultivars in Verticillium resistance. The percentage of dead plants ranged from 0 to 12.50%. Dolce cv. recorded the highest percentage of dead plants (12.50%) followed by Eggizi (10.00%), while Frantoio, Arbequina, Verdal and Cairo 7 cvs. were the lowest (0-5).

These results are in agreement with Lopez-Escudero *et al.*, (2004) they reported that dead plants were found in 7 of the 23 studied cultivars, the percentage of dead plants ranged from 50% in the very susceptible cv. to (0.00) in Frantoio cv.

Moreover, Latife and Mehmet (2011) in study on 71 olive cultivars reported that the PDP ranged from 100% in Memecik cv. to 2.5% in D36 cv. while there were no. dead plants observed in Arbequina and Leccino cvs.



**Fig. 4:** Percentage of dead plants of the studied olive cvs.

*Categories of cultivars resistance:*

According to the obtained results the studied cultivars could be classified into the following resistance categories:

1. Resistance cultivars: Frantoio, Arbequina, Verdal and Cairo 7 cvs.
2. Moderately susceptible: Picual, Manzanillo, Teffahi, Chemlali, Koroneiki and Chemlali cvs.
3. Susceptible: Dolce, Eggizi and Coratina cvs.

Ciccarese *et al.*, (2002) classified olive cultivars on the base of external symptoms severity. The most resistance cultivars were DA 12 I, followed by Coratina, Yusti and Cima di Bitonto, while Nocellara de Belice, Bosana and Leccino cvs. considered as susceptible cultivars.

According to Porras-Soriano *et al.*, (2003) Cornicabra olive cv. classified as highly susceptible, while Frantoio, Lechin and Empeltre found to be very resistant Arbequina cv. displayed a medium resistant. Similar results reported by Lopez-Escudero *et al.*, (2004), Latife and Mehmet (2011) and Sanei and Razavi, (2011).

There was an obvious variation in classification of resistance categories in olive cultivars, Manzanillo cv. classified as extremely susceptible (Latife and Mehmet, 2011), Moderate susceptible (Sanei and Razavi, 2011), while Lopez-Escudero *et al.*, (2004) classified Manzanillo as a resistant cultivars.

This may be attributed to the difference in the virulent of the pathogen; RAPD-PCR also enabled the characterization of two pathotypes, (Pérez-Artés *et al.*, 2000). Usually infections caused by D pathotype lead to the plant death whereas infections by ND pathotype can result in a complete symptom remission (Mercado-Blanco *et al.*, 2002).



**Fig. 5:** Showed the re isolation of the disease of *V. dahliae*.



**Fig. 6:** Showed the re isolation of the disease of *V. dahliae*.

## References

- Agrios, G.N., 2005. Plant Pathology. Fifth Ed. Elsevier Academic Press, 984 pp.
- Bellahcene, M., Z. Fortas, J.P. Geiger, A. Matallah and D. Henni, 2000. Verticillium wilt in olive in Algeria: geographical distribution and extent of the disease. *Olivae*, 82: 41-43.
- Biris, D.A. and C.C. Thanassouloupoulos, 1980. Field trials for chemical control of Verticillium wilt of olives. In: Proceedings of the 5 Congress of the Mediterranean Phytopathological Union, Patras, pp: 54-55.
- Booth, J.A., 1970. Verticillium albo-atrum. In: Chiarappa, L. (Ed.), Crop loss assessment methods: FAO manual on the evaluation and prevention of losses by pests, diseases and weeds. Common Wealth Agriculture Bureaux., Farnham Royal, UK, pp: 50-51.
- Ciccarese, F., A. Ambrico, O. Longo and D. Schiavone, 2002. Search for resistance to Verticillium wilt and leaf spot in olive. *Acta Hort.*, 586: 717-720.
- Cirulli, M. and G. Montemurro, 1976. A comparison of pathogenic isolates of *Verticillium dahliae* and sources of resistance in olive. *Agric. Conspectus Scientifics*, 39: 469-476.
- Cirulli, M., C. Colella, M.D. Amico, M. Amenduni and G. Bubic, 2008. Comparison of screening methods for the evaluation of olive resistance to *Verticillium dahliae* kleb. *J. Plant Pathol.*, 90(1): 7-14.
- Hartmann, H., W.C. Schnathorst and J. Whisler, 1971. Oblonga, a clonal olive rootstock resistant to verticillium wilt. *California Agric.*, 25: 12-15.
- Erten, L. and M. Yildiz, 2011. Screening for resistance of Turkish olive cultivars and clonal rootstocks to Verticillium wilt. *Phytoparasitica* 39: 83-92.
- Latife, E. and Y. Mehmet, 2011. Screening for resistance of Turkish olive cultivars and clonal rootstocks to Verticillium wilt. *Phytoparasitica*, 39(1): 83-92.
- López-Escudero, F.J. and M.A. Blanco-López, 2007. Relationship Between the inoculum density of *Verticillium dahliae* and the Progress of Verticillium Wilt of Olive. *Plant Disease*, 91(11): 1372-1377.
- Lopez-Escudero, F.J., C. Del Rio, J.M. Caballero, M.A. Blanco-López, 2004. Evaluation of olive cultivars for resistance to *Verticillium dahliae*. *Eur. J. Plant Pathol.*, 110: 79-85.
- Mercado-Blanco, J., D. Rodríguez-Jurado, E. Pérez-Artés and R.M. Jiménez-Díaz, 2002. Detection of the defoliating pathotype of *Verticillium dahliae* in infected olive plants by nested PCR. *European J. Plant Pathol.*, 108: 1-13.
- Mercado-Blanco, J., M. Collado-Romero, S. Parrilla-Araujo, D. Rodríguez-Jurado and R.M. Jiménez-Díaz, 2003. Quantitative monitoring of colonization of olive genotypes by *Verticillium dahliae* pathotypes with real-time polymerase chain reaction. *Physiol.Molecul. Plant Pathol.*, 63: 91-105.
- Mstat, C., 1989. Users guide: a microcomputer program for the design, management and analysis of agronomic research experiments. Michigan University, East Lansing, MC, USA.
- Pagg, G.F., B.L. Brady, 2002. Verticillium Wilts, CABI Publishing, New York, pp: 357.
- Pérez-Artés, E., M.D. García-Pedrajas, J. Bejarano-Alcázar and R.M. Jiménez-Díaz, 2000. Differentiation of cotton-defoliating and nondefoliating pathotypes of *Verticillium dahliae* by RAPD and specific PCR analyses. *European Journal of Plant Pathol.*, 106: 507-517.
- Hartmann, H., W.C. Schnathorst and J. Whisler, 1971. Oblonga, a clonal olive rootstock resistant to verticillium wilt. *California Agric.*, 25: 12-15.
- Porrás Soriano, A., M.L. Soriano Martín, A. Porrás Piedra, 2003. Grafting olive cv. Cornicabra on rootstocks tolerant to *Verticillium dahliae* reduces their susceptibility. *Crop Prot.*, 22: 369-374.
- Rodríguez-Jurado, D., M.A. Blanco-Lobez, H.F. Rapaport and R.M. Jimenez-Diaz, 1993. Present status of Verticillium wilt of olive in Andalusia (Southern of Spain). *EPPO Bull.*, 23: 513-516.
- Sanei, S.J. and S.E. Razavi, 2011. Reaction of some olive cultivars to *verticillium dahliae* isolates agent of vascular wilt: A comparative study. *Amer. J. Exper. Agric.*, 1(4): 320-330.

- Schnathorst, W.C. and G.S. Sibbett, 1971. The relations of strains of *Verticillium albo-atrum* to severity of *Verticillium* wilt in *Gossypium hirsutum* and *Olea europaea* in California. *Plant Disease Reporter.*, 55: 780-782.
- Sesli, M., E. Onan, S. Oden, H. Yener and E.D. Yegenoglu, 2010. Resistance of olive cultivars to *Verticillium dahliae* *Scientific Research and Essays.* 5(12): 1561-1565.
- Snedecor, G.W. and W.G. Cochran, 1980. *Statistical Methods.* Oxford and J.B.H. Bub Com. 6<sup>th</sup> Edition.
- Tjamos, E.C., 1981. Virulence of *Verticillium dahliae* and *V. albo-atrum* isolates in tomato seedlings in relation to their host of origin and the applied cropping system. *Phytopathol.*, 71: 99-110.
- Tjamos, E.C., D.A. Biris and E.J. Paplomatas, 1991. Recovery of olive trees from *Verticillium* wilt after individual application of soil solarization in established olive orchards. *Plant Dis.*, 75: 557-562.
- Waller, A. and Duncan, D.B., 1969. Multiple ranges and multiple tests. *Biometrics*, 11: 1-24.