Jordan Journal of Agricultural Sciences, Volume **4**, No.**4**, 2008

Effect of Post-Planting Soil Solarization and Solar Chamber on *Verticillium* Wilt of Olive

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ABSTRACT

The objective of this experiment was to determine the effectiveness of single post-planting soil solarization and solar chamber for control of *Verticillium* wilt of olive. Soil solarization was applied for 3 months starting from mid July in olive orchards under desert plain and up-land environmental conditions in Jordan. A completely closed solar chamber was applied for 12 and 24 hours light to cover individual *V. dahliae*-infected trees in an up-land olive orchard in mid-August. Disease incidence and severity were significantly reduced in solarized trees compared to the non-solarized controls. Percentage of fungal isolations was significantly reduced among solarized trees as compared with non-solarized ones and was diminished to less than 10 % at the two sites. Both recovery and symptoms remission due to soil solarization exceeded natural recovery, which occurred in lower ratios among control trees than among solarized trees. Treatment of *Verticillium*-infected trees with solar chamber in August for either 12 hours or 24 hours exposure was efficient in reducing the disease development to a significant level. Twenty-four hours solar chamber treatment significantly reduced disease severity and percentage of *V. dahliae*-infected olive branches as compared to the control from mid-October to mid-January. Effect of solar chamber treatment for 24 hours did not differ significantly from that of 12 hours.

Keywords: Wilt, Olive, *Verticillium*, Solarization, Solar chamber.

INTRODUCTION

Several fungal diseases attack olive trees causing great crop losses. In Jordan, the following fungal diseases were reported: *Verticillium* wilt caused by *V. dahliae* Kleb. (Naser, 1996), "Peacock eye spot" caused by *Cycloconium oleaginum* Cast. (Mamluk *et al.*, 1984), "Leaf spot" caused by *Alternaria* spp. (Mamluk *et al.*, 1984), and "Leaf mold" caused by *Cladosporium* spp. (Qasem, 1970). *Verticillium* wilt of olive is well known in all Mediterranean countries. It was acute in irrigated olive orchards of highly productive and susceptible olive cultivars (Tjamos, 1993) and caused high economic losses to olive trees (Al-Ahmad and Hamidi, 1984; Thanassoulopoulos *et al.*, 1979; Naser, 1996; Tjamos *et al.*, 2000).

Several approaches have been used to control the disease in established olive orchards including chemical control (Tawil *et al*., 1992; Tawil and Abdin, 1994), soil solarization (Tjamos *et al*., 1991), solar chamber (Al-Ahmad and Duksi, 1994), cultural practices (Al-Ahmad, 1993), integrated control (Abu-Qamar and Al-Momany, 2001 and 2002) and biological control (Tjamos, 1993). Among the evergreen fruit tree species used in a study performed in the Jordan Valley, olive was able to

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Received on 18/4/2007 and Accepted for Publication on 29/5/2008.

survive the post-plant soil solarization treatment (Abu-Gharbieh *et al*., 1998). Post-planting soil solarization in California achieved transient recovery of *V. dahliae*-infected pistachio (Ashworth and Goana, 1982). The high soil temperature in mulched soil caused no detrimental effect on the root system. Several workers in California (Wilhelm and Taylor, 1965), Greece (Thanasoulopoulos *et al*., 1979) and Spain (Rodriguez-Jurado *et al*., 1993) reported the phenomenon of natural recovery of olive tree from Verticillium wilt. Usually, *Verticillium*-infected trees may recover after 2-3 years of symptom expressions (Wilhelm and Taylor, 1965). An innovative technique (solar chamber) for controlling *Verticillium* wilt in the above ground parts of olive trees was developed by Al-Ahmad (1993). In this technique, an individual *V. dahliae*-infected tree was covered for few days with clear plastic chamber to raise atmospheric air temperature around the tree to a detrimental level for the fungus without affecting the tree health. Results of an experiment revealed that 80 % of solarized trees were free of the disease, and it was impossible to isolate the fungus one year after treatment (Al-Ahmad and Duksi, 1994).

The objective of this study was to determine the effectiveness of single post-planting soil solarization and solar chamber treatments for control of *Verticillium* wilt in infected young olive trees.

MATERIALS AND METHODS

Pathogen Isolation and Identification

Samples of 2-year-old olive branches showing wilt symptom were collected from olive trees, cultured on selective media (Ausher *et al*., 1975) and incubated for two weeks at 22°C. Fungal growth was examined microscopically to determine the genus and species of olive wilt pathogen by using taxonomic key (Issac, 1967). The pathogenicity of the fungus was tested on olive seedlings.

Assessment of Disease Development

Two locations were selected for assessment of the disease severity and percentage of *V. dahliae*-infected branches. The first location was in Al-Baqa'a region in the up-lands. The second was in Al-Halabat region in the desert plains. Nabali was the main olive cultivar in both locations. In Al-Baqa'a location, 5-years old olive trees were grown in clay soil and drip irrigated during summer, six hours once every two weeks. While in Al-Halabat location, 5-years old olive trees were grown in silty loam soil and drip irrigated six hours twice weekly.

A scale of visual estimation of disease severity (Tjamos and Paplomatas, 1991) was used depending on foliar symptoms such as leaf yellowing, rolling and defoliation of infected branches. The following index was used where; 0 = tree free of infection, 1 = 1-20 % of the tree was infected (very slight infection), 2 = 21-40% (slight infection), 3 = 41-60% (intermediate infection), 4= 61-80% (severe infection), 5 = 81-100% (nearly dead to dead tree). The overall assessment of disease severity was expressed as the sum of all numerical ratings divided on the total number of trees, multiplied by 100% and divided on the maximum disease severity category, which was five.

Disease incidence was determined as the extent of stem colonization by recording the percentage of infected branches per tree within the whole plot expressed as the number of positive isolations of the pathogen per branch divided on the total number of collected branches. Six 30-cm-length branches were randomly taken from each infected tree, cut into 0.5-1.0cm length pieces, surface sterilized into 0.5% NaOCl for 2 minutes and plated on the selective medium. After 2-week incubation at 22°C, the percentage of stem pieces in which the pathogen had developed was calculated.

Both disease incidence and severity were taken monthly in both locations. Data were analyzed statistically using Duncan’s multiple range test (P=0.05) (Steel and Torrie, 1980).

Soil Solarization

Soil solarization was applied for a period of 3 months, starting on 15 July and ending in mid-September where plastic mulches were removed. The experiment was laid out in a complete randomized block design consisting of five replications of solarized and non-solarized treatments and carried out in the two locations. Each plot was composed of five trees. Transparent polyethylene (PE) sheets (0.1 mm thick, 3 m length and 2 m wide and ultraviolet light stabilized) were used to cover the soil around tree trunk. The soil was first plowed, leveled and irrigated to field capacity. Before soil solarization, experimental plot surface was manually cleared of weeds, debris and large stones to avoid damaging plastic sheets. The inner edges of the sheets were joined, stapled and attached to the tree trunks. The outer edges of the sheets were buried in furrows to secure the plastic mulch, to prevent movement by the wind, to capture sun energy and to maintain soil moisture. The irrigation tubes remained under PE sheets to enable irrigation. Soil temperatures were registered manually monthly at noontime using the simple needle thermometers placed at a depth of 5 cm during the whole solarization period.

Solar Chamber

Two solar chambers were made, each of 1.5x1.5x2.5m wood framework, covered with transparent plastic sheets (0.1 mm thick and UV-stabilized). The chambers were placed over 5-6 years old diseased olive trees at Al-Baqa'a location alone on 15 August (the hottest month during summer). The lower edges of the chambers were buried in furrows to secure them, to prevent movement by the wind and to maintain temperature. Thus, they were completely closed to prevent air exchange between the inside and outside of the chamber. Two treatments were made including coverage with solar chamber for 12 light hours and for 24 light hours. Five diseased olive trees were used for each treatment and another five were kept unsolarized as a control. Atmospheric air temperature was manually registered monthly at noontime with a thermometer placed inside the chamber during solarization period.

RESULTS

Isolation and Identification of Olive Wilt Pathogen

The fungus was isolated from wilted olive branches collected from Al-Baqa'a and Al-Halabat locations. Microscopic examinations and pathogenicity test indicated that *V. dahliae* was the causal agent of olive wilt. White cottony mycelial growth was observed after 7 days of incubating the infected plant materials on the selective media. As a result of microsclerotia and dauermycelia formation, the color of the colony converted to dark black within 2 weeks after planting. Early symptoms of infection started on the leaves of young shoots from late winter to early spring. Leaves rolled downward along the main axis and become yellowish green. After two weeks, the leaves become brown in color. Defoliation of leaves took place within one month. Longitudinal section in the vascular system of wilted olive branches revealed no or slight discoloration of the vascular tissue.

Soil Solarization

The maximal monthly temperature in the solarized soil fluctuated from 48-56°C at Al-Halabat location and from 46-52°C at the 5-cm depth at Al-Baqa'a location and was about 6-12°C higher than that in the control trees (data not shown). Disease symptoms were similar in control and solarized trees. Disease recovery was common in the form of new vegetative growth in stems and branches. Many diseased plants produced new suckers arising from the crown.

Disease severity of *Verticillium* wilt was significantly lower in solarized than non-solarized trees. The lowest disease severity was 4 % in mid-August and mid-September at Al-Baqa'a location and 4 % in mid-September to mid-November at Al-Halabat location (Table 1). This indicated that recovery and symptoms remission among the solarized trees exceeded the natural recovery, which was much less in the control.

Disease incidence was significantly reduced in solarized trees compared to the non-solarized controls. Percentage of fungal isolations from infected branches was significantly reduced among solarized trees as compared with non-solarized ones and was diminished to 6.7 % in mid-September and mid-October at Al-Baqa'a location and to 3.3 % from mid-July till mid-November at Al-Halabat location. Proportion of *V. dahliae*-free branches in the solarized trees was higher than that in the control trees. Average relative percentage of infected branches was reduced by 56 % and 48 % at Al-Halabat and Al-Baqa'a locations, respectively (Table 1).

Solar Chamber

Twenty four hours solar chamber treatment significantly reduced disease severity and percentage of *V. dahliae*-infected olive branches as compared to the control from mid-October to mid-January at Al-Baqa'a location. Effect of solar chamber treatment for 24 hours did not differ significantly from that of 12 hours. Treated individual trees appeared wilted after the treatment, but they tended to recover after a short time. Some injuries occurred to foliar parts of treated trees due to high temperature stress induced by the solar chamber. After mid-January, due to the high activity of the fungus and new stem infections which took place, no significant differences between solar chamber and the control treatments were noticed (Table 2).

DISCUSSION

Verticillium wilt symptoms in experimental plots were similar to those previously described (Naser, 1996). In our experiments, solarization consistently increased soil temperatures related to values reached in control plots. The increase in the maximum soil temperature was relatively higher at Al-Halabat which has hotter climate during summer since it is located in the desert plains than Al-Baqa'a which is located in the up-lands of Jordan. However, increases of 6 to 12°C were reached. These values are similar to those previously reported (Tjamos and Paplomatas, 1991; Lopez-Escudero and Blanco-Lopez, 2001). The treated olive trees in both locations were able to survive the high temperature with no injury. In another study conducted in the Jordan Valley, among the evergreen fruit tree species used, olive, citrus and guava were able to survive the post-plant solarization treatment that effectively reduced populations of the vascular wilt fungus *Fusarium oxysporum* and Citrus nematode *Tylenchulus semipenetrans* in the soil (Abu-Gharbieh *et al.*, 1998).

In general, trees do not overcome injuries and infections by healing damaged or infected tissues, but by isolating the damaged part and replacing it by new functional tissues (Hiemstra, 1998). Recovery is a particularly common feature of wilt in olive and has also been reported in other fruit-tree species. The effect of soil solarization was expressed as increased recovery and symptoms remission in the growing season. Both recovery and symptoms remission due to soil solarization exceeded natural recovery, which occurred in less ratios among control trees than among the solarized trees. The rate of recovery of trees in solarized soil significantly exceeded natural recovery of untreated control trees which could be attributed to lack of root re-infections. Microsclerotia of *V. dahliae* were nearly eliminated in the soil around treated trees, whereas propagules of *Talaromyces flavus*, a known antagonist of *V. dahliae*, not only survived solarization but also increased in treated soil as compared with untreated control soils (Tjamos, 1993). Covering the soil with 0.05 mm transparent polyethylene film for 12 weeks around the infected 15-20 years old trees, reduced drastically the number of *V. dahliae* microsclerotia in the soil (Skoudridakis, 1993). Plastic mulching can also prevent leaves from becoming incorporated with soil thus reduced the inoculum in soil. In perennial species like olive and maple, large quantities of inoculum can be similarly released when leaves containing microsclerotia and hyphae are shed from diseased trees (Tjamos and Botseas, 1987; Naser, 1996). Moreover, detached leaves may be blown considerable distances thus serving to disperse the pathogen.

The efficacy of solarization seems to be lower on woody than on herbaceous hosts due, in part, to residual inoculum that remains viable after solarization and to difficulties of mulching around woody plants (Lopez-Escudero and Blanco-Lopez, 2001). The residual non-controlled populations might be responsible for the inoculum build up due to lower temperatures occurring in shaded zones, escape of microsclerotia present in deeper clay layers or viable microsclerotia that remained at the borders of solarized plots. Additionally, in the experiment, drip irrigation might have an influence on the increase of inoculum density. Thus, around wet areas, where the samples were taken, defoliated leaves may have provided an inoculum source (Tjamos and Botseas, 1987; Tjamos, 1993; Grinstein *et al*., 1995; Naser, 1996; Lopez-Escudero and Blanco-Lopez, 2001). The beneficial effect of soil solarization could be maximized by earlier application (May) and combining solarization with the addition of fungal antagonists to *V. dahliae*.

The plastic chamber is an innovative technique for controlling *Verticillium* wilt of olive. Al-Ahmad (1993) has developed this simple solar chamber. The air temperature in the solar chamber was raised to 55°C. It was not possible to isolate *V. dahliae* from infected trees after 15-20 days of treatment. Results of another experiment indicated that 80% of solarized trees were free of the disease, and it was impossible to isolate the fungus one year after treatment (Al-Ahmad and Duksi, 1994). In our experiment, treating *Verticillium*-infected trees with solar chamber for either 12 hours or 24 hours exposure during August was inefficient in reducing the disease development to a significant level. This might be attributed to the insufficiency of solar chamber exposure period. It might need longer exposure but should be started in less hotter months to avoid the heavy foliar injury. Delaying the application of solar chamber to September or in early May might be helpful. This agreed with Al-Ahmad (1993) who suggested that the exposure period has to be sufficiently long for many days to ensure effectiveness of the treatment. Some injuries occurred to foliar parts of treated trees due to high temperature stress induced by solar chamber that might restrict the length of the treatment and limit the use of solar chamber for controlling *Verticillium* wilt of olive.

Individual tree soil solarization can be practical and economical for controlling *Verticillium* wilt in irrigated olive orchards. Considering the relatively low application cost of soil solarization, the control by solarization of other soil-borne diseases and weeds and the increased growth response, soil fertility and crop production and quality, solarization- at least in locations with extended periods of solar radiation and high light intensity- can play a useful role in crop protection programs for the control of *Verticillium* wilt, as well as other soil-borne diseases, at low cost and without causing imbalances to the soil ecosystem.

Table (1): Effect of post-planting soil solarization on percentages of disease severity and *V. dahliae*-infected olive branches at Al-Baqa'a and Al-Halabat locations in Jordan.

Location	Al-Baqa'a				Al-Halabat			
Parameter	Average monthly disease severity %		Average monthly disease incidence %		Average monthly disease severity %		Average monthly disease incidence %	
Treatment/ Date	solarization	control	solarization	control	solarization	control	solarization	control
Mar. 15	80 a*	76 a	76.7 a	83.3 a	76 a	80 a	53.3 b	56.7 b
April 15	56 b	56 b	56.7 b	66.7 b	72 a	80 a	40 c	70 a
May 15	60 b	48 c	50 b	46.7 d	60 b	76 a	60 a	73.3 a
Jun. 15	40 c	40 cd	33.3 c	40 de	60 b	68 b	26.7 d	56.7 b
Jul. 15	16 ef	32 d	16.7 d	40 de	24 d	44 d	3.3 f	23.3 c
Aug. 15	4 g	36 d	10 d	33.3 e	8 e	40 de	3.3 f	16.7 d
Sep. 15	4 g	36 d	6.7 d	33.3 e	4 e	28 fg	3.3 f	6.7 e
Oct. 15	8 g	52 bc	6.7 d	36.7 e	4 e	20 g	3.3 f	13.3 de
Nov. 15	8 g	56 b	10 d	46.7 d	4 e	36 ef	3.3 f	20 cd
Dec. 15	12 fg	48 c	10 d	43.3 d	8 e	44 d	10 ef	23.3 c
Jan. 15	12 fg	60 b	16.7 d	30 e	12 e	56 c	10 ef	70 a

Location	Al-Baqa'a				Al-Halabat			
Parameter	Average monthly disease severity %		Average monthly disease incidence %		Average monthly disease severity %		Average monthly disease incidence %	
Treatment/ Date	solarization	control	solarization	control	solarization	control	solarization	control
Feb. 15	24 de	60 b	13.3 d	56.7 c	20 d	68 b	16.7 e	73.3 a
Mar. 15	28 d	64 ab	30 c	83.3 a	32 c	72 ab	23.3 de	76.7 a
Mean	27.1 a**	51.1 b	25.9 a	49.2 b	29.5 a	54.8 b	19.7 a	44.6 b

* Columns of solarization and control followed by the same letters are not significantly different at 0.05 probability level using Duncan's multiple range test (Soil solarization was performed from mid-June to mid-September).

** Means of each parameter/ location within row followed by the same letters are not significantly different at 0.05 probability level using Duncan's multiple range test.

Table (2): Effect of solar chamber on percentages of disease severity and *V. dahliae*-infected olive branches at Al-Baqa'a location in Jordan.

Location	Al-Baqa'a					
Parameter	Average Monthly Disease Severity %			Average Monthly Disease Incidence %		
Treatment /Date	Solar chamber 24 hours	Solar chamber 12 hours	control	Solar chamber 24 hours	Solar chamber 12 hours	control
Mar. 15	65 a	75 a	75 a	87.5 a	79.2 a	87.5 a
April 15	65 a	60 b	65 b	66.7 b	75 a	70.8 b
May 15	60 a	45 c	50 c	50 c	41.7 d	45.8 cd
Jun. 15	50 b	35 d	45 cd	41.7 d	54.2 c	41.7 cd
Jul. 15	30 d	30 de	30 e	41.7 d	33.3 ef	37.5 de
Aug. 15	25 de	20 fg	25 e	29.2 e	33.3 ef	16.7 h
Sep. 15	10 g	15 gh	45 cd	8.3 fg	4.2 g	20.8 gh
Oct. 15	10 g	10 h	40 d	12.5 f	8.3 g	25 fg
Nov. 15	10 g	10 h	45 cd	4.2 g	8.3 g	37.5 de
Dec. 15	15 fg	25 ef	45 cd	12.5 f	4.2 g	33.3 ef
Jan. 15	20 ef	25 ef	45 cd	12.5 f	4.2 g	33.3 ef
Feb. 15	45 c	50 c	50 c	45.8 cd	25 f	45.8 cd
Mar. 15	55 b	60 b	65 b	62.5 b	62.5 b	70.8 b
Mean	35.4 a**	35.4 a	48.1 b	36.5 a	33.3 a	43.6 b

* Columns of solar chamber 24 hours and 12 hours and control in each parameter followed by the same letters are not significantly different at 0.05 probability level using Duncan's multiple range test (Application of solar chamber was performed in mid-August).

** Means of each parameter/ location within row followed by the same letters are not significantly different at 0.05 probability level using Duncan's multiple range test.

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مكافحـة ذبول الزيتون الفيرتيسيليومي باستخدام الحجرة البلاستيكية وتشميس التربة بالبلاستيك

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ملخـص

تهدف الدراسة إلى تحديد فعالية تشميس التربة بالأغطية البلاستيكية واستخدام الحجرة البلاستيكية في مكافحة ذبول أشجار الزيتون- الصنف النبالي البلدي- المتسبب عن فطر الفيرتيسيليوم. تمت تغطية التربة المحيطة بجذع أشجار الزيتون بشرحات بلاستيكية لمدة ثلاثة شهور ابتداء من منتصف تموز في موقعين أحدهما في البقعة ممثلاً للمناطق المرتفعة والثاني في الحلابات ممثلاً للمناطق الصحراوية في الأردن. كما استخدمت الحجرة البلاستيكية في منتصف شهر آب لمدة 12 ساعة إضاءة و 24 ساعة إضاءة في تغطية أشجار زيتون مصابة في موقع البقعة. لقد انخفضت نسبة الإصابة وشدة الإصابة بالمرض في الأشجار المعاملة بالتشميس معنوياً مقارنة بالأشجار غير المعاملة؛ فقد انخفضت نسبة العزل الفطري من الأشجار المعاملة إلى اقل من 10 % في كلا الموقعين. وكانت قدرة الأشجار المعاملة على استعادة الحيوية والاستشفاء بشكل أسرع مما هو عليه في الأشجار غير المعاملة. وقللت تغطية أشجار الزيتون المريضة بالحجرة البلاستيكية لمدة 24 ساعة من شدة الإصابة بالمرض ونسبة الأفرع المصابة معنوياً بالمقارنة مع الشاهد، وذلك للفترة الممتدة من منتصف تشرين الأول ولغاية منتصف شهر كانون الثاني. ولم تظهر الدراسة فروقاً معنوية بين معاملات الحجرة البلاستيكية سواء كانت لمدة 12 ساعة أو 24 ساعة.

الكلمات الدالة: الزيتون، الذبول الفيرتيسيليومي، التشميس، الحجرة البلاستيكية.

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تاريخ استلام البحث 2007/4/18 وتاريخ قبوله 2008/5/29.