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Investigations on Alternative Control Measures of Verticillium Wilt of Olive

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ABSTRACT

The effectiveness of different compounds to be likely used against wilt disease caused by *Verticillium dahliae* Kleb. on olive was studied in pot trials. Aliette (fosetyl-Al, %80 WP), ISR-2000, pine sawdust, broccoli residue and Perlka (calcium cyanamid, (%20,6 N and %38 Ca and %11 C) granule) were used as test materials. Fosetyl-Al was applied both on soil and plant while ISR-2000 was sprayed on plant only. The rest were applied as pre-sowing treatment into soil. The plants were planted either in inoculated soil directly or in inoculated soil after soaking the plants in spore suspension of the pathogen.

Disease development on the plants was recorded at certain intervals after inoculation. The average of disease intensity on control plants was 78 %. The effectiveness of the test materials were generally below 50% and dual inoculation caused lower protection. The broccoli residue in inoculated pot soil without pre-inoculation of the plants could bring out an effectiveness of 56%. The possible application of the test materials to control of the Verticillium wilt in the practice was discussed.

Key words: *Verticillium dahliae*, olive, Aliette, ISR-2000, sawdust, broccoli residue, Perlka.

INTRODUCTION

Turkey occupies an important place among the olive and olive oil producing countries of the world (Akay, 1996). As a result of modern techniques and advancements in the sector, olive production has increased and is spreading day by day. In the past few years, Verticillium Wilt, caused by *Verticillium dahliae* (Kleb.) has arisen and its spread is showing an increasing trend. This increasing trend is disturbing the olive growers in Turkey as a whole and especially in Aegean Region, the potential olive growing region of Turkey. Saydam and Copcu (1972) determined that disease was especially prevalent in lower laying lands causing partially or complete drying of the twigs and branches. In the other studies (Benlioglu et al., 2000; Yolageldi et al., 2003) the incidence of the disease was found to be much higher in the fields where other susceptible hosts of the pathogen such as cotton and vegetables were previously grown or in orchards where flooding was practiced for irrigation.

INVESTIGATIONS ON ALTERNATIVE CONTROL MEASURES OF VERTICILLIUM WILT OF OLIVE

Verticillium wilt is difficult to control. Studies on the improvement of resistant or tolerant varieties and rootstocks indicate some hopeful results (Erten, 2004).The cultural practices such as careful soil cultivation, drip irrigation, avoidance of cultivation of the susceptible host plants such as solanaceous vegetables and soil solarization are the main measures to combat the disease .Up to now no fungicides are available, which could stop or lower the disease development in trees sufficiently.

The present study was conducted in screen house as pot trials with the objective to determine the effect of Aliette, ISR-2000, sawdust (pine wood dust), broccoli residues and Perlka on the control of wilt disease of olive. The experiment was carried out in Department of Plant Protection, Faculty of Agriculture, Aegean University, Bornova-Izmir in 2001-2003.

MATERIAL AND METHOD

Plant material: Transplants variety "Manzanilla" obtained from Olive Research Institute, Bornova/Izmir were grown in perlite and used during the study.

Pathogen: Two *V. dahliae* isolates were used;

1. The isolate "**Vz-5**", obtained from culture stock of Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection.
2. The isolate "**No.1**" obtained from culture stock available in Olive Research Institute, Izmir.

Test Material: Test materials used in the experiment and some of their properties are given in Table 1 below.

Table 1. Test Materials and their properties

Material	Trade name	Firm	Active ingredient (%)	Form.	Doses used in experiment
Fungicide	Aliette	Aventis	Fosetyl-Al 80%	WP	- 0.028 gr/1kg soil - 449 gr/ 100 lt. water (leave application)
Plant Activator	ISR-2000	Seres (Improcop)	*Yucca schidigera* plant extract 10% + Alfa amino nitrogen (fruit extract) 300mg/lt	EC	500 ml/100 lt water (leave application)
Sawdust	-	-	Pine wood	-	0,815 gr/1kg soil
Broccoli	-	Ege U. Hort. Department	Fresh broccoli plant,blended	-	80 gr/1kg soil
Fertilizer	Perlka	Gentrans	Calcium Cyanamid (20,6% N and 38% Ca and 11% C)	Granule	400 kg/ha (0,16 gr/1kg soil)

Plant Growing

A mixture prepared from sand + orchard soil + peat (5: 3.5: 1.5) and desinfected with methyl bromide was used in 1 kg pots which are 13 cm diameter (with 13 cm Φ). The roots of olive plants grown in perlite material were washed with tap water and two

plants were transplanted per pot on 02.06.2003. Before transplantation, the roots of one plant were dipped in inoculum suspension ($1x10^8$ spore/ml) for ten minutes as explained in following chapter, the other one remained untreated. After then, the shoots tips of the transplants were cut uniformly in order to encourage the leave growth. Experiments were conducted in a controlled environment at 24 °C with 16/8 hour day/ night period.

Inoculum preparation

V. dahliae isolates were grown on PDA at 25 °C for 15 days and then cultured on natural medium to obtain inoculum for the soil mixture or on PDA as inoculum for root inoculation.

Natural medium: Sharply chopped barley straw was moistened with water and then put into 0.5 liter glass milk bottles in such a way that 75% volume of the bottles were filled with the material. The openings of the bottles were clogged with cotton plug covered with aluminum foil and sterilized in autoclave at 120 °C for 40 minutes. The young colony of the pathogen grown on PDA in petri plates was added at a dose of ¼ petri per bottle and bottles were incubated at 25 °C for one month. During the inoculation period the bottles were regularly shaken at an interval of 4-5 days so that the mycelium may cover the straw fully. At the end of growth period the straw pieces were covered with the black microsclerotia of the pathogen. The bottles were opened then in a sterile environment and the material was transferred on to sterilized aluminum foil and was dried at room temperature for four days. The dried material was utilized as inoculum in the soil mixture (Onan, 1993).

Inoculum preparation for the root inoculation of the olive transplants: A spore suspension of the pathogen was prepared by adding sterile water containing Tween 80 to the petri dishes with growing colony. After shaking on a tube shaker spore concentration was adjusted with the help of a haemocytometer as $1x10^8$ spore/ml. (Çelebi, 2002).

Inoculation

The soil inoculation was carried out at the time of transplantation of plants to the pots in all the treatments except the treatments containing Perlka and broccoli residues where the inoculation was done at least 30 days before the transplantation. A total of 7.5 g inoculum mixture (5 g inoculum of Isolate No.1 and 2.5 g from isolate no. Vz-5) per pot was applied. Inoculation was not done to the soil of the control pots of all the treatments.

In the dip method, the roots of the plants, after washing with tap water, were dipped in the spore suspension for 10 minutes just before the transplantation and transplanted to the pots containing inoculated soil, so that each pot had two plants, one directly transplanted, other one after dipping in spore suspension.

The control plants were washed with tap water and transplanted after dipping them in sterile water for 10 minutes.

Application of Test Substances

Aliette: This fungicide was used as soil and foliar treatment (Anonymous, 2001).

Soil treatment: Aliette was applied to the soil at time of transplantation of plant at dose of 0.028 g per pot with water.

Foliar Application: On appearance of first symptoms Aliette was applied at dose 4.49 gr/lt water to the leaves.

ISR-2000: The plants were dipped in ISR-2000 solution in sterile water at dose of 5 ml/liter and kept there for 15 minutes before planting to pots. The second treatment was done 15 days after planting by applying the same dose on foliage (Tosun, 2001, personal communication).

Sawdust: The sawdust, obtained from the pine wood, was incorporated in to the soil at the time of inoculation with pathogen at a dose of 0.815 g per pot and the transplants were planted (Anonymous, 1998; Bourbos, 1997).

Broccoli Residue: The broccoli material finely chopped was incorporated into the soil at the time of inoculation with pathogen at a dose of 80 g per pot. The cuttings were planted in the treated pots 30 days later (Subbarao-Krishna et al, 1999).

Perlka: This test material was used at dose of 160 mg for 1 kg soil. The treatment was done 30 days before the transplantation and pots were watered with the interval of 4-5 days till the transplantation of plants (Kacar, 1986.

Evaluation: The development of disease symptoms on the leaves were evaluated by using the following 0-4 scale (Tjamos at al., 1991):

0: No disease symptoms; **1:** Wilt symptoms on the leaves up to 25%; **2:** Wilt symptoms on the leaves up to 50%; **3:** Wilt symptoms on the leaves up to 75%; **4:** Wilt symptoms on the green parts up to 80% or more

Statistical Analysis: The data obtained from the experiments were analyzed by using SPSS 9.05 statistics programme for Windows. The means were compared by using Duncan Multiple Range Test (P=0.5). The square root transformation was done to all the % values.

RESULTS

Disease development on plants transplanted direct to the inoculated soil and effect of test materials

The experiment was carried out during March- September 2003 and first symptoms were observed six week after transplantation. The growth and development of the shoots and leaves blocked , color changed from normal to greenish yellow and wilting symptoms appeared. A general irregular inward bending, curling of drying leaves and withering of the shoots were also observed.

The disease intensity was determined twice, firstly on 01.08.2003 and secondly on 21.08.2003. The data obtained is shown in Table 2.

The disease intensity was found to be the highest in positive control pots whereas a clear cut decrease in disease intensity in pots treated with test materials was observed.

In the second evaluation the disease intensity was found to increase in all treatments except Aliette-soil. In case of ISR-2000 treatment, disease intensity slightly decreased.

The efficacy of treatments was determined on the basis of second evaluation. The broccoli residue treatment was found to be the most effective (56%) compared with other test substances, but statistically no significant difference was observed between different treatments with respect to average disease intensity.

Table 2. Effects of different test materials on disease intensity (%) of olive transplants transplanted direct to inoculated soil.

Treatment	Disease Intensity (%)			Efficacy %
	01.08.03	21.08.03	Average	
Negative Control	0	0	0	-
Positive Control (Pathogen)	75,00	81,25	78,13 a	-
Pathogen + Aliette- leave	50,00	56,25	53,13 a	32,00
Pathogen + Aliette- Soil	43,75	43,75	43,75 a	44,00
Pathogen + ISR 2000	56,25	50,00	53,13 a	32,00
Pathogen + Saw Dust	31,25	56,25	43,75 a	44,00
Pathogen + Broccoli Residue	31,25	37,50	34,38 a	**56,00**
Pathogen + Perlka	56,25	81,25	68,75 a	12,00

Note: Means followed by the same letters are not significantly different according to the least significant difference test (P=0.05)

Disease development on plants transplanted to the inoculated soil after dipping in the spore suspension and effect of test materials

On the plants which were dipped in the spore suspension before transplanting to the pots with inoculated soil, symptoms were observed much earlier i.e. four weeks after transplantation. The leave color of infected plants changed from normal to greenish yellow and wilting symptoms appeared, accompanied with curling lengthwise and inward and drying. The growth of shoot stopped and their color changed to light green .The xylem transmission vessels gained a light brown color.

The disease intensity was also determined twice, firstly on 01.08.2003 and secondly on 21.08.2003. The data obtained is shown in Table 3.

In case of dip inoculation method the disease intensity was higher as compared with the soil inoculation and the efficacy of test materials was also found to be low accordingly. The isease intensity in plants of positive control and in plants treated with Aliette and sawdust was high and remained unchanged in second evaluation. In other

treatments, intensity of disease has increased over time. Statistically no significant difference was found among different treatments. However, Aliette-leave was found to have a higher efficacy of 42.31%.

Table 3. Effect of different test materials on disease intensity (%) in olive plants transplanted to inoculated soil after dipping in spore suspension

Treatment	Disease Intensity (%)			Efficacy %
	01.08.03	21.08.03	Average	
Negative Control	0	0	0	-
Positive Control (Pathogen)	81,25	81,25	81,25 a	-
Pathogen + Aliette- Leave	37,5	56,25	46,88 a	42,31
Pathogen + Aliette- Soil	75	75	75 a	7,69
Pathogen + ISR 2000	43,75	56,25	50 a	38,46
Pathogen + Saw Dust	75	75	75 a	7,69
Pathogen + Broccoli Residue	50	68,75	59,38 a	26,92
Pathogen + Perlka	75	81,25	78,13 a	3,85

Note: Means followed by the same letters are not significantly different according to the least significant difference test (P=0.05)

DISCUSSION AND CONCLUSIONS

In this study Aliette, ISR-2000, sawdust, broccoli residues and Perlka was used to determine their efficacy for the control of Verticillium wilt of olive in a pot trial. One group of olive plants were planted directly to infected soil while the other group was first dipped in the spore suspension of pathogen and then planted to the infected soil. Disease intensity was measured 60 and 80 days after inoculation.

In plants which planted directly to infected soil, disease intensity was high in positive control plants and increased slightly, from 75% to 81,25% with time (Table 2). In plants treated with test materials, average disease intensity changed between 68,75 - 34,38% and their efficacy in percent was not high enough and remained below 50%, except broccoli (56%). In our opinion, the efficacy of a test material must not be less than 50%, if it is to be used for control of the pathogen in future experiments or in the orchard. In this respect, no study was found in literature.

In case of treatment of plants with spore suspension before transplanting in inoculated soil, the disease intensity was high (81%) and remained unchanged in the second evaluation. This indicates that an additional inoculation could not contribute to increased disease intensity in positive control plants. However, effectiveness of test materials was much lower as compared to the previous method of inoculation. Only Aliette-leave (42,31%) and ISR-2000 (38,46%) treatments were found a little effective (Table 3). The efficacy of test materials especially used to reduce the population of pathogen in soil was found to be significantly lowered by additional dip-inoculation. In

other words, the direct introduction of pathogen in to the roots decreased the efficacy of the test materials.

These results reveal that the only promising organic material for the control Verticillium wilt of olive seems to be broccoli residues. In literature broccoli residues has not been tested before for the control of Verticillum wilt of olive. However, Shetty et al. (2000) found that broccoli displayed resistance against *V. dahliae* infection in an field experiment. Xiao et al. (1998) and Subbarao et al. (1999) reported that incorporation of broccoli residues to the infected soil or rotation of crops susceptible to Verticillium wilt with broccoli can be one of the best methods to control this disease.

According to Xiao et al. (1998) the soil population of V. *dahliae* reduced 94%, in the field where broccoli was cultivated for two consecutive seasons whereas the inoculum amount increased 5 folds when cauliflower, susceptible host plant, was grown in the same period.

Subbarao-Krishna et al. (1999) reported that chopped broccoli residue application to an infected soil with pathogen at dose of 200 kg per 36 m^2 area reduced microsclerotia of V.*dahliae* as much as or more than the application of chloropicrin and metham sodium.

Broccoli residues not only have negative effect on the survival of resting soil microsclerotia of *V. dahliae* but also have inhibiting effect on root colonization potential of living microsclerotia (Shetty et al., 2000).

In the recent years cultivation and consumption of broccoli in Turkey has an increasing trend. Considering this fact and also the above mentioned reports, it seems possible to say that broccoli or broccoli residues could help the olive growers to reduce the disease incidence in the olive orchards economically. There may be several ways to use this plant as an organic amendment in olive orchards:

1. Broccoli cultivation for a couple of years in suspected areas , e.g. in old cotton fields before installation of a olive orchard
2. Inter-cropping cultivation of broccoli in infected orchards.
3. Broccoli cultivation under the canopy of olive trees.
4. If broccoli cultivation is not possible in olive orchards, its residues can be transported from other broccoli fields.

Broccoli residues gained as leaves and also roots after harvest must be througly chopped using a cultivator before incorporation into soil.

The low efficacy of Aliette as foliar application can be attributed to the low dose of this test material used in our experiment because application of fosetyl-Al at a dose of 1.2 and 1.68 kg/ hl (= 1,5 and 2,1 kg Aliette / hl) in water has been reported to impede the wilt symptoms and to convert the yield of infected trees to the normal in fruit bearing trees of olive orchards (Anonymous, 2002; Fodale et al., 2002). In this experiment, 0,449 kg Aliette / hl in water have been used (Table 1)

The use of sawdust has been reported to encourage the growth of antagonist microorganisms in the soil against *V.dahliae* and is included in the control program keeping in view its particular impact (Wilhelm and Taylor, 1965, in Onogur et al., 2001). The use of sawdust at a dose of 10 kg per tree is also recommended for effective control of the disease in olive (Anonymous, 1998; Bourbos, 1997). Sawdust needs specific duration for its decay effectiveness in the soil, but in our experiment the expected efficacy was not observed due to plantation of olive transplants just after its application. The incomplete decay of sawdust in pot experiment can be considered as a reason for its low or no efficacy.

ÖZET

ZEYTİN VERTICILLIUM SOLGUNLUĞUNUN ALTERNATİF YÖNTEMLERLE MÜCADELESİ ÜZERİNDE ARAŞTIRMALAR

Bu araştırmada, zeytinde *Verticillium dahliae* Kleb.'in neden olduğu Solgunluk Hastalığı'na karşı savaşımda kullanılabilecek maddelerin etkililikleri saksı denemeleriyle araştırıldı. Denemelerde Aliette (fosetyl-Al, %80 WP), ISR-2000 (bitki aktivatörü), çam bıçkı talaşı, brokoli kalıntısı ve Perlka (kalsiyum siyanamid, (%20,6 N,%38 Ca ve %11 C) granül) kullanıldı. Bu maddelerden Aliette toprağa ve bitkiye uygulanırken, ISR-2000 bitkiye, diğerleri ise dikim öncesi toprağa verildi. Bitkiler ya doğrudan veya spor süspansiyonuna daldırıldıktan sonra etmenin sklerotları ile bulaştırılmış toprağa dikildiler.

Bitkilerde hastalık şiddeti inokulasyondan sonra belli aralıklarla belirlendi. Kontrol bitkilerinde ortalama % 78 oranında hastalık şiddeti saptandı. Test maddelerinin etkililikleri, genel olarak %50 seviyesinin altında kaldı. Bitkilerin önce spor süspansiyonuna daldırıldıktan sonra bulaşık toprağa dikilmeleri halinde bu etkinlik daha da azaldı. Brokoli kalıntısının ön inokulasyon yapılmadan doğrudan bulaşık toprağa dikilen bitkilerde %56 oranında etkililik göstermesi dikkati çekti. Makalede test maddelerinin pratikte uygulanabilirliği ayrıca tartışıldı.

Anahtar Sözcükler: *Verticillium dahliae*, zeytin, Aliette, ISR-2000, bıçkı talaşı, brokoli kalıntısı, Perlka.

LITERATURE CITED

AKAY, Z., 1996. Zeytinyğı Sektöründe Son Gelişmeler. **Izmir Ticaret Borsası Dergisi 7:** 25

Anonymous, 1998. Proceedings of the International Seminar on Olive Growing, Chania, Crete, Greece, 18-24 May 1997.

Anonymous, 2001. http://www.ipm.ucdavis.edu/PMG/r280190211.html.

Anonymous, 2002. ttp://www.actahort.org /books/586/586_157.htm.

Benlioğlu, S., M.Demirtaş ve H.Ulusal, 2000. Aydın Ilinde zeytin ağaçlarında görülen kurumalarla ilgili yayınlanmamış survey raporu. Adnan Menderes Üniversitesi Ziraat Fakültesi, Bitki Koruma Bölümü, Aydın.

Bourbos, V.A., 1997. Organic olive oil; production, tecniques, restrictions and perspective. National Politicies In The Olive Oil Sector. UZK (IOOC). 108/Doc. No.4

Çelebi, Ö. 2002. Ege ve Marmara Bölgesi Zeytin Fidanlıkları Ve Bazı Damizlık Zeytin Bahçelerinde Verticillium Solgunluğunun Yaygınlığı, İzolatların Patojenisiteleri ve Optimum Gelişme Sıcaklıkları. Yüksek Lisans Tezi. Adnan Menderes Üniversitesi, Fen Bilimleri Enstitüsü, Aydın

Onan, E. 1993. Effect of Soil Solarization on the Viability of *Verticillium Dahliae* Kleb. Microsclerotia In Aegean Region Of Türkiye. **J.Turkish Phytopath. 22:** 85-93

Erten, L. 2004. Bazı zeytin çeşit ve anaçlarının Verticillium Solgunluğuna (*Verticillium* dahliae Kleb.) duyarlılıklarının belirlenmesi. Doktora tezi. Ege Üniversitesi, Fen Bilimleri Enstitüsü, Bornova_İzmir.

Fodale, A.S., Mulè, R..,Tucci, A. and Cappello, A., 2002. Foliar Treatments with Phosetyl-Al to Control *Verticillium Dahliae* Kleb. in Olive Trees. Proc. 4th IS on Olive Growing .Acta Hort. 586**.** ISHS 2002: 733-736

Kacar, B., 1986. Gübreler Ve Gübreleme Tekniği, T.C.Ziraat Bankası Yayınları NO:2 Ankara 473s.

Onoğur, E., L. Yolageldi, C. Tunç ve I. Yıldırım., 2001. Batı Anadolu Zeytin Ağaçlarında Solgunluk Hastalığının Yaygınlığı Ve Etmeninin Saptanması. E.Ü.Ziraat Fakültesi Bitki Koruma Bölümü, Tariş Ar-Ge, Tübitak-Togtag. Bornova-Izmir

Saydam, C. and M. Copcu, 1972. Verticillium wilt of olives in Turkey. J.Turkish Phytopathol., **1** (2): 45-49

Shetty, K.G., K.V. Subbarao, O.C. Huisman and J.C. Hubbard., 2000. Mechanism of broccoli-mediated Verticillium wilt reduction in cauliflower. Phytopathology, **90** (3): 305-310.

Subbarao-Krishna,V., C. Hubbard-Judith, T. Koike-Steven, 1999. Evaluation Of Broccoli Residue Incorporation Into Field Soil For Verticillium Wilt Control In Cauliflower.Plant Disease, **83** (2) 124-129.

Tjamos, E.C, Biris, D.A. and Paplomatas, E.J. 1991. Recovery Of Olive Trees With Werticillium Wilt After Individual Application Of Soil Solarization In Established Olive Orchards. Plant Diasease 75:557-562

Tosun,N. 2001, personal communication. Ege Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü.

Wilhelm, S. and J.B. Taylor, 1965. Control of Verticillium wilt of olive through natural recovery and resistance. Phytopathology **55:**310-316.

Xiao,C. L., K.V. Subbarao, K.F. Schulbach, S.T. Koike, 1998. Effects of Crop Rotation and irrigation on *Verticillium dahliae* microsclerotia in soil and wilting cauliflower. Phytopathology. **88** (10) 1046-1055

Yolageldi, L., E. Onogur, C. Tunç, 2003. Present status of Verticillium wilt of olive in Western Anatolia and some factors affecting the disease prevalence. J. Turkish Phytopathology, **32** (1):31-40