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Effect of Soil Solarization in Controlling **Verticillium** Wilt of Cotton in Aegean Region of Türkiye

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ABSTRACT

*Soil solarization was effective in controlling **Verticillium** wilt of cotton by mulching with polyethylene sheets for six weeks. Treatment resulted in greatly reduced natural populations of **Verticillium dahliae** microsclerotia, which were positively correlated with significant reductions in disease severity.*

INTRODUCTION

Verticillium wilt is a limiting factor in the production of cotton (**Gossypium hirsutum** L.) in some areas of the Aegean Region of Türkiye. Since the first report of **Verticillium** wilt of cotton in the Aegean Region by Iyriboz in 1941 (Iyriboz, 1941), the disease has caused variable losses. Between the years of 1970 and 1976, average yield reductions of 11, 8% were reported (Uygun et al., 1978). Cultural practices, environmental conditions and the concentration of propagules of **Verticillium dahliae** Kleb. in soil influence the occurrence and severity of wilt. Once the pathogen has been introduced into a field it may survive for years in the soil.

Soil disinfestation by chemical fumigants for controlling **Verticillium** wilt can be effective but is of uncertain economic benefit due to the associated high costs. Despite significant progress in development of resistant cultivars, economic loss remain substantial. At present no control practices other than cultivar selection and crop rotation are commercially employed.

Soil solarization (heating soil using a cover of clear polyethylene film to trap solar energy during a fallow period) is a nonchemical alternative to chemical fumigation (Katan, 1981). Control of a number of pathogens by solarization, including **Verticillium dahliae** (Pullman et al., 1981 a, 1981 b; Tjamos and Paplomatas, 1988), **Rhizoctonia solani** (Elad et al., 1980; Katan, 1980; Pullman et al., 1981 b), **Fusarium oxysporum** (Katan, 1981), **Sclerotium rolfsii** (Grinstein et al., 1979) have been reported. Soil solarization has also been shown to be effective in Aegean Region on the viability of **Verticillium dahliae** Kleb. microsclerotia (Onan, 1993). In addition, solarization offers potential costs reduction over chemical fumigation without residue or toxicity problems.

This study was conducted to determine the effectiveness of soil solarization in reducing **Verticillium** wilt of cotton in the field.

MATERIALS AND METHODS

Application of solarization

Soil solarization was conducted on 9 July 1992 in a clay loam field selected for its history of severe **Verticillium** wilt in Cotton Research Institute, Nazilli. Most plant remnants were removed and the soil was ploughed, rotovated, irrigated and divided into 12 plots (5x10 m). Three days after irrigation, six plots were tightly covered with transparent polyethylene sheets (100 µm thick). Six plots were left untarped and used as untreated controls. The polyethylene was left in place for six weeks and the maximum values of temperatures of non-solarized soil were measured by inserting thermometers at soil depths of 5,10 and 20 cm. The polyethylene was removed on 25 August.

Quantitative assessment of Verticillium dahliae populations

Microsclerotia of **V. dahliae** were quantified from shovel samples at soil depths of 5-20 cm. Samples were randomly collected from 10 sites in each experimental plot (3 kg per plot) on 6 July 1992 before the solarization. Second and third soil assays were carried out after six weeks (25 August 1992) and ten months (14 June 1993) respectively. Triplicate 15-g samples of air-dried soil were treated by wet sieving as described by Huisman and Ashworth (1974) with the following modifications: the soil was washed through sieves of mesh 150 and 42 µm; the residure retained by the 42 µm sieve was washed for 30 s with 40 g/l NaOCl, transferred to 50 ml tubes and made up to 30 ml with sterile distilled water. The soil particles were left to precipitate for 30 min and excess water was removed by aspiration. The final 10 ml residue was spread uniformly over 10 agar plates containing a verticillium-selective growth medium (Ausher et al., 1975) and incubated for 21 days at 18 C°. **Verticillium dahliae** colonies were counted.

Verticillium wilt assessment

Sayar 314 cultivar of cotton, which is susceptible to **V. dahliae,** was sown in each experimental plot on 4 June 1993. The effectiveness of solarization in controlling **Verticillium** wilt was evaluated on 7 October 1993. Disease severity was assessed for each plant on O-4 scale (O= 0%, 1= 1-33%, 2= 34-66%, 3= 67-100%, 4= dead plant) according to the percentage of affected foliage in acropetal progression (Blanco Lopez et al., 1989).

RESULTS

Effects of solarization on the viability of V. dahliae microsclerotia

Populations of **V. dahliae** microsclerotia were either eradicated or greatly reduced and remained very low at the end of six weeks in solarized plots. Population reductions (%) in solarized and unsolarized plots are seen in Table 1.

Table 1. Effect of six weeks of soil mulching with polyethylene sheets on the viability of **V. dahliae** microsclerotia (at Nazilli, 1992).

Experimental plot	Solarized plots: Mean number of microsclerotia* pre-solarization (6 July)	Solarized plots: Mean number of microsclerotia* post-solarization (25 August)	Population reduction (%)	Unsolarized plots: Mean number of microsclerotia* 6 July	Unsolarized plots: Mean number of microsclerotia* 25 August	Population reduction (%)
1	115	16	86.0	123	108	12.1
2	96	2	97.9	103	81	21.3
3	68	0	100.0	93	88	5.3
4	112	6	94.6	101	92	8.9
5	75	3	96.0	69	58	15.9
6	83	0	100.0	118	103	12.7
Mean	91.5	4.5	95.7	101.1	88.3	12.7

x ;per 15 g soil

A pronounced population reduction in the solarized plots was seen by mulching: 86.0 to 100.0% reduction. Populations in the unsolarized plots remained high level during six weeks (Table 1).

The mean maximum temperatures recorded in bare soil between 9 July and 25 August were 44.6 C°, 33.1 C°, and 31.5 C° at depths of 5 cm, 10 cm and 20 cm respectively.

Ten months after solarization (14 June 1993), mean number of microsclerotia in soil samples from each experimental plot is given in Table 2.

Table 2. Effect of soil solarization on survival of **Verticillium dahliae** microsclerotia (ten months later, 14 June 1993, Nazilli)

Experimental plot	Experimental plots: Mean number of microsclerotia per 15 g soil	Unsolarized plots: Mean number of microsclerotia per 15 g soil
1	11	112
2	6	87
3	0	79
4	9	98
5	0	63
6	0	71
Mean	4.3	85

It follows from Table 2 that the untreated plots had high populations during ten months, and the reduction was also evident in solarized plots.

Effect of solarization on disease severity

Solarization drastically reduced disease severity compared to the untreated controls. Effect of solarization in the plots varied between 84% and 100% on disease severity (Table 3).

Table 3. Effect of soil solarization on disease severity of **Verticillium**-susceptible Sayar 314 cotton plants (at Nazilli, 1993).

Experimental plot	Solarized plots Disease severity (%)	Unsolarized plots Disease severity (%)	Effect of solarization (%)
1	10.0	62.5	84.0
2	5.0	52.5	90.4
3	0.0	47.5	100.0
4	7.5	55.0	86.3
5	0.0	37.5	100.0
6	0.0	42.5	100.0
Mean	3.7	49.5	93.4

DISCUSSION

Verticillium dahliae Kleb., which causes vascular wilt, is one of monocyclic pathogens; i.e. those that complete only one cycle of pathogenesis in one cropping season. The amount of disease induced by a monocyclic pathogen in a single season is a function of several interacting factors: size and distribution of the pathogen population, the inherent ability of the pathogen to induce disease host factors, and environmental influences.

Disease caused by monocyclic pathogens are managed most effectively by techniques that reduce initial inoculum. Soil solarization is an example of such a technique. Pullman et al (1981 b) demonstrated a 90% reduction in **Verticillium dahliae** propagules at 37 C at 46 cm in moist field soil after solarization. Those data correlate well with ours from this study after solarization for six weeks. **V. dahliae** microsclerotia were reduced mean 95,7 % (from 91.5 to 4.5 microsclerotia per 15 g soil) at soil depths of 5-20 cm (Table 1).

Soil moisture greatly affects sensitivity of the resting structures to heat treatment. In various experiments carried out in a controlled-temperature water bath, moistened

sclerotia-inoculum of **V. dahliae** were eradicated by 100% after an incubation period of 1 hour at 50 C°. In air-dried soil, viability of **V. dahliae** was reduced only partially by 50 C° for 6 hours or at 55 C° for 1 hour (Katan et al., 1976). It was found that temperatures achieved at the upper soil layers by mulching in Aegean Region were about 10 C° higher than in the uncovered plots (Onan, 1993). Considering temperatures in the bare soil (44.6 - 33.1 C°) in this study, it could be said that temperatures achieved by mulching were in the range of those found to be lethal to the pathogens (Nelson and Wilhelm, 1958).

Soil solarization could keep microsclerotia populations at low levels throughout the ten-months experimental period (Table 2). The long-term effect of soil solarization has been attributed to the possible survival and increase of heat-resistant antagonists. Martyn and Hartz (1986) have shown a sharp reduction in populations of **F. oxysporum** f.sp. **niveum** in solarized soils and a significant reduction of total disease incidence in **Fusarium**-susceptible watermelon plants. They also reported increases in populations of saprophytic **Fusarium** spp. in solarized compared with non-solarized plots. Stapleton and DeVay (1982) have shown an increase in populations of fluorescent pseudomonads, along with an increase of **Penicillium** and **Aspergillus** in solarized compared to untreated plots. Also, Tjamos and Paplomatas (1988) have found an increase of **Talaromyces flavus** in solarized plots. It could be said that such a phenomenon in the cotton field may be at least partially responsible for about one-year effect of solarization.

Solarization was effective on wilt symptoms and reducing disease severity in **Verticillium**-susceptible Sayar 314 cotton plants (Table 3). It is easily seen that the effect on disease severity related to the low inoculum level in solarized soils.

Consequently, our results demonstrate that soil solarization for six weeks may be effective on **Verticillium** wilt of cotton under the Aegean Region conditions. Further research is needed to determine the lasting effects of solarization on populations of **V. dahliae** and the effects of long-term solarization on wilt disease severity since control measures with long-lasting effects are most desirable to the farmer because of the substantial cost reductions involved. In addition, it will be interesting to determine heat-resistant antagonists in the solarized fields.

ÖZET

EGE BÖLGESİ'NDE PAMUKTA VERTİSİLYUM SOLGUNLUĞUNU ÖNLEMEDE TOPRAK SOLARİZASYONUNUN ETKİSİ

Ege Bölgesi koşullarında toprak solarizasyonunun pamukta Vertisilyum solgunluğunu önlemedeki etkisini belirlemek için tarla koşullarında yürütülen çalışmadan elde edilen veriler, toprak solarizasyonunun oldukça etkili olduğunu göstermiştir.

Toprak solarizasyonu, altı haftalık süreç içerisinde topraktaki mikrosklerot populasyonunu ortalama %95.7 geriletmiştir. Gerileyen mikrosklerot populasyonu bir yıl sonraki pamuk ekim dönemine dek aynı düzeyde kalmıştır.

Toprak solarizasyonu uygulanan parsellerde solgunluk şiddeti düşük düzeyde, kontrol parsellerde solgunluk şiddeti yüksek düzeylerde ortaya çıkmıştır. Toprak solarizasyonu pamuk bitkilerinde solgunluk şiddetine ortalama %93.4 oranında etkili olmuştur.

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Analysis of **Agrobacterium tumefaciens** Population in West Anatolia, Their Biological Control and Search for Natural Antagonists

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ABSTRACT

*Crown gall caused by **Agrobacterium tumefaciens** is a problem in west Anatolia nurseries especially stone fruits, pear, apple and rose. It is also problem on the fruit tree, rose and grapevine. But this disease was not studied on grapevine both nurseries and vineyards in this study. A great majority of agrobacteria isolated from galled plants in west Anatolia belong to biovar 2 and are sensitive to agrocin 84. Results of biological control trials on tomatoes have shown that K 84 has some affect on **A.tumefeciens** strains resistant to agrocin 84 **in vitro**.*

*In an attempt to find other strains which could be used as agents of biological control, 840 strains of agrobacteria isolated from soil samples were tested for bacteriocin production against pathogenic strains. One strain was highly effective to pathogenic strains **in vitro** but not **in vivo**.*

INTRODUCTION

Crowngall caused by **Agrobacterium tumefaciens** is a widewspread disease in Türkiye and constitutes a serious problem especially for nurseries of stone and pome fruit trees, but also for nurseries of grapevine and for the cultivation of grapevine in vineyard. The disease manifest itself in the form of hyperplasias, which withdraw nutrients from the plant and interfere in the transport of water. As with most bacterial plant diseases, crown gall is diffucult to control by chemical bactericides.

Kerr (1972) in Australia developed a biological control method for **A.tumefaciens,** based on the use of **A.radiobacter** strain K 84, isolated from the rhizosphere of diseased plants. This strain possesses a high antagonistic capacity, both chemically (production of agrocin 84) and mechanically (competiton for infection sites) (Cooksey and Moore, 1980). Today the strain K 84 is used successfully in many countries for rose peach and other stone fruit trees (Bazzi and Mazzucchi, 1978; Garrett, 1979; Moore, 1979). However, this method is not effective for cultures like grapevine, chrysanthemum, apple, pear and species of **Rubus,** which are generally attacked by agrobacteria that are insensitive to the strain K 84 (Grimm and Vogelsanger, 1983; Grimm

and Sule, 1981). In addition transconjugant resistant agrobacteria, although rare, may arise (Panagapoulos **et al**; 1979).

In our research we have studied the distribution, biotype, pathogenicity and the sensitivity of our isolates of **A.tumefaciens** to agrocin 84 **in vitro** and **in vivo.** Moreover, we have evaluated the antogonistic capacity of a group of agrobacteria which produce antibiotic compounds.

MATERIALS and METHODS

A hundred-fourteen gall samples were collected from diseased plants including peach (51), cherry (13), plum (16), apricot (11), almond (2), pear (11), apple (5), rose (4) and quince in western Anatolia orchards from June to September 1992, and from galled plants in nurseries in İzmir, Manisa and Aydın provinces at winter months in 1992 and 1993.

Isolation, pathogenicity and identification: Galls were surface sterilized for 30 second in 70% ethanole and rinsed in sterile distilled water. Each gall sample was plated on three plates of the King medium B (King **et al.**, 1954). Colonies that produced fluorescein or were other wise atypical of **Agrobacterium** were discarded.

Stems of 4-5 wk old tomato plants (cv. Pierra) were inoculated with all **Agrobacterium** isolates (330 isolates) A 48-hr. old NA (Difo) culture was spread with a loop on the stem surface and introduced into the stem by wounding it lightly with a sterile needle. Three tomato plants were inoculated per culture, kept for 2 wk in a growth chamber at 26-28°C and then scored for tumor development.

The 330 isolates judged typical **Agrobacterium** were tested for 3. Ketolactose reaction, growth on 2% NaCl, Litmus milk, acid from erythritol, alkaline from L-tartarate and maximum growth temperature as described by Kerr and Panagopoulos (1977).

Isolation of bacteriocinogenic agrobacteria from soil: Agrobacteria were isolated from 135 samples taken from areas where crown gall incidence was high Soil samples were diluted in sterile distilled water (1 g. in 100 ml) and 0.1 ml added to three plates containing both the selective medium of Brishane and Kerr (1983) I A (Biovar 1) and 2 E (Biovar 2). For every soil, up to 60 colonies resembling **Agrobacterium** were selected from each medium and tested for bacteriocin production. They were transferred by spot inoculation to plates containing Stonier's Agar medium (Stonier, 1960), 20 colonies perplated. The plates were incubated for 48 h and then each used as a master plate from which three replica plates. These replica plates were incubated for 48 h, the bacteria killed with choroform and seeded with two indicator strains on seperate plates. Results were recorded after 48 h. Any strain which caused marked inhibition an indicator strain was subcultured from the master plate, purified and retested for inhibitory properties. If still positive, the strains were checked for the salient characters of **Agrobacterium.**

Sensitivity to agrocins:

The method of Stonier (1960) was used to test the the sensitivity of pathogenic **Agrobacterium** isolates to isolate K 84 and to ATT/87_4 (own bacteriocinogenic Agrobacterium isolate). 200 µg biotin (vitamin H) per litre was added to the medium (Kerr and Panagopoulos, 1977).

Test for biological control:

Tomato tests: Twenty-five pathogenic isolates taken from various host were tested for their susceptibility to control by Kerr 84 and ATT/87_4 in the greenhoue. The isolates were mixed in Sterile distilled water in 1:1, 1:3 and 1:10 proportions antagonists to pathogen. Cultures were grown at 27°C for 48 hr suspended in sterile distilled water to give approximately 10^8 cells per milliter and then mixed. The mixture was injected to the tomato stem by using a 1-ml. syringe. Tumor production or control was rated 2 wk after inoculation.

RESULTS and DISCUSSION

Isolation, identification and distribution of Agrobacterium isolates:

A total of 331 isolates were obtained, 196 of which (59.3%) were determined as pathogen, 135 (40.7%) as nonpathogen. The proportion of pathogenic to nonpathogenic isolates varied considerably in gall samples from orchards and nurseries. Fresh galls from young plants obtaiened were better sources of pathogenic isolates than from mature trees in orchards.

A total of 196 pathogenic isolates were obtained, 43 of which (21.9%) were classified as biovar 1, 153 (78.1%) as biovar 2 and nine as biovar 3. The results are shown in Table 1.

Lopez **et al.** (1987) also reported that 31.1% of isolated were biotype 1, 61.8% biotype 2 and 7.1% biotype 3.

Agrocin sensitivity:

Sensitivity to agrocin 84 was determined in stonier medium (Stonier, 1960). Isolates that were sensitive to Kerr 84 varied in their sensitivity; this was indicated in Stonier plates by differences in diameter of the inhibition zone (0.0-6.8 cm). Figure 1 shows some of these reactions. Sensitivity to agrocin 84 was found in 91.8% of isolates while 8.2% were found resistant. Mostly pear isolates were found resistant to Kerr 84.

In addition some of non-virulent isolates tested for agrocin sensitivity **in vitro** were in great part in sensitive to Kerr 84. Psallidas (1988) reported that all Greek isolates of biotype 1 and all but 3 isolates of biotype 2 were sensitive to biological control with the antagonistic bacterium **A.radiobacter** Strain K 84. Nevertheless, it was reported that relatively high proportion of tumor-inducing **Agrobacterium** isolates from South Carolina (36%) and Tennessee (24%) were resistant to the K 84 (Alconero, 1980).

Table 1. Characteristics of pathogenic **A. tumefaciens** isolates

Host	No.of isolates	Characters						
		3-keto lactose	2% NaCl	Max.growth temp.	Litmus milk	Acid from erythritol	Alkali E.tartarate	Biovar
Peach	60	-	-	29-31	Acid	+	+	2
	17	+	+	37-40	Alk	-	-	1
Cherry	22	-	-	29	Acid	+	+	2
	7	+	+	37-39	Alk	-	-	1
	24	-	-	29-30	Acid	+	+	2
	5	+	+	38	Alk	-	-	1
Apricot	16	-	-	29	Acid	+	+	2
	6	+	+	37-38	Alk	-	-	1
Almond	1	-	-	29	Acid	+	+	2
	2	+	+	37	Alk	-	-	1
Pear	12	-	-	29-30	Acid	+	+	2
	6	+	+	37-39	Acid	-	-	1
Apple	6	-	-	29-30	Acid	+	+	2
Quince	3	-	-	29-30	Acid	+	+	2
Rose	6	-	-	29-30	Acid	+	+	2

Isolation of bacteriocinogenic agrobacteria from soil:

Eight hundred and forty colonies resembling **Agrobacterium** were isolated from 135 soil samples and tested for production of bacteriocin against strains ATG/38 (sensitive to Kerr 84), ATG/20 (insensitive to Kerr 84) and ATG/16 (insensitive to Kerr 84). The capacity of 207 non virulent strains to produce antibiotic which are active against indicator strains was detected **in vitro.** Inhibition zones diamater were varied between 0.2-6.4 cm. Especially, one soil sample (obtained Selçuk) yielded many bacteriocinogenic isolates. Five of all these isolates were very active against indicator isolates and consitituted inhibition zones changing from 5.0 to 6.4 cm. One biotype 1 strain (designated ATT/87_4) produced a bacteriocin which inhibited 130 pathogenic strains. Eighteen pathogenic strains (mostly biovar 1) were weakly sensitive to ATT/87_4. It was interesting to determine Kerr 84 strain was sensitive to ATT/87_4. Antibiotic producing agrobacteria are found in nature, although not frequently.

Biological control

Table 2 lists the response of tomato plants to inoculation with 19 representative pathogenic isolates aplied alone and mixed in various proportions with antagonists (Kerr 84 and ATT/87_4) it also lists (Table 2) the response of the isolates to antagonist strains in the Stonier tests (stonier, 1960).

Our results show that isolates which had no inhibition zone in Ştonier plates were not controlled at 1:1 and some 1:3 (pathogen/antagonist) ratios in tomatoes. a 1:10 ratio was needed to control these isolates in tomatoes. Some agrocin sensitive strains were produced very small galls at 1:1 ratios in tomatoes. Conversely, Alconero (1980) reported that when there was no inhibition on the plates, there was good development of galls in both peaches and tomatoes, whatever the proportion of antagonist to test culture. On the other hand, it was reported that K 84 had some effect on **A.tumefaciens** strains resistant to agrocin 84 (Lopez **et al.**, 1988).

Although, all of selected virulent strain were sensitive to ATT/87_4 strain **in vitro,** they were produced galls at 1:1 and 1:3 ratios in tomatoes, only reduced the tumors size. It was reported antibiotic producing agrobacteria were found in nature, although not frequently; none of them, however, showed **in vivo** the same antagonistic properties as the strain K 84 (Raio and Zoina, 1992).

However, we think, it will be usefull to investigate the biological control capacity of this antagonistic strain against crown gall in natural infested soils. As is known, there is a complete correlation between inhibition **in vitro** and successful biocontrol **in vivo** (Psallidas, 1988).

Studies have been continiued on formulation and standartization of inoculant, and the studies on effectivity of inoculant in naturally infested soils.

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Table 2. Gall development in tomato plants inoculated with antagonist Kerr 84 (left) and ATT/87_4 (right) and 19 representative **A. tumefaciens** isolates that differed in sensitivity to antagonists in Stonier plate tests.

Isolate	Host	Alone	1:1	1:3	1:10	Stonier inhibition dia. (cm)	Alone	1:1	1:3	1:10	Stonier inhibition diam. (cm)
ATG78	Pear	+	+	w	vw	0	+	+	w	vw	6.2
ATG/16	Apricot	+	w	--	-	0	+	+	-	-	6.2
ATG/20	Pear	+	+	-	-	0	+	w	-	-	6.0
ATG/21	Peach	+	+	w	-	0	+	+	-	-	6.4
ATG/24	Plum	+	w	-	-	0	+	+	vw	-	6.2
ATG/17	Apricot	+	w	-	-	5.8	+	w	vw	-	6.0
ATG/26_1	Peach	+	+	-	-	6.2					
ATG/31_3	Plum	+	-	-	-	6.6	+	vw	-	-	6.0
ATG/35_1	Pear	+	+	w	-	5.6	+	+	w	-	6.0
ATG/67_2	Peach	+	-	-	-	6.8	+	+	w	-	5.0
ATG/68_4	Peach	+	-	-	-	7.2	+	+	w	-	5.4
ATG/90_1	Peach	+	w	vw	-	5.6	+	w	vw	-	6.0
ATG/93_1	Pear	+	w	vw	-	6.0	+	+	vw	-	6.0
ATG/96_1	Apricot	+	w	-	-	6.5	+	+	w	-	5.5
ATG/107_3	Cherry	+	-	-	-	7.0	+	+	vw	-	6.0
ATG/87_2	Apple	+	-	-	-	6.6	+	w	vw	-	5.4
ATG/66_6	Plum	+	-	-	-	7.2	+	w	-	-	6.2
ATG/33_5	Cherry	+	w	vw	-	5.6	+	w	vw	-	5.2

\+ : good gall development
w : weak gall
vw : very small gall development
\- : no galls

ÖZET

BATI ANADOLU'DAKİ A.TUMEFACIENS POPULASYONUNUN İNCELENMESİ, BİYOLOJİK KONTROLU ve DOĞAL ANTAGONİSTLER ÜZERİNDE ARAŞTIRMALAR.

Agrobacterium tumefaciens'in neden olduğu kök kanseri hastalığı özellikle sert çekirdekli meyve fidanlıklarının yanı sıra elma, armut ve gül fidanlıklarında da ciddi bir problemdir. Ayrıca meyve ağaçları, gül ve asmalarda da önemli enfeksiyonlar oluşturmaktadır. Ancak bu çalışma kapsamında asma fidanlıkları ve bağlar bu hastalık yönüyle ele alınmamıştır.

Batı Anadolu Bölgesinden toplanan galli bitkilerden izole edilen **A.tumefaciens** izolatlarının büyük çoğunluğu Biovar 2 özelliği göstermiş ve yine büyük çoğunluğu **in vitro** koşullarda agrocin 84 (Kerr 84)'e duyarlı bulunmuşlardır.

Domateslerde yürütülen biyolojik kontrol denemeleri sonucunda **in vitro**'da agrocine dayanıklılık gösteren izolatlarında Kerr 84 tarafından az da olsa kontrol edildiği belirlenmiştir.

Biyolojik kontrol ajanı olarak yararlanılabilecek izolatların elde edilmesi amacıyla, toprak örneklerinden izole edilen 840 agrobacter **in vitro** koşullarda bacteriocin üretimi yönünden testlenmiştir. Agrocin ürettiği belirlenen izolatlar içinden seçilen 1 izolat, **in vitro** koşullarda patojen izolatlara karşı yüksek etkili bulunmasına karşı, **in vivo** testlerde aynı düzeyde etkili olmamıştır.

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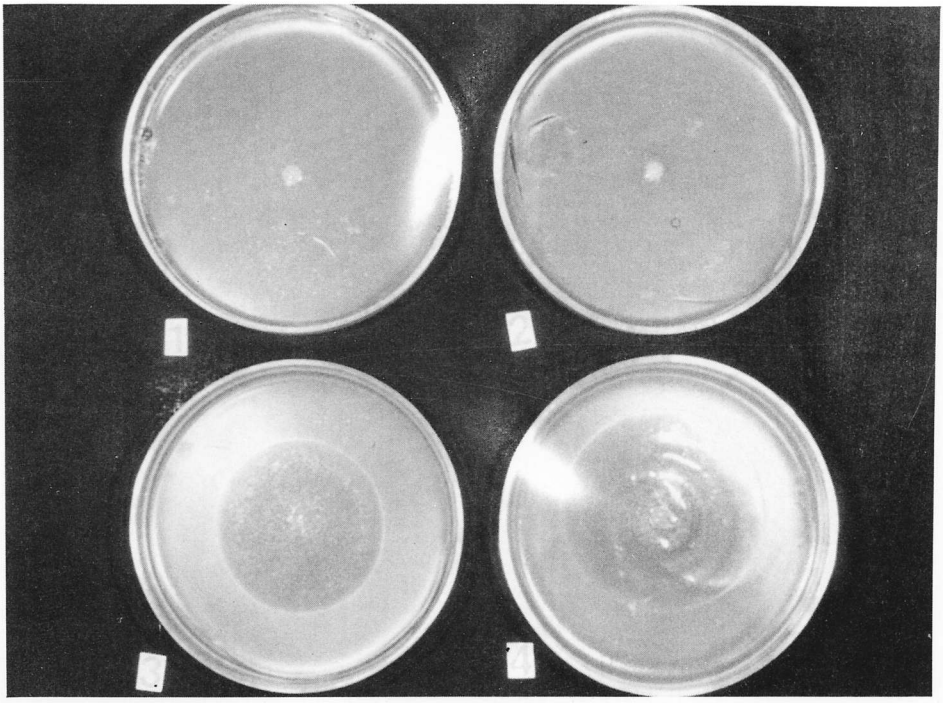

Fig. 1. Sensitivity of *Agrobacterium* isolates to K 84. Isolate 1 is a resistant pathogenic isolate to K 84. Isolate 2 is very weakly sensitive to K 84. Isolate 3 and 4 are pathogenic and very sensitive to K 84.

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Effect of Fluorescent Pseudomonads on Fusarium Wilt of Watermelon*

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ABSTRACT

Seventy eight out of 164 Pseudomonas strains, isolated from healthy melon and watermelon roots, were influorescent characteristics Thirty strains out of 78 showed antagonistic effect against ***Fusarium oxysporum*** *f.sp.* ***niveum*** *(FON)* ***in vitro.*** *It has been proved that siderophore effect is the causal mechanism of inhibition of colonial development of FON* ***in vitro.*** *Seventeen fluorescent Pseudomonas strains (12 were positive and 5 were negative* ***in vitro*** *antagonism test) were selected at random for* ***in vivo*** *test in pots. Fluorescent Pseudomonas strain 28 reduced the disease severity up to 85% in pots.*

INTRODUCTION

Suppression of the diseases by antagonistic bacteria has been shown in several studies: fluorescent Pseudomanads tested for their suppressiveness on Fusarium wilt of radish (LEEMAN **et al.** 1990), on Thielaviopsis root rot of tobacco seedlings (REDDY and PATRICK 1990), and on **Fusarium oxysporum** f.sp.**radicis-lycopersici** of tomato (REDDY **et al.** 1990) showed positive results. On the other hand, fluorescent Pseudomonads showed an additive effect on the biological control of Fusarium diseases when inoculated together with non-pathogenic Fusarium strain (LEMANCEAU and ALABOUVETTE 1990).

In recent years, iron competition between Fusarium and fluorescent Pseudomonads has been subjected to some study on suppression of Fusarial wilt diseases of cucurbitaceaus plants (WELLER 1988). It has also been observed that some siderophore producing members of fluorescent Pseudomonads showed an inhibitive effect against **F. oxysporum** f.sp. **melonis** on melon in Türkiye (BORA **et al.** 1992). The siderophores produced by fluorescent Pseudomonads carry the ferric ions into the bacterial cells. This causes iron deficiency in the rhizosphere, and spore germination abilitiy of the pathogen reduces (KLOEPPER and SCHROTH 1981, SCHER and BAKER 1982).

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F.oxysporum f.sp. **niveum** is widespread and serious in all the watermelon growing area in Türkiye. In this study, the isolates of siderophore producing Pseudomonads were screened in respect to their antagonistic effect on FON **in vitro,** then the isolates tested for their efficacy to reduce the severity of watermelon wilt in pots.

MATERIALS and METHODS

Isolation and Screening of Fluorescent Pseudomonads.

The fields of melon and watermelon plants were visited in August when the disease symptoms are the most remarkable. Only the healthy plant roots were collected and fluorescent Pseudomonads (FP) were isolated from the cleaned and washed surfaces of these roots on to King's medium B (GEELS and SCHIPPERS 1983).

All the isolates of FP strains were grown on King's medium B for two days and sprayed by the conidial suspension of FON (10^5 CFU/ml) for their antagonistic effect. Five days later the inhibition zones between the antagonistic bacteria and the Fusarial colonies were measured by 0-5 scale (GEELS and SCHIPPERS 1983). The tests were repeated for 3 times.

Tests for Siderophore Effect.

Role of the siderophore effect on the inhibition of Fusarial development by the bacterial isolates was proved by adding 80 µMFeCl/L into the King's medium B. Annulment of the inhibition showed that the isolate produces siderophore (ELAD and BAKER 1985).

Soil Bacterization.

Soil provided from the watermelon field was previously inoculated by the pathogen (FON) cultured on wheat seeds for ten days at the rate of 4 g/L soil, then sprayed by the suspension (10^7 CFU/ml) of antagonistic bacteria. Ten seeds of watermelon cv. Washington were sown into each pot, 3 seedlings were left after emerging in each pot for the observations.

Measurement of Wilt Severity.

Thirty days after sowing, severity of the wilt was measured by 0-4 scale of SUNG and HUANG (1984). According to the scale: (0) no disease. (1) 20% or less of the vines wilted, (2) 50% of the vines wilted, (3) 70% of the vines wilted and (4) plants died.

RESULTS and DISCUSSION

Screening of Bacterial Isolates *in vitro:*

Of the 164 bacterial strains isolated from the helthy roots 78 were identified as fluorescent Pseudomonads (FP). Thirty out of 78 FP isolates showed antagonistic effect on the colony development of FON. The results are shown on Table 1.

Table 1. Antagonistic Effect of 30 FP Isolates On The Colony Development of FON*

Isolate Number	Source of Isolate	Inhibition of FON** (0-5)	Isolate Number	Source of Isolate	Inhibition of FON** (0-5)
2	Watermelon-Ödemiş	3.33	69	Melon-Kırkağaç	5.00
12	Melon-Soma	3.00	72	Watermelon-Kırkağaç	3.00
14	Watermelon-Tire	3.75	98	Melon-Akhisar	2.00
17	Watermelon-Bayındır	3.91	99	Watermelon-Akhisar	1.00
19	Watermelon-bayındır	3.00	100	Melon-Akhisar	2.10
20	Watermelon-Manisa	3.50	101	Melon-Akhisar	2.00
23	Watermelon-Cumaovası	2.83	109	Melon-Kepsut	1.00
28	Watermelon-Torbalı	3.61	125	Watermelon-Gönen	2.00
29	Watermelon-Torbalı	3.50	128	Melon-Bayındır	2.00
30	Melon-Torbalı	3.16	140	Watermelon-Bandırma	2.10
31	Watermelon-Tire	3.08	141	Melon-Bandırma	1.25
32	Watermelon-Tire	2.61	148	Melon-Balıkesir	1.00
33	Melon-Tire	4.25	155	Melon-Menemen	1.35
35	Melon-Tire	3.41	158	Watermelon-Menemen	1.75
65	Watermelon-Saruhanlı	4.00	160	Melon-Menemen	1.55

* Antagonistic effect was measured by the scale of 0-5

** Mean value of 3 plates with 4 measurements in each.

As shown on Table 1, the rates of the antagonistic effect of 30 FP isolates ranged between 1.00-5.00. Twelve of the isolates shown on Table 1 and 5 of the ineffective isolates were selected at random for the **in vivo** tests in pots.

Because of annulment of the inhibitive effect when $FeCl_3$ was added to the plates it can be thought that the inhibition of the Fusarial colony development resulted by siderophore effect of the antagonistic FP isolates **in vitro.**

Suppression of Fusarium Wilt of Watermelon in Pots.

Seventeen FP isolates selected at random were tested **in vivo** for their suppressive effect on wilt in pots with 3 replications. On 30. day after seeding, the wilt severity was measured by the scale. The results are shown on Table 2.

Table 2. Suppression of FON by Antagonistic Fluorescent Pseudomonads via Soil Bacterization.

Isolate Number	Source of isolate	Wilt severity (%)*	Suppressive Effect on FON (%)***
Control	-	55.55 **a	0.00
2	Watermelon	30.33 ab	45.40
14	Watermelon	55.55 a	0.00
17	Watermelon	22.22 ab	60.00
20	Watermelon	33.33 ab	40.00
28	Watermelon	8.33 c	85.00
30	Melon	25.00 ab	54.99
33	Melon	27.77 ab	50.00
38/1°	Watermelon	19.44 ab	65.00
41/1°	Watermelon	27.77 ab	50.00
62°	Melon	50.00 ab	9.90
63°	Watermelon	19.44 ab	65.00
65	Watermelon	22.22 ab	60.00
67°	Watermelon	55.55 ab	0.00
69	Melon	44.44 a	20.00
72	Watermelon	22.22 ab	60.00
109	Melon	44.44 ab	20.00
155	Melon	22.22 ab	60.00

* Mean of 3 pots with 3 plants in each.

** Values followed by the same letter are not significantly different (P: 0.05) according to LSD test.

*** As percentage ratio to the control.

° Ineffective isolates **in vitro** test.

As it is shown on Table 2, fluorescent Pseudomonas strain 28 showed a very high (85%) suppressive effect on Fusarium wilt of watermelon in pot test (Figure 1.). FP strain-28 also showed a good inhibitive effect **in vitro** test (Table 1).

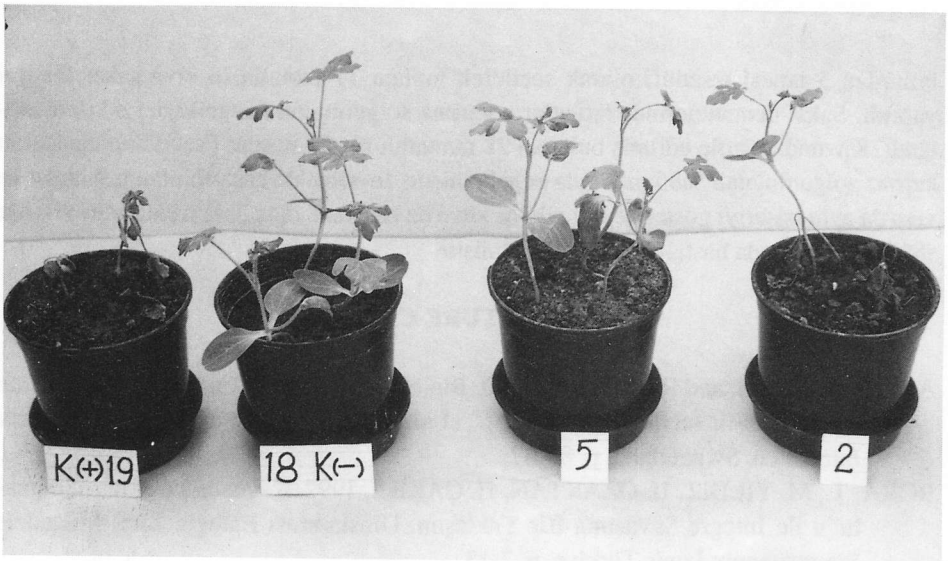

Figure 1. Fluorescent Pseudomonas 28 (previously coded as number 5) suppressed the Fusarial wilt of watermelon in pots, while Pseudomonas 14 (previously coded as number 2) was ineffective. K (+): positive control, K (-): negative control.

On the other hand, five strains found ineffective **in vitro** inhibition assay such as 38/1, 41/1, 63 and 155, suppressed the disease up to 65% **in vivo.** On the contrary, strain - 69 which showed the highest efficacy **in vitro,** could suppress the disease not more than 20% **in vivo.** This contraversial relation between the results of **in vitro** and **in vivo** tests has been reported elsewhere (AXELROOD and RADLEY 1990).

ÖZET

FLUORESENT PSEUDOMONASLARIN KARPUZ FUSARIUM SOLGUNLUĞU ÜZERİNE ETKİSİ

Bu çalışma 1992-1993 yıllarında yapılmıştır. Kavun ve karpuz tarlalarından solgunluk göstermeyen bitkilerin köklerinden King-B besiyerine yapılan izolasyonlarda 164 Pseudomonas izolatı elde edilmiştir. Bunların aynı besiyerinde geliştirilen kolonilerinin ultra viyola ışık altından geçirilmesiyle 78 tanesinin fluoresent özellikte olduğu anlaşılmıştır. Bu 78 izolatın karpuz solgunluk etmeni **Fusarium oxysporum** f.sp. **niveum** (FON) un koloni gelişmesine etkisi **in vitro**'da araştırıldı. Bunlardan 30 izolatın FON'un koloni gelişimini 0-5 ıskala değerlerine göre 1.00-5.00 arasında engellediği saptanmıştır. King-B besiyerine $FeCl_3$ ekleyerek yapılan testlerde antagonistik fluoresent Pseudomonas izolatlarının tümünde antagonistik özelliğin sideroforetkiye bağlı olduğu kanıtlanmıştır. Bu izolatlardan 12 tanesi ile **in vitro**'da FON'u engellemeyen izo-

latlardan 5 tanesi tesadüfi olarak seçilerek toplam 17 izolatla **in vivo** saksı testleri yapıldı. Saksı denemelerinde izolatların karpuz solgunluğunu engelleyici etkileri saptandı. Kavundan izole edilmiş bulunan 28 numaralı bir fluoresent Pseudomonas izolatı karpuz solgunluğunu %85 oranında engellemiştir. **In-vitro**'da başarılı olan her izolat **in vivo**'da aynı başarıyı göstermediği gibi **in vitro**'da başarısız olan bazı izolatlar **in vivo**'da %50-%65 arasında hastalığı engelleyebilmiştir.

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Development of **Ascochyta rabiei** (Pass.) Labr. in the Leaves of Susceptible and Resistant Chickpea Cultivars

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ABSTRACT

*Process of infection of susceptible (ILC 629) and resistant (65C830) chickpea plants by **Ascochyta rabiei** (Pass.) Labr. and histological changes in the resistant and susceptible chickpea cultivars to Ascochyta blight were studied by light microscopy. Race 1 of **A. rabiei** was used in this study.*

*Spores of **A. rabiei** began to germinate 12 h after inoculation and developed a polar germ tube. Appressoria formation was observed 48 h after inoculation. Leaves of susceptible plants were penetrated by the fungus directly through the cuticle, the fungus then expanded subepidermally. Penetration usually occurred on the 3rd. and 4th. day after inoculation. Rate of penetration in the leaves of susceptible cultivar was higher than in the resistant cultivar. The epidermal cells began to collapse on the 4th. day after inoculation. Development of leaf spots and fungal pycnidia were observed 5-7 days post inoculation. In the very early stages of infection (24-48 h) leaves of resistant cultivar exhibited areas with strong autofluorescence. In the later stages of the infection (4-6 days after inoculation) fluorescent areas developed into small necrotic spots all over the leaflet. Leaves of the resistant cultivar were infected by the fungus to less than 10%.*

INTRODUCTION

Chickpea (**Cicer arietinum L.**) is an important grain legume crop in Türkiye. The most damaging disease of chickpea, an important legume crop of dryland agriculture is Ascochyta blight, caused by **Ascochyta rabiei** (Pass.) Labr. (teliomorph: **Mycosphaerella rabiei Kov.**). The disease has been reported from twenty-six countries (Nene, 1982), Ascochyta blight is most severe in Western Asia, North Africa, Southern and Eastern Europe and the Nothwestern region of India and Pakistan (Singh and Reddy, 1983). **Ascochyta rabiei** infects all aerial parts of the plant and can be distinguished by characteristic circular necrotic lesions on both leaflets and pods. The fungal pycnidia are normally observed as concentric bands within these lesions. Brown elongated lesions forming on the stem and petiole of chickpea can girdle the stem, leading to breakage and subsequent death of the dislocated branch. If the main stem is girdled at the collar region the whole plant dies (Nene, 1982).

The pterocarpan phytoalexins as medicarpin and maackiain have been identified as the main phytoalexins of this plant (Kunzru and Sinha, 1970) and these two phytoalexins have an important role in the antifungal defence mechanism of **Cicer arietinum** (Dolar and Gürcan, 1993).

Although there is adequate information on the life cycle, biology and control of pathogen (Kaiser, 1973; Nene, 1982), information on the mode of infection and invasion of host tissues with associated histological changes is not adequate. Pandey et al. (1985 and 1987) studied on the mode of infection of stems and leaves of susceptible chickpea plants but development of the disease in the leaves of resistant plants was not studied by them.

In the present work, the process of infection of Ascochyta blight has been studied in detail by histological means using a resistant and a susceptible variety of chickpea.

MATERIALS and METHODS

1. Cultivation of Fungi:

Utilized in this study, the race 1 of **Ascochyta rabiei** (isolate Af. 1) was determined from different isolates of **A. rabiei** collected from different location in Türkiye (Dolar and Gürcan, 1992). It was routinely grown on Chickpea Seed Meal Dextrose Agar (CSMDA) medium. Petri plates were incubated for 14 days at 20±2°C with an illumination of 12 hours per day by 2 white fluorescent tubes (F20T12/CW).

2. Plant Material:

The seeds of the resistant cultivar (65C830) and the susceptible cultivar (ILC 629) were surface sterilized with sodium hypochlorite (1%) for 5 min and washed 3 times with sterile distilled water. The seeds of two cultivars were sown in 15 cm pots containing sterilized Pro-Mix Bx. Plants were grown for fifteen days at 22±1°C under a photoperiod of 12 hours per day with white fuorescent light (14 850 lux). The plants were watered daily and fertilized twice a week with a dilute solution of 20-20-20 (N-P-K).

3. Inoculation of Plants:

All aerial parts of the plants were sprayed uniformly with spore suspension ($1.2x10^6$ spores/ml) of **A. rabiei.** Control plants were sprayed with sterile distilled water. After spraying, the plants were covered with transparent polyethylene bags for four days to maintain leaf wetness and incubated in a growth chamber with a 12 hours photoperiod (light intensity 14 850 lux) and day and night temperatures of approximately 20°C and 18°C respectively.

4. Microscopy:

Samples of leaflet were taken at 24 h intervals up to the nineth day. Harvested leaflets were fixed and stained by boiling for several minutes in lactophenol-cotton blue/ethanol (1:2, v/v). Tissue was cleared by storing in chloral hydrate solution (2.5 g

ml^{-1} water, volume required to cover tissue) for at least 12 h before mounting in 50% glycerol (Higgins, 1982). Microscopic examination was facilitated by the use of Nomarski interference contrast optics.

Rate of spore germination as well as formation of appressoria, rate of penetration, quantification of necrotic spots, together with quantification of leaf spots and fungal pycnidia formation were determined microscopially. Spore germination rate represents the ratio of ungerminated to germinated spores in a defined leaf area. Rate of appresoria formation is given as percentage of the final maximum amount of appressoria in a defined leaf area. The rate of necrotic spots, leaf spots and fungal pycnidia formation is given by the ratio of leaves with symptoms to all leaves inoculated. The rate of penetration was determinated as the ratio of penetrated leaves to all leaves inoculated.

RESULTS

Fungal development on the leaf surface (i.e. germination of spores, development of hyphae and appressoria formation) was found to be essentially identical for plants of the susceptible (ILC 629) and the resistant (65C830) cultivar (Fig. 1). Germination of conidia of **A. rabiei** on leaf surface was observed 12-24 h after inoculation. Germination included swelling of the spores and development of a polar germ tube. On both cultivars fungal germ tubes elongated and formed ramifications (2-3 days). These hyphal branches developed special modifications at their tip, which could be regarded as appressoria. The leaves of the susceptible cultivar showed that fungal penetration occurred directly through epidermis. Penetration through stomata was not observed in this study. The fungus then spread subepidermally inside of the leaf. Hyphae grew parallel between epidermal cells and palisade parenchyma cells. Percentage of penetration in the leaves of the susceptible cultivar was found significantly different from resistant cultivar.

Initial events comprising spore germination, germ tube elongation and penetration required about 3 or 4 days. Up to this stage no morphological symptoms were distinguishable, as all the inoculated leaves appeared normal. Between 4th and 5th day after inoculation, the hyphae gradually started colonizing in subepidermal ground tissue (mesophyll) of susceptible host. The epidermal cells collapsed, cells of palisade and spongy parenchyma lost their shape and their organization (Fig. 2). The first morphological symptom was recorded as yellow specks on the leaves after 4 or 5 days which coincided with the formation of subepidermal mycelial aggregates. Between 5th and 6th day, these mycelial aggregates enlarged in size and started differentiating pycnidia (Fig. 2). Mature pycnidia were observed on the leaf surface after 6th and 7th day as black dots. Enlargement of mycelial aggregates and pycnidial differentiation coincided with increase in size of lesions. These lesions covered large areas of the leaf, sometimes the whole leaflet. In about 73% of all inoculated leaves of susceptible cultivar included leaf spot and pycnidia 7-8 days after inoculation (Fig. 1).

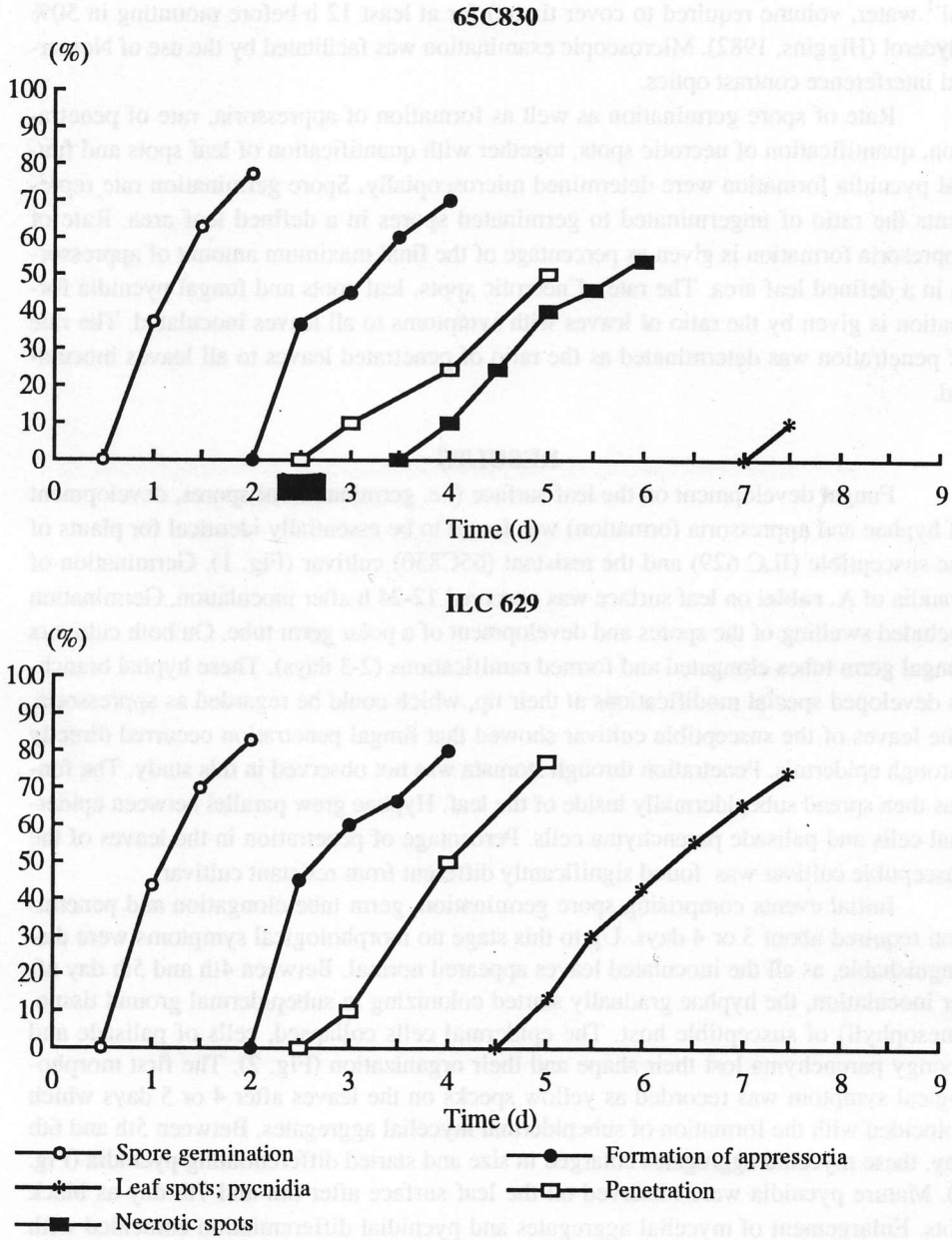

Fig. 1. Percentage of spore germination, appressoria formation and penetration of **Ascochyta rabiei** and type of disease symptoms on the leaves of resistant (65C830) and susceptible (ILC 629) chickpea cultivar to Ascochyta blight.

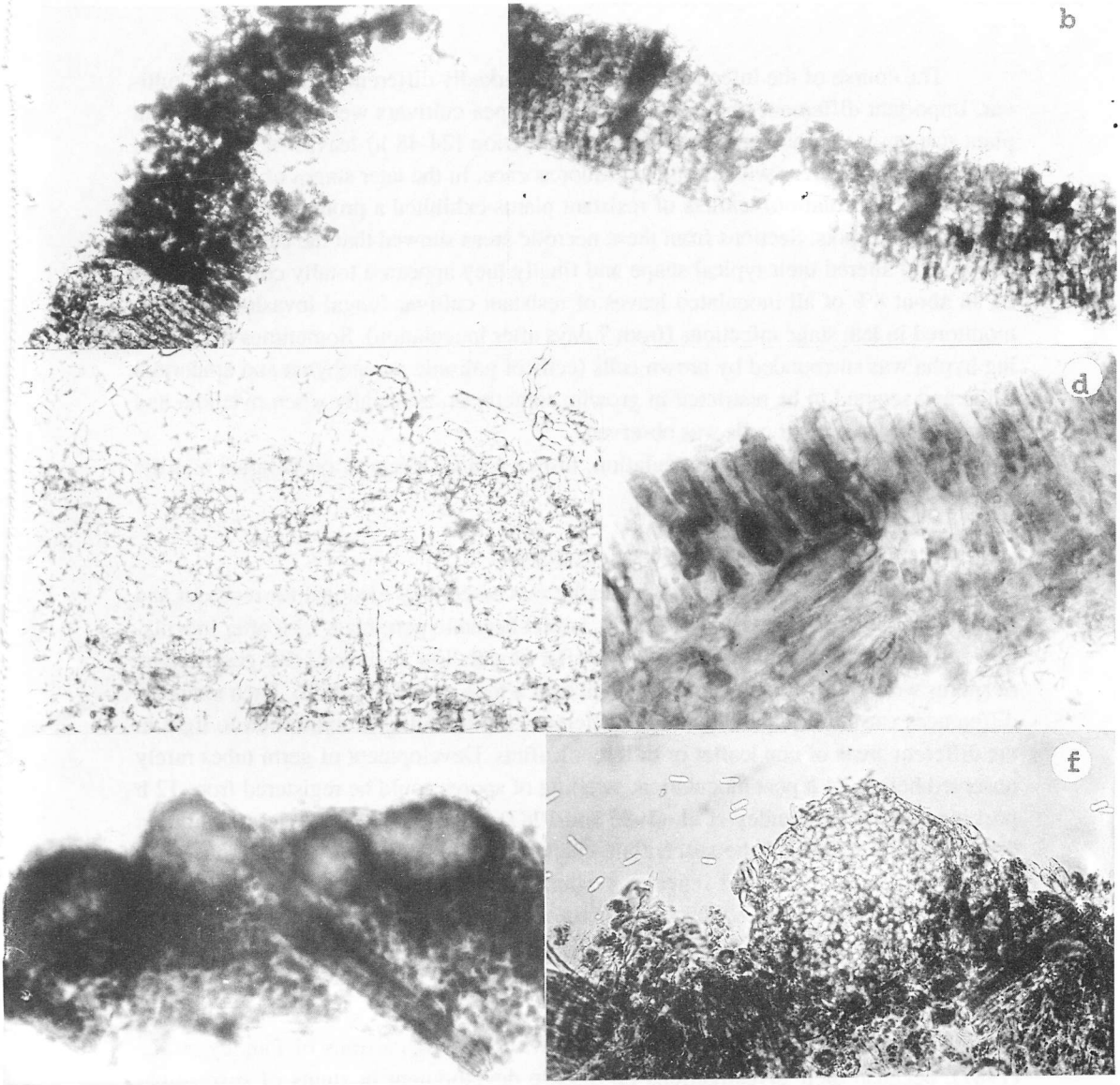

Fig. 2. (a) Cross section of infected leaflet of the susceptible cultivar (4 days post inoculation) showing brown discolouration and disorganization of the epidermal cells (400 x).
(b) Cross section of a necrotic spot in a leaflet of the resistant cultivar (4 dpi). The cells had turned brown and appeared compressed (400 x).
(c) A section of the susceptible cultivar (5 dpi) showing intercellular growth of the fungal hyphae and formation of sub-epidermal mycelial aggregate. Fungus causes extensive damage to parenchymatous tissues (625 x).
(d) The hypersensitive reaction in the leaflet of the resistant cultivar (5 dpi). Cell death is limited and the dead cells is surrounded by the living cells (625 x).
(e) A section through a leaflet of the susceptible cultivar (6 dpi). Fungal hyphae have aggregated to form pycnidia (400 x).
(f) Cross section of a leaflet of the susceptible cultivar (8 dpi) showing the mature pycnidium and pycnidiospores (400 x).

The course of the infection process was markedly different in the resistant cultivar. Important differences between the two chickpea cultivars were seen in the initial plant responses. In the very early stages of infection (24-48 h) leaves of the resistant cultivar exhibited areas with strong autofluorescence. In the later stages of infection (4-6 days after inoculation) leaflets of resistant plants exhibited a pronounced number of small necrotic spots. Sections from these necrotic areas showed that the cells had turned brown, had altered their typical shape and finally they appeared totally collapsed (Fig. 2). In about 8% of all inoculated leaves of resistant cultivar fungal invasion could be monitored in late stage infections (from 7 days after inoculation). Sometimes the invading hypha was surrounded by brown cells (cells of palisade parenchyma and epidermal cells) and seemed to be restricted in growth, sometimes, especially when pycnidia had formed, no browning of cells was observed.

Four or six days after inoculation, brown lesions occured on petioles and the branches drooped down.

DISCUSSION

Fungal development outside the leaflet was essentially identical for resistant and susceptible cultivars (Fig. 1). Spores of **A. rabiei** began to germinate 12 h after inoculation and germination contiuned up to 48 h after inoculation. It showed that germination of spores was not coordinated and extended over a long period (12-48 h). Such temporal differences can be explained by slight differences in humidity or exhibition to light in the different areas of one leaflet or different leaflets. Development of germ tubes rarely observed before 24 h post inoculation, swelling of spores could be registered from 12 h post inoculation on. Pandey **et al.** (1985 and 1987) determinated spore germination on the stems and leaflets of the susceptible chickpea plants 12 h after inoculation. They also observed germ tube and fungal mycelium on leaf surface 36 h after inoculation. Pandey **et al.** (1985) reported that spore germination, germ tube elongation and penetration required about 3 days. Results in this paper are in agreement with the observation of Pandey **et al.** (1985).

The observed formation of appressoria has to be regarded as the first step in penetrating the host tissue. This result disagree with the observations of Pandey et al. (1987), because their investigations on disease development in stems of susceptible chickpea plants suggested that the fungus penetrated the host tissue directly, without the formation of an appressorium. The development of appressoria, however, has also been observed in the interaction of **Ascochyta pisi** and **Pisum sativum** (Heath and Wood 1969).

Fungal penetration occured through the wall of epidermal cells presumably without invading the cells. These finding are in agreement with the observation of Pandey **et al.** (1987), who reported that germ tubes of **A. rabiei** penetrate the stem tissues at the

juncture of two epidermal cells. It was not demonstrated, so far, that cell wall degrading enzymes were involved in this penetration process. Heath and Wood (1969) suggested that in the lesions caused by **Ascochyta pisi** and **Mycosphaerella pinodes** cellwall degrading enzymes were active for some time during infection and colonization.

The resistant cultivar 65C830 showed during the early stage of infection autofluorescent spots all over the leaflet. 4-6 days after inoculation leaflets of resistant cultivar covered with local necrotic spots corresponding to the earlier fluorescent areas. Spores or their germination fluid can elicit one or several defense mechanisms, which render the cells to turn brown and necrotic. This is characteristic for a hypersensitive reaction that significantly contributed to resistance in many plant-pathogen interactions. The autofluorescent spots over infected area on leaflet could be correlated with the accumulation of phytoalexin (medicarpin and maackiain) in these area. But it is not yet clear whether the phytoalexins accumulate in the autofluorescent spots. However several workers reported that hypersensitive reaction connected with the production of a phytoalexin and accumulated phytoalexins at the interface between the living and dead cells (Hargreaves and Bailey, 1978; Bailey, 1982, 1983, 1987). The amounts of phytoalexin accumulation in the infected tissue allow to differentiate between resistant and susceptible cultivars, because medicarpin and maackiain are much higher in resistant cultivar (Dolar and Gürcan, 1993).

The results of this study showed that some factors as early metabolic activity (autofluorescence) and active cell death (necrotic spots) contribute to the resistance of cultivar 65C830 to **A. rabiei.**

ÖZET

DAYANIKLI VE DUYARLI NOHUT ÇEŞİTLERİNİN YAPRAKLARINDA Ascochyta rabiei (Pass.) Labr.'IN GELİŞMESİ

Nohut antraknozu [**Ascochyta rabiei** (Pass.) Labr.]'na dayanıklı (65C830) ve duyarlı (ILC 629) nohut çeşitlerinin enfeksiyonu ve hastalık nedeniyle bitkilerde meydana gelen histolojik değişimler ışık mikroskobuyla incelenmiştir. Bu çalışmada **A. rabiei**'nin 1 numaralı ırkı kullanılmıştır.

Ascochyta rabiei'nin sporları inokulasyondan 12 saat sonra çimlenmeye başlamışlar ve her biri 1 polar çim tübü oluşturmuştur. Apressorium oluşumu ise inokulasyondan 48 saat sonra gözlenmiştir. Duyarlı çeşidin yapraklarında fungus direk olarak kütikuladan giriş yapmış ve daha sonra epidermis altında ilerliyerek gelişmesine devam etmiştir. Penetrasyon çoğunlukla inokulasyondan sonraki 3. ve 4. günlerde olmaktadır. duyarlı çeşidin yapraklarında gözlenen penetrasyon oranı dayanıklıdakinde gözlenenden çok daha yüksek bulunmuştur. İnokulasyondan sonraki 4. günde epidermiş hücreleri çökmeye başlamıştır. Yaprak lekeleri ve piknit gelişmesi inokulasyondan 5-7 gün sonra görülebilmiştir. Enfeksiyonun erken döneminde (24-48 saat) dayanıklı çeşidin yaprak-

larında kuvvetli fluoresan parlaklıkta alanlar gözlenmiş ve enfeksiyonun geç devresinde (inokulasyondan 4-6 gün sonra) bu fluoresan alanların olduğu yerlerde küçük nekrotik lekeler gelişmiştir. Dayanıklı çeşidin yapraklarının %10'un dan azı fungus tarafından enfekte edilmiştir.

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Detection of Tomato Spotted Wilt Virus in Tobacco and Tomato Cultuvars by Enzyme Linked Immunosorbent Assay

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ABSTRACT

In this study, recent epidemic situation of tomato Spotted Wilt Virus (TSWV) has been determined in the several tobacco and tomato cultuvars on the bases of indexing and ELISA tests. Mechanical sap inoculated ***Petunia hybrida*** *Minstrel test plants displayed local necrotic lesions (LNL) 4 days after the inoculation.* ***Nicotiana glutinosa*** *plants developed typical large LNL forming concentric necrotic zones systemic patterns. The result of ELISA test revealed that, TSWV has made epidemy in some tobacco and tomato growing areas. According to our tests and observation results, TSWV outbreaks in Izmir and Manisa were associated with seed transmission of the virus. The virus-free tomato seeds should be used for complete eradication of the virus and controlled thrips population level in the early thrips detection in the seedbed and in the fields.*

INTRODUCTION

Tomato spotted wilt virus (TSWV) (Lycopersicum virus 3) is known World wide distributed in temperate and suptropical regions (Mickovski, 1981; Samuel et al. 1930; Smith, 1932). It has very wide host range such as in 166 plant species in 34 families (Anonymous, 1970, Smith, 1951, 1957), causing serious economic losses in important vegetable, legume, solanaceous ornamental crops in the fields and the green houses (Pritchard, 1949; Matteony **et al.**, 1988). Previous study (Azeri, 1981) demonstrated that, TSWV caused economic damage and losses in tobacco fields in Çanakkale between 1979-1981 due to the favourable climatic conditions and high population abundance of the most efficient vector of TSWV, **Thrips tabaci** (Lindeman). TSWV incidens in tobacco fields in Çanakkale (Kalkım, Akçakoyun, Karabey) were between 80-100%. Tomato crops also were found infected with TSWV in the vicinity of the infected tobacco fields.

Since 1981, it has been experimentally proved by indexing tests that, the mentioned virus was present and caused epidemy in tobacco growing regions, Çanakkale (Yenice), Balıkesir (Bigadiç), Manisa (Gördes, Demirci, Kula), Uşak and Samsun (Bafra). In recent years, TSWV has been caused serious diseases in some tomato crop fields in İzmir and Manisa. The most efficient TSWV vector, Onion thrips, **Thrips tabaci** (Lindeman) is widespreded in Türkiye and responsible for spreading of TSWV in the fields and greenhouse crops (Lodos, 1981; Azeri, 1981). TSWV has also been reported seed transmitted in tomato by the seed testa (Anonymous, 1970).

In this present study, ELISA has been used to detect TSWV in some commercially tobacco and tomato growing regions.

MATERIALS and METODS

Indexing tests with herbaceous. Indexing of TSWV with herbaceous plants have been continously carried out in June and July 1993. Leaf samples collected from the infected tobacco and tomato crops from the various growing areas including İzmir, Manisa, Çanakkale, Balıkesir, Uşak and Samsun have been indexed with herbaceous **Petunia hybrida** Minstrel and **Nicotiana glutinosa** L.. Leaf tissues were triturated in 0,02 M phosphate buffer pH= 7,4 (for stabilizing of the virus). The prepared inoculum were rubbed onto carborandum dusted (500-mesh) leaves of the above mentioned herbacecus plants. The symptom observations started after 2 days from the sap inoculations on the test plants. Inoculated test plans kept in a climate controled chamber at 25°C (Klinkowski and Uschdra, 1952; Anonymous, 1970; Azeri, 1981; Chrisochoou, 1981; Gibbs et al., 1970).

Seed transmission of TSWV from tomato seeds: Seed samples collected from the diseased tomato plants in İzmir (Menemen) and Manisa (Turgutlu) severly affected by TSWV (Showing typical leaf and fruit symptoms of TSWV) have been tested by indexing tests. The seeds have been obtained from totally 10 tomato fields and collected from 20 diseased plant fruits. Totally 10 seed test groups belong to the 10 fields contained 50 seeds in each groups were used in the sap inoculation test.The seed portions of each samples were triturated seperately with pestle in 0,02 M PO_4 buffer, containing the same buffers as used in the sap inoculation tests. The prepared inoculum were rubbed on carborandum dusted leaves of **N.glutinosa** and **P.hybrida** host plants.

Field observations have been made in June and July 1993 in the tobacco and tomato growing areas in İzmir and Manisa. Tobacco and tomato plants were investigated for characteristic symptoms of TSWV. Leaf samples from the virus suspected or diseased plants were collected for ELISA test. Leaft samples from the disease affected tobacco plants have also been braught and collected from Çanakkale, Balıkesir and Samsun (Bafra) for the tests.

Enzyme-linked immunosorbent assay: The double antibody sandwich ELISA method was used to detect TSWV in tobacco and tomato crops. The Anti-TSWV-IgG (BR-01 Lettuce Type, polyclonal antiserum) and Anti-TSWV-AP-Conjugate obtained from Loewa Biochemica GmbH. The young shoot leaf samples were collected in summer (In June and July 1993). The leaf samples taken from 5-10 plants were collected in the same plastic bag and accepted one sample. Totally 87 samples from the 11 different regions have been tested as seen in table 1. The leaf samples were homogenized in PBS-0,5 Tween 20-PVP-extraction buffer at a dilution of 1/10 (W/V) pH= 7.4. The buffer was freshly prepared and egg-albumin was added in it just before the use (Bioreba AG-Basel/Schweiz).

Anti-TSWV-IgG and Anti-TSWV-AP-conjugate were used in 1:500 dilution. ELISA test procedure was carried out with polystyrene microtitre plates in accordance with ELISA procedure outlined by Clark and Adams (1977), and recommendation by Loewa Biochemica GmbH., München. ELISA reactions were measured spectrophotometrically at 405 nm using an Multiskan Plus MK 11 ELISA reader, and positive readings were confirmed by visual observations. A samples was considered TSWV-positive if the $A_{405\ nm}$ values were greater than "$\bar{x}$ + 3 S" value of extraction buffer control values.

RESULTS AND DISCUSSION

Symptomatology of TSWV. Symptoms on the tobacco leaves obtained from Çanakkale (Yenice and Kalkım), Balıkesir (Bigadiç) and Samsun (Bafra) were showing severe systemically necrotic rings with a central spots, large plaque like lessions composed of concentric zones or necrotic tissue, necrotic lines mainly along the side of the vein (Fig. 1. A-B). These symptoms on the tobacco plants were similar of the severe TSWV isolates as previously reported (Azeri, 1981).

During the field survay and observations in June and July 1993 in the tomato fields in İzmir and Manisa, the most noticed characteristic symptoms of TSWV were found the leaflets were covered with concentric rings, and small dark spots on the yough levels, mosaic and distortion of the young leaves, slightly downward and inward rolling and bronzing of the leaves, tip degeneration and tip and stem necrosis, stunting (as showen in Fig. 2.) chlorotic and necrotic ring spots, numerous concentric rings, yellow and white spots in the red colored ripe fruit, reduction in fruit set were showing on the infected tomato fruits.

Indexing Results. In the sap inoculated **Nicotiana glutinosa** plants developed typical large local necrotic lesions 4 days after the sap inoculations. These lesions increased in size and formed concentric necrotic zones about 3 to 4 mm in diameter (Fig. 3). After these typical symptoms, **N.glutinosa** plants showed lethal systemic necrosis and died in 7 or 10 days as previously reported by Smith (1951), Gumpf and Weathers (1972), Kohler and Klinkowski and Uschdra (1954), Gibbs et al. (1970) and Azeri

(1981). After 4 days from the sap inoculations, **Petunia hybrida** test plants produced typical local necrotic lessions of TSWV as showen in fig 4. The severe local necrotic circular rings spots, about 3-4 mm in diameters developed on the inoculated leaves as previously reported by Smith (1951; 1957), Ivancheva (1959), Gibbs et al., (1970), Chrisochoou (1981), Mickovski (1981), Azeri (1981). TSWV isolates from the severly infected tomato plants obtained from Manisa-Turgutlu showed very severe symptoms on petunia. According to the indexing tests, TSWV isolates from the diseased tomato plants were found very severe and have high infectivity level on the herbaceous hosts.

Seed transmission test. ın the sap inoculated **N.glutinosa** and **P.hybrida** test plants developed typical and very severe leaf symptoms of TSWV as described in fig 3 and 4. The result of the seed transmission tests has been demonstrated that, seed transmissibility for this prevalent virus (TSWV) was found as previously reported (Anonymous, 1970).

ELISA. The obtained results from the ELISA test revealed that, the most of the tobacco and tomato samples collected from the experiment locations given in Table 1 and Fig. 5 (histograms) found infected with TSWV. The extinction values of the tobacco samples from Samsun (Bafra), Çanakkale and Balıkesir were very high (between 2.128 - 3.539 nm). Because of the cool climatic conditions and high thrips populations in Çanakkale (Azeri, 1981) and Samsun, the infectivity level were higher then the other hat climatic places (as showen in table 1). It has been reported by the most investigators that, epidemiology of TSWV is directly related with the habits and the ecology of its vector thrips (Azeri, 1981; Chrisochocu 1981; Taraku, 1989; Cho et al., 1988). In addition, TSWV is known to be seed transmissible by seed testa of tomato (Anonymous, 1970). According to our tests and fields observations, seed transmission of the virus was responsible from widespread damage of TSWV in our tomato growing areas. The disease became epidemic and caused damages generally in June and July throughout the experiment places given in this study. Manisa region is one of the most important commercially tomato growing center of Türkiye. In this respect, growing healty tomato crops is main aim of the most growers. Using virus free seed is the first step protecting and making safe of tomato growing. For this purpose, treating seeds with Natrium-3-phosphate, dry-air treatment, cloramin T, HCL acid and Na-hipoclorid was found decrease virus infections in important degree (as previously reported by Erkan and Delen, 1985).

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Table 1. ELISA detection of TSWV in the leaf samples extracts collected from infected tobacco and tomato plants in the several locations (In June and July 1993) of the Aegean Region and Samsun (Bafra).

Local. (a) No:	Field And Sample No:	Plant Name (b)	Ab. Va[c]	Local. No:	Field And Sample No:	Plant Name (b)	Ab. Va	Local. No:	Field And Sample No:	Plant Name (b)	Ab. Va	Local. No:	Field And Sample No:	Plant Name (b)	Ab. Va
2/1		Tob.	2.640 (+)	10/6		Tob.	0.024 (-)	3/9		Tom.	1.029	7/8		Tom.	0.550
2/1		Tob.	2.128	10/6		Tob.	0.090 (-)	3/9		Tom.	1.125	7/9		Tom.	0.090
2/2		Tob.	2.825	10/7		Tob.	0.100 (-)	3/10		Tom.	1.154	8/1		Tom.	0.622
2/2		Tob.	2.864	10/7		Tob.	0.120 (-)	3/19		Tom.	1.136	8/2		Tom.	0.062 (-)
2/3		Tob.	2.904	11/1		Tob.	0.052 (-)	3/19		Tom.	0.952	8/2		Tom.	0.075
2/3		Tob.	2.696	11/1		Tob.	0.117 (-)	3/20		Tom.	0.724	9/1		Tom.	0.040
2/4		Tob. (+ Cont.)	3.230	11/2		Tob.	2.693	3/20		Tom.	0.959	9/1		Tom.	0.051
2/4		Tob. (+ Cont.)	3.539	11/2		Tob.	2.310	3/21		Tom.	0.930	9/2		Tom.	0.022
2/5		Tob.	2.845	11/3		Tob.	0.020 (-)	3/21		Tom.	0.960	9/3		Tom.	0.033
2/5		Tob.	2.345	11/3		Tob.	0.012 (-)	4/1		Tom.	0.422	9/4		Tom.	0.092
2/6		Tob.	0.790	11/4		Tob.	0.553	4/1		Tom.	0.490	9/4		Tom.	0.080
3/11		Tob.	1.103	11/4		Tob.	0.457	4/2		Tom.	0.925	3/18		Pep.	0.970
3/11		Tob.	1.542	11/5		Tob.	0.553	4/3		Tom.	0.876	3/18		Pep.	1.188
3/12		Tob.	1.133	11/5		Tob.	0.510	4/3		Tom.	0.966	7/2		Pep.	0.016
3/13		Tob.	0.731	11/6		Tob.	0.375	4/4		Tom.	0.927	7/2		Pep.	0.011
3/13		Tob.	0.967	11/7		Tob.	0.044	4/4		Tom.	1.121	11/13		Tom + Pep.	0.013
3/14		Tob.	0.918	11/7		Tob.	0.012	4/5		Tom.	1.124	11/13		Tom + Pep.	0.022
3/14		Tob.	1.342	11/8		Tob.	0.018	4/5		Tom.	0.950				
3/15		Tob.	1.027	11/8		Tob.	0.050	5/1		Tom.	0.840				X values
3/15		Tob.	0.744	11/9		Tob.	0.027	5/2		Tom.	0.904				0.005
3/16		Tob.	1.225	11/10		Tob.	0.458	5/2		Tom.	1.069				0.000
3/16		Tob.	1.023	11/11		Tob.	0.034	3/27		Tom.	1.093				0.000
3/17		Tob.	0.711	11/11		Tob.	0.054	3/27		Tom.	0.995				0.057
3/17		Tob.	0.903	11/12		Tob.	0.056	6/2		Tom.	0.710				0.016
3/22		Tob.	0.918	11/12		Tob.	0.067	6/4		Tom.	0.481				0.026
3/23		Tob.	1.342	11/14		Tob.	2.332	6/4		Tom.	0.615				0.000
3/24		Tob.	1.233	11/14		Tob.	2.694	6/5		Tom.	0.845				0.060
3/25		Tob.	1.237	11/15		Tob.	2.307	6/5		Tom.	0.756				0.004
6/1		Tob.	0.075 (-)	11/15		Tob.	2.390	6/6		Tom.	0.418				0.005
6/1		Tob.	0.090 (-)	3/1		Tom.	1.007	6/7		Tom.	0.074 (-)				0.0024
6/3		Tob.	0.854 (+)	3/1		Tom.	1.143	6/7		Tom.	0.070 (-)				0.087
10/1		Tob.	0.083 (+)	3/2		Tom.	1.021	7/1		Tom.	0.040 (-)				0.096
10/1		Tob.	0.100 (-)	3/3		Tom.	1.201	7/1		Tom.	0.070 (-)				0.085
10/2		Tob.	0.082 (-)	3/4		Tom.	1.003	7/3		Tom.	0.421				0.050
10/2		Tob.	0.079 (-)	3/5		Tom.	0.949	7/4		Tom.	0.824				
10/3		Tob.	0.100 (-)	3/6		Tom.	1.101	7/5		Tom.	0.486				
10/3		Tob.	0.117 (-)	3/6		Tom.	1.006	7/5		Tom.	0.551				
10/4		Tob.	0.045 (-)	3/7.		Tom.	1.139	7/6		Tom.	0.442				
10/4		Tob.	0.110 (-)	3/7		Tom.	1.402	7/6		Tom.	0.402				
10/5		Tob.	0.472	3/8		Tom.	0.904	7/7		Tom.	0.253				
10/5		Tob.	0.340	3/8		Tom.	0.745	7/8		Tom.	0.510				

(a) Localities No, 2: Samsun (Bafra);3: Manisa (Turgutlu); 4: Manisa; 5: İzmir (Menemen);
6: Manisa (Muradiye); 7: Salihli; 8: Manisa (Turgutlu);
9: Manisa (Saruhanlı);
10: Çanakkale;11:Balıkesir (Bigadiç).

(b) Tob.: Tobacco
Tom.: Tomato

(c) Absorbance Value.

x values: Extraction buf. Control values for 2 plates.

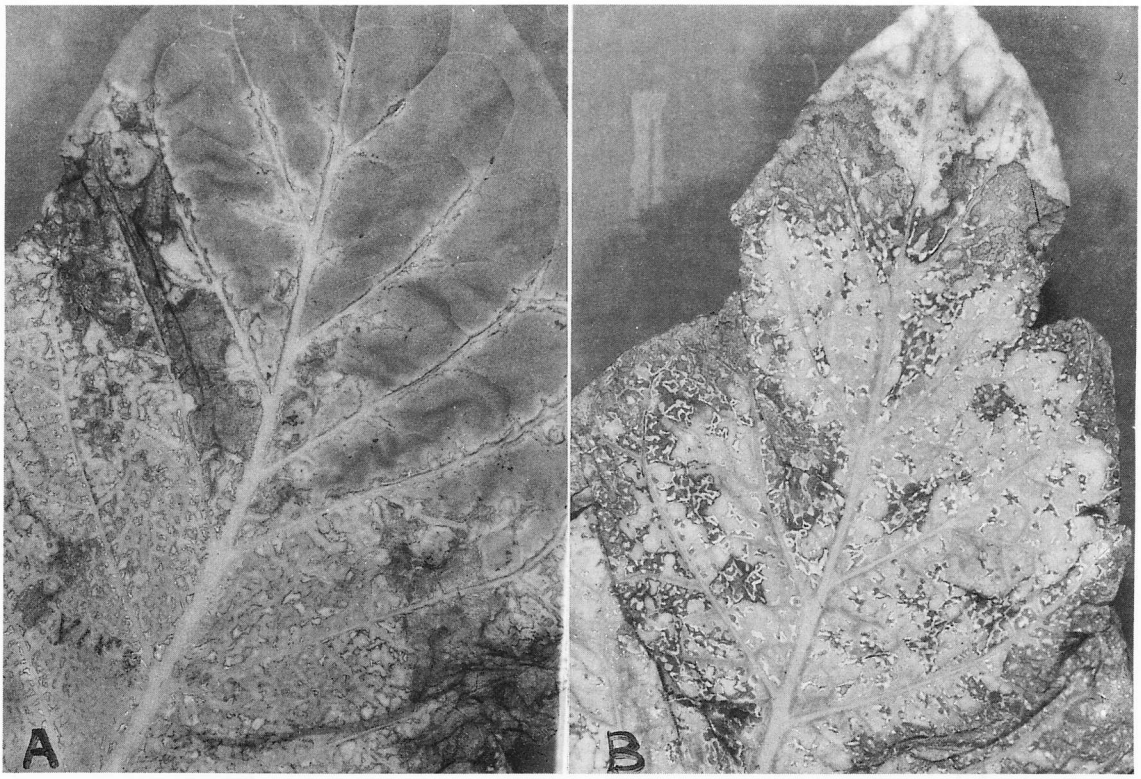

Figure 1. Leaves of tobacco plants (A-Virginia K 326, B-maden K-188/35) infected with TSWV, showing typical systemic leaf symptoms.

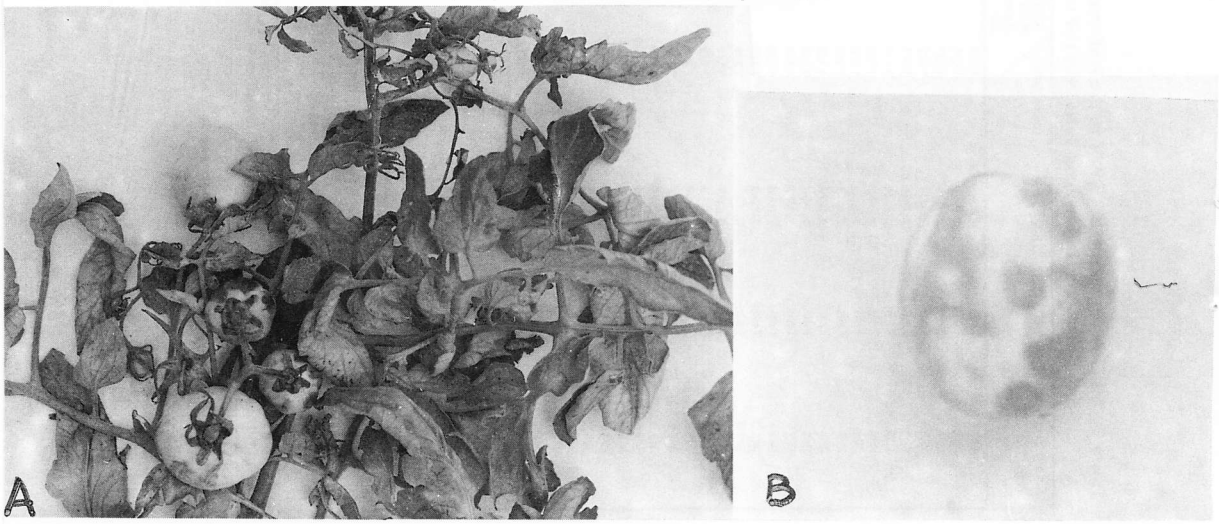

Figure 2. TSWV symptoms on infected tomato plants (A) and tomato fruit (B).

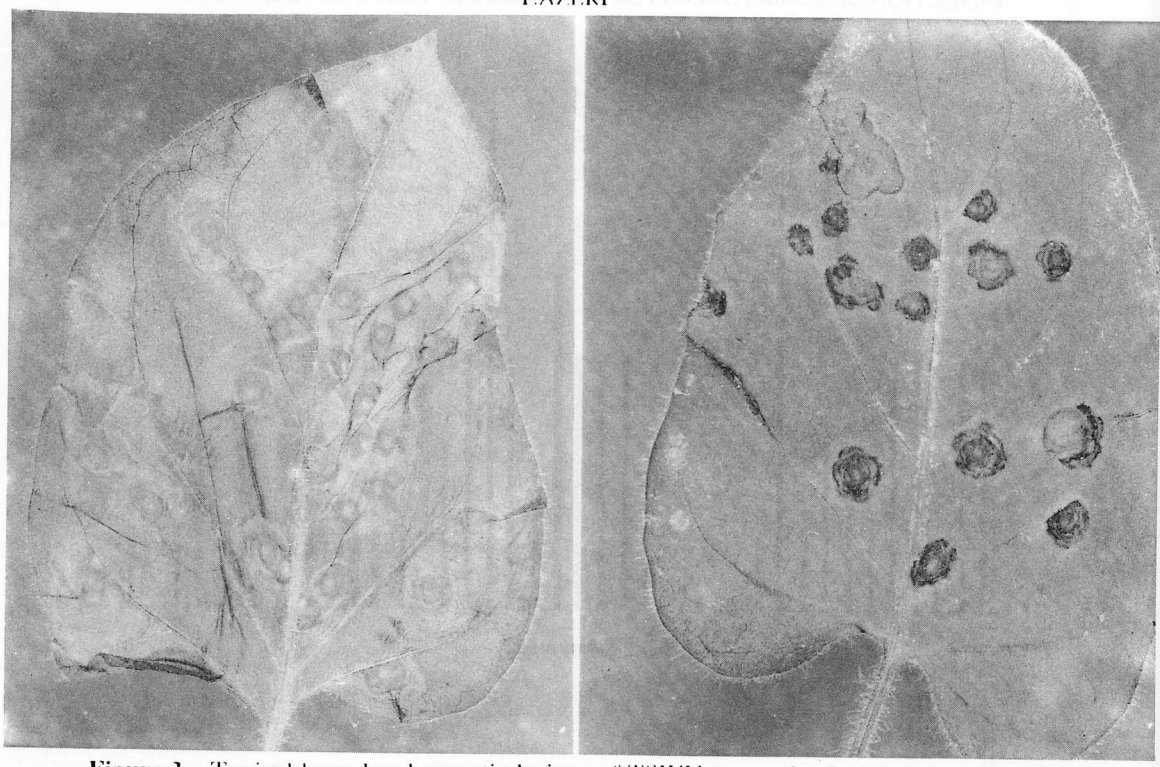

Figure 3. Typical large local necrotic lesions of TSWV on mechanical sap inoculated **Nicotiana glutinosa** leaves (Forming concentric necrotic zones can be seen on the leaves).

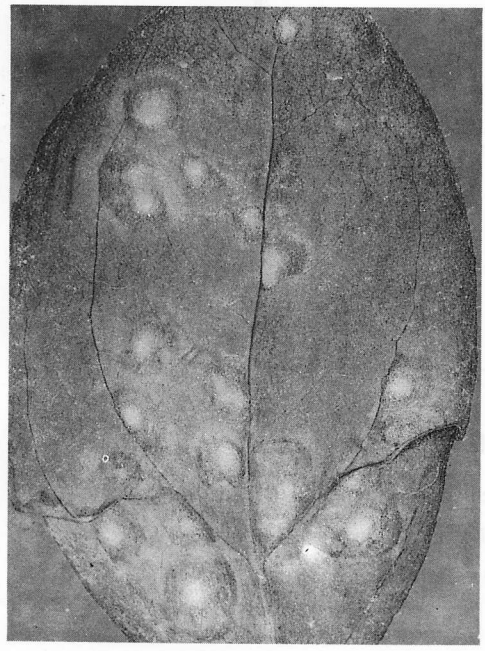

Figure 4. Typical local necrotic circular rings spots of TSWV on **Petunia hybrida** indicator plant 4 days after sap inoculations.

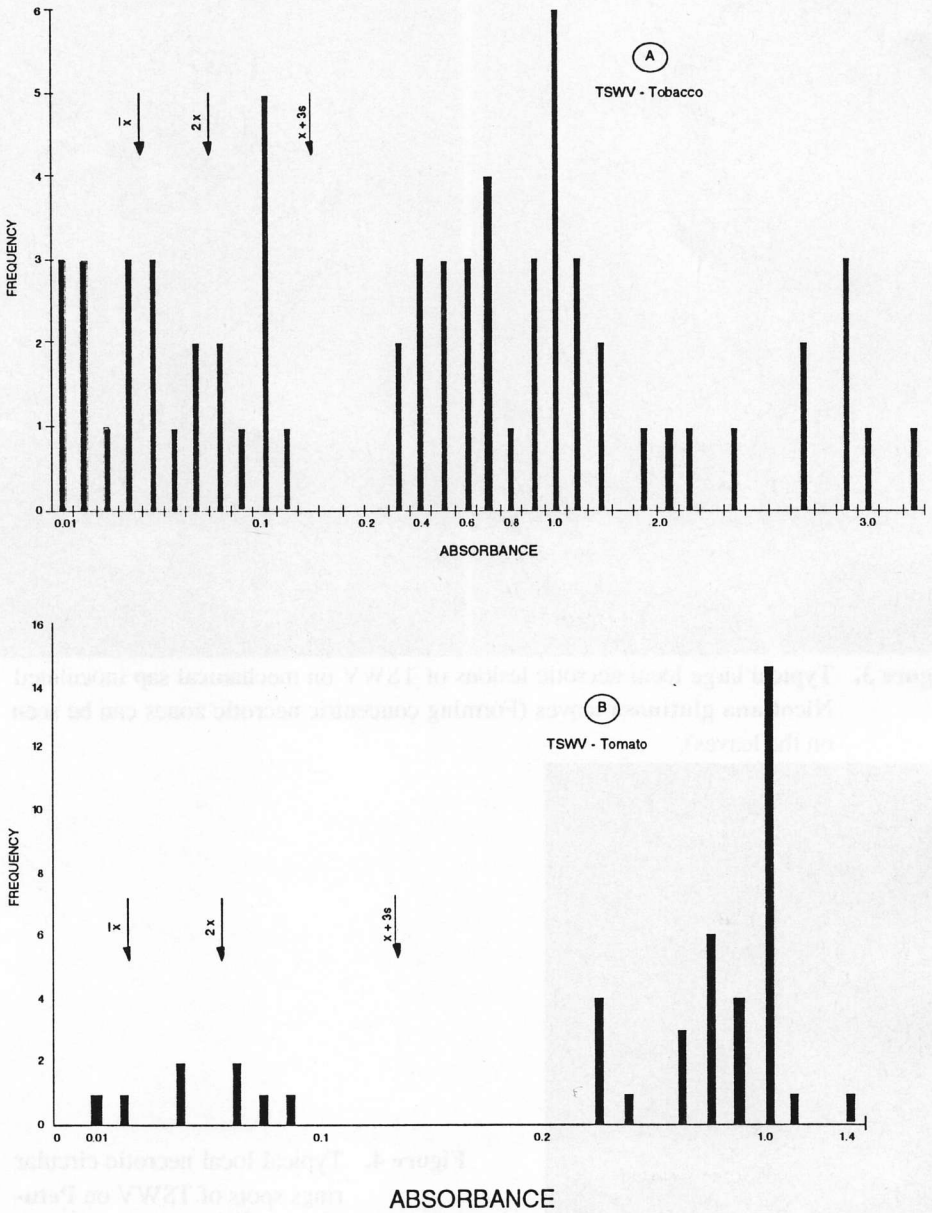

Fig. 5. Histogram of ELISA results of TSWV in tobacco (A) and in tomato (B); Arrows indicate: $\bar{x}$ = 0.034 (Average value of 15 Extraction buff. Control samples); " $\bar{x}$ + 3S" = 0.139; S (Standart deviation of 15 Ext. buff. Cont. Samp.) = 0.035.

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Toxicity of *Bacillus thuringiensis* on *Plutella xylostella* and *Drosophila melanogaster*

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ABSTRACT

*The effect of **Bacillus thuringiensis** on **Plutella xylostella** and **Drosophila melanogaster** was investigated. The whole bacterial solution, the upper phase of the bacterial solution after centrifugation was taken and the hydrophobic and hydrophilic exotoxin and endotoxin of the bacteria were used for this purpose. At the all treatments the bacterial subtances were not found effective on the third instar larvae of cypermethrin resistance strain of **P. xylostella**. But the whole bacterial substance and exotoxin of bacteria treatments were recognized effective on the second instar larvae with 32 % and 40 % mortality respectively. But hydrophobic and hydrdphilic exotoxin and endotoxin of bacteria did not affect to the cypermethrin resistance of **P. xylostella**. The whole bacterial solution was effective on the **D. melanogaster**. The percent of mortality for whole bacterial solution treatment was determined as 26.85 %.*

INTRODUCTION

Bacillus thuringiensis is a complex divisible into >20 varieties or H- serotypes by serological and biochemical tests. These produce several insecticidal toxins, two of which are used in agriculture the δ - endotoxin and β - exotoxin.

Krieg (1971) reported an exotoxin which is toxin to diamond back moth, **P.xylostella.** β - exotoxin: Beers of **B. thuringiensis** were recognized to contain a water- soluble, heat stable toxin highly toxic to larvae of several species of flies. δ - endotoxin: This δ - endotoxin in the crystal of **B. thuringiensis** has an activity spectrum limited, to the Lepidoptera, mosquitoes, chironomids and blackflies (Dulmage 1981).

Bacillus thuringiensis and its endotoxins are used for control of **D. melanogaster.** The thermostable exotoxin in **B. thuringiensis** was discovered by Mc Connel and Richards (1959) and increased the insecticidal use of **B. thuringiensis.**

The exotoxin preparations replaced the chemical insecticides in trials for fly control in the early ten years but some researchers claimed that the exotoxin was lethal to mice so its commercial use was delayed. However, Department of Microbiology University of Helsinki, showed that allergic, toxic or cytogenetic damage caused by exotoxin in used to control insect pests are negligible (Carlberg 1973).

Carlberg and Lindström (1981) recorded that early application of exotoxin is effective against the comparatively small housefly population of that time.

The purpose of this research is, to investigate of the toxicity of **Bacillus thuringiensis** on **Plutella xylostella** and **Drosophila melanogaster.**

MATERIALS AND METODS

Investigation toxicity of **Bacillus thuringiensis** on cypermethrin resistance strain of **Plutella xylostella:**

P.xylostella was obtained from Dr. White. This cypermethrin resistance strain was collected from Indonesia in 1982. **Bacillus thuringiensis** was supplied from The Department of Agricultural and Environmental Science, University of Newcastle upon Tyne.

Chinese cabbage leaves (~ 200 mg weight and 3x3 cm) and 2nd 3rd instar larvae were used for this purpose. Then, the series of bioassay experiment were applied as follows: (Dr. R.M. Wilkins, 1993, personal communication)

1. The whole bacterial solution ($2x10^{10}$ cell/ml) thawed at room temperature (the bacterial suspension was taken from freezer and used directly).
2. The whole bacterial solution was centrifugated and the supernatant (the upper phase) was filtered with filter 0.2 μm pore size,
3. The hydrophobic and hydrophilic exotoxin of bacteria were seperated by etil asetate processing,
4. The whole bacteria was centrifugated and the bacterial pellet was broken down by probe sonicator and the endotoxin of bacteria was released.

Investigation toxicity of *Bacillus thuringiensis on Plutella xylostella*:

Both sides of each leaf were painted with. 0.1 ml of the described above solution by using a fine brush. The leaves were allowed to dry under the laboratory conditions. After drying the leaves were put into a damp filter paper in a petri dish. Then 3rd instar larvae that had been starved for 2h were released in each petri dish. The same experiment were set up with 2nd instar larvae. All the petri dishes were painted for 48h at 24±1°C in a 14h light-10h dark photoperiod. This experiment were carried on with 6 replicates, for each treatment.

The leaves were removed and the amounts of dead larvae were counted after 48h.

Investigation toxicity of *Bacillus thuringiensis on Drosophila melanogaster*:

1. The Drosophila food was prepared by 50 g yeast, 50 g glucose, 15 g agar and 600 ml distilled water. And the adults were rared in this medium.
2. The **Bacillus thuringiensis** was obtained from The Department of Agriculture and Environmental Science, University of Newcatle upon Tyne.
3. The subtance of bacteria were added into the **Drosophila** food as following concentration and were proportioned to the each tubes.

The experiment was designed as a 6 replicates for each treatment.

Treatments and concentrations:

a.	whole bacterial solutions	2.5%v/v
b.	freeze dried exotoksin of bacteria	0.1% w/v
c.	the solution including endotoksin of bacteria	50.0% v/v
d.	the substance including hydrophilic exotoxin of bacteria	0.3% w/v
e.	the subtance including hydrophobic exotoxin of bacteria	0.3% w/v
f.	control (with Drosophila food)	
g.	control (with minimum salt medium)	50.0% v/v

Minimum salt medium:

Amonium sulphate	0.5 g
Dipotassium hydrogen sulphate	2.0 g
Dihydrogen potassium sulphate	0.2 g
Magnesium sulphate	0.05 g
Trace elements	1.0 ml
H2O	999.0 ml
Glucose	2% w/v
	1000.0 ml

Drosophila food were prepared with subtances described above, proportioned the each tubes, then **D. melanogaster** adults were put into the tubes. After 24h, they were taken and when the larvae became pupae, number of dead larvae and pupae were counted. Then percent of mortality was determined. All the tubes were incubated at 24±1 C.

RESULTS

Toxicity of **Bacillus thuringiensis** on cypermethrin resistance strain of **Plutella xylostella:**

The percent of mortality and dry weight eaten were represented in Table 1. As seen in Table 1, at the all treatments the bacterial substances were not found effective on the 3rd instar larvae of cypermethrin resistance strain of **P. xylostella.** But the whole bacterial subtance and exotoxin of bacteria treatments were recognised effective on the 2nd instar larvae with 32% and 40% mortality respectively. But hydrophobic and hydrophilic exotoxin and endotoxin of bacteria did not affect to the cypermethrin resistance of **P. xylostella.**

In this experiments, 3rd instar larvae consumed all the leaves in each replication in the each treatment. But dry weight eaten of 2nd instar larvae was 0.0039±0.001 for the whole bacterial solution experiment and 0.0049±0.001 for the exotoxin of bacterial treatment.

Table 1. The mortality and dry weight eaten in the toxicity experiment on cypermethrin resistance strain of **P. xylostella.**

Treatments	Mortality %		Dry weight eaten (g)	
	2nd ins. larvae	3rd ins. larvae	2nd ins. larvae	3rd ins. larvae*
whole bacterial solution	32.0	18.3	0.0039±0.0010	-
Exotoxin of bacteria	40.0	10.0	0.0049±0.0010	-
Hydrophobic exotoxin of bacteria	5.0	3.3	0.0087±0.0002	-
Hydrophilic exotoxin of bacteria	3.3	3.3	0.0056±0.0005	-
Endotoxin of bacteria	3.3	3.3	0.0064±0.0010	-
Control	0.0	0.0	0.0073±0.0020	-

*All the leaves were consumed by 3rd instar larvae

Toxicity of *Bacillus thuringiensis* on *Drosophila melanogaster*:

The concentration of **Bacillus thuringiensis** substances that we used could not affect on the **D. melanogaster** larvae except the whole bacterial solution. The percent of mortality for the whole bacterial solution treatment was determined as 26.85%.

DISCUSSION

According to our results, the whole bacterial substance and exotoxin of **B. thuringienis** treatments were found effective on the 2 nd instar larvae of **P. xylostella** with 32% and 40% mortality respectively.

While the β - exotoxin is a broad-spectrum poison, the δ - endotoxin in the crystal of **B. thuringiensis** has an activity spectrum limited. Krieg (1971) also reported an exotoxin, toxic to mice and to diamondback moth (**P.xylostella**) in supernatants of beers of **B.thuringiensis.**

The differences between the amount of the dry weight eaten, may be due to the mortality differences of the individuals sharing the nutrients. Higher mortality ratio (40%) was observed though the nutrient consumption (0.0049±0.001) was more, at the characters both fed with the whole bacterial subtance or exotoxin, during the second instar larvae period where exceed feeding was present.

The whole **Bacillus thuringiensis** solution can recognize effective to the **Drosophila** adults. But the higher concentration of exotoxin **Bacillus thuringiensis** of might be affected. Our results confirm the results of Carlberg and Lindström (1981).

Carlberg and Lindström (1981) reported that endotoxin of **B. thuringiensis** was effective on the **D. melanogaster,** but our results did not confirm with their results.

ÖZET

BACİLLUS THURİNGİENSIS'İN PLUTELLA XYLOSTELLA VE DROSOPHİLA MELANOGASTER ÜZERİNDEKİ TOKSİSİTESİ

Bacillus thuringiensis'in **Plutella xylostella** ve **Drosophila melanogaster** üzerindeki etkisi araştırılmıştır. Bakteri solüsyonu, santrifüjden sonra bakteri solusyonunun üst fazı, bakterinin hidrofobik ve hidrofilik eksotoksini ve endotoksini bu amaçla kullanılmıştır. Denemede hiçbiri, cypermethrine dayanıklı **P.xylostella**'nın 3. dönem larvalarına etkili bulunmamıştır. Fakat bakteri solüsyonu ve bakterinin eksotoksini 2. dönem larvalarda sırasıyla %32 ve %40 ölüm meydana getirmiştir. Hidrofobik ve hidrofilik eksotoksini ve endotoksini ise cypermethrine dayanıklı **P. xylostella** larvalarına etkili olmamıştır. **B. thuringiensis** solusyonunun, **D. melanogaster** erginlerinde %26.85 ölüme neden olduğu belirlenmiştir.

ACKNOWLEDGEMENTS

I am grateful to Dr. Richard Wilkins for obtaining the **Bacillus thuringiensis** isolate and Adi Basukradi for the cypermethrin resistance strain of diamond back moth.

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New Records

Distribution of Rhizomonia in Sugar Beet Growing Areas of Türkiye

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ABSTRACT

In 1987, yellowing was observed on foliage of sugar beets in Tosya and Erbaa region. In cooperation with the Ankara Sugar Institute and Sugar Factories Head Office, investigations were carried out in the fields. Yellowing and browning of the vascular system and root bearding symptoms were observed. Also, narrowing of the bottom region of the root, resembling to radish, and small tumors were present.

ELISA tests were performed and in addition to the Beet necrotic yellow vein virus, Beet soil-borne virus serotype 2 was found. Occasionally serotype I was also present.

Table 1. Sugar beet growing areas affected by Rhizomonia

Factory and Region:	
Adapazarı:	Planted area 5.000 decare
	İzmit (Eski Adapazarı yolu), İkizce, Çaltıcak, Acıelmalık)
Alpullu:	Planted area 60.000 decare
	Babaeski (Katranca, Çiğdemli, Kuleli and Taptık)
	Keşan (Çamlıca, Mahmutköy, Bahçeköy, Kocadere, Yaylagöre)
	Lüleburgaz (Büyükkarıştıran, Turgutbey, Yancıklar Sarıcali-Ergene ovası)
	Pehlivanköy (Ergene ovası), Hayrabolu (Çıkrıkçı), Alpullu Merkez (Mandıra, E-gene ovası, Lahana), Edirne (Kirişhane, Döllük)
	Saray, Çerkezköy, Çatalca
	Uzunköprü (Hamidiye, Çöpköy, Uzunköprü)
Ankara:	Planted arsa 3.000 decare
	Polatlı Porsuk
Eskişehir:	Planted area 3.000 decare
	Sivrihisar (İlören)

Kastamonu: Planted area 22.000 decare
Boyabat (Sinop yolu, Yenice, Cuma, Kayalı)
Fab. Merkez (Ayvalı-Kastamonu)
Ilgaz (Merkez, Tosya)
Taşköprü (Etem mahallesi, Emerce)

Turhal: Planted area 8.000 decare
Niksar (Buzkoy, Direkli and Yeşilhisar)
Tokat (Çamağzı)
Turhal Merkez (Samurçay, Kızoğlu, Çengel Kurudere)

Türkiye'de Şekerpancarı Ekiliş Alanlarında Rhizomonia

1987 Yılında Tosya ve Erbaa bölgesi şekerpancarı ekim alanlarında yer yer renk açılması şeklinde dikkati çeken hastalık etmeni üzerinde Şeker Fabrikaları Genel Müdürlüğü ve Ankara Şeker Enstitüsü ile işbirliği sonucu tarlalarda yürütülen incelemelerde kök sakallanması; kökün kuyruk kısmının üzerinde birden daralarak turp şeklini alması, kuyruk yüzeyinin tümörcüklerle kaplanması kökün iletim dokularının sararması ve kahverengileşmesi gibi simptomlar görülmüştür.

Yapılan ELİSA testleri sonucu şekerpancarlarında Beet necrotic yellow vein virus yanında Beet soil-borne virus seratip 2 ve nadiren de seratip 1 bulunmuştur.

Tablo 2. Rhizomonia ile bulaşık ekim alanlarımız

Fabrika ve bölgesi:

Adapazarı: Ekim alanı 5.000 dekar
İzmit (Eski Adapazarı yolu), İkizce, Çaltıcak, Acıelmalık)

Alpullu: Ekim alanı 60.000 dekar
Babaeski (Katranca, Çiğdemli, Kuleli ve Taptık)
Keşan (Çamlıca, Mahmutköy, Bahçeköy, Kocadere ve Yayla göre)
Lüleburgaz (Büyükkarıştıran, Turgutbey, Yancıklar ve Sarıcali, Ergene ovası)
Pehlivanköy (Ergene ovası), Hayrabolu (Çıkrıkçı), Alpullu Merkez (Mandıra, Ergene ovası, Lahana), Edirne (Kirişhane ve Döllük)
Saray, Çerkezköy ve Çatalca
Uzunköprü (Hamidiye, Çöpköy ve Uzunköprü

Ankara: Ekim alanı 3.000 dekar
Polatlı Porsuk kıyıları

Eskişehir: Ekim alanı 3.000 dekar
Sivrihisar (İlören)

Kastamonu: Ekim alanı 22.000 dekar

Boyabat (Sinop yolu, Yenice, Cuma ve Kayalı)
Fab. Merkez (Ayvalı-Kastamonu)
Ilgaz (Merkez, Tosya)
Taşköprü (Etem mahallesi ve Emerce)

Turhal: Ekim alanı 8.000 dekar
Niksar (Buzkoy, Direkli ve Yeşilhisar)
Tokat (Çamağzı)
Turhal Merkez (Samurçay, Kızoğlu ve Çengel Kurudere

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