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Investigations on Fungistasis with Respect to Wilt Diseases in Important Cultivated Soils of the Western Aegean Region (*)

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ABSTRACT

In the Ege Region of Turkey, the fungistatic effects of the soils from the fields of where pepper, tomato, muskmelon, watermelon and cotton cultivated were investigated against to the fungal pathogens **Fusarium oxysporum** f.sp. **melonis, F. oxysporum** f.sp. **lycopersici, F. oxysporum** f.sp. **niveum, Phytophthora capsici** and **Verticillium dahliae.** Nine out of the eightynine examined soil samples were fungistatic ranging between 25 % and 93,3 %. The fungistasis had microbiological origin. Ten antagonistic microorganisms with different levels of antagonistic effects against to these five fungal pathogens were isolated. According to identifications, four isolates of them were from the genus **Penicillium** (three **P. cyclopium**), other four isolates from the genus **Streptomyces** (two **S. gougeroti**) and remaining two isolates from the bacterial genus **Coccus.** The fungistasis have been affected by the organic matter and total nitrogen contents of the soils. Furthermore four of the nine fungistatic soils were rich in clay mineral montmorillonit and other five in illit. In vivo tests as well, amongst the antagonistic isolates three **Penicillium,** two **Streptomyces** and bacterium were obtained highly effective.

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INTRODUCTION

In Turkey the first attemp to investigate fungistasis had been carried out in 1973 (7). In this first study the fungistatic effects of soil samples from Bornova and Menemen towns of İzmir were measured against to six **Fusarium** and one **Verticillium** species, and the reason of the fungistasis was attached to antagonistic microorganisms. Further in another study, the phenomenon was also investigated in same region in more detail, and the study was carried out in soils from the cotton fields where wilt disease were not seen (13). While in this second study the reasons were investigated to explain with the soil analyses that cotton wilt disease was not occurred (**Verticillium dahliae** Kleb. the causal agent of the disease). According to the results, the occurence of the wilt associated with pH; actual humidity; the population level of the causal agent; P, K, Na and Mg contents, which are the factors in the soils.

Louvet et.al. in France serially investigated the effects of the soil fungistasis against to **Fusarium oxysporum** f.sp. **melonis** (Leach and Curr.) Snyder and Hansen the causal agent of muskmelon wilt (1, 2, 3, 4, 16, 21, 22). In the first of these researches, it was put forward that the pathogen could not cause the disease although it was present in the soils, and that the microbiological activities in the soil were responsible for this result (16). In the second part of the study fungistatic soil samples were the origin of the fungistasis. Inthat studys fungistatic soil samples were exposed to 50°C for 30 minutes, and it was obtained that the soil fungistasis was continued under this condition but not at 55°C for same period (21). After the latter heat treatment, important reductions in the microbiological populations were found out, especially in the saprophytic Fusarium populations, and from this result it was concluded that the origin of the fungistasis could be based on the competition amongst the **Fusaria.** Also the same conclusion was revealed by **in vitro** tests showed that the ionizing radiation doses of 250 and 500 Krd caused complete destroying of the fungistasis.

There is not a definite harmony amongst the ideas of the investigators about the effects of soil types on fungistasis. However, Lockwood (1964) reported from the literature that, the fungistasis in the different type of soils occurred in the following decreasing order: heath soil > garden soil > clay soil > leaf mold > peat (no inhibition). Hence, it may appear that most effective fungistasis may be present in the heath soil (15).

The fungistasis is increased by the effects of the high levels of moisture, organic matter, temperature, and also high microbial acti-

vities in the layers between the surface and 15 cm depths of the soil (6). However the members of the Genus **Phytophthora** may be survive in spite of the fungistasis which is especially caused by the microbiological activity, because they are generally able to live in fairly deep layers of the soils (6).

Up today the works have provided a definite explanation that there was an effect of the antagonistic microorganisms on the soil fungistasis, and in addition to this it has also revealed that the fungistasis has associated with the type, chemical composition, pH and moisture of the soils.

In the Ege Region of Turkey the wilt diseases are of primary importance on some cultivated plants. Amongst these, wilt diseases of musk-melons, water-melons, cotton and tomatoes, and root and colar rot (**Phytophthora** blight) of peppers are most remarkable diseases.

In this research, the fungistatic properties and its reasons were investigated in the soils from the fields where above mentioned diseases were not naturally occur. The aim of the investigation was to control the diseases in question with application of this natural fungistasis of the soil. **In vitro** and **in vivo** tests were carried out in the study, but **in vivo** tests were as pot experiments level and they were not field experiments.

MATERIAL and METHODS

In the research, **Fusarium oxysporum** f.sp. **lycopersici** (Sacc.) Snyder et Hansen-Race 2; **F. oxysporum** f.sp. **melonis** Snyder et Hansen-Race 1, 2; **F. oxysporum** f.sp. **niveum** (E.F. Smith) Snyder et Hansen, **Verticillium dahliae** Kleb.; and **Phytophthora capsici** Leon., were used as the pathogen test organisms. The first fungal isolate was from West Germany, and other four isolates were from the Ege Region. The sensitive varieties for each fungal agent were used as the plant materials in the study. These respectively used varieties were **Lycopersicon esculentum** cv. Campell-33, **Cucumis melo** cv. Hasanbey, **Citrullus vulgaris** cv. Sugar Baby, **Gossypium hirsitum** cv. Cooker-100 A/2, and **Capsium annuum** cv. Çarliston (from the Horticultural Section of the University of Ege).

Total eighty-nine soil samples were taken from the rhizospheres of the fields where primarily the wilting and drying symptoms were not occured and secondarly the wilting less occured. These samples were taken from the surface to the 20 cm depths of the soils and they were used in the fungistasis tests and other analyses.

The soil samples taken to the laboratory were dried in air for 12 hours, and they were used in the preliminary elimination tests

for fungistasis by the Agar Disc Technique (11, 12). The germination percentages of microconidia of **Fusarium** isolates and conidia of **V. dahliae** isolate and Zoospores of **P. capsici** were criterions in the soil fungistasis tests. The soils were selected as fungistatic which they inhibited the spore germinations more than 25 percent.

In the soils which selected as fungistatic the analyses were carried out for texture (8), pH, cation exchange capasity, exchangeable cations, clay minerals (10), organic matter (20), calcium carbonate and some microelements (24), soluble salts (25), available water percentage, field (water) capasity and wilting point (23).

Further more, the microbial compositions of these fungistatic soils were found out and the isolations were made by the Dilution Plate Count Method (9, 19). In the isolations, 25 g air-dry soil from each selected sample were diluted $1/10^5$ times for fungi, $1/10^6$ times for bacteria and actinomycetes, and then the diluted soil suspensions were added to the spesific media were next to the solid phase. In the isolation studies, the spesific media were Martin's medium for fungi, Yeast Extract Agar for bacteria and Küster-William's medium (27) for actinomycetes.

A series of **in vivo** glass jar and/or pot experiments in climatic chambers with the soil samples showing fungistasis were conducted to prove fungistasis, to determine the activities of antagonistic microorganisms and the effects on the development of the diseases of the different levels of the fungistatic soils. In these experiments the inocula were prepared from the PDA cultures of the pathogens, as suspensions in sterile distilled water and the suspensions were given taking into account the cultures of 1/2 petri dish per pot.

The fungistatic soils were fumigated with methyl bromide and were autoclaved at 120°C for 30 minutes to obtain an approach to the origin of the fungistasis, and then they were tested one by one against to the causal agent of the disease which they were found effective against to itself in the preliminary elimination. This was done to determine whether the fungistasis was broken or not by the treatments.

In vitro antagonism tests to measure the antagonistic effect were carried out by means of double inoculations both of the pathogens and the antagonists into the same petri dishes containing PDA medium. The colonial diameters and inhibited colonial growths of the pathogens were taken as the measures for the effectiveness of the **in vitro** antagonism.

RESULTS

Nine out of the total eighty-nine soil samples were selected as fungistatic in the preliminary elimination tests. These nine fungistatic soil samples were obtained 4 from water melon, 3 from musk melon and 2 from cotton fields. None of the total 37 soil samples which were obtained from the fields of pepper and tomato, showed the fungistatic effect. Table 1. shows the effect of 9 fungistatic soil samples on the test pathogens in percentages.

Table 1. Inhibitive Effect of 9 Fungistatic soil Samples on Spore Germination of the Pathogens (%)*

Soil Samples	Pathogens**				
	F.o.l.	F.o.m.	F.o.n.	P.c.	V.d.
K_2S : Muskmelon Field, Denizli	0	28	0	0	0
K_2H : Muskmelon Field, Denizli	0	31	0	0	26
K_5 : Muskmelon Field, Buldan-Denizli	42	67	0	33	93,3
P_1 : Cotton Field, Manisa	0	0	0	0	30
P_{19} : Cotton Field, Bayındır-İzmir	0	53	0	0	28
Kz5 : Watermelon Field, Salihli-Manisa	0	0	0	0	55,6
Kz8 : Watermelon Field, Tire-İzmir	0	0	0	0	60,4
Kz9 : Watermelon Field, Bayındır-İzmir	0	0	0	0	25,4
Kz12 : Watermelon Field, Bayındır-İzmir	0	0	0	0	47,8

* Each value is the average of 80 countings: 5 Petri dishes x 4 agar discs x 4 microscopic fields.

** Abbreviations show the wilt pathogens of tomato, muskmelon, Watermelon, Pepper and cotton, respectively.

The results of the physical and chemical analysis of the fungistatic soil samples are shown on Table 2.

Table 2. Some Properties of the Fungistatic Soil Samples..

Soil Properties	Soil Samples								
	Kz5	Kz8	Kz9	Kz12	P_1	P_{19}	K_2S	K_2H	K_5
pH	7,45	7,32	7,05	6,70	7,22	6,12	7,34	7,40	7,32
$CaCO_3$ (%)	9,98	1,35	1,58	1,35	7,33	1,34	38,2	34,0	38,2
Clay (%)	12,7	24,7	10,7	6,7	14,4	12,4	30,2	23,2	31,2
Silt (%)	32,1	54,1	28,1	30,1	28,0	25,0	57,0	57,0	55,0
Sand (%)	55,2	21,2	61,2	63,2	57,6	62,6	12,8	19,8	13,8
Soluble Salt (%)	0,03	0,03	0,07	0,02	0,05	0,08	0,09	0,08	0,10
CEC (me/100 g)	12,0	15,8	6,5	0,4	20,2	11,8	15,1	15,1	24,1
Na+ (me/100 g)	0,08	0,13	0,05	0,06	0,28	0,11	0,13	0,14	0,14
K+ (me/100 g)	0,41	0,30	0,21	0,14	0,88	0,14	0,87	0,40	1,03
Ca++ (+) Mg++ (me/100 g)	11,57	15,37	6,24	5,20	19,04	11,45	14,10	14,56	22,93
Avail, K (me/100 g)	7,2	8,5	5,0	4,2	30,5	6,5	36,5	15,0	40,5
Avail, P (ppm)	6,44	25,20	53,76	47,04	0,28	7,00	0,28	0,98	0,70
Total N (%)	0,063	0,112	0,070	0,056	0,056	0,007	0,070	0,063	0,154
Org. Matter (%)	0,72	1,40	0,46	0,31	0,72	0,93	0,93	0,52	2,38
Total Cu (ppm)	20	40	30	29	50	25	38	18	38
Total Zn (ppm)	23	97	54	36	90	70	115	100	95
Total Mn (ppm)	268	588	496	424	575	650	540	520	450
Total Fe (ppm)	1,52	3,06	2,42	2,04	2,95	2,58	2,85	2,70	2,80
Field Capasity (%)	7,20	23,90	7,65	8,18	15,06	11,93	22,90	25,19	35,09
Wilting Point (%)	2,87	6,37	2,83	2,83	7,23	4,62	10,20	9,91	19,21
Avail, Water (%)	4,33	17,53	4,82	5,80	7,83	7,31	12,70	15,28	15,88
Clay Mineral (%) *	İ>K>M	İ>K	İ>K>M	İ>K>M	M>İ>K	İ>K	M>İ>K	M>İ>K	M>İ>K

* İ=İllit, K=Caolinit, M=Montmorillonit

The fungistatic effect was broken when the soils were treated with methyl bromide and atoclaved (with the exception of Kz9 and P_{19} for methyl bromide).

In order to show the fungistatic effect experimentally, the artificial inoculation tests were made by using the pots with appropriate hosts on the nine soil samples. The results are in Table 3.

Table 3. Effect of the Fungistatic Soil on the wilt of muskmelon (K), tomato (D), and Cotton (P) and on the root and colar rot of pepper (B), (%)*

Control and Fungistatic Soils	Hosts x Pathogens			
	D x F.o.l.	K x F.o.m.	P x V.d.	B x P.c.
Inoculated Control	39,20	100,00	63,00	100,00
Uninoculated Control	0,00	0,00	11,70	0,00
K_5	8,80	9,50	64,20	100,00
K_2H	—	20,70	22,50	—
K_2S	—	8,80	—	—
P_1	—	—	23,70	—
P_{19}	—	10,00	52,00	—
Kz5	—	—	28,50	—
Kz8	—	—	59,00	—
Kz9	—	—	38,00	—
Kz12	—	—	33,50	—

* Each value is the average of ten pots and shows the disease incidence on pepper and disease severity on the others.

The ranges of colony countings in the 9 fungistatic soils were : 50×10^3/g - 152×10^3/g; 164×10^4/g - 772×10^4/g; $3,2 \times 10^4$/g - 52×10^4/g for fungi, bacteria and for actinomycetes respectively.

The cotton and two watermelon field soils were the poorest in respect to microbial population but all the soil samples from muskmelon field were the richest.

The fungal, actinomycetal and bacterial colonies isolated from the soils were tested, **in vitro**, for their antagonistic effect. Eventually, ten most effective antagonistic isolates were obtained: 4 **Penicillium**, 4 **Streptomyces** and 2 bacteria. Three isolates of **Penicillium** were identified as **P. cyclopium** Westling, two isolates of **Streptomyces** were identified as **S. gougeroti** (Duche) Waksman and Henrici and the bacteria were **Coccus.**

The level of the antagonistic effect of these antagonists against their test organisms was determined in **in vitro** and **in vivo** conditions. The antagonists and pathogens were cultured together in Petri dishes with three replications in the tests of **in vitro**. The percentages of growth inhibition in the colonies of the pathogens were estimated daily in proportion to the controls. **In vivo** tests were conducted in pots with 10 replications and with 5 plants in each pot. The values were expressed as average of the disease severity in the case of muskmelon and cotton wilt, and, as average of the disease incidence for **Phytophthora** on pepper. In the case of muskmelon wilt test, the pathogen and the antagonists were incubated on the wheat straw for a period of 48 hours before inoculation. The results of the test were given in Table 4.

Table 4. The percentages of the Inhibitive Effect of Antagonists in the tests of **In vivo** and **In vitro.**

Treatments and Antagonists	Pathogens						
	V.dahliae		F.o.melonis		P.capsici		F.o. lycopersici[1]
	In Vitro	Cotton	In Vitro	Muskmelon	In Vitro	Pepper	In Vitro
Control	0,00	1,00	0,00	0,00	0,00	4,00	0,00
Inoculated Control	—	49,00	—	60,50	—	28,00	—
All Antagonists	—	—	—	19,50**	—	—	—
All Actinomycetes	—	26,00*	—	—	—	—	—
All Bacteria	—	26,00*	—	—	—	—	—
All Penicillia	—	13,00**	—	—	—	4,00*	—
S. gougeroti (C_1)	82,50	7,00**	97,50**	37,50**	—	—	—
S. gougeroti (C_6)	57,50	14,00**	97,82	48,00*	—	—	—
Streptomyces sp (C_{43})	62,50	49,00	93,39	56,50	—	—	93,05
Streptomyces sp (C_{167})	50,00	36,00	—	—	—	—	—
Coccus sp (B_{55})	75,00	20,00**	—	—	—	—	—
Coccus sp (B_{59})	75,00	7,00**	—	—	—	—	—
P. cyclopium (P_3)	87,50	16,00**	100,00	57,00	100,00	40,00	97,22
P. cyclopium (P_7)	95,00	12,00**	100,00	39,00**	—	—	97,22
Penicillium sp (P_8)	90,00	18,00**	97,82	44,00**	100,00	—	—
P. cyclopium (P_{38})	95,00	30,00**	94,56	32,00**	—	10,00	100,00

[1] The results of **in vivo** test were unavailable because of decreasing virulence of the pathogen in the case of tomato wilt.

* Significant at the level P 5 %

** » » » » P 1 %

Table 5 shows the results obtained by the test conducted with the different doses of fungistatic K_5 soil (from the muskmelon field).

Table 5. The Effect of the Different Doses of Fungistatic K_5 Soil on Muskmelon and Cotton Wilts and Root and Collar Rot of Pepper (%)[1]

Doses of the K_5 Soil (%)	Severity of the Cotton Wilt (%)	Incidence of the Muskmelon Wilt (%)	Incidence of the root and Collar Rot of Pepper (%)
0	35	100	24
25	16*	60**	100
50	20*	20**	96
100	34	8**	80

[1] Average of 5 pots with 5 plants in each.

DISCUSSION

There was a correlation between the fungistatic effect and the content of total N and organic matter of the fungistatic soils (Table 2). The coefficient of dependence between the fungistatic effect and these two factors were 0,82907 and 0,83389, respectively. In other words, the more increase the N and organic matter of soil, the higher effect of fungistasis. As it was previously suggested by the others (6, 14, 26), this is due to the phenomena of saprophytic competition, antagonism and pathogen starvation which are resulted from the increased microbial activity in the soil. The findings, of which the fungistatic soils were rich in montmorillonit and illit minerals, coincided with the other research workers (6, 16, 17, 18). The presence of the two clay minerals especially montmorillonit in the soil results the increased population and activity of antagonistic actinomycetes and bacteria.

Since the fungistatic effect dissapeared by autoclaving or methyl bromide application it can be suggested that the fungistasis is in microbial nature. Only in the two soil samples treated with methyl bromide there was no change in the fungistatic effect. This may be due to the selective effect of methyl bromide. Indeed, it was reported that the soil may rich in the population of Actinomycetes (6) and Penicillium (16) after disinfection with methyl bromide.

As it is shown in Table 3, the

results, obtained from the **in vivo** and **in vitro** tests and conducted in order to prove the fungistasis, were coincided with each other. Only in the case of K_5 soil inoculated with **V. dahliae,** the fungistatic effect was zero in vivo while it was the highest in **in vitro.** It can be suggested that the root exudations of cotton plant inhibit the fungistasis in the field soil of muskmelon. In fact, Arjunaro (5) had proved that root exudation of cotton plant inhibited the fungistatic effect of wilt-resistant soil against **Fusarium vasinfectum.** In the case of **P. capsici** decreasing in fungistasic effect of K_5 soil in **in vivo** test may due to rapid infection ability of the pathogen (16).

The results of quantitative analysis on the microbial content of fungistatic soils showed that the cotton field soils were the poorest one in this respect. The soils obtained from watermelon fields were in second order. The muskmelon field soils were the richest in respect to this microbial content.

The findings obtained from **in vitro** tests made by using the antagonistic isolates were confirmed by the results of **in vivo** tests (Table 4). Some results may be concluded from this table; the antagonistic isolates were more effective when inoculated together in comparison with single antagonist. C_1 **(S. gougeroti),** B_{59} **(Coccus** sp.) and P_7 **(P. cyclopium)** were the most effective isolates in decreasing the severity of cotton wilt. C_1 and P_{38} **(P. cyclopium)** were the most effective antagonistic isolates in decreasing the severity of muskmelon wilt. On the other hand, in the case of root and collar rot of pepper when the antagonistic isolates were given singly the effect was not significant, but when the isolates of P_3 and P_{38} were given together the effect was high.

Even the dose of fungistatic K_5 soil as low as 25 % (mixed with garden soil) decreased the severity of muskmelon wilt at least 40 % (Table 5). When the dose increased up to 50 % the disease incidence was only 20 %. Similar results were obtained by Louvet and his co-workers (16) with the fungistatic muskmelon field soil in France. The results, obtained by the pathogen. **F. oxysporum** f.sp. **melonis** were not valid for the tests with **V. dahliae** and **P. capsici.** This may be due to the rapid infection property of two pathogens.

These results need to be supported by the field experiments before recommending for the biological control program.

Ö Z E T

BATI EGE'NİN ÖNEMLİ TARIM TOPRAKLARINDA SOLGUNLUK HASTALIKLARI AÇISINDAN FUNGİSTASİS ARAŞTIRMALARI

Ege Bölgesinde biber, domates, kavun, karpuz ve pamuk tarlası topraklarında **Fusarium oxysporum** f. sp. **melonis, F.o.f. sp. lycopersici, F.o.f. sp. niveum, Phytophthora capsici** ve **Verticillium dahliae**'ya karşı fungistatik etki araştırıldı. Toplam 89 toprak örneğinden 9tanesi % 25 - % 93,3 arasında, fungistatik bulundu. Fungistasisin kaynağı mikrobiyaldi. Patojenlere değişik düzeylerde etkili 10 antagonist izole edildi. Bunlardan 4 izolat **Penicillium** (3'ü **P. cyclopium**), 4 izolat **Streptomyces** (2'si **S. gougeroti**) ve 2 izolat da **Cossus** genusundandı. Toprağın organik madde ve toplam azot içeriği fungistasisi etkilemekteydi. Ayrıca 9 toprağın 4 tanesi montmorillonit, 5 tanesi de illit kil minerallerince varsıldı. Antagonistlerden 3 **Penicillium**, 2 **Streptomyces** ve 1 **Coccus in vivo** testlerde de yüksek derecede etkili bulundu.

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A Study of the Prevalence, Pathogenicity and Physiological Races of Fusarium Wilt of Watermelon and the Effect of Macroelements Nutrition of Host on Disease Development in Relation to the Production of Pectolytic Enzymes[1]

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ABSTRACT

194 isolates of **Fusarium**, including 76 **Fusarium oxysporum**, were obtained from wilting watermelon plants in the Aegean Region. Among the **F. oxysporum** isolates, differences were demonstrate in their pathogenicity to watermelon, but none of the isolate was pathogenic to other cucurbits. Six local cultivars of watermelon when tested against 5 selected isolates, were found to be susceptible.

Two pathogenic races of **F.o.f.s.p. niveum** (race «o» for two isolates; race «1» for one isolate) identified, but at least one and probably more additional pathogenic races were present among the other remaining 20 tested isolates.

A significant decrease in the disease index was observed as the concentration of nitrogen and calcium in a nutrient solution was increased. An increase in concentration of potassium slightly decreased the disease index whereas increasing phosphorus concentrations had no significant influence on wilt.

The amounts of two pectic enzymes, polygalacturonase and pectin methylesterase, were higher in most diseased plants, which had recieved the minimum nitrogen than in those of given the highest level of nitrogen.

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INTRODUCTION

Fusarium oxysporum f.sp. **niveum** (E.F.Sm) Snyd. and. Hans. is an important wilt causing soil-borne pathogen of watermelon. The disease caused by the pathogen is fairly destructive for the crop and is reported from almost all the countries where watermelon is being grown (5).

In Turkey the disease was first recorded in 1969 and was thought to be caused by **Fusarium spp.** (1). Another study from Aegean region showed the percentage of disease incidence and the isolations of same **Fusarium** spp. from diseased plants, but no pathogenicty tests were being performed with the isolated species (6). However it was being investigated in Marmara region that **F. oxysporum** f.sp. **niveum** was the causal agent of watermelon wilt in Turkey (14).

The purpose of this study was to investigate thoroughly 1) the occurence and distribution of watermelon wilt organism, 2) pathogenicity of the causal agent, 3) host races of the pathogen and 4) resistance or susceptibility of watermelon cultivars of local origin.

Two pathogenic races of **F. oxysporum** f.sp. **niveum** are reported from the world (7, 9). One race of this pathogen has been identified from the isolates of Marmara region (14). So it was also one of the objective of the present study to identify the races present in Aegean region.

It is well known that certain macroelements when provided to plants as an inorganic nutrition, also effect the incidence or severity of certain diseases. There are various reports available where these inorganic materials were used to control certain wilt diseases. Fusarium wilt of watermelon was found to be decreased by adequate supply of nitrogen and calcium to host (17). Calcium nutrition is also known to inhibit disease development in tomato (12, 32). Contrary to this, potassium nutrition resulted in an increase in tomato and muskmelon wilt diseases (4, 31). So it was considered worthwhile to investigate the effects of nitrogen, potassium, calcium and phosphorus nutrition of host on disease development.

Plenty of literature is available which clearly show that pectolytic enzymes produced in Fusarium wilt disease syndrome and involved in pathogenesis. Amongst the various attempts to inhibit or rather to modify the activity of pectic enzymes, by alterations in the nutrition of the host is also being considered. This was practically demonstrated in **Fusarium** wilt of tomato where the disease severity was reduced when pectolytic enzymes activity was inhibited by calcium nutrition of the host (12).

So it was planned to measure the activity of pectolytic enzymes in a series of plants which will be most and least diseased as the results of nutrient applications.

This study was financed by The Scientific and Technical Research Council of Turkey and was conducted at Dept. of Phytopathology and Agricultural Botany, Faculty of Agriculture of Aegean University, İZMİR during the years of 1976-1979.

MATERIAL and METHODS

The area of investigation was all the eight districts of Aegean region for isolating the **Fusaria,** Peptone-PCNB medium was used for isolation (25), however for preparations of inocula Czapeck-broth and to measure enzymes activity, a solid medium of DINGLE et al. (11) was being employed. The seeds of watermelon cultivar «Sugar Baby» were obtained from local seed production station whereas the seeds of cultivars used in differentiating the races of the pathogen were recieved from the seeds of six cultivars of local origin were obtained from the Botany department, Faculty of Science (İzmir).

A collection of wilted watermelon plants was obtained from each sub-district of the eight districts of Aegean region on the basis of area under cultivation of watermelon crop. **Fusarium** isolates were obtained by direct plating the surface disinfected stem pieces on media. Inoculum prepared in Czapeck-dox broth was in the form of spore suspension containing required cencentration of conidia and an equal amount of this suspension was introduced to the base of seedlings to be tested.

For evaluating the pathogenic potential of all 76 **Fusarium oxysporum** isolates, small glass bottlers containing sterilized garden soil were used to raise the seedlings. The seedlings were inoculated at the stage of first true leaves. The experimental design was randomized block design with three replicates and 5 seedlings in each replication.

The isolates which showed at least 40 % pathogenicity were selected for subsequent studies. These 23 isolates were tested on young as well as on old plants seperately with 5 replications of 5 plants, each, experiment was also conducted to see whether the isolates are pathogenic to muskmelon cv. «Hasan Bey» and to cucumber cv. «Dere».

The cultivars of watermelon used to differentiate the pathogenic races of **F. oxysporum** f.sp. **niveum** were «Sugar Baby», «Charleston Grey» and «Calhoun Grey». To evaluate their reaction in the seedling stage to 23 isolates, the inoculations were made by dipping the slightly cut roots into the inocu-

lum. However evaluating the reaction of older plants of these cultivars to 9 selected isolates, the plants were inoculated by direct pouring the inoculum around stem base.

The degree of susceptibility or resistance of 6 watermelon cultivars of local origin was tested against 6 selected isolates.

The experiments regarding the effect of 4 macroelements on disease development were conducted in sand cultures. The nutrient solutions used were nitrogen (62.5, 125, 250 ppm.), potassium (70, 280, 560 ppm.), calcium (40, 160, 320 ppm.) and phosphorus (20, 80, 160 ppm.) and 125, 140, 80 and 40 ppm. levels of nitrogen, potassium, calcium and phoshorus respectively were used in a control series and each treatment also contained this optimum levels of elements in addition to other necessary microelements. The plants were fed on respective nutritions upto 30 days and then inoculated with a typical isolate (Fon 21) by pouring method. Final evaluation of the disease percentage was recorded 32 days after inoculation. The experiment was designed according to the randomized block design with 5 replications and 10 plants in each replication.

In vivo, pectolytic enzymes activity was measured from the plants showing maximum disease incidence, i.e.at the first level of nitrogen and from those showing minimum disease incidence at the third level of nitrogen from amongst all the nutritions treatments. The activity of pectin methylesterase was measured by the titration method of GÜNDEL (15). This provided an activity rate in terms of 0, 01 N NaOH used to bring the reaction mixture of 1 % pectin and enzyme extract again to pH 7 after on specific interval of time. A cup-plate method of DINGLE et al. (11) was employed to measure the activity of polygalacturonase and pectin transeliminase.

All the experiments were performed in an environmental room with controlled light, temperature and relative humidity considered to be optimum for growth of host plants. The necessary statistical analysis for the experiments were made in the «IBM» computer center of this University.

RESULTS

426 diseased samples were collected from 142 fields of the region and as the result of isolations 194 isolates of **Fusarium** were obtained. Among the total isolates, 76 were **F. oxysporum**, 64 **F. solani**, 27 **F. equiseti** and the rest were grouped under unidentified **Fusarium** isolates. The number of fields visited, fields with **F. oxysporum** and the number of other **Fusarium** spp. found in each district of the region

is shown in table 1. It was also observed that the number of total Fusaria isolated from irrigated and nonirrigated fields vary considerably in some districts but for the regional total, this figure was almost equal in irrigated or unirrigated fields. In contrast, the numbers of **F. oxysporum** were much higher in irrigated fields than in non-irrigated fields of the region.

All 76 **F. oxysporum** isolates were subjected to pathogenicity test and they showed a variation in pathogenicity (0-100 %) on susceptible watermelon cultivar «Sugar Baby». The number of isolates tested from each district and the degree of pathogenicity shown in table 2 and the pathogenic behaviour of some of these isolates was shown in Fig 1.

Twenty three isolates tested for their pathogenic potentials on young host plants and were found to be all pathogenic; pathogenicity ranged 36-100 %. Table 3 shows the pathogenicity percentage of individual isolates as they are grouped accurding to LSD test. Isolate No 31 exhibited maximum pathogenicity and was found to be most aggressive isolate (Fig. 2). These isolates when tested on more mature plants of the host, it was observed that although initial wilt symptoms appear a little late but overall pathogenicity is also less than on young plants, but all the isolates were pathogenic and phenomenon of adult plant resistance is lacking in this host pathogen interaction.

Table 2. Districtwise distribution of **F. oxysporum** isolates and pathogenicity exhibited by them.

District	No. of isolates tested	Pathogenicity (%)		
		0-19 %	20-39 %	40-100 %
İzmir	34	9	6	19
Manisa	9	6	2	1
Aydın	12	1	10	1
Çanakkale	10	7	2	1
Denizli	3	2	0	1
Balıkesir	3	2	1	0
Muğla	2	2	0	0
Uşak	3	1	2	0
Regional total	76	30	23	23
Occurence %		39.4	30.2	30.4

Table 1. The distribution and % occurence of **Fusarium** spp. isolated from different district of Aegean region.

District	No of fields visited	No of fields with **F.** oxysporum	% age occurrence	No of total Fusaria isolated	% age occurence	No of **F. oxysp.** isolated	% age occurence	No of **F. equiseti** isolated	% age occurence	No of Unidentified Fusarium isolated	% age occurence	No. of Unidentified Fusarium isolated	% age occurence
İZMİR	41	26	63.4	65	33.5	34	44.7	20	31.2	4	14.8	7	25.0
MANİSA	37	7	18.9	42	21.6	9	11.8	18	28.1	11	40.7	4	14.8
AYDIN	15	8	53.3	30	15.4	12	15.7	9	14.0	5	18.5	4	14.8
ÇANAKKALE	15	8	53.3	22	11.3	10	13.1	5	7.8	2	7.4	5	18.5
DENİZLİ	12	2	16.6	10	5.1	3	3.9	3	4.6	2	7.4	2	7.4
BALIKESİR	10	3	30.0	9	4.6	3	3.9	2	3.1	1	3.7	3	11.1
MUĞLA	7	2	29.0	13	6.7	2	2.6	7	10.9	2	7.4	2	7.4
UŞAK	5	3	60.0	3	1.5	3	3.9	0	0.0	0	0.0	0	0.0
REGIONAL TOTAL	142	59	41.5	194	—	76	39.2	64	32.9	27	13.9	27	13.9

Table 3. The grouping of **F.o. niveum** isolates on the basis of their pathogenicity on young host plants.

Serial No.	Isolate No.	Disease incidence %	Transformed value	Groups
1	31	100	90.000	A
2	1	92	79.3740	AB
3	18	88	76.8407	ABC
4	19	88	74.0610	ABC
5	15	82	71.5277	BCD
6	13	90	66.2147	BCDE
7	40	80	66.2147	BCDE
8	34	76	63.6814	BCDEF
9	21	68	61.8463	CDEF
11	9	64	56.3074	DEFG
11	2	60	54.0000	EFGH
12	12	60	54.0000	EFGH
13	22	60	51.2203	EFGHI
14	17	56	48.6870	FGHI
15	66	56	48.4611	FGHI
16	7	48	43.8463	GHI
17	11	48	43.6204	GHI
18	20	48	41.5389	GHI
19	53	44	41.5389	GHI
20	49	44	41.3130	GHI
21	4	40	39.0056	HI
22	8	36	36.4723	I
23	10	36	36.4723	I

LSD 5 % : 17.2871

Wilt susceptible cucumber cultivar «Dere» and muskmelon cultivar «Hasan bey» were found to be resistant towards all 23 isolates tested. This gave an experimental proof that causal organism of watermelon wilt is **F. oxysporum** f.sp. **niveum** and that it is a specialized pathogen for watermelon only.

In the tests for differentiation of races, cultivar «Sugar Baby» was found to be most susceptible towards all the isolates. Two isolates were found as non-pathogenic to Cvs. «Charleston Grey» and «Calhoun Grey» but highly pathogenic to Cv. «Sugar Baby» were designated as race «0» whereas one isolate pathogenic to Cvs. «Sugar Baby» and «Charleston Grey» but non-pathogenic to Cv. «Calhoun Grey» was designated as race «1» of the

pathogen, Remaining 20 isolates were almost equally pathogenic to all the three cultivars used. Test results regarding the pathogenicity of 23 isolates on cultivars used are shown in table 4 and figure 3 and 4 show the differential reaction of two isolates (Fon 4 and Fon 21) on three cultivars. Two isolates of race «0» and one isolates of race 1, along with 6 isolates amongst the remaining 20 isolates were tested again on morernature plants of the three cultivars. Results were exactly the same as obtained with young plants.

Three isolates grouped in two races along with 2 additional isolates of **F. oxysporum** f.sp. **niveum** were tested on 6 local cultivars of the host. The disease incidence was high (minimum 85.3 %) in all the cultures and no differences were observed in the pathogenic potential of isolates used.

The experiments regarding the effect of host nutrition on disease development revealed the following results.

Increasing concentration of nitrogen led to a gradual decrease in the disease index i.e. at first level of the element (N_1), the disease incidence was 98 %; significant decrease occurred at the second level (67.4 %) and at third level, the disease incidence fell to 50.8 % which was not significantly different from the disease incidence value at second level of nitrogen. The disease incidence % along with the other statistical data for three concentrations each of 4 macroelements is depresented in table 5. Figure 5 shows the behaviour of plants at three levels of nitrogen.

Table 4. Variation of pathogenicity among 23 isolates of **F. oxysporum** f.sp. **niveum** tested on young plants of three race differentiating cultivars of watermelon (32 days after inoculation).

Isolate No.	wilt disease %		
	Sugar Baby	Charleston Grey	Calhoun Grey
1	86.6	73.3	93.3
2	93.3	0.0	0.0
4	93.3	0.0	0.0
7	93.3	86.6	66.6
8	100.0	60.0	93.3
9	93.3	66.6	53.3
10	100.0	80.0	73.3
11	93.3	60.0	66.6
12	93.3	86.6	73.3
13	93.3	80.0	86.6
15	100.0	46.6	0.0
17	100.0	80.0	93.3
18	100.0	93.3	100.0
19	86.6	80.0	100.0
20	86.6	46.6	53.3
21	86.6	80.0	86.6
22	93.3	86.6	66.6
31	100.0	66.6	73.3
34	93.3	66.6	100.0
40	93.3	100.0	100.0
49	100.0	93.3	100.0
53	100.0	93.3	80.0
66	93.3	66.6	80.0

Table 5. The effects of different levels of macroelements nutrition on percentage of wilt.

Elements	Concentration in nutrient solution (ppm.)	Percent wilt	Transformed value	Group category (LSD test)
Nitrogen	62.5	98.0	86.3130	A
	125.0	67.4	55.9619	B
	250.0	50.8	45.4750	B
Potassium	70.0	98.1	86.4885	A
	280.0	93.3	76.6723	A
	560.0	79.1	63.7256	B
Calcium	40.0	98.0	86.3130	A
	160.0	82.8	66.1082	B
	320.0	54.2	47.2613	C
Phosphorus	20.0	92.5	75.7450	A
	80.0	94.3	79.2900	A
	160.0	98.1	86.4885	A

At the lowest level of potassium (70 ppm.), the disease incidence was 98.1 % and fell slightly but significantly at second level, but at third level of the element (560 ppm.), the disease incidence was 79.1 % which was statistically different from the other two values (table 5).

At the increasing levels of calcium in the nutrient solutions the disease incidence was 98.0, 87.8, 54.7 % respectively. These figures were found bo te statistically different at 5 % level.

At all the three levels of phosphorus the disease incidence was high and no differences could be observed on the disease incidence on high or low levels of phosphorus.

The results achieved from the methods applied for each of the three groups of pectic enzymes are summarized as follows:

Production of polygalacturonase (PG) was measured in the form of diameter of reaction ring on test medium. The PG level in the most diseased plants (N_1 treatment) was considerably greater than in the least diseased plants (N_3 treatment) as the diameter of leaction ring 6.7 and 1.3 mm. respectively. No trace of this enzyme was detected in healthy plants with the same treatments, (table 6). The activity of pectin methylesterase was measured in the form of 0,01 N NaOH used to bring the reaction mixture of pectin and crude enzyme extract again to pH 7.

Table 6. Production of pectin methylesterase and polygalacturonase in plant extracts prepared from N_1 and N_3 fertilizer treatments.

Plant extract	PME vol.of NaOH used (ml.)	PG Diameter of reaction ring (mm.)
N_1 inoculated plants	52.2	6.7
N_1 uninoculated plants	9.1	0.0
N_3 inoculated plants	30.8	1.3
N_3 uninoculated plants	4.3	0.0
Boiled extracts (control)	0.0	0.0

As shown in table 6, the extracts from diseased plants with N_1 treatment consumed 52.2 ml., whereas the extract from diseased plants with N_3 series consumed 30.8 ml. NaOH respectively. The extracts from healthy plants with N_1 and N_3 treatments consumed 9.1 and 4.3 ml. NaOH respectively; heat inactivated extracts from either treatments did not reveal any PME activity.

The production of pectin Transeliminase could not be detected in any plant extract.

DISCUSSION

From 142 fields comprising 426 samples, only 194 isolates of Fusaria were obtained. The number of Fusarial isolates are comparatively less, if compared with the studies of RIED (27) and that of JOFFE and PALTI (16). This is partly due to differences in the isolation techniques and partly due to the fact, that 42 fields were apparently wilt free out of 142 fields visited. So naturally the number of Fusaria isolates were less.

Presence of **F. oxysporum, F. solani, F. equiseti** along with some other Fusarium species in the diseased plant tissue is also well known by the studies of BORA and ÖZKUT (6) in the Aegean region of Turkey and that of JOFFE and PALTI (16) in Israel. The number of **F. oxysporum** isolates were comparatively more than other species, which is considered as normal, because this species is considered to be a causal agent of watermelon wilt. As regards of the distrubition of this species, it can be said, as a result of the present study, that it is being found in every district of Aegean region. In Izmir district, its occurence was the highest. This may be due to cultivation of watermelon in some sub-districts of Izmir more extensively and may be grown every year in the same fi-

elds. So population of this species become increased in the soil. In the present study the number of **F. oxysporum** isolated from irrigated fields were much more, than in the non-irrigated fields. PALTI and JOFFE (21) have also found the same correlation between the irrigation status of the fields and the number of **F. oxysporum** isolates.

In a series of three pathogenicity tests, it was clearly observed that great variations of pathogenicity were exist among the different izolates of **F. oxysporum**. This observation corresponds with the studies of other authors in which they established that the isolates of watermelon wilt **Fusarium** collected from different localities differ considerably in pathogenicity in green house tests.

It as a matter of general observation that in the field, the susceptible crop of watermelon is being attacked by the pathogen at any stage of the development of plant. This field observation was confermed in the pathogenicity tests on young as well as on more mature plants, and was found that adult plant resistance does not occur in watermelon wilt disease.

JOFFE and PALTI (16) in Israel showed that some isolates from wilted cucurbits including watermelon are pathogenic to other cucurbits as well. Our results are in age reement with that of the majority of other workers (2, 7, 20, 28) as no pathogenicity could be found for all the 23 isolates from watermelon when tested on same wilt susceptible cultivars of muskmelon and cucumber. As the result of this study, the identity **F. oxysporum** f.sp. **niveum** was established as a causal organism of watermelon wilt in Aegean Region of Turkey and that it is specialized pathogen for watermelon.

According to the race differentiation system of CRALL (9) and CIRULLI (7), 2 of the total 23 isolates of **F.o.**f.sp. **niveum** clearly exhibited pathogenic reaction on watermelon cultivars used as differentials and included in race «0» of **F.o.**sp. **niveum** whereas one other isolates was classified under race «1» of the pathogen. As the remaining 20 isolates were almost equally pathogenic to the three cultivars used so it is probable that in addition to the two races of the pathogen mentioned one or probably more races are present among the isolates used. Presence of additional races at least in Some Mediterranean countries is well reflected by the studies of NETZER (22) in Israel in which the author found that local isolates and one from Greece ware pathogenic to cultivars «Charleston Grey» and «Calhoun Grey». However ARMSTRONG and ARMSTRONG (2) could not find any differential reaction between two cultivars towards many isolates of U.S. origin, so authors do not accept the pre-

sence of two pathogenic races of **F. oxysporum** f.sp. **niveum.**

Differential resistance among cultivars against various isolates do exist in U.S.A. (3), but the results of present study showed that 6 cultivars of local origin are highly susceptible towards 5 isolates. Therefore it will be worthwile if large number of cultivars of local origin should be screened for their resistance or susceptibility against local isolates whichs may help in determining the presence of some other races of the pathogen and this will also help in evolving wilt resistant cultivars of watermelon.

The results on the effect of macroelement nutritions of host on disease developmet showed that about 50 % damage due to wilt can be avoided by providing more nitrogen to watermelon plant. WEI et al. (30) almost found the same results but NO_3-form of nitrogen was found to inhibit disease development in watermelon more pronouncedly (17). Increasing potassium nutrition from 70 to 560 ppm. inhibited the disease development, but not as much as nitrogen, For cotton **Fusarium** wilt EL-GINDI et al. (13) got a substantial decrease in disease incidence only at 1000 ppm of potassium level. It is expected that the more increase in K concentration, the more wilt disease in watermelon might have been obtained.

The increasing calcium nutrition, significantly increased the resistance of plants. This effect of calcium is found consistent with studies on other **Fusarium** wilt (12). It is also being said that by raising the pH of soil by liming, considerable reduction in the development of Watermelon and cucumber wilt has been achieved (17). In this study the pH of growth medium for plant at every level of calcium ranged from 6.5 to 6.8 so it is not clear that disease reductnon actually occurs due to increased soil pH.

For Fusarium wilt, of muskmelon WENSLEY and Mc KEEN (31) proved that phosphorus nutrition has no effect on disease development. The results of the present study also gave the same results and KIRALY's (18) views are hereby supported that the action of phosphorus on disease resistance is variable and is not very clear.

On the basis of data obtained from the investigations on pectolytic enzymes activity it was being demonstrated that pectin methylesterase and polygalacturonase were present in diseased plants. Presence of both the enzymes more in the plants of N_1 fertilizer treatment as compared to plants of N_3 treatment. As the plants of former treatment were more suscebtible to wilt than the latter treatment, so differences in production of enzymes may have arised from the differances in colonization of wilt

organism in plant tissue of both the series. PME is normally found in the tissues of healthy plants whereas PG is not. Therefore the increase of PME following infection and the presence of PG only in the diseased tissue was due to production of the enzymes by the pathogen, as already found in other Fusarium-wilt diseases (10, 19, 26, 29).

The presence of these two enzymes in diseased tissue ot watermelon plants although could not determine their role in wilt disease development, if the results of this investigation are compared with other investigations on Fusarium wilt of tomato, cotton and banana (10, 21, 29, 33), then it seems that PME and PG of watermelon wilt disease complex do play some role in disease development. NISHIMURA (23) is also of the view that pectolytic enzymes are one of the most important factors in Fusarium wilt of watermelon disease symptoms.

With the method used, the presence of pectin-transeliminase in diseased or non-diseased plant tissue could be demonstrated COOPER and WOOD (8) found this enzyme in culture filtrate of **F. oxysporum** f.sp. **lycopersici** and no other report was available regarding its presence or absence for other wilt disease. It is presumed to be absent in watermelon wilt disease.

Ö Z E T

EGE BÖLGESİNDE KARPUZ FUSARIUM SOLGUNLUĞU ETMENİNİN PATOJENİSİTESİ, IRKLARI, HASTALIK İLE MAKROBESİN ELEMENTLERİ VE PEKTOLİTİK ENZİM İLİŞKİLERİ ÜZERİNDE ARAŞTIRMALAR

TOAG/351 numarayla TÜBİTAK tarafından desteklenen bu çalışma, 1976-1979 yıllarında Ege Üniversitesi Ziraat Fakültesi Fitopatoloji ve Zirai Botanik Kürsüsünde yürütülmüştür.

Karpuz solgunluk etmeni **Fusarium oxysporum** f.sp. **niveum**'un Ege Bölgesindeki bulunuşu, yaygınlığı ve fizyolojik ırklarının saptanması, araştırmanın ilk bölümünü oluşturmuştur. İkinci bölümde ise, konukçunun 4 makroelement'le beslenmesinin hastalık gelişimine ve **in vivo**'da iki uç beslenmenin pektolitik enzim oluşumuna etkisi araştırılmıştır.

Araştırmadan elde edilen önemli sonuçlar şunlardır:

Ege Bölgesinin 8 iline ait karpuz tarlalarındaki hastalıklı bitkilerden izole edilen 194 **Fusarium** izolatının 76'sı **Fusarium oxysporum** olarak tanılanmıştır. Tarlaların sulanması hem solgunluğun hem de izole edi-

len **F. oxysporum** sayısının artmasına neden olmuştur.

Solgunluğa duyarlı «Sugar Baby» karpuz çeşidiyle kontrollu koşullarda 76 **F. oxysporum** izolatıyla yürütülen denemelerde, izolatların patojenisitelerinin % 0 - % 100 arasında değiştiği görülmüştür.

Seçilen 23 **F. oxysporum** f.sp. **niveum** izolatının hem karpuz fidelerinde ve hem de gelişmiş bitkilerde patojenik oldukları ve bu izolatların diğer Cucurbitaceae üyelerini hastalandırmadığı, dolayısıyla karpuza özelliştikleri saptanmıştır. Bu testler sonunda, Ege Bölgesinde karpuzda solgunluk etmeninin **Fusarium oxysporum** f.sp. **niveum** olduğu sonucuna ulaşılmıştır.

«Sugar Baby», «Charleston Grey» ve «Calhoun Grey» karpuz çeşitlerinin kullanıldığı etmenin fizyolojik ırklarını saptama çalışmalarında, 2 ve 4 nolu izolatların ırk (0) ve 15 nolu izolatın ırk (1) tipi reaksiyon verdikleri görülmüştür. Hem fide dönemindeki ve hem de gelişmiş bitkilerde yürütülen denemelerde benzer sonuçlar alınmıştır. Buna karşın 23 izolattan 20 tanesinin, her üç çeşidi de hastalandırdıkları saptanmıştır. Bu bulgu, bizi etmenin bilinen iki fizyolojik ırkının varlığına ek olarak, Ege Bölgesinde ek bazı ırk veya ırkların olabileceği düşüncesine götürmüştür.

6 yerel karpuz çeşidi ve değişik ırk gruplarından seçilen 5 yerel **F.o.niveum** izolatıyla yürütülen denemede, tüm çeşitlerin hemen hemen eşit ve yüksek derecede duyarlı oldukları saptanmıştır.

Makrobesin elementleriyle yürütülen denemede, azot ve kalsiyumun artan seviyelerinin hastalık çıkışını önemli derecede engellediği görülmüştür. Azotun N_1 seviyesinde (62,5 ppm.) hastalık yüzdesi % 98.0 iken, bu değer N_3 de (250 ppm.) % 50.8'ye düşmüştür. Yine Ca_1 seviyesinde (40 ppm.) % 98 hastalık çıkışına karşılık, Ca_4 de (320 ppm.), bu değer % 54.2 olarak saptanmıştır. Buna karşın denemede yer alan Potasyum ve Fosfar beslenmesinin hastalık gelişimini engellemesi yönünde önemli bir etki yapmadığı ortaya konmuştur. En az (N_1) ve en yüksek (N_3) hastalık çıkışının olduğu iki seviyede beslenmiş bitkilerde, hem Poligalakturonaz (PG) ve hem de Pektin Metil Esteraz (PME) enzimlerinin varlığı saptanmıştır. Ancak N_1 uygulamasının yapıldığı bitkilerde, N_3 uygulamasının yapıldığı bitkilere oranla, daha yüksek bir Poligalakturonaz aktivitesi bulunmuştur. Sağlıklı N_1 ve N_3 bitkilerinde bu enzimin saptanabilir izine rastlanılmamıştır. Pektin Metil Esteraz (PME) enzimi, sağlam bitkilerde de bulunabilmesine karşın, elde edilen değerler hastalıklı bitkilere oranla daha düşük olmuştur. Pektin Trans Eliminas (PTE) enzimi ise deneme koşullarında hiç bir bitki ekstraktında saptanmamıştır.

Araştırmadan elde edilen kesin sonuçlar şöyle sıralanabilir:

— **Fusarium oxysporum** f.sp. **ni-**

veum Ege Bölgesinde Karpuz solgunluğunun etmenidir ve karpuzda özelleşmiştir.

— Ege Bölgesinde 0 ve 1 nolu ırklara ek olarak yeni ırk veya ırklar söz konusudur.

— Yerel kaynaklı bazı karpuz çeşitleri etmene karşı yüksek derecede duyarlıdırlar.

— Kalsiyum ve Azot'un artan seviyeleri hastalık gelişimini engellemektedir.

— Hasta dokuda Pektin Metil Esteraz (PTE) ve Polifalakturonaz (PG) enzimleri fungus tarafından salgılanmaktadır.

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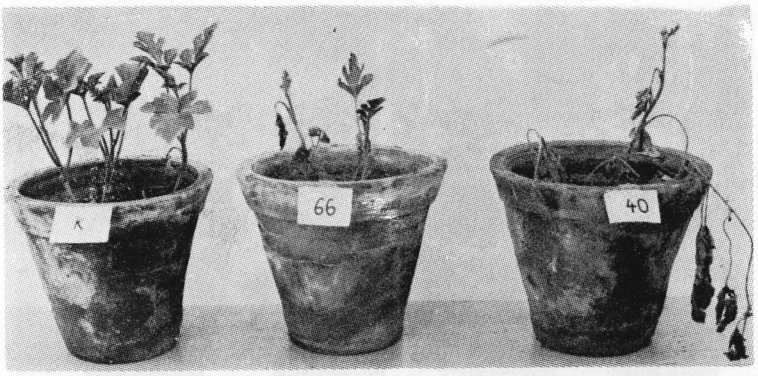

Fig.1. Patogenicity of some **F. oxysporum** isolates on Sugar Baby Watermelon Variety (25 days after inoculation)

Fig. 2. Patogenicity of **F.o.**sp. **niveum** isolates No. 2 and No. 31 on Sugar Baby Watermelon Variety (25 days after inoculation).

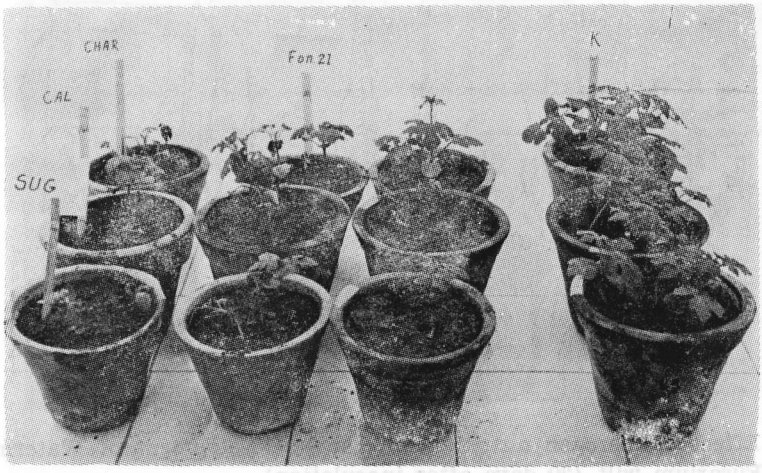

Fig. 3. Differential reactions of **Fusarium oxysporum** f.sp. **niveum** isolate No. 21 on three watermelon cultivars (18 days after inoculation).

Fig. 4. Differential reactions of **Fusarium** o.f.sp. **niveum** isolate No. 4 on three watermelon cultivars (18 days after inoculation).

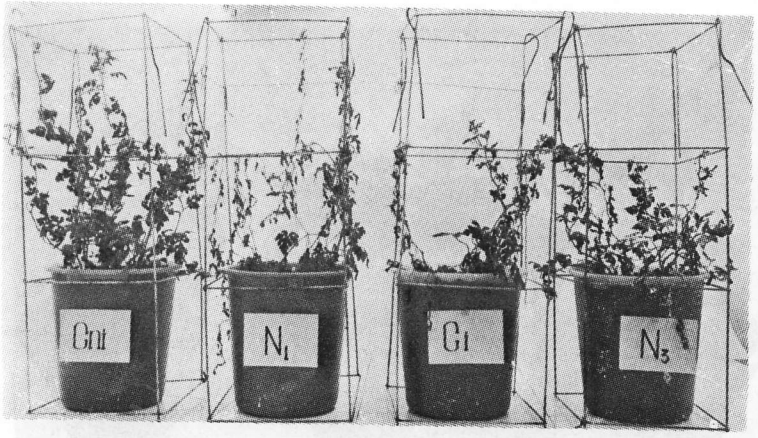

Fig. 5. Effect of nitrogen nutrition on disease development of Watermelon Fusarium wilt (20 days after inoculation)
Cni : non inoculated control.
Ci : inoculated control.

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Fungicide Resistance of Some Fungal Pathogens Isolated From Greenhouses in Turkey*

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ABSTRACT

The south and south-west coastal area of Turkey has a hot climate and several vegetable crops are grown early in greenhouses. These economically important crops are subjected to some fungal pathogens. In spite of an intensive spray programme with fungicides, growers increasingly complained about the reduced effect of several chemicals. In view of this, pathogen isolates obtained from greenhouse crops were tested in respect of sensitivity **in vitro** against the fungicides concerned, namely carbendazim, thiram and mancozeb. As the results of these tests, six out of twelve **Botrytis cinerea** isolates were able to grown on agar media containing 1,5 mg/ml carbendazim; one out of four **Rhizoctonia solani** isolates, and three out of six **Sclerotinia sclerotiorum** isolates were able to grow on agar containing 0,005 mg/ml carbendazim. On the other hand, three **Cladosporium** spp. isolates were able to grow on 1 mg/ml of carbendazim containing agar medium.

Some reduced sensitivity to mancozeb and thiram was noticed in isolates from **B. cinerea** and **Cladosporium** spp. Similarly reduced sensitivity to thiram was also found in isolates of **S. sclerotiorum.**

INTRODUCTION

Turkey has very favourable natural conditions for plant growing, for this reason agriculture has a great economical importance. The south and south-west coastal area of Turkey has a hot climate and have many greenhouses for early growing vegetable production. According to the statistics of 1981, totally 47.500 decars of greenhouses

* Supported by the Faculty of Agriculture, University of Ege.

located in this part of the country (11). The early vegetable crops especially tomato, cucumber, pepper, eggplant, beans are produced in these greenhouses have high economical importance. These economically important crops are subjected to some fungal pathogens. The main pathogens of these crops are: **Botrytis cinerea, Clodosporium** spp., **Sclerotinia sclerotiorum, Fusarium oxysporum, Pseudoperonospora cubensis, Rhizoctonia solani** and powdery mildews. Although benzimidazoles and dithiocarbamates are sprayed intensively for controlling these pathogens, but now growers began to suffer from the ineffectiveness of these chemicals.

The purpose of this study is to prove the variation of sensitivity of some fungal pathogens against some chemicals.

MATERIAL and METHODS

In the study, following fungicides were used: carbendazim (Derosal, 60 % WP), thiram (Pomarsol Forte, 80 % WP) and mancozeb (Dithane M-45 special, 80 % WP).

To determine the variation of sensitivity against above mentioned chemicals **B. cinerea, R. solani, S. sclerotiorum** and **Cladosporium** spp. isolates were tested. The isolates of these fungal pathogens obtained from the vegetable growing greenhouses of the south and south-west coastal area of Turkey.

The experiments were conducted according to the randomizing plot design with five replications.

RESULTS

Sensitivity of twelve **B. cinerea** isolates were given in Table 1.

According to Table 1, three isolates of **B. cinerea** couldn't grow on the agar media amended with 0,001 mg/ml carbendazim, but 6 isolates could grown on 1,5 mg/ml carbendazim containing media. Although 2 isolates grew very slightly on the 1,5 mg/ml thiram, 5 isolates grew on 1,5 mg/ml mancozeb.

Three **B. cinerea** isolates which could grow on 1 and 1,5 mg/ml carbendazim (B—IV and B—VII) and on 1,5, 2,5 and 5 mg/ml mancozeb were seen in Fig. 1 and 2.

Sensivity of four **R. solani** isolates to carbendazim and thiram were summarized in Table 2.

Table 1. Sensitivity of **Botrytis cinerea** isolates to carbendazim, thiram and mancozeb.

Isolate No.	Host	Half Diameter of Colony at 5th Day (mm.)											
		Chemical and Concentrations (Micron g. a.i./ml.)											
		Control	Carbendazim					Thiram			Mancozeb		
		0	1	5	500	1000	1500	1500	2500	5000	1500	2500	5000
B—I	Tomato	8.00	20.10	17.25	14.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B—II	Tomato	29.90	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B—III	Cucumber	43.79	44.00	43.88	44.50	17.91	21.62	0.00	0.00	0.00	0.00	0.00	0.00
B—IV	Pepper	37.40	28.75	36.50	33.55	38.50	34.45	0.50	0.00	0.00	5.30	0.30	3.60
B—V	Tomato	39.05	35.15	35.00	33.40	27.16	16.16	0.00	0.00	0.00	0.00	0.00	0.00
B—VI	Eggplant	33.67	36.00	36.70	24.80	29.18	27.90	0.00	0.00	0.00	0.00	0.00	0.00
B—VII	Eggplant	36.89	38.85	31.90	35.20	33.56	28.35	0.00	0.00	0.00	12.80	10.05	5.56
B—VIII	Cucumber	22.00	39.75	34.50	17.85	16.55	10.85	0.20	0.00	0.00	5.65	1.95	4.45
B—IX	Tomato	45.00	0.90	0.80	1.05	0.20	0.00	0.00	0.00	0.00	3.25	4.80	3.70
B—X	Cucumber	20.40	0.20	0.00	5.00	0.00	0.00	0.00	0.00	0.00	1.85	1.95	5.40
B—X1	Strawbery	39.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B—XII	Tomato	43.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 2. Sensitivity of **Rhizoctonia solani** isolated from tomato to carbendazim and thiram*

Isolate No.	Half Diameter of Colony at 5th Day (mm.)			
	Control	Carbendazim (micron g. a.i./ml.)		
	0	1	5	50
R—1	44.30	45.00	0.00	0.00
R—II	41.60	45.00	0.00	0.00
R—III	45.00	45.00	0.00	0.00
R—IV	45.00	45.00	45.00	0.00

* : No colonial growth on thiram.

As obvious from Table 2, only one **R. solani** isolate grew profusely on 0,005 mg/ml carbendazim, but no colonial growth obtained on thiram.

Variation of sensivity in **S. sclerotiorum** isolates against carbendazim, thiram and mancozeb were given in Table 3.

Table 3. Sensitivity of **Sclerotinia sclerotiorum** isolates to carbendazim, thiram and mancozeb*

Isolate No.	Host	Half Diameter of Colony at 5th Day (mm.)				
		Chemical and Concentrations (micron g. a.i./ml).				
		Control	Carbendazim		Thiram	
		0	1	5	1500	2500
SS I	Cucumber	45.00	42.18	42.75	0.20	0.22
SS II	Lettuce	45.00	0.00	0.00	0.00	0.00
SS III	Cucumber	45.00	0.00	0.00	0.00	0.00
SS IV	Eggplant	45.00	0.00	0.00	0.00	0.00
SS V	Cucumber	45.00	40.63	40.41	0.00	0.00
SS VI	Cucumber	45.00	45.00	45.00	0.00	0.00

* : No colonial growth on mancozeb.

The figures in Table 3 show that, three out of six isolates were able to grow on PDA containing 0,005 mg/ml carbendazim. Although one **S. sclerotiorum** isolate grew very poorly on thiram containing media, there was no growth observed on mancozeb amended media.

Behaviour of **Cladosporium** spp. isolates on different concentrations

of carbendazim, thiram and mancozeb containing PDA were summarized in Table 4.

According to Table 4, all **Cladosporium** spp. isolates could grow in 1 mg/ml carbendazim, 5 mg/ml thiram, and in 2,5 mg/ml mancozeb containing media.

The growth of **C. fulvum** isolate on carbendazim, thiram and mancozeb concentrations were given in Fig. 3 and 4.

Table 4. Sensivity of **Cladosporium** spp. to carbendazim, thiram and mancozeb.

Isolate No.	Host	Half Diameter of Colony at 11th Day (mm.)										
		Chemicals and concentrations (micron g. a.i./ml.)										
		Control	Carbendazim				Thiram			Mancozeb		
		0	1	50	500	1000	1500	2500	5000	1500	2500	5000
C—I (**C. fulvum**)	Tomato	18.83	17.85	17.25	16.65	17.95	9.95	0.80	0.50	0.90	0.60	0.00
C—II (**Cladosporium** sp.)	Watermelon	19.87	15.91	24.33	22.79	1.70	0.12	1.43	0.81	0.43	1.18	0.00
C—III (**Cladosporium** sp.)	Cucumber	11.82	9.37	7.25	12.25	10.75	0.54	0.25	0.41	0.25	0.15	0.00

DISCUSSION

From the results of this study it is obvious that, resistance to carbendazim has reached to very high levels in some fungal isolates which were obtained from greenhouse grown vegetables in Turkey. There are many studies on the spontaneous or acquired resistance of **B. cinerea** (6, 7, 8, 15, 16), **R. solani** (4, 9), **S. sclerotiorum** (2) and **Cladosporium** spp. (17, 18) isolates to benzimidazoles.

In Turkey, benzimidazole compounds began to registrated in 1975 (19). Since then, these group of chemicals are being used intensively for controlling the pathogenic fungi on vegetables and fruit trees (3). For this reason, successive applications of benzimidazole fungicides effected the sensitivity of some pathogenic fungal isolates in the greenhouses. NEMLİ (12) was recorded that, an isolate of **B. cinerea** which was obtained from greenhouse was sensitive to 1 ppm benomyl. In this study the isolate B-1 was obtained from the same greenhouse and it was found to be resistant to 0,5 mg/ml carbendazim (Table 1).

Field resistance to dithiocarbamates are seldom (13, 14). For example the reduced sensitivity of **B. squamosa** (10), **R. bataticola** (1) and **Sclerotinia** sp. (13) to this group of chemicals were reported. Moreover, it was found that in our recent study **F. oxysporum** f.sp. **cucumerinum** has an adaptation ability to thiram (5). As reported before, in this present study it was also noticed that certain isolates showed some reduced sensitivity to dithiocarbamates.

Ö Z E T

TÜRKİYE'DE SERALARDAN İZOLE EDİLEN KİMİ FUNGAL PATOJENLERİN FUNGİSİDLERE DAYANIKLIĞI

Ülkemizin sıcak bir iklime sahip güney ve güney batı kıyılarında sebze seraları geniş bir alan kaplamaktadır. Ekonomik yönden de büyük önem taşıyan bu seralarda yetiştirilen sebzeler, kimi fungal etmenlerce zararlandırılmaktadır. Bu hastalık etmenlerini önleme amacıyla yoğun fungisid uygulamaları yapılmasına karşın, yetiştirici, kullandığı kimi ilaçların etkinlerinin giderek azaldığından yakınmaktadır.

Bu çalışma, kimi hastalık etmenlerinin seralarda yoğun kullanılan benzimidazole grubu sistemik fungusidleri temsilen carbendazim'e ve dithiocarbamate grubu klasik fungusidleri temsilen de thiram ve mancozeb'e değişen duyarlılıklarını saptamayı amaçlamıştır.

Yapılan laboratuvar çalışmaları sonucu, değişik konukçulardan izole edilen 12 **Botrytis cinerea** izolatından 6 tanesinin 1,5 mg/ml, 4 **Rhizoctonia solani** izolatından 1 ta-

nesi ile 6 **Sclerotinia sclerotiorum** izolatından 3 tanesinin ise 0,005 mg/ml carbendazim içeren ortamda gelişebildiği ortaya konmuştur. Ayrıca, bir **Cladosporium fulvum** izolatıyla, 2 **Cladosporium** spp. izolatının da 1 mg/ml carbendazim dozunda gelişimini sürdürebildiği görülmüştür. Sözü edilen fungal etmenlerin dayanıklı olmayan izolatları ise 0,001 mg/ml carbendazim içeren ortamda gelişememişlerdir. Diğer yandan **B. cinerea** ve **Cladosporium** spp. izolatlarının kimileri mancozeb ve thiram'a, kimi **S. sclerotiorum** izolatlarının da thiram'a duyarlılıklarının azaldığı yine bu çalışmayla ortaya konmuştur.

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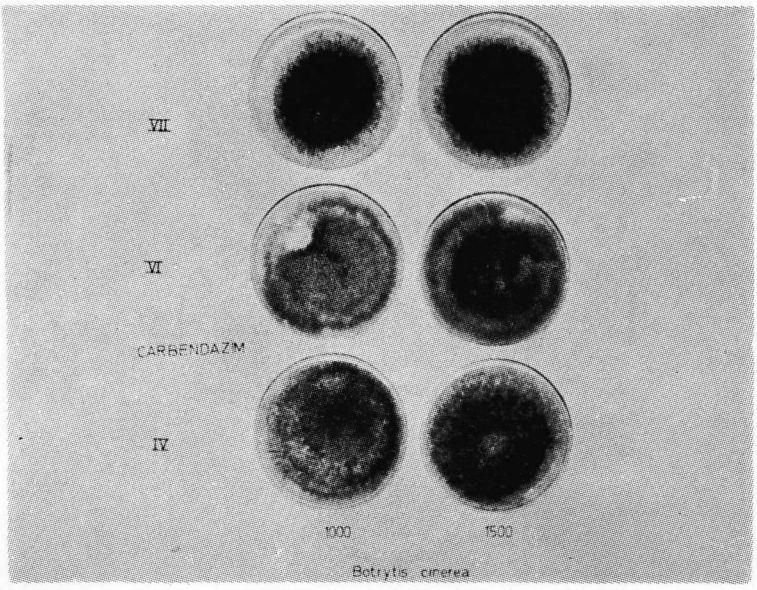

Fig. 1. Growing of three **B. cinerea** isolates, B. VII (top) B-VI (middle) and B-IV (bottom), on 1 and 1,5 mg/ml carbendazim containing PDA.

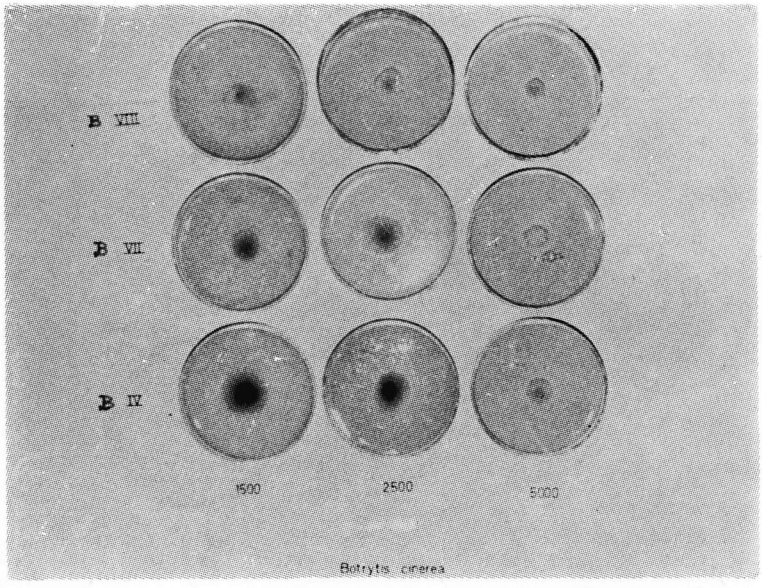

Fig. 2. Growing of three **B. cinerea** isolates, B-VIII (top), B-VII (middle) and B-IV (bottom), on 1,5, 2,5 and 5 mg/ml mancozeb containing PDA.

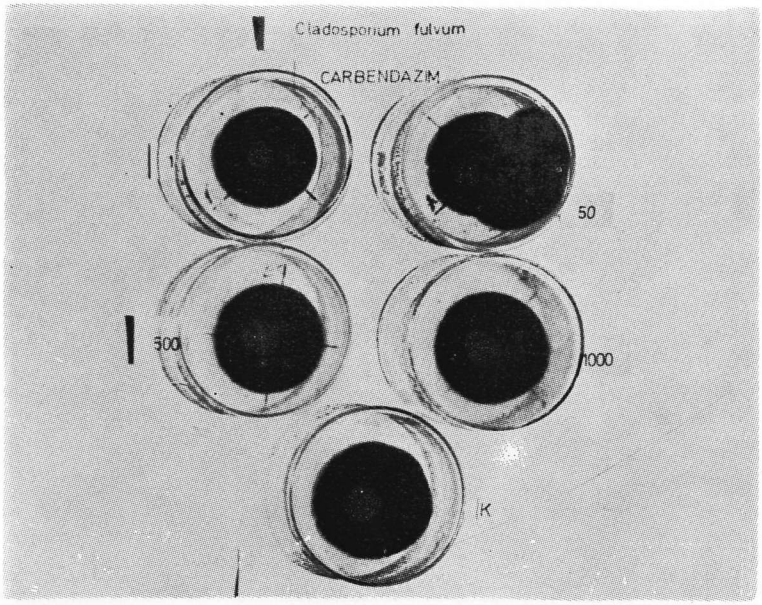

Fig. 3. Growth of **C. fulvum** isolate on 1 (left at the top), 0,05 (rifht at the top), 0,5 (left in the middle), 1 (right in the middle) mg/ml carbendazim containing media. (K=Control, at the bottom).

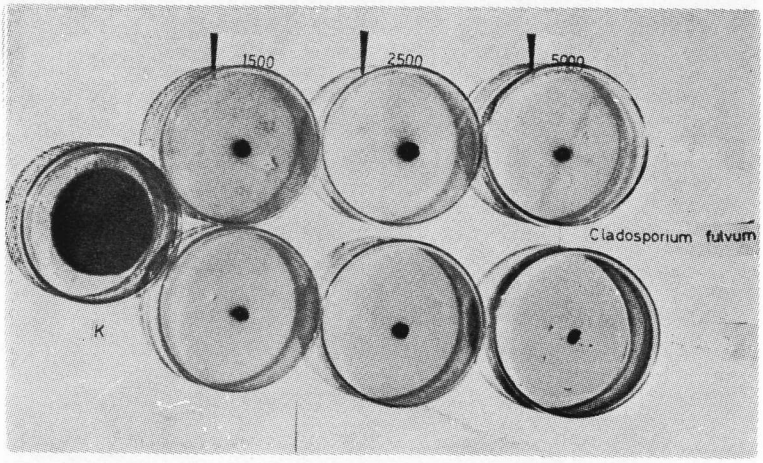

Fig. 4. Growth of **C. fulvum** isolate on 1,5, 2,5 and 5 mg/ml thiram (top) and mancozeb (bellow) containing media (K: Control, at left).

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Investigations on the Effects of Some Cultural Applications and Antagonistic Fungi on **Rhizoctonia solani** Kühn. and **Verticillium dahliae** Kleb. in the Aegean Region.

I- Effects of Crop Rotation and Fertilizations :

Emel SEZGİN* Ayhan KARCILIOĞLU* and Ümit YEMİŞÇİOĞLU**

ABSTRACT

The effects of different rotation systems and fertilizers on the damping-off and wilt diseases of cotton have been investigated under the field conditions, in addition that, the effects of these cultural practices on **Rhizoctonia solani** Kühn and **Verticillium dahliae** Kleb. were studied in vitro. Also, the effects of rotation and fertilizers on the rhizosphere of cotton and the population of **R. solani** and **V. dahliae** in this rhizosphere were investigated.

INTRODUCTION

Cotton is a very important industrial plant for Turkey. There are two serious diseases of cotton in Ege Region. One of them is damping-off which leads to damage during the seedling stage. **R. solani** is a major agent causing the seed rot and pre and post emergence damping-off cotton throughout the cotton growing areas of Turkey (KARCILIOĞLU, 1976). The other disease is Verticillium wilt, which is the most important disease responsible for the crop losses in cotton fields. **V. dahliae** had been found to be associated with the wilt dise-

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ase in a frequency of 96,8 % (KARACA et al, 1970).

R. solani and **V. dahliae** are soil-borne pathogens. The occurence and severity of soil-borne pathogens are dependent upon the interaction of many factors in the physical and biological environments. SOLOVEVE and VASILEV (1961) reported that a single rotation with rice in fields of 108-F cotton variety, heavily infested with wilt, reduced incidence of the disease. It was found that the crop rotation with maize, **Sorghum cernuum**, Lucerne, oats, barley, rye and others had an effect on reducing **V. dahliae** infection on cotton (KONONOVA, 1965; YUNUSOV and KURBANOV, 1968; SULTANOV and RAKHIMBAEU, 1968).

This study had been conducted to find the effects of crop rotation and fertilization on damping-off and wilt diseases of cotton and on the rhizosphere of cotton plant.

MATERIAL and METHODS

Field experiments : Field experiments were designed according to Randomised Block Desing with four replications. Assesments of field experiments were done at seedling stage as «diseased» and «healty» plants for damping-off disease and «0-3» scale was used at green boll stage for wilt disease.

Crop-rotation experiments : Seven different rotation systems were tested. These systems were two years cotton, one year wheat + potatoes, two years cotton, one year wheat + maize; two years cotton, one year wheat + sesami; two years cotton, one year wheat + bean; two years cotton, one year wheat + watermelon; one year wheat, two years cotton, two years alfalfa and continuously cotton. The plot size was 500 m².

Fertilization experiments : In these tests, Nitrogen (Urea 45 %; 100 Kg/ha.); K_2SO_4 50 % (60 Kg/ha.), Urea + K_2SO_4 (100 + 60 Kg./ha.); Green manure (Common vetch 15000 Kg/ha.) and barn yard manure (40000 Kg/ha.) were used. The plot size was 32 (10 X 3,2) m².

Laboratory experiments : In laboratory tests, PDA, water-agar media and sucrose nitrate and Ozapeck's solutions were used as culture media. Cultures were incubated at 22°C and 24°C temperatures. Each treatment was replicated ten times.

The effects of leaf and root extracts of rotation plants and the manures on the growth of **R. solani** and **V. dahliae** were investigated on PDA by measuring the dimater of their colony. Also, to determine the effect of the extracts of rotation plants and the manures on dry weight of **R. solani** and **V. dahliae** the colonies of fungi in czapeck's and SN solutions were weighed respectively. Leaf and root extracts

were added to the media 1:10 ratio. The manures were used at field dosages and five times more.

Rhizosphere experiments : Soil samples were taken from all plots in May and September according to the methods of MEREDITH (1940). The soil-plate technique and MARTIN (1950), media were used to determine rhizosphere fungi. The soil plates were incubated for 5 days at 24°C; then each colony was counted. The fungi were identified as genus. The populations of **V. dahliae** and **R. solani** in the rhizosphere were investigated according to NADAKAVUKAREN and HORNER (1959) and PAPAVIZAS and DAVEY (1967) methods respectively.

RESULTS and DISCUSSION

Rotation experiments : The severity of wilt disease on the various rotation systems were given in Fig. 1.

In rotation experiments, disease incidence and severity changed according to the cropping plants. Also, in all of the rotation systems the severity of wilt disease was lower than continuously cotton plots, and the populations of **V. dahliae** in continuously cotton grown plots was higher than rotation plots. SULTANOV and RAKHIMBAEU (1968) reported that the greatests numbers of microsclerotia of **V. dahliae** were found in the rhizosphere of cotton grown continuously. In our experiments the higher population of **V. dahliae** was found in continuously cotton grown plots too. The most significant decrease incidence of wilt occured on one year wheat, two years cotton, two years alfalfa and two years cotton, one year wheeat + maize plots. Also, alfalfa soils contained the lowest number of colonies of **V. dahliae** (fig 2). Many tests confirmed the importance of crop rotation in depressing **V. dahliae** in the soil. For example, in a previous study it was found that three years alfalfa, three years cotton, two years cotton, one year maize and one year cotton, one year sesami + wheat cropping reduced the incidence of wilts (KAYMAK et al., 1967). These results were confirmed with the results obtained by YOUNG et al. (1959), USMANOV (1968) and SIDOROVA (1974). Different disease severity developed in these variously cropped soils may be associated with population of **V. dahliae** in soils. Also, the population of **V. dahliae** changed according to the rotation plants and seasons. The spring population was higher than the autumn (Fig. 2).

SIDOROVA (1974) reported that, the most significant decrease in population of **V. dahliae** and consequently the lowest infection of cotton occured after legume crops (pea, clover), than after cereals (barley, rye, maize).

In laboratory tests the root and leaf extracts of alfalfa retarded the growth and dry weight of **V. dahliae** 37,12 % and 12.37 % respectively. On the other hand, the root extract of alfalfa inhibited the development of microsclerotia (Fig. 3).

The numbers of the colonies of **R. solani** were found to be quite high and almost alike in all of the rotation systems and continuously cotton grown plots (Fig. 2). This may be due to the fact that the rotation crops were also the hosts of **R. solani**. The population of **R. solani** was higher in rotation plots except continuously cotton grown and wheat + potato plots in spring than in autumn. The highest population of **R. solani** was isolated from wheat + bean plots in spring. On the other hand the rotation systems did not significantly affect the damping-off disease. If the pathogen has a very large number of possible hosts such as **R. solani** it wilt become very nearly impossible to establish a favorable rotational pattern (STEVENS, 1960).

Fertilizers experiments : In our tests the fertilizers in used formulations and dosages increased the damping off disease when compared with control. The effect of a specific form of nitrogen on soilborne pathogens has been observed for many years. Root rot of bean is reduced with NO_3–N and increased with NH_4–N (HUBER and WATSON, 1974). AFANASIEV and CARLSON (1942) emphasized that the form of nitrogen as well as the amount is important in determining the severity of black root-rot **(R. solani)** of sugarbeets. In their studies the number of diseased plants was doubled with NH_4–N compared with NO_3–N. NO_3–N reduces Rhizoctonia root rot of bean compared with «Slow release» urea or NH_4–N fertilizers (HUBER and WATSON, 1974), Also, the barn yard manure increased the damping-off disease. BOYLE (1956) reported that **Sclerotium rolfsii** and **R. solani** will not function as pathogens on peanuts unless the soil contains a supply of organic matter.

The effect of fertilizers on the population of **R. solani** in the soil were different from their effect on damping off disease on the field. The population of **R. solani** occured more frequently in spring than in autumn. The highest population was isolated from urea and barn yard manure amended plots, Also, urea retarded the growth and dry weight of **R. solani** in laboratory tests. The lowest population was isolated in K_2SO_4 amended plots both in spring and in autumn (Fig. 4). There are many factors involved in the change of **Rhizoctonia** population in the soil. It was demonstrated that the fertilizers affected on the total numbers and types of soil fungi (KAUFMANN and WILLIAMS, 1974). NH_4–H increased the saprophytic growth of **Rhizoctonia** population in the soil. It was demonstrated that the

fertilizers affected on the total numbers and types of soil fungi (KAUFMANN and WILLIAMS, 1974). NH_4–N increased the saprophytic growth of **R. solani** so, the persistance of **R. solani** in the soil was increased (DAVEY and PAPAVIZAS, 1963).

In our laboratory tests, urea significantly increased the growth and dry weight of **R. solani** according to the control-Although, K_2SO_4 reduced the number of **R. solani** colonies both in spring and in autumn, but the incidence of disease was higher than control. It may be suggested that K suppressed the population of **R. solani** but did not affect its virulence. On the other hand, **R. solani** was not the only causal agent of damping-off disease in the field. Also, ZYNGAS (1963) found that, high K significantly suppressed the disease but combined with low or high levels of P reduced pre-emergence but not post-emergence damping-off disease. In laboratory tests, K_2SO_4 affected growth of **R. solani**. High K reduced growth and dry weight of **R. solani** 6,39 % and 35,68 % respectively.

Fertilizers decreased the severity of wilt diseases when compared with control except N + K and K. Urea caused significant decrease in the incidence and development of wilt disease in cotton plants. MIRPULATOVA (1961) reported that urea (1 gr/drill) 20 days before sowing was more effective than 6 fungicides reducing infection to 11 % judged by external symptoms compared with 60 and 89 % without urea. Also, urea decreased the population of **V. dahliae** in soil both in spring and in autumn (Fig. 4). In vitro conditions urea decreased growth and dry weight of **V. dahliae** 12.42 % and 58,87 respectively and prevented the production of microscloroti. RANNEY (1962) reported that the growth of **V. albo-atrum** markedly changed when urea was the sole N source and vegetative growth shifted from the normal white aerial mycelium to production of microsclerotia and yeast-like growth. He suggested that perhaps, an increased concentration of urea in the rhizosphere and in the plant was preventing penetration or reducing the growth and spread of the fungus. K, green manure and barn yard manure + treatments caused decreases on the severity of wilt disease. HAFEZ **et al.** (1975) found that K treatments significantly decreased the disease of wilt on cotton plants. Also, in the plots amended with K_2SO_4 and green manure the numbers of **V. dahliae** colonies were lower than control plots both in spring and in autumn in control rhizosphere. In the laboratory tests the normal field and high dosages of K reduced the growth and dry weight of **V. dahliae** 4.34 % and 22,04 % respectively according to the control. In the plots amended with green manure the incidence of wilt was

lower than control it may be due to increasing the numbers of the antagonistic fungi, especially **Myrothecium** spp. MARUPOV (1974), reported that, spore preparations of **T. lignorum** suppress development of **V. dahliae** in soil and their effectiveness is increased after green manure.

Rhizosphere experiments : In rotation experiments, the total mycoflora were affected in accordance with the cropping plants. Total numbers of fungi were greater in spring than in autumn in all of the rotation systems. In contrast, total numbers of genera were higher in autumn than in spring rhizosphere of cotton. There were 44 genera identified from rotation plots. These fungi were indicated in Table 1. Among these fungi **Preussia fleischhakii** (Auersw.) Cain, **Pestalotia truncata** Lev. and **Pithomyces** sp. were new species for Turkish mycoflora. The prevalence of the fungi affected by crop was summarized Fig. 5.

Greater numbers of groups of Aspergilli, Penicillia and Fusaria were isolated from the rotation and continuously cotton grown plots. It was found that, these are common for soils and rhizosphere in many countries (MENON and WILLIAMS, 1957; PARKINSON et al; 1963; BAGGA, 1970; SARIBAY, 1974). The groups of Aspergilli and Penicillia were isolated in the highest number in spring than in autumn. Wheat + bean and watermelon plots soils contained the highest number of colonies of total Penicillia. Fusarium spp. were isolated abundantly from all of the rotation plots except the alfalfa soil in autumn. The highest numbers of total Fusaria occured in wheat + corn and wheat + sesami plots. The **Mucorales** and species of **Trichoderma** + **Gliocladium** + **Myrothecium** + **Chaetomium** occured commonly in autumn rhizosphere (Fig. 5).

The rotation plants had an effect on the cotton rhizospher flora both qualitatively and quantitatively. WILLIAMS and SCHMITTHENNER (1962) reported that, the type of cropping seemed an important factor in determining soil fungus populations. MENON and WILLIAMS (1957, investigated changes in mycofloras in the laboratory and greenhouse. They found that the highest numbers of colonies of **Penicillium funiculosum** series and **P. purpurogenum** series were isolated from corn soil; **Fusarium** colonies were the highest in alfalfa soil and the lowest in corn soil. Also, in our experiments, Penicillia significantly increased in potatoes, bean and watermelon plots after wheat in spring. On the other hand, the numbers of colonies of antagonistic fungi were obtained relatively high from rotation plots in May and September. This effect could be the result of cropping various plants.

Table I. Fungi isolated from rotation plots (Number of colonies per gram of soil)

FUNGUS	SPRING							AUTUMN						
GENERA	continuously cotton	wheat + potatoes	wheat + corn	wheat + sesami	wheat + bean	wheat + watermelon	wheat + alfalfa	continuously cotton	wheat + potatoes	wheat + corn	wheat + sesami	wheat + been	wheat + watermelon	wheat + alfalfa
PHYCOMYCETES														
Rhizopus	16	9		9	5	3	6	6		2	6	10	9	12
Mucor	15	2	13	3	15	7	9	16	5	23	11	22	4	44
Actinomucor	1			1				5		1	13			
Pythium							2	1		1				
Mortierella					1		1							12
ASCOMYCETES														
Chaetomium		15	1	4	2				1	1	1		4	4
Gymnoascus				2										
Sordaria												1		
Monilia	1	1					1							
Preussia												1		
DEUTEROMYCETES														
Aspergillus	259	334	128	113	47	82	176	57	31	15	131	144	258	115
Penicillium	87	13	32	162	77	838	100	4	54	3	3	17	8	9

(Table I. Continuing)

Fusarium	27	4	22	52	52	34	59	73	39	116	73	51	21	68
Botryotrichum	9		5	1	10		3	10		4	10	17	17	4
Trichoderma	6	6	8	8	4		7	12	17	9	14	8	4	18
Ulocladium	6		3	2	7		5	4	22					2
Cylindrocarpon	4							7						
Alternaria					1			21	42	16	39	13	8	2
Cladosporium								8		3	14	2	2	
Helminthosporium							1	3		1				9
Stemphylum				2				2			2	1		
Epicoccum								1					2	
Gliocladium	1		1	2	8	5	11		3				5	1
Stachybotris	1		1				2							8
Darotomyces	1		2							2				
Cephalosporium									8					7
Phoma			4						1		1		1	
Pestalotia									1					
Verticillium			1	1			2						3	
Humicola			3								9			2
Dreschlera							1			1				
Cladorrhinum										1				
Myrothecium		6	6				7				3	18		6
Scopulariopsis		3		1										
Papulospora		1											2	
Botrytis							2							
Curvilaria							2			1		3		8

(Table I. Continuing)

Scylalidium					2								
Steril			1		4	4	3			10		5	
Periconia				1									
Pithomyces			3						1				
Macrophomina			1							1			
Chalaropsis			1										
Torula								1				1	
Metarrhizium								2					
Heterosporium				1									

Soil character : Clay - loam

Table II. Fungi isolated from the plots amended with fertilizers and control plots (Number of colonies per gram of soil)

FUNGUS GENERA	SPRING						AUTUMN					
	Control	Urea	K_2SO_4	Urea + K_2SO_4	Green manure	Barn yard. manure	Control	Urea	K_2SO_4	Urea + K_2SO_4	Green manure	Barn yard. manure
PHYCOMYCETES												
Mucor		9	5	8	5	13	34	18	24	43	53	41
Rhizopus	1	1			2		3	8	6	2	18	
Actinomucor		2	4	8		2	1	1	3		2	
Pythium							1		4			
ASCOMYCETES												
Chaetomium	2											
Pyrenoma	2											
Pseudeurotium		6								1		
DEUTEROMYCETES												
Aspergillus	169	229	212	221	141	139	79	59	112	69	49	121
Fusarium	114	146	172	62	148	50	77	60	18	37	23	53
Penicillium	91	128	181	77	26	82	4	4	15	8	5	11
Trichoderma	40	41	44	39	35	51	31	23	10	10	11	8
Humicola	13	30	34	16	13	29	26	13	9	8	12	20

(Table II. Continuing)

Myrothecium	10	4	27	4	70	3	1		2	2	2	7
Ulocladium	7	1	4	11	19	12						
Gliocladium	7	5	7	5	5	9		3				3
Botryotrichum	3	6	2	13	7	2		3			2	6
Stemphylum	3	13	11		1	5		1				
Verticillium	3	13	3		3	1						
Cephalosporium	2	4	7	2	6	4						
Alternaria	1		2	1	6	10		3	1	1		
Macrophomina	1											
Phoma	1	5	3									8
Stachybotris		2	2				3		1			
Pestolatia			5	1	1		3	10				
Cladosporium								3				
Cylindrocarpon					4	1		20	4			13
Epicoccum		1										
Papulospora		1										
Helminthosporium		2				2						
Curvilaria			1									
Dreschlera			1						6			
Steril		7	1	1					3			4

Soil character : Clay

Many workers, (SIU SINDEN; 1951; ZYNGAS; 1963; KAUFMAN, WILLIAMS; 1964; ASKAROVA, MAMADALIEN, 1966; HUBER, WATSON, 1974) reported that soil microorganizms were greatly influenced by various fertilizers. In our experiments it was determined that the fertilizers have qualitative and quantitative effects on mycoflora. Total numbers of fungi and genera were greater in spring than in autumn. Potassium fertilization had a greatest effect on the total numbers of soil fungi in spring and it was followed by urea, green manure and N + K respectively. Urea and Potassium fertilization had the greatest effect on composition of the soil fungus population both in spring and in autumn. Barn yard fertilization was caused decrease of total numbers of soil fungi in spring according to the other fertilizers and control however, it was caused increase of total numbers of soil fungi it autumn according to the others.

Thirtytwo genera were identified from the plots amended with fertilizers and control plots. These were shown in Table II. Among these fungi **Scytalidium lignicola** Pesante, **Ramichloridium schulzeri** (Sacc.) de Hoog var. **schulzeri, Humicola grisea** Thraaen. were new species for Turkish mycoflora.

The prevalence of the fungi affected by fertilizers was summarized in Fig. 6.

In the plots amended with fertilizers, the species of **Aspergillus, Penicillium** and **Fusarium** were most prevalent as in the rotation experiments. These species, especially Penicillia, increased at most in spring. Also, they were higher than control in spring. Total Aspergilli increased significantly with urea. K_2SO_4 treatments also, increased the total numbers of Penicillia and Fusaria, the numbers of **Trichoderma, Gliocladium, Chaetomium** and especially. **Myrothecium** species showed a significant increase with green manure according to the control and other fertilizers.

KAUFMANN and WILLIAMS (1964) were investigated the effect of mineral fertilization (N-P-K) and soil reaction (pH) on total numbers and types of soil fungi in field and laboratory conditions. They found that, numbers of **Verticillium** sp. decreased, but numbers of **Myrothecium** sp. increased under N fertilization. Also, in field studies, the response of **P. funiculosum** series to potassium fertilization was significantly greater at high level than low level.

ÖZET

EGE BÖLGESİ PAMUK TARLALARINDA UYGULANAN BAZI KÜLTÜREL İŞLEMLER İLE ANTAGONİSTİK FUNGUSLARIN PAMUKLARDA HASTALIK ETMENLERİNDEN **Rhizoctonia solani** Kühn. VE **Verticillium dahliae** Kleb'a OLAN ETKİLERİNİN ARAŞTIRILMASI

1. Münavebe ve Gübrelerin Etkileri :

Çalışmada Menemen Bölge Toprak-Su Araştırma Enstitüsünce sürdürülen ve pamuk tarımı için önerilecek münavebe sistemlerinin yine pamuk tarımında kullanılan çeşitli gübrelerin pamuğun önemli hastalıklarından olan Çökerten ve Vertisilyum solgunluğu hastalıkları ile pamuk rizosferi üzerindeki etkileri tarla ve laboratuvar koşullarında araştırılmıştır.

Sonuç olarak buğday + yonca münavebesi ile Azot (üre) ve çiftlik gübresinin solgunluk hastalığını azaltıcı etkileri olduğu saptanmıştır. Denenen münavebe sistemleri ve gübreler çökerten hastalığı üzerinde etkili görülmemiştir.

Münavebe bitkileri ve gübreler pamuk rizosferini nicel ve nitel yönden etkilemişlerdir. Yeşil gübre antagonistik fungus sayısında özellikle **Myrothecium** türlerinde artışa sebep olmuştur.

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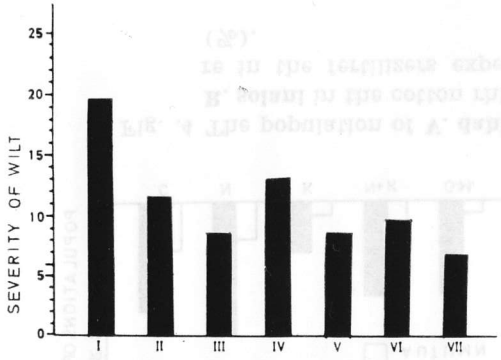


Fig. 1. The severity of wilt disease in the various rotation systems (%).

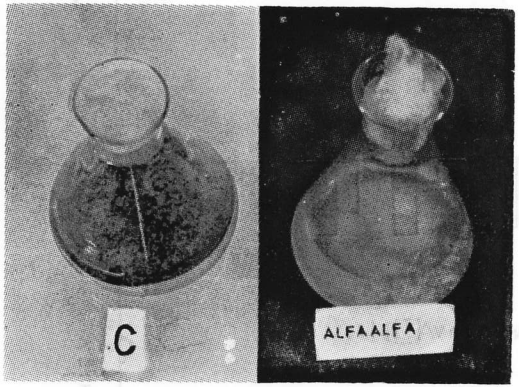
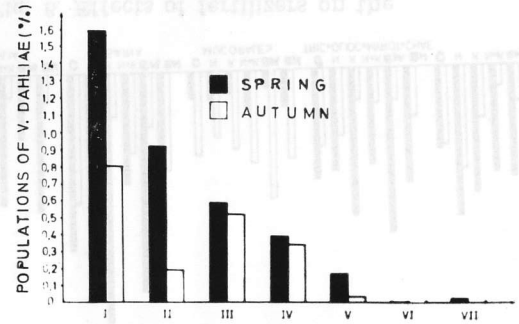


Fig. 3. The microsclerotial development of **V. dahliae** in the roct extract of alfalfa.



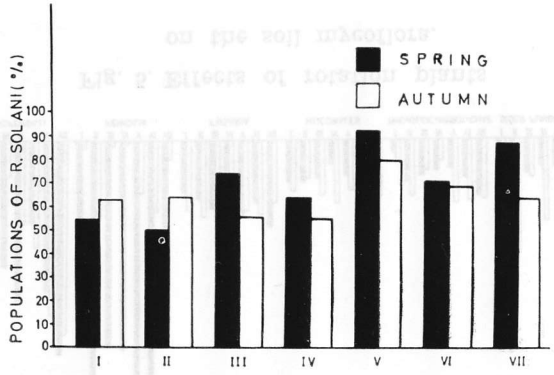


Fig. 2. The population of **V. dahliae** and **R. solani** in cotton rhizosphere in the rotation experiments (%).

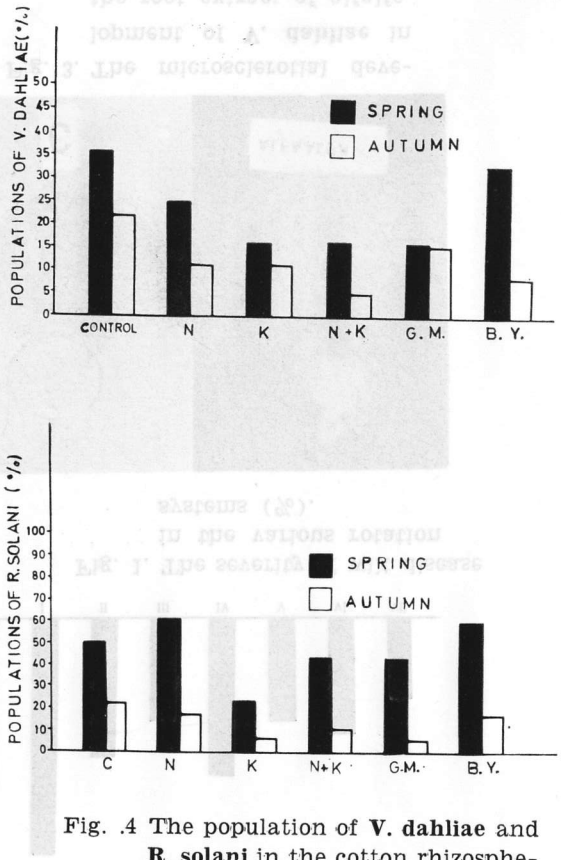


Fig. .4 The population of **V. dahliae** and **R. solani** in the cotton rhizosphere in the fertilizers experiments (%).

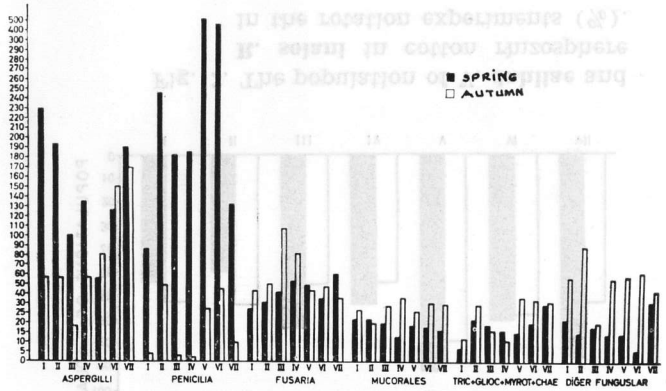


Fig. 5. Effects of rotation plants on the soil mycoflora.

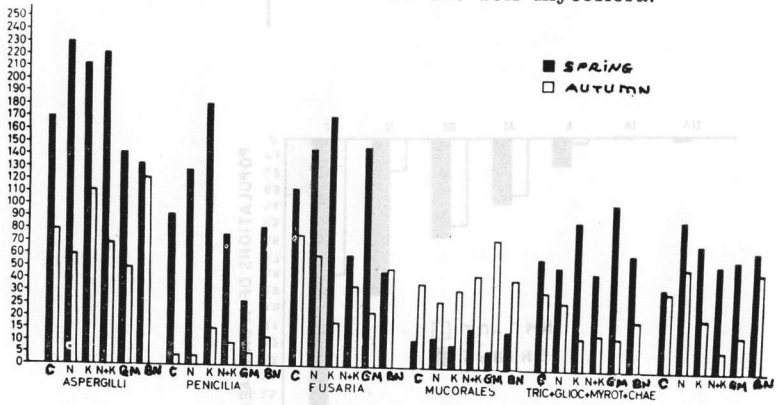


Fig. 6. Effects of fertilizers on the soil mycoflora.

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Investigations on the Determination of Susceptibility of Some Cotton Varieties Against Cotton Wilt Disease Caused by **Verticillium dahliae** Kleb.

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ABSTRACT

Susceptibilities of the twenty cotton varieties against **Verticillium dahliae** Kleb. were investigated by the field and pot experiments. Taşkent-1, Taşkent-2 and Taşkent-3 were found to be the most tolerant among the twenty cotton varieties against cotton wilt disease.

INTRODUCTION

Cotton has been growing in about 250.000 ha areas in the Aegean region. In this region, the most important problem of cotton is the cotton wilt disease. This disease has been observed here and there for about 40 years but it has gradually increased recently and threatened cotton growing due to the fact that the sensitive varieties were grown and the rotation and the sufficient cultural control were not carried out.

The cotton wilt was first reported by İyriboz (1941) from Kırkağaç in Turkey and the pathogen was determined as **Verticillium** sp. Studies were carried out to determine the causal agent in our region and the results showed that it was **V. dahliae** (Karaca et al, 1971).

The present study was done with the twenty cotton varieties during 1975-1977.

MATERIAL and METHODS

Del Cerro, DPL 15/21, Aleppo-1, Acala 4-42 (Uganda), Caroline Queen, Nazilli 66-100 (72-17), Nazilli 66-100, Acala 3080, Acala S.J.1, Taşkent-2, Taşkent-3, Coker 310, Coker 312, Coker 417, Coker 5110,

Coker 100 A/2, 149-F, Paymaster 18, Sealand 542 cotton varieties were tested.

1. The pot experiments

Each treatment was replicated seven times and the pots, 50 cm. diameter, were used. The inoculum prepared according to Zunnunov (1962) was added in to each pot and then cotton seeds were sown. Five cotton-seedlings were left in eachpot when they had four leaves.

The final observations were done at the end of the vegetation period according to the vascular browning of the cotton plants.

2. The field experiments

Field experiments were set up according to the randomized block desing with three replications in a naturally infested field in Regional Cotton Research Institute in Nazilli during 1976-1977.

In order to obtain a uniform infestation of the field, the inoculum prepared according to Zunnunov (1962) was given in course of the sowing. The plots were made by 2 rows each was 10 m. long. The disease intensity and severity for the each variety in the plots were estimated by using 0-3 scale. The statistical significance of the difference among the varieties was calculated by applying the Analysis of Variance test.

The pot and field experiments were repeated for three and two years respectively.

RESULTS

1. Pot experiments :

The average rates of the incidence of **V. dahliae** are shown in Table 1.

Table 1. The average rates of the incidence of **V. dahliae** for the cotton varieties tested in 1975-1976 and 1977 (%)

Varieties	1975	1976	1977
DPL 15/21	100	100	94
Caroline Queen	100	100	100
Sealand 542 X69-2	100	100	91
Aleppo-1	100	82	51
Coker 100 A/2	93	100	77
Paymaster	100	94	62
Coker 312	96	97	85
Coker 417	96	88	74
Coker 5110	93	48	62
149 F	93	85	77
Del Cerro	90	94	65
Nazilli 66-100 (72-17)	81	85	77

Table 1. Continuing)

Nazilli 66-100	75	82	68
Coker 310	83	85	71
Acala S-J-1	70	68	97
Acala 4-42	70	88	60
Acala 3080	63	77	94
Taşkent-1	56	48	31
Taşkent-2	63	42	57
Taşkent-3	80	48	45

From the above table, it is clear that Taşkent-1, Taşkent-2 and Taşkent-3 are more tolerant as compared to the all twenty cotton-varieties.

The rates of the incidence of **V. dahliae** according to the vascular browning and the disease severity by 0-3 scale are given in Table 2.

Table 2. The rates of the incidence of **V. dahliae** and the disease severity of the cotton varieties.

Varieties	Disease incidence (%) Years		Disease severity (%) Years	
	1976	1977	1976	1977
D.P.L. 15/21	100	96,6	91,4	53,3
Sealand 542	99,4	93,3	81,6	35,3
Caroline Queen	99,4	93,3	96,3	62,0
149 F	94,6	66,6	65,8	29,2
Coker 310	90,6	76,6	59,4	37,7
Paymaster 18	92,8	66,6	67,9	36,7
Coker 417	94,6	63,3	67,2	33,3
Aleppo-1	93,3	66,6	56,4	37,0
Coker 100 A/2 (75-151)	92,4	66,6	62,5	38,0
Del Cerro	84,9	73,3	50,9	38,7
Coker 5110	89,1	63,3	57,7	32,3
Coker 312	89,5	56,6	57,3	37,4
Nazilli 66-100 (72-17)	88,5	50,0	49,5	31,7
Nazilli 66-100	82,9	53,3	43,5	33,7
Acala 3080	65,5	50,0	37,0	31,7
Acala S.J.I.	68,5	46,6	31,7	23,7
Acala 4-42	81,5	43,3	39,7	31,3
Taşkent-3	6,6	10,0	3,1	3,7
Taşkent-2	13,5	6,6	6,4	1,0
Taşkent-1	9,8	3,3	4,9	0,3

Statistical analysis showed that Taşkent-varieties were followed by Acala S.J.1, Acala 3080, Acala 4-42 in 1976 and 149 F, Acala 4-42, Acala 3080 in 1977.

DISCUSSION

Twenty cotton varieties were tested by the pot and field experiments for the determination of susceptibilities against cotton wilt disease caused by **V. dahliae** in Aegean Region.

Taşkent-1, Taşkent-2, Taşkent-3 were found more tolerant than the other cotton-varieties by both pot and field experiments in three successive years. Milkovski and Bozhinov (1977) has also reported that Taşkent varieties were rather tolerant.

Although Acala S.J.1. and Acala 3080 showed low disease intensity in the pot experiments in 1975-1976, they showed high disease intensity in 1977. In the field experiments as well, Acala S.J.1. and Acala 3080 indicated low disease intensity and severity. Acala 4-42 generally seems more tolerant than the other varieties. It is reported to be tolerant by Garber and Houston (1967) as well.

According to the both pot and field experiments, it seems that DPL 15/21, Caroline Queen, Sealand 542 and Coker 100 A/2 were the most sensitive as compared to the other varieties. Besides, 149 F, Aleppo 1, Paymaster, Coker 312, Coker 310, Coker 417 and Del-Cerro were also found to be sensitive.

As the result of the present sutudy, twenty cotton-varieties were tested by pot and field experiments between the years of 1975-1977. Taşkent-1, Taşkent-2, Taşkent-3 were found to be tolerant. We came to a conclusion that Acala 3080, Acala S.J.1. Acala 4-42 (Uganda) should be taken in consideration but other varieties should not.

Ö Z E T

BAZI PAMUK ÇEŞİTLERİNİN **Verticillium dahliae** Kleb. FUNGUSUNUN NEDEN OLDUĞU SOLGUNLUK HASTALIĞINA KARŞI DUYARLILIKLARININ SAPTANMASI ÜZERİNDE ARAŞTIRMALAR

Ege Bölgesi pamuklarının en önemli sorunu Pamuk Solgunluk hastalığıdır. Hastalıktan korunmanın en etkin yolu kültürel önlemlerin yanısıra dayanıklı çeşitler yetiştirmektir. Bu çalışmanın amacı hastalığa dayanıklı, verimli ve bölge koşullarına uygun pamuk çe-

şitlerini elde etmek için yapılacak ıslah ve melezleme testlerine materyel teminidir.

Çalışma 1975-1977 yılları arasında saksı ve doğa'da yürütülmüştür. Denemelere muhtelif 20 pamuk çeşidi alınmış, bu çeşitlerin **V. dahliae**' ye duyarlılıkları saptanmıştır.

Saksı çalışmalarında saksılar yulafla üretilen inokulum ile bulaştırılmış, vegetasyon devresi sonunda bitkiler tek tek kesilerek sayımları yapılmıştır.

Tarla denemeleri Nazilli Bölge Pamuk Araştırma Enstitüsünün hastalıkla bulaşık bir tarlasında yürütülmüştür. 0-3 skalasına göre sayım yapılmış, çeşitlerin hastalığa yakalanma oranları ve hastalık şiddetleri bulunmuştur.

Denenen 20 pamuk çeşidi arasında Taşkent-1, Taşkent-2 ve Taşkent-3 çeşitleri pamuk solgunluk hastalığına tolerant olarak bulunmuşlardır.

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Investigations on the Inhibition of Potato Virus X (PVX) Infectivity by Some Plant Extracts

I. The Effectiveness of Extracts from Various Plant Species on Potato Virus X Infection and the Effects of Certain Factors on the Inhibitive Activity of Plant Extracts.

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ABSTRACT

In this study, the inhibitory effects of the extracts obtained from 18 different species of plants which were at two different stages of their vegetations on the infection of PVX were studied by test plants. According to the results, it was determined that the extracts of **C. annuum, V. vinifera** and **A. sterilis** plants at the actively growing stage and those of **C. annuum, P. avium** and **P. persica** plants at the end of vegetation inhibited PVX infection at high level. Among these extracts, it was found that the inhibitive activity of the extracts of **C. annuum** plants at both stages were not greatly influenced due to some factors. According to the findings obtained from this study, it can be thought that the inhibitors in these extracts have effect on the host plants rather than on virus. It was proved that proteins and phenolic substances in **C. annuum** extracts acted on the inhibition of PVX together.

INTRODUCTION

Potato is an important crop not only for food but also for industrial purposes, as it is rich in carbohydrates and proteins. The fact that the human nutrition have been established on especially starchy nutrients in the developing and under-developed countries increases the value of potato. Potato which makes it possible to obtain dry matter 2,7 times more than wheat per decar becomes a necessary crop for our country having to import wheat some years (46).

Potato the economical value of which has been mentioned above in a few words is extensively grown in Turkey. As seen in the production of every plant, various diseases, pests and weeds can give rise to the important crop losses in potato production. Among the potato diseases which cause crop losses of over 30 % in world potato production (9), potato virus diseases have more importance in comparison to others. Potato Virus X (PVX) is one of the world-wide and well-known potato virus diseases and was first described by Smith in 1931 (5). Some researchers indicate that PVX was considered as harmless to potato plants due to the fact that it generally causes latent infection or produces sometimes the scarcely visible symptoms on potato plants (4). Thus, PVX spreads easily by tubers obtained from plants taken into consideration as noninfected with this virus. On the other hand, it is known to all that PVX can also spread naturally by contact between healthy and infected plants during the cultural practices.

Since the descriptions of PVX and other virus diseases were made, numerous investigations have been carried out as to prevent them by means of the different control measures. In many instances, the use of resistant varieties, heat therapy and meristem cultures have proved to be suitable but, however, they can certainly produce definite results in control of virus diseases (6, 26, 40). For this reason, in some countries such as West Germany, Czechoslovakia, India, the U.S.A., Japon and England, the studies have been oriented for control of virus diseases by the natural materials (stuffs). In these natural materials, plant sap or extracts which are very simple to obtain and to apply occupy the first place.

It is known that some viruses transmitted by sap are, however, non-transmissible from certain host plants to others. This is generally due to the existence of a substance or substances called «inhibitor» in the sap of these host plants which prevents or inhibits the infection of virus when inoculated to a susceptible plant (3, 24). The presence of inhibitor (s) in plant sap or extracts was first taken into notice by Allard in 1914 (2). Upto now, many studies were made on the inhibition of especially Tobacco Mosaic Virus (TMV) infection (10, 17, 34, 42, 43) and the infections of other viruses (1, 20, 23, 32, 35, 39) by plant extracts. The number of the investigations ever made concerned with the inhibition of PVX infection is less. In the studies carried out on this matter, it was found that the extracts obtained from the leaves of **Capsicum annuum** L., **C. frutescens** L., **Chenopodium album** L., **C. amaranticolor** Coste and Reyn., **Datura metel** L., **D. stramonium** L., **Pelargonium hortorum** Bailey, **Pha-**

seolus vulgaris L., **Solanum tuberosum** L., **Spinacia olaracea** L. and **Trifolium repens** L. inhibited the infection with PVX (7, 11, 13, 14, 16, 27, 32, 39, 45). Moreover, some researchers determined that the bark extracts from some treas such as **Azadirachta indica** Juss., **Ficus species, Prunus persica** (L.) Batsch. and **Pyrus communis** L. were inhibitory on PVX infection (36, 37, 38, 44).

In the present study, the extracts obtained from 18 various species of plants at two different stages of their vegetations were tested for their inhibitory action on the infectivity of PVX. In addition, the effects of certain factors on the inhibitive activity were studied in some extracts promising to inhibit PVX infection.

MATERIAL and METHODS

The plant species used and the inhibitory effects on the infection of PVX of which have been examined in this study are as follows: **Avena sterilis** L., **Beta vulgaris** L., **Capsella bursa-pastoris** (L.) Medik., **Capsicum annuum** L., **Chenopodium amaranticolor** Coste and Reyn., **Citrus unshiu** Marc., **Datura stramonium** L., **Ficus caria** L., **Gossypium hirsitum** L., **Medicago sativa** L., **Prunus avium** L., **P. persica** (L.) Batsch., **Sinapis arvensis** L., **Solanum tuberosum** L. (Alpha-susceptible to PVX), **S. tuberosum** L. (Marijke-resistant to PVX), **Spinacia oleracea** L., **Vicia faba** L. and **Vitis vinifera** L.

In the present study, the bark samples were taken out from trees of only **P. avium** and **P. persica** while the leaf samples were collected from species of the remaining 16 plants. The bark and leaf samples were obtained from the healthy individuals of the be forementioned plants at two different stages of their vegetations (at the actively growing stage and the stage before the end of vegetation.) These samples were stored in deep-freezer at —30°C until used.

The strain 3 of PVX was used as inoculum throughout this study and was maintained in **Nicotiana tabacum** L. var. xanthi-nc plants.

Chenopodium amaranticolor test plants were used in local lesion assays for PVX and they were initially grown from seeds in 10 cm. clay pots at a temperature of 20-24°C in a glasshouse. Two or three weeks after sowing, individual seedlings were transplanted to 10 cm. plastic pots at the rate of one plant per pot. The test plants were usually ready for inoculation about two weeks after transplantation. At this time, they had 4-6 nodes. In all experiments, the upper four or five fully expanded leaves were used. In this study, **Gomphrena globosa** test plants were used to determine the existence of PVX in

extracts to be tested for the inhibitive activity. These plants as well were grown as mentioned above.

The plant extracts and inoculum were prepared as previously described by Firscher und Nienhaus (11) Lal et al. (17) and Singh (34, 35, 36).

To prepare the extracts to be used in the studies, each sample was homogenized with distilled water (1:1, w/v) in a homogenizer (20.000 rpm) for 5 minutes, the pulp filtered through double layer of muslin and the fluid extract was clarified by centrifugation (3.000 rpm for 30 min. at 4°C). The resulting supernatant was tested for the presence of PVX on **G. globosa** test plants and then, was used in studies.

The inoculum containing PVX was obtained by grinding the young sistemically infected leaves of **N. tabacum** var.xanthi-nc plants in a mortar with the addition of 1 ml. 0,02 M phosphate buffer, pH=7,2 for each gram of leaf material, pressing the pulp through double layer of muslin and centrifuging the extracted sap at 3.000 rpm for 30 min. at 4°C.

The inhibitory effects of the extracts under test on PVX were investigated according to half-leaf method (7, 11, 17, 34, 35). The extracts or extracts subjected to various treatments were mixed with inoculum (PVX) in equal proportions and 10 minutes after mixing, they were inoculated on 10 half leaves of **C. amaranticolor** test plants using celite as an abrasive.

The corresponding half leaves were inoculated with inoculum into which the distilled water was added at the ratio 1:1 (v/v) as control. In all experiments, the amount of inoculum and the number of strokes were constant. After inoculations, the leaves were rinsed with and the inoculated test plants were placed in a room which was illuminated for 16 h per day, with the light intensity of 4000-5000 lux and the temperature maintained 22 $\mp$ 2 C. The number of local lesions on each half leaf were counted within 6-9 days after inoculations and the inhibition (%) for each sample was estimated according to the following formula (28) :

$$\text{Inhibition (\%)} = 100 \left(1 - \frac{\text{number of lesions on treated half leaves}}{\text{number of lesions on control half leaves}} \right)$$

Prior to the statistical analysis, the data was transformed to angles from percentages. Then, the results were anlysed according to the Analysis of Variance test and L.S.D. test were applied when necessary.

Considering the results obtained from the experiments, plant extracts as of three which highly inhibited the infection of PVX at

high level at both of sampling stages were selected for using in the further steps of the investigation. The following experiments were carried out in order to study the effects of some factors (dilution, heating, storing **in vitro**, desiccation, pH and, the place and time of application) on the inhibitive activity of these selected extracts. Moreover, with the two plant extracts which inhibited PVX infection at the highest level at both of sampling stages, the attempts were made to isolate soluble proteins (18, 29, 30) and phenolic compounds (8, 15, 25, 41) and the inhibitory actions of these substances on the infection of PVX were investigated.

RESULTS and DISCUSSION

1. Inhibition of PVX infection by extracts from various plant species.

The results presented in Table 1 show that the extracts of **C. annuum, V. vinifera** and **A. sterilis** plants at the actively growing stage and those of **C. annuum, P. avium** and **P. persica** plants at the stage before the and of vegetation inhibited the infection of PVX at higher levels in comparison with others.

The promising results which we obtained to inhibit the PVX infection by the extracts of **C. annuum** at both stages and **P. persica** plants are agreeble with the records of the researches carried out earlier (7, 11, 27, 31, 36, 38). Although there are not any reports on the inhibition of PVX infection by the extracts obtained from plants of **V. vinifera** and **P. avium**, it was experimentally found that they inhibited the infection of some viruses such as Tobacco Ring Spot Virus (Tob RSV), Tomato Ring Spot Virus (Tom RSV) and Alfalfa Mosaic Virus (AMV) (15, 21, 22).

As shown in Table 1, the plant extracts of **C. bursa-pastoris** and **V. faba** at the actively growing stage and, the extract from **B. vulgaris** plant at the stage before the end of vegetation augmented the PVX infection. The results that obtained on the effectivenes of these three extracts and other extracts on the infectivity of PVX, in this study are in agreement with the results of the other studies on the same subject (7, 13, 14, 27, 31, 36, 37, 39, 45).

In the present study, the effectiveness, of the extracts obtained from the plants of **A. sterilis, B. vulgaris, C. bursa-pastoris, C. unshiu, G. hirsitum, M. sativa, P. avium, S. arvensis** and **V. vinifera** on the infectivity of PVX are investigated for the first time.

The different levels of effectivenesses produced by plant extracts in these studies on the infectivity of PVX are presented in Fig. 1.

Table 1. The inhibitory effects of some plant extracts on the infectivity of PVX.

Actively growing stage		Stage before the end of vegetation	
Extract	Inhibition (%)[1]	Extract	Inhibition (%)
C. annuum	85,95 a[2]	**C. annuum**	92,67 a
V. vinifera	76,38 b	**P. avium**[3]	89,73 ab
A. sterilis	72,69 bc	**P. persica**[3]	88,20 b
C. unshiu	63,69 c	**G. hirsitum**	82,83 c
C. amaranticolor	49,59 d	**C. amaranticolor**	82,51 c
S. oleracea	49,20 d	**D. stramonium**	80,28 cd
F. carica	46,67 d	**F. carica**	75,98 de
C. hirsitum	38,54 de	**S. oleracea**	72,49 ef
S. tuberosum «Narijke»	33,49 e	**M. sativa**	68,50 fg
P. avium[3]	29,44 ef	**V. vinifera**	67,46 fg
P. vulgaris	20,97 fg	**A. sterilis**	67,34 fg
P. persica[3]	17,86 gh	**S. tuberosum** «Narijke»	65,85 g
M. sativa	15,50 gh	**C. bursa-pastoris**	59,55 h
S. tuberosum «Alpha»	15,39 gh	**S. tuberosum** «Alpha»	54,97 h
S. arvensis	13,82 hi	**V. faba**	48,23 i
D. stramonium	6,02 i	**C. unshiu**	24,52 j
C. bursa-pastoris	—5,54 j	**S. arvensis**	24,28 j
V. faba	—7,15 j	**B. vulgaris**	—13,81 k

[1] Figures indicate the average of inhibition (%) obtained from 10 replications

[2] Statistical groups

[3] The bark extracts of these plants were used in the experiments.

2. The effects of certain factors on the inhibitive activity of the some plant extracts.

Since the extracts of **C. annuum**, **V. vinifera** and **A. sterilis** plants at the actively growing stage and those of **C. annuum, P. avium** and **P. persica** plants at the stage before the end of vegetation appear to be most effective in inhibiting the PVX infection, further studies were made with only these six extracts. The results of studies that carried out to determine the effects of some factors, given below, and the inhibitive activity of these extracts are summarized in Table 2.

Dilution: The plant extracts were 10^{-4} with distilled water. Equal volumes of PVX were added to each dilution and these mixtures were inoculated to the leaves of test plants.

The results in Table 2 show that the dilution decreased the inhibitive property of all extracts under

test. At a dilution of 10^{-2}, the extracts from only **C. annuum** plants at both stages inhibited the infection of PVX appreciably as also indicated by Blaszczak et al. (7) and Rao and Raychaudhuri (27). Moreover, it was reported that, in general, the plant extracts can be diluted to 10^{-1} and 10^{-2} with distilled water without losing their inhibitory activity (7).

Heating: The extracts were placed in the glass tubes and heated for 10 minutes in a water bath at various temperatures. After heating, the tubes were inmediately cooled in running tap water. The cooled extracts were mixed with equal volumes of PVX and inoculated.

Table 2. The effects of certain factors on the inhibitive activity of the some plant extracts.

	Inhibition (%)[1]					
	Actively growing stage			Stage before the end of vegetation		
Factors	**C.annuum**	**V.vinifera**	**A.sterilis**	**C.annuum**	**P.avium**[2]	**P.persica**[2]
Dilution						
Undiluted	85,95 a[3]	76,38 a	72,69 a	92,67 a	89,73 a	88,20 a
10^{-1}	82,77 b	38,41 b	67,90 a	72,37 b	51,42 b	61,33 b
10^{-2}	62,98 c	26,71 c	26,43 b	61,47 c	22,07 c	32,06 c
10^{-3}	26,69 d	14,45 d	24,08 b	19,52 d	10,53 d	28,02 c
10^{-4}	3,88 e	8,33 e	13,94 c	6,50 e	7,05 e	8,32 d
Heating (°C)						
Unheated	85,95 b	76,38 a	72,69 a	92,67 b	89,73 a	88,20 a
40	91,76 a	66,96 b	7,01 b	93,64 ab	43,61 b	56,91 b
50	87,42 b	66,77 b	3,16 cd	94,54 a	43,88 b	57,44 b
60	86,80 b	67,07 b	2,26 d	83,81 c	43,07 b	54,70 b
70	74,98 c	68,08 b	2,11 d	72,67 d	24,42 c	46,64 c
80	65,36 d	67,00 b	2,12 d	60,20 e	19,43 d	32,93 d
90	54,66 e	66,64 b	1,72 d	59,92 e	9,05 e	5,95 e
100	50,54 e	26,65 c	4.70 c	61,38 e	6,86 f	5,14 e
Storing in vitro (in days)						
Unstored	85,95 b	76,38 a	72,69 a	92,67 a	89,73 a	88,20 b
2	84,00 bcd	19,99 e	70,45 ab	92,39 a	90,37 a	92,31 a
4	85,71 bc	46,92 b	64,46 bc	91,35 ab	87,27 b	91,50 a
6	85,46 bc	32,84 c	58,03 c	91,48 ab	85,10 c	91,13 a
8	81,29 d	26,26 d	57,73 c	89,61 b	84,84 c	89,53 b
10	82,00 cd	11,94 f	21,96 e	91,73 ab	86,36 bc	89,32 a
100	92,93 a	26,69 d	32,70 d	54,56 c	39,82 d	51,75 c

Table 2. (continuing). The effects of certain factors on the inhibitive activity of the some plant extracts.

Desiccation						
Control	85,95 a	76,38 a	72,69 a	92,67 a	89,73 a	88,20 a
Desiccated	68,97 b	24,24 b	18,00 c	45,86 c	15,86 b	17,71 b
After 1 week	65,94 b	17,99 c	18,25 c	46,37 c	6,26 c	10,30 c
After 2 weeks	54,72 c	6,64 d	38,87 b	54,60 b	8,30 c	5,59 d
After 4 weeks	57,65 c	7,15 d	35,26 b	52,73 b	—8,01 d	—6,61 e
pH						
Original pHx	85,95 c	76,38 a	72,69 a	92,67 a	89,73 a	88,20 a
Original pH+2	93,52 a	37,66 b	21,62 c	91,43 ab	40,54 c	48,36 b
Original pH+4	92,80 ab	29,01 c	12,27 d	92,18 ab	26,93 e	41,43 c
Original pH+2	92,21 ab	30,80 c	15,76 d	92,11 b	46,49 b	16,87 d
Original pH+4	91,19 b	33,94 bc	33,66 b	89,41 c	31,28 d	47,18 b
	6,35x	4,30x	5,20x	5,60x	4,50x	4,70x
Application place of extract						
Upper surface	85,95 b	76,38 a	72,69 a	92,67 b	89,73 a	88,20 a
Lower surface	92,99 a	17,49 b	16,36 b	95,23 a	46,93 b	29,37 b
Application time of extract						
48 hr before inoculation	42,75 c	—11,56 g	22,45 b	37,95 d	10,68 g	12,57 g
24 hr before inoculation	43,40 c	3,68 g	22,87 b	44,56 c	10,76 fg	14,77 e
12 hr before inoculation	54,83 b	10,44 d	22,84 b	39 92 cd	13,65 f	18,41 d
4 hr before inoculation	52,50 b	19,26 bc	20,50 b	42,23 cd	30,13 c	39,82 b
2 hr before inoculation	56,12 b	9,14 d	23,28 b	52,11 b	41,68 b	42,86 b
The same time with virus	85,95 a	76,38 a	72,69 a	92,67 a	89,73 a	88,20 a
2 hr after inoculation	32,84 d	22,76 b	20,50 b	19,77 e	24,65 d	22,86 c
4 hr after inoculation	25,29 e	18,62 c	19,14 b	15,67 f	18,23 e	22,72 c
12 hr after inoculation	22,11 e	10,37 d	12,87 c	10,04 g	13,13 f	13,78 ef

Table 2. (continuing). The effects of certain factors on the inhibitive activity of the some plant extracts.

24 hr after inoculation	13,19 f	6,04 e	13,63 c	9,87 g	13,46 f	12,72 e
48 hr after inoculation	7,56 g	4,96 ef	12,00 c	9,42 g	12,87 fg	8,09 g

[1] Figures indicate the average of inhibition (%) obtained from 10 replications

[2] The bark extracts of these plants were used in the experiments.

[3] Statistical groups.

According to the figures in Table 2, heat treatments caused the decrease of the inhibitive activity in all extracts. It was determined that the inhibitory effects of the extracts of **C. annuum** at both stages and **V. vinifera** plants did not totally lose by heating at 100°C (Table 2). The present study, as well as other reports (7, 12, 39) indicated that heating almost completely destroyed the inhibitive activity in some extracts and not in others. Blaszczak et al. (7) reported the inhibitors of PVX in **Capsicum** species that resist dilution also resist heating, but this is not true for all extracts.

Storing **in vitro**: The extracts to be tested were stored in stoppered vials at room temparature (18-20°C) at different intervals of time. At the end of these periods, the stored extracts were mixed with equal volumes of PVX and inoculated to leaves of test plants.

As it can be seen in Table 2, during 20 days of storage, no change of inhibitory action was found in the extracts, except for **V. vinifera** and **A. sterilis**, but, when they were stored for 100 days, only the extract of **C. annuum** plant at the actively growing stage did not lose its ability to inhibit the infection of PVX. The mentioned extract of **C. annuum** plant was more inhibitory than control on PVX (Table 2) as also indicated by Blaszczak et al. (7) and Singh and Singh (38) previously.

Desiccation: The plant extracts were desiccated at room temperature (18-20°C) in a desiccator. After 62 days, all extracts under test completely dried. The plant extracts were made upto their original volumes with distilled water at four different times (when desiccated and 1, 2, 4 weeks after this) and tested for the inhibitory activity by mixing in equal proportions with PVX.

The results presented in Table 2 show that, although they were desiccated, the extracts of **C. annuum** plants at both stages inhibited the virus infection by 57, 65 and 52, 73 percent, respectively whereas the extracts of **P. avium** and **P. persica** plants augmented the infection.

Feldman (10) and Marchoux (19) also reported that desiccation did not affect much the inhibitory activity of seed and leaf extracts obtained from **C. annuum** plants.

pH: The original pH values of the plant extracts under test were increased and reduced at the levels of 2 or 4 using 1 N sodium hydroxide and 0,1 M citnic acid. After 20 minutes, the pH values of these extracts were readjusted to their original values by the same solutions. The extracts subjected to the different pH values were mixed with equal volumes of PVX separetely and inoculated to tests plants.

As it is shown in Table 2, the changes in pH did not affect the inhibitory action of the extracts of **C. annuum** at both stages, but considerably reduced the inhibition of PVX by other four extracts. Feldman (10) also found that the seed extract of **C. annuum** was inhibitory to TMV at pH values ranging between 1,0 and 12,0.

Application of plant extracts to lower surfaces of leaves:

The plant extracts were applied to lower leaf surfaces of test plants by muslin cloth saturated with extracts. The upper surfaces of the same leaves were inoculated with PVX.

The results given in Table 2 show that when the extracts from **C. annuum** plants at both stages were applied to the lower leaf surfaces of test plants, their ability to inhibit the infection of PVX was increased. On the other hand, it was observed that the other four extracts in this study greatly lost their inhibitory effects on PVX infection as the result of same applications (Table 2). It follows from the findings of the present work and present work and previous reports (10, 19, 20) that the inhibitory activity in the extracts of **C. annuum** plants is translocatable between lower and upper surfaces in a leaf and also, from a leaf to another opposite leaves, as vertical in former and lateral in latter, respestively.

Application of plant extracts before and after inoculation with virus: The plant extracts were applied on half leaves of test plants at intervals of 2, 4, 12, 24 and 48 hours before and after PVX inoculation. The corresponding half leaves were treated with distilled water.

As shown in Table 2, it was found that the inhibitory activity in extracts decreased rapidly with the number of hours elapsed after applications when the extracts were applied to test plants after virus inoculation. From these results, it became obvious that the inhibitors in the extracts did not induce a resistance to the virus infection in the plants as also indicated by Yoshizaki and Murayama (47). When the extracts in the present work were applied 48 hours (the longest period tested) before virus inoculation, the highest inhibition on PVX infection was obtained from the extracts of **C. annuum** plants at both stages (42,75 and 37,95 per cent respectively) as

shown in Table 2. In our opinion, inhibitors in extracts used in this study combine with the infectible sites (33) which exposedor created on the leaves by an abrasive or wounding and as a result of this combination, the entry points of virus into plants are blocked or destroyed. On the other hand, it was found that when some extracts were applied to the lower leaf surfaces and the upper surfaces were inoculated with virus, there was no change in the inhibition of PVX by these extracts (Table 2). According to these results, it can be thought that inhibitors in **C. annuum** plant extracts can move in the host plants and act by altering the metabolism of the host cells so that they are no more suitable for virus establishment. Moreover, Feldman (10) and Marchoux (19) reported that the inhibitors in **C. annuum** plants influenced the susceptibility of the host plants to TMV and thus, inhibited the virus infection.

In conclusion, it can be said that inhibitors in the extracts used in this study have effect on the host plants rather than on virus and prevent the infection.

The attempts to determine the chemical nature of inhibitors in **C. annuum** plants: Several attempts were made to isolate the inhibitory substances in the extracts from **C. annuum** plants. For this reason, the methods used previously by other workers were modified in compliance with the instruments and material available. The obtained results from this study show that soluble proteins and phenolic compounds in **C. annuum** extracts acted on the inhibition of PVX infection together (Table 3).

Table 3. The inhibitory action of some substances isolated from **C. annuum** plant extracts on PVX infection.

The substances isolated	Inhibition (%)[1]	
	Actively Growing Stage	Stage before the end of vegetation
	C. annuum	**C. annuum**
Control[2]	5,37	6,69
Soluble proteins	73,58	54,38
Phenolic compounds	21,54	61,24

[1] Figures indicate the average of inhibition (%) obtained from 10 replications

[2] *The plant extract soluble proteins of which were denatured and* phenolic compound of which were removed.

In the previous studies, Marchoux (19), Fischer und Nienhaus (11) and Rao and Raychaudhuri (27) determined that the inhibitory power in **C. annuum** plants was connected with proteins and phenolic substances. Moreover, the sharp or gradual reduction of inhibition with dilution and heat treatments in extracts indicates the presence of the different inhibitory substances in the tested extracts, as also reported by Singh (35).

According to the results from the studies conducted on tests plants, it can be found that the before mentioned factors affected less the inhibitory activity in **C. annuum** plants than in other plants. Although the promising results are being obtained with **C. annuum** plants to prevent the PVX infection, some considerations must be taken into account for practical purposes. Firstly, the studies to be carried out as to the intake of the extracts by plants, transportations of them in plants and their mode of action can provide some facilities for protecting the plants from virus diseases. Secondly, it as necessary to study the effectiveness of the extracts on the virus infections in natural conditions for their practical usage. The second part of this work will deal in more detail with this subject.

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Ö Z E T

BAZI BİTKİ EKSTRAKTLARI İLE PATATES X VİRUSU (PVX)'NUN İNFEKSİYON OLUŞTURMA YETENEĞİNİN ENGELLENMESİ ÜZERİNDE ARAŞTIRMALAR

I. Değişik Bitkilerden Elde Edilen Ekstraktların PVX İnfeksiyonuna Etkileri ve Bitki Ekstraktlarının Engelleyicilikleri Üzerinde Bazı Faktörlerin Etkileri.

Bu çalışmada 18 değişik bitkiden vejetasyonlarının farklı iki evresinde elde edilen ekstraktların, PVX infeksiyonuna olan etkileri test bitkilerinde araştırılmıştır. Deneme sonuçlarına göre, aktif gelişme evresindeki **C. annuum, V. vinifera** ve **A. sterilis** ile gelişmenin yavaşladığı evredeki **C. annuum, P. avium** ve **P. persica** bitkilerinden elde edilen ekstraktların PVX infeksiyonunu yüksek düzeyde engelledikleri saptanmıştır. Bu ekstraktlar arasında, her iki evredeki **C. annuum** eks-

traktlarının engelleyiciliklerinin, bazı faktörlerden fazla etkilenmediği bulunmuştur. Elde edilen bulgulara göre, bu ekstraktlardaki inhibitörlerin virustan çok konukçu bitki üzerinde etkili oldukları düşünülmektedir. PVX inhibisyonunda, **C. annuum** ekstraktlarındaki proteinlerin ve fenolik bileşiklerin birlikte rol oynadıkları saptanmıştır.

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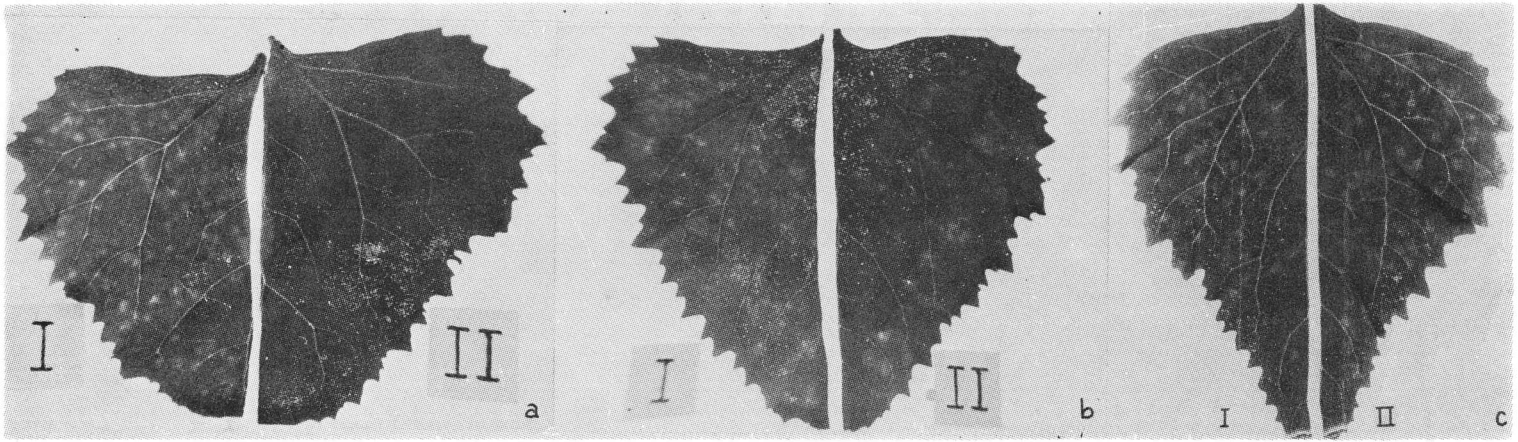

Figure 1. The different types of effectivenesses exposed by plant extracts used in the studies on PVX infection.

a. Inhibition (at high level)
b. Inhibition (at low level)
c. Augmentation

I : PVX+Distilled water (Control)
II : PVX+Plant extract.

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Rew Record

A New Host of **Verticillium dahliae** Kleb. in Turkey.

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The typical symptoms of **Verticillium** wilt on American grapevine rootstocks were first observed in Manisa during a study in September 1981. The symptoms were characterized by stunting, yellowing and inward rolling marginal drying and wilting of the leaves of the 2 year-old 41B and 5BB rootstocks. The browning on the cross section of the shoots of the affected rootstocks was also another characteristic symptom of the disease. The disease incidence observed in vineyard was 15 percent.

Isolation studies were made by using standart methods and **Verticillium dahliae** Kleb. was isolated.

ÖZET

TÜRKİYE'DE VERTICIILLIUM DAHLIAE KLEB'İN YENİ BİR KONUKÇUSU

Manisa'da 1981 yılı Eylül ayında yapılan bir çalışma sırasında Amerikan asma anaçlarında tipik solgunluk belirtileri dikkati çekmiştir. Gelişme geriliği gösteren 2 yaşındaki 41B ve 5BB anaçlarının yapraklarında sararma, kenarlardan başlıyan kuruma ve içe doğru bükülmeler karakteristik **Verticillium** solgunluğu görünümündeydi. Hasta sürgünlerin enine kesitlerinde görülen iletim borularındaki kahverengimsi renk değişikliği de söz konusu hastalığı karakterize eden bir diğer belirtiydi. Böyle bitkilerin söz konusu bağdaki bulunuş oranı % 15 civarındaydı.

Belirti gösteren bu anaçlardan yapılan izolasyon çalışmaları, hastalık etmeni fungusun **Verticillium dahliae** Kleb. olduğunu ortaya koymuştur.

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