ISSN 0378 - 8024

THE JOURNAL OF TURKISH PHYTOPATHOLOGY

PUBLISHED BY THE TURKISH PHYTOPATHOLOGICAL SOCIETY

Volume : 24 Number : 1 Jan : 1995

TURKISH PHYTOPATHOLOGICAL SOCIETY

The Journal of Turkish Phytopathology is published by Turkish Phytopathological Society and issued twice or three times a year to from volume. The subscripton rate per volume is $ 21.00

EGE ÜNİVERSİTESİ BASIMEVİ

BORNOVA - İZMİR, 1995

THE JOURNAL OF TURKISH PHYTOPATHOLOGY

TURKISH PHYTOPATHOLOGICAL SOCIETY

VOL. 24 JAN NO. 1

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J. Turk. Phytopath., Vol. 24, No. 1, 1-5, 1995 ISSN 0378 - 8024

Recovering of Virus Free Grapefruits by Shoot-Tip Grafting and Thermotherapy at Two Temperature Regimes

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ABSTRACT

Seven grapefruit varietes found to be infected with stubborn, exocortis, psorosis and cachexia as determined by biological indexing and/or Elisa were treated by two different techniques to eliminate virus and virus-like diseases. One group was handled with thermotherapy at 40°C and Shoot-Tip Grafting (STG) and the second group with thermotherapy set at 25-35°C plus STG.

Stubborn and exocortis were eliminated by thermotherapy at 40°C plus STG by 100%, but the psorosis group was eliminated by only 62%. Lower temperature during thermotherapy had no influence on the elimination of stubborn and exocortis but delimination rate of psorosis decrased to only 48%.

INTRODUCTION

Citrus virus and virus-like diseases cause very important production losses in all Turkish citrus growing areas (Çınar *et al.*, 1993). They cause decline loss of vigour, low yield and quality as well as a short commercial life of trees. In Türkiye, they are the main limitations for the development of the citrus industry.

To improve productivity and to control diseases it is necessary to provide and to use healthy and high quality trees in new plantations. The production of such trees requires at least a sanitation program but works only properly if also a quarantine and certification program is included.

Objective of a sanitation program is to recover healthy plants from selected local or imported cultivars. Pathogen free tested plants can be obtained by STG **in vitro** with or without thermotherapy. STG is effective for elimination of all virus and virus-like pathogens and produce true type plants without juvenile characters (Navarro *et al.*, 1975, Navarro, 1988). There are many factors that influence the recovery of healthy plants by STG, especially shoot tip size and temperature (Navarro *et al.*, 1976; Navarro, 1981).

In this paper we present the recovery of healthy grapefruit cultivars by STG and different temperature regimes during thermotherapy.

MATERIALS AND METHODS

In this study seven different grapefruit cultivars were used: Budwood of Duncan, Marsh Seedless, Star Ruby, Ruby Red and Henderson were collected from the citrus germplasm collection at the Çukurova University, and Rio Red and Rey Ruby from commercial orchards in Abdioğlu near Adana.

Collected budwood were propagated on ten, one year old sour orange seedlings for each variety. Each sour orange was planted in a three liter container filled with a mixture of sand, peat and pumice (1: 1: 1:). Propagations were kept in a partly shaded greenhouse cooled by evaporation coolers at 25-28°C temperature.

Virus and virus-like diseases in the source plant were diagnosed by biological indexing according to Roistacher (1991) and Elisa for Stubborn disease following the protocol of Saillard and Bove (1983). Antisera for the detection of **Spiroplasma citri** was prepared and provided by Dr. J.M. Bove, INRA BORDEAUX. At least four indicator plants were inoculated for each disease and source tree and one healthy control and one positive control, if available, were added. Test trees were indexed for citrus tristeza virus (CTV) on Mexican lime. To detect and identify psorosis groupe, Madame Vinous indicator plants were used. After inoculation all indicator plants were grown under cool temperature (25°C maximum day and 20°C minimum night). The citrus exocortis viroid (CEVd) was identified on Etrog citron (Arizona 861 S-1) and citrus cachexia viroid (Ca) on Parson's Special mandarin kept under warm conditions (35°C day and 27°C night).

The success of two temperatures regimes in combination with STG was tested on all varieties. For this purpose, each of ten propagated plants were divided into two groups. One group was pre-treated at 25-35°C, 60% relative humidity and 16 hours artificial light (10.000 Lux) in climatic chamber for 8 weeks. After this period, thermotherapy was at 40°C at a day time (16 hours) and 30°C during night time for another 8 weeks in the same climatic chamber (CALAVAN *et. al.*, 1972). The second group were placed in greenhouse, adjusted to 25-35°C, 60% relative humidity for 16 weeks.

Following these thermotherapy treatments all plants were defoliated to force growth of new shoots. New shoots were used in vitro STG following the method of Murashige *et al.* (1972), and Navarro *et al.* (1975). 3-6 weeks after STG the young shoots were directly grafted onto well established rough lemon seedlings according to De Lange (1978) and kept at 25-22°C (d/n) temperature in a greenhouse for 3-4 months. Three plants from each variety were selected and reindexed as describe above.

RESULTS AND DISCUSSION

No field symptoms attributable to virus and virus-like diseases were observed on the 7 selected grapefruit varieties. However, according to biological indexing and Elisa the trees were infected with several diseases (Table 1).

Table 1. The presence of virus and virus-like diseases in seven selected grapefruit varieties as diagnosed by biological indexing and Elisa.

	Psorosis	Exocortis	Cachexia	Tristeza	Stubborn
Duncan	+	-	-	-	+
Marsh Seedless	+	-	-	-	-
Star Ruby	+	+	+	-	-
Ruby Red	+	-	-	-	-
Rio Red	+	-	+	-	-
Henderson	+	+	-	-	-
Rey Ruby	+	+	-	-	-

(-) healthy, (+) infected

According to the reindexing results, there was no effect of different temperature regimes during thermotherapy on recovery of stubborn and exocortis free plants. All plants on which thermotherapy and STG were applied, were found free exocortis and stubborn. In opposite, different rates of elimination of the psorosis group were obtained using two different temperatures regimes.

Psorosis was eliminated in 13/21 plants (62%) if warmer temperature was applied during thermotherapy and in 10/21 plants (48%) if kept at cooler temperature (Table 2). No cachexia symptom was observed on Parson's Special in reindexing after eight months. To detect mild cachexia symptom, the plants will be diagnosed again after 18 moths. The results of this indexing are forthcoming.

Table 2. Eliminating rates of plants from virus and virus-like diseases by two temperature regimes and STG.

	Rates of Eliminating (%)	
	Cooler temp. + STG	Warmer temp. + STG
Psorosis	47.61	61.90
Exocortis	100	100
Stubborn	100	100

Already Navarro *et al.* (1980) studied the influence of temperature on elimination of Psorosis-like pathogens (PLP). They obtained only 7-10% PLP free plants if the source plants for STG were kept at 18-25°C. However, this percentage increased up to 69% when source plants were held at 27-32°C. The elimination of exocortis xyloporosis-cachexia, tristeza were not affected by this temperature. Using the routine procedure of the Citrus Variety improvement Program in Spain (Navarro, 1993) more than 90% of plants recovered by STG were free of virus and virus-like pathogens including those that are difficult to eliminate (Navarro *et al*, 1988).

Navarro *et al.* (1976) proved that increasing shoot tip size resulted in high incidence of succesful grafts but also lower number of healthy plants recovered. The relatively low number of virus free plants obtained from thermotherapy at warm temperature and STG in our experiments may be due to the use of too large shoot tips. However, if source plants of shoot tip grafting were defoliated and kept at warm temperature, the number of recovery of healthy plants would be considerably higher compared to those plants held at relatively cool temperature.

ÖZET

SÜRGÜN UCU AŞILAMA VE İKİ FARKLI TERMOTERAPİ ISI UYGULAMASI İLE VİRÜSTEN ARİ GREYFURT ELDE EDİLMESİ

Bu çalışmada farklı yedi altıntop çeşidinde (Marsh Seedless, Duncan, Star Ruby, Ruby Red, Rio Red, Rey Ruby ve Henderson) bulunan virüs ve virüs benzeri hastalık etmenlerinin, termoterapi ve **in vitro** Sürgün Ucu Aşılama (SUA) yöntemlerinin kombine edilerek uygulanması ile arındırma oranlarının saptanması amaçlanmıştır. Ayrıca termoterapi olarak adlandırılan sıcaklık uygulamasında bitkiler SUA'ya alınmadan önce, iki farklı sıcaklık uygulanmış ve bu sıcaklıkların arındırmaya olan etkisi de karşılaştırılmıştır.

Çalışmanın ana kaynağını oluşturan materyallere, uygulanan biyolojik indeksleme ve serolojik tanı metodu (Elisa) ile stubborn, psorosis, exocortis ve cachexia etmenleri tesbit edilmiştir. Bu patojenlerin arındırılmasına yönelik olarak yapılan çalışmada termoterapi ve sıcak kabin + **in vitro** SUA yöntemleri uygulanmıştır. Elde edilen bitkilerde arındırma oranlarını saptamak amacıyla, tekrar biyolojik indeksleme ve Elisa yapılmıştır. Exocortis ve stubborn, termoterapi veya 32°C sıcaklık uygulaması ile **in vitro** SUA'nın kombine edilmesi sonucu %100 oranında arındırılırken psorosis termoterapi + **in vitro** SUA ile %62 oranında, 32 °C 'lik sıcaklık uygulaması + **in vitro** SUA ile %48 oranında arındırılmıştır.

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J. Turk. Phytopath., Vol. 24, No.1, 7-14, 1995 ISSN 0378 - 8024

Virus Diseases of Vegetables in Greenhouses in İzmir and Muğla

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ABSTRACT

According to the survey results, it was found that the rate of existence of mosaic virus was 7.3 % on tomato, 2.4 % on pepper, 3,6 % on cucumber and 0,3 % on eggplant and their widespread area was 30.7-100 %, 70.5 - 100%, 37,7-50% and 100% respectively in greenhouses in İzmir and Muğla.

During the surveys in İzmir in 1992, in Muğla in 1993 and 1994, the leaf samples were collected from vegetables showing virus-like symptoms in the period of flowering and fruiting. The leaf samples were investigated by elisa test, the samples giving positive results by elisa test then were subjected to mechanical inoculation.

As a result of mechanical inoculation tests, tobacco mosaic virus, tomato mosaic virus + potato x virus on tomatoes; tobacco mosaic virus on pepper and cucumber mosaic virus on cucumbers have been found in İzmir.

As a result of Elisa test, tomato spotted wilt virus, tomato ring spot virus and tomato black ring virus on tomatoes; tomato ring spot virus on peppers; tomato ring spot virus and tomato black ring virus on cucumbers and tomato black ring virus on eggplants have been found in Muğla. Tomato ring spot virus and tomato black ring virus are new records for Türkiye.

INTRODUCTION

The Marmara, Aegean with Mediterranean coats are the most important greenhouse areas of Türkiye and there is about 85.331 da of the greenhouse area in these regions and the production of these regions reached about 750 tons in 1990. Production of vegetables in the greenhouses is about tomato 85 %, cucumber 27 %, pepper 80 % and eggplant 5 % (Yüce 1990).

The greenhouses have mainly been established in İzmir and Muğla provinces of Aegean Region. Vegetables in the greenhouses are infected with the most virus diseases. Tomato greenhouses have been infected with TMV in Antalya (Demir and Erdiller 1980). Tekinel *et al* (1969) have been found tomato mosaic virus and cucumber mosaic virus on tomatoes and potato x virus on pepper in İçel. As a result of study done by Güllü and Çalı (1991), tobacco mosaic virus and cucumber mosaic virus on toma-

toes, peppers and cucumbers, tomato mosaic virus + potato x virus on tomatoes; eggplant mosaic virus on eggplant were found in the greenhouses of Adana.

The present study has been carried out in order to find out the virus diseases affecting tomato, pepper, cucumber and eggplant in the greenhouses in İzmir (1992) and Muğla (1993-1994).

MATERIALS and METHODS

Leaves of tomato, pepper, cucumber and eggplant showing virus like symptoms were collected from greenhouses in İzmir and Muğla (Table 1). In February and May at least 169 samples were collected in 1992, 1993 and 1994. The total samples included 136 from tomato, 15 from pepper, 10 from cucumber, 8 from eggplant diseased leaves were homogenized in a mortar with addition of a little (w/v:1/10) 0,01 M Phosphate buffer (pH. 7.0) than small amount of 500 mesh carborandum powder was added to the inocula as an abrasive before inoculation of test plants (**Chenopodium amaranticolor, C. quinoa, Nicotiana glutinosa, N. clevelandii, N. tabacum** "White Burley" **Datura stramonium, Gomphrena globosa**). The test plants were observed after inoculation for symptom development. Methods of surveys and determination of disease ratio were carried out according to Bora and Karaca (1970).

All plants were tested serologically for the presence of tomato mosaic virus, tomato black ring virus, tomato ring spot virus and tomato spotted wilt virus by Elisa, using a polyclonal antibody with detection kits of Loewe firm. The results were evaluated at 405 nm, absorbance values over 0.10 were considered positive (Clark 1981).

Reagents

Coating reagent: Dilution of the anti, TBRV, TRSV and ToMV-IgG with coating buffer 1/200 and TSWV 1/500 (v/v).

Antibody-AP. Conjugate reagent: Dilution of the antibody-AP-conjugate with sample buffer TSWV 1/500 others 1/200 (v/v).

Substrate reagent: Dilution of 4-nitrophenylphosphate with sample buffer 1/1000 (w/v).

RESULTS and DISCUSSION

According to the survey results, it was found that the rate of existence of mosaic virus was 7.3% on tomato, 2.4% on pepper, 3.6% on cucumber and 0.3% on eggplant and their widespread area was 30.7-100%, 70.5-100%, 37.7-50% and 100% respectively in greenhouses in İzmir and Muğla (Table 1).

Mechanical inoculations were applied to the test plants and 24 virus isolates were identified from 34 samples (tomato, pepper and cucumber) in İzmir (Table 2). 11 tobacco mosaic virus and 3 tomato mosaic virus + potato x virus were found from 19 tomato samples. According to the survey results, one of the most important virus diseases is tomato mosaic virus + Potato x virus on tomato for greenhouses in İzmir. Same virus disease was identified on tomatoes, peppers and cucumbers in Türkiye by Heper, (1974); Yorgancı (1975), Erkan and Yorgancı (1983),Güllü and Çalı, (1991), Nogay and Yorgancı (1984), Tekinel *et al*, (1969), Yılmaz and Davis, (1985).

117 tomato, 7 pepper, 3 cucumber and 8 eggplant samples collected from greenhouses in Muğla were made by Elisa tests; Tomato mosaic virus; tomato black ring virus and tomato ring spot virus antisera were used (Table 3).

Tomato mosaic virus were obtained from 16 tomato and 2 pepper samples on Elisa test. Same samples have been made mechanical inoculation tests. Tomato mosaic virus produced necrotic local lesions on **C. amaranticolor** and systemic mosaic on **C. quinoa, N. glutinosa** and **N. tabacum** "WB". These results have also been corfirmed by literature (Hollings and Huttinga, 1976).

Tomato black ring virus were obtained from 40 tomato, 1 pepper and 1 cucumber samples by Elisa test, mechanical inoculation tests have been applied to same samples. Tomato black ring virus produced chlorotic local lesion on **C. quinoa** and necrotic local lesion and systemic mosaic on **N. clevelandii** and **N. tabacum** "WB". These symptoms were similar to that of previous report (Murant, 1970).

Tomato ring spot virus were found from 28 tomato, 2 pepper, 1 cucumber and 1 egglant samples by Elisa test. The results of the mechanical inoculation tests, tomato ring spot virus produced cholorotic local lesion on **C. amaranticolor** and **C. quinoa**, systemic mosaic on **N. tabacum** "WB". These results were agreement with those in literature (Stace-Smith, 1970).

Elisa tests were made on 28 tomato samples collected daily from Fethiye by using antiserum of tomato spotted wilt virus. Tomato spotted wilt virus was obtained from 21 tomato samples.

By this study it has ben determined that tomato ring spot virus and tomato black ring virus on tomatoes, tomato ring spot virus on peppers, tomato ring spot virus and tomato black ring virus on cucumbers and tomato black ring virus on eggplants have been present. These diseases are new records for Türkiye.

Table 1. Incidence and prevelance of mosaic virus in İzmir and Muğla (1992-1993).

		Total area of greenhouses (da)				Number of examined greenhouses				Number of examined samples				Incidence rates of mosaic virus (%)				Prevelance rates area of mosaic virus (%)			
Survey Area		T	P	E	C	T	P	E	C	T	P	E	C	T	P	E	C	T	P	E	C
İZMİR	Konak	174,41	72,1	-	140,2	13	8	-	8	19	8	-	3	8,3	4,5	-	5,3	30,7	70,5	-	37,7
	Menderes	21	-	-	435,4	1	-	-	9	-	-	-	3	10	-	-	0,77	100	-	-	44,4
	Seferihisar	31,2	2	-	64	2	-	-	2	-	-	-	1	1,5	-	-	5	100	-	-	50
MUĞLA	Bodrum	38	-	-	-	3	-	-	-	3	-	-	-	0,6	-	-	-	33,3	-	-	-
	Dalaman	250	-	-	-	6	-	-	-	6	-	-	-	-	-	-	-	65	-	-	-
	Fethiye	5520	50	200	-	60	3	5	-	79	7	8	3	6,9	2	1,8	-	66	100	100	-
	Köyceğiz	260,5	6	3,5	-	6	-	-	-	10	-	-	-	17,1	-	-	-	75	-	-	-
	Milas	180	53	40	-	4	-	-	-	4	-	-	-	7,5	-	-	-	75	-	-	-
	Ortaca	1516	-	-	-	15	-	-	-	15	-	-	-	16	-	-	-	46,6	-	-	-
Average														7,3	2,4	0,3	3,6				

T - Tomato
P - Pepper
E - Eggplant
C - Cucumber

Table 2. Reactions of test plants used for identification of the virus diseases on tomato, pepper and cucumber.

Test Plants	Virus diseases			
	tomato (Tobacco mosaic virus)	tomato (Tobacco mosaic virus + potato x virus)	pepper (Tobacco mosaic virus)	cucumber (Cucumber mosaic virus)
C. amaranticolor	CLL	CLL	CLL	NLL
D. stramonium	NLL	NLL, Mo	NLL	NLL
G. globosa	NLL	NLL	NLL	NLL
N. glutinosa	NLL	NLL	NLL	Mo
N. tabacum "WB"	Mo.VN	Mo	Mo.VN	Mo

CLL - Clorotic local lesion
NLL - Necrotic local lesion
Mo - Mosaic
VN - Vein Necrosis

Table 3. Elisa reactions of TSWV, TBRV, TRSV and ToMV in the leaf sample extracts collected from tomato, pepper, cucumber and eggplant in the Aegean Region (405 nm)

Tomato spotted wilt virus				Tomato blacük ring virus				Tomato ring spot virus				Tomato mosaic virus			
Plate No	Sample No	Ab.Va	Cont. Ab.Va	Plate No	Sample No	Ab.Va	Cont. Ab.Va	Plate No	Sample No	Ab.Va	Cont. Ab.Va	Plate No	Sample No	Ab.Va	Cont. Ab.Va
1	103/1	0,010	0.00 6	1	1/1 C	0,107	0,034	1	0/10	0,183	0,000	1	0/6	0,111	0,009
	103/2	0,169	0,002		1/2 E	0,119	0,028		1/1 P	0,587	0,020		0/11	0,308	0,017
	103/3	0,106	0,009		1/3 E	0,105	0,023		37/1	0,144	0,001		1B	0,119	0,007
	111/1	0.602	0,001		31/2	0,156	0,021		41/1	0,267	0,011		1P	0,110	0,006
	111/2	0,698			40/5	0,339			41/2	0,192			40/1	0,146	
	111/3	0,268			42	0,106			56/1	0,686			41/1	0.137	
	111/4	0,299			44	0,135			56/2	0,638			62	0.136	
	112/1	0,304			52	0,142			73/2	0,122			73/2	0,201	
	112/2	0,108			53	0,183			80/1	0,119			80/2	0,166	
	112/3	0,110			54	0.214			94/2	0,179					
	112/4	0,162			76/4	0,126			94/3	0,174		2	0	0,114	0,004
	113/1	0,533			84	0,105							1/2 C	0,235	0,005
	113/2	0,424			87/1	0,119		2	0-1	0,152	0,037		1/2 E	0,124	
	113/3	0,366			89	0,127			0-3	0,111	0,027		2/3 P	0,103	
	113/4	0,135			90/1	0,138			0-6	0,168	0,028		27/2	0,151	
	114/1	0,258			90/6	0,107			0-7	0,121	0,009		30	0,237	
	114/2	0,122			91/2	0,110			0-10	0,225			53	0,117	
	114/3	0,169			92/1	0,134			0-11	0,106			79	0,114	
	114/4	0,242			93/3	0,177			0-12	0,315			90/1	0,123	
	115/1	0,218							0-13	0,174			90/4	0,282	
	115/2	0,126							0-14	0,186			90/4	0,408	
				2	30	0,134	0,024		1B	0,203			90/6	0,324	
					40/5	0,576	0,007		3/3 E	0,116			91/1	0,375	
					40/8	0,190	0,016		37/1	0,114			91/3	0,137	
					65	0,168	0,001		40	0,118			92/1	0,379	
					76/4	0,223			50	0,114			92/2	0,176	
					90/6	0,153			56	0,278			92/4	0,584	
					93/3	0,259			57	0,110			93/1	0,221	
									62	0,148			93/2	0,324	
				3	10/1	0,100	0,005		67	0,178			93/4	0,160	
					4/3	0,124	0,010		68	0,136			1/3	0,225	
							0,007		73/2	0,160					
							0,021		80/1	0,211		3	4/3	0,243	0,027
									92/3	0,232			1/2	0,100	0,006
									94/1	0,158					0,017
															0,015

P: Pepper E: Eggplant C: Cucumber

ÖZET

İZMİR VE MUĞLA İLLERİNDEKİ SERALARDA YETİŞTİRİLEN SEBZELERDE SAPTANAN VİRÜS HASTALIKLARI

İzmir ve Muğla ilinde; domates, biber, hıyar ve patlıcan seralarında survey çalışmaları yapılmıştır. Mozayık belirtisi gösteren virus hastalıklarının, hastalık oranları domateslerde %7.3, biberde %2.4, hıyarda %3.6 ve patlıcanda %0.3, yaygınlık oranları ise; domateste %30.7-100 arasında, biberde %70.5-100, hıyarda %37.7-50 ve patlıcanda %100 olarak belirlenmiştir. İzmir ilinde toplanan örneklerde yapılan mekanik inokulasyonlar sonucunda; domateslerde tütün mozayık virusu (tobacco mosaic virus), çift viruslu çizgi hastalığı (tomato mosaic virus + potato x virus), biberlerde tütün mozayık virusu, hıyarlarda hıyar mozayık virusu (cucumber mosaic virus) saptanmıştır. Muğla ilinde yapılan surveylerden alınan örneklere yapılan Elisa testi sonucunda; domateslerde domates lekeli solgunluk virusu (tomato spotted wilt virus), domates mozayık virusu (tomato mosaic virus), domates siyah halka virusu (tomato black ring virus) ve domates halka leke virusu (tomato ring spot virus) saptanmışıtr. Biber ve hıyar örneklerinde domates halka leke virusu ve domates siyah halka virusu, patlıcanda ise domates siyah halka virusu tesbit edilmiştir. Domates halka leke virusu ve domates siyah halka virusu Türkiye için ilk kayıttır

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J. Turk. Phytopath., Vol. 24, No.1, 15-22, 1995 ISSN 0378 - 8024

Evaluation of Some Chickpea Cultivars for Resistance to **Ascochyta rabiei** (Pass.) Labr., **Fusarium oxysporum** and **Fusarium solani** in Türkiye

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ABSTRACT

Reactions of six chickpea cultivars against three races (race 1, 4 and 6) of ***Ascochyta rabiei*** *(Pass.) Labr. and two Fusarium species (****Fusarium oxysporum*** *and* ***Fusarium solani****) were determined. The cultivar ILC 195 and ILC 482 were found resistant to race 1 and 4 of* ***A. rabiei*** *but tolerant to race 6. The cultivar Canıtez 87 and one local cultivar, named Spanish were susceptible to all three races of* ***A. rabiei.*** *The hybrid cultivar 65C830xICP114 was resistant to race 1 and susceptible to the other two races. Eser 87 was revealed as resistant to race 1 and 4 but susceptible to race 6. All of the six chickpea cultivars were found highly susceptible to* ***F. solani*** *and* ***F. oxysporum.*** *But plant mortality in the cultivar ILC 482 inoculated with* ***F. oxysporum*** *compared with other five chickpea cultivars was lower (60%).*

INTRODUCTION

Chickpea (**Cicer arietinum** L.) is the second most important pulse crop of Turkey after lentil. In 1989, it was sown in 818.000 ha and 796.000 tons were harvested. Despite the importance of chickpea, however, the national average is only 858 kg/ha (Anonymous, 1992). Chickpea is attacked by several serious pathogens. Among these, blight caused by **Ascochyta rabiei** (Pass.) Labr. is the most important in Turkey. Besides this, wilt and black root rot caused by **Fusarium oxysporum** and **Fusarium solani** are very serious diseases. Soran (1975) found out that **Fusarium acuminatum** Ell. and **Fusarium oxysporum** were the most important root rot agents under field conditions. Maden (1987) determined that **A. rabiei** (56.42%), **F. oxysporum** (50%) and **F. solani** (14.28%) were present on the chickpea seed samples brought from the important chickpea producing area of Turkey. Fusarium wilt caused by **F. oxysporum** Schlechtend.: Fr. f. sp. **ciceris** (Padwick) Matuo & K. Sato and Ascochyta blight are major constraints to chickpea production in the Mediterranean region and Indian s ubcontinent (Jiménez - Dìaz **et al.,** 1991). Ascochyta blight is a factor limiting chickpea production in Turkey. However, precise information on yield losses and varietial resistance to these pathogens are lacking.

Nene (1988) has emphasized the need to develop chickpea cultivars with combined resistance to several of the important diseases. Varieties possesing combined resistance/tolerance to these diseases will be very useful in minimizing the losses caused by them.

Dolar and Gürcan (1992) demonstrated the existence of three races of **A. rabiei** in Turkey. The same researchers determined the resistance of seven chickpea cultivars to three races (race 1, 4 and 6) of **A. rabiei** (Dolar and Gürcan 1992). In this work the same six chickpea cultivars were considered but evaluation of resistance was based on a different disease score than used by the previous workers. Besides, the resistance of the cultivars to Fusarium wilt and root rot (**F. oxysporum** and **F. solani**) was determined.

MATERIALS and METHODS

1) Plant Material:

Six chickpea cultivars; three registered (Eser 87, Canıtez 87, ILC 482), one pro-registered (ILC 1995), one hybrid 65C830 x ICP114 and one local cultivar (named as Spanish) were used for determination of resistance to three races of **A. rabiei** and two Fusarium species (**Fusarium oxysporum** and **F. solani**). Chickpea cultivars were obtained from different Research Centers in Turkey, one cultivar (ILC 482) from Diyarbakır Agricultural Research Institute, three cultivars (Eser 87, Canıtez 87, 65C830 x ICP114) from Eskişehir Agricultural Research Institute and cultivar ILC 195 from Menemen Agricultural Research Institute. In addition, one local cultivar, beige colored and large seeded that originated from Spain was collected from farmer populations in Çorum.

Seeds of each cultivar were surface-sterilized with sodium hypochloride (1%) for 3 min and washed 3 times with sterile distilled water. Eight seeds were sown in 15 cm diameter pots containing sterilized soil, river-bed sand, peat moss (1:1:1, v/v). Plants were grown in growth room at 25 ± 2°C with a relative humidity of 25-50% and illuminated for 12 h per day with white fluorescent light (light intensity 11.000 lux).

2. Fungal Material:

The three races (1,4 and 6) of **A. rabiei** reported from Turkey (Dolar and Gülcan, 1992) and two Fusarium species (**F. oxysporum** and **F. solani**) were used in present study. Isolates of **A. rabiei** were maintained on CSMDA (Chickpea - Seed Meal Dextrose Agar). Petri plates were incubated for 14 days at 20±1°C with 12h photoperiod of near UV light. Spore suspensions of $1.2x10^6$ spores/ml were prepared using sterile distilled water.

Isolates of **F. oxysporum** and **F. solani** were obtained from wilted plants of chickpea collected from chickpea growing areas in Ankara province. Single spore isolations of the cultures were obtained and identified according to Booth (1971) using slide culture method (Booth, 1977). The pathogenicity of isolates of **F. oxysporum** and **F.**

solani were tested using susceptible cultivar AUG 424 obtained from NIAB (Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan).

One of the most virulent isolates of each Fusarium species was selected for this experiment and maintained on PDA (Potato Dextrose Agar). Petri plates were incubated under the same conditions as **A. rabiei** for 7 days.

3. Inoculation of Plants:

Fifteen days old plants for each cultivar were inoculated with the three races of **A. rabiei** by employing five pots of eight plants for each race. All areial parts of the plants were sprayed uniformly with spore suspensions ($1.2x10^6$ spores/ml) of **A. rabiei.** Control plants were sprayed with sterile distilled water. After spraying, the plants were covered with transparent polyethylene bags for 4 days to maintain leaf wetness and incubated in a growth room at 23±2°C with a 12 h photoperiod (11.000 lux.)

To produce the inoculum of **F. oxysporum** and **F. solani,** 100 g. of sand: chickpea meal (90 g sand + 10 g chickpea flour + 20 to 25 ml distilled water) in 250 ml erlenmeyer flasks was sterilized and inoculated with a 1 cm diameter plug from the margin of actively growing 7 day-old fungus on PDA. Flasks were incubated for 14 days at 25 ± 2°C with an illumination of 12 h per day. The inoculum of each flasks was mixed with the 2 kg sterile soil in each pot. The soil in the pots was lightly watered after the inoculum was incoporated. Sowing was done 4 days later, to allow establishment of the fungi. Eight seeds of each cultivar were sown in inoculated soil in the plastic pots. Control plants were grown in a comparable mixture of noninfested sand + chickpea flour (90 + 10 g) and autoclaved soil (Reddy **et al.,** 1988; Kunwar **et al.,** 1989; Haware **et al.,** 1992). Plants were grown in a growth room at 25 ± 2°C with a 12 h photoperiod of fluorescent light at approximately 11.000 lux. Each treatment was replicated 4 times, a 15 cm plastic pot with eight seedlings forming a replication.

4. Disease Assessment:

Disease assessment for **A. rabiei:** Twenty one days after inoculation, plants were rated on a scale of 1 to 9 where 1: no visible lesions on any plants; 3: lesions visible on less than 10% of the plants, no stem girdling; 5: lesions visible on up to 25% of the plants, stem girdling on less than 10% of the plants but little damage; 7: lesions on most plants, stem girdling on less than 50% of the plants resulting in the death of a few plants; 9: lesions profuse on all plants, stem girdling on more than 50% of the plants and death of most plants (Singh **et al.,** 1981). Those plants rated from 1 to 4, from 4.1 to 5.5, from 5.6 to 9 were considered resistant, tolerant and susceptible, respectively (Jamil **et al.,** 1993).

Disease assessment for Fusarium species: The observations on mortality because of wilt and root rot were recorded at 15 days intervals up to 45 days. Disease reactions were çlassified according to the percentage of dead plant (Haware and Nene, 1982) as resistant (0-20%), moderately resistant (21-50%) and susceptible (51-100%) (Jıménez-Dìaz **et al.,** 1991).

RESULTS and DISCUSSION

Six chickpea cultivars in Turkey were evaluated for resistance to Fusarium wilt and Ascochyta blight under controlled conditions. Disease reactions of these cultivars to races 1, 4 and 6 of **A. rabiei** are given in Table 1.

Cultivars Canıtez 87 (19/1-5) and local cultivar were categorized as susceptible to three races (1, 4 and 6) of **A. rabiei.** The hybrid cultivar 65C830xICP114 was resistant to race 1 but susceptible to race 4 and 6. Three cultivars, namely Eser 87 (65C830), ILC 482 and ILC 195 were resistant to races 1 and 4. The cultivar Eser 87 was susceptible to race 6 whereas other two cultivar (ILC 482 and ILC 195) were tolerant to race 6. Large differences occurred in the reactions of the six selected chickpea cultivars to infection by races of **A. rabiei.** None of the cultivars was rated as immune.

Singh and Reddy (1990) determined that the cultivar ILC 482 was resistant to race 1 but susceptible to races 4 and 6. This cultivar was found susceptible (disease rating 6, on 1-9 scale) to 4 isolates of **A. rabiei** by some workers (Hussain and Malik, 1991). On the other hand, in another work it was moderately resistant (disease rating 4, on 1-9 scale) to an isolates of the pathogen (Reddy and Singh, 1993). But they did not report which race of **A. rabiei** were used in their study. In this paper, ILC 482 was found resistant to races 1 and 4 and tolerant to race 6. The reasons of these differences among the results of the studies mentioned before could be varying factors such as plant age, inoculum concentration, isolates or races of **A. rabiei.**

Table 1: Reaction of six chickpea cultivars to three races of **Ascochyta rabiei** 21 days after inoculation.

	Disease Rating[1]					
	Cultivars Name					
Races	Canıtez 87	Local Cultivar	65C830 X ICP114	Eser 87	ILC 482	ILC 195
1	S (6.5)	S (7.5)	R (4.0)	R (3.5)	R (3.1)	R (3.0)
4	S (8.5)	S (8.5)	S (6.5)	R (3.0)	R (3.5)	R (3.5)
6	S (8.7)	S (8.5)	S (8.0)	S (6.0)	T (5.5)	T (4.5)

1 R (Resistant) = 1.0 – 4.0
T (Tolerant) = 4.1 – 5.5
S (Susceptible) = 5.6 – 9.0

The cultivar ILC 195 was found resistant to race 1, 4 and tolerant to race 6 of **A. rabiei** in this study. Singh **et al.** (1984) reported that the cultivar ILC 195 had showed resistant reaction in six of eleven countries (Algeria, India, Greece, Jordan, Lebanon, Morocco, Pakistan, Spain, Syria, Tunisia and Turkey). However Açıkgöz and Demir (1988) reported that ILC 195 had been resistant to all of the 25 isolates of **A. rabiei** used in their experiment. Furthermore, ILC 195 was also found to be resistant by some workers while Eser 87 was tolerant cultivar and Canıtez 87 was susceptible cultivar to **A. rabiei** (Dalkıran **et al.,** 1988). All the above mentioned researchers utilized a population of **A. rabiei** for determination of resistant cultivars and, in their studies, it is not clear which races of **A. rabiei** they used.

All of the six chickpea cultivars were found susceptible to **F. solani** and **F. oxysporum** in this study (Table 2).

Table 2: Reaction of six chickpea cultivars to **Fusarium oxysporum** and **Fusarium solani** 45 days after inoculation.

	F. oxysporum		F. solani	
Cultivars Name	Percent[1] Mortality	Reaction[2] Class	Percent Mortality	Reaction Class
Canıtez 87	100.00	S	100.00	S
Local Cultivar	91.66	S	100.00	S
65C830 X ICP114	100.00	S	100.00	S
Eser 87	95.83	S	100.00	S
ILC 482	60.00	S	100.00	S
ILC 195	80.00	S	100.00	S

1 Average of four replications (8 plants replication^{-1})

2 R = Resistant (0-20% mortality)
MR = Moderately Resistant (21 - 50% mortality)
S = (Susceptible) (51-100% mortality)

These chickpea cultivars showed highly susceptible reaction (100% mortality) to **F. solani.** Small differences were observed in the disease reaction of six chickpea cultivars to **F. oxysporum.** The cultivar Canıtez 87, 65C830 x ICP114, Eser 87 and local cultivar were found highly susceptible to **F. oxysporum.** Plant mortality in ILC 195 (80%) and ILC 482 (60%) was lower than in the other chickpea cultivars. Especially, disease reaction of ILC 482 compared with other five chickpea cultivars was lower. None of the cultivars were resistant or moderately resistant to **F. solani** and **F. oxysporum.**

Many workers have studied on sources of resistance to wilt (Ahmad and Sharma, 1990; Reddy **et al.,** 1990; Jıménez - Dìaz **et al.,** (1991) determined that one Spanish kabuli landrace (PV 60) with large, white seeds was highly susceptible to Fusarium wilt. In this study, local cultivar, named as Spanish was found highly susceptible to **F. oxysporum** and **F. solani.**

Fusarium wilt and Ascochyta blight are important diseases of chickpea producing countries in the world. For this reason, researchers have tired to develop chickpea cultivars with combined resistance to these important diseases. Unfortunately registered and proregistered cultivars in Turkey did not show a combined resistance against two of the diseases, specially the resistant cultivars ILC 195 and ILC 482 to most of the races of **A. rabiei.** However, this conclusion was based one isolate of both **F. oxysporum** and **F. solani** whereas **F. oxysporum** f. sp. **ciceri** has 6 races (Phillips, 1988). For the pathogens having races, such as **F. oxysporum** f.sp. **ciceri,** the reactions of the cultivars should be assesed against the known races too.

ACKNOWLEDGMENT

The author is thankful to Dr. F. F. Jamil (Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan) and Diyarbakır Agricultural Research Institute, Eskişehir Agricultural Research Institute, Menemen Agricultural Research Institute, Turkey for providing the seeds required for this study.

ÖZET

TÜRKİYE'DEKİ BAZI NOHUT ÇEŞİTLERİNİN *Ascochyta rabiei* (Pass.) Labr., *Fusarium oxysporum* ve *Fusarium solani*'ye DAYANIKLILIĞININ SAPTANMASI

Ascochyta rabiei (Pass.) Labr.'nin üç ırkına (ırk 1, 4 ve 6) ve iki Fusarium türüne (**Fusarium oxysporum** ve **Fusarium solani**) karşı altı nohut çeşidinin gösterdiği reaksiyon bu çalışmada saptanmıştır. ILC 195 ve ILC 482 **A. rabiei**'nin 1 ve 4 nolu ırkına dayanıklı, 6 nolu ırkına ise tolerant bulunmuştur. Canıtez 87 ve İspanyol olarak isimlendirilen lokal çeşit üç ırka da duyarlı olarak tespit edilmiştir. Hibrit çeşit olan 65C830 x ICP114 ırk 1'e dayanıklı diğer iki ırka duyarlı reaksiyon göstermiştir.

Eser 87 ırk 1 ve 4'e dayanıklı, ırk 6'ya duyarlı bulunmuştur. Denemede kullanılan altı nohut çeşidinin hepsinin **F. solani** ve **F. oxysporum**'ye yüksek derecede duyarlı olduğu saptanmıştır. Fakat ILC 482'deki bitki ölümü diğer beş nohut çeşidi ile kıyaslandığında düşük (%60) bulunmuştur.

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J. Turk. Phytopath., Vol. 24, No.1, 23-28, 1995 ISSN 0378 - 8024

Determination Of The Reactions Of Some Carnation Cultivars Against Carnation Rust (**Uromyces caryophyllinus** (Schr.) Wint.)*

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ABSTRACT

*In this study, the most popular 17 carnation cultivars grown in Türkiye were tested against **Uromyces caryophyllinus** (Schr.) Wint.*

As a result of these observations; Nibbio, Desio, Gallimuraylia, Kortina and Isac cultivars showed resistance to rust (< 20%); Indios, Calypso, Astor, Gastellero Nobbi, Fanbio and Kontinent cultivars showed moderate susceptible cultivars (20-50%); White Sim, White Calypso, Raggio di Sole, Bianco New Nobbi, Irene and Aurigo cultivars were susceptible (>50%).

INTRODUCTION

One of the most popular cutting flower is carnation which takes places 50% of cutting flower produce in Türkiye(Hatipoğlu, 1982).

The most important fungal pathogen of carnation is carnation rust (**U. caryophyllinus**). This pathogen is especially common in glasshouse and causes big damage (Pirone, 1970; Fletcher, 1984; Sezgin and Esentepe, 1986).

Fungicides are used for control of **U. caryophyllinus.** But these fungicides cause a decrease of natural characteristic of ornamentals and cause environmental pollution. On the other hand, these chemicals don't absolutely inhibit the progress of this disease. It was not obtained any successful control of this rust by using **Verticillium lecani** and **Darluca filum.** Thus, improvement of a resistant variety is important against this pathogen.

The aim of this project is to determine the reactions of commonly grown carnation cultivars against **U. caryophyllinus** in Türkiye.

According to disease reactions cultivars were grouped in different categories. For example, Pape (1964) classified the cultivars as resistant and susceptible. According to

* This work was supported by Research foundation of Ankara University.

Pavel *et al* (1977) disease rates above 20% were classified as susceptible, on the other hand, disease rates below 20% were classified as resistant. According to Semina and Shestachenco (1981) there are four groups (immune, resistant, moderately susceptible, highly susceptible). Sezgin and Esentepe (1986) recognized three groups (less than 20% resistant, 20% - 50% moderate, more than 50% susceptible). But while some cultivars were determined as susceptible in one experiment, the same cultivar was determined as resistant in another study. For example, while White Sim cultivar was determined resistant according to Pape (1964), the same cultivar was determined susceptible according to Pavel *et al* (1977).

In Türkiye, there is one study which investigated six carnation cultivrs in this respect (Sezgin and Esentepe 1986). It was determined Astor, Floriana and Lena cultivars as susceptible and Alicetta and Ernesta as moderate susceptible cultivars and Minirosa cultivar as resistant cultivar.

MATERIALS and METHODS

Seventeen carnation cultivars were obtained from Atatürk Horticultural Research Institute and Yalova Flower Growing and Marketing Co-operative.

The inoculum material is consisted of rust uredospores which were collected from greenhouses located at Turkish Grand National Assembly and President's Palace.

Single spore isolations were made to obtain uniformity of inoculum. Uredospores were collected with a brush from diseased leaves and were incubated at 20°C for 72 h in a moist chamber. After that, spores and talc powder (twice of spore amount) were added to steril water. Whole part of Astor plant which is known as susceptible was inoculated with this suspension by brush and incubated in a moisture chamber for 48 h. Then, this pot which contained plant were taken to greenhouse with a 18-20°C and 80-90% relative humudity. After 15-20 days inoculation, a single big pustule is selected from Astor plant and monospore isolation was made. This uredospores can be acceptable as enough purity which is used reaction experiments. These uredospores were multiplied and were put deep-freeze at glass tube, until use.

After 5-7 days of planting carnation, these plants inoculated with rust uredospores.

The inoculum was sprayed by a small glass sprayer until whole plant soaked. Five ml suspension was sprayed to each pot and then these pots were remained in a growth chamber 48 h which contained 100% humidity. Spor suspension was prepared (density $1x10^5$ spor/ml) according to Spencer (1981). For this purpose, 12 mg uredospores was mixed with 24 mg talc and 100 ml steril water. Control group was sprayed with a mixture which did not contain uredospor. Then plants stayed in the greenhouse on 80-90% relative humudity and 25 ± 5°C.

Result was evaluated according to the 0-4 scale of Semina and Shestachenko (1981).

0. immune plant: no necrotic spot,
1. high resistance: The 10% of leaves and stem covered by waxy and necrotic spots,
2. less resistance: The 25% of leaves and stem covered by pustule
3. moderately susceptible: The 50% of leaves and stem covered by rust spores and pustule at the yellowish leaves
4. susceptible: The 50% of leaves and stem damaged by rust and died.

Observation was made after 50 days inoculation and this result was evaluated. Scale value was transformed to % disease severity for statistical analysis (Toros and Maden 1991). Analysis of variance was performed according to randomized block design and different groups were determined using Duncan's test (Düzgüneş *et al.* 1983).

RESULTS and DISCUSSION

Carnation cultivars have shown different reactions against the rust isolate in our experiment. First pustules started 15-20 days after inoculation and the number of pustules increased steadily. More pustules were observed on young leaves (Figure 1).

Figure 1. Pustules on young leaves of carnation plant.

After 50 days of inoculation, disease severity in carnation cultivars, were presented in Table 1.

Table 1: After 50 days of inoculation, disease severity in carnation cultivars, inoculated with **U. caryophyllinus** uredospores.

Cultivars	Disease severity (%)		
White Sim	92.50	A	a*
White Calypso	73.75	AB	ab
Raggio di Sole	72.50	AB	abc
Bianco New Nobbi	57.50	BC	abcd
Aurigo	52.60	BCD	bcde
Irene	52.50	BCD	bcde
Indios	50.00	BCD	bcde
Calypso	48.75	BCD	bcde
Astor	38.75	BCDE	bcdef
Gastellero Nobbi	38.75	BCDE	bcdef
Fanbio	27.50	CDEF	cdefg
Kontinent	21.25	DEF	defg
Nibbio	20.00	EF	defg
Desio	18.75	EF	efg
Gallimuraylia	12.50	F	fg
Kortina	5.00	F	g
Isac	5.00	F	g

* Figures followed by different capital letters differ significantly at $p<0.05$ and small letters differ significantly at $p<0.01$

After 50 days of inoculation, although 1% difference among white Sim, Raggio di Sole, White Calypso and Bianco New Nobbi was not significant, this ratio was significant in comparison to the other cultivars. Therefore, this formed as a susceptible group.

As seen in table 1, result of statistic analysis, it may possible to classified to the specices it will be artifical. Because of the wide varyate it is difficult to mathc with standart scale.

For this reason, it is possible that one cultivar that could be classified as moderate susceptible in our classification, could be classified as susceptible or resistant in another experiment. For example, White Sim cultivar is resistant according to Pape

(1964); however, the same cultivar was listed as susceptible by Pavel (1977) and Spencer (1983). A similar situation was observed using Astor cultivar. In their study Sezgin and Esentepe (1986) classified the Astor cultivar as susceptible, on the other hand, the same cultivar was found as moderate susceptible in our study (Table 1). The variation in the experimental conditions and the differences in evaluation of the results by researchers can be counted for these differences.

We can divide, the reactions to three group with respect to the disease severity of the carnation cultivars, the group with above of 50% disease were susceptible, and 20-50% were moderately susceptible, and below of 20% were resistant.

As a result of our observations; the groups were defined as fallow.

Susceptible cultivars; White Sim, White Calypso, Raggio di Sole, Bianco New Nobbi, Aurigo, Irene cultivars.

Moderately susceptible cultivars; Indios, Calypso, Astor, Gastellero Nobbi, Fanbio, Kontinent cultivars.

Resistant cultivars; Nibbio, Desio, Gallimuraylia, Kortina, Isac cultivars.

Even though, we have three groups based on disease reactions, for the reasons explained above, cultivars can deviate from these groups.

ÖZET

BAZI KARANFİL ÇEŞİTLERİNİN KARANFİL PASI (Uromyces caryophyllinus (Schr.) Wint'NA KARŞI REAKSİYONLARININ SAPTANMASI

Ülkemizde en çok yetiştirilen 17 karanfil çeşitinin karanfilin en yaygın hastalığı olan karanfil pası **Uromyces caryophyllinus** (Schr.) Wint'e karşı reaksiyonları araştırılmıştır.

Bu gözlemler sonucunda; Nibbio, Desio, Gallimuraylia, Kortina ve Isac çeşitleri dayanıklı çeşitler olarak (<%20), Indios, Calypso, Astor, Gastellero Nobbi, Fanbio ve Kontinent çeşitleri orta hassas çeşitler olarak (%20-50), White Sim, White Calypso, Raggio di Sole, Bianco New Nobbi, Aurigo and Irene çeşitleri hassas çeşit olarak gözlenmiştir.

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J. Turk. Phytopath., Vol. 24, No.1, 29-37, 1995 ISSN 0378 - 8024

Plant Parasitic Nematodes Associated with Citrus in the East Mediterranean Region of Türkiye

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ABSTRACT

In a survey on plant parasitic nematodes in the East Mediterranean region of Turkey, 16 nematode species were found associated with citrus. Out of these, only four species, ***Helicotylenchus pseudorobustus*** *(Steiner) Golden,* ***Rotylenchulus macrodoratus*** *Dasgupta, Raski et Sher,* ***Tylenchorhynchus goffarti*** *Sturhan, and* ***Tylenchulus semipenetrans*** *Cobb are able to infect citrus. About 90% of the orchards sampled were infected with the citrus nematode,* ***T. semipenetrans****. The infestation rate was considerably high as 62.5% of the examined orchards revealed population densities above the economic treshold.* ***T. semipenetrans*** *infestation level was very low in citrus nurseries or in young orchards but strongly increased with the age of the citrus plants.*

INTRODUCTION

Yield losses in citrus production caused by nematodes estimated 8.7-14.2% on world basis (Cohn, 1972; Sasser, 1987). The citrus nematode, **Tylenchulus semipenetrans** Cobb is one of the most harmful nematodes of citrus. It inhabits in all countries of the world where citrus is grown. **T. semipenetrans** is considered as the causative agent of the slow decline in citrus (Decker, 1969; Tarjan & O'Bannon, 1984; Duncan&Cohn, 1990).

Besides **T. semipenetrans,** citrus is attacked by several other nematode species, e.g., **Belonolaimus longicaudatus** Rau, **Pratylenchus brachyurus** (Godfrey) Filipjev& Schuurmans Stekhoven, **P. coffeae** (Zimmerman) Filipjev & Schruurmans Stekhoven, **P. vulnus** Allen & Jensen, **Radopholus citrophilus** Heuttel, Dickson & Kaplan (Duncan & Cohn, 1990).

There is few knowledge about plant parasitic nematodes associated with citrus in the East Mediterranean region as well as in other citrus production areas of Türkiye. Kıray (1963), Yüksel (1966) and Özkut (1970) reported on the occurrence of the citrus nematode. Elekçioğlu (1992), Elekçioğlu & Uygun (1994) and Elekçioğlu et al. (1994) gave some information on occurrence and distribution of nematodes in citrus of this region.

The aim of this study was to determine the importance, distribution and the population densities of nematodes associated with citrus in the East Mediterranean region.

MATERIALS AND METHODS

In a survey on plant parasitic nematodes soil and root samples were collected from 110 orchards in the entire citrus growing area in the East Mediterranean region since 1989. The dominant structure of nematode species found in 110 citrus orchards and the population densities of the citrus nematode in 40 selected orchards that differ in age were determined between 1992-1994. Soil samples were collected by mixing at least 25 (for large orchards 30-40) individual core-samples by following a W-shaped path. Root samples were collected usually from 8-10 trees of each orchard. Vermiform nematodes were obtained from soil and roots by using a modified Baermann-funnel technique (Hooper, 1986a, 1986b). To extract sedentary females roots were stained with acid fuchsin-lactoglycerol:

10 ml acid fuchsin (1%) + 90 ml lactoglycerol (1750 ml lactic acid + 126 ml glycerol + 124 ml water).

Stained semiendoparasitic females of citrus nematode were counted directly from roots. The population densities of nematodes were measured from 100 g soil and 1 g roots. Nematodes were mounted in glycerol and identified to species and family levels.

RESULTS AND DISCUSSION

During this survey, 16 nematode species were identified in citrus orchards. Furthermore, some undetermined species belonging to the genera **Helicotylenchus, Heterodera, Paratylenchus, Pratylenchoides, Trophurus,** and **Tylenchus** and some species of the families Dorylaimidae and Mononchidae were found in citrus orchards in the East Mediterranean region since 1989 (Table 1).

From these species, only four have been reported from citrus: **Helicotylenchus pseudorobustus** (Steiner) Golden in California, Iran and Nigeria (Fortuner, 1985); **Rotylenchulus macrodoratus** Dasgupta, Raski et Sher in the Mediterranean countries (Lamberti, 1981) and in Florida (Tarjan & O'Bannon, 1984); **Tylenchorhynchus goffarti** Sturhan in Libya (Siddiqui et al., 1987) and **T. semipenetrans** in all citrus growing countries (Decker, 1969; Tarjan & O'Bannon, 1984; Duncan & Cohn, 1990). The impact on citrus is known only for **T. semipenetrans,** whereas for other the three above mentioned species the importance as citrus parasite has not been determined yet. In spite of, that large population densities of **H. pseudorobustus** were found in some orchards in Erdemli in the soil of sour oranges, damage on roots could not be observed. The population densities of all other species listed in Table 1 were low. It is not clear whether they are parasites of citrus or not.

The citrus nematode was found an important parasite of citrus in this region and widely distributed throughout the region wherever citrus was grown (Fig. 1). 90 of 110 citrus orchards investigated were infested with this parasite (Tab. 1). Population densi-

ties ranged 0-1212 females/g root mass and 0-39000 juveniles + males / 100 g soil. Tree age greatly affected the population size of **T. semipenetrans** (Fig. 2). Most of the trees in citrus nurseries appeared to be less or not infected. However, in orchards elder than 2 years the infestation rates increased dramatically up to 12600 juveniles and males/100 g soil. Also the number of females/g root mass was considerably low in nurseries but reached on average 720 females/g root mass in orchards elder than 15 years. This is in coincidence with findings of Decker (1969), who stated that infestation is not apparent in young citrus seedlings and the degree of infestation increases with orchard age.

In those 40 orchards which were studied bore in detail, only 37.5% revealed infestation rates below the economic threshold of 1600 juveniles + males / 100 g soil (Fig. 3). Thus, about 2/3 of all citrus orchards may respond economically to nematicide treatment or in other words may increase yield considerable according to an estimation by Garabedian **et al.** (1984). The density of females was low in 47.5% of the orchards, moderate in 25% and high in 27.5% of all orchards.

The population density of **T. semipenetrans** in orchards may differ from tree to tree, e.g., in a 17 years old and furrow irrigated orchard, 50% of trees were investigated. Nearly 98% of trees were infected by the citrus nematode, but its population densities were not uniform on all trees (Elekçioğlu, unpublished data).

Citrus damage by nematodes will be severe, if management strategies are not done properly; in other words if trees are under nutrition, or other environmental stress. Before planting or replanting citrus orchards the soil should be analyzed for citrus nematodes. These analyses will provide information about the potential nematode damage. From these data it is possible to develop management strategies. The citrus nematode larvae can remain viable in the soil over five years after removal of infested trees (Decker, 1969). Baines **et al.** (1962) obtained living larvae from soil having no host plants of **T. semipenetrans** for nine years; the citrus nematode may live on the viable roots of the removed citrus plants.

Citrus nematode migration from infected to non-infected trees rarely occurs, except where roots overlap or the nematodes are moved by drainage or irrigation water (Meagher, 1967). Drip irrigation may prevent nematode migration from infected trees to non-infected trees. Citrus orchards being one, two, and four years old and established in an area where no citrus had been grown before, were investigated for the occurrence of **T. semipenetrans** in 1994. Both, the citrus nematode and other citrus parasitic nematodes were not observed in neither of these orchards (Elekçioğlu, unpublished data).

These findings indicate that it is possible to keep an citrus orchard almost nematode free if suitable cultivation techniques and quarantine measures are applied.

Table 1. Dominant structure of plant parasitic nematodes associated with citrus in the East Mediterranean region of Türkiye in 1989-1994.

Nematode species	No. of times present in 110 orchards	Location
Aphelenchoides bicaudatus	48	widespread in whole region
Aphelencus avenae	65	widespread in whole region
Ditylenchus myceliophagus	46	widespread in whole region
D. valveus	10	Mersin
Helicotylenchus pseudorobustus	12	Adana, Erdemli
Helicotylenchus sp.	1	Kozan
Heterodera sp.	1	Balcalı (Adana)
Merlinius brevidens	15	Adana, Kozan, Mersin, Tarsus
M. microdorus	8	Balcalı, Erdemli, Dörtyol
Paratylenchus sp.	1	Erdemli
Pleciorotylenchus striaticeps	1	Kozan
Pratylenchoides alkani	2	Erdemli
Pratylenchoides sp.	1	Erdemli
Pratylenchus scribneri	12	Adana, Erdemli
P. thornei	23	Adana, Mersin, Dörtyol
Rotylenchulus macrodoratus	15	Adana, Erdemli, Dörtyol
R. parvus	14	Balcalı, Kozan, Erdemli
Trophurus sp.	1	Erdemli
Tylenchorhynchus brassicae	6	Balcalı, Mersin
T. goffarti	10	Adana, Dörtyol
Tylenchulus semipenetrans	90	widespread in all citrus areas
Tylenchus sp.	-*	Adana, Mersin, Hatay
Dorylaimidae species	-	Adana, Mersin, Hatay
Mononchidae species	-	Adana, Mersin, Hatay

* not determined

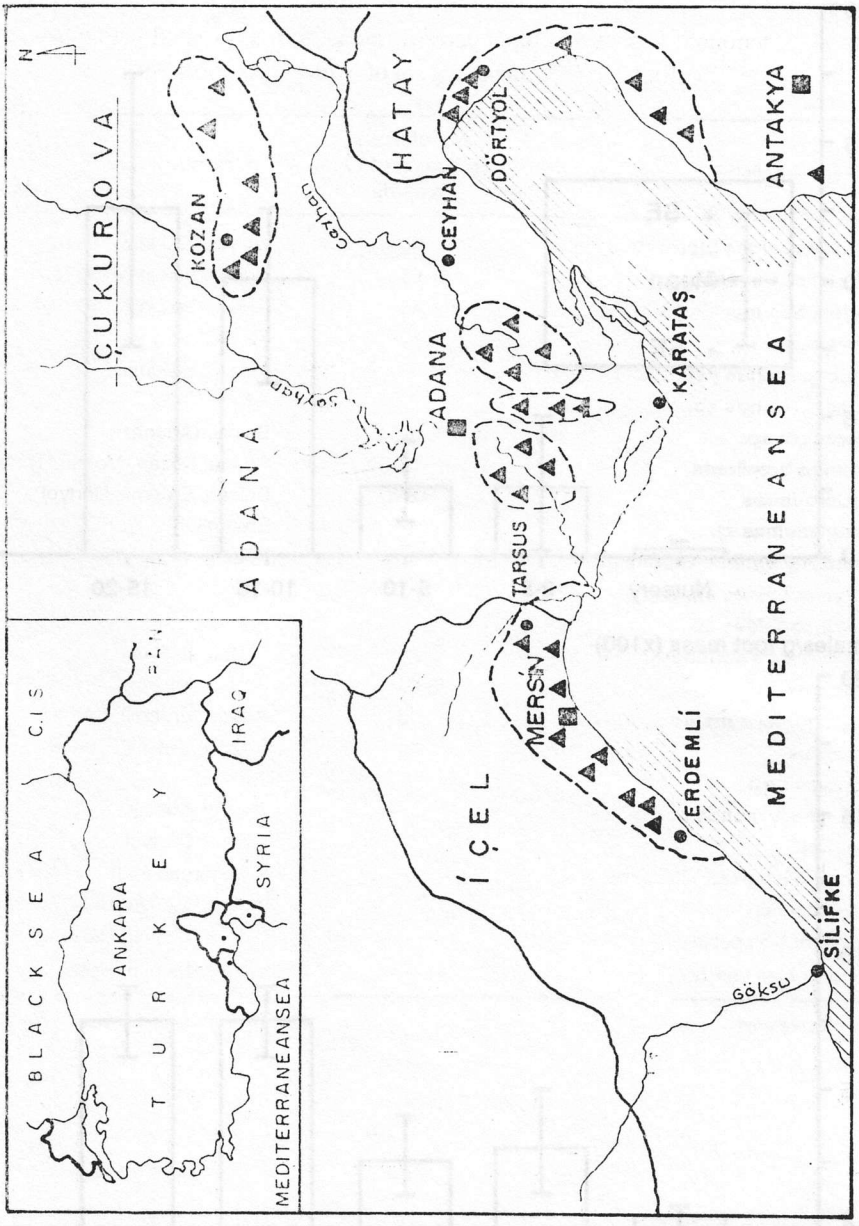

Fig. 1. Distribution of **Tylenchulus semipenetrans** in the East Mediterranean region of Türkiye

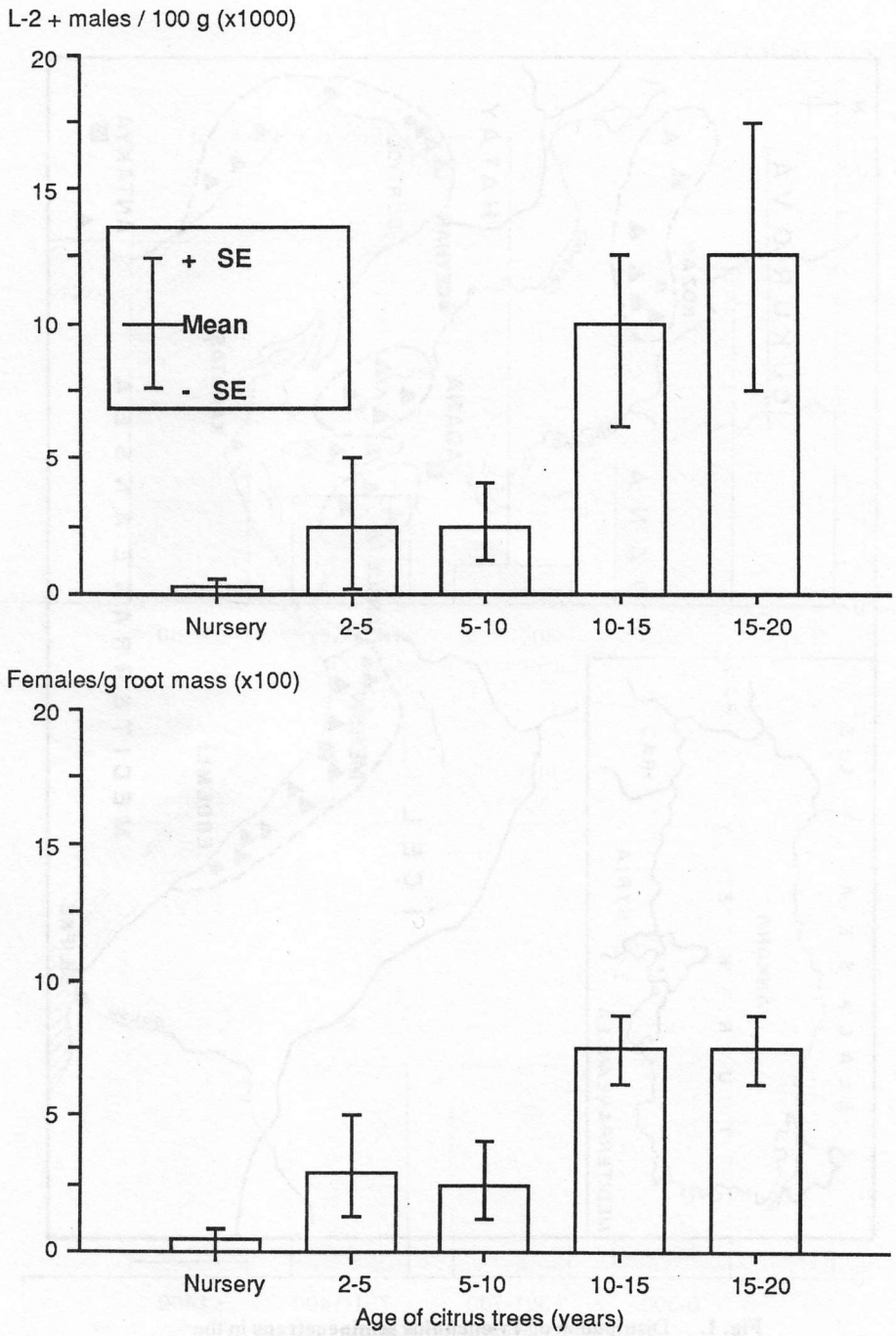

Fig. 2. Population densities of **Tylenchulus semipenetrans** and females in citrus orchards in terms of tree ages in the East Mediterranean region of Türkiye in 1992-1994

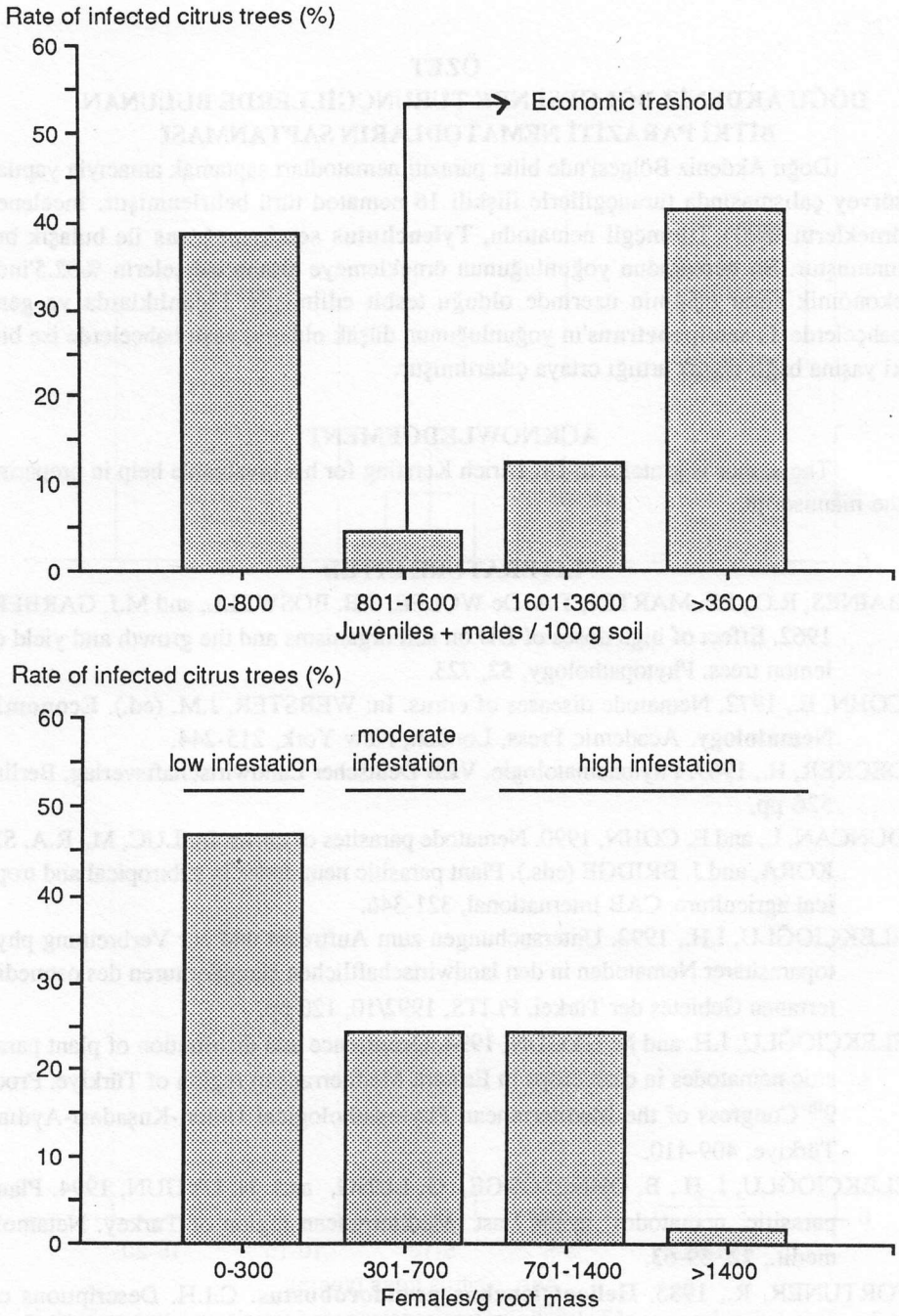

Fig. 3. Frequency of different population densities of **Tylenchulus semipenetrans** attacking citrus trees in the East Mediterranean region of Türkiye in 1992-1994. Indicated levels of economic threshold and infectation are taken from Garabedian *et al.* (1984).

ÖZET

DOĞU AKDENİZ BÖLGESİ'NDE TURUNÇGİLLERDE BULUNAN BİTKİ PARAZİTİ NEMATODLARIN SAPTANMASI

Doğu Akdeniz Bölgesi'nde bitki paraziti nematodları saptamak amacıyla yapılan sörvey çalışmasında turunçgillerle ilişkili 16 nematod türü belirlenmiştir. İncelenen örneklerin %90'ı Turunçgil nematodu, **Tylenchulus semipenetrans** ile bulaşık bulunmuştur. Bu nematodun yoğunluğunun örneklemeye alınan bahçelerin %62.5'inde ekonomik zarar eşiğinin üzerinde olduğu tesbit edilmiştir. Fidanlıklarda ve genç bahçelerde **T. semipenetrans**'ın yoğunluğunun düşük olduğu, yaşlı bahçelerde ise bitki yaşına bağlı olarak arttığı ortaya çıkarılmıştır.

ACKNOWLEDGEMENT

The author is grateful to Dr. Ulrich Kersting for his invaluable help in preparing the manuscript.

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J. Turk. Phytopath., Vol. 24, No.1, 39-42, 1995 ISSN 0378 - 8024

The Effects of Some Herbicides on Wild Black Cumin (**Nigella arvensis L. var. glauca Boiss.**) in Wheat Fields

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ABSTRACT

The application of Terbutryn + Triasulfuron at the rate of 15 + 2.4 g. (a.i.)/da at 5-6 leaves stage of wild black cumin and tillering stage of wheat did not control ***Nigella arvensis*** *L. var.* ***glauca Boiss.*** *Tribenuron-methyl 7.5 g. (a.i.)/da, Tribenuronmethyl+Thifensulfuron-methyl 0.5+1g (a.i)/da and Metasulam – N + EHE 6 + 630 g (a.i)/da dosages controlled* ***N. arvensis*** *59.25%, 52.5% and 57.5%, respectively. On the other hand, Chlorsulfuron 0.75 g. (a.i.)/da and 2.4-D Isooctyl ester 108 g. (a.i.)/da gave 87.5% and 96.75% control of the weed, respectively.*

INTRODUCTION

Davis (1965) has reported that many places in Türkiye have wild black cumin even in non-crop lands, steppes and agricultural fields.

Recently, it was determined by our observations that, the amount of wild black cumin has increased in the cereal crop fields. On the other hand, farmers have pointed out that some herbicides which have been used in cereal fields do not show any effect on wild black cumin.

There is no reference related with the chemical control of **Nigella arvensis** in literature. With this experiment, the effects of some herbicides used in cereal fields were investigated.

MATERIALS AND METHODS

This research was carried out as post emergence in a farmer's field in Yenikent region of Ankara province in 1994.

Chlorsulfuron (Glean 75 DF), Tribenuron-methyl (Granstar), Terbutryn + Triasulfuron (Logran extra 64 WG), Tribenuron methyl + Thifensulfuron – methyl (Harmony extra), Metasulam - N + EHE (Sansac) and 2,4 - D Isooctyl ester (Agro-D ester) were tested at 5-6 leaves stage of the weed at the following dosages shown in Table 1.

Table 1. The herbicides tested, active ingradients and percentages, and application dosages for **Nigella arvensis**

Herbicides	Active ingradient and percentage	Rate of application g (a.i.)/da
Glean 75 DF	Chlorsulfuron, 75	0.75
Granstar	Tribenuron-methyl, 75	7.5
Logran extra 64 WG	Terbutryn+Triasulfuron, 60+4	15+2.4
Harmony extra	Tribenuron-methyl+Thifen-sulfuron-methyl, 25+50	0.5+1
Sansac	Metasulam – N + EHE, 5 + 542.4	6+630
Agro-D ester	2,4-D Isooctyl ester, 48	108

Experiment was established at randomised experimental block design at 4 replicates with 6 characters in a field of a farmer. Plot sizes were arranged as 3x2 m. Untreated stripes of 0.5 m among the parcels and 1 m among the blocks were left. Application was made in a clear and windness weather. Before the application, the density of wild black cumin in the field was determined according to percentage of covered area.To determine the effectiveness of herbicides to wild black cumin, observations were made in the first week and 14th, 21th and 30th days after applications. Calculations were made basicly on the last day's observation data.

RESULTS

The effect of herbicides on **Nigella arvensis** is shown in Table 2.

Table 2. The effectiveness of the herbicides on **Nigella arvensis**

Herbicides	Application dosage g (a.i.)/da	Effectiveness in replicates (%) I	II	III	IV	Mean* effectiveness (%)	
Chlorsulfuron	0.75	95	85	89	90	87.5	a
Tribenuron-methyl	7.5	50	55	45	40	59.25	b
Terbutryn+Triasulfuron	15+2.4	15	0	30	15	15	c
Tribenuron-methyl+ Thifensulfuron-methyl	0.5+1	45	50	55	40	52.5	b
Metasulam-N+EHE	6+630	50	55	45	30	57.5	b
2,4-D Isooctyl ester	108	98	95	96	97	96.75	a

* The differences between the values reciving the same letter are not statistically significant (p = 0.05)

As it can be seen in Table 2, Terbutryn + Triasulfuron did not control the wild black cumin. On the other hand, Tribenuron - methyl, Tribenuron - methyl + Thifensulfuron - methyl and Metasulam - N + EHE had very little effect on the weed according to the evaluation method in Anonymous (1981). Other than that, Chlorsulfuron 0.75 g. (a. i.)/da and 2,4-D Isooctyl ester 108 g. (a.i.)/da dosages controlled **N. arvensis** in percentages of 87.5 and 96.75, respectively. The effects of both Clorsulfuron and 2,4-D ester appliations were not statistically important ($P > 0.05$), Whereas the effect differences between these two herbicides and the other four were found to be significantly important ($P < 0.05$).

DISCUSSION

Chemical control of **Nigella arvensis** L. var. **glauca Boiss.** by herbicides was performed for the first time with this research.

Besides 2,4-D compounds, several newly developed herbicides have begun to be used widely in wheat fields. However, by our research, it was determined that these new herbicides did not effect wild black cumin sufficiently. This proved the reason of farmers complaints about these new herbicides. Because, especially Tribenuron-methyl, Terbutryn + Triasulfuron, Tribenuronmethyl + Thifensulfuron - methyl and Metasulam - N + EHE which are recommended for broad foliage weeds had very little effect on **Nigella arvensis.** 2,4 - D esters showed to be the most effective on wild black cumin. On the other hand the effectiveness of Chlorsulfuron was within acceptable limits according to the evaluation method in Anonymous (1981).

These data determined in our experiment are very interesting. Because, an increase in **N. arvensis** population can happen in the case of continue to use these new herbicides. On the other hand there is no more study carried out on the chemical control of wild black cumin.

This weed is not very common in cereal fields in Middle Anatolia region, but increasing day by day. If chemical control does not start on this weed, it might be create problem in the near future. For that reason more researches should be carried out on the chemical control of **Nigella arvensis** as soon as possible.

ÖZET

BAZI HERBİSİTLERİN BUĞDAY TARLALARINDAKİ YABANİ ÇÖREKOTU (Nigella arvensis L. var glauca Boiss.)'NA ETKİLERİ

Yabani çörekotu (**Nigella arvensis L. var glauca Boiss.**'na 5-6 yapraklı devrede ve buğdayın kardeşlenme döneminde uygulanan Terbutryn + Triasulfuron'un 15 + 2.4 g (a.m.)/da dozu bu yabancıotu etkilememiştir. Tribenuron - methyl 7.5 g (a.m.)/da, Tribenuron - methyl + Thifensulfuron - methyl 0.5 + 1 g (a.m.)/da ve Metasu-

lam - N + EHE'in 6 + 6.630 g. (a.m.)/da dozları (**Nigella arvensis**'i sırasıyla %59.25, %52.5 ve %57.5 oranında kontrol etmiştir. Chlorsulfuron'un 0.75 g (a.m.)/da ve 2.4-D Isooctyl ester'in 108 g (a.m.)/da dozlarının bu yabancıota sırasıyla, %87.5 ve %96.75 oranında etkili olduğu belirlenmiştir.

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The Effects of Root Exudates of **Boreava orientalis** Jaub et Spach on The Root Growth of Wheat Seedlings

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ABSTRACT

*This study was carried out to determine the effect of root exudates of **Boreava orientalis** Jaub et Spach on wheat seedlings under laboratory conditions. The seeds of **B. orientalis** were obtained from Gölbaşı district of Ankara. The seeds coats were eliminated and then surface disinfection was made with hipoclorit for 2%. The seeds of **B. orientalis** were germinated in tubes. Tubes contained water agar for 1% and one seed of **B. orientalis**. The seedlings of **B. orientalis** were removed from agar in tubes on 20th day after sowing and disinfected wheat seeds were sown instead of them. Each tube contained one wheat seed. The root lenghts of wheat seedlings were measured on tenth day after sowing. The data were evaluated using t-test. The variation between treated and control is important at 1% level. Root exudates of **B. orientalis** inhibited root growing of wheats.*

INTRODUCTION

B. orientalis is a member of Cruciferae family and common in Western Asia and Greece (Hegi, 1918). Davis (1965) determined Iran and Türkiye as a motherland of this genus.

B. orinetalis is a common weed among cereal in the Central Anatolia (Göksel, 1956). It is also common in the other parts of Türkiye. Davis (1965) found out **Boreava aptera** Boiss and Heldr. in Burdur and Antalya Provinces.

Özer (1975) determined germination potential of **B. orientalis** seeds using TTC-test. Güncan (1982) determined necessity of removing of the fruit coat of seeds and the inhibition effect of light on the seed germination. He obtained that the germination of seeds were minimum at 1-30°C, optimum at 15-25°C and maximum at 30-35°C. These results showed that **B. orientalis** adapted to cool parts of Türkiye as Central and Eastern provinces. Taştan *et al* (1991) determined optimum temperatures between 5-20°C.

There is not any research on the effects of root exudates of **B. orientalis** on wheat in Türkiye and in the world.

B. orientalis seeds were obtained from Gölbaşı district of Ankara. Seeds in fruits were seperated and surface disinfection was made with hipocloride for 2% and

rinsed 10 minutes with sterilized distilled water (Güncan, 1982).

Clean seeds were sown into tubes and these tubes contained water agar for 1%. These tubes were kept at 10°C and dark condition in incubator (Taştan *et al.*, 1991). Replication number was 23. The seedlings of **B. orientalis** were removed from tubes on 20th day after sowing and disinfected wheat seeds were sown instead of them. Each tube contained one seed. The root lengths of wheat seedlings were measured on 10th day after sowing, the data were evaluated using t-test.

RESULTS

The results are shown in Table 1, the variation between treated and control is important at 1% level. Root exudates of **B. orientalis** inhibited root growing of wheats. The comparison between control and treated wheat seedlings for 10 days are shown at Figure 1.

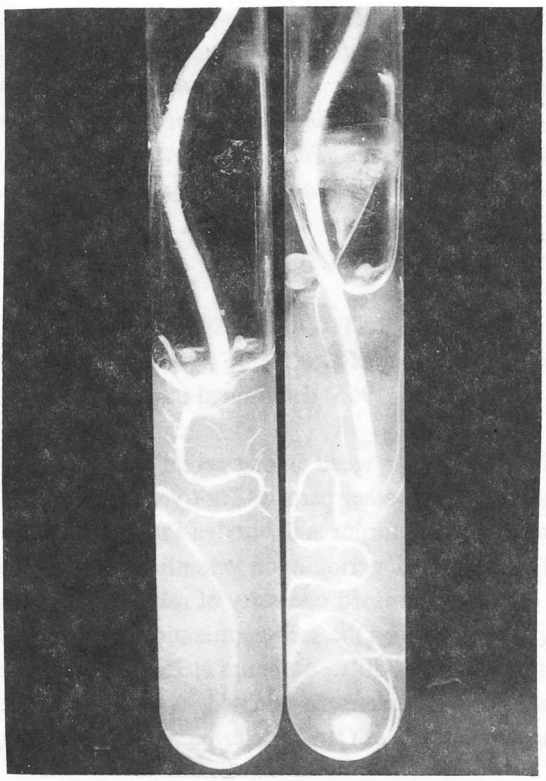

Figure 1. Right control, left treated wheat seedlings for 10 days

Table 1. Root lengths of wheat seedings (mm)

Replicates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Total	Average
Treated	7	3	0	0.5	5	1.5	0	3	3	8	8	4	0.5	2	3	0	0.5	12	5	4	10	13	1	94	4.08
Control	2	8.5	12	10	1	8	7	17	10	4	13	6	15	2.5	18	0.5	9	11	1.9	1	6	8	7	178.4	7.75

t =3.257xx> $t_{\%1}$ = 2.819

Root lengths of control were more than root lenghts of treated.

DISCUSSION

Fruit coats of seeds were removed like Güncan (1982) did. Optimum temperature was accepted 10°C like Taştan et al. (1991). The effects of root exudates of **B. orientalis** on the root lengths of wheat seedlings were found out important. The root exudate of **B. orientalis** inhibited root lengths of wheat seedlings. Root lengths differences between treated and control were important and significance was 1% when statistical analysis was made.

ÖZET

SARIOT (Boreava orientalis Jaub et Spach) UN KÖK SALGILARININ BUĞDAY FİDELERİNİN KÖK GELİŞMESİNE ETKİLERİ

Bu çalışma **B. orientalis**'in kök eksudatlarının laboratuvar şartlarında buğday çimlerinin gelişmesine etkilerini araştırmak amacıyla yapılmıştır. Sarıot tohumlarının kabuğu soyularak yüzey dezenfeksiyonu yapılmıştır. Tüplerdeki %1 lik su agarda sarıot tohumları çimlendirilmiş, çimler 20 günlük iken tüplerden alınmış yerine buğday tohumları ekilmiştir. Her tübe bir tohum konularak 23 tekrarlı çalışılmıştır. Buğday çimleri 10 günlük iken kök uzunlukları ölçülmüştür. Veriler t-testi ile karşılaştırılmıştır. Muameleli ve control arasındaki varyasyon %1 seviyesinde önemli bulunmuştur. Sarıotun kök eksudatları buğday fidelerinin kök uzunluklarını engellemiştir.

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