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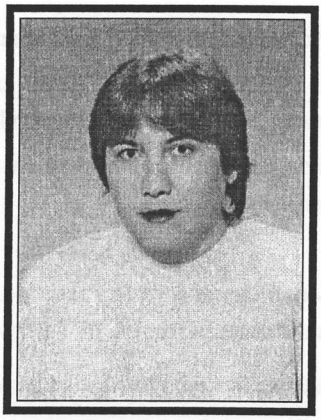
CONTENTS



Assoc. Prof. Yeter CANIHOŞ
(1962 - 2002)

Yeter Canıhoş was an Associated Professor in the Department of Plant Protection, Faculty of Agriculture at the University of Çukurova, Adana / Turkey until passed away in 9th December, 2002.

Yeter obtained a B.S. degree in 1985, and M.Sc. degree in 1988 from the same university. After she get her M.Sc. degree, she started her academic carrier as a research assistant in Plant Protection Department, University of Çukurova. She conduced her Ph.D. study on "The Use of Bacterial Chitinase and B-1,3- Glucanase Genes to Engineer Disease Resistance in Lettuce" from Biochemistry and Biological Science, Wye College, University of London, England in the years of 1990 - 1994. She got a position as assistant professor in 1997, associated professor in 1998 in the same department of Çukurova University.

She has been involved in many research projects in Turkey and abroad. Most of her work involves the epidemiology and control of fungal diseases of citrus and specially induced resistance in lettuce, cucurbits, eggplant and cotton.

Yeter had been in Israel for 6 weeks to attend in a course on "Protected Cultivation on High Value Crops", in Volcani Center and had been in U.S.A. for 4 months work on "Epidemiology and Molecular Characterization of Alternaria Brown Spot on Citrus" in the University of Florida, Citrus Research and Education Center, Lake Alfred, 1997.

During her academic career, she had been conducted 10 project as a leader and/or partner, 6 of them from Turkish Scientific Council and 4 of them from University of Çukurova Research Foundation. She attended 11 congresses national or international, in Canada, USA and Israel. Out of her scientific work she had 28 publications, including 14 papers in foreign language. The main subjects which she work on could be summarized as below:

- The infestation ratio of bunt and yield losses in South East Anatolia Region wheat growing area,
- The usage of antagonist *Trichoderma* species against to Gummosis diseases on lemon tress,

- Integrated control of *Sclerotium rolfsii* on groundnut,
- The role of bacterial citinase and β-1,3 genes on the resistance mechanism against to *Bremia lactucae* and *Botrytis cinerea* on lettuce,
- To encourage the induce resistance mechanism to the pathogens by using herbicides and some inducers,
- Wild mushrooms,
- The distribution, epidemiology and variety reactions of citrus diseases *Alternaria* brown spot in East Mediterranean Region of Turkey.

In spite of her unexpected diseases her passing was sudden and early. Yeter was married and have two sons.

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Determination of the Effect of Wilt Disease Caused by *Verticillum dahliae* Kleb. on Some Physiological and Technological Properties in Cotton

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ABSTRACT

The aim of this study is to determine the relationship between some physiological and fiber technological characterishes of cotton seed and wilt disease caused by *Verticillum dahliae* Kleb. The study was carried out in a farmer's field which was naturally infected by the pathogen in Diyarbakır in 1998.

The Carmen, Delta Pine 90, KAT 92 and SG-501 cotton varieties were used in the experiment. At the end of the season, one kg of cotton seed yield was collected from the healthy and diseased plant for each variety. The weight of 100 seeds, the percentage of germination of seeds, ginning percentage, fiber brightness, yellowness degree of fiber, fiber fineness, fiber length, fiber elongation, fiber strength, and fiber uniformity were examined in the laboratory.

The examined characters were found to be significantly different between healthy and diseased plants for all cotton varieties, except for fiber strength. The weight of 100 seeds, percentage of seeds germination, yellowness degree of fiber, fiber fineness, fiber length, fiber elongation, fiber strength, and fiber uniformity were determined to be higher in healthy plants, but the ginning percentage and fiber brightness were found to be higher in the diseased plants.

Key words: Cotton, Wilt Disease, *Verticillium*

INTRODUCTION

Cotton, as an agricultural and industrial crop, has a significant importance in Turkey. There has been an increase in following the development in irrigation opportunities in the Southeastern Anatolian region. In this region where the cotton production is 882 154 tons per year, and it is 43.6 % of our country's cotton production (Akyıl, 1999). A 118 % increase in Turkey's cotton production by means of the GAP project (Tekinel, 1989) is expected.

One of the most important issues that affects the cotton agriculture is the wilting disease caused by *Verticillium dahliae* fungus. In Turkey, the disease was firstly found by Iyriboz (1941) in Manisa, and later Karaca et al. (1971) and Esentepe (1974) indicated that the disease was common in the Aegean and Mediterranean regions, respectively. Sağır et al. (1992), also, in their studies on cotton diseases carried on in the Southeastern region, found out that the most important disease in the region was the wilting disease and that the prevalence rate of the disease was 79.28 % and the avarage disease rate was 16.27 %.

In some other, (Uygun et al., 1978; Aydemir et al., 1978; El-Zik, 1985; Esentepe et al., 1986; Bejanaro-Cazar et al., 1997), it was determined that there was a close relationship between the disease severity and the yield loss, and that the disease caused significant cotton yield loss and had negative effects on the industrial characteristics of the cotton fiber.

V. dahlae fungus also causes diseases in many vegetables, leguminous plants, ornamentals, industrial plants, orchards, and wild plants besides in cotton plant (Saydam and Copçu, 1972; Saydam and Copçu, 1973; Kocatürk and Karcılıoğlu, 1979; Bhat and Subbaro, 1999).

The fungus is a soil pathogen; however, there is no economic chemical control against it. Therefore, in order to reduce the damage of the disease, the idea of growing more tolerant varieties has been suggested besides the cultural practices (El-Zik, 1985; Sezgin, 1985; Sezgin et al., 1985; Karcılıoğlu et al., 1992; Sağır and Tatlı, 1995; Oran et al., 1994; Melero Vera et al., 1995; Sağır and Başbağ, 1998; Xiao et al., 1998).

Although there are many studies on the cotton wilting disease in our country, only a few studies were found in a literature study'ıng the effects of wilting disease on the cotton seed physiology and the fiber technology.

This study was carried out in order to find out under the conditions of Diyarbakır the effects of wilting disease on 100 seed weight, seed germination rate, cotton gin yield, and the physiologic and industrial characteristics of cotton fiber under Diyarbakır province conditions.

MATERIALS and METHODS

This study was conducted in a grower's field which was infected by the disease (*V. dahliae)* in the Dicle River valley in Diyarbakır in 1998. The experiment was set up in randomized blocks designed with four replications, and the cotton varieties such as Carmen, Delta Pine 90, KAT 92 and SG-501 were used in the study.

The ground fertilizer carrying 7 kg/da pure nitrogen and 8 kg/da pure phosphor were used and the sowing was held on 25th April 1998. The second fertilizer was 4 kg/da

nitrogen with the first irrigation and 3 kg/da nitrogen with the second irrigation was used.

The plants were analized for the disease by cutting them at the crown level four times from randomly chosen four locations in every parcels for every species at the end of the growing season on 21st October 1998. One kilo of cotton from the diseased and the healthy plants were brought separately to the laboratories.

The laboratory studies were made as four replications. The fiber yield and 100 seed weight of each varieties were calculated after the aplication of cotton gin for both cotton species. Later, the germination rate was determined by germinating these seeds at 22°C in an incubator. The fiber brightness, the yellowness degree, the fiber elasticity, the fiber fineness, the fiber strength, the fiber length and the fiber uniformity of each cotton variety were examined by the "High Volume Instruments (HVI)" in the quality analysis laboratories of Akyıl Tekstil A.Ş. in Diyarbakır.

The variance analysis for each characteristics investigated by the MSTAT C programs on the computer was used to determine the different characteristics after establishing the angle values of the results given as percentages.

RESULTS and DISCUSSION

The results of 100 seed weight, the germination rate, the cotton gin yield, the fiber brightness, the yellowness degree, the fiber fineness, the fiber length, the fiber strength, the fiber elasticity, and the fiber uniformity of both varieties, infected by the wilting disease and the healthy varieties as Carmen, Delta Pine 90, KAT 92 and SG-501, are given in Table 1.

As it can be seen from the table, the features showed statistically differences except the fiber strength, and 100 seed weight of four varieties were found out to be less than the healthy plants. The lowest 100 seed weight (6.39 g) was found in the infected Delta Pine 90 plants; however, KAT 92 plants had the highest 100 seed weight (10.34 g). The results optained about the seed weights confirmes the results of Aydemir et al. (1978). However, the researchers mentioned above states that the seed gained from the infected plants were undersized.

The varieties showed differences on the cotton gin yield, and the highest yield was gained as 42.61% from the infected Delta Pine 90, while the lowest yield was gained as 38.16% from the healthy SG-501 plants. In general, the cotton gin yield of infected plants were found out to be higher.

The germination rate of the infected and the healthy plants showed significant differences for all varieties. In the study, however, the lowest germination rate was found out as 51.75% in the infected SG-501 plants and the highest as 90.25% in the

Table 1. 100 Seed Weight, Ginning Percentage, Germination Rate and Some Technological Properties of Fiber Obtained From Healthy and Diseased Plants of Cotton Varieties Such as Carmen, Delta Pine 90, KAT 92 and SG-501.

Properties Examined	Plants	Cotton Varieties			
		Carmen	Delta Pine 90	KAT 92	SG-501
100 Seed Weight (g)	Diseased	8.49 B	6.39 C	8.66 B	8.52B
	Healthy	9.80 A	7.93 B	10.34 A	9.81 A
Ginning Percentage (%)	Diseased	40.56 BC	42.61 A	39.71 BCD	38.16 D
	Healthy	39.90 BCD	41.25 B	39.26 CD	38.78 D
Germination Rate (%)	Diseased	60.41 CD	58.50 CD	80.16 ABC	51.75 D
	Healthy	85.25 AB	90.25 A	87.75 A	71.25 BCD
Fiber Brightness (%)	Diseased	78.15 A	79.08 A	78.00 AB	76.85 AB
	Healthy	79.30 AB	75.72 B	77.77 AB	76.00 B
Fiber Yellowness	Diseased	7.51 D	8.11 BC	8.40 AB	8.00 BC
Degree (%)	Healthy	7.70 CD	8.68 A	8.00 BC	8.12 BC
Fiber Fineness	Diseased	3.10 B	2.75 B	3.08 B	3.08 B
(Micronair)	Healthy	3.80 AB	3.45 AB	3.88 A	3.88 AB
Fiber Length (mm)	Diseased	29.05 AB	28.75 AB	27.54 C	28.86 AB
	Healthy	29.60 A	29.12 AB	28.54B	29.19 B
Fiber Elasticity (%)	Diseased	6.48 ABC	6.30 BC	6.70 AB	6.05 C
	Healthy	6.60 AB	6.60 AB	6.83 A	6.53 AB
Fiber Strength (g/tex.)	Diseased	20.48 A	19.58 A	19.60 A	20.10 A
	Healthy	21.13 A	20.98 A	19.95 A	20.78 A
Fiber Uniformity (%)	Diseased	44.45 BC	43.65 C	44.03 C	43.73 C
	Healthy	45.48 AB	44.25 BC	43.85 C	46.10 A

healthy Delta Pine 90 plants. According to these results, it was found that the wilting disease had significant effects on germination.

The reason for the fact that the seed weight of the infected plant seeds were lower than those of the healthy plant seeds was that the seeds does not grow well due to the disease itself rather than being infected. However, Karaca et al. (1973), reported that, *V. dahliae* fungus was carried by the cotton seeds in their study in A.R.

The fiber brightness degrees of all varieties were found to be higher for the infected plants than the healthy plants. However, the infected and healthy plant fibers of KAT 92 were in the same group in terms of this feature. The fiber brightness is a very important feature in terms of technology. The fact that the fiber brightness of the infected plants was found to be much higher was due to the fact that the bolls opened earlier because of the disease.

The yellowness degree of the fibers gained from the infected varieties as Carmen, Delta Pine 90 and SG-501 were found to be lower than the other healthy varieties.

However, the results of KAT 92 were just the opposite. The textile industry prefers the low degree of the fiber yellowness. It was quite interesting that the fiber yellowness degrees of the infected varieties as Carmen, Delta Pine 90 and SG-501 were low.

The infected plant fibers of each of four cotton varieties used in the study were found to be thinner than the healthy plants. The thickest fibers (3.88 mic.) were found in the healthy KAT 92, while the thinnest fibers (2.75 mic.) were measured in healthy Delta Pine 90 plants. The findings about the fiber fineness were compatible with those of Aydemir et al. (1978).

The fiber length gained from the healthy plants of four cotton varieties studied was found out to be longer than that of the diseased plants. The longest fiber (29.60 mm) was gained from the healthy Carmen plants, but the shortest fiber (27.54 mm) was obtained from the diseased KAT 92 plants.

The fiber elasticity gained from the healthy plants of all varieties was found to be higher than that of the diseased plants. The lowest value (6.05%) was found in the diseased SG-501 plants, whereas the highest value (6.83%) was obtained from the healthy KAT 92 plants.

The values of the varieties, and the healthy and the diseased plants were statistically placed in the same group in terms of the fiber strength. The lowest fiber strength value (19.58 g/tex.) was found from the healthy Delta Pine 90 plants and the lowest value (21.13 g/tex.) from Carmen plants.

The fiber uniformity of the healthy Carmen, Delta Pine 90 and SG-501 plants was found to be higher than those of the diseased plants. However, the fiber uniformity of both healthy and diseased KAT 92 plants was placed in the same group. The lowest uniformity (43.65%) was found in diseased fibers of Delta Pine 90 plants and the highest (46.10%) in diseased SG-501 plants.

Although there has been a lot of researches on wilting disease in Turkey, the relationship between the disease and the technological characteristics of the fibers have not been fully investigated yet. However, Aydemir et al. (1978), in their study in the Aegean region, indicated that the fibers of the diseased plants were very thin. In the study by El-Zik (1985), this issue was discussed. The researcher found out that there was a negative but significant relationship between the disease and the fiber length, between the fiber yield and the fiber strength; a positive relationship between the fiber elasticity and the fiber uniformity; and that there was no relationship between the cotton gin yield and the fiber fineness.

As a result, it was found out in the study carried under Diyarbakır province conditions, that the wilting disease was effective on the technological characteristics of the fibers. It would be advisable to focus on this issue during breeding studies and to sustain these studies by using various varieties.

ÖZET

SOLGUNLUK HASTALIĞI (*Verticillum dahliae* Kleb.)' NIN PAMUĞUN BAZI FİZYOLOJİK VE TEKNOLOJİK ÖZELLİKLERİNE ETKİSİNİN BELİRLENMESİ

Bu çalışma, solgunluk hastalığı ile pamuk tohumunun bazı fizyolojik ve lif teknolojik özellikleri arasındaki ilişkiyi belirlemek amacıyla1998 yılında Diyarbakır'da yapılmıştır. Çalışma, hastalık ile yoğun bulaşık bir üretici tarlasında,Carmen, Delta Pine 90, Kat 92 ve SG-501 pamuk çeşitleri kullanılarak yürütülmüştür. Mevsim sonunda 4 tekrarlı olarak her çeşide ait hastalıklı ve sağlıklı bitkilerden 1 kg pamuk toplanarak; 100 tohum ağırlığı, tohumların çimlenme oranı, çırçır randımanı, lif parlaklığı, lif sarılık derecesi, lif inceliği, lif uzunluğu, lif elastikiyeti, lif kopma dayanıklılığı ve lif uniformitesi özellikleri incelenmiştir. Laboratuar analizleri sonucunda elde edilen bulgulara göre, lif kopma dayanıklılığı hariç her 4 pamuk çeşidinde de hasta ve sağlam bitkilerden elde edilen tohumların ağırlığı, tohumların çimlenme oranı ve çırçır randımanı ile incelenen diğer teknolojik lif özellikleri istatistiki olarak farklılık göstermiştir. Lif kopma dayanıklılığı hariç, incelenen tüm özellikler pamuk çeşitlerinde hastalıklı ve sağlıklı bitkilerde farklılık göstermiştir. Hasta bitkilerden elde edilen 100 tohum ağırlığı, tohum çimlenme oranı, lif sarılık derecesi, lif inceliği, lif uzunluğu, lif elastikiyeti, lif kopma dayanıklılığı ve lif uniformitesi sağlıklı bitkilere göre daha düşük; çırçır randımanı ve lif parlaklığı ise hastalıklı bitkilerde daha yüksek bulunmuştur.

Anahtar Kelimeler: Pamuk, Solgunluk, *Verticillium*

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Detection of Cucumber Mosaic Virus (CMV) and Zucchini Yellow Mosaic Virus (ZYMV) in Squash in Erzurum, Erzincan and Artvin Provinces by Serological and Biological Methods

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ABSTRACT

This study was conducted in order to determine viral agents on squash fields in Erzurum (Dumlu, Ilıca), Erzincan (Üzümlü) and Artvin (Yusufeli) districts in 1999-2001 time period. In this study, total 90 squash leaf samples from Erzurum (48), Erzincan (24), Artvin (18) provinces were collected in June and August of 1999, 2000, and 2001. They have been tested by DAS-ELISA kits specific to cucumber mosaic virus (CMV) and zucchini yellow mosaic virus (ZYMV). According to the results of ELISA, all samples were positive for ZYMV infection whereas no CMV was detected in the same samples collected from the same districts. We observed that disease varied from year to year and the number of infected plants increased within the year.

Key words: CMV, ZYMV, Squash, ELISA

INTRODUCTION

Turkey has quite a proper climatic conditions for vegetable growing. However, vegetable production is mainly restricted in planting area and plant cultivars due to climatic conditions in Eastern Anatolia.

The squash production in Erzurum, Erzincan and Artvin provinces is less than other regions. According to 1998 statistics, the total squash production of Erzurum, Erzincan and Artvin are 977, 1459, and 62 ton, respectively (Anonymous, 2000). Nevertheless, there are number of factors that decrease yield and quality of squash. Virus diseases are one of these factors limiting quality and quantity. So far, more than 30 virus diseases have been reported to cause yield lost in cucurbits economically. ZYMV and CMV are reported as the most destructive virus diseases of cucurbits worldwide (Gibbs and Harrison, 1970; Lovisolo, 1980; Lisa and Lecoq, 1984; Davis, 1986). The previous studies performed in Turkey have reported the existence of ZYMV

and CMV in squash (Özalp, 1964; Yılmaz and Davis, 1985; Ertunç, 1992; Vargün and Ertunç, 1994; Yılmaz et al., 1994; Uçar and Ertunç, 1998).

ZYMV is a member of potyvirus group and transmissible by inoculation of sap or in non-persistent manner by aphids, causes reduction in plant growth, yellow mosaic and distortion in leaves and malformation in fruits (Lisa et al., 1981; Yılmaz et al., 1994; Zitter, 1996). CMV belongs to cucumovirus group, has a wide host range, is transmitted by many aphid species in the non-persistent manner and is readily transmissible by inoculation of sap, causes severe plant stunting, yellow mosaic, malformation in cucumber and other cucurbits (Gibs and Harrison, 1970; Zitter et al, 1996).

However, there have been no attempts to study the virus diseases; causing yield losses in squash growing in eastern Anatolian region of Turkey, including Erzurum Erzincan and Artvin provinces.

This study was conducted to determine virus diseases caused by DAS-ELISA in squash plants growing in Dumlu, Ilıca (Erzurum); Üzümlü (Erzincan) and Yusufeli (Artvin) districts and to monitor symptoms in squash plants grown under greenhouse conditions.

MATERIALS and METHODS

This study was conducted during June through August in 1999, 2000, and 2001. Squash plants showing virus-like symptoms (mosaic, malformation) collected from Dumlu, Ilıca (Erzurum); Üzümlü (Erzincan) and Yusufeli (Artvin) districts were used in this study. Total 90 squash samples were collected from Dumlu (30), Ilıca (18), Üzümlü (24), and Yusufeli (18) and stored in –30°C.

Samples were tested serologically by using direct DAS-ELISA kit specific for CMV and ZYMV viruses (Clark and Adams, 1977). ELISA kits were specific to CMV and ZYMV and supplied from Agdia (Agdia Company, Elkhart, USA). Sample preparation was performed as described by the manufacturing company. After dispensing 100 ml from each sample into the well of a 96-well microtiter plates, the plates were kept at room temperature for 2 hours and then washed with wash buffer 6 times. (Elx50 Auto Strip Washer Bio-Tek Instruments, Inc. B-2610 Wilrijk, Belgium). An aliquot of 100 ml alkaline phosphates conjugated was added to each well. The plates were incubated for 2 hr at room temperature and washed as described above. 100 µl PNP (P-nitrophenyl phosphate buffer) was added into each well and the plates were incubated for 40 min. Absorbans was measured at 405 nm by using a microplate autoreader (Elx800 Universal Microplate Reader Bio-Tek Instruments, Inc. B-2610 Wilrijk, Belgium).

Three samples taken from each region were inoculated into three squash (*Cucurbita pepo* L. Cv "Sakız") plants grown in a greenhouse by mechanical inoculation

method. Mechanical inoculation was done 12 days after the formation of cotyledon leaves of seedling and development of symptoms was monitored for 6 weeks.

RESULTS and DISCUSSION

We found that virus disease were limited to certain planting areas and observed that virus spreading in vegetation season increased in these planting areas. There were few plants infected by the virus in two farmer's fields in Erzincan and five farmer's fields in Dumlu (Erzurum) at the end of June in 1999. However, approximately all of the plants in these fields where infection by the diseased at the end of the season. Similar phenomenon were observed in Ilıca (Erzurum) and Yusufeli (Artvin) in 2000.

The reason of increase in virus spreading in same planting areas would be the transmission of viruses by vectors or by means of field applications such as harvesting and hoeing. Transmission of viruses by seeds could explain why plants in some areas were infected locally in some years. The distribution rate of this virus was relatively higher in the fall compared to spring. It has been reported that ZYMY is distributed by seeds and mechanical means as non-persistent with vectors such as *Myzus persicae* and *Aphis gossypii* and many weeds is known to be hosts for ZYMV (Uçar and Ertunç, 1998; Zitter et al., 1996). It may be explained by low rate of virus infected fields and disease incidence, at the beginning of season, but increasing by the end of season.

ELISA results demonstrated that all samples taken from squash plants were positive for ZVMV. No CMV was detected in the same samples. In squash plants ZYMV caused symptoms like yellow-green mosaic on leaves, fruit deformation, and stunting squash plants were observed both under field and greenhouses conditions. Distortion of leaves and malformation of fruits were observed in ZYMV infected plants (Figure 1, 2). The symptoms of ZYMV observed in this study is in accordance with the observations reported in many other studies (Lisa et al., 1981; Yılmaz and Davis, 1985; Yılmaz et al., 1994; Vargün and Ertunç, 1994;1995; Zitter, 1996; Uçar and Ertunç, 1998).

Overall evaluation of these results showed that the existence and distribution of ZYMV in squash were relatively limited to region. This result may be explained by the lack of an alternative host.

ZYMV was determined first time by this study in Erzurum, Erzincan and Artvin provinces.

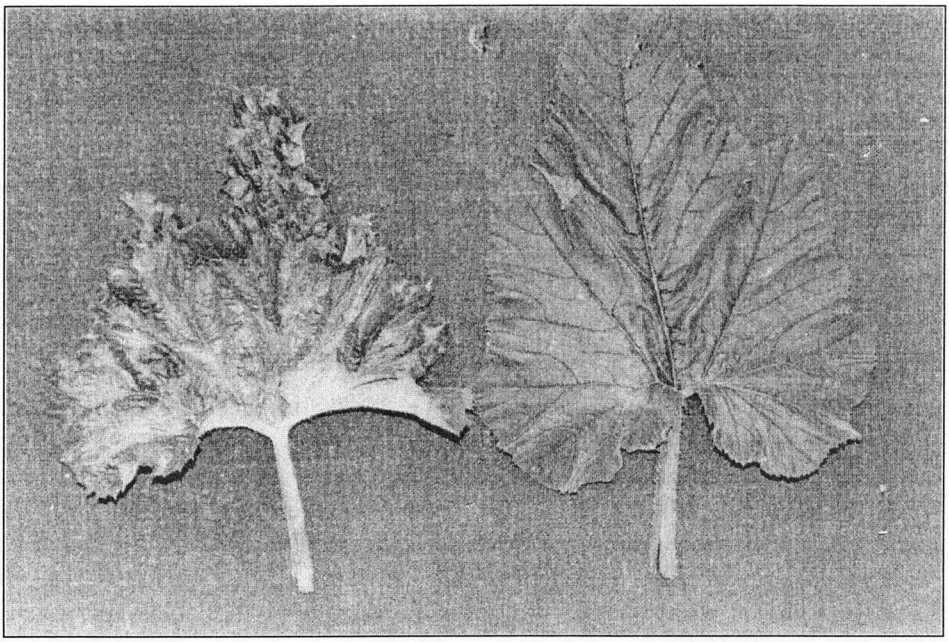

Figure 1. A) ZYMV symptoms on a squash plant leaf 21 days after inoculation B) A healthy squash plant leaf.

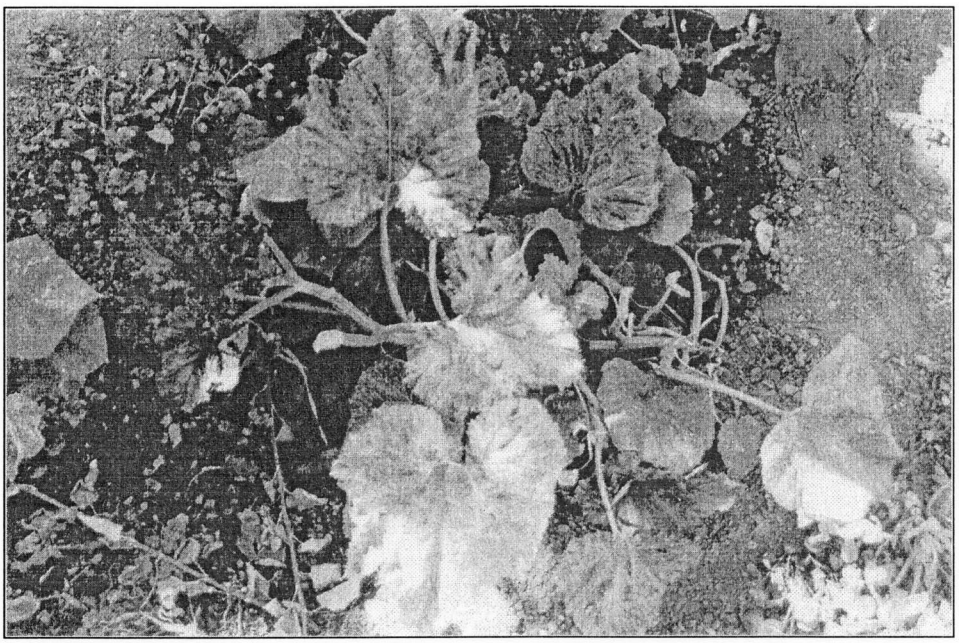

Figure 2. ZYMV symptoms on a squash plant grown under field conditions.

ÖZET

ERZURUM, ERZİNCAN VE ARTVİN İLLERİNDEKİ KABAK EKİM ALANLARINDA ZYMV VE CMV'NİN SEROLOJİK VE BİYOLOJİK OLARAK BELİRLENMESİ

Bu çalışma 1999-2001 yılları arasında Erzurum (Dumlu, Ilıca), Erzincan (Üzümlü) ve Artvin (Yusufeli) illerinde kabaklardaki viral etmenleri belirlemek amacıyla yapılmıştır. Çalışmada, Erzurum'dan 48, Erzincan'dan 24 ve Artvin'den 18 olmak üzere Haziran ve Ağustos aylarında virüslere özgü simptom gösteren bitkilerden toplam 90 yaprak örneği alınmıştır. Alınan yaprak örnekleri Hıyar mozayik virüsü (CMV) ve Zucchini sarı mozayik virüsüne (ZYMV) ait spesifik DAS-ELISA kitleri ile testlenmişlerdir. ELISA testi sonucunda örneklerin tamamının ZYMV ile enfekteli olduğu belirlenirken CMV saptanamamıştır. Çalışmada hastalık seyrinin ekim alanından ekim alanına, yıldan yıla değişim gösterdiği ve yıl içerisinde hastalıklı bitki sayısında artışların olduğu gözlenmiştir.

Anahtar Kelimeler: CMV, ZYMV, Squash, ELISA

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The Effects of Chemical Control of Powdery Mildew (*Erysiphe betae*) on Yield and Quality of Susceptible and Tolerant Sugar Beet Varieties

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ABSTRACT

In this study, the effects of the chemical control of powdery mildew on yield and quality of susceptible and tolerant sugar beet varieties were investigated. The study was carried out in Ankara and Konya, representing Central Anatolia region, in 1997-1998. The disease appeared in mid August in 1997 and at the end of July in 1998 at the earliest. All plots except the untreated ones in both trial sites were treated one time in 1997 and 2 times in 1998 with of 15-20 days intervals.

Tridemorph application has increased the root yield by 3.9-10.3% (2.44-4.64 t/ha) and the extractable sugar yield by 5.6-10.4% (0.62-0.80 t/ha). Treatment with Nuarimol gave a 1.2-6.4% increase in Nuarimol gave increases in the root yield by 1.4-5.1% (0.84-2.31 t/ha) and the extractable sugar yield (0.13-0.75 t/ha). In both treatments slight increases were obtained in sugar content as 0-2.6% (0-0.55 °Z) and in extractable sugar content as 0-3.2% (0-0.59 °Z).

The tolerant variety, "Hülya", was superior in root yield by 2-14.7% and extractable sugar yield by 3.6-16.1% to the susceptible variety, "Fiona". The quality differences between the varieties were low and can be neglected.

These results indicate that root and extractable sugar yield of sugar beet by Tridemorph application against the disease was better than Nuarimol application. In addition, not only fungicide applications but also sowing of tolerant varieties in powdery mildew control showed a better performance in root and extractable sugar yield of sugar beets. But there were no important changes in quality parameters of sugar beets.

Key words: Powdery mildew, *Erysiphe betae*, sugar beet, tolerant sugar beet variety, chemical control

INTRODUCTION

Powdery mildew [*Erysiphe betae* (Vañha) Weltzien] is second important foliar disease after Cercospora leaf spot on sugar beet in Turkey and it occurs in all the regions of the country, except East Anatolia.

Keskin (1974) and Göbelez (1976) obtained 8-18% (4.8-7.87 t/ha) increases in root yield, 0-2% (0-0.33 °Z) in sugar content, and 9-20% (0.88-2.27 t/ha) in sugar yield by using Enovit Super in control of the disease in Konya.

Özgör (1995) reported that the disease causes 5-10% losses in root yield at the beginning time of the infection and advised that sprayings should be started with systemic fungicides such as Calixin, Bayleton, Bayfidan, Trimidal and Sumi when the symptoms of the disease first occur in fields.

In Spain Ayala and Bermejo (1995) reported that powdery mildew was almost completely controlled by fungicides and that the disease control resulted by a 16% increase in sugar yield when Impact-R was used and 12.5% when Punch CS and Alto Combi were used.

In Spain Ayala and Gordo (1998) determined that the disease correlated with root yield of 86%, sugar content of 70% and sugar yield of 83% negatively, but α-AmN content of 76%, Na content of 73% and K content of 27% positively.

Bayford (1996) reported that powdery mildew leads to losses of root yield of 2-15 % and that fungicides which have good effects on the disease such as Sulphur, Bayleton, Bayfidan and Calixin were generally used to control the disease in many countries in the world.

Powdery mildew, causes a significant loss in root and sugar yield in sugar beet in many countries. These losses range from country to country, from region to region and furthermore, even in places where there is a little different climate in the same region. In this study, by sowing a tolerant sugar beet variety and by applying effective and common fungicides against powdery mildew right after the time of the infection, the effects of the disease control on the yield and the quality of sugar beet were investigated in detail under Ankara and Konya conditions, representing the Central Anatolia.

MATERIALS and METHODS

Powdery mildew appears in about middle of summer season every year according to climatic conditions in the Central Anatolia where sugar beet is extensively grown. Farmers do not take any precaution. That's why the trials were conducted in Ankara (Etimesgut Trial Station of Sugar Institute) and Konya (Alakova Trial Station of Sugar Institute), representing the Central Anatolia, in 1997-1998.

The trials were carried out in split-plot design with two factors and 4 replications. One of the factors was fungicide treatments, and the other was sugar beet varieties. The fungicides, which were untreated (control) and treatments of Calixin (75% Tridemorph, 0.5 l/ha) and Trimidal (9% Nuarimol, 0.2 l/ha), were applied to main plots. The varieties, called Fiona (susceptible sugar beet variety) and Hülya (tolerant sugar beet variety)

were applied to subplots in the trials. The treatments of all main plots and subplots were at randomly chosen.

Sugar beet seeds were sown in 5 cm seed spacing and 45 cm row width. Fungicides were applied with a knapsack sprayer having hollow cone nozzle of No. D3 in the volume of 444 l/ha. In both trials sites, the first sprays were done when the symptoms of powdery mildew appeared and 15-20 days later the second sprays were done.

The roots of sugar beet plants were weighed and analysed. In everyplot after being harvested. The results were evaluated in terms of root yield, sugar content (%), µ-AmN (%), Na (%), K (%), extractable sugar content, and extractable sugar yield.

Table 1. The date of sowing, treatment, and harvesting in the trials of Ankara and Konya in 1997 and 1998.

Site	Trial management	Years and the date	
		1997	1998
Ankara	Sowing	21.05.97	04.05.98
	First spraying	15.08.97	24.07.98
	Second spraying	-	13.08.98
	Harvesting	31.10.97	03.10.98
Konya	Sowing	02.04.97	16.04.98
	First spraying	20.08.97	24.07.98
	Second spraying	-	13.08.98
	Harvesting	17.10.97	03.10.98

RESULTS

The results of the trials conducted in Ankara and Konya are given in Table 2 and 3.

The difference between Tridemorph and Nuarimol applications against powdery mildew and untreated was not statistically significant in the study done in Ankara in 1997. However, Nuarimol and Tridemorph gave quite high increases in the root yield of 2.31-3.37 t/ha and in the extractable sugar yield of 0.31-0.66 t/ha respectively. Powdery mildew tolerant variety (Hülya) was superior to susceptible variety (Fiona) in root yield by 7.08 t/ha and extractable sugar yield by 1.30 t/ha. The superiority of Hülya to Fiona in root and extractable sugar yield was statistically significant but there were no differences between the two varieties in terms of the quality parameters, which are sugar, ∝-AmN, Na, K and extractable sugar contents.

In 1998 in the same are, only Tridemorph application gave a significant increase in the extractable sugar yield of 0.80 t/ha. No differences were found statistically important between the other parameters in either Tridemorph nor Nuarimol. However, a

Table 2. The effects of chemical control of powdery mildew on yield and quality parameters of two sugar beet varieties. Fiona and Hülya, under Ankara conditions.

	Treatment and variety		Root yield	Sugar content	Na	K	AmN	Extractable Sugar content	Extractable Sugar yield
			t/ha	°Z	mmol /100	g	beet	°Z	t/ha
1997									
Untreated	Fiona		51.88	20.49	1.16	5.38	1.63	17.80	9.24
	Hülya		57.38	20.49	1.05	5.50	1.29	17.83	10.23
Average of Untreated			54.63	20.49	1.10	5.44	1.46	17.82	9.73
Calixin	Fiona		54.90	20.48	1.03	5.27	1.18	17.91	9.83
	Hülya		61.10	20.68	1.11	5.74	1.34	17.91	10.95
Average of Calixin			58.00	20.58	1.07	5.50	1.26	17.91	10.39
Trimidal	Fiona		52.18	20.15	1.16	5.31	1.37	17.51	9.14
	Hülya		61.70	20.55	1.18	5.75	1.42	17.75	10.94
Average of Trimidal			56.94	20.35	1.17	5.53	1.40	17.63	10.04
Average of Fiona			52.98	20.37	1.11	5.32	1.39	17.74	9.40
Average of Hülya			60.06	20.57	1.11	5.66	1.35	17.83	10.70
LSD for the average		%5							
of the fungicides		%1							
LSD for the average		%5	2.31						0.39
of the varieties		%1	3.32						0.55
1998									
Untreated	Fiona		42.08	19.16	1.18	3.92	2.19	16.92	7.12
	Hülya		48.00	19.59	1.11	4.25	1.97	17.28	8.26
Average of Untreated			45.04	19.38	1.14	4.09	2.08	17.10	7.69
Calixin	Fiona		47.63	19.18	1.30	3.92	1.90	16.92	8.04
	Hülya		51.73	19.50	1.08	4.01	1.57	17.32	8.95
Average of Calixin			49.68	19.34	1.19	3.96	1.73	17.12	8.49
Trimidal	Fiona		42.63	19.24	1.09	3.88	2.07	17.05	7.26
	Hülya		52.08	19.19	1.05	4.29	1.90	16.89	8.79
Average of Trimidal			47.35	19.21	1.07	4.09	1.99	16.97	8.02
Average of Fiona			44.11	19.19	1.19	3.91	2.05	16.96	7.47
Average of Hülya			50.60	19.43	1.08	4.18	1.81	17.16	8.67
LSD for the average		%5							0.47
of the fungicides		%1							0.71
LSD for the average		%5	2.69						0.34
of the varieties		%1	3.87						0.49

Table 3. The effects of chemical control of powdery mildew on yield and quality parameters of two sugar beet varieties. Fiona and Hülya, under Konya conditions.

Treatment and variety			Root yield t/ha	Sugar content °Z	Na mmol /100	K g	AmN beet	Extractable Sugar content °Z	Extractable Sugar yield t/ha
1997									
Untreated	Fiona		56.28	19.52	0.50	3.55	1.29	17.72	9.97
	Hülya		63.23	20.00	0.42	3.30	1.03	18.34	11.58
Average of Untreated			59.75	19.76	0.46	3.43	1.16	18.03	10.78
Calixin	Fiona		58.70	19.80	0.47	3.57	1.18	18.01	10.57
	Hülya		67.80	19.73	0.44	3.25	1.16	18.06	12.24
Average of Calixin			63.25	19.76	0.46	3.41	1.17	18.04	11.40
Trimidal	Fiona		56.18	19.69	0.48	3.46	1.17	17.94	10.08
	Hülya		65.00	19.70	0.42	3.25	1.10	18.05	11.73
Average of Trimidal			60.59	19.69	0.45	3.35	1.13	17.99	10.91
Average of Fiona			57.05	19.67	0.48	3.53	1.22	17.89	10.21
Average of Hülya			65.34	19.81	0.43	3.27	1.09	18.15	11.85
LSD for the average of the fungicides		%5							
		%1							
LSD for the average of the varieties		%5	1.88						0.42
		%1	2.70						0.60
1998									
Untreated	Fiona		62.75	20.74	0.71	4.87	1.21	18.42	11.53
	Hülya		62.93	20.88	0.51	4.51	0.93	18.78	11.82
Average of Untreated			62.84	20.81	0.61	4.69	1.07	18.60	11.68
Calixin	Fiona		65.55	21.05	0.49	4.99	0.80	18.81	12.33
	Hülya		65.00	21.09	0.55	4.52	0.81	18.98	12.34
Average of Calixin			65.28	21.07	0.52	4.76	0.81	18.89	12.33
Trimidal	Fiona		62.65	21.28	0.55	4.87	0.97	19.04	11.93
	Hülya		66.83	21.44	0.52	4.52	0.85	19.34	12.92
Average of Trimidal			64.74	21.36	0.54	4.69	0.91	19.19	12.43
Average of Fiona			63.65	21.02	0.58	4.91	0.99	18.75	11.93
Average of Hülya			64.92	21.13	0.53	4.52	0.86	19.03	12.36
LSD for the average of the fungicides		%5							0.59
		%1							0.90
LSD for the average of the varieties		%5							
		%1							

rather high root yield of 2.31 t/ha in Nuarimol treatment and 4.64 t/ha in Tridemorph treatment were obtained. A considerably high extractable sugar yield (0.33 t/ha) was also gained by Nuarimol application. Hülya was superior to Fiona in root yield of 6.49 t/ha and extractable sugar yield of 1.20 t/ha. The superiority of Hülya to Fiona in terms of root and extractable sugar yield was statistically significant. On the other hand, the differences between Hülya and Fiona were unimportant in terms of the quality parameters as 1997.

Although no statistically important differences were found between Tridemorph and Nuarimol treatments in terms of the yield and the quality parameters of sugar beet in Konya in 1997, considerable increases in root yield (0.84-3.50 t/ha) and in extractable sugar yield (0.13-0.62 t/ha) were obtained in both applications, which were firstly Tridemorph and secondly Nuarimol. Hülya was superior to Fiona in root yield by 8.29 t/ha and extractable sugar yield by 1.64 t/ha. The superiority of Hülya to Fiona in root and extractable sugar yield was statistically significant. But in contrast, there was no difference between the varieties in quality parameters.

In 1998 in the same place, was sprayed with Tridemorph and Nuarimol against the disease and gave statistically significant increases in the extractable sugar yield by 0.65 t/ha and 0.75 t/ha respectively. Despite no statistically important differences were determined between Tridemorph and Nuarimol treatments in root yield, sugar content, and extractable sugar content, quite high root yield ranging from 1.90 to 2.44 t/ha was obtained. Hülya was noticeably superior to Fiona in root yield of 1.27 t/ha and extractable sugar yield of 0.43 t/ha although the superiority of Hülya to Fiona in the term of all the parameters was unimportant statistically.

DISCUSSION

Powdery mildew control gave significantly increases in root yield of 3.9-10.3% and extractable sugar yield of 5.6-10.4% with Tridemorph application and in root yield of 1.4-5.1% and extractable sugar yield of 1.2-6.4% with Nuarimol application under Ankara and Konya conditions in 1997 and 98. The best results were obtained by Tridemorph application. Slight increases were gained in sugar content of 0-2.6% and extractable sugar content of 0-3.2% in both treatments.

In this study (survey) carried out in Ankara and Konya provinces the chemical control of powdery mildew resulted by some increase in root yield, extractable sugar yield and sugar content like results of the work carried out by Keskin (1974) and Göbelez (1976) in Konya. At the same time, root yield increases of 3.9-10.3% obtained by chemical control of the disease in the study resembles the root yield loses of 2-10% and 2-15% reported respectively by Özgör (1995) in Turkey and by Byford (1996) in European countries.

By using Sulphur in powdery mildew control, connected with different severe infections of the disease in the climatic conditions which changed according to countries and regions, gave increases:

- in sugar beet root yield by 6% and 9%, sugar content by 3% and 7%, and sugar yield by 9% and 16% in S. Costanzo and Recanati respectively in Italy (Cioni et al. 1996)
- in root yield by 4-7%, sugar content by 0.5-2%, and sugar yield by 5-11% in England (Asher and Williams 1996).

The results gained in Italy and England resemble approximately the results obtained in this trial in Turkey.

In general evaluation of this study, chemical control of powdery mildew, which was firstly Tridemorph and secondly Nuarimol, increased significantly the root and extractable sugar yield of beet and changed the quality parameters of beet as low as unimportant in Ankara and Konya.

In comparasion of susceptible variety (Fiona) with the powdery mildew tolerant variety (Hülya) in average untreated and applications of Tridemorph and Nuarimol Hülya was superior in root yield by 13.4-14.7% and extractable sugar yield by 13.8-16.1% in Ankara, and in root yield by 2.0-14.5% and extractable sugar yield by 3.6-16.1% in Konya. There was a little difference between both varieties in quality parameters as low as unimportant.

According to these results, Hülya showed a better performance in only root and extractable sugar yield then Fiona under Central Anatolian conditions.

ÖZET

KÜLLEME HATALIĞI İLE KİMYASAL ŞAVAŞIMIN HASSAS VE TOLERANT ŞEKER PANCARI ÇEŞİTLERİNİN VERİM VE KALİTESİ ÜZERİNE ETKİLERİ

Bu çalışmada, Küllemeye karşı yapılan ilaçlı savaşımın şeker pancarının verim ve kalitesi üzerindeki etkisi incelenmiştir. Çalışma **İç Anadolu Bölgesi** şartlarında 1997 ve 1998 yıllarında Ankara ve Konya'da yapılmıştır. Hastalık, 1997'de en erken Ağustos ortasında, 1998'de ise en erken Temmuz sonuna doğru çıkmıştır. Her iki deneme yerinde kimyasal mücadele, hastalığın görülmesi ile başlamış ve 15-20 gün ara ile 1997'de birer ve 1998'de ikişer ilaçlama yapılmıştır. Deneme yerlerinde Tridemorph uygulamasında şeker pancarının kök veriminde %3.9-10.3 (2.44-4.64 t/ha) ve arıtılmış şeker veriminde %5.6-10.4 (0.62-0.80 t/ha), Nuarimol uygulamasında ise şeker pancarının kök veriminde %1.4-5.1 (0.84-2.31 t/ha) ve arıtılmış şeker veriminde %1.2-6.4 (0.13-0.75 t/ha) oranlarında

önemli artışlar sağlanmıştır. Her iki ilaçlamada da pancarın şeker varlığı %0-2.6 (0-0.55 °Z) ve arıtılmış şeker varlığında %0-3.2 (0-0.59 °Z) arasında değişen oranlarda küçük artışlar elde edilmiştir. Külleme tolerant Hülya çeşidi, hassas çeşit Fiona'ya kıyasla, şeker pancarının yalnızca kök veriminde %2-14.7 (1.27-7.08 t/ha) ve arıtılmış şeker veriminde %3.6-16.1 (0.43-1.30 t/ha) oranlarında daha üstün bir performans sergilemiştir. Çeşitler arasında kalite bakımından önemsenmeyecek kadar küçük farklar bulunmuştur. Bu sonuçlara göre, Külleme hastalığının kontrolünde Nuarimol uygulamasına göre Tridemorph uygulaması daha yüksek kök ve arıtılmış şeker verimi sağlamıştır. Hem ilaçlı mücadelede hem de tolerant çeşit ekiminde, şeker pancarının kök ve arıtılmış şeker verimleri artmış, kalite değerleri ise değişmemiştir.

Anahtar Kelimeler: Külleme, *Erysiphe betae*, Şeker pancarı, tolerant şeker pancarı çeşidi, kimyasal mücadele

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Determination of Virus Diseases on Tomato and Cucumber Grown in Greenhouses in Erzurum and Artvin Provinces by ELISA

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ABSTRACT

During 2000-2001, a survey study was conducted in the greenhouses of Uzundere, Tortum, İspir, Olur, Ilıca (Erzurum) and Yusufeli (Artvin) districts located in the eastern Anatolian region of Turkey.

Suspected leave samples collected from tomato and cucumber plants showing virus-like symptoms (mosaic, bronzing, chlorosis, malformation, rolling of leaves, bush growth) were tested by DAS-ELISA (Double Antibody Sandwich-Enzyme Linked Imminosorbent Assay). The results of ELISA tests showed the incidence of TMV on tomato samples taken from the greenhouses in Uzundere, Tortum, İspir, Olur, Ilıca and Yusufeli was 1.73%, 1.76%, 1.41%, 1.62%, 2.48% and 1.02%, respectively. ToMV was detected at the rate of 0.18% and 1.45% in the tomato samples taken from Yusufeli and Ilıca, respectively, and TSWV was only observed in 0.22 % of the tomato samples in Ilıca. None of TRSV, PVX and CMV were detected in the samples were tested in this study. On the other hand, CMV was only detected in 4.3% of cucumber samples collected from Ilıca.

Key words: Virus diseases, ELISA, tomato, cucumber

INTRODUCTION

Turkey has got a quite proper climatic conditions for vegetable growing. According to 1998 statistics, the total vegetable growing area of Turkey is 785 000 ha, and total vegetable production is approximately 20 216 295 tons (Anonymous, 1998). Tomato (*Lycopersicon esculentum* Mill.) has ranked the first and cucumber (*Cucumis sativus* L.) ranked in the forth place among the vegetable crops grown in Turkey. The economic value of tomato and cucumber productions is approximately equal to 45% of total vegetables value propagated in Turkey (Anonymous, 1998).

Greenhouses is a profitable activity due to production of high-cash-crop throughout the year (Günay, 1980). Therefore, greenhouse production of vegetables has become a common practice of growers in the Mediterranean, Aegean, and Marmara Regions, where environmental conditions are suitable for vegetable production more

than once a year. Last years, greenhouse production has been well accepted by growers in microclimatic locations like in eastern Anatolian region (Ölez, 1986).

Tortum, Uzundere, Olur, Oltu, and İspir districts in Erzurum province; Yusufeli district in Artvin are microclimatic locations. Thus, greenhouses industry is developing and spreading steadily among the growers who wants to earn extra income (Kurt, 1996). But, there are a number of factors causing decrease in yield and quality of tomato and cucumber. Virus diseases are one of these factors limiting the quality and quantity in tomato and cucumber production in the greenhouses and fields. So far, more than 30 virus diseases have been reported to cause economic yield losts in tomato and cucumber production alone or in combination (Smith, 1972; Lovisola, 1980).

The previous studies performed in Turkey have reported the existence of TMV, PVY PVX, TSWV, TBRV, TRSV, TYLCV, ToMV on tomato and CMV on cucumber grown in greenhouses (Çiçek and Yorgancı, 1990; Abak et al., 1991; Erkan et al., 1991; Güldür et al., 1991; Nogay, 1991; Yılmaz et al., 1991; Yorgancı and Erkan, 1991; Fidan, 1993, Güldür and Yılmaz, 1994; Yorgancı et al., 1994; Güldür et al., 1995; Özgöz et al., 1995). However, there has been no attempts to study the virus diseases, causing yield losses in the greenhouses located in eastern Anatolian region of Turkey, including Tortum, Uzundere, Olur, Oltu, and İspir districts in Erzurum province; and Yusufeli district in Artvin.

In this study, existence and distribution of virus diseases on tomato and cucumber grown in eastern Anatolian region were determined by of DAS-ELISA (Double Antibody Sandwich-Enzyme Linked Imminosorbent Assay).

MATERIALS and METHODS

Suspected leaves of tomato and cucumber showing virus-like symptoms (mosaic, bronzing, chlorosis, malformation, rolling of leaves, bush growth) were collected from greenhouses in Tortum, Uzundere, Olur, İspir, Ilıca (Erzurum) and Yusufeli (Artvin) districts. Total 90 tomato samples being 20 from Tortum, 26 from Uzundere; 10 from Olur, 6 from İspir, 7 from Ilıca, 21 from Yusufeli, and 22 of cucumber samples collected from Ilıca were directly tested with DAS-ELISA.

Methods of surveys and determination of disease ratio were carried out according to the method described by Bora and Karaca (1970). A total 162 tomato samples were collected from Tortum (30), Uzundere (39), Olur (15), İspir (9), Ilıca (10), Yusufeli (35) and 34 cucumber samples from Ilıca in June and September of 2000 and June of 2001, and then kept in –20°C deep freeze for long term storage to use in further tests. Samples of tomato were tested serologically by using ELISA kit specific for TMV, CMV, ToMV, PVX, TRSV and TSWV viruses, and cucumber samples were tested by CMV specific kit DAS-ELISA (Clark and Adams, 1977). All of the kits used in this study were supplied

from Agdia (Agdia Company, Elkhart, USA). Sample preparation was performed as described by the manufacturing company.

In all experiments, a negative control (extraction buffer) and a positive control were included. After dispensing 100 µl from each sample into the well of a 96-well microtiter plate, the plates were incubated at room temperature for 2 hrs and then washed with wash buffer for 6 times. (Elx50 Auto Strip Washer Bio-Tek Instruments, Inc. B-2610 Wilrijk, Belgium). An aliquot of 100 ml alkaline phosphates conjugated was added to each well. The plates were incubated for 2 hr at room temperature and washed as described above. 100 µl PNP (P-nitrophenyl phosphate buffer) was added into each well and the plates were incubated for 40 min. Absorbans was measured at 405 nm with microplate autoreader (Elx800 Universal Microplate Reader Bio-Tek Instruments, Inc. B-2610 Wilrijk, Belgium).

RESULTS and DISCUSSION

The incidence of viruses detected in the tomato and cucumber samples were summarized in Table 1.

The results showed that TMV was present in all greenhouses visited in this study. Distribution rate of TMV was different for each location. ToMV, TSWV and CMV were only found in Yusufeli and Ilıca. Nevertheless, PVX and TRSV were not detected in any locations.

Table 1. The incidence of viruses in tomato and cucumber samples collected from greenhouses in 6 different district located in Erzurum and Artvin provinces of Turkey.

District	TMV (%)	ToMV (%)	TSWV (%)	TRSV (%)	PVX (%)	CMV (%)
Yusufeli	1.73	0.18	-	-	-	-
Tortum	1.76	-	-	-	-	-
Uzundere	1.41	-	-	-	-	-
Olur	1.62	-	-	-	-	-
İspir	2.48	-	-	-	-	-
Ilıca	1.02	1.45	0.22	-	-	4.30

ELISA results have been detected in tomato plants with of deformation, severe mosaic; slight mosaic; top leaf curving, bronz spots and stunting symptoms in plant which were infected by TMV, ToMV and TSWV, respectively.

The symptoms of TMV, ToMV and TSWV observed in this study were confirmed by many other researches (Ie, 1970; Hollings and Huttinga, 1976; Verhoven and Roenshorst, 1992; Jones et al., 1993; Zitter, 1993; Blancard, 1994; Kaminska and Korbin, 1994; Pereira and Cortes, 1994; Kaminska, 1996).

The percentage of TMV determined in tomato samples collected from Yusufeli, Tortum, Uzundere, Olur, İspir and Ilıca were %1.73, 1.76, 1.41, 1.62, 2.48 and 1.02, respectively. The distribution rate of this virus was relatively high in the autumn compared to spring. The increase in distribution rate of TMV showed that the virus spread by mechanical means during practices in the growing season (Agrios, 1997). TMV was found in all greenhouses and in all seasons suggesting that the primary source of infection may be farmers who smoke in the greenhouses. Furthermore, the spread of this virus through seed and mechanical means was reported in some other studies (Çiçek and Yorgancı, 1990; Zitter, 1993; Yılmaz et al., 1998).

The greenhouses in Yusufeli and Ilıca were determined to be infected by ToMV at the rate of 0.18 % and 1.45 %, respectively. TSWV was only detected at the rate of 0.22 % in the spring of 2000 in Ilıca. Both of these viruses were observed in some greenhouses in the first counting, but not in other greenhouses and in later countings. These findings are indicating that these viruses are introduced to the region from other places via seeds or seedlings.

These results are supported by the evidence of ToMV is a seed-borne and sap-transmissible pathogen reported in similar studies (Erkan et al., 1991; Hassan et al., 1993; Hassan, 1995).

CMV was only found in 4.3 % of cucumber samples collected from Ilıca in the first counting. It was shown that CMV has symptoms of mosaic, leaf yellowing, stunting and early death on cucumber plants, which were recorded by Gibbs and Harrison (1970) and Zitter et al. (1993).

Overall evaluation of the results showed that the existence and distribution of virus diseases in the region was relatively limited. It may be explained by the lack of alternative host or vectors of these viruses in the region.

TMV, ToMV, TSWV and CMV were first time determined in this study in the eastern Anatolian region of Turkey.

ÖZET

ERZURUM VE ARTVİN İLLERİNDEKİ SERALARDA DOMATES VE HIYARDAKİ VİRÜS HASTALIKLARININ ELISA İLE BELİRLENMESİ

Survey çalışmaları 2000-2001 yıllarında Uzundere, Tortum, İspir, Olur, Ilıca (Erzurum) ve Yusufeli (Artvin) ilçesindeki seralarda yapılmıştır. Seralardaki domates ve hıyar bitkilerinde virüs benzeri simptom (mozaik, bronzlaşma, kloroz, deformasyon, yapraklarda kıvrılma, gelişme geriliği) gösteren bitkilerden yaprak örnekleri alınmış ve DAS-ELISA ile testlenmişlerdir. ELISA sonucunda Uzundere, Tortum, İspir, Olur, Ilıca (Erzurum) ve Yusufeli (Artvin) ilçelerinden alınan domates örneklerinin sırasıyla %1.73, %1.76, %1.41, %1.62, %2.48 ve %1.02 oranında TMV ile enfekteli olduğu saptanmıştır.

ToMV sadece %0.18 ve %1.45 oranında Yusufeli ve Ilıca ilçesinde; TSWV ise yalnızca %0.22 oranında Ilıca'da belirlenmiştir. TRSV, PVX ve CMV'e ise domateslerde rastlanılmamıştır. Diğer taraftan CMV sadece Ilıca'da %4.3 oranında hıyarlarda saptanmıştır.

Anahtar Kelimeler: Virus hastalıkları, ELISA, domates, hıyar

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Studies on the Effect of Calcium and a *Pseudomonas fluorescens* Isolate to Control *Botrytis cinerea* Pers. On Tomato

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ABSTRACT

This study was carried out to investigate the effects of different calcium sources, (CaH_2O_2, $CaSO_4$, $Ca(NO_3)_2$, $CaCl_2$ and CaO used as foliar fertilizers (Manvert-Kalsiyum, Calbit, Aminoqualent- Ca) and an antagonistic microorganism *Pseudomonas fluorescens* 144 on gray mould caused by *Botrytis cinerea* on greenhouse grown tomatos.

The effect of some calcium salts on the growth of *B. cinerea* was determined by adding calcium salts to PDA and PDB media in vitro test. CaH_2O_2 at the dose of 600 mg/ml inhibited growth of *B. cinerea* by 20.50% on PDA and 91.50% PDB.

The greenhouse tests were carried out on bean and tomato plants. The most effective results were obtained by the application of calcium salts three times on the bean plant. The effectiveness of CaH_2O_2 was found 72.5% when used at a rate of 0.5% dose. The combination of calcium salts, increasing the resistance of plant, and antagonist were also applied which was found to decrease the disease severity by 70.93% and 94.50% on tomato and bean plants, respectively.

The CaH_2O_2 treatment was found highly effective in all the experiments.

Key words: *Botrytis cinerea*, *Pseudomonas fluorescens*, antagonist, Calcium

INTRODUCTION

Tomato is a significant crop in greenhouse vegetable cultivation, in Turkey. Greenhouse microclimatic conditions are essentially warm and humid. Gray mold caused by *Botrytis cinerea* Pers. is a major problem in greenhouse production of vegetables including tomato (Jarvis, 1992). The pathogen primarily infects leaves or fruits. Gray mold epidemics is a serious problem in vegetable greenhouses and causes finishing of a crop earlier in the season. In general, growers use fungicides to control gray mold diseases.

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The frequent development of resistantcy of to common fungicides in *B. cinerea* populations and desire to reduce pesticide use have led the efforts for exploring alternative measures in disease management. As the researches show it may be unrealistic to expect and achieve successful control of gray mold with the help of a single method such as, the application of fungicides (Gullino, 1992). Rather, the control will increasingly rely on an interaction of different methods to strenghten the resistance of the host plant or weaken the development of the pathogen.

The physiological status of the plants significantly influences their susuceptibility to pathogen infections. Calcium is usually found in high concentrations in plant cell walls. It's responsible for the integrity of membranes and the production of the cell wall. The presence of this element is associated with delayed senescens of plant tissues by delaying the softening of fruit flesh (Ferguson, 1984), thus making it less susceptible to degradation by the enzymes of *B. cinerea*. Supplementation of the plant with calcium can be useful in reducing the severity of gray mold.

Fertilization with calcium reduced Fusarium wilt of tomato (Corden, 1965), muskmelon (Spiegel et al., 1987), and also susceptibility of gray mold disease on bean and tomato. This decrease was attributed to the inhibition of polygalacturanase and other pectic enzymes (Bateman & Lumsden, 1965).

Biological control of gray mold is another aspect of the disease control. Several biocontrol agents has been reported for their effectiveness against *B. cinerea. Bacillus pumilis* and *Pseudomonas fluorescens* were found to be effective as standard dichlofluonid sprays against *B. cinerea* on strawberry (Yıldız, 1990, Yıldız, 2000, Swadling & Jeffries, 1996).

In this study, several calcium salts and a Pseudomanas isolate which were isolated and found effective in another study supported by Tubitak (Turkish Scientific and Research Council) were investigated in reducing the disease incidence (Yıldız, 2000).

MATERIALS and METHODS

Materials

An Iprodione resistant isolate of *B. cinerea* used in the trials has been obtained from the tomato greenhouses in Fethiye province. *Pseudomonas fluorescens* was also isolated on tomato and found out to be effective against *B. cinerea* according to our previous research results (Yıldız, 2000). PDA (Potato Dextrose Agar), PDB (Potato Dextrose Broth) and King B (King et al., 1954) media were used in the *in-vitro* tests.

The plant material used in the trials were faba bean (*Vicia faba*) and tomato (*Lycopersicum esculentum* cv. Rio grande).

The pot soil was sterilized before transplanting the plants. The calcium salts, found effective in the previous studies were selected. The commercially used foliar

fertilizers containing calcium were also selected. As CaO is the only commercially used fertilizer, the other calcium salts were also added to the tests. The properties of calcium salts and commercial fertilizers are summarized in Table 1.

Table 1. The foliar fertilizers, calcium salts and their ingredients.

Calcium sources	Ingredients (%)		Commercial Name
	Ca	N	
Aminoquelant - Ca	8	4.89	Bioiberica S.A.
Manvert - Calcium	14.4	8.3	Agrochem A.S.
Calbit – C	15		Valagro
Calcium Salts	g / mol weight	% Ca	
Calcium sulphate $CaSO_4.2\ H_2O$	172.14	23.237	Riedel de Haen
Calcium nitrate $Ca(NO)_2.4H_2O$	236.15	16.938	Riedel de Haen
Calcium Chloride $Ca\ Cl_2$	110.99	36.039	Merck
Calcium hydroxide CaH_2O_2	74.10	53.981	Merck

Methods

In-vitro treatments

A series of experiments were conducted to investigate the effects of the foliar fertilizers and calcium salts on the colony growth of *B. cinerea*. A dose series containing five doses (60, 150, 300, 400, 600 ppm) were arranged in such a way that the calcium contents of the foliar fertilizers and pure calcium salt may remain stable. The foliar fertilizers were applied directly and others were used after mixing them with 50 ml sterilized water, as 600, 400, 300, 150 and 60 ppm dose series, to the 50 ml PDA media at 45-50°C. After pouring the medium to the plates, three (0.5 mm) discs of agar covered with the mycelium of *B. cinerea* from 7 day old culture were placed on the petri plates. After incubation at 27°C, for three days, the mycelial growth of the pathogen was measured.

The same doses were used in determination of effects of calcium sources on mycelium weight of the pathogen. The calcium sources were added to the erlenmayers containing, 50 ml of PDB as five different doses, and water three discs from 7 day old *B. cinerea* were added to the erlenmayers. The erlenmayers were incubated in the growth chamber on the rotary shakers for 9-10 days. The mycelium was dried in an incubator at 40°C for 3 days. The dry weight of the mycelium was measured after the drying period.

***In-vivo* tests**

A series of tests were carried out to determine the effects of the calcium sources on disease rate. The first tests were conducted on broad bean plants. The treatments found effective were than applied to tomato plants. *In vivo* tests were conducted in greenhouse grown plants which were grown in the pots. A surfectant–sticker (Rozamin-vet, 30 cc/100 l.) was added to calcium sources. Calcium salts dissolved in water were applied to the plants until the whole plants were covered with the suspension.

The foliar fertilizers were applied in two different forms and doses, a recommended dose (0.5%) and the dose found effective in the primary tests (400 ppm), twice and thrice during the trials with one week intervals.

The combinations of the different calcium sources and doses and antagonist found effective on broad bean plants were tested on the tomato plants too. The similar calcium sources and doses were applied to tomato plants. The treatments were repeated three times. Three tomato plant were used in the tests and the treatments were repeated thrice.

Inoculum Preparation

Antagonistic *Pseudomonas fluorescens*, was applied to both broad bean and tomato plants after the last application of calcium source to the plants. The bacterial culture grown on King B medium was added to King B Broth after 48 h. The cultures were shaken at 90 rpm for 48 h. at approximately 25°C. The cultures were than centrifuged at 4000 rpm for 10 minutes. The supernatants of the culture was poured down and the pellet was resuspended to a concentration of 10^8 cells/ml in sterile water.

B. cinerea was grown on PDA. Suspensions of conidia ($1x10^5$ conidia/ml) of *B. cinerea* were prepared from 10-14 days old cultures by scraping the sporulating culture in sterile distilled water containing 1% carrot juice and jelatin and filtering through three layered cheesecloth.

The antagonist suspension was applied 48 h. before inoculation of the pathogen. The pots were covered with polyethylene bags to obtain higher relative humidity and incubated for 10 days (Delen et al., 1984, 1988).

The leaf samples were collected after evaluating the treatments. The leaf samples were wrapped in newspaper, labelled and dried in a drying cabin for 48 h at 70°C. Dry plant material was ground in the mill . Dry ashed samples were analyzed for Ca content by using atomic absorption spectrophotometer.

The evaluation of the disease intensity was made by using 0-5 scale for broad bean plants (Anonymous, 1983; Delen et al., 1988); and 0-4 scale for tomato plants (Anonymous, 1996). Statistical analyses were performed to reveal the differences between the treatments.

RESULTS and DISCUSSION

In-vitro Tests

The effects of Calcium salts on the colonial growth of *B. cinerea*

The effects of the different calcium sources on *B. cinerea* were tested in a series of experiment *in-vitro*. The colonial growth of the pathogen were measured for three days. Two tests were conducted in-vitro and percent growth rate of colony was calculated according to the control. The LSD results and groups are given in Table 2.

Table 2. The effects of different calcium sources on the colonial growth of *B. cinerea*.

Calcium sources	600 ppm	400 ppm	300 ppm	150 ppm	60 ppm
Control	100.0 C	100.0 C	100.0 CD	100.0 BC	100.0 B
CaH_2O_2 %0.5	79.543 A	82.379 A	81.055 A	88.381 A	89.967 A
$CaSO_4$ %0.5	89.362 B	92.791 B	98.242 BCD	100.998 D	98.925 B
Ca $(NO)_3$ %0.5	106.17 D	101.386 C	94.753 B	98.171 BC	91.795 A
$CaCl_2$ %0.5	87.799 B	100.625 C	97.013 BC	102.633 D	90.858 A
CaO (Man-Ca) 300 cc/100 lt	104.019 CD	97.182 BC	102.599 D	95.71 B	99.332 B

(P > 0.05) The means followed by the same letters in the columns are not significantly different. Means of two tests were compared by LSD at P < 0.05.

Almost all of the sources have affected the colonial growth of *B. cinerea*. CaH_2O_2 at a dose of 600 ppm has given a good result with 79.54% colonial growth (20.54% effectiveness).

The effects of the calcium sources on the mycelial quantity of *B. cinerea*

The effects of the different calcium sources on the mycelial quantity of *B. cinerea* were tested on the serially diluted PDB media. The dry mycelium mass were weighed and calculated as a percentage rate. The results of these tests are given in Table 3.

Table 3. The effects of the different calcium sources on the mycelial mass of the *B. cinerea* (mean of two tests).

Dose of the calcium	CaH_2O_2 %0.5	$CaSO_4$ %0.5	$Ca(NO_3)_2$ %0.5	$CaCl_2$ %0.5	CaO 300 cc/100 lt Man-Ca
Control	100.0 B	100.0 B	100.0 B	100.0 A	100.0 A
600 ppm	8.543 A	90.652 B	84.259 AB	95.046 A	135.405 B
400 ppm	11.306 A	69.369 A	69.444 A	100.874 A	99.604 A
300 ppm	16.332 A	98.324 B	76.852 AB	97.970 A	99.404 A
150 ppm	68.844 B	99.803 B	95.833 B	92.671 A	96.404 A
60 ppm	86.935 B	93.141 B	99.792 A	92.792 A	93.804 A

(P> 0.05)

In-vivo Tests

The effects of the Calcium salts on the disease ratio

In two tests with broad bean plants, calcium sources were used alone. In further tests with the broad bean plants, the calcium sources were combined with *P. fluorescence*. The calcium sources were applied to the plants twice and thrice in the broad bean tests. Most of the calcium sources were found to affect the disease severity. Higher efficacy of CaH_2O_2 was also observed in-vivo trials. When *P. fluorescence* were applied to the plants, the disease severity was decreased. The number of application of the Calcium sources has also increased the effectiveness of the treatments. Trials on the tomato plants, calcium sources were used three times and combined with the antagonist. Results of the tests are given in Table 4.

Table 4. The disease rate of the broad bean and tomato plant applied three times of calcium.

	Calcium sources + *P. fluorescens*[1]				Calcium sources[2]	
	Tomato		Broad bean		Broad bean (mean of 2 experiment)	
Treatments	Dis. severity (%)	Effectiveness (%)	Dis. severity (%)	Effectiveness (%)	Dis. severity (%)	Effectiveness (%)
Control	89.233	-	31.722	-	25.473	-
P. fluorescens	16.806	81.166 AB	-	-	-	-
CaH_2O_2*	4.536	94.905 A	9.222	70.93 A	7.075	72.46 A
CaH_2O_2**	25.925	72.058 BC	12.338	61.11 AB	12.531	51.05 BCD
$CaSO_4$*	25.524	71.382 BC	11.000	65.33 A	9.913	61.88 ABC
$CaSO_4$**	10.900	87.773 A	13.790	56.53 ABC	11.452	55.43 BC
$Ca(NO_3)_2$*	36.642	58.920 CD	13.278	58.14 AB	12.672	50.58 BCD
$Ca(NO_3)_2$**	34.628	61.177 CD	15.250	51.93 ABCD	13.333	46.44 CDE
$CaCl_2$*	40.415	54.691 D	13.195	58.41 AB	12.577	50.92 BCD
$CaCl_2$**	24.919	70.946 BC	15.917	49.83 ABCD	15.569	38.38 DE
Man-Ca**	11.711	86.863 A	20.194	36.34 CD	11.625	55.22 BC
Man-Ca**	16.203	81.829 AB	15.619	50.76 ABCD	17.966	30.47 E
Amin-Ca*	14.584	83.645 AB	21.388	32.58 D	21.875	14.11 E
Amin-Ca**	6.480	92.726 A	18.322	42.24 BCD	9.186	64.50 AB
Calbit*	25.807	71.063 BC	14.876	53.11 ABCD	12.121	51.89 BCD
Calbit**	17.036	80.895 AB	12.667	60.07 AB	12.190	51.13 BCD

($P > 0.05$) * recommended dose, ** 400 ppm dose 1. Calcium sources was combined with the antagonist, 2. Calcium sources was applied alone.

The disease rate in treatments applied three times was lower than the two times applications. Tomato showed better results in all of the treatments than broad bean plants, CaH_2O_2 was the most effective treatment when applied three times. These are supported by *in-vitro* trials. CaH_2O_2 was the most effective treatment at 0.5% dose on the tomato plants (Figure 1).

Effects of calcium salts on the amount of calcium of tomato plants

To determine effects of the calcium sources on the calcium contents in tomato plants, calcium analysis of the leaves were done after preping the samples. The results of this analysis is shown at Table 5.

Table 5. The effects of different calcium sources on the total calcium content of tomato.

Treatment	Amount of Ca (%)
Control (-)	2.483 ABC
Control (+)	2.266 BC
CaH_2O_2*	3.645 A
CaH_2O_2**	2.798 BC
$CaSO_4$*	2.562 CD
$CaSO_4$**	2.167 E
$Ca(NO_3)_2$*	2.660 C
$Ca(NO_3)_2$**	2.778 BC
$CaCl_2$*	3.035 B
$CaCl_2$**	2.680 C
Man-Ca*	2.503 CD
Man-Ca**	2.621 C
Amin-Ca*	2.778 BC
Amin-Ca**	2.798 BC
Calbit*	2.680 C
Calbit**	2.483 CDE

(P > 0.05) * recommended dose, ** 400 ppm dose

Some differences between the calcium amounts and the treatments were observed. The highest calcium amount was determined by the application of 0.5% dose of CaH_2O_2. However, there was no significant difference between the controls, the calcium amounts in all treatments except $CaSO_4$ was found higher than controls.

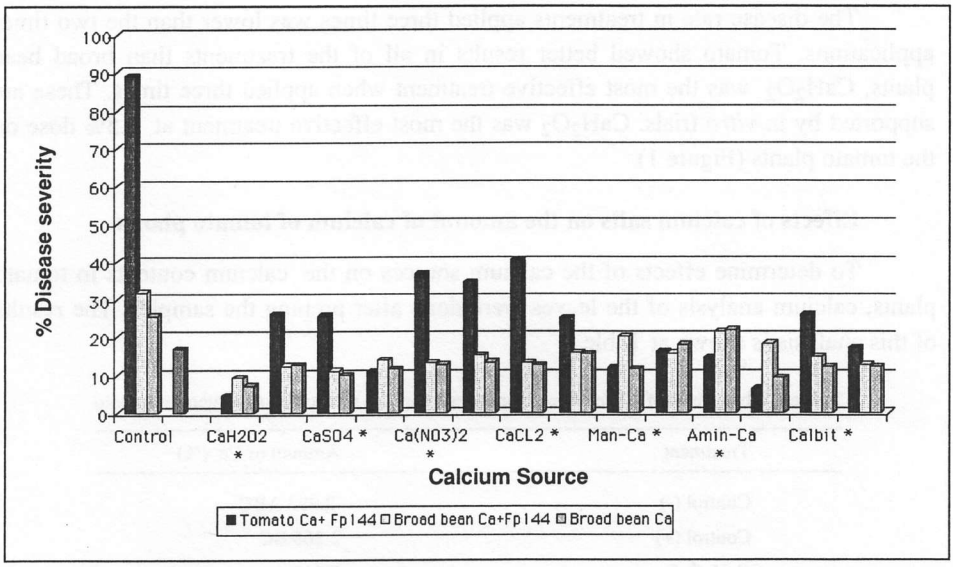

Figure 1. The results of three time applications of Ca sources on tomato and broad bean plants.

Discussion

The major aim of the fertilizers usage on these plants is to increase plant growth and yield. The effect of the fertilization on disease incidence was revealed by several researches. It is possible to influence the microflora on both the phyllosphere and rhizosphere with the aid of fertilization. Calcium induces the resistance in the plants. The disease incidence was diminished by the application of calcium before the infection.

A lot of microorganisms isolated from different parts of plants has antagonistic effects on pathogens.

It was shown that calcium nutrition effectively controlls gray mold of roses (Elad and Volpin, 1988; Volpin and Elad, 1991), bean, and tomato (Elad and Volpin, 1993). A *P. fluorescence* isolate was found out to be effective (Yıldız, 2000) in combination with the several calcium sources in another study supported by TUBITAK. This antagonist reduced disease severity (86.73%) significantly when applied on tomato. The results obtained in the previous study and our results are similar (81.17%).

Reduction of the gray mold disease was observed with application of 0.1 mM of $CaSO_4$, $Ca(NO_3)_2$, and $CaCl_2$ in-vitro studies (Elad & Volpin, 1993). Biggs et al., (1997), investigated the effect of several calcium salts on growth and infection by *Monilia fructigena* in peach trees.

When, $CaCl_2$, $Ca(NO_3)_2$, CaO, $CaSO_4$, CaH_2O_2, compounds were added to PDA medium, the pathogen growth rate was reduced significantly.

Same effects were obtained with PDB medium. In our study, CaH_2O_2, $CaCl_2$ and $CaSO_4$ showed different effects with 20.50%, 12.20% and 10.64%, respectively. The effect on the liquid medium was much higher and CaH_2O_2, $CaCl_2$ and $CaSO_4$ were found to be 91.50%, 30.63% and 30.55% effective, respectively (Table 4). The pH values of calcium sources except CaH_2O_2 were about equal to neutral. Different doses of the CaH_2O_2, increased the pH of the medium with a 12.5 on 600 ppm dose rate. The inhibition of the pathogen growth *in-vitro* conditions, confirms previous works (Volpin & Elad, 1991). The effects of calcium both at fertilization and postharvest on the susceptibility of the rose flowers were investigated in some studies (Volpin & Elad, 1991), and calcium at a concentration of 3.5 mM was found to reduce the severity of disease by 55% under greenhouse conditons. The application of Calcium nitrate (Calnit) reduced the pathogen infection by 35 – 50% at the greenhouse plants of pepper, eggplant, and cucumber (Elad et al, 1993). Fertilization of the bean plants with $Ca(NO_3)_2$, reduced the gray mold by 70% and severity of fruit ghost spot of tomato plants by 45% (Elad and Volpin, 1993).

The combination of calcium fertilization with the antagonist has a higher effect on the broad bean plants than only calcium application on the pot trials in greenhouse. It was observed that the three times application of the calcium is more effective than the two times application on broad beans. CaH_2O_2 was the most effective treatment both on broad bean and tomato plants for reducing the gray mould disease; this is consistant with an earlier report (Elad & Volpin, 1993).

In this study, the effect of CaH_2O_2 in reduction of gray mold was found *in-vitro* with 20.50% at PDA and 91.50% at PDB. In the tests conducted with the calcium sources *in-vivo*, CaH_2O_2 application on broad bean reduced the disease severity by 70.93% when applied three times, combination of the CaH_2O_2 and antagonist reduced the disease severity by 72.50%. The combination of CaH_2O_2 and antagonist on tomato plants reduced the disease severity by 94.91% on tomato.

In this study, combination of the cultural and biological methods was used together to control the disease. The detailed study on calcium should be tested in future for a better control of gray mold.

ÖZET

KALSİYUM İLE *PSEUDOMONAS FLUORESCENS*'İN DOMATESLERDE *BOTRYTIS CINEREA* PERS.'YA ETKİSİ ÜZERİNDE ARAŞTIRMALAR

Bu çalışmada, sera domateslerinde *Botrytis cinerea*'nın neden olduğu kurşuni küf hastalığına karşı farklı kalsiyum kaynaklarının ve antagonistik mikroorganizmanın etkisi araştırılmıştır. Kalsiyum kaynakları olarak CaH_2O_2, $CaSO_4$, $Ca(NO_3)_2$, $CaCl_2$ ve yaprak gübresi olarak kullanılan CaO (Manvert-Kalsiyum, Calbit, Aminoqualent- CA) ve biyolojik kontrol ajanı olarak bir *Fluorescent pseudomonas* izolatı kullanılmıştır.

In vitro testlerde PDA ve PDB besi yerlerine farklı kalsiyum tuzları eklenerek *B. cinerea*'nın gelişimi üzerinde etkileri araştırılmıştır. CaH_2O_2 600 mg/ml dozunda *B. cinerea* gelişimini PDA'da %20.50 ve PDB'de %91.50 oranında azaltmıştır.

Sera denemeleri bakla ve domates bitkileri ile yapılmıştır. Bakla bitkisinde kalsiyum tuzlarının 3 kez uygulaması en iyi etkiyi vermiştir. Bu denemede CaH_2O_2 %0.5 dozunda %72.50 etki sağlamıştır. Bitkide dayanıklılık artırıcı olan kalsiyum ve antagonistin birlikte uygulamalarında sırasıyla %70.93 ve %94.50 oranında hastalık şiddeti azaltılmıştır. CaH_2O_2 uygulamaları bütün denemelerde en iyi sonucu vermiştir.

Anahtar Kelimeler: *Botrytis cinerea, Pseudomonas fluorescens,* antagonist, kalsiyum

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Wilt Disease of *Nigella sativa* in Turkey

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Black cumin (*Nigella sativa* L.) is an annual plant and a member of the Ranunculaceae family. Its seeds are used in food industry and as a spice. It has also medicinal uses. In Turkey, it is primarily grown in Afyon, Burdur and Isparta provinces (Baytop, 1985).

In the summer of 2000, severe wilting and dying of *N. sativa* plants grown in Burdur-Gölhisar region of Turkey was observed. Plants were at the flowering/seed forming stage. Root and crown rot symptoms and in some plants blackening of the inner stem was observed. Isolation of the pathogens were made by placing diseased plant segments on PDA (Potato Dextrose Agar) and Corn Meal Agar. *Fusarium oxysporum* and *Macrophomina phaseolina* were consistently isolated from diseased regions. For pathogenicity test, soil inoculation method was used (Kunwar et al., 1989; Ahmad and Sharma, 1990; Jiménez-Diaz et al., 1983; Mihail, 1992).

Studies are conducted in a controlled growth room in order to determine the pathogenicity of this fungi. The tests were terminated 60 days after sowing. Wilt and root rot severity was assessed using a 0-3 scale (0: no disease, 1: yellowing of leaves and wilting, 2: root and hypocotyl tissue covered with lesions, 3: complete death). Reisolations from the infected hypocotyl and root tissues were made on PDA. *Fusarium oxysporum* and *Macrophomina phaseolina* were consistently isolated from diseased areas. No fungal development was observed on the control plants.

Wilt symptoms started to appear approximately one month after the inoculation with *F. oxysporum*. Gradually most of the plants showed wilt symptoms and died. In diseased plants, root development was severely impaired and thinning of the roots and brown discoloration were commonly observed (Figures 1 and 3). Disease severity value for *F. oxysporum* inoculated plants was 80%.

In pots inoculated with *M. phaseolina*, approximately one and a half month later, blackening of the crown and dying of the plants were observed in some plants. Most of the plants showed a blackening of the roots. Root system of the diseased plants were also severely impaired (Figures 2 and 3). Disease severity value for *M. phaseolina* inoculated plants was 50%.

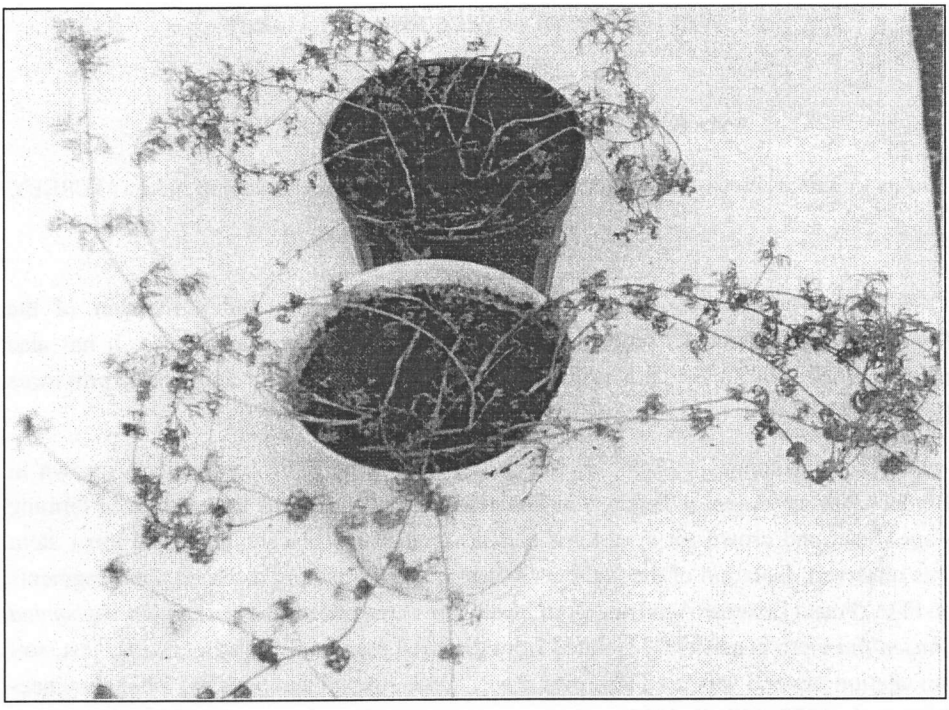

Figure 1. Control (front) and *Fusarium oxysporum*-inoculated plants (back) two months after inoculation.

This is the first report on *F. oxysporum* and *M. phaseolina* causing diseases on *N. sativa* in Turkey. It appears that these fungi are responsible for the wilt disease occurring in *N. sativa* grown areas. These fungi were also reported from India. Dubey (1995), proposed the name *F. oxysporum* f.sp. *nigella* for the *N. sativa* pathogen. Sinha and Singh (1994) reported *N. sativa* as a new host for *M. phaseolina*. Our results confirm their findings.

TÜRKİYE'DE *NIGELLA SATIVA*'NIN SOLGUNLUK HASTALIĞI

Çörekotu (*Nigella sativa* L.) Ranunculaceae familyasında yer alan tek yıllık bir kültür bitkisidir. Tohumları gıda sanayiinde, baharat ve ilaç olarak kullanılmaktadır. Türkiye'de en fazla Afyon, Burdur ve Isparta illerinde yetiştirilmektedir (Baytop, 1985).

2000 yılı yazında, Burdur-Gölhisar bölgesinde yetiştirilen çörekotu bitkilerinin çiçeklenme ve tohum oluşturma döneminde şiddetli solgunluk ve ölüm gözlenmiştir. Hastalıklı bitkilerde kök ve kökboğazı çürüklük belirtileri görülmüş ve bazı bitkilerde gövde içinde siyahlaşma olduğu gözlenmiştir. Patojenlerin izolasyonu, hastalıklı bitki parçalarının PDA (Patates Dekstroz Agar) ve Mısır Unu Agara alınmasıyla yapılmıştır.

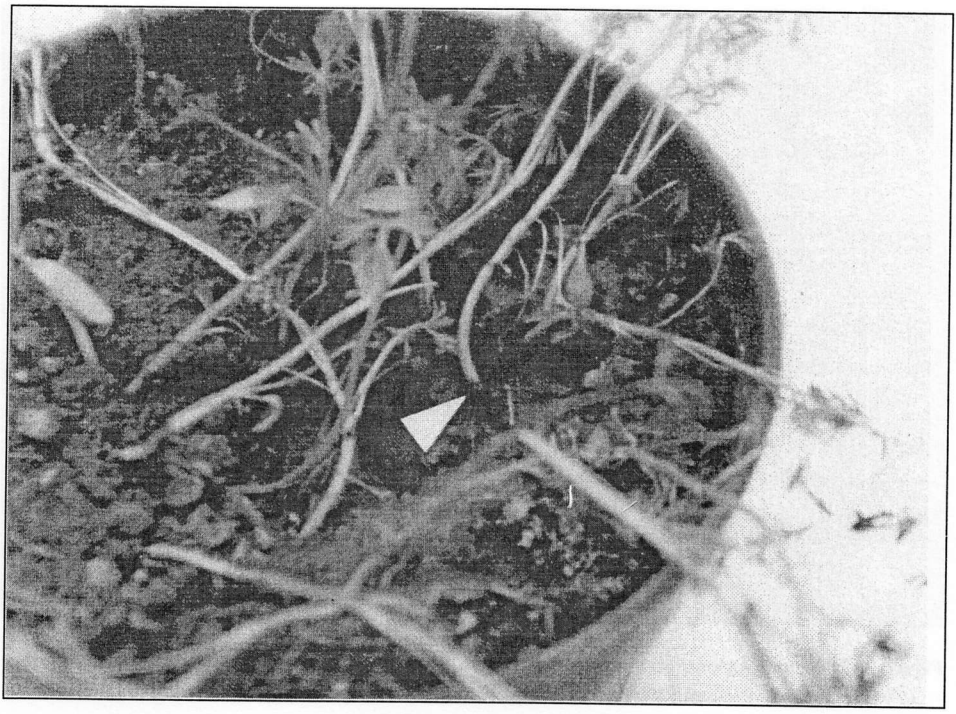

Figure 2. Blackening of the crown of *Nigella sativa* plants two months after inoculation with *Macrophomina phaseolina*.

Hastalıklı bölgelerden sürekli olarak *Fusarium oxysporum* ve *Macrophomina phaseolina* izole edilmiştir. Patojenisite testi için toprak inokulasyon metodu kullanılmıştır (Kunwar ve ark., 1989; Ahmad ve Sharma, 1990; Jiménez-Diaz ve ark., 1983; Mihail, 1992).

Bu fungusların patojenisitelerini belirlemek üzere yapılan çalışmalar kontrollü bitki yetiştirme odasında yürütülmüştür. Testler ekimden 60 gün sonra sona erdirilmiştir. Solgunluk ve kök çürüklüğü şiddeti, 0-3 ıskalası (0: hastalık yok, 1: yapraklarda sararma ve solgunluk oluşumu, 2: kök ve hipokotil dokularında lezyonlar, 3: ölüm) kullanılarak belirlenmiştir. Enfektelihipokotil ve kök dokularından PDA ortamına reizolasyonlar yapılmıştır. Hastalıklı bölgelerden *F. oxysporum* ve *M. phaseolina* izole edilmiştir. Kontrol bitkilerinden yapılan izolasyonlarda hiçbir fungal gelişme olmamıştır.

F. oxysporum ile inokulasyondan yaklaşık bir ay sonra solgunluk belirtileri görülmeye başlamıştır. Bitkilerin çoğu zamanla solgunluk belirtileri göstermiş ve ölmüştür. Hastalıklı bitkilerde kök gelişimi belirgin bir şekilde zayıflamış ve köklerde incelme ve kahverenginde renk değişimi yaygın olarak gözlenmiştir (Şekil 1 ve 3). *F. oxysporum* ile inokule edilen bitkilerdeki hastalık şiddeti %80 olmuştur.

M. phaseolina ile inokule edilen saksılarda, yaklaşık 1-1.5 ay sonra bazı bitkilerde kökboğazında siyahlaşma ve ölüm gözlenmiştir. Bitkilerin çoğunda kök ve kökboğazında

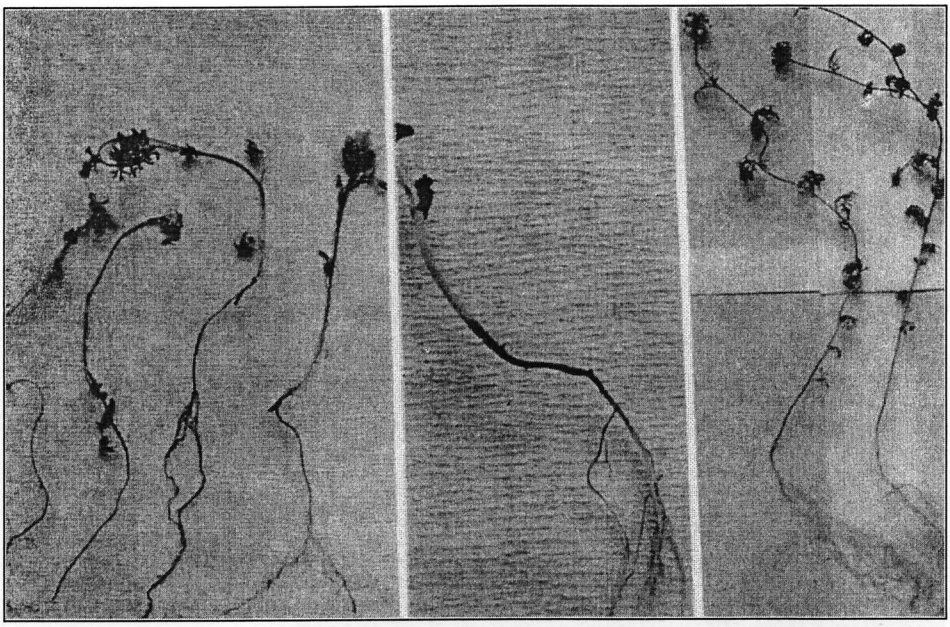

Figure 3. Root rot symptoms observed in *Nigella sativa* roots two months after inoculation with *Fusarium oxysporum* (left), and *Macrophomina phaseolina* (middle), and healthy control roots (right).

siyahlaşma görülmüştür. Aynı zamanda hastalıklı bitkilerin kök sistemlerinde şiddetli zayıflık ortaya çıkmıştır (Şekil 2 ve 3). *M. phaseolina* ile inokule edilen bitkilerdeki hastalık şiddeti %50 olmuştur.

Bu çalışma ile Türkiye'de ilk defa *F. oxysporum* ve *M. phaseolina*'nın çörekotlarında hastalık oluşturduğu tespit edilmiştir. Bu funguslar çörekotu yetiştirilen bölgelerde meydana gelen solgunluk hastalığından sorumlu olan patojenler olarak görülmektedir. Bu funguslar aynı zmanda Hindistan'dan da rapor edilmişlerdir. Dubey (1995), *N. sativa* patojeni için *F. oxysporum* f.sp. nigella ismini önermiştir. Sinha ve Singh (1994), çörekotunu *M. phaseolina*'nın yeni bir konukçusu olarak rapor etmiştir. Bu bilgiler bizim sonuçlarımızla paralellik göstermektedir.

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Induction of Resistance in Chickpea to *Ascochyta* Blight [*Ascochyta rabiei* (Pass.) Labr.] By Salicylic Acid*

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ABSTRACT

In this study the role of the exogenous salicylic acid (SA) application in forming resistance to against *Ascochyta rabiei* (Pass.) Labr. in chick peas was investigated. SA was applied to seed and foliage of the susceptible chickpea cultivar (Canıtez-87). Besides, in vitro effect of SA on mycelial growth, spore germination and germ tube growth of *Ascochyta rabiei* was determined. With the application of SA to seeds, infection by *A. rabiei* was reduced at a rate of 5.23-23.44% as compared to controls. The highest inhibition was observed in plants with 1 mM SA application. One application of SA to foliage 2 days before the inoculation with pathogen, reduced the infection more as compared to applications on other days and at 0.8 mM, inhibition rate of disease severity was found out to be 48.15%. Also, 2, 3, 4 and 5 times more SA application with 2 days intervals and inhibition rate was observed as 46.10% with the twice 5 mM SA application. In almost all cases the most effective dose was found out to be 5 mM. SA also was applied following inoculation with the pathogen and disease severity reduction values at one day after inoculation ranged from 2.55 to 43.37%. The most effective dose was 2.5 mM.

Fungitoxicity studies revealed that application of 7.5 mM SA under in vitro conditions inhibited the colony growth, spore germination and germ tube growth.

Key words: *Ascochyta rabiei* (Pass.) Labr., *Ascochyta* Blight, Chickpea, Salicylic Acid (SA) Systemic Acquired Resistance (SAR)

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the most extensively grown legume crop in Turkey, where it is cultivated on 625.000 ha in 1999 (Anonymous, 2000). *Ascochyta* blight, caused by *Ascochyta rabiei* (Pass.) Labr. (Telemorph: *Didymella rabiei* (Kovacheski) v. Arx)), is the most serious disease of chickpea and causes substantial economical loss in favourable environmental conditions (Karahan, 1968; Smithson et al., 1985; Singh and Reddy, 1990). The pathogen infects all aerial parts of the plants and often causes breaking

* This study was carried out between 1999 and 2001 as Msc. thesis

of stems and death of plant parts above lesions. Since effective chemical control measures have not been developed, the use of resistant cultivars is the most effective and economic way of controlling the disease (Kaiser et al., 1973; Singh and Reddy, 1989). However, there is a continuous need for new disease resistant cultivars due to the appearance of new races of the pathogen (Nene and Reddy, 1987; Singh and Reddy, 1991).

Some plants are able to restrict the spread of fungal, bacterial or viral pathogens to a small area around the point of initial penetration where a necrotic lesion appears. This protective cell suicide is referred to hypersensitive reaction (HR). In addition to local defence responses, many plants develop an increased resistance against subsequent pathogen infection in uninfected tissues. This enhanced disease resistance is termed systemic acquired resistance (SAR) (Ross, 1961). Commonly associated with HR and SAR is the expression of a set of defence–related genes, such as the genes encoding pathogenesis-related proteins (PR proteins) (Ward et al., 1991; Uknes et al., 1992). The production of these proteins seems to be a part of a general defence system against pathogens. SA is shown to be an endogenously synthesised compound critical for the induction of SAR signalling pathway (Malamy et al., 1990; Metraux et al., 1990; Raskin, 1992; Gaffney et al., 1993; Ryals et al., 1994). The accumulation of SA is also associated with the subsequent induction of genes including those encoding PR-proteins (Yalpani et al.,1991; Ward et al., 1991). Exogenously SA application has been reported to induce resistance to pathogens as well as expression of SAR genes (White, 1979; Ward et al., 1991; Uknes et al., 1992). Besides, it was reported that SA or its derivatives affected culture growth and enzyme activity of pathogens to a various degree, depending on concentrations and pathogen (Çökmüş and Sayar, 1991; Elad, 1993; Palva et al., 1994; Coquoz et al., 1995; Spletzer and Enyedi, 1999). However, SA has been used to induce SAR in a variety of plants such as tobacco, tomato, potato and cucumber (Wieczorek, 1993; Rasmussen et al., 1991; Elad, 1993; Cohen, 1994; Narusaka et al., 1999), no attempts have been made to examine the effects of SA on disease susceptibility in chickpea up till now.

For this reason, the effect of the exogenously application of SA on *Ascochyta* blight which is the most important disease of chickpea has been investigated. The present study describes the role of the SA on the acquired resistance to chickpea against *Ascochyta rabiei*.

MATERIALS and METHODS

Fungal material

Isolate of *Ascochyta rabiei* (Race-6) used in this study was obtained from Assoc. Prof. Dr. F. Sara DOLAR (Ankara University, Agriculture Faculty, Plant Protection Department). Ascochyta rabiei was grown on chickpea seed meal–dextrose agar (CSMDA: 4% chickpea flour, 2% dextrose, and 2% agar in 1 l distilled water) in 9 cm glass petri dishes (Gowen, 1986). The dishes were incubated at 22±1°C with 12 h NUV (near ultraviole light).

Plant material

The susceptible chickpea cultivar (Canıtez-87) seeds used in this study were obtained from Ankara University, Department of Plant Protection. Seeds were surface-sterilized with 1% sodium hypochlorite solution for two minutes and washed 3 times with distilled water. Eight seeds were sown in 10 cm pots containing sterilized soil, river-bed sand, manure (1:1:1, v/v) mixture, and thinned to five per pot after germination. Four pots were used for each treatment and plants were grown for 15 days at 23±2°C with 12 h photoperiod (light intensity, 171 µmoles sec^{-1} m^{-2}).

Inoculation of plants

Spore suspensions of *A. rabiei* were prepared from 15 days-old cultures using sterile distilled water. The spore suspension was filtered through cheesecloth (3 layers) to remove mycelial fragments and it was adjusted to a concentration of $6x10^5$ spores per ml. Twenty days after sowing, aerial parts of plants were sprayed (to run-off) with spore suspensions using a pressure hand sprayer. Control plants were sprayed with sterile distilled water (SDW). After inoculation, plants were covered with transparent polyethylene bags to maintain leaf wetness and incubated for 21 days in a growth room as described above. The polyethylene bags were removed after 3 days.

Application of SA to plants

Salicylic acid (Şifa Chemical Company) suspensions were adjusted to pH=6.5 with 0.1 M NaOH and applied in both seed and foliage. In seed treatment, seeds were soaked in different concentrations of SA suspension (0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1, 2, 4 and 8 mM) for 1 h before sowing. Chickpea plants were grown for 20 days and, then inoculated with *A. rabiei* as described above.

In plant treatments, SA was applied to foliage in three different ways as once and several times before inoculation and after inoculation. Eight different concentrations of SA (0.025, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 mM) were sprayed on 20 days-old plants once using a pressure sprayer 1, 2, 3 and 4 days before inoculation. Similarly, SA (0.25, 0.5, 1, 2, 3, 4, 5 and 6 mM) was sprayed several times (2, 3, 4 and 5) to foliage at 2 day intervals and the plants were inoculated for 2 days after last application. In addition, different concentrations of SA (0.5, 1, 2.5, 5, 7.5 and 10 mM) were sprayed to plants 1, 3 and 5 days after inoculation.

Fungitoxicity tests

The fungitoxic effect of SA on growth of *A. rabiei* was tested in CSMDA medium amended with SA concentrations ranging from 0 to 15 mM. These plates were inoculated with a 7-mm agar plug from the peripheries of 15 days old cultures and incubated at 22±1°C and 12 h photoperiod (UV). The colony diameter was measured 7, 14 and 21 days after inoculation. A conidial suspension of *A. rabiei*, containing 100 spores in 5 µl was prepared with SDW. Different concentrations of SA (2.5, 5, 7.5, 10, 12.5 and 15 mM)

were incorporated in Czapek-Dox broth (50%) medium in erlen mayers and suspended with ultrasonic sound wave. 45 µl of each SA doses and 5 µl of spore suspension were put in wax pencil rings on sterile glass slides which are inside a petri plate humidity chamber. 5 µl of spore suspension plus 45 µl of medium served as the control. The slides were incubated at 22±2°C and the spores were killed and fixed at 16 hours by adding acid fucsin in lacto phenol. The germination and germ tube length of 100 spores for each slide was recorded. Each treatment including four slides and 400 spores were examined in total (Dolar ve Gürcan, 1993).

Disease assessment

Plants were scored at weekly intervals for three weeks on a scale (Singh et al., 1981) of 1 to 9. All statistical analyses were performed with MSTAT software statistical package. Significance was determined at $P<0.05$, using Fisher's Least Significant Difference Test and Duncan's Multiple Range Test. Each experiment was repeated at least two times.

RESULTS and DISCUSSION

Seed treatment

The results of investigation, undertaken to determine if application of SA on seed induces SAR in chickpea against subsequent infection are shown in Table 1. The concentrations of SA, ranging between 0.025 and 0.8 mM did not significantly reduce disease severity as compared with control plants. However, the higher concentrations of SA reduced infection by *A. rabiei* between 14.38 and 23.44%. In addition, no significantly difference in the concentrations of SA, ranging between 0.8 and 8 mM was observed. The highest inhibition was accomplished by providing 1 mM dose of SA.

Table 1. Effect of seed treatment with SA on infection of *A. rabiei*.

SA-concentrations (mM)	Disease severity (%)[a]		Percentage of inhibition
0	84.44±2.22	a	-
0.025	80.02±7.04	ab	-5.23
0.05	79.79±4.24	ab	-5.50
0.1	75.16±4.52	abc	-10.99
0.2	75.05±4.96	abc	-11.12
0.4	74.69±5.26	abc	-11.54
0.8	70.04±4.45	bc	-17.05
1	64.64±11.92	c	-23.44
2	72.29±3.92	bc	-14.38
4	70.00±0.49	bc	-17.10
8	68.68±7.98	c	-18.66

[a]Means followed by the same letter are not significantly different (P>0.05)

The reason that much inhibition did not get in treatment of seed with SA may be because SA systemically does not act upwards in chickpea or loses effectiveness during period between its application and inoculation. Besides, SA may not be adequate to induce resistance, converted to a various of its derivatives. Exogenously applicated SA was converted to β-O-D-glucosylSA by an SA-inducible glucosyltransferase (SA-Gtase) in tobacco leaves (Enyedi et al., 1992) and in rice shoots (Silverman et al., 1995).

Application of SA on plant before inoculation

The effect of one time SA-application on foliage before inoculation on challenge infection is given in Table 2. In treatment of foliage with 0.025, 0.05 and 0.1 mM doses of SA 4 days before inoculation, the disease severity significantly increased, however, the other doses did not significantly change in response to infection by *A. rabiei* as compared with control plants. SA treatments 1 and 3 days before inoculation did not cause an apparent change in disease severity. In application of SA 2 days before inoculation, while the lower doses from 0.4 mM did not significantly affected infection, 0.4 mM and 0.8 mM doses of SA reduced disease severity to 43.43 and 37.03%, respectively.

Table 2. Effect of one application of SA on plants before inoculation with *A. rabiei* on disease severity.

SA concentrations (mM)	Disease severity (%)[a] SA application days before challenge inoculation			
	4th	3rd	2nd	1st
0	71.42±2.91 cdefg	-	-	-
0.025	95.83±4.41 (+34.17)[b] a	73.50±13.73 (+2.91) bcdef	77.77±6.42 (+8.89) bcde	79.01±932 (+10.62) bcd
0.05	87.65±4.89 (+22.72) b	70.76±12.94 (-0.92) cdefg	78.88±6.73 (+10.44) bcd	79.08±4.81 (+10.72) bcd
0.1	98.69±2.26 (+38.18) a	73.73±13.81 (+3.23) bcdef	61.90±4.39 (-13.32) efg	82.01±6.00 (+14.82) bcd
0.2	83.33±5.55 (+16.67) bc	79.08±2.26 (+10.72) bcd	60.49±7.70 (-15.30) fg	66.66±3.22 (-6.66) defg
0.4	68.42±6.63 (-4.20) cdefg	83.30±1.89 (+16.63) bc	43.43±19.35 (-39.19) hı	71.42±5.53 (0.00) cdefg
0.8	69.75±12.5 (-2.33) cdefg	72.22±4.89 (+1.12) cdefg	37.03±6.36 (-48.15) I	68.05±4.72 (-4.71) cdefg
1.6	70.25±4.36 (-1.63) cdefg	71.80±8.08 (+0.53) cdefg	55.50±10.90 (-22.29) gh	70.30±2.68 (-1.56) cdefg

[a]Means followed by the same letter are not significantly different (P>0.05)
[b]The values in parenthesis are percentage of inhibition (-) or stimulation (+) over control

These results showed that application of SA 2 days before challenge inoculation was more effective than other days and 0.8 mM dose of SA was the most effective dose. This agrees with the other studies, reporting the of need two days between SA application and challenge inoculation to induce SAR (Cohen, 1994; Spletzer and Enyedi, 1999; Fenquan and Jinsheng, 2000). It is unclear if the resistance, obtained in this research was due to induction of PR-protein accumulation or to other enzyme activities. Generally, chitinase accumulation as well as PAL activity increases at 24-48 hours after SA treatment and PR proteins, involved in SAR signal pathway accumulate locally in treated tissues, depending on SA concentration (Yalpani et al., 1991; Palva et al., 1994; Coquoz et al., 1995; Lawton et al., 1995; Narusaka et al., 1999; Fengquan ve Jinsheng, 2000). Similarly, some researchers reported that exogenously application of SA prior to inoculation conferred resistance to plants against some viral, bacterial and fungal diseases (Rasmussen et al., 1991; Weete, 1992; Elad, 1993; Wieczorek, 1993; Cohen, 1994; Narusaka et al., 1999).

Application of SA several times to foliage significantly reduced the infection of *A. rabiei* (Table 3). Inhibition values of infection on plants were found between 14.78 and 29.10% by five times SA application. The highest inhibition was determined at 5 mM.

Table 3. Effect of several times application of SA on plants before inoculation with *A. rabiei* on disease severity.

SA concentrations (mM)	Disease severity (%)[a] Number of application			
	5	4	3	2
0	84.31±2.88 a	-	-	-
0.25	71.85±2.57 (-14.78)[b] bcd	70.37±7.40 (-16.54) bcdef	72.22±3.34 (-14.34) bc	70.37±1.85 (-16.54) bcdef
0.5	66.66±2.22 (-20.94) bcdefgh	75.75±7.25 (-10.16) ab	64.10±2.83 (-23.98) cdefghı	64.81±7.23 (-23.13) cdefghı
1	62.96±3.39 (-25.33) cdefghı	61.11±1.11 (-27.52) defghıj	70.94±4.59 (-15.86) bcde	68.25±3.30 (-19.05) bcdefg
2	64.44±8.01 (-23.57) cdefghı	71.71±3.68 (-14.95) bcd	61.31±3.77 (-27.27) defghıj	55.55±0.00 (-34.12) hıjk
3	60.68±11.56 (-28.03) defghıj	65.07±6.65 (-22.82) cdefghı	62.96±4.20 (-25.33) cdefghı	50.61±2.82 (-39.98) jk
4	61.11±1.11 (-27.52) defghıj	63.62±7.48 (-24.53) cdefghı	68.88±7.28 (-18.31) bcdef	58.97±7.78 (-30.06) fghıj
5	59.78±1.39 (-29.10) efghıj	55.55±16.4 (-34.12) hıjk	60.78±3.11 (-27.91) defghıj	45.45±4.09 (-46.10) k
6	66.66±6.94 (-20.94) bcdefgh	56.94±6.70 (-32.47) ghıj	59.25±5.60 (-29.73) fghıj	53.53±4.71 (-36.51) ıjk

[a]Means followed by the same letter are not significantly different (P>0.05)
[b]The values in parenthesis are percentage of inhibition (-) over control

Inhibition rates of infection changed from 10.16 to 34.12% at four times, 14.34 to 29.73 % at three times, 16.54 to 46.10 % at twice application. In almost all cases the most effective dose was found to be 5 mM. The highest inhibition is determined on plants treated twice with SA. Çökmüş and Sayar (1991) reported that seven applications of SA (0.36 to 7.24 mM) to tomato plants at 2 day intervals prior to inoculation with *Pseudomonas syringae* pv. tomato reduced disease severity up to 81.0%. Inhibition rate of disease severity was found as 48.15% by one application of 0.8 mM SA to foliage two days prior to inoculation while this ratio was 46.10% in the plants treated twice with 5 mM SA. Hence, one application of 0.8 mM SA was sufficient to reduce infection by *A. rabiei*.

Application of SA on plant after inoculation

Treatment with 0.5 mM of SA one day after inoculation did not inhibit *A. rabiei* infection while the higher doses significantly reduced disease severity between 30.81 and 43.37% (Table 4). The most effective dose of SA was found out to be 2.5 mM when applied to plants one and also three days after inoculation. There was no effect of SA on disease severity 5 days after inoculation.

Table 4. Effect of SA application on plants after inoculation with *A. rabiei* on disease severity.

SA-concentrations (mM)	Disease severity (%)[a]		
	SA application days after challenge inoculation		
	1st	3rd	5th
0	86.00±2.61 bcd	-	-
0.5	83.80±3.98 (-2.55)[b] bcd	90.85±5.96 (+5.64) ab	89.07±9.23 (+3.57) ab
1	59.50±7.30 (-30.81) fg	77.70±5.44 (-9.65) cde	89.70±1.42 (+4.30) abc
2.5	48.70±3.78 (-43.37) g	65.60±3.91 (-23.72) efg	88.80±7.87 (+3.25) abc
5	53.90±8.16 (-37.32) g	71.23±11.32 (-17.17) def	88.80±2.11 (+3.25) abc
7.5	57.40±11.57 (-33.25) fg	83.72±12.62 (-2.64) abcd	87.60±2.09 (+1.86) abc
	52.70±3.99 10(-38.72) g	88.00±9.41 (+2.33) abc	95.20±1.31 (+10.69) a

[a]Means followed by the same letter are not significantly different (P>0.05)
[b]The values in parenthesis are percentage of inhibition (-) or stimulation (+) over control

The spores *A. rabiei* start to germinate on the leaflets and stems 12 h post inoculation and penetrate the plants within 72-96 h, forming germ tubes and appressoria

(Pandey et al., 1987; Höhl et al., 1990; Dolar, 1994). Hence, treatment with SA one day after inoculation may exert fungitoxic influence on spores or reduce infection of *A. rabiei* by stimulating accumulation of medicarpin and mackiain phytoalexins in plants (Weigand et al.,1986; Dolar and Gürcan, 1993).

Fungitoxic effect of SA on *A. rabiei*

Inhibition rate of mycelial growth of *A. rabiei* was found out as 3.77 and 17.09% when grown in medium containing 2.5 and 5 mM SA, respectively (Table 5). 7.5 mM dose of SA completely inhibited culture growth. Spore germination and germ tube growth were not affected with 2.5 and 5 mM doses of SA. However, application of 7.5 mM SA reduced spore germination rate by 98.7% and only few spores formed in the germ tube (Table 6). Spore germination was completely inhibited by doses higher than 7.5 mM. We concluded that SA has antifungal activity against *A. rabiei.*

Table 5. Effect of SA on mycelial growth of *A. rabiei.*

SA-concentrations (mM)[c]	Colony diameter (mm)[a]		
	Days		
	7th	14th	21st
0	32.87±0.62 a	60.25±0.64 a	82.62±0.47 a
2.5	32.00±0.91 (-2.64)[b] a	57.12±2.59 (-5.19) a	79.5±3.02 (-3.77) b
5	21.37±2.25 (-34.98) b	44.12±2.83 (-26.77) b	68.5±2.38 (-17.09) c
7.5	0.0±0.0 (–100.00) c	0.0±0.0 (–100.00) c	0.0±0.0 (–100.00) d
10	0.0±0.0 (–100.00) c	0.0±0.0 (–100.00) c	0.0±0.0 (–100.00) d
12.5	0.0±0.0 (–100.00) c	0.0±0.0 (–100.00) c	0.0±0.0 (–100.00) d
15	0.0±0.0 (–100.00) c	0.0±0.0 (–100.00) c	0.0±0.0 (–100.00) d

[a]Means followed by the same letter are not significantly different (P>0.05)
[b]The values in parenthesis are percentage of inhibition (-) over control
[c]Each column was separately analysed with LSD test

Our findings are similar to the result of Elad (1993) who reported that SA (10 mM) inhibited mycelial growth (42%) and conidia germination (52%) of *Botrytis cinerea.* However Çökmüş and Sayar (1991) reported that treatments with 3.62 mM SA reduced the disease severity of *Pseudomonas syringae* pv. tomato by 71.7-81.0% but culture growth of *P. s.* pv. *tomato* was not affected at concentrations between 0.36 and 7.24

mM. Besides, it was shown that SA did not inhibit mycelial growth of *Phytophthora infestans* (1-10 mM) and *Alternaria solani* (0-200 µM) (Coquez et al., 1995; Spletzer ve Enyedi, 1999).

Table 6. Effect of SA on spore germination and germ tube growth.

SA-concentrations (mM)	Germination rating (%)	Percentage of inhibition[a] of germination	Germ tube length (µm)
0	89.50±2.32	-	38.82±4.40
2.5	88.00±2.68	-1.68	34.85±4.40
5	86.16±3.43	-3.74	29.44±3.24
7.5	1.16±1.29	-98.71	7.50±1.58
10	0.00±0.00	-100	0.00±0.00
12.5	0.00±0.00	-100	0.00±0.00
15	0.00±0.00	-100	0.00±0.00

[a]The values are percentage of inhibition (-) over control

The results of this study indicated that treatment of plants with SA increased resistance against *Ascochyta* blight in chickpeas. As an alternative to the development of resistant cultivar, it may be possible to utilise a scheme of inducible plant defences to provide protection against *Ascochyta* blight in chickpea. In resistance to *A. rabiei*, PR proteins as β- 1,3 glucanase and chitinase, and pterecarpon phytoalexins are thought to play an important role (Weigand et al., 1986; Vogelsang and Barz, 1990; Dolar and Gürcan, 1993). However, the action of SA in induction of resistance to *A. rabiei* is not understood. Future studies are needed to understand the mechanisms involved in SAR signal transduction pathway. Generally, resistance induction studies related with SA application are carried out in vitro but in vivo studies are limited. In a study performed with different potato cultivars in field trials, once SA treatment caused increased susceptibility in the susceptible cultivar to *Phytophthora infestans* whereas SA caused a slight increase resistance in resistant and moderately resistant cultivars (Quintanilla and Brishammar, 1998). Also, SA application significantly reduced the incidence of *Botrytis cinerea* storage rots (Poole and McLeod, 1994). More studies related with induction of resistance with SA application under field conditions are needed.

ÖZET

NOHUTLARDA *ASCOCHYTA* YANIKLIĞINA [*Ascochyta rabiei* (Pass.) Labr.] KARŞI DAYANIKLILIĞIN SALİSİLİK ASİT UYGULAMASI İLE UYARILMASI

Ascochyta rabiei (Pass.) Labr.'ye karşı nohut bitkilerinin dayanıklılığında salisilik asit (SA) uygulamalarının rolü bu çalışmada araştırılmıştır. Salisilik asit duyarlı nohut çeşidinin (Canıtez-87) tohum ve toprak üstü organlarına uygulanmıştır. Bunun yanısıra

salisilik asidin in vitro' da *A. rabiei*'nin koloni gelişimi, spor çimlenmesi ve çim borusu uzunluğuna olan etkisi de tespit edilmiştir.

Tohuma salisilik asit uygulamasında *A. rabiei* enfeksiyonu %5.23 ile %23.44 oranında engellenmiş olup en fazla engelleme 1 mM SA uygulanan bitkilerde görülmüştür. Bitkinin toprak üstü organlarına SA uygulamasında, inokulasyondan 2 gün önce bitkilere bir kez SA uygulanmasının diğer günlere kıyasla hastalık gelişimi üzerinde daha etkili olduğu ve hastalık şiddetinin 0.8 mM'da %48.15 oranında engellendiği saptanmıştır. İnokulasyondan önce 2 günlük aralıklar ile birkaç (2, 3, 4 ve 5 kez) kez SA uygulamasında ise 2 kez 5 mM SA uygulanan bitkilerdeki hastalık şiddetinde %46.10 oranında azalma sağlanmış ve hemen hemen tüm uygulamalarda en etkili dozun 5 mM olduğu tespit edilmiştir. İnokulasyondan sonra SA uygulamasında, *A. rabiei* ile inokulasyondan 1 gün sonra bitkilere SA uygulanmasının hastalık şiddetini %2.55 ile %43.37 oranında azalttığı ve en etkili dozun 2.5 mM olduğu tespit edilmiştir.

Fungitoksisite denemelerinde 7.5 mM SA uygulamasının in vitro'da *A. rabiei*'nin koloni gelişimini, spor çimlenmesini ve çim borusu gelişimini tamamen durdurduğu saptanmıştır.

Anahtar Kelimeler: *Ascochyta rabiei* (Pass.) Labr., *Ascochyta* Yanıklığı, Nohut, Salisilik Asit (SA), Sistemik Kazandırılmış Dayanıklılık (SKD)

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