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CONTENTS

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Control of Wet Bubble Disease of Microwave Treatments

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ABSTRACT

In this study, the possible use of microwave power against wet bubble disease in mushrooms was investigated. The efficacies of four different microwave treatments were tested both on pathogen inoculated and noninoculated casing soil. All of the treatments were able to inhibit the disease completely on pathogen inoculated soil with a significant ($P \leq 0.05$) increase in yield. Although, the mean yield obtained from the growing bags of this treatment was significantly less than that of control. Treating the noninoculated casing soil with microwave at 60°C for 10 minutes gave more satisfactory results as compared to other 3 treatments.

Key words: *Mycogene perniciosa*, Wet Bubble, Microwave, Thermal treatments

INTRODUCTION

Cultivated mushroom growing is limited by several diseases (Fletcher et al., 1989). Wet bubble disease caused by *Mycogone perniciosa* (Magn.) Delacr) is one of the most serious disease in mushroom growing. Recently, the mushroom industry has been suffering from an epidemic of wet bubbl disease both in Turkey and in some other countries (Fidan et al., 1998; İlhan and Tezcan, 2000; Gea et al., 2000).

The usual commercial practice for the management of the diseases of mushroom is the use of synthetic fungicides and disinfectants. However, the use of sythetic chemicals on mushroom is becoming more difficult to justify in the recent years. Many factors, have contributed to the implementation of strategies for reducing dependency on fungicides. These include: the enhanced proliferation of resistant strains of pathogens due to improper and prolonged use of fungicides, thereby diminishing their efficacy (Van Zaayen, 1982; Fletcher and Adas, 1992; Swatton, 1993; Flegg, 1993; Adie and Grogan, 2000) and the puplic concern about fungicide residues on harvested mushrooms because the cultivated mushrooms is harvested once a day or every couple of days (Bora and Özaktan, 2000; Bhatt and Singh, 2000). Therefore, an interest among the scientists in finding 'non-chemical' control methods that can replace the conventional methods (chemical control) has been increasing.

Previous studies in finding alternative control methods indicated that the scientists have focused on biological control of mushroom diseases by using several

antagonist microorganisms. Although, some of these studies indicated succesful results against some mushroom pathogens it should be considered that, the common point in all of these studies is that biological control agents used against *M. perniciosa* has an insulficient effect compared to others under in vivo conditions (Bora et al., 1998; Bhatt and Singh, 2000; Singh et al., 2000).

M. perniciosa is a soil inhabiting fungus and it is believed that the casing soil is the main source of infection (Atkins, 1961). The commercial practice to inhibit this disease is treating the casing soil with a disinfectant such as formalin. However, the use of this chemical is not enough to reduce the incidence of disease to commercially acceptable levels. Also, the procedure of treating tho soil with formalin takes at least 7 days causing a time loss and extra labor cost for growers (Stoller, 1981).

The overall objective of this study was to examine the possible use of microwave power against wet bubble disease in mushrooms. The main idea in using microwave was to heat the casing soil until the pathogen could not survive. The main advantage of using microwave was rapid and uniform heating of casing soil as compared to steam or hot air treatments due to the special physical properties of soil.

MATERIALS and METHODS

Microwave Set-Up. The experimental set-up is a pilot-scale 2450 MHz microwave system consisting essentially a MW generator (Sariem, 0-1.2 kW) that was connected to a multimode MW cavity through a circulator, a directional coupler, and a three stup tuner (Fig. 1).

The microwave cavity incorporated an auxiliary hot air heating unit with a electric heater capable of delivering up to 3 kW. A fan blew the hot air across the cavity and another fan discharged the hot air from the cavity. The temperature within the casing soil was recorded by using a PT100 thermocouple, with 5 minutes intervals, during microwave treatment. Uniformity of microwaves in the cavity was enhanced by a turntable (35 cm in diameter, 2 rpm).

Fungal Culture. *M. perniciosa* was obtained from the infected caps of mushrooms found in growing houses with severe infections and cultured on Potato Dextrose Agar. Spore suspensions were prepared by removing the spores (chlamydospores) from srorulating edges of 3-week-old culture with a bacteriological loop, and suspending them in sterile distilled water. Spore concentrations were determined with a hemocytometer and adjusted to 10^5 conidia per ml.

Effect of Microwave on Wet Bubble Disease. Preliminary experiments, conducted to evaluate the effects of different microwave treatments on yield losses of mushrooms caused by *M. perniciosa have* indicated that heating the casing soil with 4 different

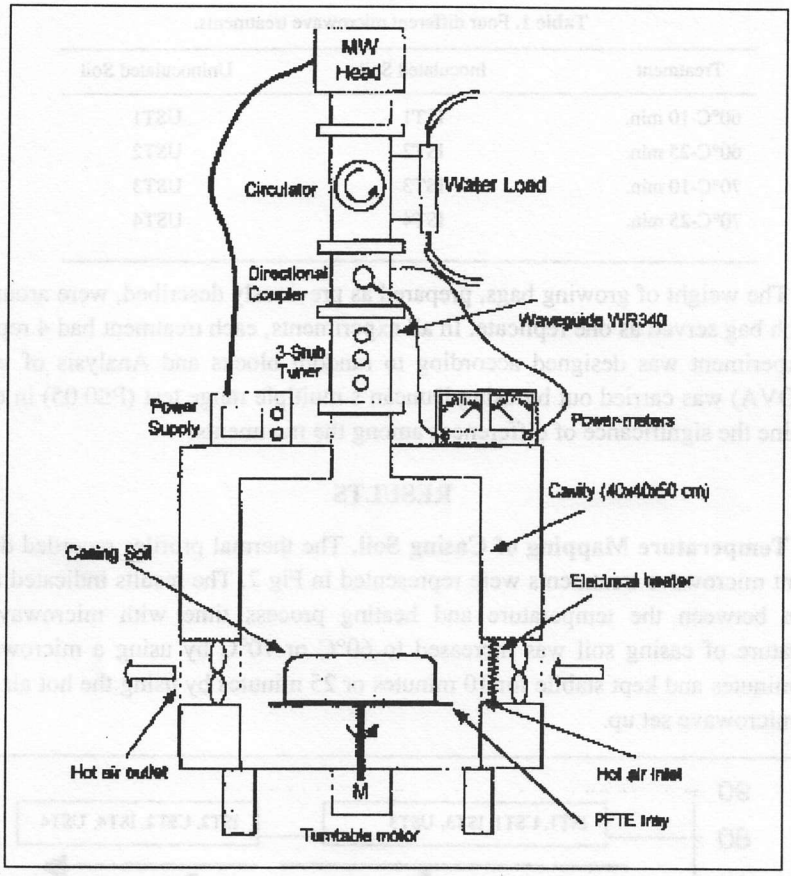

Figure 1. Experimental Microwave Set-up.

microwave treatments was able to reduce the yield losses effectively. These results were the basis of our decision to conduct further experiments with 4 different microwave treatments. The compost used in the experiments was commercially prepared and pasteurized by a mushroom company. All of stages of compost preparation, such as spawning with Sylvan 512 strain of *Agaricus bisporus* and running, were performed as it was done in the commercial practice. A commercial company also provided the casing soil used for the experiment. The raw casing soil was inoculated with spore suspension of (10^5 spores ml^{-1}) of *M. perniciosa* prior to the microwave treatments. Inoculated casing soil was incubated in a saturated atmosphere for 3 days and then, heated in microwave for 4 different treatments as citied in Table 1. Treated soil was cased on to compost after being kept at room temperature for 24 hours. Uninoculated casing soil was also treated in microwave. Following the treatments and casing, mushroom were grown as it is done in commercial growing houses.

Table 1. Four different microwave treatments.

Treatment	Inoculated Soil	Uninoculated Soil
60°C-10 min.	IST1	UST1
60°C-25 min.	IST2	UST2
70°C-10 min.	IST3	UST3
70°C-25 min.	IST4	UST4

The weight of growing bags, prepared as previously described, were around 5 kg and each bag served as one replicate. In all experiments, each treatment had 4 replicates. The experiment was designed according to random blocks and Analysis of variance (ANNOVA) was carried out by using Duncan's multiple range test ($P \leq 0.05$) in order to determine the significance of differences among the treatments.

RESULTS

Temperature Mapping of Casing Soil. The thermal profiles recorded during 4 different microwave treatments were represented in Fig 2. The results indicated a linear relation between the temperature and heating process time with microwave. The temperature of casing soil was increased to 60°C or 70°C by using a microwave for 15-20 minutes and kept stabile for 10 minutes or 25 minutes by using the hot air system of the microwave set up.

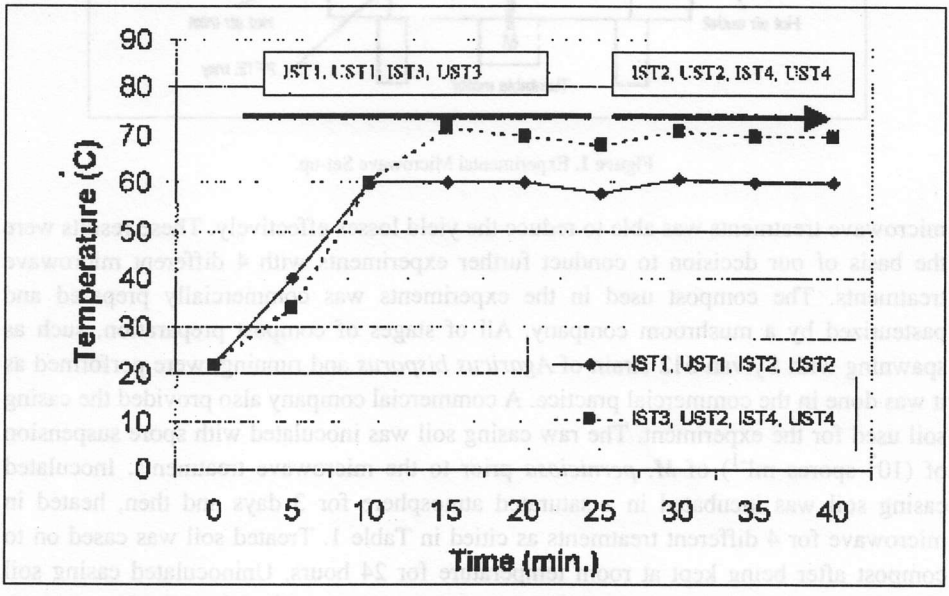

Figure 2. Thermal profiles of casing soil during four different treatments. IST1, UST1 (60°C-10 min.), IST2, UST2 (60°C-25 min.), IST3, UST3 (70°C-10 min.), IST4, UST4 (70°C-25 min.).

Effect of Microwave on Wet Bubble Disease. The amount of yield obtained by the pathogen inoculated and treated soil was shown in Table 2 while the amount obtained by noninoculated and treated soil are shown in Table 3.

Table 2. The amount of yields obtained from inoculated casing soil.

Treatments	Yields (g) Growing Bags				
	1	2	3	4	MEAN*
Control	1070	55	118	153	349.0b
IST1	686	994	718	1130	882.0a
IST2	1273	1065	935	1153	1106.5a
IST3	1144	1144	1353	1222	1215.7a
IST4	1104	1000	1084	1250	1109.5a

IST1, UST1 (60°C-10 min.), IST2, UST2 (60°C-25 min.), IST3, UST3 (70°C-10 min.), IST4, UST4 (70°C-25 min.)
*Means followed by different letters are significantly different according Duncan's Multiple Range Test (P≤0.05).

The results shown in Table 2. indicates that all of the microwave treatments were able to inhibit the wet bubble disease as compared to control. There were no significant differences among 4 different microwave treatments. No disease symptoms (sclerodermoid) of *M. perniciosa* in any of the growing bags treated with microwave were observed. In contrast, the sclerodermoid mass recorded in the control bags was 685.5 g totally.

Table 3. The amount of yields obtained from noninoculated casing soil.

Treatments	Yields (g) Growing Bags				
	1	2	3	4	MEAN*
Control	1445	1427	1370	1486	1432a
UST1	832	1291	1056	838	1004b
UST2	505	461	416	587	492.3c
UST3	981	875	840	767	865.7b
UST4	874	937	859	832	875.5b

IST1, UST1 (60°C-10 min.), IST2, UST2 (60°C-25 min.), IST3, UST3 (70°C-10 min.), IST4, UST4 (70°C-25 min.)
*Means followed by different letters are significantly different according Duncan's Multiple Range Test (P≤0.05).

Treating the noninoculated casing soil by microwave at 60°C for 10 minutes gave more satisfactory results as compared to other 3 treatments although the mean yield obtained from the growing bags of this treatment was significantly less than that of control.

DISCUSSION

The results of this study revealed that microwave treatments have potential to control on wet bubble disease of mushrooms. Several in vitro studies have shown that thermal treatments have the potential to inhibit or kill the mycelium and spores of *M. perniciosa* (Dar and Seth, 1994; Jufang et al., 1997). The application of microwave energy is a known method of achieving rapid heating, which is already widely used to inhibit the deterioration caused by microorganisms on fruits and foods (Karabulut, 2001).

The results shown in Fig. 1. indicated us that it was possible to keep the casing soil at high temperatures by using microwave treatment. This was a successful point for us to test the effect of microwave treatments against *M. perniciosa* since we have seen that it was very difficult to heat the casing soil uniformly by using conventional heating methods such as stem and hot air due it its physical properties.

The effect of microwave treatments on inhibiting pathogens inoculated into casing soil are shown in Table 2. It was evident that all of tho treatments were able to inhibit the disease completely, and there was a proportional increase in yield as compared to controls.

The effect of microwave treatments on noninoculated casing soil, was also tested. The results given in Table 3. indicated that the microwave treatments could have some adverse effects on the yield. The yield obtaned from the treated bags was significantly less that that of control. We have observed similar results in our preliminary experiments. This may be due to the adverse effect of microwave on the beneficial microflora of casing soil. It has been known that there were some beneficial microorganisms inside the casing soil that suppress the pathogens, stimulate the growth of mushroom, and help the formation of pinhead (Özaktan and Bora 1994; Singh et al., 2000). An adverse effect of microwave on this beneficial microflora will reduce the yield while inhibiting the pathogens.

In conclusion, our results show that microwave treatments have a potential on cntrolling th wet bubble disease of mushrooms. In our future studies, we intend to find on optimum temperature and duration for microwave treatments in order to control the pathogens without effecting the beneficial microflora of casing soil and the yield. Using the microwave technology together with the biological control agents which are in effective against M. perniciosa as stand alone treatments can help us to form an integrated approach on controlling diseases of mushroom. New researches and scientists should be focused on these points.

ÖZET

KÜLTÜR MANTARI ISLAK KABARCIK HASTALIĞINA KARŞI MİKRODALGA ENERJİSİNİN KULLANILMA OLANAĞININ ARAŞTIRILMASI

Bu çalışmada kültür mantarının ıslak kabarcık hastalığına karşı mikrodalga enerjisinin kullanılma olanağı araştırılmıştır. Dört farklı mikrodalga uygulamasının etkisi hem patojen inokule edilen örtü toprağında hem de herhangi bir inokulasyon yapılmamış örtü toprağında denenmiştir. Patojen inokule edilen toprakta bütün uygulamalar hastalığı tamamen engellemiş ve ürünü önemli düzeyde ($P \leq 0.05$) arttırmıştır. İnokulasyon yapılmamış toprağın 60°C'de 10 dakika süreyle mikrodalgayla ısıtılması sonucu diğer 3 uygulamaya oranla daha başarılı sonuçlar alınsa da, bu uygulamanın kullanıldığı torbalardan elde edilen ortalama ürünün kontrol uygulamasından elde edilene oranla daha düşük olduğu bulunmuştur.

Anahtar kelimeler: *Mycogene perniciosa*, Islak Kabarcık, Mikrodalga, Isıl Uygulamalar

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Isolation of Bacteria Antagonistic to Some Fungal Pathogens Causing Root Rot of Bean From the Rhizoplane and Investigation on Their Potential for Biological Control

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ABSTRACT

In this studyix hundred and twenty different bacterial isolates were screened *in vitro* for their ability to inhibit vegetative growth of *Fusarium solani* f.sp. *phaseoli*, *Macrophomina phaseolina* and *Rhizoctonia solani*, causal agents of root rot of bean. Bacterial isolates were obtained from bean rhizoplane by dilution plate technique. Seven flourescent *Pseudomonas* isolates found to be antagonistic against all the pathogens tested *in vitro*, have been tested under greenhouse conditions for their ability to control the root rot disease. Bacterial isolates showing antagonistic activity *in vitro* were applied as drench (10^7 cfug^{-1} soil) to pathogen-infested soil. Pathogens were cultured on the corn meal-wheat bran medium. After 24 hours, each pot was planted with 10 seeds. Treatments were replicated 4 times in a randomized plot design. Healthy and diseased plants were recorded 4 weeks after planting. Isolate number M61 on *F. solani* f.sp. *phaseoli*, M61 and M15 on *M. phaseolina*, and M75 on *R. solani* provided significant (P=0.05) reduction in the root rot incidence compared with the others. No correlation was found between *in vitro* and *in vivo* effectiveness. These antagonistic isolates will be identified and evaluated in the further studies.

Key words: Antagonism, bean, fluorescent Pseudomonas, rhizoplane, root rot

INTRODUCTION

Dry bean, *Phaseolus vulgaris* L., is an important vegetable crop in Turkey, and it is susceptible to root pathogens. Root rot disease caused by *Fusarium solani* f.sp. *phaseoli* (Mart.) Appel and W., *Macrophomina phaseolina* (Tassi.) Goid., *Rhizoctonia solani* Kuehn and *Pythium ultimum* Trow. is widespread. *P. ultimum* and *R. solani* cause pre-emergence damping-off; *F. solani* and *M. phaseolina* cause post-emergence root rot (Burke and Kraft, 1974; Hall, 1983). *R. solani* and *F. solani* f.sp. *phaseoli* are the primary pathogens in seedling and older stages of bean, respectively. Disease complex causes serious yield losses especially in consecutive plantings (Buonassisi et al., 1986).

Crop rotation does not provide sufficient control against the disease. Also while Tolerant cultivars are beneficial in relation to disease control (Burke and Kraft, 1974),

but growers prefer mostly the susceptible cultivars giving high yield. Some fungicides can control the disease; however, inconsistency of protection is frequently encountered (Brent, 1987).

Various soil bacteria including *Bacillus, Pseudomonas, Serratia, Clostridium* and *Azotobacter* have been shown to reduce soil-borne fungal diseases when applied to seed, to soil or inoculated to root (Cook and Baker, 1983). In addition, rhizobacteria can enhance the growth and yield of many crops even in the absence of plant pathogens (Weller, 1988, Reddy et al, 1990). Several studies on the biological control of this disease complex has been carried out so far. Most of the studies were done under laboratory and greenhouse conditions. Of biocontrol agents, bacterial antagonists were frequently preferred to control pathogens (Elad and Chet, 1987; Reddy et al., 1993; Sunchez et al., 1994; Pleban and Chet, 1995). A number of biopesticide were developed in recent years as a result of research on the subject. Indeed, some of the commercial products, in which bacterial agents are the active ingredients, are now in commercial use.

In this study, 620 bacterial isolates of different origins were screened out for their antagonistic activity in vitro and their ability to control root rot disease of bean caused by *R. solani, F. solani* f.sp. *pahseoli*, and *M. phaseolina* under greenhouse conditions

MATERIALS and METHODS

Isolation of rhizoplane bacteria and screening them for antagonistic activity

Rhizoplane bacteria were isolated from healthy bean roots of 400 samples collected from the fields in Meram, Konya. Special care was taken that as much soil as possible remained at the root system in order to avoid a drying out of the roots before further processing in the laboratory. Each sample was put in a sterile plastic bag in the field. Isolations were carried out according to the method by Rouatt and Katznelson (1961). The roots were first freed from the adhering coarse soil particles. One hundred roots from each location were combined to form one mixed sample. Exactly 16 g root mass was washed three times with 0.5 L sterile demineralized water so that all residual soil particles were removed. To remove soil residue, sample was placed in a 250-ml flask with 90 ml of 0.1% proteose-peptone (PP) solution and 20 g glass beads (Ø2 mm) and shaken vigorously for 10 min on a shaker. Then a dilution series with a 0.1% proteose-peptone solution, was prepared. Of the first dilution level (10^{-1}), 0.1 ml was taken and transferred to 9 ml PP solution and mixed 5 min in order to obtain a uniform mixture. These steps were repeated until dilution level 10^{-4}, of which then 0.05 ml was spread out on the King's –B and Nutrient agar culture medium. Forty petri dishes were prepared per experimental sample. Dishes were incubated at 27°C for 72 h. A total 620 bacterial colonies having different morphological properties were chosen to assay their antibiotic efficacy against *R. solani, M. phaseolina* and *F. solani* f.sp. *phaseoli*.

Inhibitory effects of bacterial isolates on mycelial growth of the pathogens were tested by using the streak method. Each isolate was streaked at one end of a petri plate.

After three days, a mycelial plug (5 mm diameter) taken from the edge of an actively growing fungal colony was placed on the other end. Plates were incubated at 25°C in darkness for 7-10 days. Bacterial isolates producing inhibition zones, more than 0.5 cm, were choosen for further studies.

Pot experiments

The pathogenic isolates of *M. phaseolina, R. solani* and *F. oxysporum* f.sp. *phaseoli* were obtained from infected bean roots and maintained on PDA slants. They were then grown separately in flasks containing corn meal- wheat bran mixture moistened with potato-saccharose broth at 25°C for 30 days. Soil infestation was achieved by incorporating these stock cultures separately into the pot soil, consisting of sand and farm manure at equal rate, in the ratio of 1/19 (v/v). Bacterial isolates having antagonistic activity were grown on PSY culture medium (200 g potato, 5 g saccharose, 2 g yeast extract (Oxoid), 1 L tap water) for 4 days. Pot soil, artificially infested with pathogens, was drenched with bacterial suspensions (10^7 cells g^{-1} soil.). Control pot soils were not inoculated. After 24 hours, each pot (Ø 25, length 22 cm) was planted with 10 seeds and irrigated as needed.

Experimental design was a randomized plot with four replicates. Disease incidence for *M. phaseolina* and *F. solani* f.sp. *phaseoli* was calculated, basing on healthy and infected plants, four weeks after planting.

A 0-4 scale was used for *R. solani*, where 0= no lesion on the root base, 1=one lesion, 2= two lesions, 3= two larger or more than two lesions and 4= dead plant.

Effectiveness of the bacterial isolates was calculated by applying Tawsend-Heuberger to Abbott formula.

RESULTS and DISCUSSION

A total of 620 bacterial isolates from the rhizoplane of healthy plants were tested to find out the antagonistic ones against *R. solani, F.solani* f.sp. *phaseoli* and *M. Phaseolina*. Among them, only 7 isolates showed antagonistic activity towards all test pathogens *in vitro* (Table 1). Isolates M46, M15 and M51 possessed the strongest inhibition on mycelial growth of *F. solani* f.sp. *phaseoli* while M/61 showed the weakest effect. In the same way, M46, M15 and M75 on *M. phaseolina*, M46, M75 and M61 on *R. solani* provided significant (P=0.05) antagonistic effects on the mycelial growth compared with the others. In addition, only M46 showed antagonistic activity towards all pathogens tested. The effectiveness of M161, M61 on *M. phaseolina* and M15, M51 and M142 on *R. solani* was not significantly stronger than that of others. It was found that bacterial isolates showed different antagonistic activity against the pathogens tested. This result was in agreement with previous studies regarding biological control *in vitro* (Elad and Chet, 1987; Reddy et al., 1993). Isolates showing antibiotic activity to all pathogens tested were chosen to assay ability to suppress the pathogens in the pot experiment.

Table 1. The width of inhibition zones (cm) formed in the consequence of antagonistic activity of bacterial isolates *in vitro*.

Strain No.	*F. solani* f.sp. *phaseoli*	*M. phaseolina*	*R. solani*
M46	2.3 a*	3 a	2.2 a
M75	1 e	2.1 bc	1.8 b
M142	1.3 d	1.8 d	0.6 d
M161	1.7 c	1.5 e	1.5 c
M51	2 ab	2.1 c	0.7 d
M15	2 b	2.3 b	0.6 d
M61	0.6 f	1.5 e	1.6 bc

P=0.05

*: Values followed by same letters in a column are not significantly different (P < 0.05). Values (means.etc) were compared using LSD, DMR or Student's t etc etc...

The bacterial isolates showing antagonistic activity *in vitro*, provided differential suppression on the disease incidence caused by *R. solani*, *F. solani* f.sp. *phaseoli* and *M. phaseolina*. All isolates except for M46 and M75 reduced the disease incidence caused by *Fusarium solani* f.sp. *phaseoli* by 2.08-41.3% (Table 2). Isolate M61 was found to be the most effective against *F. solani* f.sp. *phaseoli* and *M. phaseolina*. M75 provided the best control against *R. solani* while M142 showed the lowest disease incidence in pot experiment.

Table 2. Effect of the isolates showing antagonistic activity *in vitro* on the disease incidence caused by *Fusarium solani* f.sp. *phaseoli*, *Macrophomina phaseolina* and *Rhizoctonia solani*.

	Disease incidence and protective effect of the isolates					
	F. solani f.sp. *phaseoli*		*M. phaseolina*		*R. solani*	
Characters	Disease incidence %	Protection %	Disease incidence %	Protection %	Disease incidence %	Protection %
Control	92		96		86	
M46	92	0	92	4.16 bc*	75.5	12.20 bc*
M75	92	0	94	2.08 d	60.5	29.65 a
M142	90	2.17 d*	94	2.08 d	83	3.49 cd
M161	88	4.34 cd	90	6.25 bc	82	5.23 cd
M51	82	10.86 bc	84	12.50 b	82	4.65 cd
M15	68	26.08 b	60	37.50 a	81.5	8.00 cd
M61	54	41.30 a	66	31.25 a	69	19.76 b

P=0.05

*: Same as in previous table.

There was no positive correlation between the antagonistic activity of bacterial isolates *in vitro* and the ability to reduce the disease incidence in the greenhouse. Similar results were obtained in some other studies regarding biological control (Reddy et al., 1993).

There are many ways in which an antagonistic organism can operate in biological control: colonization, competition and antibiosis. It was thought that antagonistic activity by these isolates resulted from antibiosis, involving siderophore *in vitro* study. But it should be remembered that antibiotics are not the sole prerogative of selected antagonists. In pot experiment they may use one or a few of these mechanisms of biological control. However these mechanisms are affected by many factors such as substrate, soil pH, temperature and structure, and adaptation of biocontrol agents to plant roots and soil. One of the biocontrol mechanisms affected in soil environment is antibiosis. Antibiotic production in soil is affected by many factors such as substrate, soil pH, temperature and structure. Standardized and preferred culture medium is generally used for *in vitro* studies, whereas this situation is quite different in soil environment.

Some soils are carbon limited. In such soils, microorganisms are dormant because of carbon or nitrogen limitation, and may not produce antibiotics (Campbell, 1985). On the contrary, in soil, amended with organic matter or other readily available carbon sources, it could be produced in detectable amounts by antagonistic microorganisms. The other nutrient source is the root exudates which could allow sufficient nutrients for antibiotic production. However, in soil environment, antibiotics may adsorbed on to clay or organic colloids, which may concentrate small amounts. It is also possible that microbial antibiotics could be rapidly broken down by enzymes in the soil.

The other way, in which an antagonistic organism can operate, is microbial competition. Competition occurs when two (or more) organisms require the same thing and the use of this by one reduces the amount available to the other. Thus micro-organisms may compete for nutrients: one organism gets most of the nutrients and grows, while other has insufficient access and dies. This is known for both carbon and nitrogen sources. Competition is also possible for oxygen and space. An essential point of the definition is the deprivation of one of the organisms. Here if it is considered that these introduced biocontrol agents caused antagonistic activity against the pathogens by competition, some isolates had not sufficiently competed towards some pathogens tested. However it is difficult to find out that which mechanisms are used by antagonists in biological control in soil.

Because of factors mentioned above, antagonistic activity obtained from *in vitro* might not be shown *in vivo*. In greenhouse studies, strains showing antagonistic activity could possibly have these features: Biocontrol agents might produce antibiotics; well adapted to bean roots; have potential to colonize and protect roots; induce resistance of host. These seven isolates showing the ability to control the disease could use one or a

few of these features. Isolates giving good protection will be identified and evaluated in studies.

ÖZET

FASULYE KÖK ÇÜRÜKLÜĞÜNE NEDEN OLAN BAZI PATOJENLERE KARŞI ANTAGONİSTİK RHIZOPLANE BAKTERİLERİNİN İZOLASYONU VE BİYOLOJİK KONTROL POTANSİYELLERİNİN ARAŞTIRILMASI

Fasulyede kök çürüklüğüne neden olan etmenlerden *Fusarium solani* f.sp. *phaseoli*, *Macrophomina phaseolina* ve *Rhizoctonia solani*'ye karşı laboratuvar koşullarında 640 adet bakteriyel izolat denenmiştir. Bakteriyel izolatlar fasulye bitkisinin rhisoferinden dilusyon tekniği ile elde edilmiştir. Yedi flourescent *Pseudomonas* izolatın *in vitro*'da test edilen tüm patojenlere karşı antagonistik ve laboratuvar koşullarında kök çürüklüğü hastalığını kontrol edebilme yeteneğinde olduğu bulunmuştur. *In vitro*'da antagonistik aktivite gösteren izolatlar patojenle bulaştırılmış toprağa ıslatma şeklinde (107 cfug-1 toprak) uygulanmıştır. Patojenler mısır unu- buğday kepeği ortamında kültüre alınmıştır. Antagonist uygulamadan yirmi dört saat sonra her bir saksıya 10 tohum ekilmiştir. Deneme tesadüf parseller deneme desenine göre dört tekerrürlü olarak yürütülmüştür. Ekimden dört hafta sonra hastalıklı ve sağlıklı bitkiler kaydedilmiştir. M61 nolu izolat *F. solani* f.sp. *phaseoli* (%41.30), M61 ve M15 *M. phaseolina* (% 37.50, %31.25) ve M75 nolu izolat *R. solani*'ye karşı (%29.65) diğer izolatlara göre kök çürüklüğü çıkışında önemli ölçüde azalma sağlamıştır. İzolatların *in vivo* ve *in vitro* etkinliği arasında bir ilişki bulunmamıştır. Bu antagonistik izolatlar teşhis edilecek ve ileriki çalışmalarda değerlendirilecektir.

Anahtar Kelimeler: Antagonizm, fasulye, fluorescent *pseudomonas*, rhizoplane, kök çürüklüğü

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Detection of Squash Mosaic Comovirus (SqMV) of Cucurbits by Biological, Serological and Advanced Techniques Methods in Çukurova Region in Turkey*

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ABSTRACT

Squash mosaic comovirus was identified by serological and biological assays, SDS-PAGE, electron microscopy and dsRNA analysis methods in the imported melon seeds of different firms.

The virus causes systemic mosaic, leaf deformation, ringspot on the infected melon, cucumber and squash plants. The isometric virus particules in 28 nm diameter were observed under electron microscopy. The molecular weigth of coat protein components was found as 42kDa, 22kDa and 5kDa by SDS-PAGE. RNA of virus was also charecterized by dsRNA analysis method.

This is the first report on the detection of SqMV in detail in the seed by using advanced techniques in Turkey.

Key words: Squash Mosaic Virus, ELISA, Purification, Double-Stranded RNA (dsRNA)

INTRODUCTION

The Eastern Mediterranean Region is known as an important region of polyculture type of agriculture of various crops and is designated as a main vegetable growing area in Turkey. The vegatable production having annual yield with 1.265.680 tons in Adana province is equal to the 6.25% of total production of Turkey (Anonymous, 1998).

Turkey is one of the most important countries in the world for the production of watermelon (*Citrillus vulgaris* L.), melon (*Cucumis melo* L.) cucumber (*Cucumis sativus* L.) and squash (*Cucurbita pepo* L.). Adana province takes an important place with 807.199 tons of production in it (Anonymus, 1998). However the yield is not going paralel with growing area due to yield losses caused by virus. With respect to the infection;

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Zucchini Yellow Mosaic Potyvirus (ZYMV), Watermelon Mosaic Potyvirus (WMV), Cucumber Aphid-Borne Yellow Virus (CABYV), Cucumber Mosaic Cucumovirus (CMV), and Papaya Ringspot Potyvirus (PRSV) and Cucumber Vein Yelloving Virus are reported as prevalent virus diseases of Cucurbitaceae plants in Mediterranean Region (Yılmaz et al., 1989, Yılmaz et al., 1992, Lecog et al., 1994).

The farmers have complained about yield reduction and deformations in melon and squash. When we have visited the plants in the fields we have observed that the infected plants expresses mosaic type of symptoms on the leaves and the vines The pattern of dissemination suggested that the causal organism of diseases might be a seed-borne virus.

The objective of this study is to identify the causal organism by biological and serological assays and molecular techniques and Electron Microscopy.

MATERIALS and METHODS

The research was conducted in the Çukurova Region in 1999-2002. The infected plant samples were collected from cucurbit fields at the different locations in the region. The seeds were taken from the mature fruits of infected plants and were grounded in the 0.02 M phosphate buffer (PH: 7.0) containing 0.1% 2-mercaptoethanol with a mortar and pestle an later mechanically inoculated to the previously carborandum dusted leaves of *Cucumis melo, Cucurbita pepo, Cucumis sativus, Citrillus lanatus, Chenopodium amaranticolor* Costa&Reyn, *Chenopodium quinoa* Wild., *Datura stromarium* L., *Nicotiana glutinosa* L., *Nicotiana tabacum* L. "Xanthi", *Nicotiana tabacum* L. "Samsun NN", *Nicotiana tabacum* L. "Samsun" The symptoms expression were periodicaly observed and recorded.

Another investigation was carried out to detect the rate of seed transmission of the commercial seeds stocks of firms .Those seed samples were sown in the steril media containing of 1:1:1 sand:soil:manure pasture in pots. After germination of seedlings the young leaves were harvested and sequiezed and inoculated to the indicator plants.

Serological Tests

The Serological tests were applied to determine the infectivity rate of seed transmission in which 2200 seeds were used. During the research 4 local and 18 imported cultivars were employed.

For this purposes one hundred seeds of each cultivar of local and imported *Cucumis melo, Citrillus vulgaris, Cucumis sativus, Cucurbita pepo* and *Citrillus lanatus* were used for the tests.

Double antibody sandwich enzyme linked immunosorbend assay (DAS-ELISA) and Immunodiffusion test were used for testing of the crude seeds extracts and infected

young leaves against to SqMV, CMV, WMV, ZYMV and MNSV. The antisera were obtained from Dr.Lecog (Station de Pathologie Vegetale Domaine INRA-FRANCE). Polystyrene microtitre plates were coated with gamma globulin at a concentration of 1 µg/ml. Plant samples were applied at a dilution of 1/5 (W/V) in phosphate buffered saline (PH:7.0), containing 0.05% Tween-20 and 2% PVP (PVP-40). IgG-congugated was applied at the concentration of 1 µg/ml. Alkaline phosphatase congugate was used at a 1/1000 dilution. Results were determined spectrofotometrically at 405 nm medispes ESR 200 ELISA plate reader (Clark and Adams,1977).

The SDS-immunodiffusion tests were conducted in a medium consisting of 0.75% Nobel agar (Difco), 0.5% Sodium azide (Merck) in 0.07M phosfate tampon (PH:7.4) (Ball,1990).

Samples from infected tissue (1g) was extracted in 1ml 0.1M phosfate buffer (PH:7.2). This extract was centrifuged in 5.000 rpm for 5 minutes and aqueous phase was taken. Added 250 µl SDS 10% Per ml aqueous phase. The extracted was incubated at room temperature for 45 minutes. Sample was tested against SqMV antiserum (at dilution 1/20) in a medium consisting of 0.85%NaCI, 5% BSA in 0.05M Tris (PH:7.2).

Virus Purification

The infected young leaves were used in the purification. The leaves were homogenized (1/2,w/v) with 0.1M phosphate buffer (PH:7.0) in a blender. The extract was filtered through a double layer of steril cheesecloth and clarified by centrifugation in a JA-21 rotor at 10.000 rpm for 20 minutes. Butyl alcohol added to the mixture to reach 8% concentration of the supernatant which was later stirred for at least 30 minutes at +4°C and then the coagulated green debris and pelet were discarded after centrifugation at 10.000 rpm for 20 minutes. As stirring of the liquid in the cold room for 30 to 40 minutes. 8% of PEG 6.000 and 4% of NaCI were added to the supernatant for precipitation of virus. Mixture was then centrifuged at 10.000 rpm for 30 minutes. The pellet was removed and resuspanded in 0.1M phosphate buffer (PH:7.0) and clarified by centrifugation at 10.000 rpm for 10 minutes in the same rotor. The PEG precipitation was repeated two more times to increase concentration of the virus (Albersio et al., 1975).

SDS-PAGE

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used for electrophoretic analysis of SqMV coat proteins (Laemelli, 1970).

Electron Microscopy

Purified virus preparation were examined under electron mycorcopy. The grids were stained with the 2% of Uranyl acetate pH 4.5 (Milne, 1993).

Double-Stranded RNA Analysis

dsRNA analysis was performed acording to Valverde et al., (1990).

The melon leaves were grinded in STE buffer and 10% SDS, bentonite (from a 2% aqueous suspension), and then 1x STE-saturated phenol added to the extract and it was shaken well for 30 minutes. The mixture was centrifuged at 8.000g for 15 minutes, and the supernatant was saved and 95% ethanol were added to the supernatant. After preparation of the two cellulose columns, the sample was added to the first column and the liquid discarded from the column. The column was flushed with 1x STE containing ethanol (16.0%v/v) and kept for refilling the column until all the buffer is used. The column was drained completely and the liquid was discarded. 2.5 ml of 1x STE was added and drained completely. 10.0 ml of 1xSTE was added to the column and later collected 10.0 ml in a centrifuge tube. 2.1 ml of 95% ethanol were put into the sample. The procedure for filling up the sample and flushing of the second column is the same as that of first column except. 6.0 ml of 1x STE was added to the second column and later this amount was collected in a centrifuge tube. 0.5 ml of 3.0 M sodium acetate (pH 5.5) and 20.0 ml of 95% ethanol were added to each sample. The sample was stored for 2 hr at -20°C for the precipitate of dsRNA and then the sample was centrifuged at 8.000 g for 25 minutes. The ethanol was removed and the tube placed upside down for draining about 15 minutes. 200 µl of EG buffer was added to each tube and mixed well for resuspention of dsRNA. dsRNA was stored at -20°C until used.

Electrophoresis was performed in 1.0% agarose gel and 5% polyacrylamide gel.

RESULTS and DISCUSSION

Investigation was conducted under the controled greenhouse conditions. After germination of seeds it was observed that 5% of the young plants were found to be infected with virus.

SqMV is a wordwide spread virus in cucurbits and it has multicomponent particules. It is seed (Lockhart et al., 1982) and beetle (*Acalymma trivittata; A. thiemei thiemei; Diabrotica undecipunctata undecipunctata; D. bivitula; Epilachna chrysomelina; E. paenulata*) transmitted virüs (Nolan et al., 1984).

Seed tranmission rate of virus was reported 10% and 35% in *Cucumis sativus* and *Cucurbita pepo* respectively however it is not transmitted by pollen (Alvarez and Campbell, 1978; Nolan and Campbell, 1984).

The fruit of infected plants are reduced in size and weight (Powell et al., 1970).

The virus causes systemic mosaic, leaf deformation, ringspot by mechanical inoculation on the infected melon, cucumber and squash plants in the greenhouses. The

symptoms are mainly mosaic type and may appear 12-15 days after inoculation on the leaves (Figure 1). This type of symptom is also observed in the fields. But the magnitude of severety of symptom may change with the host species.

The virus did not cause symptoms on *Citrillus lanatus, Chenopodium amaranticolor* Costa&Reyn, *Chenopodium quinoa* Wild., *Datura stromarium, Nicotiana glutinosa, Nicotiana tabacum* "Xanthi", *Nicotiana tabacum* "Samsun NN", *Nicotiana tabacum* "Samsun".

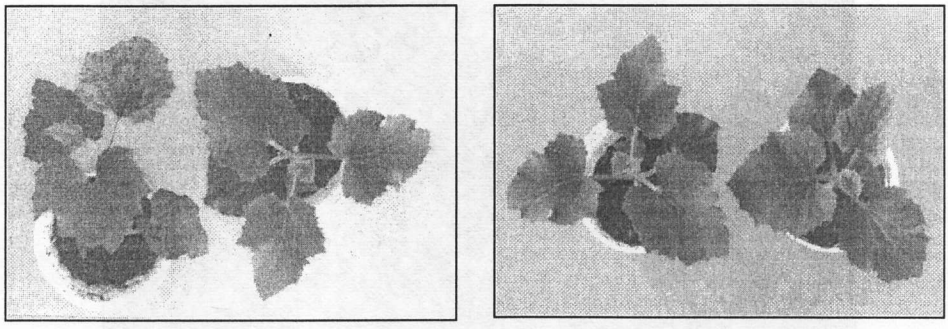

Figure 1. Mosaic Type of symptom on two different melon species obtained from firms (1 firm a; infected b; healthy), (2 the other firm a; healthy b; infected).

Serological Test

SqMV was only detected virus in the seeds of two melon cultivars by ELISA test. The absorbance value of infected and healthy plant samples were recorded at 405 nm as 1.777 and 0.258 respectively.

The other melon varieties used in the experiments were found to be virus free.

The antisera of CMV, WMV-2, ZYMV and MNSV did not react against the seeds of cucurbits species.

Virus Purification and SDS-PAGE

SqMV was purified by PEG precipitation technique from the susceptible melon cultivar. The moleculer weight of coat proteins components of virus was detected as 42 kDa, 22 kDa and 5kDa by SDS-PAGE. Our results reveal aggrement with the report of Rice et.al., (1954).

Electron Microscopy

The picture of virus particles is not clearly fixed on the electron micrograph, but the polyhedral virus particles (Figure 2) were observed under electron microscopic examination. The diametre of the particles were measured about 28 nm in diameter.

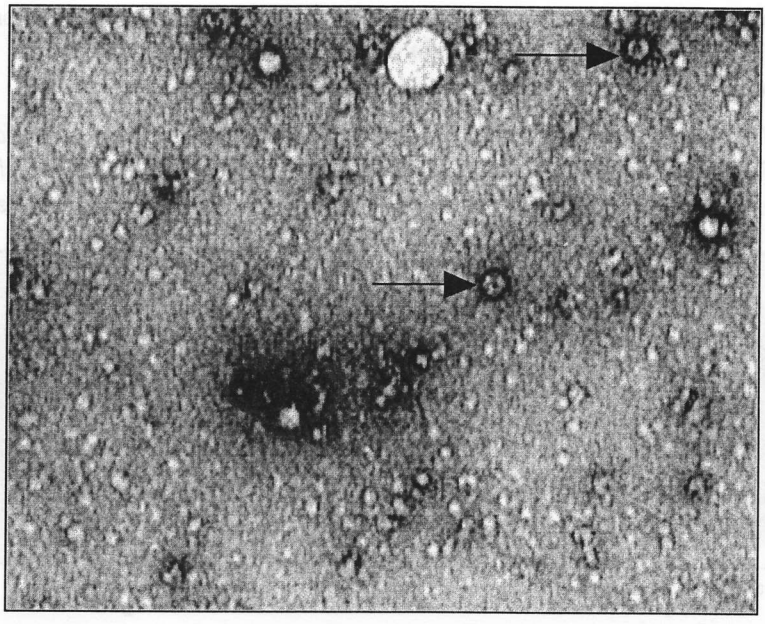

Figure 2. Electron Micrograph of Squash mosaic comovirus (SqMV) After Partial Purification (Bar is equeal to 100 nm).

Double-Stranded RNA Analysis

The dsRNA analysis was used to diagnose SqMV in the young leaves of seedling previously obtained from the infected seed and the mechanically inoculated to melon leaves. This procedure has been developed by Valverde et al., (1990) as reliable diagnostic method of virus identification. By using of this method, SqMV was detected in the infected plant materials. After isolation of dsRNA of SqMV, one band was observed on the agarose gel (Figure 3). However in the case of using 5% polyacrylamide gel, the two distinguished dsRNAs bands were determined. The reason for the differences in occurence of number of bands between agarose and polyacrylamide gels is directyl related to the gel not to the virus itself.

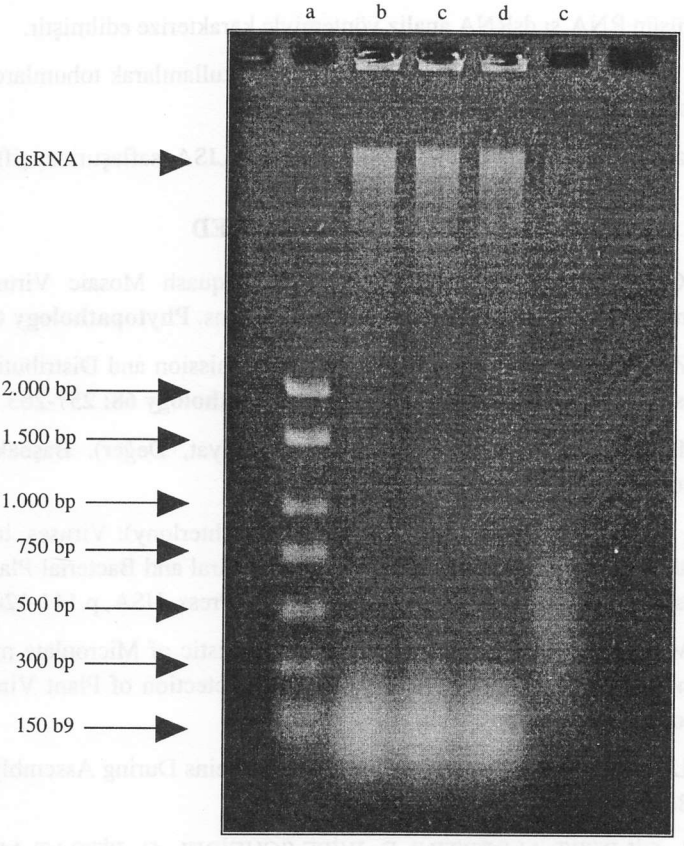

Figure 3. Agarose gel Electroforesis of dsRNA of SqMV extracted from melon. a; marker b; infected seedling obtain from the seed c-d; mecanicaly inoculated melon e; healthy.

ÖZET

ÇUKUROVA BÖLGESİNDE CUCURBITACEA'LERDE GÖRÜLEN SQUASH MOSAIC COMOVIRUS (SqMV)'UN BİYOLOJİK, SEROLOJİK VE İLERİ TEKNİKLERLE TANILANMASI

Squash mosaic comovirus (SqMV) farklı firmaların ithal ettiği kavun tohumlarında serolojik, biyolojik, SDS-PAGE, elektron mikroskobu ve dsRNA analiz yöntemi kullanarak saptanmıştır.

Virüs kavun, kabak ve hıyar bitkilerinin yapraklarında deformasyon, sistemik mozaik ve halkalı lekeler şeklinde simptomlar oluşturmaktadır.

Elektron mikroskobu ile yapılan incelemelerde virüs partiküllerinin 28 nm çapında izometrik yapıda olduğu gözlenmiştir. Kılıf proteinlerinin de SDS-PAGE yöntemiyle 42kDa, 22kDa and 5kDa ağırlığında olduğu saptanmıştır.

Virüsün RNA'sı dsRNA analiz yöntemiyle karakterize edilmiştir.

Bu araştırma ülkemizde moleküler teknikler kullanılarak tohumlarda SqMV'nün tanılanması ile ilgili ilk çalışmadır.

Anahtar Kelimeler: Kabak mozaik virüsü, ELISA, saflaştırma, çift iplikli RNA

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First Report of Citrus Exocortis Viroid on Grapevine (CEVd-g) in Turkey

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ABSTRACT

Grapevine (*Vitis vinifera* L.) is an important crop in the world. A number of viroids have been described from different grapevine growing countries. According to structural analysis of viroids 6 distinct grapevine viroids were determined up to now (Semancik et al., 1987).

A survey to identify grapevine viroids in Turkey was conducted with several grapevine varieties at East Mediterranean region. Vein banding, yellow vein, leaf rolling, yellowing and small leaves were observed as symptoms. 184 samples were collected from 1350 decare areas and samples were tested for the presence of grapevine viroids by sPAGE (sequential poliacrilamide gel electrophoresis) analysis and RT-PCR (Reverse Transcriptase-Polimerase Chain Reaction).

According to sPAGE analysis, 62 samples were found to be infected by one or more viroid and there was no viroid band found in 122 samples. CEVd-g was found in 4 out of 184 samples. This viroid was found with GYSVd-1 (Grapevine yellow speckle viroid-1), GYSVd-2 (Grapevine yellow speckle viroid-2) and HSVd-g (Hop-stunt viroid-grapevine) as a mix infection.

CEVd-g band was also observed after mechanic inoculation from Etrog citron (*Citrus medica*), *Gynura aurantiaca* and tomato (*Lycopersicon esculentum*) plants in sPAGE gels.

The sPAGE and RT-PCR (Figure 1) data clearly showed that CEVd-g was present on grapevine samples in Turkey.

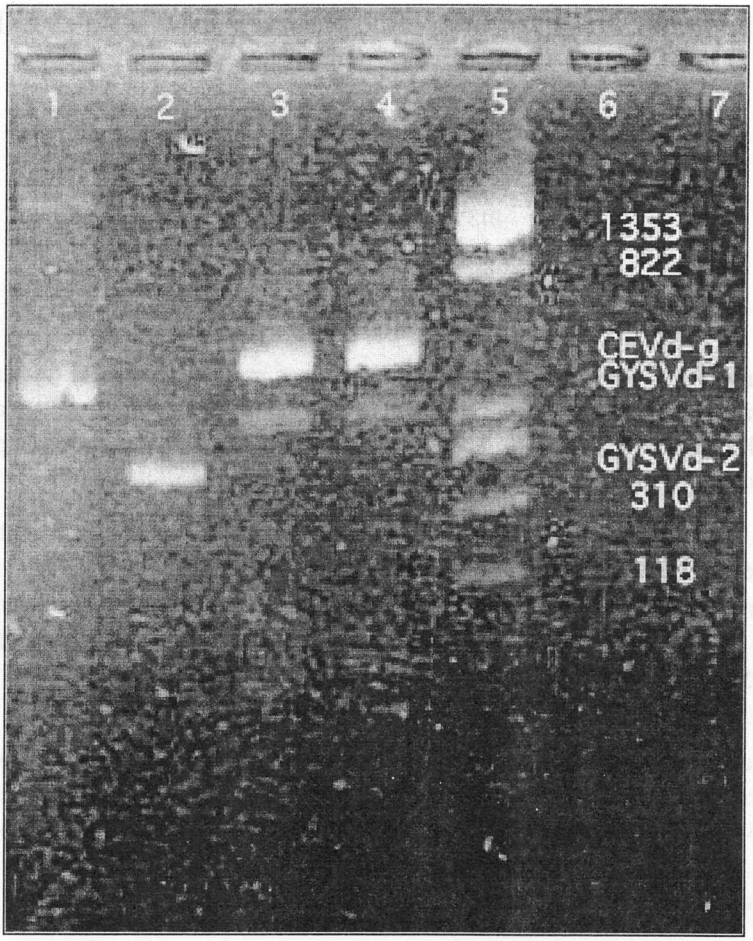

Figure 1. RT-PCR results of grapevine viroids. Lane 1; GYSVd-1, Lane 2; GYSVd-2, Lane 3 and 4; CEVd-g in grapevine samples from East Mediterranean Region of Turkey, Lane 5; Marker ϕX174 / HaeIII.

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In-vitro Untersuchungen Über Phytoalexinabbau Durch die an Hafer Kompatible und Inkompatible Isolate von *Gäumannomyces graminis*

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ZUSAMMENFASSUNG

Phytoalexine gehören zu den physiologischen Abwehrfaktoren von Pflanzen gegenüber verschiedenen Krankheitserregern. Phytoalexine aus Haferpflanzen wurden mit dem Namen Avenalumin bezeichnet und spielen eine wichtige Rolle in der Resistenz gegen pilzliche Krankheiten. Mit der vorliegenden Arbeit wurde festgestellt, daß *Gäumannomyces graminis* auch in der Lage sind, Avenalumine *in vitro* abzubauen, die kompatiblen spezies können dies schneller und vollständiger als die inkompatiblen. Bei den unbehandelten Varianten wurde die Endkonzentration konstant gehalten.

Stichwörter: Hafer, Phytoalexin, Avenalumin, Avenaluminabbau, *Gäumannomyces graminis*

EİNLEİTUNG

Unter den Abwehrfaktoren von Pflanzen gegen Pilzbefall scheint den Phytoalexinen eine besondere Rolle zuzukommen (Bailey & Mansfield, 1982). Sie sind definiert als niedermolekulare, antimykotisch wirksame Substanzen und gehören verschiedenen Stoffklassen an. Viele Pflanzen sind in der Lage, mehrere Phytoalexine – auch unterschiedlicher Struktur – zu synthetisieren (Jersch, 1986). Phytoalexinenbildung in der Nähe des Infektionsortes wird durch Elicitoren ausgelöst; eine Translokation in weiter enfernte Gewebepartien scheint nicht stattzufinden (Keen, 1971; Heath & Wood 1971). Die Auslösung ihrer *de novo*-Synthese völlig unspezifisch (Bailey, 1982). In Gramineen konnten ausser Momilacton A und B aus Mais lange Zeit keine Phytoalexine nachgewiesen werden. Erst 1981 konnten MAYAMA und Mitarbeiter (1981) nach der Infektion mit *Puccinia coronata* f. sp. *avenae* Phytoalexine aus Haferblättern isolieren und chemisch charakterisieren. Die hydrophile, stickstoffhaltige phenolische Verbindungen wurde mit dem Namen Avenalumin bezeichnet. Deren Rolle in der Resistenz wurde von vielen Wissenschaftlern gegen *Drechslera* spp., *Fusarium* spp. und *Erysiphe graminis* f. spp. untersucht und nachgewiesen (Jersch, 1986; Steinhauer, 1992; Çetinkaya 1995).

Der Rückgang der Avenaluminkonzentration in den Pflanzenteilen nach fortschreitender Inkubations- oder Induktionsdauer gab Anlaß zu Abbauversuchen mit Krankheitserregern. Ein Abbau der Avenalumine durch die Pilze *in vivo* ist durchaus möglich. Da Phytoalexine nicht unbedingt Endprodukte eines pflanzliches Stoffwechselweges sein müssen, können sie auch in der Pflanze weiteren Umwandlungen unterliegen (Van Etten et al., 1982).

Das Ziel dieser Arbeit war festzustellen, in wieweit die an Hafer kompatiblen und inkompatiblen *Gäumannomyces graminis* Isolate die Avenalumine in vitro abbauen können.

MATERIAL und METHODEN

Avenalumin 1 und 2 wurden von der Schering AG Berlin zur Verfügung gestellt. Die Versuche mit *Gäumannomyces graminis* wurden unter sterilen Bedingungen in GH-(Glukose-Hefeextrakt) Nährlösung mit einer Endkonzentration von 200 µl/ml in jeweils 5-fachen Wiederholungen durchgeführt (Steinhauer, 1982). GH besteht aus 10 g. Glukose, 2 g Hefeextrakt und 1000 ml Wasser. Avenaluminlösungen wurden mit den an Hafer kompatiblen und inkompatiblen *Gäumannomyces graminis* Isolaten inokuliert und bei 20°C und Dauerdunkelheit inkubiert. Nach 2, 3 und 4 Tagen sowie in kürzeren Zeitintervallen wurden Proben steril entnommen und auf ihren Gehalt an Avenalumin 1 bzw. 2 mittels HPLC analysiert. Die Proben wurden auf einer RP18 Säule, Merck Lichrospher 100, Teilchengröße 5 µm, 250 x 4 mm mit Vorsäulenkartusche 4 x 4 mm bei einer Flußrate von 2 ml/min aufgetragen (Çetinkaya, 1995).

ERGEBNISSE

Im Vergleich zu unveränderten Werten der nicht inokulierten Kontrolle war das Avenalumin bei den kompatiblen *G. graminis* var. *avenae* Isolaten 73 und 74 nach 2 Tagen weitgehend und nach 3 Tagen vollständig abgebaut (Abb. 1). Von den inkompatiblen var. *tritici* war Isolat Nr. 75 gleichermaßen aktiv. Bei den beiden Isolaten von *G. graminis* var. *tritici* Nr. 76 und 77 war ein deutlich verzögerter Abbau zu erkennen.

Bei einem ähnlichen Ansatz mit Avenalumin 2 wurden vergleichbare Abbauraten festgestellt (Abb 2). Durch die kompatiblen *G. graminis* var. *avenae* (73 und 74) wurde Avenalumin 2 innerhalb von 2 Tagen vollständig abgebaut. Gleiches gilt für die inkompatible var. *tritici* 77. Bei den restlichen Isolaten *G. graminis* var. *tritici* (75 und 76) war erst nach vier Tagen ein vollständiger Abbau festzustellen.

Um den Verlauf Avenalumin 1 besser erkennen zu können wurden in einer Untersuchung mit gleichen Versuchsbedingungen in kürzeren Intervallen Proben gezogen und analysiert.

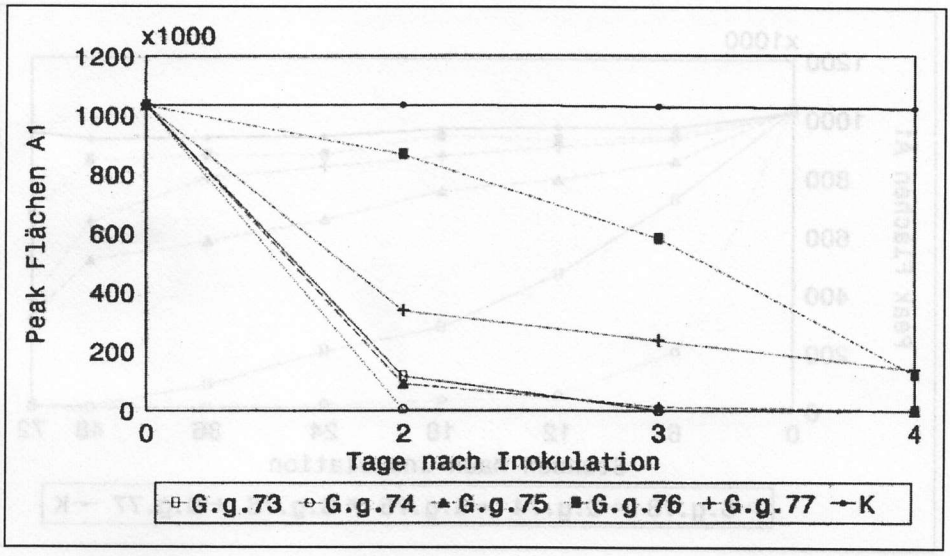

Abb. 1. Abbau von Avenalumin 1 in wässriger Lösung durch an Hafer kompatible *G. graminis* var. *avenae* (73, 74) und inkompatible var. *tritici* (75, 76, 77).

K: Unbehandelte Kontrolle
G. g. 73: var. *avenae* 73
G. g. 74: var. *avenae* 74
G. g. 75: var. *tritici* 75
G. g. 76: var. *tritici* 76
G. g. 77: var. *tritici* 77

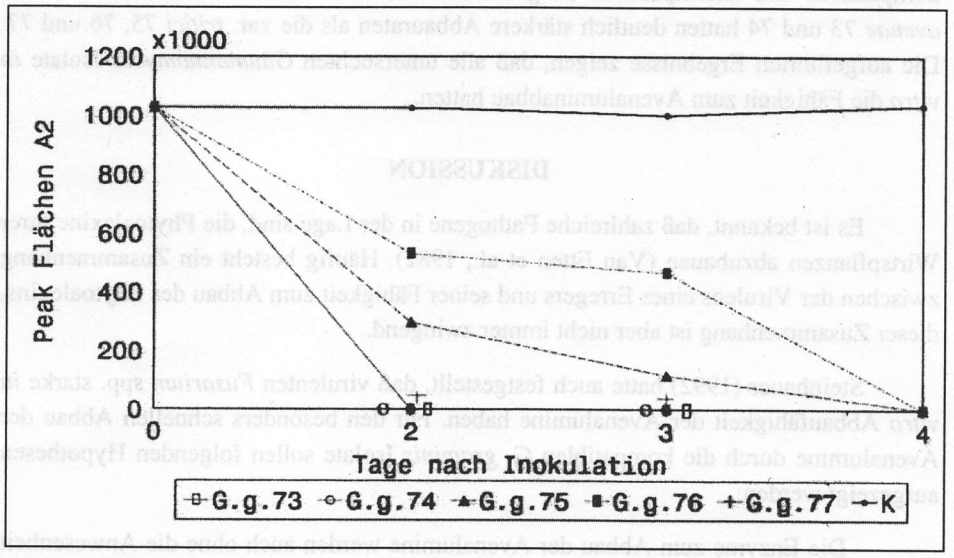

Abb. 2. Abbau von Avenalumin 2 in wässriger Lösung durch an Hafer kompatible Isolate von *G. graminis* var. *avenae* (73, 74) und inkompatible var. *tritici* Isolate (75, 76, 77).

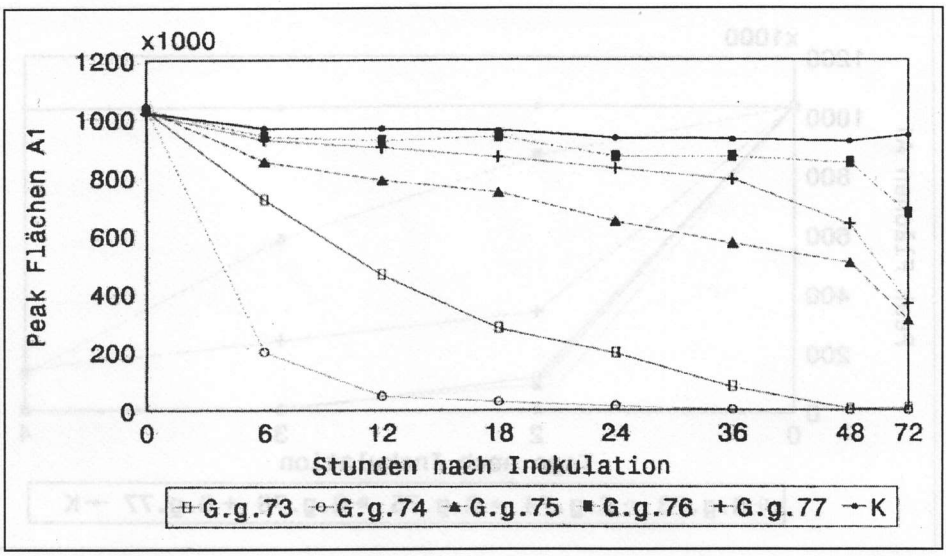

Abb. 3. Abbau von Avenalumin 1 in wässriger Lösung in kürzeren Zeitintervallen durch an Hafer kompatible *G. graminis* var. *avenae* (73, 74) und inkompatible var. *tritici* Isolate (75, 76, 77) (Mittelwerte einer Untersuchung mit jeweils 5 Wiederholungen).

In diesem Ansatz war eine deutlichere Differenzierung zwischen den an Hafer kompatiblen und inkompatiblen *G. graminis* Isolate zu erkennen (Abb. 3). Die var. *avenae* 73 und 74 hatten deutlich stärkere Abbauraten als die var. *tritici* 75, 76 und 77. Die aufgeführten Ergebnisse zeigen, daß alle untersuchten *Gäumannomyces* Isolate *in vitro* die Fähigkeit zum Avenaluminabbau hatten.

DISKUSSION

Es ist bekannt, daß zahlreiche Pathogene in der Lage sind, die Phytoalexine ihrer Wirtspflanzen abzubauen (Van Etten et al., 1982). Häufig besteht ein Zusammenhang zwischen der Virulens eines Erregers und seiner Fähigkeit zum Abbau des Phytoalexins, dieser Zusammenhang ist aber nicht immer zwingend.

Steinhauer (1992) hatte auch festgestellt, daß virulenten *Fusarium* spp. starke *in vitro* Abbaufähigkeit der Avenalumine haben. Für den besonders schnellen Abbau der Avenalumine durch die kompatiblen *G. graminis* Isolate sollen folgenden Hypothesen aufgezeigt werden:

- Die Enzyme zum Abbau der Avenalumine werden auch ohne die Anwesenheit der Phytoalexine gebildet und an die Umgebung abgegeben, sie sind also eher unspezifisch und könnten ihre Hauptaufgabe bei anderen Stoffwechselaktivitäten haben (Steinhauer,

1992). Es ist durchaus möglich, daß die avenaluminabbauenden Enzyme in Zusammenhang mit der Pathogenese der *Gäumannomyces graminis* Varietäten stehen.

- Da nur die stark virulenten Isolate mit höheren Konzentrationen der Avenalumine in Kontakt kommen, hat sich auch nur bei diesen im Laufe der Evolution die Abbaufähigkeit besonders gut entwickelt.

Die Vertreter der Varietät *avenae*, die sich an Haferwurzeln erwiesen hatte, zeigten einen sehr starken Abbau der Avenalumine *in vitro*. Die Vertreter der schwach virulenten Varietät *tritici* waren zwar auch dazu in der Lage, Avenalumin abzubauen, aber wesentlich langsamer und ineffektiver.

Im Rahmen vorliegenden Arbeit war es nicht möglich, den Metabolismus der Avenalumine näher zu bearbeiten.

ÖZET

YULAF'A UYUMLU VE UYUMSUZ *GÄUMANNOMYCES GRAMINIS* İZOLATLARININ YULAF FİTOALEKSİNLERİNİ YIKMA YETENEKLERİ ÜZERİNDE *IN VITRO* ARAŞTIRMALAR

Fitoaleksinler çok sayıda hastalık etmenine karşı bitkilerin fizyolojik savunma faktörleri içinde yer alırlar. Yulaf tarafından oluşturulan fitoaleksinler "Avenalumin" adı ile anılmakta ve fungal patojenlere karşı dayanıklılıkta rol oynamakta ancak özellikle uyumlu konukçu-patojen ilişkisinde yıkıma uğruyabilmektedirler. Bu çalışma ile yulaf ve buğdaya özelleşmiş *Gäumannomyces graminis* varyetelerinin *in vitro* koşullarda Avenaluminleri yıkma yeteneğinde olduğu saptanmıştır. Bu olgu yulafa uyumlu *Gäumannomyces graminis* izolatlarında uyumsuz olanlara oranla daha hızlı ve maddenin tümünün yıkımı şeklinde gerçekleşmiştir. Herhangi bir uygulama yapılmayan varyantlarda fitoaleksin konsantrasyonu sabit kalmıştır.

Anahtar Kelimeler: Yulaf, Fitoaleksin, Avenalumin, Avenalumin yıkımı, *Gäumannomyces graminis*

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Reactions of Different Potato Cultivars Against to Early Blight Disease

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ABSTRACT

By this study the reactions of some potato cultivars, having different maturity features, against early blight disease caused by *Alternaria solani* Sorauer was investigated. Early maturity (Resy), middle-early maturity (Marfona, Pasinler 92 and Granola), middle-late maturity (Agria) and late maturity (Caspar) cultivars of potato were chosen to determine of *A. solani* on the different maturity cultivars. Middle-early maturity cultivars were determined as the more susceptible than the middle late-maturity cultivar. It was established the most susceptible variety is Caspar; the susceptible variety is Pasinler 92, the moderately susceptible varieties are Granola, Marfona and Resy and the moderately resistance variety is Agria according to this study. Relation between appearance of the initial lesions of *A. solani* and maturation were researched and was seen that initial lesions were apparent on the earlier and middle-early cultivars of potato earlier than the late and the middle-late cultivars.

Key words: Potato (*Solanum tuberosum*), maturity, early blight disease (*Alternaria solani*), cultivars

INTRODUCTION

Potato (*Solanum tuberosum*) is known to be a major significance in human nutrition ranking fourth in world production after wheat, maize and rice. Potatoes are grown on over 44 million acres in more than 125 countries, with annual production of about 425 million tons (Rowe, 1993). Yield losses of potato crop in the potato growing countries was determined to be 31.5% due to insect, weed and diseases. In this yield decrease, diseases were responsible of 21.8%, according to FAO datum in 1993 year (Eken et al., 2000). There are many pathogenic microorganisms such as fungi, bacteria and viruses, which can cause important yield losses. Early blight disease, caused by *Alternaria solani* Sorauer, is seen wherever potatoes are grown. The importance of the disease is known as a limiting factor in potato production particularly under warm and humid conditions. There are several control measures to avoid the disease. In recent years the use of resistant cultivars has been proposed as an effective method to reduce the economic losses (Brandolini, 1992). Secor and Gudmestad (1999) reported that the

use of resistant or tolerant varieties was to effective than fungicides against to *A. solani*. Also previous studies showed that formation of resistance mechanism might be influenced from several factors such as environmentally, climatic, morphologically, biochemical and genetically (Johanson and Thurstan, 1990; Langsdorf et al., 1990; Lynch et al., 1991; Thirthamolappa and Lohithaswa, 2000). Maturity properties of cultivars are also involved those factors above mentioned. The results of other studies relating to maturity properties of potato cultivars showed that there was a strong correlation between maturity and resistance and early-maturing cultivars were susceptible more than late-maturing cultivars against early blight disease (Pelletier and Fry, 1990; Christ, 1991; Wastıe et al., 1994).

In recent years important yield losses are due to early blight disease caused by *A. solani* in potato growing areas of Van province (Demir et al., 2002). The objective of this study was to determine reactions of some potato cultivars having different maturity features, to *A. solani* and to propose resistant or tolerant cultivars to potato growers.

MATERIALS and METHODS

Material

Plant materials and pathogen isolate

The cultivars of Marfona, Pasinler 92, Caspar, Granola, Agria and Resy were used as plant materials. Tubers were supplied from Institute of East Anatolia Agricultural Research (Erzurum/Türkiye). Maturity and yield properties of the cultivars were given in Table 1 (Anonymous, 1988).

Table 1. Maturity and yield features of potato cultivars used in the study.

Cultivars	Properties
Resy	Early maturity, middle productive
Marfona	Middle-early maturity, highly productive
Pasinler 92	Middle-early maturity, highly productive
Granola	Middle-early maturity, highly productive
Agria	Middle late-maturity, highly productive
Caspar	Late maturity, highly productive

For the inoculation of cultivars AS-2004 isolate of *A. solani* which having highly virulence was used as inoculum (Demir et al., 2002).

Methods

Reproducing of *A. solani* and preparation of spore suspension

Tomato juice agar (T) (Tomato juice 200 ml, agar 20 g., distilled water 800 ml.) was used as culture media in the growth *A. solani* (Benlioğlu and Delen, 1996). Petri

plates containing T were kept in the incubator for 6 days in the dark at 23 °C and then 12 h light at 26 °C and at least 12 h dark at 18 °C for inducing sporulation (Benlioğlu and Delen, 1996). The spore suspension was prepared by agitating the spores with jets of sterile distilled water containing 0.1% Tween 80 on the plate. The suspension was centrifuged at 5500 rpm for 15 min; the supernatant was removed and then pellet was resuspended with 1 ml sterile distilled water. 5 µl of spore suspension were pipetted and the number of spores per ml were calculated under the light microscope (Benlioğlu and Delen, 1996). Spore suspension was prepared as concentration of 10^6 spores/ ml (Langsdorf et al., 1990).

Inoculation

For the breaking of dormancy of seed tubers and to promote sprout development from eyes of tubers were plunged into water containing 0.1% gibberellic acid for 10 min. Then tubers were kept in dark and light for 15 days period respectively. Potato tubers with sprouts were planted into the pots of 25 cm in diameter containing sterilized mixture (1:1:2, sand, manure, soil). After 55 days planting, spore suspension of *A. solani* was sprayed on leaves of young potato plants and only sterile distilled water on the leaves of control plants. The experiment was designed according to randomized plot with five repetitions.

Evaluation of disease severity

The inoculated plants were kept in growth chamber (80±5% relative humidity) at 25/18 °C day/night regime during harvest. The potato plants were observed periodically and initial lesions of *A. solani* were recorded regularly. Disease severity of inoculated plants were also recorded using a scale of 0-7, with 0= no lesions, 1= trace to %1, 2= 1-5%, 3= 6-10%, 4= 11-25%, 5= 26-50%, 6= 51-75%, and 7= 76-100% of foliage covered with lesions (Christ, 1991). Disease severity of potato cultivars in the pots was recorded 28 days after inoculation using scale of 0-7. Reaction levels were determined and for this, scale of 0-100 (0% disease severity= resistance, 1-25%= moderately resistance, 26-50%= moderately susceptible, 51-75%= susceptible, 76-100%= most susceptible) was arranged based on our experience. Collected data were subjected to analysis at variance and Duncan multiple range test.

RESULTS

The observations were made soon after inoculation of potato plants and the appearance of initial lesions and disease severity were recorded as below (Table 2).

The initial lesions of Resy, Marfona, Pasinler 92, Granola, Caspar and Agria were recorded according to the scale of 0-7. Disease severity of all potato cultivars was recorded between 19.02 % - 22.03% (Table 2). 5 weeks after inoculation infection level

of all cultivars was determined and the susceptibility of each cultivar was revealed according to the scale of 0-100. Severities of each variety were determined at the first and second observation dates which were summarized in Figure 1.

Table 2. Appearance date of initial lesions and disease severity on potato cultivars

Cultivars	Appearance of initial lesions	Disease severity (%)
Resy	5 days after inoculation	19.51 a *
Marfona	7 days after inoculation	22.03 ab
Pasinler 92	7 days after inoculation	20.45 a
Granola	8 days after inoculation	21.81 ab
Caspar	10 days after inoculation	20.36 a
Agria	10 days after inoculation	19.02 a

* Means a column followed by the same letter are not significantly different (P< 0.05) according to Duncan's multiple range test

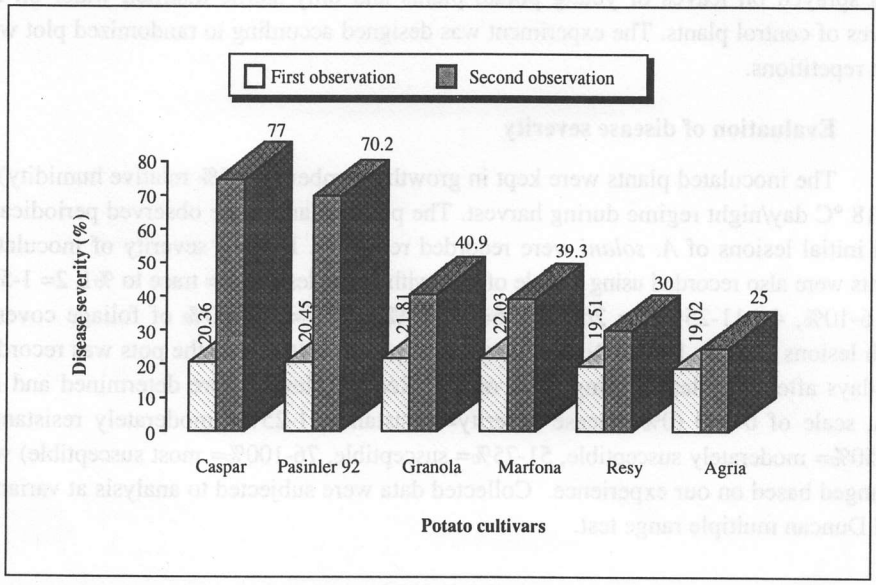

Figure 1. Infection level of each cultivar determined at the first and second observations.

The results showed that there was an increase at the disease severity between the first and second observation dates on the all potato cultivars. Disease severities and levels of resistance or susceptibility of cultivars to *A. solani* was also given in Table 3.

Table 3. Disease severities (%) and resistance and/or susceptibility of each cultivar to *A. solani*

Cultivars	Disease severity (%)	Levels of resistance or susceptibility
Caspar	77. 00 a *	Most susceptible
Pasinler 92	70.20 b	Susceptible
Granola	40.90 c	Moderately susceptible
Marfona	39.30 c	Moderately susceptible
Resy	30.00 d	Moderately susceptible
Agria	25.00 e	Moderately resistance

* Means a column followed by the same letter are not significantly different ($P< 0.05$) according to Duncan's multiple range test.

As shown in Table 3 all cultivars were included into different statistical group except Granola and Marfona. The most susceptible cultivar was determined as Caspar (77.00%), the susceptible cultivar was determined as Pasinler 92 (70.20%), the moderately susceptible cultivars were determined Granola, Marfona and Resy (40.00%, 39.30%, 30%, respectively), and the moderately resistance cultivar was determined as Agria (25%).

DISCUSSION

Each cultivar reacted differently. A parallelism was observed between maturation and disease severity among all the cultivars except Caspar. Early maturity (Resy) and middle early maturity (Marfona, Granola and Pasinler 92) cultivars of potato were found more susceptible compared to middle late maturing cultivar (Agria) (Table 3). Johanson and Thurstan (1990) and Christ (1991) also reported that the correlation of early blight resistance and maturity is indeed very strong under controlled field conditions, however, this correlation mechanism is not clear. Some researchers found that some tuber isozymes were more accumulated in the later maturing cultivars than earlier maturing cultivars and they thought this reason might be effective on disease resistance (Johanson and Thurstan, 1990). Disease resistance to early blight was investigated as genetically by Brandolini (1992). He revealed that the clones of earlier maturing potato cultivars were the most slanting towards high susceptibility compared to the clones of later maturing cultivars. Besides in this research the initial lesions of *A. solani* appeared on the earlier cultivars earlier than later cultivars (Table 2). Our results confirm previous studies about the initial appearance of *A. solani* on the different cultivars (Franc et al., 1988; Shtienberg and Fry, 1990). These researchers also related to initial lesions with resistance properties of cultivars against *A. solani* and observed that initial lesions of early blight appeared later on resistant or moderately resistant (or later-maturing) cultivars compared to susceptible (or earlier-maturing) cultivars.

According to the results of this investigation maturity properties of potato cultivars are most effective on the early blight disease and it is kept under strict control on the

later maturing cultivars. For this reason we think that later or middle late-maturity cultivars of potato (i.e., Agria) may be recommended to growers to take yield and adaptation features into consideration and in this way it possible to reduce yield losses due to early blight disease.

ÖZET

FARKLI PATATES ÇEŞİTLERİNİN ERKEN YANIKLIK HASTALIĞINA KARŞI REAKSİYONLARI

Bu çalışmada farklı olgunlaşma özelliklerine sahip bazı patates çeşitlerinin erken yanıklık hastalığına neden olan *Alternaria solani* Sorauer'ye karşı reaksiyonları belirlenmiştir. Çalışmada erkenci (Resy), orta erkenci (Marfona, Pasinler 92, ve Granola), orta geççi (Agria) ve geççi (Caspar) patates çeşitleri kullanılmıştır. Erkenci ve orta erkenci patates çeşitlerinin hastalığa daha duyarlı, orta geççi çeşitlerin ise daha dayanıklı oldukları tespit edilmiştir. Bu patates çeşitlerinden Caspar çok duyarlı, Pasinler 92 duyarlı, Granola, Marfona ve Resy orta duyarlı, Agria ise orta dayanıklı çeşitler olarak belirlenmişlerdir. Ayrıca *A. solani*'nin ilk lezyon çıkışları ile olgunlaşma arasındaki ilişkisi de araştırılmış ve ilk lezyonların erkenci ve orta erkenci çeşitlerde geççi ve orta geççi çeşitlerden daha önce ortaya çıktığı gözlenmiştir.

Anahtar Kelimeler: Patates (*Solanum tuberosum*), olgunlaşma, erken yanıklık hastalığı (*Alternaria solani*), çeşit

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Distribution of Ascochyta Blight in Some of the Important Chickpea Grown Provinces of Turkey

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ABSTRACT

Surveys were done in some of the important chickpea grown regions of Turkey in 2001 and 2002 in order to determine the distribution of chickpea blight. Disease incidences and severities were 0.83%, 0.43%, 5.65%, 3.12% and 18.43%, 12.06% in the South Eastern Anatolian provinces of Kahraman Maraş, Adıyaman and Diyarbakır respectively in 2001. The figures for Ankara and Eskişehir provinces in central Anatolia were 1.59%, 0.91% and 7.23%, 5.50% respectively in 2002. The figures were low in the Mediterranean province of Antalya, which were 1.82% and 0.96% respectively. In the eastern Aegean provinces of Denizli, Uşak, Kütahya; the disease incidence and severity were 20.86%, 14.81%; 0.68%, 0.22% and 1.02%, 0.45% in 2002 respectively. In both of the years the surveyed areas had a relatively dry growing season.

Key words: Chickpea, *Ascochyta rabiei*, ascochyta blight, survey

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most extensively grown legume crops in Turkey, the acreage and production being 622.214 ha and 548.000 tons respectively (Anonymous, 2000). Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labr. (teleomorph, *Didymella rabiei* (Kov.) v. Arx) is one of the most important diseases affecting this crop all over the world where it is grown (Nene, 1982). The disease mainly affects all the above ground parts of the plants, causing lesions mostly on stems and breaking them. It is started by infected seeds and diseased debris remained in the field. Ascochyta blight can be effectively controlled by using tolerant or resistant cultivars (Nene and Reddy, 1987) and for breeding resistance distribution of the pathotypes has to be known.

Distribution of Ascochyta blight in Turkey has been investigated several times in different places at limited areas specially in the southern provinces (Yücel and Güncü, 1991). Some authors determined infections in seeds obtained from various places and found that more than 50% of the seed samples were infected by the pathogen (Maden

et al., 1975; Maden, 1987). There has not been an extensive survey study for the last 10 years nor a collection of isolates. Three races (races 1,4 and 6) were determined from the isolates collected from various places (Dolar and Gürcan, 1992) and outbreak of the other races can be expected. For this aim collection of a wide range of isolates is needed. This work was undertaken to determine the incidence and distribution of the disease and to have a collection of isolates from the main chickpea grown regions of Turkey.

MATERIALS and METHODS

Disease surveys were done at podding stage and approximately 2.5% of the chickpea sown fields of 9 provinces which have 34.70% of the total acreage of chickpea were visited (Figure 1). Thirty plants from the four corners and the centre from every field were counted and rated by 1-9 disease scale of Singh et al (Singh et al., 1981) and number of diseased plants and average disease scale were calculated. Average disease scale was converted to percent disease severity, the scale value of nine being 100%. The mean disease severity of the field was calculated by the 5 measured values. Disease percentages of disease incidence or severity of a district, a province and a region were calculated according to the formula below;

$$\text{Disease incidence or severity of a district} = \frac{\Sigma \text{ (Percent incidence or severity of a field x area (da))}}{\text{Sum of the area of the district (da)}}$$

From every diseased field, a disease sample of 10-15 plants was collected and isolation of the pathogen was made. For isolation of the pathogen; samples were surface sterilized in 1% NaOCl for 2 minutes and aseptically removed disease portions were placed on Chickpea Seed Meal Dextrose Agar medium (CSMDA) (g/l: chickpea meal 40,

Figure 1. The surveyed provinces of Turkey in 2001 and 2002.

dextrose 20, agar 20). After sufficient incubation pure cultures were prepared and then single spore isolations were made. Every single spore isolate was transferred to Microbank vials (Pro-Lab, Diagnostics, Canada) and stored in -80 °C.

RESULTS and DISCUSSION

In 2001, most of the Anatolian plateau did not get much rain during the growing season and we only found Ascochyta blight in the South Eastern provinces of Anatolia (Table 1, Figure 2). In 2002, surveys were made in the other provinces of Anatolia. In the first year, the highest disease incidence and severity were determined in Diyarbakır (18.43 and 12.06% respectively) where mostly early sowing of chickpea was observed, and it was followed by Adıyaman (5.65 and 3.12% respectively). In a previous study, Yücel and Güncü (1991), calculated the disease incidence of K. Maraş province as 8.02%, where we found 10 times less incidence. This low rate can be attributed to the dry season and late sowing in 2001.

Table 1. Disease incidences and severities of Ascochyta blight of chickpea in some provinces in Turkey

Provinces	Acreage in the Province (da.)	Survey Areas (da)	Disease Incidence (%)	Disease Severity (%)
K. Maraş*	32.917	762	0.83	0.43
Adıyaman*	24.134	755	5.65	3.12
Diyarbakır*	37.382	1715	18.43	12.06
Ankara**	21.003	850	1.59	0.91
Eskişehir**	16.204	217	7.23	5.50
Kütahya**	23.299	416	1.02	0.45
Uşak**	22.406	318	0.68	0.22
Denizli**	12.719	219	20.86	14.81
Antalya**	25.903	478	1.82	0.96
Total	215.967	5730	7.94	5.11

* The figures belong the year 2001
** The figures belong the year 2002

In the second year, the highest disease ratio was observed in Denizli (20.86 and 14.81% respectively) and it was followed by Eskişehir (7.23 and 5.50% respectively). Very low disease incidence was observed in Kütahya, Uşak and Antalya provinces. These figures may not represent a long period of disease distribution because of the draught in the two years. Another reason for those regions having low disease occurrence is the practice of late sowing of chickpea by the farmers. Disease incidences and severities in 9 provinces in 2001 and 2002 of Turkey were 7.94 and 5.11% respectively.

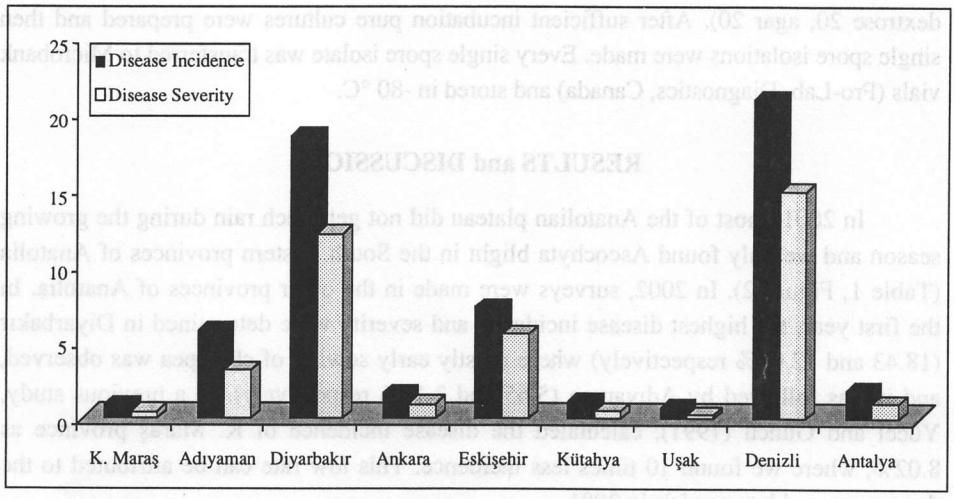

Figure 2. The values of disease incidence and severity of Ascochyta blight in 9 provinces.

The distribution of areas having various percentages of disease incidences is shown in Table 2. It can be easily seen that most of the infested areas showed up to 5% disease incidence. Very few fields had high percentage of disease incidences. There was a strong positive correlation between disease incidence and severity (r =0.99).

Hundred and forty seven single spore isolates of Ascochyta rabiei were achieved from the samples from various places (Table 3) and they have been kept in a deep freezer at -80 °C in Microbank vials.

Table 2. Distribution of areas (da) having various percentages of disease incidence of Ascochyta blight in the provinces

Provinces	Disease incidences (%)					
	0	0.1-5	6-10	11-25	26-50	51-100
K. Maraş*	582	180	0	0	0	0
Adıyaman*	472	187	2	29	0	65
Diyarbakır*	30	1240	6	20	104	315
Ankara**	532	277	20	19	2	0
Eskişehir**	132	8	17	40	20	0
Kütahya**	360	14	42	0	0	0
Uşak**	264	54	0	0	0	0
Denizli**	106	32	14	26	41	0
Antalya**	360	94	4	15	5	0
Total	2838	2086	105	149	172	380

* The figures belong the year 2001

** The figures belong the year 2002

Table 3. The origin of the isolates of *Ascochyta rabiei* kept at the Department of Plant Protection, Faculty of Agriculture, Ankara University

Survey area	Number	The other areas	Number
Adıyaman	19	Amasya	7
Ankara	37	Çorum	3
Antalya	6	Kayseri	1
Burdur	2	Kırşehir	2
Denizli	14	Sivas	1
Diyarbakır	30	Tokat	2
Eskişehir	11		
K. Maraş	5		
Kütahya	4		
Uşak	3		
Total	131		16

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ÖZET

ASCOCHYTA YANIKLIĞININ TÜRKİYE'DE ÖNEMLİ NOHUT YETİŞTİRİLEN BAZI İLLERDEKİ DAĞILIMI

Nohut Antraknozu hastalığının Türkiye'deki dağılımını belirlemek amacıyla 2001 ve 2002 yıllarında önemli nohut ekiliş alanlarında sörvey yapılmıştır. 2001 yılında Güney Doğu Anadolu illerinden Kahraman Maraş, Adıyaman ve Diyarbakır'da hastalık yaygınlığı ve şiddeti sırasıyla %0.83, %0.43; %5.65, %3.12 ve %18.43, %12.06 olarak bulunmuştur. 2002 yılında ise hastalık yaygınlığı ve şiddeti Orta Anadolu illerinden Ankara ve Eskişehir'de sırasıyla %1.59, %0.91 ve %7.23, %5.50, Akdeniz bölgesinde Antalya'da %1.82 ve %.96, Ege bölgesinde Denizli, Uşak, Kütahya'da sırasıyla %20.86, %14.81; %0.68, %0.22 ve %1.02, %0.45 olarak bulunmuştur. Her iki yılda da sörvey yapılan alanlarda sezonun kurak olmasından dolayı yüksek oranda hastalık görülmemiştir.

Anahtar Kelimeler: Nohut, *Ascochyta rabiei*, ascochyta yanıklığı, Türkiye

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First Report of *Phytophthora infestans* A2 mating type in Turkey

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Severe epidemics of late blight caused by the fungus *Phytophthora infestans* had occurred on tomato growing areas throughout Turkey in 1997, resulting in great economical losses in processing tomato industry. The isolates of *P. infestans* obtained from infected tomato plants in processing tomato fields during 2000 and 2001 growing seasons in Marmara region of Turkey were tested for mating type by growing the isolates together with known mating types (A1 and A2) and searching for oospores (sexual spores). The agar plugs with mycelium of the suspected isolates were placed in the center of each of two rye A and rye B plates. On one plate, agar plugs with mycelium of a known A1 strain (US940507) was placed on either side of the plate. On the other plate, the same thing was done with using a known A2 strain (US940480) (Peters et al., 1996).

Apparently, the A2 mating type was reported for the first time in Turkey. Of 160 isolates of *P. infestans* tested, 59 (36.9%) isolates were A2 mating type while 101 (63.1%) of them were identified as A1 mating type. In addition, metalaxyl sensitivity categories were also determined with the isolates. Addltlaonally the metalaxyl sensitivity categories of the isolates were also determined on tomato in Karacabey and Yenisehir (Bursa) in Turkey. Of these, 34 (21.2%) were metalaxyl-sensitive, 104 (65.0%) were metalaxyl intermediately-resistant, and 22 (13.8%) were metalaxyl highly-resistant.

Since metalaxyl-resistant *P. infestans* A2 mating types have been reported in many countries (Fry and Goodwin, 1997; Deahl et al., 1993), these findings explain why chemical treatments with phenylamide fungicides did not effectively control the pathogen when the climate conditions were condusive for development of disease in Turkey in recent years. The innovative systemic fungicides with different modes of action should be included for the future disease management strategies in processing tomato areas.

Key words: *Phytophthora infestans*, A2 mating type, metalaxyl resistance, tomato

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ÖZET

TÜRKİYE'DE *PHYTOPHTHORA INFESTANS* A2 MATING TİPLERİNİN İLK KAYDI

Türkiye'de 1997 yılında domates yetiştirilen alanlarda *Phytophthora infestans*'ın neden olduğu erken yanıklık hastalığı şiddetli epidemilere yol açmış ve salça sanayiinde büyük zararlara neden olmuştur. Marmara Bölgesi'nde 2000 ve 2001 yetiştirme sezonlarında salçalık domates yetiştirme alanlarında enfekteli domates bitkilerinden toplanan *P. infestans* izolatları, mating tiplerinin belirlenmesi amacıyla, mating tipleri (A1 ve A2) önceden bilinen izolatlarla birlikte geliştirerek ve oospor (eşeyli spor) gelişimleri gözlenerek test edilmiştir. R'ye A ve R'ye B petrilerinin her birinin merkezine şüpheli izolatların miselyumunu içeren agar diskleri yerleştirilmiştir. Bir petriye bilinen A1 ırkını içeren (US940507) miselyumlu agar diski yerleştirilmiştir. Diğer bir petri üzerine aynı şekilde bilinen bir A2 ırkı gelişen agar diski yerleştirilmiştir (US940480) (Peters et al., 1996).

Bu çalışma ile A2 mating tipi Türkiye'de ilk kez kaydedilmiştir. Testlenen 160 *P. infestans* izolatından 59 izolat (%36.9) A2 mating tipi olarak saptanırken, 101 izolat (%63.1) A1 mating tipi olarak saptanmıştır. Ayrıca, izolatların metalaxyl'e duyarlılık kategorileri de saptanmıştır. Türkiye'de Karacabey ve Yenişehir (Bursa)'de ilk kez metalaxyl'e dayanıklı ırklar saptanmıştır. Bunların 34'ü (%21.2) metalaxyl'e duyarlı, 104'ü (%65.0) metalaxyl'e orta derecede dayanıklı ve 22'si (%13.8) metalaxyl'e yüksek derecede dayanıklı olarak bulunmuştur.

Birçok ülkede metalaxyl'e dayanıklı *P. infestans*'ın A2 mating tiplerinin bildirilmesine (Fry and Goodwin, 1997; Deahl et al., 1993) paralel olarak, bu bulgular da Türkiye'de hastalığın gelişimi için iklim koşullarının uygun olduğu son yıllarda phenylamide'li fungisitlerin hastalığın kontrolünde neden tam olarak etkili olamadığını açıklamaktadır. Farklı etki mekanizmaları bulunan yeni sistemik fungisitlerin sanayi domatesi alanlarında gelecekteki hastalık yönetim stratejilerinde dahil edilmesi gerekmektedir.

Anahtar kelimeler: *Phytophthora infestans*, A2 mating tipi, metalaxyl dayanıklılığı, domates

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