ISSN 0378 - 8024

THE JOURNAL OF TURKISH PHYTOPATHOLOGY

PUBLISHED BY THE TURKISH PHYTOPATHOLOGICAL SOCIETY

Volume : 24 Number : 3 Semp : 1995

TURKISH PHYTOPATHOLOGICAL SOCIETY

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

EGE ÜNİVERSİTESİ BASIMEVİ
BORNOVA - İZMİR
1996

THE JOURNAL OF TURKISH PHYTOPATHOLOGY

TURKISH PHYTOPATHOLOGICAL SOCIETY

VOL. 24 SEMP NO. 3

CONTENTS Page

Effect of the Some Elicitors on the Capsidiol Amount in the Leaves of Peppers (***Capsicum annuum L.***) Having Different Sensitivities to Root Rot (***Phytophthora capsici*** Leon.)
A.S. ÜSTÜN 101

In Vitro Effect of A Bacterial β-1,3 Glucanase Expressed in ***Streptomyces lividans*** on ***Bremia lactucae***
Y. CANIHOŞ (DEDE) and J. BAYNON 115

Evaluation of Various Treatments of Inducing Resistance to Fusarium Wilt on Melons
K. ERZURUM and S. MADEN 121

Relations of Some Weed Species with Lettuce Big-vein Virus in Erzurum-Türkiye
E. DEMİRCİ, S. AÇIKGÖZ and M.T. DÖKEN 135

Occurrence of Fungal Parasites of Nematodes in the East Mediterranean Region of Türkiye
İ.H. ELEKÇİOĞLU and U. GÖZEL 139

Host Range and Pathogenicity of **Phytophthora** Isolates From Kale, Tyfon, and Alfalfa
A. KARAKAYA, F.A. GRAY and D.W. KOCH 145

J. Turk. Phytopath., Vol. 24, No.3, 101-114, 1995 ISSN 0378 - 8024

Effects of the Some Elicitors on the Capsidiol Amount ın the Leaves of Peppers (*Capsicum annuum L.*) Having Different Sensitivities to Root Rot (*Phytophthora capsici* Leon.)

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ABSTRACT

The purpose of this study is to compare the amounts of capsidiol produced in the leaves of the susceptible (I. S-35) and two resistant lines [PM 217 and PM 702 (=CM 334)] after treatment with different abiotic (0.1 M $CuSO_4$ and 0.1 M $AgNO_3$) and biotic [**Phytophthora capsici** Leon., **Alternaria alternata** (Fr.) **Keissler, Monilinia fructigena** (Aderh ex. Ruhl) Honey] elicitors. In general, in each of the three genotypes capsidiol has been found in the controls. However their amounts have been observed to be different than those of the inoculated leaves. For the 0.1 M $CuSO_4$ elicitor the highest amount of capsidiol has been found in the I. S-35, on the other hand in the case of 0.1 M $AgNO_3$ elicitor, less amount of capsidiol has been seen in PM 702 than I. S-35 and PM 217 in 4th day. The effect of pathogen fungus on each of the above three varieties has been different and in the resistant line (PM 702) more amount of capsidiol has been found compared to its controls. Although important differences have been found in all three genotypes for various treatment, it is concluted that the variations of capsidiol amount in resistant and sensitive genotypes do not play a key role in the investigation of the sensitivity.

INTRODUCTION

The production of phytoalexins is a well-documented response of plants to infection with a wide variety of microorganisms (Bailey and Mainsfield, 1982). The various lines of evidence supporting a role for these antimicrobial compounds in the resistance of plants to disease have been discussed in recent reviews (Bailey and Mansfiled, 1982; Darvill and Albersheim, 1984). Phytoalexins are produced by plants not only in response to interactions with fungi, bacteria, viruses, nematodes and other living organisms, but also following treatment with many chemicals (Watson and Brooks, 1984), irradiation by ultraviolet light (Mercier *et al.*, 1993).

Plant cell cultures are proving to be of increasing value as systems for the study of phytoalexin elicitation (Whitehead *et al.*, 1987; Threfall and Whitehead, 1988). Elici-

tation of terpenoid phytoalexins has been reported in callus cultures of **Capsicum annuum** (Brooks *et al.*, 1986) and cell suspension of **Nicotiana tabacum** (Whitehead *et al.*, 1988; Vogeli and Chappell, 1988) and **Capsicum annuum** (Whitehead *et al.*, 1988., 1990; Saimmaime and Coulomb, 1993) but neither the biochemistry nor the enzymology of these systems has yet been studied in depth.

Stoessl *et al.* (1972) first reported that pepper fruits produced antifungal sesquiterpenoid phytoalexin, capsidiol in response to infection by several pathogenic and non-pathogenic fungi. The relation between capsidiol concentration and speed of fungal invasion in stems of pepper cultivars susceptible or resistant to **Phytophthora capsici** L. has been assessed by Molot *et al.* (1981).

The present study was undertaken to compare resistant (PM 217 and PM 702) (Abak and Pıtrat, 1981; Pochard *et al.*, 1986; Üstün, 1993) and susceptible (İnce-Sivri-35) (Abak and Pıtrat, 1981; Üstün, 1993) cultivar of pepper to **P. capsici** with regard to the accumulation of capsidiol in the leaves during the stages of 7-8 leaves in seedlings inoculated with biotic and abiotic elicitors, after 2, 3, 4th day.

MATERIALS AND METHODS

Resistant seeds of peppers were obtained from Prof. Dr. Mas (Station d'Amelioration des Plantes Maraicheres Domanie St. Maurice, Monfavet FRANCE), susceptible seed of pepper (İnce-Sivri-35) was obtanied from Antalya (Antalya Seracılık Araştırma Enstitüsü, Türkiye). Each of three fungus isolates was obtained from Prof. Dr. Maden (A. Ü. Z. F. Bitki Koruma B., Ankara, Türkiye). For inoculation, a zoospor suspension of **Phytophthora capsici** Leon. and conidial suspensions of other two fungi (**Monilinia fructigena** and **Alternaria alternata**) were grown on V_8 juice agar at 25°C. Then zoospore suspension ($1x10^5$ zoospor per milliliter) and condial suspensions ($1x10^5$ condia suspensions ($1x10^5$ conidial per milliliter) and conidial suspensions ($1x10^5$ conidia per milliliter) were prepared as previously described (Üstün, 1993; Üstün and Ercoşkun, 1994).

Plants were sown in a plastic pot (750x160x125 mm) with a mixture of steam-sterilized loam soil: sand and peat (1:1:1). They were watered daily and fertilized four times with a dilute solution of % 0.15 N:P:K (20:20:20) until 7-8 leaves. At the 7-8 leaves stages, the seedlings were uprooted and their roots were washed in tap water and plants were transfered to liquid medium culture (Abak, 1982; Üstün, 1993). Five days later, when plants had recovered from uprooting stress, their roots were soaked during one hour in suspension of biotic and abiotic elicitors (0.1 M. $CuSO_4$, only 10 min) and control roots were soaked in sterile distile water (in 1-2 drop Tween 20) (Üstün, 1991). After inoculation, plants were then replaced in liquid culture. Plant culture, inoculations and incibations were performed in a growth chamber at 20-22°C, 65% humidity, 14 hours light. Leaf samples were taken 2, 3 and 4 days after inoculation.

In order to compare their reactions to **Phytophthora capsici** Leon. (Antalya), 0.1 ml zoospor suspension (10^5 zoospor/ml) was applied to the end of a decapitated plant stem at the seven-eight leaves stage, which was then covered with an aluminium foil cap (Üstün, 1991). The disease has been evaluated according to 1-3 scales Reifschneider *et al.* (1986).

Extraction and Estimation of Capsidiol

Frozen leaf tissues which were cut into small pieces, were weighed, extracted with cold ethanol (40%) and extracted with ethanol first by steeping overnight at 4°C and then were extracted twice more and extracted with ether as described previously (Jones *et al.*, 1975 and Stoessl *et al.*, 1972) Comparable areas of healty tissue were treated similarly. Capsidiol concentrations were determined by gas-liquid chromatography (GLC), essentially as described previously (Molot *et al.*, 1981) except that a gas chromatograph (Varian 3700) equipped with a 5 ft x 1/8 in Steel column filled with Gas Chrom Q (60-80 mesh) coated with 10% OV-1 (Üstün and Ercoşkun, 1994). Results are based on three determinations.

RESULTS

In this research with the help of micropipet we have applied a suspension of 10^5 zoospor per ml of ***P. capsici*** Leon. (Antalya) isolate onto decapitated plant stem of three different pepper genotypes showing different degrees of resistance to **P. capsici** Leon. during their 7-8 leaves seedling stage in order to look for the effects of the disease caused by this isolate. In the I. S-35 genotype the disease developed very rapidly and on the tenth day all of the 40 seedling that have been inoculated have dried up and entered the third scales. The X^2 analysis done on the same day since all of the I. S-35 have died and given results tremendously different when compared to the other two genotypes was left out of the statistical analysis (Figure 1). On the tenth day while the average scale number in PM 217 line was 1.350, the PM 702 number was 1.050 ($X^2 = 11.47$, $p < 0.01$). As time advanced the symptoms of disease in both genotypes increased and on the thirtieth day we have averagely found the scale values of 1.850 in PM 217, 1.250 in PM 702 ($X^2 = 12.77$, $p < 0.01$), (Figure 1).

The Capsidiol Contents of the Leaves (μ gr/gr fresh weight)

All the data obtained for the capsidiol amounts in different times in the leaf tissues of I.S-35, PM 217, PM 702 inoculated with different elicitors are as shown in Table 1. In the leaves of three pepper genotypes during the seedling stage 7-8 leaves, after the controlling and inoculation processes of five different elicitors, the capsidiol amounts on the leaf tissues taken at different times (2, 3 and 4 days later) have been evaluated in accordance with multifactored variance analysis. According to Duncan test the differences between genotype x elicitors, genotype x time, elicitors x time and genotype x elicitor x time interactions have been found to be statistically considerable (Table 1, $p < 0.01$).

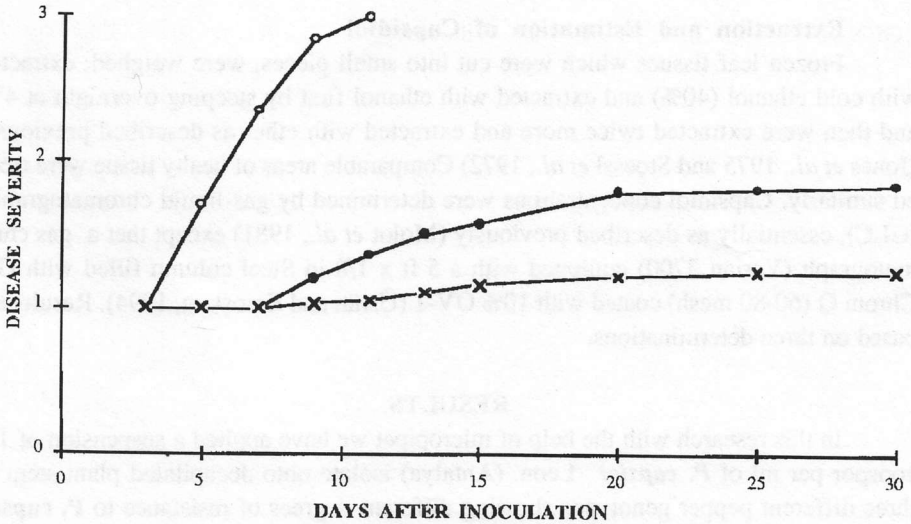

Figure 1. Disease severity curves for three pepper cultivars, I.S-35 (susceptible o—o , PM 217 (resistant o—o) and PM 702 (= CM 334) (the most resistant x—— x), inoculated with 10^5 zoospor per milliliter of **Phytophthora capsici** by using the stem apex was decapitated at the seven-eight leaves stage. Disease severity rating based on a 1-3 scale (Reifschneider *et al.*, 1986).

The Comparasion Among the Capsidiol Amounts in the Leaves Two Days After the Inoculation

When the capsidiol amounts of the leaf control samples of all three genotypes were compared the capsidiol amounts on the second day followed the order of PM 702 < PM 217 < I. S-35 ($p < 0.01$, Table 1, Figure 2). On the second day of the abiotic elicitor 0.1 M $CuSO_4$ treatment the least amount of capsidiol on the leaves was on the PM 702 line; PM 217 and I.S-35 genotypes followed it ($p < 0.01$). When each of the three genotype controls were compared (Table 1, Figure 2), they were found to be almost equal in PM 217 and lesser in PM 702 ($p < 0.01$) than the capsidiol in the control, more in I.S-35 ($p < 0.01$) than the capsidiol in the control. When abiotic elicitor 0.1 M $AgNO_3$ is used the capsidiol amounts show an increase in the order of PM 702 < PM 217 < I.S-35 (Table 1, Figure 2). Although we have found that the capsidiol amount was

Table 1. Effets of the some elicitors on the capsidiol amount in the leaves of peppers (**Capsium annuum** L.) having different sensitivities to root rot (**Phytophthora capsici** L.)

GENOTOPY	ELICITIOR	CAPSIDIOL (m gr/gr f. w)		
		2 th Day	3 th Day	4 nd Day
		$\bar{X} \pm S\bar{X}$	$\bar{X} \pm S\bar{X}$	$\bar{X} \pm S\bar{X}$
Thin-Long-35 (Susceptible)	Kontrol	58.90±0.45	38.50±2.43	76.30±3.93
	0.1 M $CuSO_4$	66.57±1.26	245.20±9.80	134.73±6.49
	0.1 M $AgNO_3$	63.97±7.80	42.90±4.90	97.90±1.20
	A. alternata	55.73±2.02	29.67±3.84	60.10±0.70
	M. fructigena	28.93±1.30	66.63±2.20	69.30±0.80
	P. capsici	23.18±0.25	41.10±1.00	97.95±5.30
P M 217 (Resistant)	Kontrol	43.50±2.80	49.67±0.22	47.60±0.22
	0.1 M $CuSO_4$	46.30±2.00	92.17±1.12	25.67±0.49
	0.1 M $AgNO_3$	51.50±0.20	78.93±0.60	75.13±0.65
	A. alternata	42.50±2.70	88.77±4.75	74.85±1.25
	M. fructigena	38.77±2.04	28.06±0.91	61.14±3.19
	P. capsici	42.20±1.21	38.37±0.82	58.64±0.24
P M 702 (= CM 334) (Resistant)	Kontrol	33.03±0.71	25.36±0.78	89.20±1.30
	0.1 M $CuSO_4$	15.37±0.71	53.80±1.93	90.77±1.41
	0.1 M $AgNO_3$	34.33±1.29	48.40±0.50	53.40±0.40
	A. alternata	65.30±0.60	37.80±1.26	28.63±1.70
	M. fructigena	67.73±1.40	35.33±0.40	56.34±6.20
	P. capsici	60.60±1..80	83.30±2.20	109.26±4.31

* Values are the means of three replicates

more in I.S-35 in 0.1 M $AgNO_3$ treatment when compared to the control treatment the difference between the two was considerable according to Duncan test at the ratio of $p < 0.05$. On the PM 217 line, on the other hand, the difference between 0.1 M $AgNO_3$ and control treatments was considerable at the ratio of $p < 0.01$; there was no statistically considerable difference in PM 702.

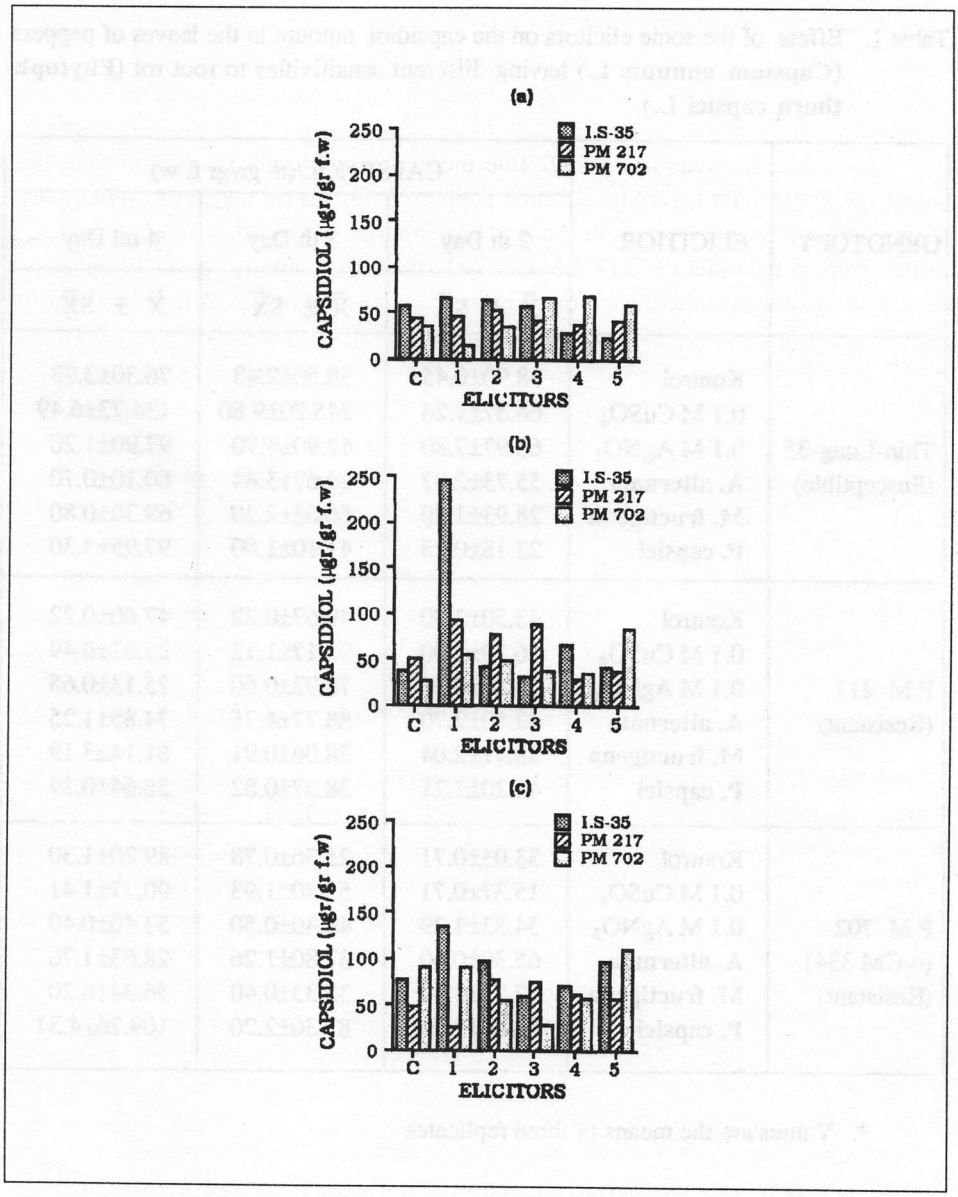

Figure 2. Effect of the some elicitors on the capsidiol amount in the leaves of peppers (**Capsicum annuum** L.) having different sensitivities to root rot (**Phytophthora capsici** Leon.) : C: Control, 1: 0.1 M $CuSo_4$, 2: 0.1 M $AgNO_3$, 3: **A. alternata**, 4: **M. fructigena**, 5: **P. capsici**, (a) Inoculated after 2th day, (b) 3th day and (3) 4nd day.

In ***A. alternata*** biotic elicitor treatment, on the second day the least capsidiol amount was found to be in PM 217, I.S-35 < PM702 followed this ($p < 0.01$, Table 1, Fig. 2). When each of the genotypes were compared with the controls the differences among the control and ***A. alternata*** samples in I.S-35 and PM 217 were not statistically significant. Whereas in PM 702 line there was much more capsidiol than the control ($p< 0.01$). The leaves of all three pepper genotypes on which ***M. fructigena*** was applied, were compared on the second day in terms of capsidiol amounts and the findings were in the order PM 217, I.S-35 < 702 ($p < 0.01$, Table 1, Fig. 2). When compared with the controls there was found to be less capsidiol in I.S-35 than the control and more capsidiol in PM 702 than the control ($p < 0.01$). It is not statistically important that PM 217 had a slight difference with the control. The leaves of all three pepper genotypes onto which ***P. capsici*** biotic elicitor was applied have been compared on the second day in terms of capsidiol amounts and the order was I.S-35 < PM 217 < PM 702 ($p < 0.01$, Table 1, Figure 2). When control and ***P. capsici*** treatments were compared in I.S-35 the capsidiol amount went down 50%, in PM 217 it stayed almost the same and in PM 702 it went up 50%. According to Duncan test during the control and ***P. capsici*** treatments of PM 702 and I.S-35 treatments the capsidiol amounts in the leaves on the second day showed a difference with $p < 0.01$. Whereas in PM 217 the difference was not found to be statistically significant.

Comparison Among the Capsidiol Contents in the Leaves Three Days After the Inoculation

When all three genotypes were compared on the third day in terms of the capsidiol content in their leaves PM 702 line controls less capsidiol than the others, I.S-35 and PM 217 followed it ($p < 0.01$, Table 1, Figure 2). In the 0.1 M $CuSO_4$ treatment each of the three genotypes was different from one another at the rate of $p < 0.01$. When the controls were compared the capsidiol content of the leaves of PM 702, PM 217 were almost twice as high on the third day and I.S-35 was even higher than they were ($p < 0.01$, Table 1, Figure 2). When leaves of all three genotypes onto which 0.1 M $AgNO_3$ was applied were compared the capsidiol amounts found were in the order of I.S-35 < PM 702 < PM 217 ($p < 0.01$, Table 1, Figure 2). When controls and 0.1 M $AgNO_3$ elicitor of each genotype were compared I.S-35 did not show a significant difference while PM 217 and PM 702 showed an increase almost twice as high ($p < 0.01$, Table 1, Figure 2).

All three pepper genotypes onto which ***A. alternata*** was were compared on the third day in terms of the capsidiol amounts on their leaves and the result was I.S-35 < PM 702 < PM 217 ($p < 0.01$, Table 1, Figure 2). When the controls were compared in I.S-35 the capsidiol amount was less than the control while in PM 217 and PM 702 lines it was more ($p < 0.01$, Table 1, Figure 2). All three genotypes on which biotic elicitor ***M. fructigena*** was applied the capsidiol amounts on the third day were PM 217 < PM 702 < I.S-35 ($p < 0.01$, Table 1, Figure 2). When compared with the controls I.S-35

showed an increase, PM 702 showed slight increase and PM 217 showed decrease ($p < 0.01$, Figure 2). The capsidiol amounts of the leaves of all three genotypes that went through ***P. capsici*** treatment were taken on the third day and the most capsidiol was found to be in PM 702, while the least was in PM 217. The differences were found more important among genotypes other than PM 217 and I.S-35 ($p < 0.01$, Table 1, Figure 2). When controls and ***P. capsici*** treatments of control and ***P. capsici*** elicitor did not show a significant difference, PM 217 line showed decrease, PM 702 showed increase ($p < 0.01$, Table 1, Figure 2).

Comparison of Capsidiol Contents of the Leaves Four Days After the Inoculation

When the capsidiol contents of all three genotypes and elicitors were compared in terms of the possible correlations, the capsidiol amounts in controls were respectively PM 217 < I.S-35 < PM 702 (p < Table 1, Figure 2). In 0.1 M $CuSO_4$ elicitor the least capsidiol was in PM 217 line while the most was in the leaves of I. S 35 ($p < 0.01$, Table 1, Figure 2). When the controls were compared I.S-35 showed more capsidiol than the control while PM 217 showed a fifty percent decrease and this was found to be statistically significant with $p < 0.01$. In PM 702 the increase was not significant (Table 1, Figure 2). In abiotic elicitor 0.1 M $AgNO_3$ treatment the capsidiol amounts changed in the order of PM 702 < PM 217 < I.S-35 ($p < 0.01$, Table 1, Figure 2). When controls were compared PM 217 showed over 50% increase, I.S-35 showed increase while PM 702 showed increase while PM 702 showed decrease ($p < 0.01$, Figure 2).

In biotic elicitor **A. alternata** treatment the change in the capsidiol amounts was PM 702 < I.S-35 < PM 217 ($p < 0.01$, Table 1, Figure 2). When compared with the controls, on the forth day, there was decrease in PM 702 line and in I.S-35 and increase in PM 217 line ($p < 0.01$, Figure 2). In **M. fructigena** treatment the capsidiol amounts of the leaves of all three genotypes on the forth day were examined and the least amount was found to be in PM 702,while the most was in I.S-35 genotype (Table 1). When the capsidiol amounts of PM 217 and PM 702 line leaves were compared, the difference was significant with $p < 0.05$. The differences among other types were significant at $p < 0.01$. When the **M. fructigena** and control treatments of all three genotypes were compared there was decrease in I.S-35 and PM 702 and increase in PM 217 ($p < 0.01$, Table 1, Figure 2). In ***P. capsici*** treatment the capsidiol amounts of the leaves of all three genotypes on the forth day were respectively PM 217 < I.S-35 < PM 702 ($p < 0.01$, Table 1, Figure 2). When the controls were compared the capsidiol amounts on the leaves of all three genotypes showed an increase ($p < 0.01$, Figure 2).

DISCUSSION

According to the findings of several researches the inoculum technique chosen, the age of the seedling, the manner of inoculum, ecological conditions, the difference

among ***P. capsici*** strains, the pepper line used and factors such as these presented us with a wide range of variables in our researches on the resistance of pepper to root rot disease (Kimble and Grogan, 1960; Karahan and Maden, 1974; Barksdale *et al.*, 1984; Barış *et al.*, 1986; Reifschneider *et al.*, 1986; Kim *et al.*, 1989; Palloix, 1986; Palloix *et al.*, 1985, 1988; Ortega *et al.*, 1985, 1986; Pochard *et al.*, 1983, 1986).

When ***P. capsici*** isolate resistance test results of three different pepper genotypes were compared with different stem edge inoculation test results we saw that I.S-35 showed a highly sensitive reaction while PM 217 and PM 702 lines showed quite resistant genotype characteristics (Figure 1).

The development of the disease was much faster in I.S-35 and after the fourth day (Figure 1) rapidly spreading stem necrosis founding in the leaves resulted in the death of all the samples inoculated with ***P. capsici*** Leon, (Antalya) ($1x10^5$ zoospor/ml) within 10 days.

In the PM 217 and 702 lines on the other hand in a few samples death takes place after the 10th and the 13th day and in the end of 30 days 30% of PM 217 line, 10% of PM 702 line die up (Figure 1). Thus we can claim that PM 702 line is more resistant than PM 217.

We observed the same situation in inoculated root of pepper seedling. When pepper seedling on which ***P. capsici*** Leon. ($1x10^5$ zoospor/ml) suspension was applied through the root were examined on the forth day I.S-35 leaves were quite pale. The other two genotypes showed less paleness and browning around the root and necrosis areas than I.S-35 although they both showed serious browning around the root. Yet the influence was found to be more than the stem edge inoculation.

We have put forth that along with the PM 217 line, advised previously for the improvement programs because of its line resistance to ***P. capsici*** isolates isolated from Turkey, PM 702 line as well can be used in improvement programs (Üstün, 1993).

Pochard *et al.* (1983) put forth that when isolates of ***P. capsici*** Leon. were inoculated to CM 334 genotyp at 22°C and 32°C, it showed more resistance than PM 217 and Fiyo 636 genotypes.

The same researchers claimed that the concept of resistance could be defined in three terms. The capacity to accept fungus, the capacity of fungus development inside and stability. With respect to these characteristics PM 217 line proved stable to all isolates and high heat conditions.

The first inoculation techniques used to examine the existence of capsidiol in the leaves and the stem were done by the applying of different elicitors to leaves in drops. Leaves inoculated with ***A. alternata*** and ***M. fructicola*** were compared and A. alternata inoculated ones showed more capsidiol. The different inoculation technique we have used has showed that our findings (Table 1) prove that the increase depend on the genotype.

Jones *et al.* (1975) and Molot *et al.* (1981, 1984) discussed that capsidiol was an element preventing the growth and development of fungus yet they also put forth that there might be other factors behind the death of hpyhae in infected cells. The relation between the inhibition of fungus development and the capsidiol amount in the plant tissue was not very clear in their opinion.

According to Ward (1976), very high values were obtained exceeding the concentration required for total inhibition of fungi **in vitro** by several orders of magnitude. There were capsidiol in fruits as much as in the leaves (Ward and Stoessl, 1974).

Molot *et al.* (1980) in another research, treated the leaves with filtrates attained from ***P. capsici*** mycelia and found out that the protection depended on the inoculum dose, the age of the filtrate, the time spent in filtrate. They could not found any relation between the resistance developed and the capsidiol accumulation.

Molot and Mas (1985) found out that the results taken from the leaves of the seedlings that were turn out from their stems and were dipped into several elicitors were better than the cotiledon inoculation, and the stimulated resistance lasted for a long time. The seedlings dipped in the elicitors could control the harms of **P. capsici** or **P. infestans.**

Molot *et al.* (1985a)started to examine the of capsidiol in genetically controlled resistance and the resistance gained from a previous infection. According to these researchers the resistant lines could not be characterized with PM 217 and CM 334 sensitive type Yolo Wonder capsidiol amount. They claimed that this could depend both on not yet understood biochemical mechanisms and a possible second role of capsidiol. According to Pochard *et al.* (1983), the three componients of the resistance described in "receptivity", "inducibility" and "stability" were easily identified, each variety being remarkable for a particular point. In resistant lines like PM 217, L 29 and CM 334 were compared with the sensitivity and resistance to high and low temperatures the receptivity of the host changes (Pochard *et al.*, 1983; Molot and Mas,. 1985). The same researches mention the existence of at least two mechanisms related to resistance while the changes in resistance can be explained by infected stems (Molot and Mas, 1985).

With respect to the root inoculation technique we have used, the capsidiol amounts of the leaves in 7-8 leaved seedling period have been look for and in all three genotypes capsidiol was found. According to the results of control experiments other abiotic and biotic elicitor experiment controls showed results different for each genotype. The capsidiol amounts in the leaves of all three genotypes were found to be almost as high as the amounts we found in fruit samples (Üstün and Ercoşkun, 1994). Our findings, in this sense, support the findings of Ward (1976).

On the forth day all three genotypes inoculated with 0.1 M $CuSO_4$ elicitor were compared and I.S-35 leaves were found to be very pale. The other two, PM 217 and PM 207 were found to be slightly pale as well yet this was much less than the sensitive type. The 0.1 M $CuSO_4$ concentration used is quite a toxic concentration for the plant and it

affects the seedling right away. For this reason the test period for this was not 1 hour as in the other elicitors but 10 minutes. The objective here was to see the effect of this element on the capsidiol amount. In I.S-35's 0.1 M $CuSO_4$ test the harm and the capsidiol accumulation were found to be the most (Table 1). This extremity in capsidiol amount could depend on the fact that capsidiol could not turn into other elements because of the harms the plant cells went through. In resistant types the capsidiol amount was less.

Stoessl *et al.* (1977) put forth that capsidiol was a secondary metabolite substance; though the balance between its formation and use cannot be explained the over doses can be related to its use. The stem-eliciting inoculation technique we have used, different from Ward's technique (1976), have been effective on the formation of capsidiol on the leaves. Different inoculation techniques used during the research makes it difficult to interpet the findings.

We agree with Pochard *et al.* (1983) and Molot *et al.* (1985) on the opinion that capsidiol cannot be effective in resistance on its own. The gas liquid cromatographic analysis we have done shows capsidiol peaks and small peaks in each elicitor forming up with the combination of at least four or six substances. The separation, the identification and the resistance role of these substances are other focuses of research. Yet another research topic is the possibility of each of the ingredients of the elicitors affecting the cell wall to move loosely and play an active role as a fitoalexin elicitor. The existence of enzymes in this process is undeniable. The research in this subject has not yet helped us to evaluate the phenomena from a biochemical perspective.

ÖZET

BAZI UYARICILARIN, KÖK BOĞAZI YANIKLIĞINA (Phytophthora capsici L.) DUYARLILIĞI FARKLI BİBERLERİN (Capsicum annuum) YAPRAKLARINDA KAPSİDİOL MİKTARINA ETKİSİ

Bu çalışmanın amacı farklı abiyotik (0.1 M $CuSO_4$ ve 0.1 M $AgNO_3$) ve biyotik [**Phytophthora capsici** Leon., **Alternaria alternata** (Fr.) Keissler, **Monilinia fructigena** (Aderh ex. Ruhl Honey)] uyarıcılar ile inokule edilen duyarlı İnce-Sivri-35 ve iki dayanıklı hat PM 217 ve PM 702 (= CM 334)'nın yapraklarında oluşan kapsidiol miktarlarını karşılaştırmaktır. Genel olarak her üç genotipin, kontrollerinde de kapsidiol bulunmuştur. Mamafih, miktarlarının inokule edilen fidelerinkinden farklı olduğu ortaya çıkarılmıştır. 0.1 M $CuSO_4$ uyarıcısı İnce-Sivri-35'de en fazla kapsidiol birikimine neden olmuştur. Diğer taraftan, 0.1 M $AgNO_3$ uyarıcısında PM 702'de İnce-Sivri-35 ve PM 217'den dördüncü günde daha az kapsidiol olduğu tesbit edilmiştir. Yukarıda bahsedilen her üç genotipte de patojen fungusun etkisi farklıdır ve dayanıklı hat (PM 702) da kontrolleri ile karşılaştırıldığında daha fazla kapsidiol bulunmuştur. Her ne kadar uygulamalar arasında üç genotipte de önemli farklılıklar bulunmuşsa da duyarlı dayanıklı çeşit ayırımında kapsidiol miktarlarındaki değişimlerin dayanıklılığı incelemede tek bir anahtar rol oynamadığı bulunmuştur.

ACKNOWLEDGEMENT

Research was supported by the University of Ankara, Türkiye. (P. No: 88 25 00 10)

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J. Turk. Phytopath., Vol. 24, No.3, 115-120, 1995 ISSN 0378 - 8024

In Vitro Effect of A Bacterial β - 1,3 Glucanase Expressed in *Streptomyces lividans* on *Bremia lactucae*

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ABSTRACT

*The aim of this study was to carry out a critical appraisal of the use of bacterial β - 1,3- glucanase against lettuce downy mildew pathogen, **Bremia lactucae**. The enzyme was found to be effective on the germ-tubes of **B. lactucae** spores in vitro. The effects observed on the germ-tubes was similar to those of lyticase.*

INTRODUCTION

The enzyme β- 1,3-glucanase is produced by many microorganisms and has the lytic effect on glucans such as laminarin and yeast cell walls (Sivan and Chet, 1989). Plants also produce such enzymes in response to the presence of potential pathogens and it therefore presumed that they play an important role in plant defence mechanism against invading fungal organisms the cell wall of which are made of mainly either β- 1,3-glucan with some β- 1,6-linkages, chitin or cellulose. β- 1,3-glucanase might be effective in directly inhibiting fungal growth or by producing short chain β- 1,3-glucans that act as elicitors of further defence mechanisms.

The regulation and activities of chitinase and β- 1,3-glucanase of ***Trichoderma harzianum***, which is an effective biocontrol agent against fungal pathogens, have been extensively studied. It has been found that the fungus released β- 1,3-glucanase and chitinase into the medium when it was grown in liquid cultures containing laminarin, chitin or fungal cell walls as sole sources suggesting that they could be the effective ingredients during mycoparasitic interactions (Sivan and Chet, 1989; Ulhoe and Peberdy, 1991).

Chitinases obtained from ***Serratia marcescens***, ***Streptomyces griseus*** and ***Pseudomonas stutzeri*** were tested for antifungal activity. The chitinase gene from ***S. marcescencs***, a gram-negative soil bacterium, was cloned and expressed in ***Pseudomonas spp.*** The ***Pseudomonas spp.*** inhibited ***Fusarium oxysporum*** f. sp. ***redolens*** germ-tubes and reduced disease of radish caused by the same fungus (Sundheim *et al.*, 1988). This chitinase was also expressed in ***E. coli*** and when tested on ***Sclerotium rolfsii*** and ***Rhizoctonia solani*** in cotton under greenhouse condition, it was found to be effective as bio-control agent (Shapire *et al.*, 1989).

Doi *et al.* (1973) analysed the two glucanase (I and II) in the liquid culture of a

Artrobacter sp. strain YCWD3. They found that the enzyme was stable at 30°C and for 100 min but lost activity when incubated above 60°C and at pH values from 3 to 8, the optimum pH was found to be 5.5-6.5.

Since bacterial hydrolases showed encouraging antifungal activity *in vitro* and some *in vivo*, we were interested in the effects of microbial β-1,3-glucanase on the growth of ***Bremia lactucae***, which is a downy mildew pathogen of lettuce.

MATERIALS AND METHODS

Streptomyces strain

Isolates of the ***Streptomyces lividans*** strain (TK-24) that was transformed either with plasmid pIJ690 containing the β-1,3-glucanase gene in the ***Streptomyces*** expression vector or with the vector alone, here were kindly provided by Dr. Maureen Bibb (John Innes Institute/England). The β-1,3-glucanase gene was cloned from ***Arthrobacter*** sp. strain YCWD3 by Doi and Doi (1986).

S. lividans strains were grown at 30 °C on R2YE solid medium or YEME liquid medium. The solid medium containing (g per litre of distilled water): sucrose, 103; K_2SO_4, 0.25; $MgCl_2$. $6H_2O$, 10.12; glucose, 10; Difco casaminaoacids, 0.1; Difco bacto agar, 22 was prepared and autoclaved, At the time of use, the following filter sterilized solutions were added; 8 ml of 0.5% KH_2PO_4; 64 ml of 3.38% $CaC1_2$; 12 ml of 20% L-Proline; 80 ml of TES buffer (pH 7.2); 4 ml of 1N NaOH. YEME medium containing (g per litre of distilled water): Difco yeast extract, 3; Difco bacto peptone, 5; oxoid malt extract, 3; glucose, 10; sucrose, 340 was autoclaved and 2 ml of filter sterilized 2.5M $MgCI_2$. $6H_2O$: was added. Thiostrepton was included at the concentrations of 50 µg/ml and 15µg/ml in R2YE and YEME, respectively, to select for the presence of the plasmid in the **S. *lividans*** cultures.

Purification of the β-1,3-glucanase

S. lividans strains were grown in the liquid culture for 4 days with vigorous shaking. The culture was then centrifuged at 20.000 g for 1 hour at 4 °C. The supernatant was transferred into a clean flask and 697 mg ammonium sulphate/ml supernatant was added and stirred at 4 °C for 2 hours. The mixture was spun at 12.000 rpm for 15 min at 4 °C and the supernatant was dialysed against glucanase assay buffer at 4 °C overnight. The next day, the mixture was passed through a disposable desalting column and then used in a glucanase assay and pathogenity test.

Glucanase assay

β-1,3-glucanase activity was assayed by measuring the rate of reducing sugar production using laminarin (SIGMA) as a substrate. Assays were carried out by the method of Abeles and Forrence (1970) with some modifications. Two sets of microfuge tubes (2/sample) containing 100 µl assay buffer were prepared, and 20 µl of the supernatant was added to the one set and labelled (+), another one blank (-). Then 100 µl of laminarin solution (40 mg/ml) was added to all tubes, mixed and incubated 30 °C for 2

hours. The reaction was stopped by adding dinitrosalicylic reagent and heating for 5 minutes. The absorbance was measured at 540 nm wavelength. Standards of enzyme substrate were included.

***In vitro* application of the β - 1,3-glucanase to *B. lactucae* spores**

Spore suspension of ***B. lactucae*** was prepared according to the method of Maclean and Tomerup (1979). The spores were germinated in sterile distilled water on glass slides which had been cleaned with acetone and rinsed with sterile distilled water for 3 hours. After 3 hours incubation in a 15 °C incubator, 50 μl of a solution of partially purified β- 1,3-glucanase was added onto 20 μl of spore suspension. Purified supernatant from ***S. lividans*** that contained the vector only, boiled purifired β- 1,3-glucanase, different concentrations of lyticase, and sterile distilled water were included as controls. Lyticase solution, that was orginally from ***Arthrobacter luteus***, supplied from Sigma (L-8012) was prepared in sterile distilled water. After overnight incubation with the test solutions, they were examined under light microscope.

RESULTS

Glucanase activity

Glucanase activity was detectable when the β- 1,3-glucanase gene was expressed in a ***Stretomyces lividans*** secretion system. Cultures of ***S. lividans*** were grown in YEME medium, containing thiostrepton, shaking at 30 °C for 4 days. The purified samples were used in assays to monitor their ability to release reducing sugars from the long chain β-glucan laminarin. As it is seen in Table 1, the enzyme activity in the culture of ***Streptomyces*** harboring plasmid containing the glucanase gene was 10-150 times higher than in cultures of both controls, ***Streptomyces*** culture only and ***Streptomyces*** harboring vector plasmid without the β-1,3-glucanase gene.

Table 1. β-1,3-glucanase activity (the assay was repeated three times)

Samples	Glucanase activity (Lyticase units/ml culture)
Control (Streptomyces culture)	23.5
Control (Strep. harbouring vector plasmid)	423.5
Streptomyces harbouring plasmid containing the glucanase gene	3648

Effects of the β-1,3-glucanase on *B. lactucae* in vitro

Effects of different treatments on spore germination are shown in Table 2. In the presence of lyticase (supplied by Sigma) at a concentration of 0.5 unit µl water, germ-tubes of **B. lactucae** were shorter compare to control spores in distilled water and they also had swollen tips. The germ-tubes subsequently started lysing from the tips. However, when half concentration (0.25 unit/µl) was applied, about 50% of spores germinated, as in the water control, and some of them produced relatively short germ-tubes with swollen tips (Table 2). Spores in the β-1,3-glucanase solution purifired from the ***Streptomyces*** culture reacted in a similar manner to lyticase treatment (0.5 unit/µl) the germination tubes were quite short with swollen tips, also lysis of germ tubes was observed. However, spores germinated in the solution extracted from *Streptomyces* culture, which harboured the vector construct only, differed from lyticase and water controls and from the β-1,3-glucanase treatment. Germ-tubes were longer than in the lyticase and glucanase treatment and thinner than in the water control., Germ tubes from each treatment were clasified according to their shape. Four types were observed; type 1, short germ tubes with swollen tips; type 2, short germ tubes with no swollen tubes; type 3, relatively long but thinner than in water control; type 4, as in water control.

Table 2. Effect of different treatments on the germ tubes of ***B. lactuae***.

Treatments	Type1 (%)	Type2 (%)	Type3 (%)	Type4 (%)
Lyticase (0.5 unit/µl)	80	20	0	0
Lyticase (0.25 unit/µl)	20	30	0	0
Vector	0	20	80	0
Denatured β-1,3-glucanase	0	0	50	50
Purified β-1,3-glucanase	40	60	0	0
Water	0	0	0	100

DISCUSSION

This work shows that ***S. lividans*** is a very effective host for the secretion of naturally secreted proteins from ***Arthrobacter*** and such secreted products can be present at very high concentrations in the culture supernatant.

There is considerable direct and indirect evidence for the role of β-1,3-glucanases in defending plants against fungal infections (Linthorst, 1991; Broglie and Broglie,

1993). Plant β-1,3-glucanases have been purified and tested for their *in vitro* antifungal properties. The general conclusion from *in vitro* tests is that β-1,3-glucanases is effective against glucan containing fungi (Mauch *et al.*, 1988; Roberts and Selitrennkoff, 1988).

To assess the *in vitro* antifungal activity of the β-1,3-glucanases from ***Arthrobacter*** sp., the enzyme was partially purified from liquid culture of ***S. lividans*** expressing the ***Arthrobacter*** β-1,3-glucanases. The *in vitro* assay could be done only on germ tubes of ***B. lactucae***, as the fungus is an obligate parasite it was not possible to use hyphae or other structures to observe inhibition zones. Glucanase activity in β-1,3-glucanase transformed *Streptomyces* culture was about 9 fold higher than vector or untransformed controls. In the lyticase control it was clear that the germ tubes were shorter and had more swollen tips than water control. Lysis of the germ tubes was also observed in the lyticase treatment. Similar results were obtained when partially purified β-1,3-gluca-nases was applied; i.e. short germ tubes, swollen tips and lysis. However, in the vector control, germ tubes were not as short as glucanase applied ones but had swollen tips and were thinner than the water control. Therefore, the reaction was not similar to either of the other controls or the glucanase treatment. This is most probably due to the content of the culture and also the secretion of other substances from the *Streptomyces*. Therefore, it is unlikely to produce the same reaction as either controls.

Only in lyticase and glucanase, treatments was the growth of germ tubes inhibited due to swelling and lysis. In fungi, wall extension is restricted to the hyphal tip and is thought to represent a balance between synthesis and degradation of the main wall components, chitin and glucan. The newly synthesised glucan at the tip is in a nascent stage and therefore particularly susceptible to the β-1,3-glucanase or chitinase. No lysis was seen in fungal spores suggessting that their walls may be protected from glucanase digestion.

In conclusion this study showed that endo-acting β-1,3-glucanase of *Athrobacter* has direct effect on the growth of ***B. lactucae***. Therefore, β-1,3-glucanase can be used to engineer resistance to fungal pathogens in lettuce in future studies. Some studies showed that overexpression of β-1,3-glucanase did not increase the resistance of trangenic plants against fungal attack (Neuhaus *et al.*, 1991). The fungus has probably adapted to the defence mechanisms of plants, therefore it might be a good idea to express hydrolases from unrelated species in plants. The new activities represented by these hydrolases may be effective against the pathogenic fungi so that the new hydrolases cannot be overcome by the invading fungus.

ÖZET

BAKTERİ ORİJİNLİ β-1,3-GLUCANASE ENZİMİNİN MARULDA MİLDİYÖ ETMENİ *BREMİA LACTUCAE* ÜZERİNE ETKİSİ

Bu çalışma bakteriyel β-1,3-glucanase enziminin marul mildiyö etmeni ***B. lactucae***'nın gelişimi üzerine etkisini araştırmak amacıyla yapılmıştır. Enzimin *in vitro*'da ***B. lactucae*** sporlarının çimlenme tüpleri üzerinde etkili olduğu bulunmuştur. Mikroskobik incelemelerden bu etkinin lytikaz enziminin etkisine benzer olduğu gözlenmiştir.

Bu çalışma sonunda eğer bu enzimi kodlayan gen uygun bir vektör içine klonlanıp bitkiye aktarılırsa bitkinin hücre duvarında glucan içeren funguslara karşı dayanıklık kazanacağı konusunda önemli bilgiler elde edilmiştir.

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J. Turk. Phytopath., Vol. 24, No.3, 121-134, 1995 ISSN 0378 - 8024

Evaluation of Various Treatments of Inducing Resistance to Fusarium Wilt on Melons

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ABSTRACT

Applicability of induced resistance by trifluralin and microorganisms against Fusarium Wilt of melon on Yuva cv. caused by race 1.2 was evaluated. Application of trifluralin at the rate of 30 and 60 µm/ml to seeds and 0.5 and 1 µ g/g to soil decreased the disease incidence 21.5, 12.9, 39.8 and 52.0 % respectivelly after 36 days of inoculation. Soil treatment was more effective compared to seed treatment in the case of inhibition of disease. Seed applications of the two isolates of nonpathogen **Fusarium oxysporum** [(18) and (9)] and two formae speciales of it (f. sp. **lycopersici** and f. sp. **niveum**) yielded 47.3, 38.1, 29.3 and 32.1 % reduction in disease incidence while root applications of them gave 45.3, 40.1, 32.8 and 31.3 % reduction. Localized leaf infections of the first true leaf of melon by **Collectotrichum lagenarium** did not protect the plants against the disease while treatment of melon seed with culture filtrates of it gave 29.5 % disease protection. When seed treatment of nonpathogenic **F. oxysporum** isolate 18 was combined with application of trifluralin at the rate of 60 µg/ml to seeds and 1 µg/g to soil decreased the disease incidence 41.0 and 51.0 % respectivelly after 36 days of inoculation.

INTRODUCTION

Melon production in Ankara is 184 247 tons and this makes 12.28 % of the total production of Türkiye, that is 1 500 000 tons (Anonymous, 1992).

One of the most serious disease of this crop is Fusarium Wilth caused by **Fusarium oxysporum** Schlecht emend. Synd & Hans. f. sp. **melonis** Leach & Currence.

Many farmers have almost given up growing melon because of the increasing intensity of the disease, unlikely effective chemical control, infeasible rotation, presence of more than one race of the pathogen in the same place and insufficient resistant cultivars to all the races.

Four races of the pathogen; 0,1, 2, 1.2, exist. Fantino and Zengin (1974), isolated race 1.2 from wilted plants showing intensive root rot in Eastern Thrace. In the Eagean Region, Yıldız (1977) recovered tree races of the pathogen, race 1 being the most common (57 %) following race 1.2 (35 %) and race 0 (6 %). Yücel (1992) also obtained races 0, 1 and 1.2 in the Mediterrenean Region.

Most of the cultivars grown in Türkiye are susceptible to all races of the pathogen. Some local cultivars grown in a limited extent were found to be resistant against some races. Yıldız (1977), screened 48 melon cultivars against the race 1.2 and did not find any resistant one. Resistant cultivars haven't been released so far in the world also.

Induced resistance in plants has been shown by several workers to be a common response to nonpathogenic bacteria, viruses, fungi and treatment by some chemicals (Kuc, 1982; 1991). Resistance to Fusarium Wilt of melon is induced by the application of trifluralin to the soil (Grinstein et al., 1984; Cohen et al., 1986). Besides this, various experiments were designed to clarify if decrease of wilt in muskmelons induced by simultaneous or prior inoculation with less virulent strains of the pathogen or incompatible races of **Fusarium oxysporum** is due to competition with the pathogen or to induction of a resistance mechanism in the plant(Mas, 1967; Maraite, 1970; Meyer and Maraite, 1971; Mas and Molot, 1974; Molot et al., 1979; Maraite 1982).

In this research we intended to find out the applicability of some chemicals and microorganisms in control of Fusarium Wilt of melons. Trifluralin as a chemical, nonpathogen melon isolate 9 and 18 of **Fusarium oxysporum**, and two formae speciales (ff. sp. ***lycopersici*** and ***niveum***), and anthracnose pathogen **Collectotrichum lagenarium** as microorganisms were tested. In addition combination of the effective treatments was also employed.

MATERIALS and METHODS

We used an isolate of race 1.2, obtained from Polatlı, Ankara and chosen as the most virulent one on both cultivars Yuva and Charentais. Yuva which is the most widely grown and appreciated cultivar in Central Anatolia was used during our study. This cultivar was also found to be susceptible to all races of the pathogen (Yıldız, 1977).

Inoculation of the Pathogen

Fusarium oxysporum f. sp. ***melonis*** was almost always inoculated to the seedling at first true leaf emergence state by dipping the roots to the 10^6 conidia /ml suspension of it (Latin and Snell, 1986; Zink and Gubler, 1986). In the case of **Colletotrichum lagenarium** leaf treatment, inoculation of **Fusarium oxysporum** f. sp. **melonis** was done at two true leaf stage.

Melon seedlings were grown in plastic trays containing a soil mixture of 95.6 % washed river sand, 3.8 % clay and 0.6 % organic matter. Inoculated seedlings were maintained in a soil mixture of garden soil, well fermented manure and river sand (1:1:2) at 26±2°C day and 20±2°C night temperature, 50-60 % relative humidity and alternated 10 hours darkness and 14 hours light (11 000 lüx intensity).

Inoculation of the Other Fungi

Nonpathogenic isolates of **F. oxysporum** 18 and 9 were isolated from diseased melon plants, grown in naturally infected fields. **Fusarium oxysporum** f. sp. **lycopersici** (Sacc.) Snyder and Hansen was obtained from Dr. Seral YÜCEL (Plant Protection Research Institute, Adana, Türkiye). **Fusarium oxysporum** f. sp. **niveum** (E. F. Sm.) Snyder and Hansen (0667TX-HC3-13B) was obtained from Dr. Charlie BILES (Texas A & University, TX77843, USA).

Nonpathogenic Fusaria were inoculated either to seeds or to seedlings. Seed inoculation was done by dipping the seeds in suspension of 10^7 conidia/ml of the respective fungi. Seedling inoculation of the nonpathogenes was performed the same as the pathogen at the concentration of 10^7 conidia/ml.

Colletotrichum lagenarium (Pass) Ell. & Halst. was obtained from Dr. Joseph KUC (University of Kentucky, Lexington, USA). Prior inoculation of **C. lagenarium** was done with thirty 5 µl drops of conidial suspansion 10^6 conidia/ml on the first true leaf (Gessler and Kuc, 1982).

Trifluralin Treatment

Trifluralin was applied in two ways, to the soil and to the seed. Soil treatment was achieved by mixing the chemical at the rates of 0.5 - 1 µg a.i./g soil (Cohen et al., 1986; Grinstein et al., 1976; 1984). Seed treatment was performed by dipping in the emulsion at the rates of 30 and 60 µg/ml water for 1 hour.

Treatment with Culture Filtrate of Colletotrichum lagenarium

Culture filtrate of **C. lagenarium** was prepared by modifying the method of Mas and Molot (1974) for **F. oxysporum** f. sp. **melonis**. Culture filtrate of **C. lagenarium** was applied to seeds by dipping for 1 hour.

Combination of the Treatments

Combinations of effective treatments; seed treatment of non-pathogenic **F. oxysporum** isolate 18 + trifluralin at the rate of 60 µg/ml. and seed treatment of nonpathogenic **F. oxysporum** isolate 18 + trifluralin soil application at the rate of 1 µg/g were tested. These treatments were achieved in the same way as the individual treatments.

Evaluation of the Disease

Disease ratios were calculated by using 0-3 scale in which 0 : no disease symptoms; 1 : yellowing of leaves; 2: wilting ; 3 : complete death (Zink and Gubler, 1986). Disease progress was observed from 12th day after challenge inoculation till 36th day and disease ratios were calculated for every four days. Statistical analysis of each treatment was done on the 36th day on percent disease. Comparison of the treatments was done on percent disease inhibition values. The differance was evaluated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Effect of Trifluralin Treatments

Trifluralin seed treatment reduced disease ratio to some extent. Even though this reduction was statistically different from the control, it was not sufficient (Table 1).

Table 1. Disease ratios obtained 36 days after **Fusarium oxysporum** f. sp. **melonis** inoculation following seed treatment with two different rates of trifluralin.

Concentration of trifluralin (µg / ml water)	Disease ratio *(%)
o	68.8 a
30	47.3 c
60	56.0 b

* Numbers following by the same letter are not significantly different (P= 0.05)

Trifluralin soil treatment significantly reduced percent disease and there was no differance between the two rates (Table 2).

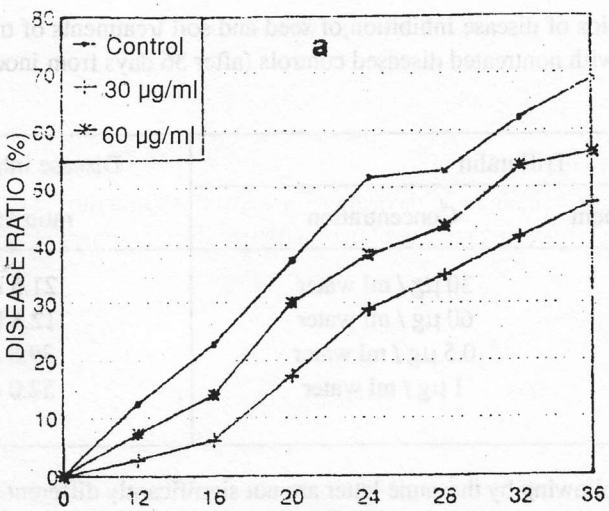
Table 2. Disease ratios obtained 36 days after **Fusarium oxysporum** f. sp. **melonis** inoculation following soil application with two different rates of trifluralin.

Concentration of trifluralin (µg / ml water)	Disease ratio *(%)
o	77.2 a
0.5	37.3 b
1	25.2 b

* Numbers following by the same letter are not significantly different (P= 0.05)

Effect of these treatments on disease progress was observed from 12th day after challenge inoculation till 36th day. There was a delay in the disease appearence and a reduction in disease percentage (Fig. 1a, 1b).

Application of trifluralin at the rate of 30 and 60 mg/ml to seeds and 0.5 and 1 mg/g to soil decreased the disease incidence 21.5, 12.9, 39.8, and 52.0 % respectivelly 36 days after inoculation, being the soil treatment markedly more effective than the seed treatment (Table 3).



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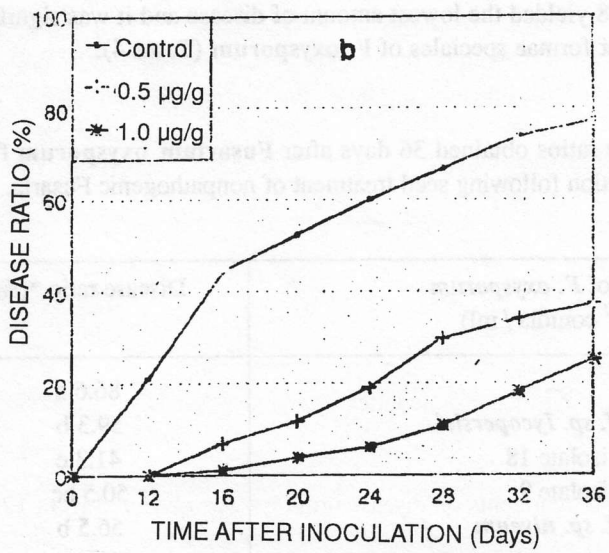

b

Fig.1. Effect on the disease progress of various treatments;
a. trifluralin seed treatment, b. trifluralin soil application.

Table 3. The ratios of disease inhibition of seed and soil treatments of trifluralin compared with nontreated diseased controls (after 36 days from inoculation)

Trifluralin		Disease inhibition
Type of treatment	Concentration	ratio *(%)
seed	30 µg / ml water	21.5 b
seed	60 µg / ml water	12.8 b
soil	0.5 µg / ml water	39.8 a
soil	1 µg / ml water	52.0 a

* Numbers following by the same letter are not significantly different (P= 0.05)

Effect of Nonpathogenic Fusaria

Seed treatment with nonpathogenic Fusaria also affected the disease and **F. oxysporum** isolate 18 yielded the lowest amount of disease and it was significantly different than the other formae speciales of **F. oxysporum** (Table 4).

Table 4. Disease ratios obtained 36 days after **Fusarium oxysporum** f. sp. **melonis** inoculation following seed treatment of nonpathogenic Fusaria.

Isolates of ***F. oxysporum*** (10^7 conidia / ml)	Disease ratio *(%)
--	86.6 a
F. oxysporum f. sp. lycopersici	59.3 b
F. oxysporum isolate 18	41.3 c
F. oxysporum isolate 9	50.5 bc
F. oxysporum f. sp. niveum	56.5 b

* Numbers following by the same letter are not significantly different (P= 0.05)

Nonpathogenic Fusaria applied to the seedlings also reduced the disease. Nonpathogenic ***F. oxysporum*** isolates reduced the disease much more (Table 5).

Table 5. Disease ratios obtained 36 days after **Fusarium oxysporum** f. sp. **melonis** inoculation following seedling treatment of nonpathogenic Fusaria.

Isolates of *F. oxysporum* (10^7 conidia / ml)	Disease ratio *(%)
--	81.1 a
F. oxysporum f. sp. lycopersici	48.3 b
F. oxysporum isolate 18	35.8 c
F. oxysporum isolate 9	41.0 c
F. oxysporum f. sp. niveum	49.8 b

* Numbers following by the same letter are not significantly different (P= 0.05)

Application of nonpathogenic Fusaria to seed and seedling delayed the disease appearence and caused a decrease in disease percentage (Fig 1c, 1d).

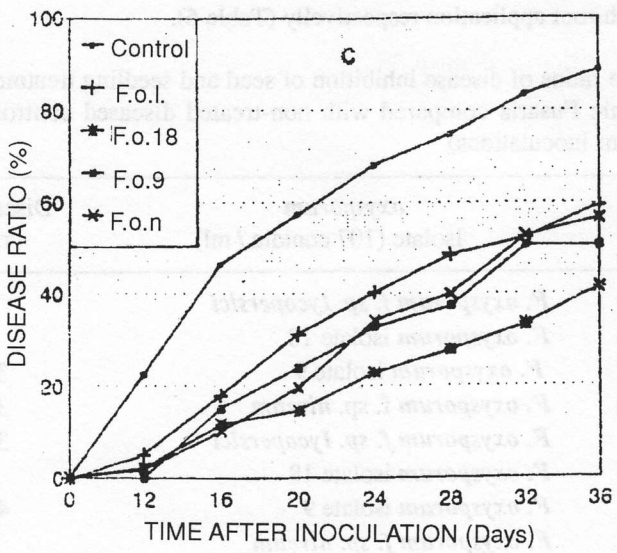

Fig. 1. Effect on the disease progress of various treatments;
c. Nonpathogenic Fusaria seed treatment,

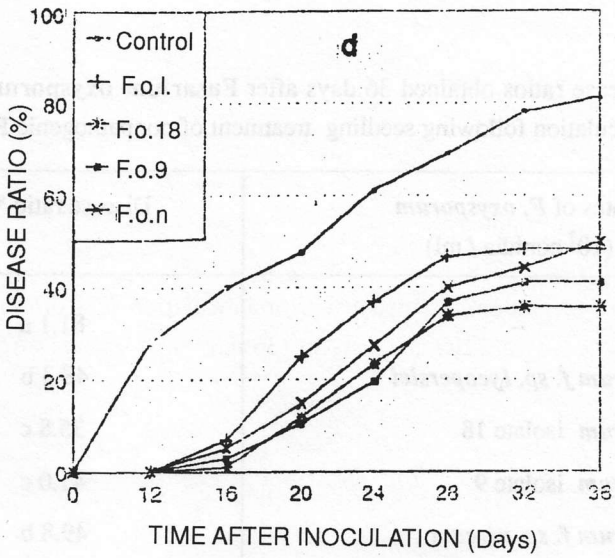

Fig. 1. Effect on the disease progress of various treatments; d. Nonpathogenic Fusaria seedling treatment,

The nonpathogenic ***F. oxysporum*** (isolate 18 and 9) and two formae speciales (ff. sp. ***lycopersici*** and ***niveum***) of ***F. oxysporum*** gave 47.3, 38.1, 29.3 and 32.1 % reduction in disease incidence with seed application and 45.3, 40.1, 32.8 and 31.3 % reduction with root application respectivelly (Table 6).

Table 6. The ratios of disease inhibition of seed and seedling treatments of nonpathogenic Fusaria compared with non-treated diseased controls (after 36 days from inoculations)

Fusarium Treatment	***oxysporum*** Isolate (107 conidia / ml)	Disease inhibition ratio* (%)
seed	***F. oxysporum f. sp. lycopersici***	29.3 c
seed	***F. oxysporum*** isolate 18	47.3 a
seed	***F. oxysporum*** isolate 9	38.1 abc
seed	***F. oxysporum*** f. sp. ***niveum***	32.1 abc
seedling	***F. oxysporum f. sp. lycopersici***	32.8 abc
seedling	***F. oxysporum*** isolate 18	45.3 ab
seedling	***F. oxysporum*** isolate 9	40.1 abc
seedling	***F. oxysporum f. sp. niveum***	31.3 bc

* Numbers following by the same letter are not significantly different (P=0.05)

Disease inhibition ratios of various treatments of nonpathogenic Fusaria were not statistically significant.

Effect of *Colletotrichum lagenarium*

Prior inoculation of ***C. lagenarium*** at two intervals did not cause any reduction in percent disease (Table 7).

Table 7. Disease ratios obtained by prior inoculation of ***Colletotrichum lagenarium*** at two different intervals (36 days after **Fusarium oxysporum** f. sp. **melonis** inoculation)

Time between two inoculations (days)	Prior inoculation (10^6 conidia/ml)	Disease ratio *(%)
3	--	91.2 a
3	***C. lagenarium***	86.2 a
7	--	84.5 ab
7	***C. lagenarium***	73.0 ab

* Numbers following by the same letter are not significantly different (P= 0.05)

Culture filtrate of ***C. lagenarium*** also reduced the percentage of the disease compared to the controls (Table 8).

Table 8. Disease ratios obtained 36 days after ***Fusarium oxysporum*** f. sp. ***melonis*** inoculation following ***Colletotrichum lagenarium*** culture filtrate application of seeds.

Treatment	Disease ratio *(%)
--	84.5
C. lagenarium culture filtrate	55.0

* Numbers following by the same letter are not significantly different (P= 0.05)

Application of culture filtrate of ***C. lagenarium*** delayed the disease appearence and caused a decrease in disease percentage (Fig. 1e)

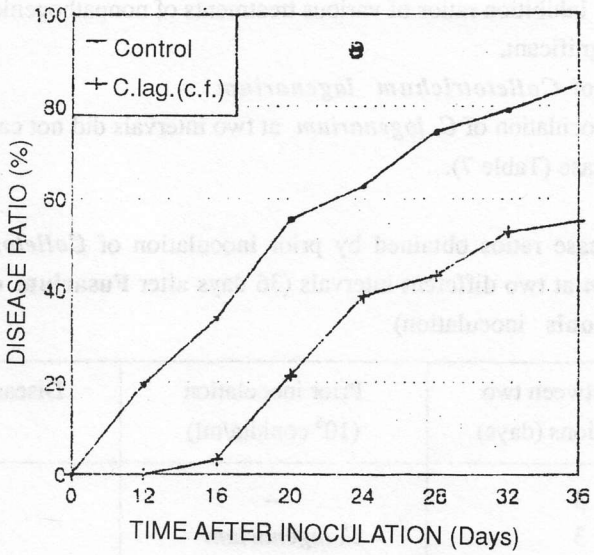

Fig. 1. Effect on the disease progress of various treatments;
e. ***C. lagenarium*** culture filtrate application of seeds,

Effect of Combination of Effective Treatments

The combination of trifluralin and nonpathogenic ***F. oxysporum*** isolate 18 also yielded comparitivelly low disease incidence but the two combinations did not differ (Table 9).

Table 9. Disease ratios obtained 36 days after **Fusarium oxysporum** f. sp. **melonis** inoculation following combination of trifluralin and ***F. oxysporum*** isolate 18.

Treatment	Disease ratio *(%)
--	80 a
Trifluralin (1µg/g) (soil) + ***F. oxysporum*** 18 (10^7 c/ ml) (seed)	29 b
Trifluralin (60 µg / ml) (seed) + ***F. oxysporum*** 18 (10^7 c/ ml) (seed)	39 b

* Numbers following by the same letter are not significantly different (P= 0.05)

Disease progress of the combined treatments also showed a different trend. Combination of trifluralin soil application and *F. oxysporum* isolate 18 seed treatment delayed the disease appearence (Fig. 1f).

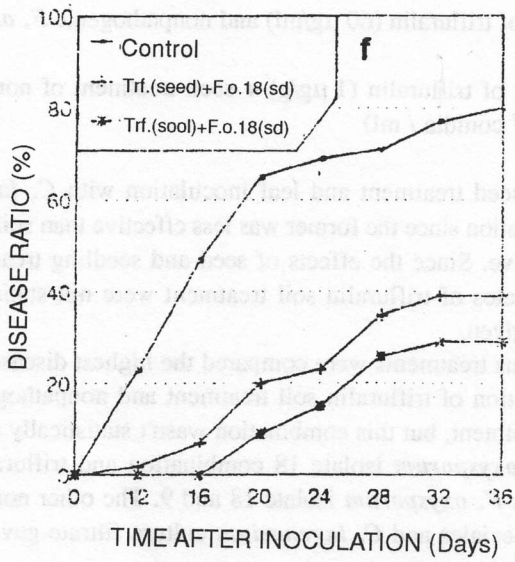

Fig. 1. Effect on the disease progress of various treatments;
f. Combination of trifluralin and *F. oxysporum* isolate 18.

Comparision of Effective Treatments

Results of the comparision of the effective treatments are summarized in Table 10.

Table 10. The ratios of disease inhibition of effective treatments compared with non-treated diseased controls (after 36 days from inoculation)

Treatment	Disease inhibition ratio *(%)
Trifluralin[1]	46.0 a
F. oxysporum isolate 18[2]	46.3 a
F. oxysporum isolate 9[2]	39.2 ab
F. oxysporum f. sp. niveum[2]	31.7 b
F. oxysporum f. sp. Iycopersici[2]	31.1 b
Colletotrichum lagenarium[3]	29.5 b
Trifluralin (seed) + *F. oxysporum* 18[4]	41.0 ab
Trifluralin (soil) + *F. oxysporum* 18[5]	51.0 a

* Numbers following by the same letter are not significantly different (P= 0.05).

1 Soil applications of trifluralin (average of 0.5-1 µg / g)
2 Average of seed and seedling application of all *F. oxysporum* streins
3 Seed treatment with culture filtrate of *C. lagenarium*
4 Seed treatment of trifluralin (60 µg/ml) and nonpathogenic *F. oxysporum* 18 (10^7 c/ml)
5 Soil application of trifluralin (1 µg/g) + seed treatment of nonpathogenic *F. oxysporum* 18 (10^7 conidia / ml)

Trifluralin seed treatment and leaf inoculation with *C. lagenarium* were not taken into consideration since the former was less effective than soil application and the latter was ineffective. Since the effects of seed and seedling treatments with *F. oxysporum* and two rates of trifluralin soil treatment were not statistically different the mean values were given.

When various treatments were compared the highest disease protection was obtained by combination of trifluralin soil treatment and nonpathogenic *F. oxysporum* isolate 18 seed treatment, but this combination wasn't statistically different from trifluralin seed and *F. oxysporum* isolate 18 combination and trifluralin soil application and nonpathogenic *F. oxysporum* isolate 18 and 9. The other nonpathogenic *F. oxysporum* formae speciales and *C. lagenarium* culture filtrate gave the lowest disease inhibition.

The results obtained under artifical conditions might be different under natural conditions. Under natural conditions, due to the higher light intensity and nutrients availability plants should be less susceptible. The lower inoculum concentration of the pathogen in nature could also favor the efficacy of the treatments. So, a higher disease reduction might be expected in naturally infestated areas.

Trifluralin soil application alone, or its combination with seed treatment with nonpathogenic *F. oxysporum* is easier to apply and should be tested in field conditions.

Seed treatment alone may face with difficulties due to attacks of other pathogens so seed treatment with fungicidies should also be considered.

Nonpathogenic isolates of *F. oxysporum* can differ in their effects, so more efficient ones can be found. Penetration and colonization of nonpathogenic Fusaria applied as seed treatment may be affected in the field conditions. Seedling inoculation is impractical for field conditions.

ÖZET

KAVUNLARDA FUSARIUM SOLGUNLUĞU'NA KARŞI BAĞIŞIKLIK KAZANDIRAN DEĞİŞİK UYGULAMALARIN DEĞERLENDİRİLMESİ

Kavunlarda, *Fusarium oxysporum* f. sp. *melonis*"in 1.2 nolu ırkının neden olduğu solgunluğa karşı savaşımda, trifluralin ve mikroorganizmalarla bağışıklık ka-

zandırma yönteminin Yuva kavun çeşidinde uygulanabilirliği araştırılmıştır. Trifluralinin 30 ve 60 µg/ml dozlarında tohum uygulaması ve 0.5 ve 1 µg/g dozlarında toprak uygulaması, hastalığı inokulasyondan 36 gün sonra sırasıyla %21.5, %12.9, %39.8 ve %52.0 oranında azaltılmıştır. Toprak uygulaması, hastalığı önlemede tohum uygulamasından daha etkili olmuştur.

Kavunda patojen olmayan ***Fusarium oxysporum***' un iki izolat (18 ve 9) ve iki "formae speciales" i (f. sp. ***lycopersici*** ve f. sp. ***niveum***) ile tohum uygulaması sonucunda hastalık sırasıyla %47.3, %38.1, %29.3 ve %32.1 oranında önlenirken, aynı izolatların kök uygulaması da solgunluk gelişimini sırasıyla %45.3, %40.1, %32.8 ve %31.3 oranında önlemiştir. İlk gerçek yapraklarda sınırlı ***Colletotrichum lagenarium*** enfeksiyonu bitkileri solgunluğa karşı korumazken, aynı fungusun kültür filtratının tohuma uygulanması hastalık çıkışında %29.5 oranında azalmaya neden olmuştur. Patojen olmayan ***F. oxysporum***' un 18 nolu izolatının tohum uygulaması, trifluralinin 60 µg/ml dozunda tohum ve 1 µg/g dozunda toprak uygulaması ile kombine edildiğinde, hastalık çıkışında inokulasyondan 36 gün sonra sırasıyla %41.0 ve %51.0 oranında azalma görülmüştür.

ACKNOWLEDGEMENT

The Authors are thankful to the University of Ankara for supporting.

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J. Turk. Phytopath., Vol. 24, No.3, 135-137, 1995 ISSN 0378 - 8024

Relations of Some Weed Species with Lettuce Big-vein Virus in Erzurum-Türkiye*

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ABSTRACT

Carduus nutans, Cichorium intybus, Taraxacum officinale were determined as the host plants of both lettuce big-vein virus and its vector **Olpidium brassicae** while **Cirsium arvense** was only the host of **O. brassicae**

INTRODUCTION

Big-vein disease of lettuce (**Lactuva sativa** L.; Asteraceae) which is prevalent in many countries as well as in Türkiye is considered as one of the serious problems of lettuces. The disease was detected in Erzurum (Döken et al., 1993) besides Aegean Region where it was initially symptomatologically determined in Türkiye by Fidan and Türkoğlu (1988). The lettuce big-vein virus (LBVV) is known to be transmitted by grafting (Campbell et al., 1961), **Olpidium brassicae** (Wor.) Dang. (Campbell and Grogan, 1963) and mechanical inoculation (Huijberts et al., 1990).

Some plant species other than lettuce were found as the hosts of LBVV. As a matter of fact big-vein symptoms have been reported from endive (**Cichorium endivia** L.) in Netherlands (Van Hoof, 1959). In California the pathogen was recovered from sowthistle (**Sonchus oleraceus** L.), showing big-vein symptoms, chicory (**Cichorium intybus** L.) and dandelion (**Taraxacum officinale** Weber.) (Campbell, 1965).

* This study is a part of the project (TBAG-1120) supported by Turkish Scientific and Technical Research Council.

MATERIALS AND METHODS

Among the Asteraceae family five weed species **Carduus nutans** L., **C. intybus, Cirsium arvense** (L.) Scop., **T. officinale** and **Xeranthemum annuum** L. which are widely distributed in Erzurum, were used in this study. Their seeds were collected from the cultivated areas in September 1993. The weed species were inoculated at seeding by placing root residues of lettuces infected by LBVV and its vector **O. brassicae**. Plants were raised in a growth chamber adjusted to 20°C and 16 hours daylength illuminated with 20.000 lux fluorescent lamps. Uninoculated healthy plants were used as control. Six weeks after the inoculation, root washings of each plant of the species were examined under microscope for the presence of **O. brassicae** zoospores.

The leaves were collected separately from each weed species then they were cooled to -20°C and grounded in 0.03 M phosphate buffer (pH 7.0) containing sodium disulphite (2.5 g/l), sodium diethyldithiocarbamate (DIECA, 5 g/l) and activated charcoal (70 g/l) without thawing. The inoculum was mechanically applied with a cheese cloth by gently wiping the carborandum dusted leaves of **Chenopodium quinoa** Willd. which proved as an excellent source of virus inoculum (Huijberts et al., 1990). After the inoculation, the leaves were rinsed with tap water and the plants were kept in growth chamber for 7 days. Then the leaves were harvested, cooled to -20°C, the crude sap obtained as mentioned above and mechanically transmitted to **Chenopodium giganteum** D. Don. Syn. **Chenopodium amaranticolor** (Coste and Reyn.) Coste and Reyn. where the virus caused chlorotic local lesions (Huijberts et al., 1990). The inoculated plants were examined daily for local lesions.

RESULTS AND DISCUSSION

Among the five inoculated weed species except **X. annuum** zoospores of **O. brassicae** were observed in the root washings of **C. nutans, C. intybus, C. arvense** and **T. officinale**. The presence of zoospores meant that they acted as hosts of **O. brassicae** and from them **C. arvense** and **C. nutans** were the first record as being the hosts of **O. brassicae**.

Seven days after the inoculation, **C. quinoa** exhibited rather large local lesions. However they usually had a diffuse border and were less pronounced due to both low virus concentration and low stability of the virus in the sap of the weed species. ***C. amaranticolor*** as a local lesion assay plant expressed tiny chlorotic local lesions on sapinoculated leaves starting 7 days after the inoculation done by using the sap obtained from ***C. quinoa*** which is a suitable host for propagation and maintenance of LBVV (Huijberts et al., 1990). The inoculum obtained from ***C. quinoa*** which was separately inoculated with the sap of ***C. nutans, C. intybus*** and ***T. officinale*** which were inoculated at seeding by ***O. brassicae*** caused local lesions on ***C. amaranticolor***. Among these threeweed species ***C. intybus*** and ***T. officinale*** were also determined

as the symptomless host of LBVV by Campbell (1965). However with the present study ***C. nutans*** was also recorded as a new symptomless artifical host.

The overwintering of LBVV and its vector ***O. brassicae*** in infected lettuce debris in soil under the severe winter conditions (Döken et al., 1994) inspired that LBVV could be also transmitted to the following vegetation period by these weed species and being perennial plants these weeds could be reservoir hosts of LBVV in Erzurum Region. So the results indicate that both climatical factors and natural vegetation in Erzurum seems to be suitable for occurrence of LBVV.

ÖZET

ERZURUM'DA MARUL İRİ DAMAR VİRUSUNUN BAZI YABANCI OT TÜRLERİ İLE İLİŞKİLERİ

Carduus nutans, Cichorium intybus, Taraxacum officinale türleri marul iri damar virusunun ve **Olpidium brassicae**'nın, **Cirsium arvense** ise sadece **O. brassicae**'nin konukçusu olarak saptanmıştır.

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J. Turk. Phytopath., Vol. 24, No.3, 139-143, 1995 ISSN 0378 - 8024

Occurrence of Fungal Parasites of Nematodes in the East Mediterranean Region of Türkiye

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ABSTRACT

In a survey of plant parasitic nematodes in the East Mediterranean region of Turkey some nematode species were found to be infected by fungal parasites, ***Catenaria anguillulae*** *Sorokin and* ***Monacrosporium ellisosporum*** *(Grove). These parasites were frequently found in samples suggesting a significant role as antagonists of nematods. This is first report on the occurrence of these fungal parasites from Türkiye.*

INTRODUCTION

Most of the control methods used against plant parasitic nematodes are either not economic and successful or cause problems in environment. The use of some nematicides was limited because of health hazards to production workers or because of their unacceptable residue levels in ground water. Other control methods, such as crop rotation and resistant crop varieties have some limitations for their use (Kerry, 1987).

There are a lot of natural enemies attaking plant parasitic nematodes in soil. These antagonists, promising for the biological control of nematodes are: plant-health promoting rhizobacteria; obligate bacterial parasites; fungal egg pathogens/parasites; predacious or trapping fungi; endoparasitic fungi; fungal pathogen/parasites of females; and endomycorrhizal fungi (Sikora, 1992a). The use of soil antagonistic potential to control of plant parasitic nematodes is an attempt for the integrated crop production (Sikora, 1992a). There are to main types of biological control: i) repeated application of antagonists to soil and ii) regulation the effectiveness of biological agents in soil ecosystem (Kerry, 1987; Sikora, 1992b).

The use of biological control bases on knowledge of antagonits in soil ecosystem. There are very few reports on natural enemies of plant parasitic nematodes in the East Mediterranean region as well as from other regions in Turkey. Enneli *et al.* (1994) reported on two strains of ***Fusarium oxysporum*** Schlecht and ***Ulocladium tuberculatum*** Simmons. Elekçioğlu (1995) gave some information on bacteria of Pasteuria

penetrans group infected plant parasitic nematodes. He found 14 nematode species infected by this bacteria including important plant parasites as: ***Tylenchulus semipenetrans*** Cobb, ***Meloidogyne incognita*** (Kofoid et White) Chitwood, ***M. javanica*** (Treub) Chitwood, ***M. arenaria*** (Nela) Chitwood, ***Pratylenchus penetrans*** (Cobb) Filipjev et Schuurmans Stekhoven and ***P. thornei*** Sher et Allen.

Because there is little information on antagonists of nematodes in the East Mediterranean region, this work aimed to determine the occurrence and host range of fungal parasites of nematodes.

MATERIAL AND METHODS

In a survey of plant parasitic nematodes in East Mediterranean region, soil and root samples were collected from various crop plants since 1993. Details on location and crop of sampled sides are given by Elekçioğlu *et al.* (1994). Nematodes were obtained from soil by using a modified Baermann funnel and centrifugal flotation technique. Nematodes infected by fungi were separated, counted, mounted and identified to species level if possible. The fungal parasites were identified after Barron (1977) and Dowe (1987).

RESULTS AND DISCUSION

Two fungal parasites were identified: a predatory fungi, ***Monacrosporium ellipsosporum*** (Grove) (Fig. 1A) and an endoparasitic fungi, ***Catenaria anguillulae*** Sorokin (Fig. 1B).

M. ellipsosporum was found on ***Aphelenchus avenae***, ***Dorylaimus*** species and on some saprophytic nematode species. This fungus was found frequently on nematodes collected in different soils from whole region since 1993. In this fungus, the nematode is staked by adhesived knobs. Only the knob itselfs is coated with adhesive. This type of trapping device is common in the higher fungi (Deuteromycetes, Basidiomycetes) (Barron, 1977). Nematodes adhere to 1-3 celled knob and eventually are caught by several knobs with subsequent penetration and destruction (Barron, 1977; Dowe, 1987).

C. anguillulae belongs to the class Chytridiomycetes and the order Blastocladiales Lagenidiales. This parasite was found on ***Longidorus*** sp., ***Aphelenchus*** spp., ***Dorylaimus*** spp. and some saprophytic nematode species. ***C. anguillulae*** was observed in many samples collected in a survey since 1993. In a peanut soil about 20-50% of ***Longidorus*** sp. were parasitized by ***C. anguillulae***. Different larval stages as well as adult females of ***Longidorus*** were parasitized by this fungus. Sayre and Keeley (1969) noted that adult nematodes are more readily parasitized than larval stages and suggested that the larger and better developed body openings of adults allow the zoospores to accumulate and penetrate.

C. anguillulae is the most commonly occurring of all the endoparasites attacking nematodes and has many hosts from eleven genera (Esser and Ridings, 1973). Barron (1977) recorded this species in over 90% of all samples processed.

In addition, two unidentified fungus were found on ***Dorylaimus*** sp. (Fig. 1C) and on ***Aphelenchus avenae*** (Fig. 1D).

There are a lot fungi in soil ecosystem that parasitize nematodes. In this study all parasitized nematodes were extracted by motility-dependent methods such as modified Baermann funnel technique. A less number of samples were extracted by using of motility-independent methods (centrifugation). Most likely, by the use of a motility-independent extraction methods more nematodes killed by parasites would have been recovered from the samples. Thus, spesific studies and surveys are necessary to determine parasitic fungus of nematodes and to judge their impact on the nematode density.

CONCLUSIONS

Nematodes live in a such complex soil ecosystem and there are always interactions with other organisms. Therefore, threefold goals are recommended: "to stimulate interest in the management of naturally occurring antagonists for biological control as opposed to the inundative release approach; to propose possible standart and innovative plant production techniques aimed at stimulation of antagonistic activity that have been neglected in favor of organisms that may be of commercial value" (Sikora, 1992a). Since most nematode species are attacked by several, sometimes highly spesific parasites, the development of a management system that enhanced such antagonist may prove useful in controlling plant parasitic nematodes.

ÖZET

DOĞU AKDENİZ BÖLGESİ'NDE NEMATODLARIN FUNGAL PARAZİTLERİNİN SAPTANMASI

Doğu Akdeniz Bölgesi'nde 1993 yılından beri yürütülen survey çalışmalarında nematodları parazitleyen iki fungus türü, ***Catenaria anguillulae*** Sorokin and ***Monacrosporium ellipsosporum*** (Grove) saptanmıştır. Nematodların önemli antagonistleri oldukları bilinen bu parazitlere incelenen örneklerde sıklıkla rastlanmıştır. Bu funguslar Türkiye'de ilk defa bu çalışma ile ortaya çıkarılmıştır.

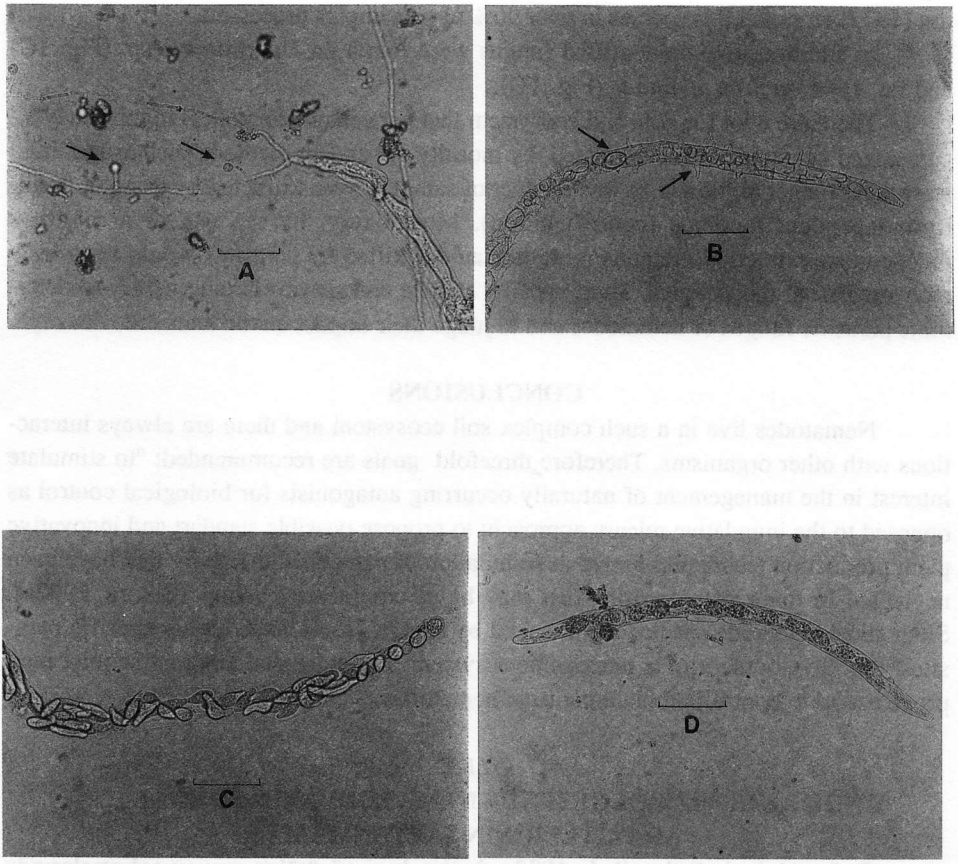

Figure 1. Photomicrographs showing A) ***Monacrosporium ellisosporum*** with adhesive knobs (arrow) parasitized ***Aphelenchus avenae***, B) ***Catenaria anguillulae*** parasitized ***Longidorus*** sp. with zoosporangia (arrow), C) Unidentified fungus parasitized ***Dorylaimus*** sp., D) Unidentified fungus parasitized ***Aphelenchus avenae*** (Scale bars A= 20 ∝ m, B-D = 100 ∝ m).

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J. Turk. Phytopath., Vol. 24, No.3, 145-148, 1995 ISSN 0378 - 8024

Host Range and Pathogenicity of **Phytophthora** Isolates From Kale, Tyfon, and Alfalfa

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ABSTRACT

Phytophthora megasperma isolated from kale and Tyfon and **P. megasperma** f. sp. **medicaginis** isolated from alfalfa, were cnoss-tested for their pathogenicity on seedlins and older plants of alfalfa and **Brassica** species in an enviromental growth chamber and greenhouse. **Phytophthora megasperma** was pathogenic on kale, rape, Tyfon, and alfalfa seedlings. Alfalfa isolate was highly pathogenic on alfalfa seedlings but it was weakly pathogenic on **Brassica** seedlings. In the older plants **Brassica** isolate was pathogenic on **Brassica** but not in alfalfa, while alfalfa isolate was pathogenic only in alfalfa. **P. megasperma** f. sp. **medicaginis** appeared to be more host spesific and it was highly virulent. **P. megasperma** appeared to have wider host range and it was less virulent.

INTRODUCTION

Brassica forage crops have desirable characteristics such as cold tolerance, rapid growth and high nutrient content (Guillard and Allinson, 1988). **Brassica** forages can be grown after alfalfa.

Phytophthora species have diverse host ranges. The **Phytophthora megasperma** group contains isolates with a narrow host range, as well as isolates with a vide host range (Erwin, 1983; Hansen and Maxwell, 1991). This important species has not been reported from Turkey.

At Powell, Wyoming (USA), a Phytophthora sp., resembling the alfalfa pathogen **P. megasperma** f. sp. **medicaginis** Kuan and Erwin, was isolated from both kale (**Brassica oleracea** L. var. **acephala** DC.) and Tyfon (**Brassica rapa** L. x **Brassica pekinensis** (Lour.) Rupr.) roots showing root rot. The field had been previously planted to alfalfa (**Medicago sativa** L.). Whether this was the P. **megasperma** f. sp. **medicaginis** that attacks alfalfa reported from Wyoming (Gray et al., 1983) or a different species was not known. Therefore, cross-pathogenicity studies were conducted in a growth chamber and in the greenhouse to determine pathogenicity as well as the experimental host range of the kale and Tyfon isolates. **P. megasperma** f. sp. **medicaginis** isolate from alfalfa was also included in several experiments.

MATERIAL and METHODS

Seedlings pathogenicity studies: The pathogenicity of **Phytophthora megasperma** isolated from kale and Tyfon, and **Phytophthora megasperma** f. sp. **medicaginis** isolated from alfalfa, was tested in a series of experiments in a controlled growth chamber with a 14/10 h light/dark cycle and 26/18ÛC temperature regime.

Small plastic pots, 10.5 cm in diameter, were used for all experiments. A pasteurized greenhouse soil mix (1/3 sand, 1/3 peat and 1/3 top soil) was used.

A completely randomized design with 3 replications was used. Two, 0.5-cm deep furrows were made in each pot and seeds of 'Premier' kale (**Brassica oleracea** L. var. **acephala** DC.), 'Emerald' rape (***B. napus*** L.), Tyfon (turnip x Chinese cabbage hybrid, **B. rapa** L. x **B. pekinensis** (Lour.) Rupr.), and 'Ranger' alfalfa (**Medicago sativa** L.) were placed into furrows. Five ml of fungal each furrow prior to seeding. The experiments with the kale, Tyfon and alfalfa isolates were repeated 5, 1, and 3 times.

To prepare the inoculum, one liter size bottles, containing 160 ml of sterilized 1/5 strength V-8 juice liquid medicum, were inoculated with two, 0.7 mm diameter mycelial plugs from 7-day-old fungal stock cultures of isolates grown on corn meal agar medium. There were two culture bottles for each isolate. After 5 days the bottles were shaken to fragment the mycelium and placed in a horizontal position for 2 weeks to allow formation of a mycelial mat. Inoculum was prepared by macedating (1 mat in 100 ml sterilized distilled water) in a Waring Blender. Approximately 4 weeks after inoculation, the number of surviring plants was recorded.

Older plants pathogenicity studies:

Kale isolate

The effect of **P. megasperma** isolated from kale on **Brassica** spp. and alfalfa. In these experiments a pasteurized greenhouse soil mix was used. Five replications (1 plant/pot) were used for each treatment. The design of the experiment was a complete randomized design. Inoculum of the kale isolate was prepared as previously described in the seedling pathogenicity studies. Four holes were in the soil at the periphery of each pot and 5 ml of fungal inoculum (sterile water for the controls) was placed in each hole. These experiments were terminated after approximately 7 months. Also on the same day, the number of surviving plants and the weight of dry shoots were recorded. Infected root pieces were removed and surface sterilized three minutes with 0.05 NaOCI and rinsed with sterile water for another three minutes. Root pieces were submerged in corn meal agar containing pimaricin (1 ml/1), penicillin (0.1 g/1), and streptomycin sulfate (0.1 g/1). Plates were examined daily for the presence of Phytophthora colonies. These experemintes were repeated once.

Alfalfa isolate

The effect of **P. megasperma** f. sp. **medicaginis** on Brassica spp. and alfalfa. These experiments were similar to the experiments described above.

RESULTS and DISCUSSION

Seedlings pathogenicity studies: In most studies **P. megasperma** isolated from kale and Tyfon significantly ($P < 0.05$) reduced seedling numbers in the inoculated groups when compared to the uninoculated controls. All crop species were affected similarly. The average percent mortality for all crops was 71.

The alflafa isolate **P. megasperma** f. sp. **medicaginis** appeared to be more host spesific and it was highly virulent towards alfalfa seedlings. The percent mortalitiy was 100. It was also weakly pathogenic to Brassica seedlings.

Older plants pathogenicity studies: After inoculation with the kale isolate of **P. megasperma**, a few of the inoculated Brassica plants died over the experimental period. Generally, the dry shoot weight of the control plants were higher than the dry matter weight of the inoculated plants. However, most of the time, these values were not statistically significant. Roots of some of the inoculated **Brassica** plants showed discoloration and root rot symptoms. The fungus was recovered from most of the inoculated **Brassica** roots. The fungus was mainly recovered from taproots showing discoloration and rot symptoms. Recovery of the fungus from the secondary roots was very low. **Phytophthora** was not recovered from the roots of control plants. All of the alfalfa plants, both in the control and inoculated groups, were survived. There was no statistically significant difference between the dry shoot weight of the control and inoculated plants. The fungus was recovered from one inoculated alfalfa root only. No **Phytophthora** was recovered from the roots of control plants.

Approximately one week after inoculation with tha alfalfa isolate **P. megasperma** f. sp. **medicaginis**, alfalfa plants showed wilting, leaf discoloration and chlorosis symptoms. Eventually a few alfalfa plants died. The remaining alfalfa plants showed severe disease symptoms and were severely stunted. All plants in the control group remained healthy. The dry shoot weights of the control plants. The fungus was recovered from most of the inoculated plants. The fungus was reisolated from tap roots frequently. The recovery rate of the fungus from the secondary alfalfa roots was approximately 50%. There were no statistically significant differences between the number of survivor plants and the dry shoot weights of the control and inoculated **Brassica** plants. The fungus was recovered from one inoculated kale root only. No **Phytophthora** was recovered from other inoculated and control plants.

Kale and Tyfon isolates both appeared to have a wide host range. These **P. megasperma** isolates appeared to be less virulent than the alfalfa isolate **P. megasperma** f. sp. **medicaginis** used in this study.

The host specificity of the alfalfa isolate **P. megasperma** f. sp. **medicaginis** has been reported previously (Erwin, 1983).

Under field conditions kale and Tyfon isolates were not isolated from alfalfa roots and alfalfa isolate was not isolated from **Brassica** roots.

For identification of the **Brassica** isolates mainly Stamps et al. (1990) key was used.

In addition to the pathological characters, **P. megasperma** from **Brassica** differed from the alfalfa pathogen morphologically. Kale and Tyfon isolates were similar and produced abundant and larger oogonia (48 and 54 μ m for the kale and Tyfon isolates, respectively). Also these isolates grew faster than the alfalfa isolate. Sporangia measurements for the kale and Tyfon isolates were 35x52 and 32x44 μ m, respectively.

Brassica isolates grew fluffy on corn meal agar in contrast to the alfalfa isolate which was granular in appearence. These **Brassica** isolates appear to be in the **P. megasperma** Drechs. group described by Hansen and Maxwell (1991).

ÖZET

BAZI BRASSICA TÜRLERİ İLE YONCADAN İZOLE EDİLEN PHYTOPHTHORA İZOLATLARININ KONUKÇULARI VE PATOJENİSİTELERİ

Brassica türlerinden izole edilen **Phytophthora megasperma** ve yoncadan izole edilen **P. megasperma** f. sp. **medicaginis** izolatı kontrollü iklim dolabında ve serada fideliklerde ve daha yaşlı bitkilerdeki patojenisiteleri yönünden karşılıklı olarak test edilmiştir. **Phytophthora megasperma Brassica** türleri ve yoncanın fidelik döneminde patojenik olarak bulunmuştur. Yonca izolatı yonca fidelerinde yüksek bir patojenisite göstermesine karşılık **Brassica** fidelerinde zayıf bir patojenisite sergilemiştir. Daha yaşlı bitkilerde **Brassica** izolatı **Brassica** bitkilerinde patojen olmasına karşılık yoncada buna rastlanamamıştır. Yonca izolatı yalnızca yoncada hastalık oluşturmuştur. **P. megasperma** f. sp. **medicaginis** konukçuya özelleşmiş ve yüksek derecede virülent bir yapı göstermiştir. **P. megasperma**'nın konukçularının daha geniş ve fungusun daha az virülent olduğu görülmüştür.

ACKNOWLEDGEMENT

We thank Dr. M. E. Matheron of the University of Arizona for confirming our **Phytophthora megasperma** identification.

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TABLE ON CONTENTS

J. Turk. Phytopathol. Vol: 24 (1995)

No.: 1

Recovering of Virus Free Grapefruits by Shoot-Tip Grafting and Thermotherapy at two Temperature Regimes
E. DEMİRER, A. ÇINAR and S. KORKMAZ 1

Virus Diseases of Vegetables in Greenhouses in İzmir and Muğla
Ü. FİDAN 7

Evaluation of Some Chickpea Cultivars for Resistance to **Ascochyta rabiei** (Pass.) Labr., **Fusarium oxysporum** and **Fusarium solani** in Türkiye
F. S. DOLAR 15

Determination of the Reactions of Some Carnation Cultivars Against Carnation Rust (**Uromyces caryophyllinus** (Schr.) Wint.)
G. AYDIN and Y. Z. KATIRCIOĞLU 23

Plant Parasitic Nematodes Associated with Citrus in the East Mediterranean Region of Türkiye
İ. H. ELEKÇİOĞLU 29

The Effects of Some Herbicides on Wild Black Cumin (**Nigella arvensis** L. var **glauca** Baiss.) in Wheat Fields
S. SÖZERİ 39

The Effects of Root Exudates of **Boreava orientalis** Jaub, et Spach on the Root Growth of Wheat Seedlings
S. ÇETİNSOY 43

No.: 2

Chemical Control and Determination of Fungal Causal Agents of Wilt Disease of Onion in Tekirdağ Province
N. ÖZER and M. ÖMEROĞLU 47

Anastomosis Groups of **Rhizoctonia solani** Kühn. and Binucleate Rhizoctonia Isolates From Various Crops in Türkiye
E. DEMİRCİ and M.T. DÖKEN 57

Biology and Chemical Control of **Melampyrum arvense** L. in cereal Fields in Central Anatolia
S. ÇETİNSOY 63

The 9th CONGRESS OF THE MEDITERRANEAN PHYTOPATHOLOGICAL UNION, Sep. 18-23, Kuşadası, Aydın, Türkiye (Papers of Keynotespeakers) 69

On the Application of Advanced Detection Techniques in Plant Pathology
A. ÇINAR and U. KERSTING 71

Biological Control of Plant Diseases: Possible Mission
E.C. TJAMOS 83

Evolving Expectations for Intergrated Disease Management: Advantage Mediterranea
J.J. STAPLETON 93

Concluding Remarks of 9th Congress of the Mediterranean Phytapathological Union
H. LECOQ 99

No.: 3

Effect of the Some Elicitors on the Capsidiol Amount in the Leaves of Peppers (***Capsicum annuum L.***) Having Different Sensitivities to Root Rot (***Phytophthora capsici*** Leon.)
A.S. ÜSTÜN 101

In Vitro Effect of A Bacterial β-1,3 Glucanase Expressed in ***Streptomyces lividans*** on ***Bremia lactucae***
Y. CANIHOŞ (DEDE) and J. BAYNON 115

Evaluation of Various Treatments of Inducing Resistance to Fusarium Wilt on Melons
K. ERZURUM and S. MADEN 121

Relations of Some Weed Species with Lettuce Big-vein Virus in Erzurum-Türkiye
E. DEMİRCİ, S. AÇIKGÖZ and M.T. DÖKEN 135

Occurrence of Fungal Parasites of Nematodes in the East Mediterranean Region of Türkiye
İ.H. ELEKÇİOĞLU and U. GÖZEL 139

Host Range and Pathogenicity of **Phytophthora** Isolates From Kale, Tyfon, and Alfalfa
A. KARAKAYA, F.A. GRAY and D.W. KOCH 145

INDEX TO VOLUME 24

A

ABAK, K. 102, 112
ABELES, F.B. 116, 120
Acremonium alternatum 86
AÇIKGÖZ, N. 19, 21
AÇIKGÖZ, S. 135
AHMAD, Q. 20, 21
ALDERMAN, S. 48, 53
ALI, A. A. 52, 53
ALLINSON, D. W. 145
Alternaria alternata 101, 105, 107, 111
Ampelomyces quisqualis 86
Anonymous 15, 21, 41, 42, 121, 133
Aphanocladium album 86
Aphelenchus avenea 140, 141, 142
Artrobacter sp. 116
Artrobacter Luteus 117
Ascochyta rabiei 15
ASHOUR, W. A. 48, 52, 59
AYDIN, G. 23

B

BAILEY, J. A. 101, 112
BAINES, R. C. 31, 36
BANDONI, R. J. 58, 62
BARIŞ, M. 109, 112
BARKSDALE, T. H. 109, 112
BARNOCZKINE-STOILOVA. E. 47, 53
BARRON, G. L. 140, 143
BAYNON, J. 115
Belonolaimus longicaudatus 29
Bifora radians 67
BONNIER, G. 65, 68
BOOTH, C. 16, 21
BORA, T. 8, 13
Boreava orientalis 43
Botrytis allii 47
BOVÉ, J. M. 73, 74, 79
Brassica sp. 145
Brassica oleracea var. *acephala* 145
Brassica pekinensis 145
Brassica rapa 145
BROGLIE, R. 118, 120
BROOKS, C. J. M. 102, 112

C, Ç

CALAVAN, E. C. 2, 5
CAMBRA, M. 73, 80
CAMPELL, R. N. 135, 137
CANIHOŞ, Y. 115
Capsicum annuum 101
Carduus nutans 135, 136
CARLING, D. E. 57, 60, 62
Catenaria anguillulae 139, 140, 141, 142
Ceratobasidium spp. 57
Cicer arietinum 15
Cichorium endivia 135
Cichorium intybus 135, 136
Cirsium arvense 135
Chenopodium amaranticolor 8, 136
Chenopodium giganteum 136
Chenopodium quinoa 8, 136
COHN, E. 29, 36
CLARK, M. F. 8, 13, 72, 80
COHEN, R. 122, 133
COLEY-SMITH-J. R. 51, 53
Collectotrichum lagenarium 121, 122
Coniothrium minitans 87
COOK, R. J. 92
Cladosporium cladosporiodes 86
Curvularia lunata 86
Cystotheco wrightii 86
ÇETİNSOY, S. 43, 63
ÇINAR, A. 1, 5, 71, 80

D

DALKIRAN, H. 19, 21
Darluca filum 23
DARVILL, A.G. 101, 112
Datura stramonium 8
DAVIS, P. H. 32, 42, 43, 46, 63, 68
DECKER, H. 29, 36
DEMİR, E. 7, 13
DEMİRCİ, E. 57, 60, 62, 135
DEMİRER, E. 1

DE LANGE,J. H. 2, 5
DOI, K. 115, 120
DOLAR, F. S. 15, 21
Dorylaimus sp. 140, 141, 142
DOWE, A. 140, 143
DÖKEN, M. T. 135, 137
DUNCAN, L. 29, 36
DÜZGÜNEŞ, O. 25, 27

E

ELEKÇİOĞLU, H. İ. 29, 36, 139, 143
EL-SHEHABY, A. I. 52, 53
ENNELİ, S. 139, 143
Epicoccum purpurescens 85
ERKAN, S. 9, 13
Erysiphe graminis f. sp. hordei 86
Erysiphe polygoni 86
ERWİN, D. C. 145, 147
Escherichia coli 115
ESSER, R. P. 141, 143

F

FANTONI, M. G. 122, 133
FERRERA, J. F. 51, 53
FİDAN, Ü. 7, 137
FLETCHER, J. T. 23, 27
FORTUNER, R. 30, 36
Fusarium acuminatum 15, 47, 48
Fusarium oxysporum 15, 17, 47, 85, 121, 122, 139
Fusarium oxysporum f. sp. *cepae* 47
Fusarium oxysporum f. sp. *lyeopersici* 121, 126, 127
Fusarium oxysporum f. sp. *melonis* 121
Fusarium oxysporum f. sp. *niveum* 121, 126, 127
Fusarium oxysporum f. sp. *redolens* 115
Fusarium solani 15, 17

G

GARABEDİANS, S. 31, 37
GARNIER, M. 73, 80
GARNSEY. S. M. 73, 80
Geranium tuberosum 67
GESSLER, C. 123, 133
Gliocladium virens 85
Gomphrena globosa 8
GÖKSEL, N. 43, 46
GÖZEL, U. 139
GRAY, F. A. 145, 148
GRINSTEIN, A. 122, 133
GUILLARD, K. 145, 148
GÜLLÜ, M. 7, 9, 13
GÜNCAN, A. 43, 46

H

HAJDU, J. 48, 53
HANSEN, E. M. 145, 148
HARDY, R. W. F. 92
HARTUNG, J. S. 74, 80
HATİPOĞLU, A. 23, 27
HAWARE, M. P. 17, 22
HEGİ, G. 43, 46, 63, 68
Helicotylenchus sp. 30
Helicotylenchus pseudorobustus 29
HENSON, J. M. 74, 80
HEPER, E. 9, 13
HOLLINGS, M. 9, 13
HOMMA, Y. 57, 62
HOOPER, D. J. 29, 37
HUIJBERTS, N. 135, 137
HUNDE, B. 21
HUSSAIN, S. 18, 21

I, İ

IISHIZAKA, N. 51, 53

J

JAMIL, F. F. 17, 22
JIMENEZ-DIAZ, R.M. 17, 22
JONES, D.R. 102, 110, 112

K

KARACA, İ. 51, 53
KARAHAN, O. 112
KARAKAYA, A. 145
KATIRCIOĞLU, Y. Z 23
KEELEY, L. S. 140
KERRY, B. R. 139-143
KERSTİNG, U. 71
KIM, J. J. 109-112
KIBLE, K. A. 112
KIRAY, Y. 29-37
KLOEPER, J. W. 92
KOCH, D. W. 145
KODAMA, F. 47,51,53
KORKMAZ, S. 1,72,80
KRANZ, J. 51,53
KUC, J. 122,133
KUNINAGA, S. 57,62

KUNWAR, I.K. 17,22

L

LACY, M. L. 47,53
Lactura sativa 135
LAMBERTI, F. 30-37
LATIN, R. X. 122,133
LEVY, L. 74,80
LIN, S. 73,80
LINTHORST, H. J. M. 118,120
Longidorus sp. 140,142
LOPER, J. E. 92
LUMSDEN, R. D. 92

M

MACLEAN, D. 117, 120
MADEN, S. 15, 22
MAKKOUK, K. M. 73, 81
MARAITE, H. 122, 134
MAS, P. M. 122,, 123, 134
MAUCH, F. 119, 120
MAUDE, R. B. 51, 53
MAXWELL, D.P. 145
MEAGHER, J. W. 30, 37
Medicago sativa 145
Melampyrum arvense 63
Meloidogyne arenaire 140
Meloidogyne incognita 140
Meloidogyne favanica 140
MERCİER,J. 101,112
MEYER, J. A. 122, 134
Microsphaera euonimi - japonicae 86
MOLOT, P.M. 102,110,122 134
Monacrosporium ellisosporum 139, 140, 141, 142
Monilinia fructigena 101,102,105,107,111
MURANT, A. F. 9, 13
MURASHIGE, T. 2, 5

N

NAIK, D.M. 47, 53
NAVARRO, L. 1, 4, 5, 77, 81
NEATE, S. 57, 62
NENE, Y.Y. 16, 22
NEUHAUS, J.M. 119, 120
Nicotiana clevelandii 8
Nicotiana glutinosa 8, 9
Nicotiana tabacum 8, 9, 102
Nigella arvensis var. glauca 39
NOGAY, A 9, 14
NOLASCO, G. 74, 81

O, Ö

OGOSHI, A 57, 60, 62
Olpidium brassiancae 135, 136
ORTEGA, R.G. 109, 113
ÖZ, S. 47, 53
ÖZER, Z. 43, 46
ÖZKUT, S 29, 37
ÖZMEN, O. 51, 53

P

PALLOIX, A 109, 113
PAPE, H. 23, 28
Paratylenchus sp. 30
PARTMETER, J.R. 58, 62
PAVEL, A. 23, 28
Penicillium oxalicum 85
PERMAR, T. A. 73, 81
Phlebia gigantea 87
PHILLIPS, J.C. 20, 22
Phytophytora capsici 101, 102, 103, 105, 107, 109, 111
Phytophytora megasperma 145
Phytophytora megasperma f. sp. medicaginis 145, 146, 147, 148
PIRONE P.P. 23, 28
Pratylenchaides sp. 30
Pratylenchus brachyurus 29
Pratylenchus coffeaa 29
Pratylenchus penetrans 140
Pratylenchus thornei 140
Pratylenchus vulnus 29
POCHARD, E 102, 109, 110, 113
PRESLEY, A.H. 48, 53
Pseudomonas stutzeri 115
Pyrenochaeta terrestris 47
Pythium butleri 47

R

Radopholus citrophilus 29
REDDY, M. V. 17, 18, 22
REIFSCHNEIDER, F. J. B. 109, 113
Rhizoctonia sp. 85
Rhizoctonia solani 47, 57, 115
RIDINGS, W. H. 141
ROBERTI, R. 48, 53
ROBERTS, W.K. 119, 120
ROD, J. 48, 53
ROISTACHER, C.N. 5, 72, 79, 81

ROSNER, A. 73, 81
Rotylenchulus macrodoratus 29 30
ROVIRA, A. D. 58 62

S

SAIKI, R.K. 74, 81
SAILLARD, C. 2, 5, 74, 81
SAIMMAIME, I. 102, 114
SASSER, J. N. 29, 37
SAYRE, R. M. 140, 143
Sclerotinia sclerotiorum 85
Sclerotium cepivorum 47, 85
Sclerotium rolfsii 85, 115
SEMİNA, S. N. 23, 28
SEMORILE, L. C. 73, 82
Serratia marcescens 115
SEZGİN, E. 23, 24, 28
SHAPIRE, R. 115, 120
SHUAG, L. S. 47, 53
SIDDIQUI, Z. A. 31, 37
SIKORA, R. A. 139, 141, 143
SIVAN, A. 115, 120
SINGH, K.B. 17, 22
SNEH, B. 57, 62
SORAN, H. 15, 22, 51, 53
SÖZERI, S. 39
SPENCER, D. M. 27, 28
Spiroplasma citri 2, 73
Sphaerotheca fuligenea 86
Sporidesmium sclerotivorum 85
STACE-SMİTH, R. 9, 14
STAMPS, D. J. 145, 148
Stephanoascus rugulosus 86
STEWARD, A. 47, 53
STOESSL, A. 102, 110, 114
SUNDHEIM, L. 115, 120
SZOLAY, F. 48, 53

T

Talaromyces flavus 85
TAKAKUWRA, M. 47, 51, 55
Taraxacum officinale 135
TARJAN, A. C. 29, 37
TAŞTAN, B. 43, 46
TEKİNEL, N. 7, 9, 14
Thanatephorus cucumeris 57
THREFALL, D. R. 101, 114
Tilletia pallescens 86
Tilletidopsis sp 86
Tilletiopsis albescens 86
Tilletiopsis minör 86
TJAMOS, E. C. 83, 92
TOROS, S. 25, 28
Trichoderma sp 85
Trichoderma harzianum 115
Trichoderma lignorum 87
Trophurus sp 30
TUNCER, G. 59, 62
Tylenchorhynchus goffarti 29, 30
Tylenchus sp. 30
Tylenchulus semipenetrans 29, 140

U-Ü

ULHOE, C. J. 115, 120
Ulocladium tuberculatum 139
Uramyses caryophyllinus 23
ÜSTÜN, A. S. 102, 110, 114

V

Van HOOF, H. A. 135, 137
Verticillium biguttotum 85
Verticillium lecani 23
VILLECHANOUX, S. 73, 82
VOGELI, U. 102, 114

W

WARD, E. W. B. 110, 114
WATSON, D. G. 101, 114
WETZEL, T. 74, 82
WHITEHEAT, I. M. 101, 102, 114
WILSON, C. L. 92

X

Xeranthemum annuum 136

Y

YANG, X. 74, 82
YILDIZ, M. 122, 134
YILMAZ. M. A. 9, 14
YORGANCI, Ü. 9, 14
YÜCE, B. 7, 14
YÜCEL, S. 122, 134
YÜKSEL, H. 29, 37

Z

ZEBZAMI, M. 73, 82
ZINC, F. W. 123, 134
ZOTE, K. K. 22

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