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CONTENTS

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A Practical Training About Resistance to Fungicides in Eyespot of Cereals (**Pseudocercosporella herpotrichoides** Fron., Deighton) at Rothamsted Experimental Station[1]

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ABSTRACT

This practical training was conducted at Rothamsted Experimental Station and supported by the British Council. The aim was to study the development of fungicide resistance in the eyespot fungus in wheat and barley. As a result of surveys by ADAS[5] in 1982 and 1983, relationships between wheat-strains (W-types) and rye-strains (R-types) and resistance to MBC (methyl benzimidazole-2-yl carbamate), MDPC (a phenyl carbamate) BAS 263 (2-(1-methoxy-2-chloro)-ethoxy-phenyl -N-methylcarbamate) type fungicides were also studied.

Parallel trials were conducted using MBC resistant isolates on agar plates and on pot plants. Negatively correlated cross-resistance between MBC and MDPC was demonstrated. A formulated MDPC and a phenyl methyl carbamate insecticide (BAS 263) were also assessed.

INTRODUCTION

The benzimidazole type fungicides (carbendazim, benomyl, thiophanate methyl) have been used in Europe to control eyespot of cereals, caused by (**Pseudocercosporella herpatrichoides** Fron., Deighton), since the mid-1970's (Hampel and Locher, 1973; Brown et al., 1984). Resistance to this type of fungicides have been known for some time from laboratory studies (Rashid and Schlosser, 1975; Olvang, 1983).

Eyespot is an important disease and caused much damage in cereals in the U.K., and serious yield losses occur most years. The benzimidazole type of fungicides are commonly applied for eyespot control. Since the mid-1970's, there has been an increasing use of MBC (methyl benzimidazole-2-yl carbamate) generating fungicides such as benomyl

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(Benlate), carbendazim (Bavistin, Derosal) or thiophanate methyl (Cercobin). In the 1982 ADAS surveys, 52 % of the winter wheat crops and 45 % of the winter barley crops received one or more applications of an MBC fungicide. Despite this widespread usage in the U.K., there was no evidence of MBC resistance in the eyespot fungus prior to 1981 (Griffin and Yarham, 1983). Recently control failures have occured following application of these fungicides, and these failures have been attributed to resistance. Control failures seem related to the intensity of previous use, which has gradually increased over the years (Bateman et al., 1985). This survey showed that resistance has been accompained by an increase in the frequency of rye (R-type) and a decline on wheat (W-type) of the pathogen (Bateman et al., 1985). Rye-types were seldom encountered prior to 1980, but in ADAS surveys carried on recently, R and W-types were present in equal proportions in winter wheat and winter barley fields. Meantime, other studies had shown that eyespot control was possible using an alternative chemical, prochloraz (Sportak), which is a sterol synthesis inihibitor, either alone or mixed with carbendazim (Sportak-Alpha) (Griffiths, 1983).

Also, recently Kato et al., 1984 have demonstrated that carbendazim resistant strains of many fungi could specifically be controlled by the related phenyl carbamate MDPC (Methyl N-(3.5-Di chloropehenyl) carbamate), and this negatively correlated cross resistance extends to eyespot (Fitt et al., 1984).

Consequently, in this work, we have monitored eyespot populations from several typical cereal growing sites. We have examined changes in the proportions of R and W-types and the effect of MDPC on both resistant and sensitive strains of the eyespot fungus.

MATERIAL AND METHODS

MBC was gift from Dupont, Stevenage U.K., streptomycin and chloramphenicol were purchased from Sigma Chemical Co., Poole, U.K. Also, MDPC and BAS 263 were gift from Basf, Germany, plastic petri dishes, plastic pots (13 cm diameters), cork borer, silver sand, sterile compost, micro pipet, the variety of wheat was ARMADA, sodium hypochloride dilution, sterile water, Mc Cartney bottles.

Assasys were based on methods already published elsewhere (Horsten, 1979; Horsten and Fehrmann, 1980; Fehrmann et al., 1982; Bateman et al., 1985). Modifications were made as follows:

Test 1

25 samples were taken from different cereal growing sites in which

resistance was suspected from previous trials (Table 1). However, in 1984 none of these sites were treated with MBC generating fungicides. Samples were stored at −20C° until use. After washing the roots, stem lesions were cut and surface sterilised in sodium hypochloride dilution (1 % available chlorine) for 5 mins and rinsed twice with sterillised water. Lesions were placed onto sterilised wet filter papers in Petri dishes, which were then sealed by sellotape to prevent drying out during exposure. To encourage sporulation Petri dishes were placed under UV (310 nm=3100 Amstrongs Phillips) at 10°C for 14 days. Then each lesions was put into a Mc Cartney bottle which contained 3 ml sterilised water contained 3 ml sterilised water containing Tween 80 (0.1 %). After shaking, each spore suspension was poured onto PDA (1/5 rate of standart PDA Oxoid = 7,8 g PDA for 1 lt water) (Horsten, 1979; Horsten et al., 1980; Fehrmann et al., 1982; Bateman et al., 1985) which contained streptomycin sulphate (0.13 mg/ml), chloramphenicol (0.1 mg/ml) to avoid contamination with MBC (0.001 mg/ml) or without. MBC was added from a stock solution in methanol (10 ml methanol with MBC for stock) and the final concentration of methanol in the PDA was 0.1 %. Then one Petri dish with MBC and one without were put in the growth room for 14 days at 20°C.

After two weeks, cultures were assessed as resistant, sensitive or without eyespot fungi. Sensitive isolates grew only on control plates, whereas, resistant isolates grew on plates containing MBC. Subcultures were made from each isolate onto PDA, in order to check whether isolates were R or W-types. R-type isolates grew in two ways. Some were dark grey with irregular feathery edges and grew rapidly with radial furrows on the surface but only at half the growth rate of W-types. Others were orange and cream with irregular feathery edges but slow growing. W-types were recognised as clear grey colonies with smooth edges but fast growing.

Test 2

The purpose of these experiments was to assess the effects of MBC, MDPC, BAS 263 and MBC+MDPC on eyespot in wheat plants. MBC (0.05 mg/ml), MDPC (0.1 mg/ml), BAS 263 (E.C. 0.0002 ml/ml) and MBC+MDPC (0.05 mg/ml+0.1 mg/ml) were for 800 ml water. For these concentrations, 40 mg MBC, 80 mg MDPC, 0.167 ml BAS 263 were solved in 4 ml methanol and then were added to 800 ml water.

The variety of wheat was ARMADA, which is susceptible to eyespot. It was grown in 13 cm diameter pots containing compost, with 12 plants per pot, for 10 days. When the young seedlings were about 3-4

Table 1. Carbendazim resistance in eyespot samples from 16 different sites.

R.E.S. Sample No.	Site	Crop	Sample date	Number of lesions					
				W-types		R-types		W+R-types	
				MBC-sens.	MBC-res.	MBC-sens.	MBC-res.	MBC-sens.	MBC-res.
1	Bungay	W.wheat	4 April	5	2	3	7	0	1
3	»	»	22 April	2	2	—	13	—	—
15	»	»	11 June	0	3	1	9	—	—
25	»	»	13 July	1	2	0	26	—	—
11	Kilham	W.wheat	—	0	0	1	2	—	—
14	»	»	11 July	0	6	6	46	1	3
22	»	»	11 July	0	0	1	26	—	—
5	Harnhill	W.wheat	18 April	0	37	0	11	—	—
16	»	»	19 July	0	9	0	25	—	—
21	Godford St. Mary	W.wheat	3 July	2	18	1	21	0	5
2	Boston	W.barley	24 April	15	1	3	4	—	—
8	Howden	W.barley	25 April	0	0	3	21	—	—
13	»	»	11 June	2	1	10	31	0	1
6	Coln St. Aldwyns	W.barley	18 April	1	22	0	1	—	—
19	Collingbourne Kingston	W.barley	3 July	6	0	0	34	—	—
10	N. Dalton	W.barley	—	—	—	—	—	—	—
12	»	»	11 June	1	0	3	5	—	—
4	Cupar	W.barley	25 April	0	0	8	0	—	—
7	Coln St. Aldwyns	W.barley	15 April	1	0	3	21	—	—
20	Hatherop	W.barley	3 July	0	0	1	32	—	—
23	Sutton-u-Brailes	W.barley	20 June	0	14	0	68	0	3
18	Stonham	W.wheat	7 August	0	0	0	33	—	—
24	Offchurch	W.wheat	20 June	1	2	3	41	—	—
17	»	»	30 July	0	1	0	2	—	—
9	—	W.barley	24 April	10	59	2	6	0	2

cm tall, they were inoculated by placing agar collars of each eyespot isolate round each coleoptile at soil level (Fig. 3). Inoculum was prepared by growing each isolate for 3 weeks in potato dextrose broth, on a shaking incubator. Then, broth cultures were homogenised under sterile conditions and 20 ml added to 40 ml sterilised water agar, cooled to 50°C and poured into Petri dishes. Collars were made by cutting concentric rings with 10 mm and 3 mm cork borers.

After inoculations, all the pot plants were covered with silver sand to keep them moist. The chemicals were applied 20 mls per pots as root drenches, at weekly intervals, on 5 occasions. Each treatment was replicated five times.

Six weeks after inoculation plants were scored as follow category numbers according to the number of successive leaf sheaths infected or penetrated and a penetration index calculated (Scott, 1971).

		Number of plants in each category									
Place of infection	:	None	Coleop.		Sheath I		Sheath II		Sheath III		INF
		Inf.	Inf.	Pen.	Inf.	Pen.	Inf.	Pen.	Inf.	Pen.	
Points	:	0	1	2	3	4	5	6	7	8	9

Test 3

This experiment compared two different formulations of MDPC on the control of eyespot caused by MBC resistant and sensitive strains. This experiment also attempted to assess the pathogenecity of isolates on rye.

Plants were grown in the same way as in test 2. They were also inoculated in the same way. In this experiment, however MDPC was also formulated as a wettable powder (50 % a.i.), which allowed it to be more readily dispersed in the test solution. Their concentrations were in the same as in the test 2. Four replicates only were used.

Assessments were made and the results analysed in the same way as in test 2.

RESULTS

The results of test 1 are following: After statistical analysis results are shown in table 2.

Table 2. Summary of results from 1984 eyespot survey.

t-test analysis (samples with < 9 **P. herpotrichoides** lesions not included)

% R-types (arcsine transformation)

	ALL CROPS	WHEAT	BARLEY
APRIL	40.7	46.7	36.3
JUNE+JULY	71.8	70.8	73.4
t	3.85	2.31	2.68
Degrees of freedom	13	9	7
P	< 0.01	< 0.05	< 0.05

% MBC-resistant (arcsine transformation)

	ALL CROPS	WHEAT	BARLEY
APRIL	64.1	70.0	59.8
JUNE+JULY	75.4	79.0	69.6
t	1.56	1.16	0.75
Degrees of freedom	13	9	7
P	> 0.1	> 0.2	> 0.3

Where $\underline{P}$ < 0.05, results for April are significantly different from those for June-July (August)

As a result of this survey, all sites had high levels of MBC-tesistance and this hardly increased from April until July. The proportion of rye-types increased considerably in this period, in both wheat and barley crops, despite being collected from untreated sites.

The test 2 resulted in as following:

The results were shown in Table 3.

Table 3. Effects of MBC, MDPC, and BAS 263 on eyespot control in pot tests.

		FUNGICIDE TREATMENTS (penetration index)					(isolate)
Isolates		None	MBC	MDPC	MBC+MDPC	BAS 263	Mean
D21	R	3.84	4.36 NS	2.33*	2.26	4.35	3.42
GP1	S	4.71	0.11***	4.89 NS	0.31	4.84	2.97
BK91	R	3.95	4.67 NS	1.38**	0.64	4.17	2.96
C20	R	4.99	5.00 NS	1.74***	2.81	4.73	3.85
D12	R	4.96	5.51 NS	2.53**	1.76	5.02	3.95
BY2GP	R	5.56	4.26 NS	1.73***	0.97	5.78	3.66
M1	S	4.23	0.17***	4.88 NS	0.67	4.75	2.94
BY2	R	4.57	4.72 NS	0.47***	0.27	4.11	2.82
M2	S	4.40	0.34***	5.02 NS	0.35	3.69	2.76
BY212	S	5.36	0.00***	5.25 NS	0.18	4.99	3.15
Mean (Fungicide)		4.65	2.91	3.02	1.02	4.64	

LSD:		5 %	1 %	0.1 %
Between two treatment means		1.17	1.73	2.86
»	» isolates means	0.43	0.58	0.76
»	» fungicides means	0.30	0.40	0.52

MDPC reduced eyespot only when MBC resistant isolates were used. A mixture of MBC and MDPC provided no benefit in addition to that of each fungicide alone. BAS 263 had no effect against all isolates used in the experiments. Isolates were different in their pathogenicity on wheat, but these differences were not correlated with resistance in any way.

Also, the results of test 3 are in table 4.

Table 4. Effects of MBC, MDPC and MDPC wattable powder on eyespot control in pot tests.

	FUNGICIDE TREATMENT (Penetration index)				
Isolates	None	MBC	MDPC	MDPC-WP	Mean
21-5 WR	3.03	3.36	1.62	1.13	2.28
21.2 W	2.08	2.98	1.60	1.90	2.14
GP 1	2.81	0.02	3.85	3.16	2.46
Mean	2.64	2.12	2.35	2.06	

LSD:	5 %	1 %	0.1 %
Between two treatments	1.21	1.83	2.95
» » fungicides	0.70	1.04	1.68
» » isolates	0.60	0.92	1.48

Table 4 shows that a wettable powder formulation of MDPC did not improve control of MBC-resistant eyespot isolates compared with pure MDPC. Two eyespot strains (BY—177M; W—100M) which had been «trained» to grow on increasing levels of MBC appeared to have lost their pathogenicity (Table 5). The unselected parents (BY—177; W—100) retained their pathogenecitiy under laboratory conditions.

Table 5. Pathogenecity of different eyespot isolates.

	Penetration index				
Isolates	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean
21—1 WR	3.20	2.00	4.10	1.00	2.57
21—2 R	2.92	4.10	1.80	2.33	2.78
BY—177	4.36	4.40	3.90	4.67	4.33
BY—177M	0.91	0.44	0.18	0.80	0.58
W—100	2.50	4.82	2.40	1.82	2.88
W—100M	0	0	0.55	0.10	0.16
Mean	2.31	2.62	2.15	1.78	

LSD: Between two isolates	5 %
	1.21

CONCLUSIONS

Negatively correlated, cross resistance of the eyespot fungus to MBC and MDPC was demonstrated;

a. In agar plate tests,

b. On plants in pots,

MBC resistant isolates had increased sensitivity to MDPC. Although higher concentrations of MDPC than of MBC were needed to control eyespot, MDPC might be useful in the field to overcome the problem of MBC resistance.

W.P. formulation of MDPC was no better than pure MDPC on pot plants. Isolates with laboratory induced resistance were less pathogenic than parent isolates maintained on fungicide-free media.

A survey of wheat and barley crops sampled in 1984 in U.K. showed a very high incidence of MBC-resistance. R-types were also common.

There was a larger proportion of R-types and MBC resistant isolates in the summer samples than in spring samples.

Because of widespread resistance to benzimidazole-type fungicides in the eyespot fungus, alternative control measures are needed. One alternative that has already been adopted involves use of prochloraz, either alone, or mixed with carbendazim. However, prochloraz is expensive to use and other measures are needed.

Rotations, incorporating crops not infected by the eyespot fungus, such as oil seed rape, vegetables, maize, varieties of beet, oats, should be introduced wherever possible. Cereal varieties with effective host plant resistance against eyespot are required from plant breeders. Further work is also needed on the mechanism of resistance to benzimidazole-type fungicides before effective strategies of fungicide use can be adopted.

Ö Z E T

ROTHAMSTED DENEME İSTASYONU' (İngiltere) NDA YAPILAN SİSTEMİK FUNGİSİTLERE DAYANIKLILIK KONUSUNDAKİ ÇALIŞMAYA AİT RAPOR

İngiltere Teknik Yardım Programı çerçevesinde, bakanlığımıza verilen burslardan birini değerlendirerek gittiğim Rothamsted Deneme İstasyonunda «Sistemik Fungisitlere Dayanıklılık» konusunda yapılan bu çalışmayı 3 bölümde gerçekleştirdim. Bunlardan ilki;

a. İnvitro koşullarda MBC ve MDPC tip fungisitlere karşı tahıl «eyespot» ırklarının negative cross resistance'ı ve dayanıklılık bakımından oranlarının saptanması amacıyla İngiltere'de tahılda eyespot hastalığı görülen yerlerden toplanan 25 adet örnek üzerinde yapılan çalışma,

b. Bu çalışmaya paralel saksı denemeleri ile birinci çalışmada kullanılan izolatlar arasında adı geçen ilaçlara karşı dayanıklılık ve duyarlılık oranlarının saptanması üzerinde yapılan çalışma,

c. İkinci çalışma temel teşkil etmek üzere, bu defa MBC tip fungisit ile MDPC tip fungisitlerin farklı iki formülasyonunun, dayanıklılık ve duyarlılık bakımından etkileri ile eyespot'un dayanıklılık gösteren ve çavdarlarda bu problemi yaratan bir ırkının laboratuvar koşullarındaki durumu üzerine kurulan çalışma şeklinde özetleyebiliriz.

Birinci çalışma sonucunda İngiltere'de tahıl ekim alanlarında buğday ve arpa tarlalarında Rye type (çavdar tipi) eyespot ırkı populasyonunun arttığı saptanmıştır. İkinci çalışmada, saksı denemeleri ile eyespot ırklarının MBC ve MDPC tip fungisitlere karşı dayanıklılık oluşturması ve oranlarının saptanmasına çalışılmıştır. Üçüncü çalışmada ise; ikinci çalışmaya paralel bitkilerin tarla koşullarının sağlandığı seralarda saksı denemeleri ile MBC ve MDPC tip fungisitlere karşı dayanıklılık oluşturmasının, ilaçların formülasyon tipleri ile ilişkisi ve çavdar bitkisinin çavdar tip eyespot ırkı ile ne derece dayanıklılık oluşturduğu üzerinde durulmuştur.

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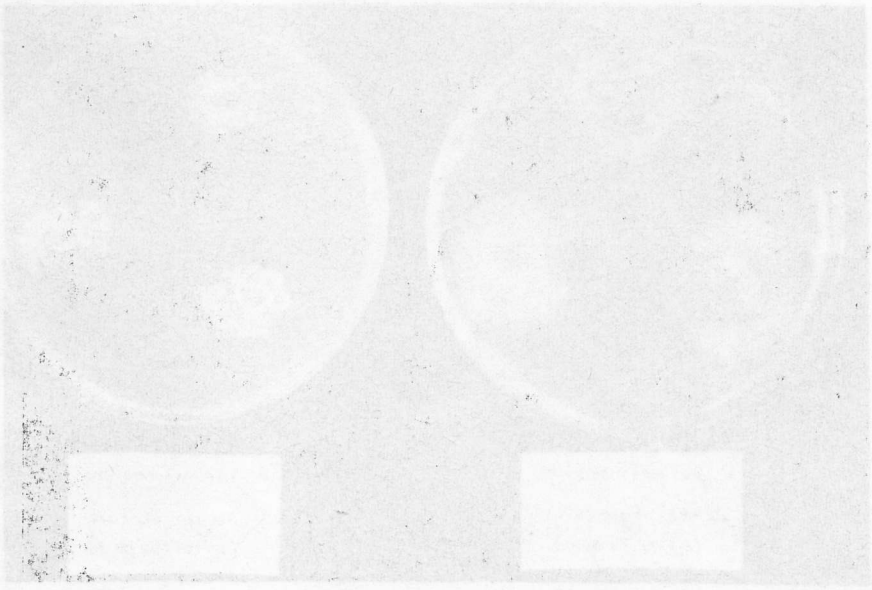

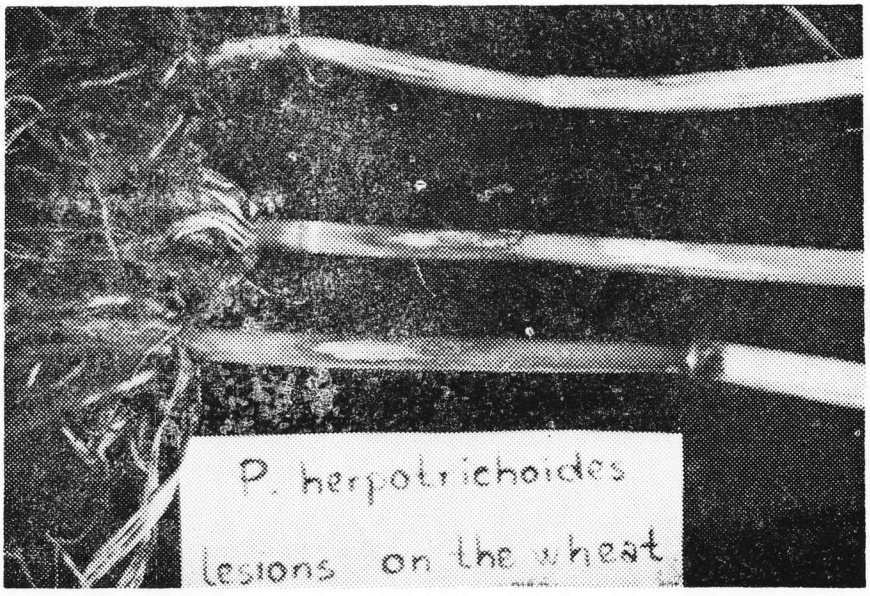

Figure.1. Eyespot disease of wheat.

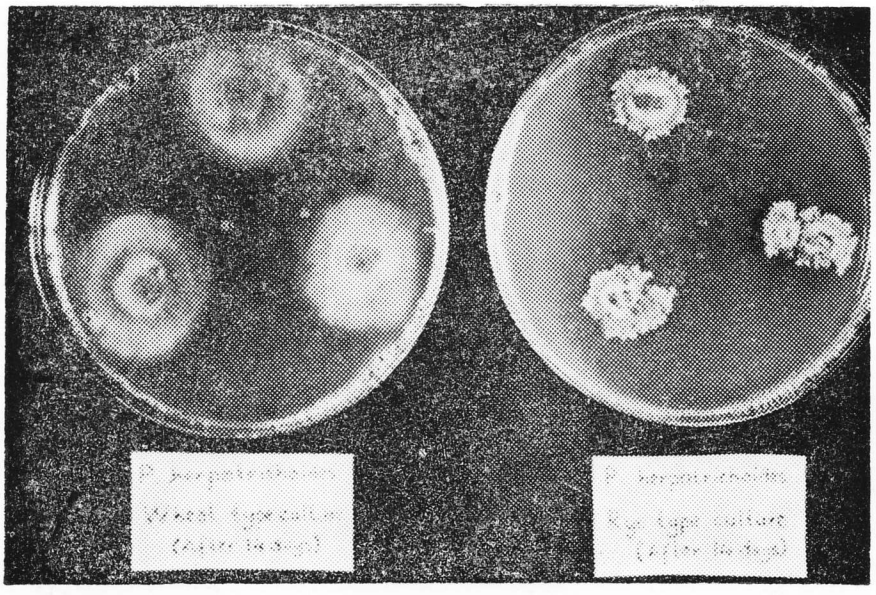

Figure.2. MDPC resistance in **P. herpotrichoides.**

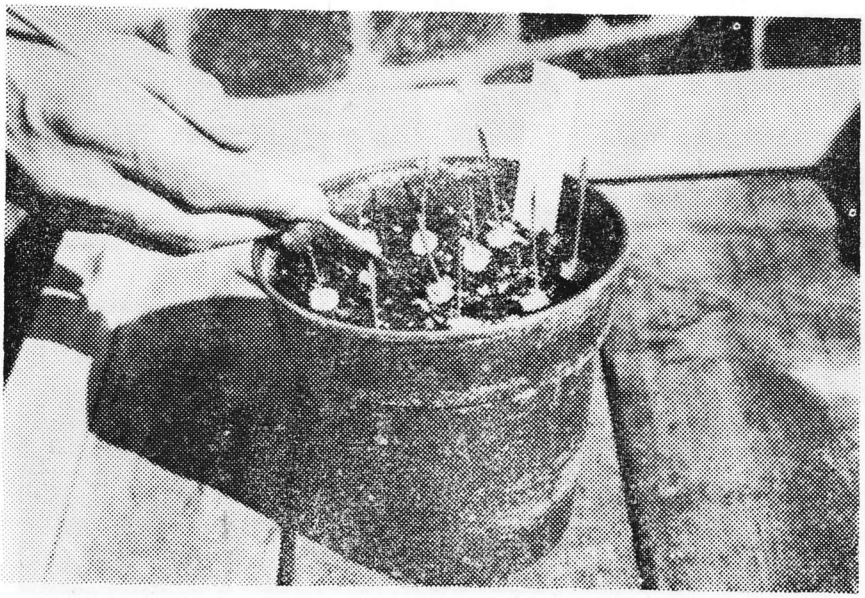

Figure.3. Assay of MBC resistance.

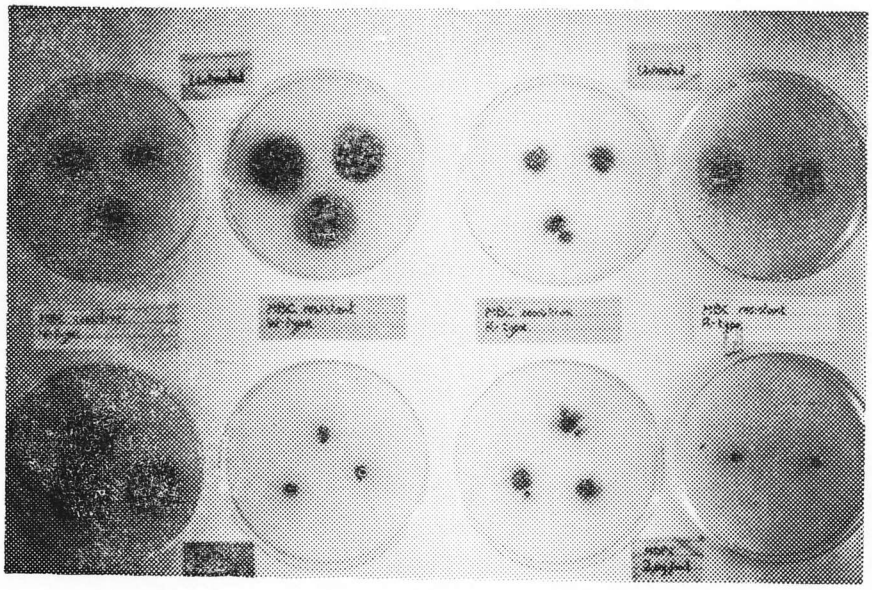

Figure.4. Wheat and rye pathotypes of **P. herpotrichoides.**

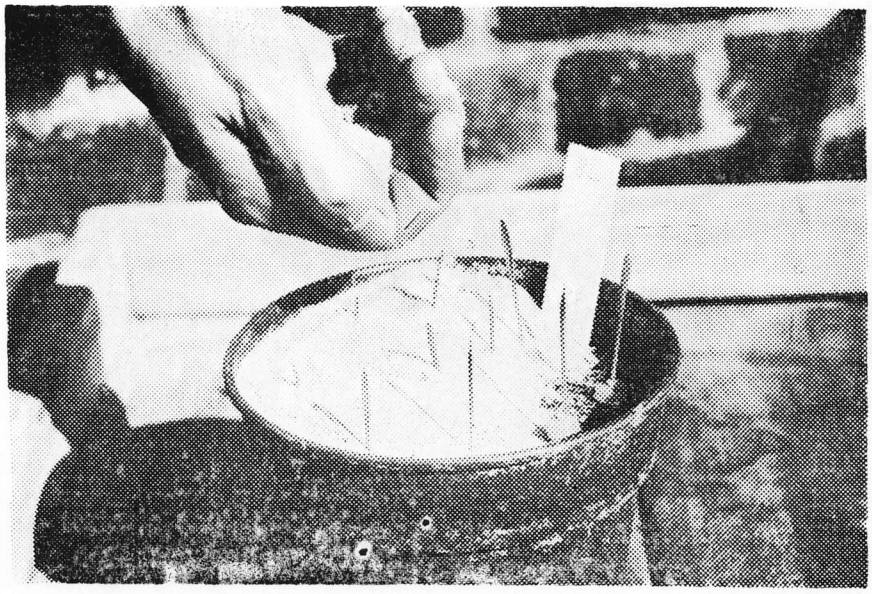

Figure.5. Covering with sand after inoculation.

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The Effects of Watermelon Mosaic Virus 1 Infection on the Physiological and Biochemical Activities of Muskmelon (**Cucumis melo** L.)

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ABSTRACT

Physiological and biochemical changes associated with watermelon mosaic virus 1 (WMV_1) in "Yuva" cultivar of muskmelon were investigated. WMV_1 isolate was collected from muskmelon field around Beypazarı-Ankara. The seedlings were grown in perlit and irrigated periodically with Arnon's nutrient solution. Respiration rate was determined at 3 days and other biochemical tests were done at weekly intervals. The results showed that WMV_1 infection increased the protein level but it reduced the level of the other physiological and biochemical activities such as respiration rate, starch, sugars (glucose, fructose and saccharose), inorganic nitrogen and total nitrogen cotents of infected plants compared with the healthy ones.

INTRODUCTION

Watermelon mosaic virus 1 (WMV_1) is one of the most destructive and widespread virus infection of muskmelon in Ankara province. Recently, it caused great decline in quality and quantity of the production. The major symptoms produced by this infection are severe mottling, deformation and blisters on the young leaves of muskmelon plants.

Biochemical changes in host plant metabolism caused by several virus infections have been elucidated (Bawden and Pirie 1952, Bawden 1959, Goodman et al. 1967). It is known that virus induced symptoms are the results of competing metabolic pathways of virus biosynthesis and normal biochemical processes of host plant (Farkas and Solymosy 1965). Recently, although more attention has been paid to the metabolic changes caused by cucurbit viruses, especially cucumber mosaic virus (Menke and Walker 1963, Sindelar et al. 1980a, 1980b, Sharma et al. 1981, Erdiller and Özyanar 1983); only the effects of WMV_1 infection on nitrogen metabolism and enzymatic activity of pumpkin plants have been investigated (Singh 1983, Singh and Bhargava 1984).

This research has been carried out in order to determine the effects of WMV_1 infection on the physiological and biochemical activities of muskmelon plant.

Virus source and inoculation: WMV_1 used was an isolate maintained in our laboratory which was found in Beypazarı-Ankara muskmelon fields.

Muskmelon cultivar (**C. melo**, "Yuva"), highly susceptible to WMV_1 was raised in galvanized iron pots 14x18.5 cm, containing sterilized perlit of 1-3 mm diameter, 5 seeds were sown to each pot. This plants were maintained in insect proof screen-cages where the mean temparature and relative humidity were 20 ± 5°C and 40-50 % respectively.

The plants were irrigated with Arnon's modified nutrient solution (Arnon 1938) at 10 days intervals. Instead of 0.05 % iron sulphate and 0.4 % tartaric acid, Sequestrine Fe-138 commercial preparation, containing 6 % iron was used.

Inoculations were made onto the cotyledons when plants were at first leaf stage with an inoculum prepared in 0.05 M phosphate buffer (pH: 7.0) at the rate of 1:1 (w/v). Control plants were left as untreated. Leaf samples were collected at 3 days intervals for the determination of respiration rates and weekly intervals for other biochemical assays.

Determination of respiration rate: Respiration rate of diseased plants and healthy controls were estimated as 0_2 consumption with theaid of Warburg manometer at 25°C, in dark (Umbreit et al. 1964). Leaf samples were collected as 200 mg for each replication in the morning and placed into the manometric flasks of the apparatus. Over the samples, 2 ml 0.1 M phosphate buffer solution (pH: 7.0) was added and 0.2 ml KOH was put in the center well of the flasks and then flasks were attached to the apparatus immediately. Equilibration of respirometer were done at 15 minutes intervals during two hours of assay period. At the end of the period, leaf samples were taken out and put into an oven at 80°C and kept there until they reached to a constant weight (Bell 1964). The respiration values of healthy and infected tissues were detected at 3 days intervals, starting from the first day after inoculation until the 33rd day. Each experiment was designed with 3 replications and the obtained values were evaluated statistically according to Erdiller (1979) and Erdiller and Özyanar (1983). 0_2 uptake was expressed as μl 0_2/mg dry weight per hour.

Estimation of chlorophyll: Chlorophyll content of WMV_1 infected and healthy leaves were detected using the method of Francis et al. (1970). Leaves, cut into small pieces, were placed in 80 % acetone (1 g of tis-

sue/10 ml) and homogenized for 2 minutes. Homogenate then was filtered through Whatman no. 1 filter paper. Residue was washed with acetone and the final volume was adjusted to 100 ml for per gram of tissue. Absorbance values of extracts were measured at 645 and 663 nm and chlorophyll a, chlorophyll b and total chlorophyll content of samples were calculated using the formulae below:

$$\text{mg chlorophyll a / g tissue} = \left[12.7\,(D_{663}) - 2.69\,(D_{645})\right] \times \frac{v}{1000\ W}$$

$$\text{mg chlorophyll b / g tissue} = \left[22.9\,(D_{645}) - 4.68\,(D_{663})\right] \times \frac{v}{1000\ W}$$

$$\text{mg total chlorophyll / g tissue} = \left[20.2\,(D_{645}) - 8.02\,(D_{663})\right] \times \frac{v}{1000\ W}$$

D : absorbance values at indicated wavelength

v : volume of extract (ml)

W : weight of the extracted tissue (g)

Estimation of carbohydrates: Biochemical changes in carbohydrate metabolism of muskmelon infected with WMV_1 have been elucidated by Anthron method of Halhoul and Kleinberg (1972) which its principle was the extraction of starch, fructosan polysaccharides and soluble carbohydrates from the same sample separately. Leaf samples were dried at 60°C until they reached to a constant weight. Dried samples were ground and 0.5 g was taken for each replication. These leaf samples were first extracted with 80 % ethyl alcohol. After the excess of the alcohol was taken off, all extracts were adjusted to 100 ml with distilled water and used for the determination of glucose and saccharose content of the samples. Absorbance values were determined at 620 nm with 1.5 % Anthron reagent (Merck-Darmstadt) in concentrated sulfuric acid. Standart solutions were prepared with glucose.

Same extraction was repeated once more with distilled water and then used for the measurement of fructose contents of the samples. In this case, standart solutions prepared with fructose. Preparation of Anthron reagent and measurement of absorbance were same as mentioned above.

The remaining sugar-free residue was treated with 52 % perchloric acid (Merck-Darmstadt) for the determination of starch content of the samples. Starch content was determined with Anthron reagent prepared as above and estimated as glucose. In all of the tests, absorbance values were determined at 620 nm with a spectronic-21 spectrophoto-

meter. All of the extracts were treated with active carbon prior the addition of Anthron reagent.

Estimation of nitrogen: The total nitrogen, inorganic nitrogen and protein contents of plants were estimated spectrophotometrically from the same sample according to the Nessler method of Johnson (1941) with slight modifications, 3 g of fresh leaves were homogenized with 15 ml 0.1 M phosphate buffer (pH: 6.0). The extract was filtered through Whatman no. 1 filter paper and was used for estimation of total nitrogen content of the plants. The proteins were precipitated by addition of 10 % trichloro acetic acid at the rate of 1:1 (v/v) to the filtrates. Following a low speed centrifugation at 5500 rpm for 20 minutes, inorganic nitrogen amount was determined from the supernatant and protein content was estimated from the precipitate.

Absorbance values were measured at 490 nm with spectronic-21 spectrophotometer. Standart solutions prepared with ammonium sulphate were used to calculate the nitrogen amounts. The exact amount of proteins were determined by multiplication of the nitrogen amount associated to host proteins by 6.25 factor.

All of the biochemical assays were done with 3 replications at least and the obtained values were analysed statistically for their importance.

RESULTS

The effects of infection on respiration

The effects of infection on the respiration of the host is shown in Fig. la. The respiration of infected plants was 12.62 % higher at 3 days after inoculation when the development of first symptoms as chlorosis have just started on true leaves, then it dropped sharply to the below of the level of healthy controls. There was no significant difference between infected and control plants on the 9th through 21st days after inoculation. During this period, the respiration rates of infected plants have reached to healthy controls. Later, it again started to decrease gradually through the end of the research period. The difference in respiration of healthy and infected plants at the end of the research period was found as significant statistically at 5 % level.

This fluctuation of respiration in diseased plants is clearly seen in Fig. 1b. In this figure, the ratios of the respiration of infected plants to healthy controls (respiration rate) were plotted against the assay period. At the end of the research period, 0_2 consumption of infected plants was about the 80 % of the healthy controls.

The effects of infection on chlorophyll pigments

The contentsof chlorophyll pigments have differed depending on the age of the plants. This was seen as a rapid increase up to 3 weeks after inoculation and then gradual decrease in chlorophyll a, chlorophyll b and total chlorophyll content of both infected and healthy plants, in Fig. 2a and 2b. On the contrary, great losses in chlorophyll a, b, and total chlorophyll contents of infected plants have occured and those were estimated as 50.10 %, 50.92 % and 50.70 % respectively, at the end of the research period. These differences were all significant at 1 % level.

The effects of infection on carbohydrates

The effects of WMV_1 infection to the carbohydrate metabolism of muskmelon were detected as gradual increase of starch content in both infected and healthy plants. Although the development of disease could not prevent the accumulation of starch, the amount detected in infected plants was 12.52 % less than of healthy plants at the end of the research period (Fig. 3).

Inoculation of muskmelon cultivar "Yuva" with the virus resulted in the lower levels of fructose during the later stages of analysis (Fig. 4a). Although a great decrease in the content of the sum of glucose and saccharose during the early stages of disease development was detected, with the advancement of the disease, it has reached to the level of the healthy plants (Fig. 4b) and no significant difference was detected at the end of the research period.

The effects of infection on nitrogen metabolism

WMV_1 infection caused a great increase in the protein content of the inoculated plants compared to the healthy controls. At the early stages of disease development, it was detected as 116.8 times more of healthy controls. Although the protein amount decreased contrarily to the disease development, it was still 94 times higher than the healthy controls at the end of the 5^{th} week (Fig. 5).

The inorganic nitrogen and total nitrogen amount of WMV_1 infected and healthy muskmelon plants differed by the same pattern (Fig. 6a and 6b). At the early stages of infection, inorganic nitrogen and total nitrogen contents of inoculated plants increased rapidly, up to 3 weeks after inoculation. Afterwards a great decline in amount of inorganic nitrogen was detected during the later stages of disease.

Although, at the early stages of disease, the amount of total nitrogen was quite high in infected plants, it continued to accumulate

through the 3 weeks after inoculation and then, started to decrease up to 19 % below the level of healthy controls at the end of research period. Those values were all significant at 5 % level statistically.

DISCUSSION

This is the first report on the metabolic alternations of muskmelon caused by WMV_1 infection. Muskmelon cultivar "Yuva" was the most susceptible cultivar, cultivated in Central Anatolia Region of Turkey, to WMV_1 infection.

The infection caused rapid increase in the respiration rate of infected plants at the early stage and then it dropped to the level of controls. It again started to decrease 3 weeks after inoculation. Similar type of respiration pattern was also observed for cucumber mosaic virus-cucumber combination (Erdiller and Özyanar 1983) and some other virus-host combinations (Goodman et al. 1967). Millerd and Scott (1962) have informed that the decrease of respiration indicates the decrease in the other metabolic functions of the host.

There is a direct relation between chlorophyll, carbohydrate content and respiration rate of the plants. Decrease in chlorophyll content reflects the decrease in net photosynthesis which means the accumulation of lower amounts of carbohydrates. Generally, the plants which contain low levels of carbohydrate, respirate less than the normal ones (Kacar 1979).

The carbon balance of diseased tissues is obviously of importance in the economy of the host. Especially, virus infections are known to cause alterations in carbohydrate levels of the tissue by influencing the rate of synthesis and/or rate of translocation (Sridhar et al. 1976). Starch accumulation and sugars synthesis were greatly affected due to WMV_1 infection. These reductions of starch and sugars levels can be attributed to the reduction of chlorophyll content of the tissues. WMV_1 infection also caused great damage in chlorophyll content of the tissues. The reduction of photosynthetic pigments is the major cause of the decrease of photosynthesis, the major metabolic process of carbohydrate production. Foster and Foster (1965) have proved that the virus infections, including WMV_1, affect root formation of muskmelon plants. This is another reason for slow photosynthetic activity of muskmelon plants. The decrease in starch accumulation was considered as the major cause of yield loss by Sindelar et al. (1980a and 1980b).

WMV_1 infection only caused great increase in protein level of the host but reduced the inorganic nitrogen and total nitrogen contents

through the end of the research period. According to Goodman et al. (1967), this may be due to the rapid virus replication so that the virus protein is included in assays together with the host protein.

Ö Z E T

KAVUN (C. melo L.)'NUN FİZYOLOJİK VE BİYOKİMYASAL FAALİYETLERİ ÜZERİNE WATERMELON MOSAİC VİRUS 1 ENFEKSİYONUNUN ETKİLERİ

Bu çalışmada watermelon mosaic virus 1'in "Yuva" kavun çeşidinde oluşturduğu fizyolojik ve biyokimyasal değişimler araştırılmıştır. WMV_1 izolatı Beypazarı-Ankara civarındaki kavun tarlalarından elde edilmiştir. Fideler perlit içinde yetiştirilmiş ve peryodik olarak Arnon' nun besi solusyonu ile sulanmışlardır. Solunum oranı 3 günlük, diğer biyokimyasal denemeler haftalık aralıklarla yapılmıştır. Elde edilen değerler, WMV_1 enfeksiyonunun, sağlamlara kıyasla enfekteli bitkilerin protein miktarını arttırdığı, buna karşılık diğer fizyolojik ve biyokimyasal faaliyetlerini, örneğin solunum oranını, nişasta, şeker (glukoz, fruktoz ve sakkaroz), inorganik azot ve toplam azot kapsamlarını düşürdüğünü göstermiştir.

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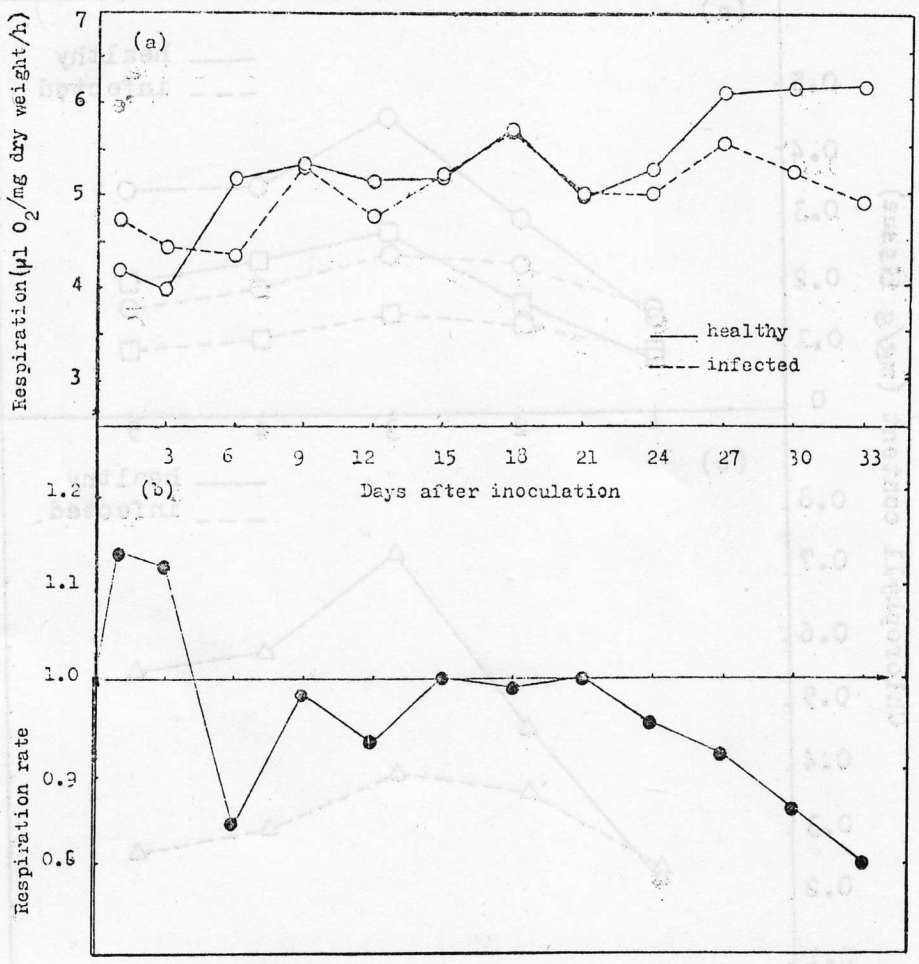

Fig. 1(a). The respiration of WMV_1 infected and healthy muskmelon leaves at times after inoculation.

Fig. 1(b). The differentiation of the respiration rate of infected leaves compared to healthy ones at times after inoculation.

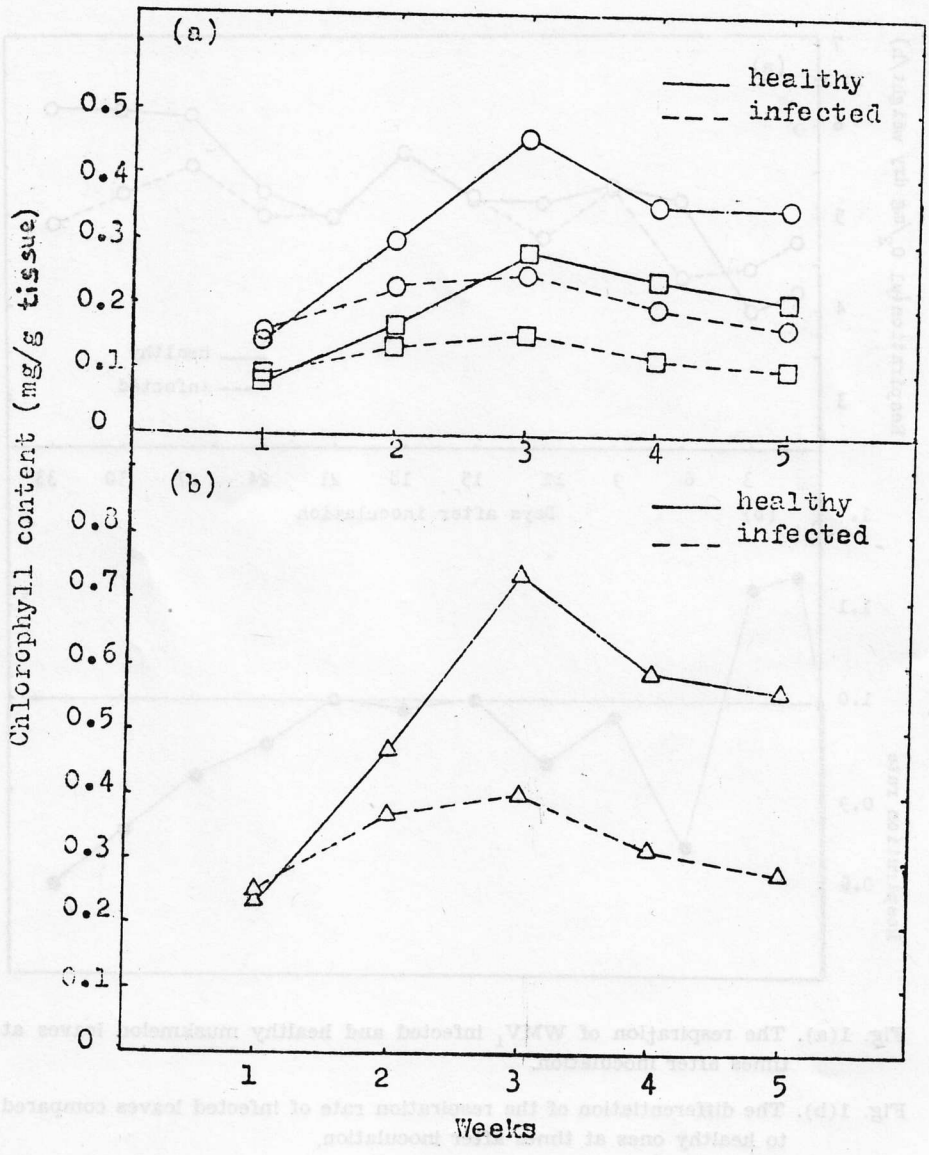

Fig. 2. Chlorophyll a, chlorophyll b and total chlorophyll content of WMV_1 infected and healthy muskmelon leaves at times after inoculation. Symbols: (a)/(O) chlorophyll a ; (□) chlorophyll b ; (b)/(Δ) total chlorophyll content.

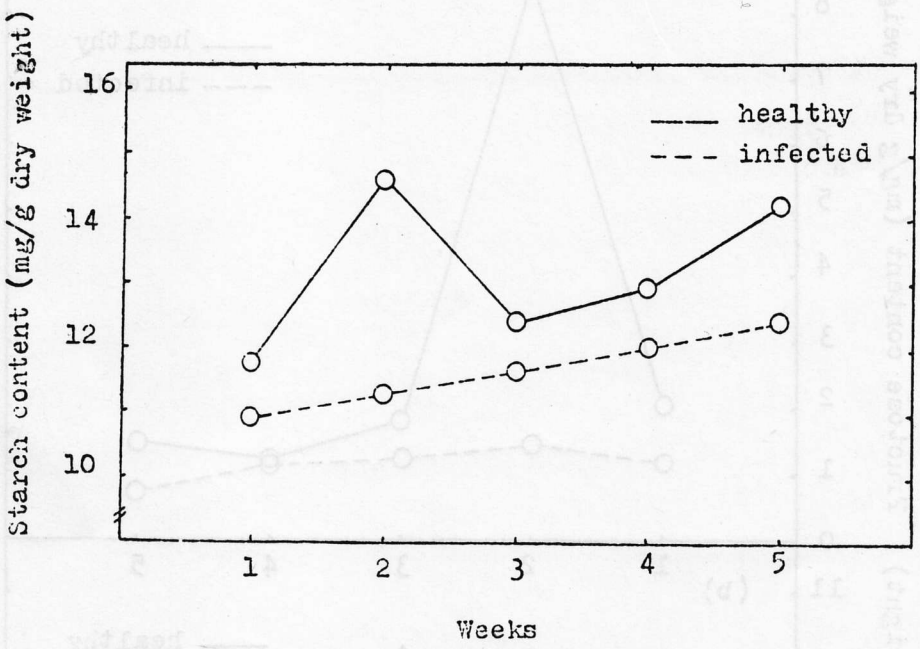

Fig. 3. Starch content of WMV_1 infected and healthy leaves at times after inoculation.

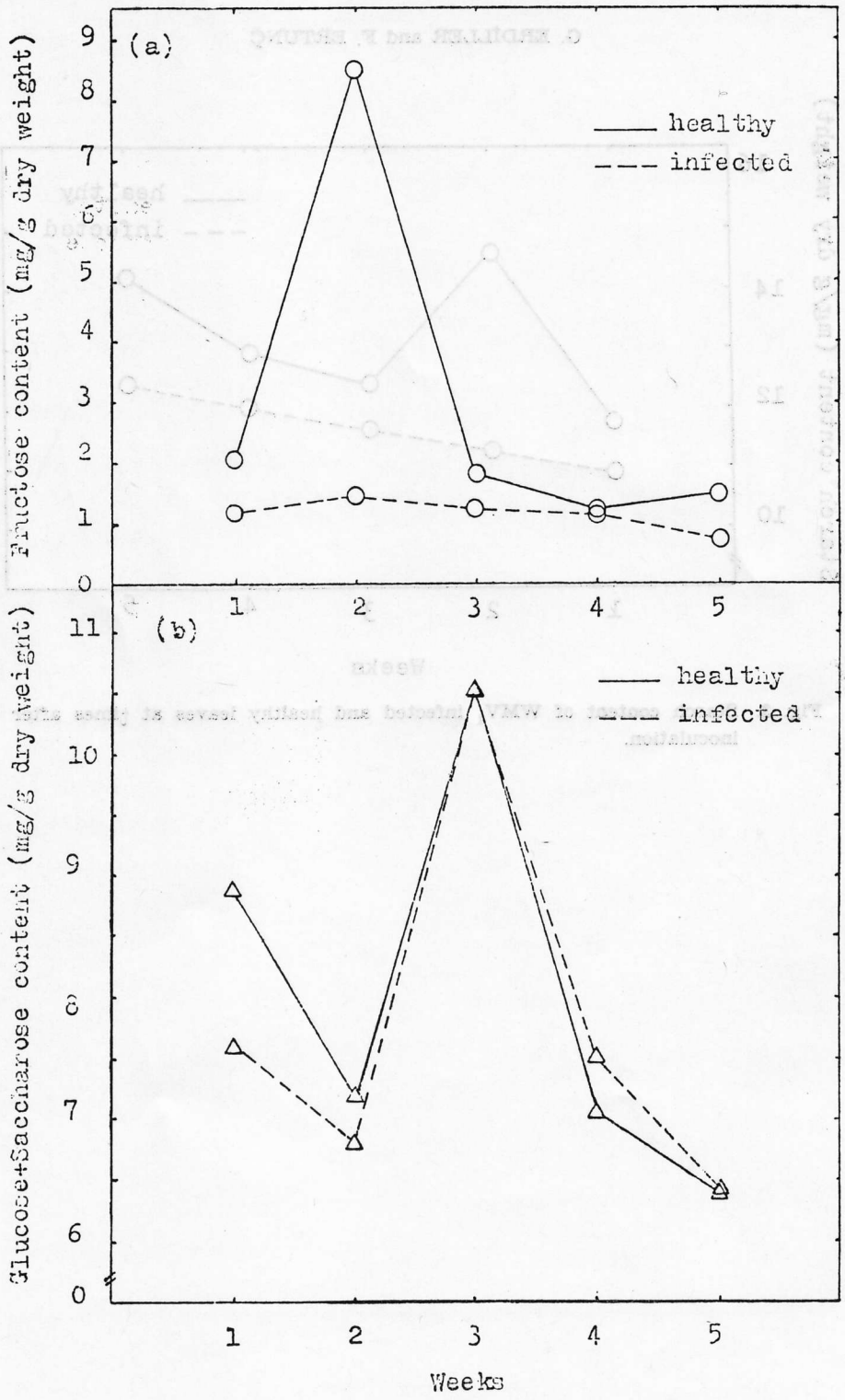

Fig. 4(a). Fructose content of WMV_1 infected and healthy leaves at times after inoculation.

Fig. 4(b). The sum of glucose and saccharose content of WMV_1 infected and healthy leaves at times after inoculation.

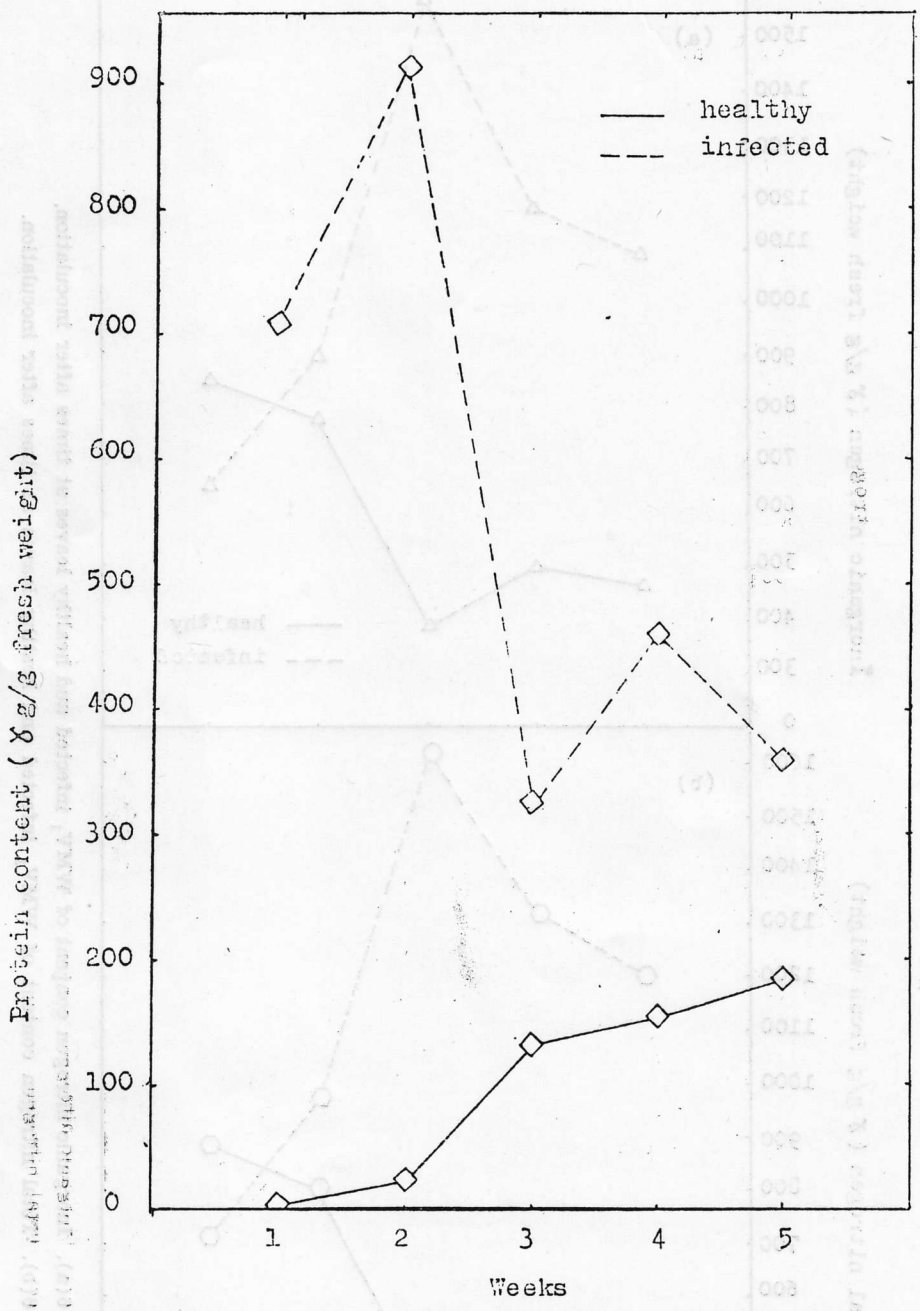

Fig. 5. Protein content of WMV_1 infected and healthy leaves at times after inoculation.

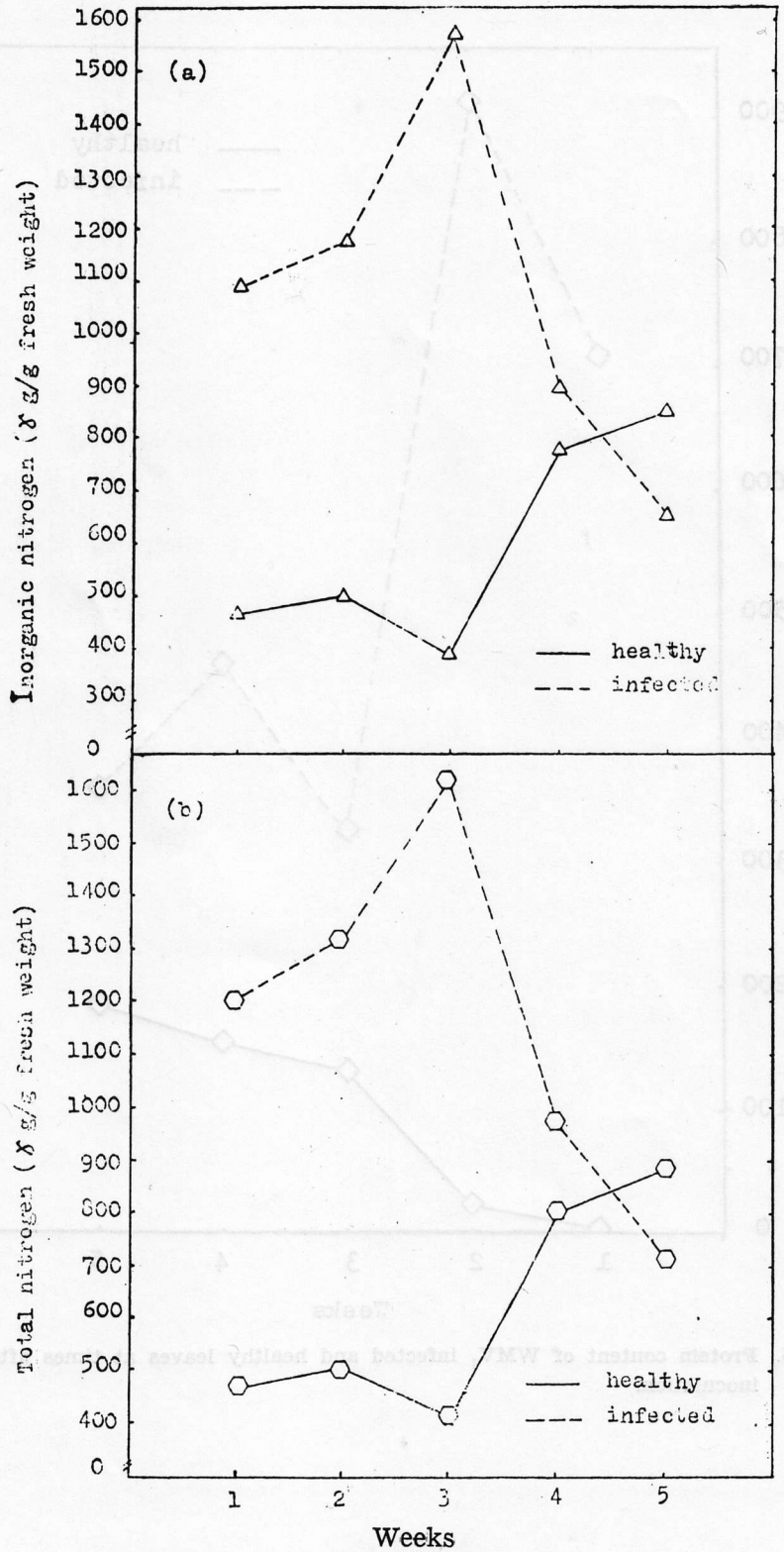

Fig. 6(a). Inorganic nitrogen content of WMV_1 infected and healthy leaves at times after inoculation.

Fig. 6(b). Total nitrogen content of WMV_1 infected and healthy leaves at times after inoculation.

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Investigations on the Stability of Tobacco Mosaic Virus (TMV) in Soil

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ABSTRACT

Recoverable infectivity from soils amended with TMV declined after 3 and 10 days of incubation. Slight fluctuations in temperature, pH and moisture caused the gradual reductions in the rates of TMV recovery from soils. The rate of TMV degradation in soils was faster at 25°C than at either 4 or 40°C. Less virus was recovered when pH values of soils were adjusted from 10,00 to 3,40. TMV degraded quicker in moist soil than in drier soil, but when the soil was flooded, TMV appeared to be stable. When introduced into autoclaved soil or soils containing streptomycin sulfate and captan, more TMV was recovered by comparison with control soil.

INTRODUCTION

In Turkey, tobacco mosaic virus "TMV" is one of the prevalent plant viruses affecting many crops including eggplant, freesia, pepper, tobacco and tomato (9, 10, 22, 23, 24). In some years, it causes serious losses in the production areas of the plants mentioned. As it is known, TMV has mainly spread by seed, seedling, insect vectors or infected plant debris in soils. Broadbent (4) and Allen (1) reported that in glasshouses infected plant debris in the soil was considered the main reservoir of virus responsible for initial infections in tomato crops. Moreover, in some studies (1, 2, 5, 6, 15, 18) it was found that a few viruses with stable structure like TMV might also remain infectious in a wide variety of soil types for the periods of certain time. However, there is little information about the influence of several edaphic factors (temperature, pH and moisture) on the survival of TMV, particularly in the extracellular state, in the soil environment as also indicated by Allen (2).

So, the present study was conducted to investigate the effects of various factors affecting the stability of TMV in soil.

MATERIALS and METHODS

In the work tomato strain (Kemalpaşa) of TMV isolated by Yorganci (22) was used. Virus was purified from systemically infected leaves of **Nicotiana tabacum** L. cv. Maden by the procedure of Schade (17) and stored at - 20°C until used. The concentration of TMV in the purified preparation was determined by a Unicam Sp 800 spectrophotometer (22).

For the present study the soil samples were collected from various tomato growing areas in some provinces (Antalya, İzmir, Manisa and Muğla).

Analyses for texture (3), pH (11), $CaCo_3$ (7), total soluble salt (19), organic matter (16) and water - holding capacity (21) were carried out on the above - mentioned soil samples.

Throughout the study, the methods previously reported by certain workers (2, 5, 6, 12, 13) were applied by modifying in accordance with the instruments and material available.

The soil sample was first air - dried and screened through 0,5 mm - mesh. 20 g. of sieved soil was placed in glass vials with screw - caps, brought to its water - holding capacity by adding sterile distilled water as needed and kept at 20 ∓ 2°C for 24 h. Later, the purified TMV was introduced into soil sample in each vial by pipetting at the rate of 10 mg in 1 ml of 0,02 M phosphate buffer (pH 7,2). The capped vials were thoroughly shaken and then, incubated at room temperature in the dark for the periods of time required.

In order to recover TMV from the soil, the soil sample in each vial was mixed with 40 ml of phosphate buffer and the soil - buffer mixture was left in a shaker for about 1 h. After being thoroughly stirred, the suspension was centrifuged at 6000 rpm for 20 min. For the infectivity assays the supernatant fluid obtained was used in inoculating six celite - dusted leaves of **Nicotiana glutinosa** L. plants. Inoculated plants were put in a room with a temperature of 22 ∓ 2°C, a light intensity of 4500 Lux and an illumination of 16 - h a day.

Considering the average number of local lesions produced on inoculated leaves within 2 to 5 days after inoculations, two soil samples were selected for using in the further steps of the investigation. Some experiments were performed to study the effect of certain factors (temperature, pH and moisture) and treatments (heat sterilization, the application of antibiotic and fungicide to soil, and the incorporation of TMV at various concentrations into soil).

The data from all assays were analysed according to Analyses of Variance and L.S.D. test was applied when necessary.

RESULTS and DISCUSSION

The results of analyses carried out on the soil samples in our study and the rates of the infectivity recovered from these soils amended with TMV after 3 and 10 days of incubation at room temperature are summarized in Tables 1 and 2, respectively.

According to the figures presented in Table 2, the rate of TMV recovery was lower in some soils (sample nos. 7, 9, 10, 18, 28, 32) after 3 and 10 days of incubation whereas in the remaining soils TMV recoverability was higher. Moreover, it became obvious from the results that the recoverable infectivity of TMV declined in all soils tested, though different in efficiency, with the time elapsed after introducing the virus into soils (Table 2) as also recorded by some researchers (2, 5, 12, 18). Furthermore ,from the data in Table 2 it can be considered that the reductions in recoverable infectivity noted in the assays could be connection with the antiviral phenomenon in the soils. Indeed, Cheo (5) came to the conclusion in his works that in general, most cultivated soils looked quite active in their antiviral activity. Although many attempts were made to correlate various soil properties with antiviral action, no important correlation could be found (Tables 1 and 2).

Since two soils (sample nos. 7 and 10) appeared to be more promising than others for the antiviral action, further studies were conducted with only these soils.

Table 1. The results of analyses on the soil samples

Soil somple no.	pH	Total soluble salt (%)	$CaCo_3$ (%)	Texture	Organic matter (%)	Water holding capacity (%)
1	7,65	0,080	8,35	Clay	2,22	36,06
2	7,55	0,030	3,84	Sand	1,29	5,60
3	7,25	0,450	10,36	Loam	1,34	19,40
4	7,45	0,060	6,68	Loam	1,03	14,04
5	7,40	0,075	13,11	Clay loam	1,70	23,17
6	4,40	0,044	1,04	Sandy loam	9,82	35,54
7	7,70	0,140	8,10	Loamy sand	1,45	15,90
8	7,50	0,041	7,18	Loam	1,45	12,65
9	7,30	0,068	5,52	Sandy loam	0,33	8,25
10	7,65	0,080	17,63	Loam	1,86	14,98
11	7,40	0,050	2,17	Sandy loam	4,76	25,06
12	6,60	0,075	0,80	Sandy loam	2,13	21,03
13	7,40	0,038	5,84	Sandy loam	1,55	15,38
14	7,20	0,037	1,04	Loamy sand	1,66	7,03
15	7,30	0,223	24,56	Loam	7,29	20,11
16	6,95	0,260	53,98	Loam	6,10	17,20
17	7,25	0,050	4,68	Sandy loam	7,50	16,00
18	7,15	0,030	9,20	Sandy loam	2,14	12,95
19	7,30	0,073	6,05	Sandy loam	3,98	13,05
20	7,40	0,074	19,84	Loam	4,65	16,01
21	7,20	0,030	66,29	Loam	2,22	16,00
22	6,90	0,054	30,60	Loam	2,07	16,07
23	6,70	0,030	0,92	Sandy loam	0,98	6,05
24	7,50	0,150	7,93	Loamy sand	2,69	8,00
25	7,65	0,090	17,54	Loamy sand	1,55	8,02
26	7,40	0,035	29,91	Sand	1,60	3,19
27	7,10	0,200	31,33	Sandy loam	1,91	9,35
28	7,30	0,200	29,49	Loam	3,41	15,07
29	7,45	0,056	3,46	Sandy loam	1,73	12,28
30	7,50	0,150	27,24	Loam	2,25	14,43
31	6,70	0,120	1,25	Clay	2,53	21,45
32	6,80	0,170	1,25	Loamy sand	2,74	10,33
33	7,20	0,078	1,46	Sandy loam	2,48	20,56
34	7,20	0,050	2,71	Loam	3,41	15,95

Table 2. The rates of TMV infectivity recovered from virus-amended soils.

Recoverable infectivity from soils amended with TMV after incubating for 3 days		Recoverable infectivity from soils amended with TMV after incubating for 10 days	
Soil sample no.	Infectivity assay*	Soil sample no.	Infectivity assay
10	33,00	10	23,50
7	41,17	7	27,83
28	45,67	9	34,83
18	48,00	28	35,00
9	50,50	32	35,00
26	51,00	18	36,00
32	51,50	29	36,00
30	51,67	27	36,83
27	52,17	26	38,00
14	53,17	22	38,50
22	53,67	15	38,50
12	54,33	19	39,67
11	56,00	34	40,00
29	56,17	12	40,33
34	56,33	11	40,50
19	58,83	30	40,83
3	59,00	20	41,17
31	59,50	14	41,33
4	61,00	21	41,83
20	61,00	4	42,00
15	61,50	3	43,17
33	63,33	17	43,33
21	64,67	31	43,50
2	66,17	33	43,67
17	66,67	2	46,50
5	66,83	8	50,33
8	67,17	16	55,17
13	73,67	5	56,17
16	75,50	13	57,83
6	77,83	6	59,67
1	98,00	1	69,00
23	106,00	24	73,00
25	107,67	25	76,17
24	111,33	23	89,83

* Figures indicate the average number of local lesions on six inoculated leaves of **N. glutinosa** and bars represent statistical groups at p=0,05 level.

The results of the experiments that were performed to determine the effects of temperature, pH and moisture on the survival of TMV in these two soils are presented in Figs. 1, 2 and Table 3.

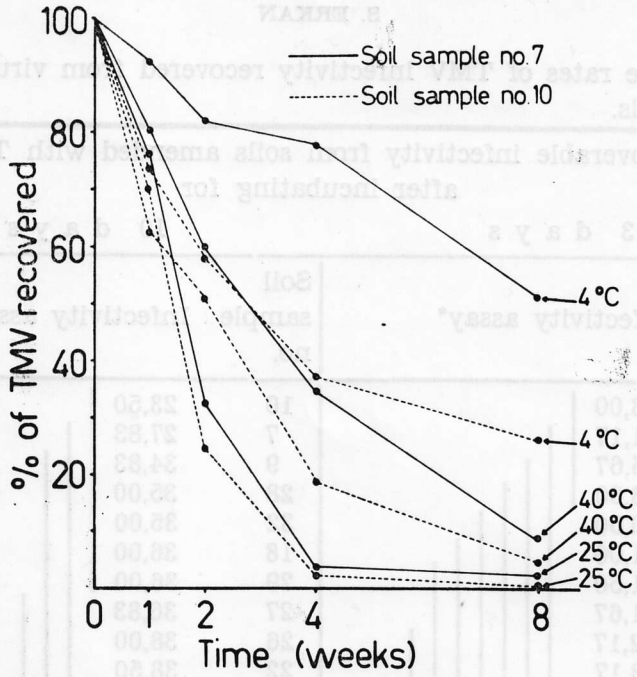

Fig.1. TMV recoveries from soils incubated at different temperatures.

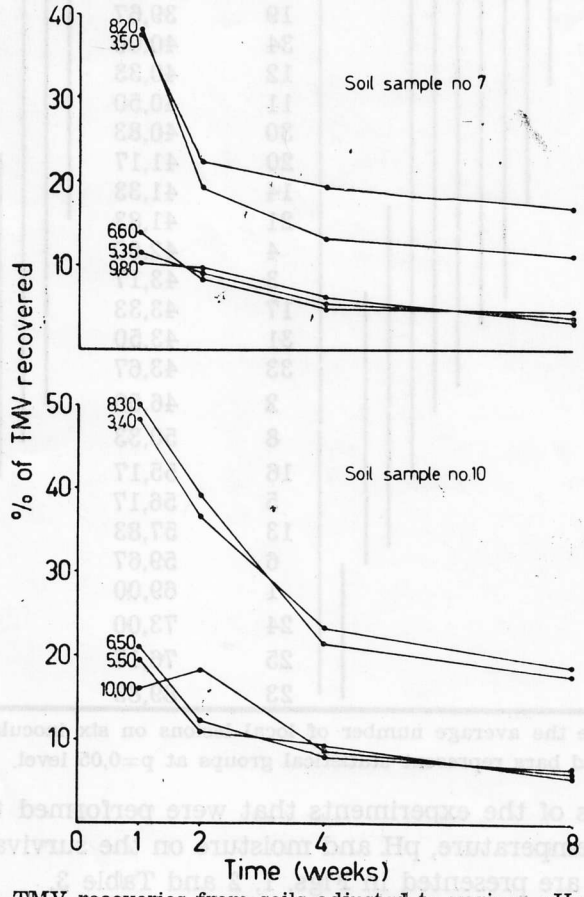

Fig.2. TMV recoveries from soils adjusted to various pH values.

Table 3. The effect of soil moisture content on TMV recovery from soils.

Soil moisture (g water) (110 g soil)	% of TMV recovered* Soil sample no. 7 Incubation period (weeks)					Soil sample no. 10 Incubation period (weeks)				
	0	1	2	4	8	0	1	2	4	8
5	100,00	33,61	22,26	11,79	6,99	100,00	41,87	22,29	7,42	9,44
10	100,00	55,00	52,07	35,42	12,07	100,00	65,37	48,04	24,04	13,51
20	100,00	58,71	63,67	36,25	20,18	100,00	55,85	48,29	29,76	17,94
30	100,00	73,36	37,14	29,68	26,18	100,00	69,25	41,62	24,54	22,37
40	100,00	72,17	49,99	38,37	23,19	100,00	66,91	53,10	20,59	23,57
50	100,00	64,12	41,43	31,01	24,54	100,00	76,82	60,12	34,17	21,05
80	100,00	82,21	67,36	45,60	38,07	100,00	84,21	54,89	39,35	40,71

* Infectivity data are expressed as mean percentages of the local lesion number on six leaves of **N. glutinosa** rubbed at time.

For the assays as to temperature, TMV-amended soils were kept atz 4°, 25° and 40°C and TMV recoveries were obtained at zero hour and afterwards at weekly intervals. As it can be seen in Fig. 1, during the two weeks of incubation the percent of recovered TMV in both of soils tested decreased more rapidly at 25°C than at either 4°C or 40°C and at 25°C TMV recoverability fell off to 2,52 and 0,75 % of the zero-hour values for two soil samples (no. 7 and 10), respectively, after 8 weeks of incubation.

To investigate the effect of pH, the original pH values of soils under test were altered by using H_2SO_4 and NaOH solutions of different normality. Later, the soils were permitted to equilibrate for 5 to 6 days. After the addition of TMV, the soils were stored at room temperature for certain weeks. As shown in Fig. 2, in general the changes on pH values caused the gradual reductions in the rates of TMV recovery and but, after the incubation period of 8 weeks more virus was recovered from both of soils at some pH values (3,50 and 8,20 for sample no. 7; 3,40 and 8,30 for sample no. 10) by comparison with those at other pH values.

To compare the rates of TMV infectivity in soils with various moisture levels, the calculated amounts of distilled water were added to the soils in glass vials and the soils were adjusted to different moisture levels on dry weight basis. Then, these soils amended with TMV were kept at 20 ∓ 2°C for 1 to 8 weeks. It follows from the date in Table 3

that the small changes in soil moisture caused large reductions in recoverable infectivity and in general, more TMV was recovered from the soils with higher moisture content. The results indicated that after 8 weeks of incubation, the rate of TMV recovery diminished faster in drier soils and when the soils were flooded the percentage of TMV recovered were 38,07 and 40,71 % for sample no. 7 and 10, respectively (Table 3).

The findings from all assays mentioned above are similar to those of some researchers (1, 2, 5, 6, 12, 13, 14, 18) who showed that the slight fluctuations in temperature, pH and moisture could greatly influence the rate of TMV survival in soils. Moreover, Cheo (5, 6) also reported that the mechanism causing the loss of virus in the soils he tested could be involved in the microbiological activity.

Considering that the possibility of some biological factors in the soilds could be responsible for their antiviral actions, further experiments were carried out in order to examine the effect of heat sterilization and the application of antibitotic and fungicide to the soils on TMV recoveries from the soils.

The soils in our study were sterilized by autoclaving at 121°C for about 1 hour in glass vials. For other treatments, streptomycin sulfate and captan were used as antibiotic and fungicide, respectively. Both of them were applied at the rates of 10 to 15 mg and 15 to 20 mg for 20 g air-dried screened soil, respectively, as suggested by Thomson (20). After the soils were brought to their water-holding capacity with the addition of distilled water as needed, TMV suspension was introduced into the vials. Following 3 weeks of incubation at 20 ∓ 2°C, TMV was recovered from soils (Table 4).

According to the results in Table 4, the recovery of TMV in autoclaved soils was higher than those in control soils and streptomycin sulfate and captan at the concentrations used in assays had less significant stabilizing influence on TMV in soils. Cheo (5) revealed that autoclaving appeared to destroy the biological activity in soils and so, this process could reduce the antiviral factors present in soils. Indeed, in a similar experiment Kegler et al. (14) reported that they failed to eliminate tomato bushy stunt virus from certain soils heated at 121°C for 2 hours.

Table 4. The effect of heat sterilization and the application of streptomycin sulfate and captan on TMV recovery from soils.

Treatments	Infectivity assay*	
	Soil sample no. 7	Soil sample no. 10
Soil (control)	17,17 a**	11,00 a
Soil (autoclaved)	27,67 b	32,83 c
Soil + Streptomycin sulfate (10 mg)	19,67 a	20,67 b
Soil + Streptomycin sulfate (15 mg)	24,50 b	18,33 b
Soil + Captan (15 mg)	18,50 a	19,00 b
Soil + Captan (20 mg)	20,33 a	21,83 b
Soil + Captan (20 mg) Streptomycin sulfate (15 mg)	20,00 a	16,33 ab

* Figures indicate the average number of local lesions on six inoculated leaves of **N. glutinosa.**

** Letters represent the statistical groups at p=0,05 level.

In tests studying the relation between the amount of TMV added to the soil and the resultant recovery rate of TMV, it was found that the percentage of TMV recovered from soils in our study rose with increasing TMV concentration in the soils after a week of incubation at 20 ∓ 2°C (Fig. 3) as also reported by Allen (1). Furthermore, Katahira and Kiriyama (12, 13) showed that when the concentration of TMV in soil increased the symptoms of infected plants appeared earlier and the percentages of diseased plants became higher.

Evidence from the present study and previous reports (1, 2, 5, 6, 12, 13, 18) brought out that when TMV was incorporated into soil its infectivity or recoverability gradually decreased depending on the levels of some factors (temperature, pH and moisture). Moreover, the results demonstrated that biological or bacterial activity could be largely responsible for reductions in infectivity. But, it is also necessary to take into consideration that the antiviral action in soil is a complicated matter and other factors such as chemical and physical forces in soil environment could also contribute to the antiviral activity. Similarly, some researchers (5, 8, 12) has indicated that soil environment may influence, both biologically and physicochemically, the adsorption and survival of viruses. Besides, Allen (2) revealed that the reduction in TMV infectivity could be associated with particle breakage and losses in and disarrangement of the capsid structure.

Although the interesting results have been obtained on the inac-

tivation of extracellular TMV in soil until now, some additional studies as to the mechanism of TMV degradation and the practical significance of the data to be acquired are required to conduct.

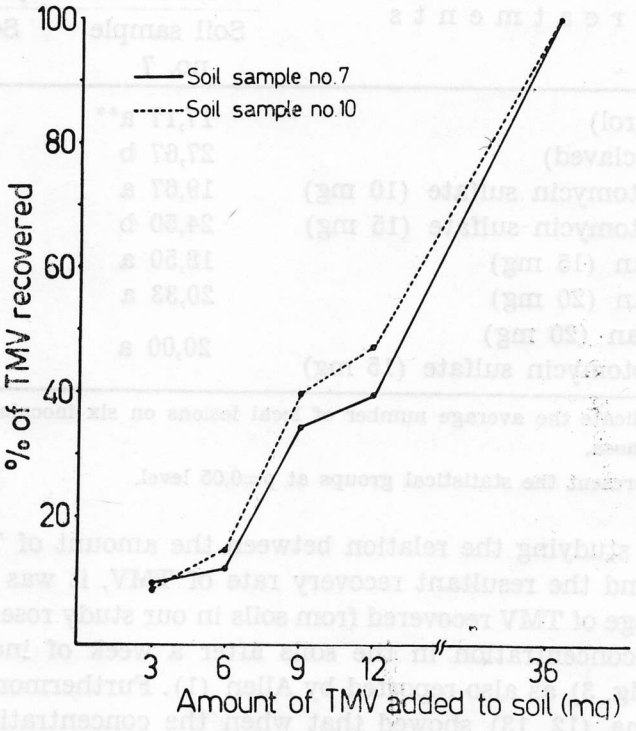

Figure 3. The relation between the amount of TMV added to soil and the recovery rate of TMV.

ACKNOWLEDGMENTS

The author would like to give his thanks especially to Dr. Murat OKTAY and I. Bülent OKUR for their kind technical assistances in the part of soil analysis of the present study.

Ö Z E T

TOPRAKTA TÜTÜN MOZAYIK VİRUSU (TMV)'NUN KALICILIĞI KONUSUNDA ARAŞTIRMALAR

Bu çalışmada tütün mozayık virusu (TMV) uygulanan topraklardan elde edilen enfeksiyöz olma yeteneğinin oda sıcaklığında 3 ve 10 günlük inkübasyon sürelerinden sonra azaldığı bulunmuştur. Sıcaklık, pH ve nem gibi faktörlerdeki küçük değişmelerin TMV'nun toprak-

lardan elde edilme oranlarında derece derece azalmalara yol açtığı gözlenmiştir. Topraklarda TMV'nun enfeksiyon oluşturma yeteneğindeki azalmanın 25°C'de 4° veya 40°C'dekine oranla daha hızlı olduğu ve çalışmadaki toprakların pH değerleri 3,40 ila 10,00 arasında değiştirildiği zaman adı geçen topraklardan daha az virus sağlandığı deneysel olarak ortaya konulmuştur. TMV'nun enfeksiyöz yeteneğinin nemli topraklarda kuru topraklardan daha seri şekilde kaybolduğu, ancak topraklar su ile doyurulduğu zaman TMV'nun daha kalıcı gibi göründüğü dikkati çekmiştir. Otoklavda sterilize edilmiş toprağa veya streptomycin sulfate ve captan içeren topraklara TMV uygulandığı zaman, bu topraklardan elde edilen enfeksiyöz TMV düzeyinin, kontrol toprağa oranla, daha fazla olduğu saptanmıştır.

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New Record

Bud Rot (**Penicillium vermoeseni** Biourge) Disease of Ornamental Palms

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It was seen that one of the palms grown as ornamental plants died due to a killing of the terminal bud and rotting of the leafstalk bases in Izmir in 1987. There were great masses of rosecoloured spores between the layers of leaves. **Penicillium vermoeseni** Biourge (**P. roseum** Link) has been isolated from the diseased leaves.

It is known that **P. vermoeseni** causes the disease on palms such as **Phoenix canariensis, Washingtonia filifera** and **Cocos plumosa** (BLISS, 1935 and 1938; MILLER, 1940; SMITH, 1940; CARPENTER et al. 1962; PROTSENKO and PROTSENKO, 1962). It was demonstrated the pathogenicity of **P. roseum** to **W. filifera** by inserting the fungus through wounds in the leaf bases of the palm (BLISS, 1938). The disease is described as producing discoloured linear streaks on the leaves of **P. canariensis.** The pinnae become yellowish brown and dying. Blister-like pustules may form on the dead leaves. The disease spreads by contact from one leaf base to another. Death of the palm occurs when the terminal bud is invaded. On **W. filifera** local necrotic areas form and enlarge rapidly on the young, tightly folded leaves in the crown of the palm. The infection spreads by contact from one folded leaf to the next one below it. Stunted and deformed leaves, retarded terminal growth, and great masses of rose-coloured spores between the layers of leaves are characteristic of the disease. On **C. plumosa** the cankers formed on the trunks may remain very inconspicuous for several years. Generally speaking, the **Penicillium** disease occurs on palms in districts with predominantly cool and moist climate.

Ö Z E T

PALMİYELERDE TOMURCUK ÇÜRÜKLÜĞÜ (**Penicillium vermoeseni** Biourge) HASTALIĞI

İzmir (1. Kordon)'de 1987 yılında yol kenarlarında süs bitkisi olarak yetiştirilen palmiyelerden birinin kuruduğu görülmüştür. Kuruyan bu ağacın büyüme noktasında pembe bir toza ve çürümeye rastlanmıştır. Ağacın büyüme noktasından alınan hastalıklı yaprak örneğinden

yapılan izolasyon sonucunda **Penicillium vermoeseni** Biourge (**P. roseum** Link) izole edilmiştir.

P. vermoeseni'nin **Phoenix canariensis, Washingtonia filifera** ve **Cocos plumosa** gibi palmiye türlerinde hastalığa neden olduğu bilinmektedir (BLISS, 1935 ve 1938; MILLER, 1940; SMITH, 1940; CARPENTER et al. 1962; PROTSENKO and PROTSENKO, 1962). Hastalık, **P. canariensis**'in yapraklarında soluk düz çizgiler oluşturmakta, yaprakçıklar sarımsı kahverengine dönüşmekte ve ölmektedir. Ölü yapraklar üzerinde kabarcıklar şeklinde püstüller oluşabilmektedir. Hastalık bir yaprağın diğerine temasıyla yayılmakta, büyüme noktası saldırıya uğradığında çürümekte ve ağacın kurumasına neden olmaktadır. Hastalık **W. filifera**'da lokal nekrotik alanlar oluşturmakta ve palmiyenin tepesindeki genç yapraklarda hızla yayılmaktadır. İnfeksiyon bir yaprağın diğerine temasıyla yayılmakta, yapraklar deforme olmakta, gelişememekte ve büyüme noktasında gelişme engellenmektedir. Yaprak tabakaları arasında gül rengindeki sporlar hastalığın karakteristiğidir. **C. plumosa**'da ise gövdede oluşan yaralar yıllarca önemsiz şekilde kalabilmektedir. Genel olarak **Penicillium** hastalığı serin ve nemli iklimin hüküm sürdüğü bölgelerde palmiyelerde önemli olmaktadır.

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NEW RECORD

New Hosts of **Eutypa lata** (Pers: Fr) Tul. in Turkey

Mualla ARI*

Eutypa lata (Pers: Fr) Tul. (Syn. **E. armeniacae** Hansf. Carter) has been only observed on grapevine so far in Turkey (ONOĞUR and ATILA, 1983). But through this study it has been come accross on plum **(Prunus domestica)** and cherry **(P. avium)** trees around the diseased vineyards in Balıkesir (Edincik-Bandırma) in May of 1987.

Pruning wound cankers, wilted and dried leaves and shoots and dieback are observed on infected branches. As the pruning wounded cankers are cut a red-brown in colour vascular necrosis wes seen. The area of necrosis horizontal and longitudinal invasion was seen easly.

Potato dextrose agar (PDA) medium has been used for isolation. Typical white mycelial colonies of **E. lata** have developed. The colonies produced the characteristic pycnidia of the imperfect form **Libertella blepbaris** (Syn. **Cytosporina** sp.) 6 weeks later.

Isolates of plum and cherry were compared with the orginal culture obtained from grapevine.

Ö Z E T

TÜRKİYE'DE (**Eutypa lata** (Pers: Fr) Tul)'NIN YENİ KONUKÇULARI

Eutypa lata (Pers: Fr) Tul. (Syn. **E. armeniacae** Hansf. Carter) Türkiye'de şimdiye kadar sadece buğda saptanmıştır. (ONOĞUR and ATILA, 1983). 1987 yılı Mayıs ayında Balıkesir (Edincik-Bandırma) ilinde söz konusu hastalığın görüldüğü bağların çevresindeki erik ve kiraz ağaçlarında da karşılaşıldı.

Hastalanmış ağaçların enfekteli dallarında, budama yara kanserleri, solmuş ve kurumuş yaprak ve sürgünler ile birlikte geriye doğru olan kurumalar gözlendi. Budama yara kanserlerinin olduğu kısımlar kesildiğinde, vaskular dokudaki kırmızımsı kahverengi nekroz görüldü. Bu nekrozlu alanın enine ve boyuna yayılması kolayca izlendi.

* Plant Protection Research Institute, Bornova, İZMİR/TURKEY

İzolasyonlar için PDA ortamı kullanıldı. **E. lata**'nın tipik beyaz renkli miselial kolonileri gelişti. 6 hafta sonra fungusun imperfekt formu olan **Libertella blepbaris** (Syn. **Cytosporina** sp.) karakteristik piknitleri oluştu.

Erik ve kirazdan elde edilen izolatlar, elimizde bulunan bağdan elde edilmiş orjinal kültür ile mukayese edildi.

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TABLE ON CONTENTS

J. Turk. Phytopathol. Volume 16 (1987)

NO. : 1

NO. : 2

NO. : 3

INDEX TO VOLUME 16

V. NATIONAL CONGRESS OF PHYTOPATHOLOGY

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