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A Suitable Medium for Production of Soluble Red Pigment by Some Strains of **Macrophomina phaseoli** (Maubl.) Ashby

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ABSTRACT

The effects of some medium ingredients, some metallic salts and some environmental conditions on the pigment production by some strains of **M. phaseoli** were investigated. The best pigment production occured on the medium which contained 30 g Potato, 1.5 g Yeast extract, 1.7 g Agar No. 3 (Oxoid, L13), 100 cc Distilled water (pH 7), in darkness, at 27°C and 3 days after inoculation.

The 10 isolates obtained from bean, pepper, potato, watermelon, chick-pea, tobacco, cotton and peanut did not produce the red coloured pigment; the 4 isolates obtained from onion, chick-pea, carnation and olive produced poorly; the 3 isolates obtained from sesame, flax and cucumber produced a dense colour on the mentioned medium under the conditions given above. It was thought that the last 3 **M. phaseoli** isolates probably belong to ssp. **sesamica.**

INTRODUCTION

An isolate of **Macrophomina phaseoli** (Maubl.) Ashby that was isolated from onion produced a soluble red pigment in PDA (Potato Dextrose Agar) under some conditions, but the two others from bean did not. It was thought that the difference in pigment production between the first isolate and the others may be one of manifestations observed easily of a variation at subspecies level.

The importance of variations in species of fungi from the point of view of phytomedicine is that, different strains of the same species can attack the culture plants at various degrees.

The 3 subspecies named as ssp. **typica**, ssp. **sesamica** and ssp. **inter-**

media in Palestine (Reichert and Hellinger, 1947) and India (Kulkarni and Patil, 1966) of a polyphagous fungus **M. phaseoli** have been determined.

Pigment production in fungi seems as a feature varying according to the conditions frequently, but pigmentation has also a hereditary aspect in many cases. Lilly and Barnett (1951) have stated that the pigments produced by fungi are determined partially by genetic factors and partially by environmental conditions; and the essential trace elements, the C and N sources, the initial pH of medium and the temperature are the important ones among the environmental factors effecting the pigment production in culture media. Leach (1971) has also stated that the blue region of spectrum and the rays near UV (ultra-violet) are effective for the stimulation of pigment production of some fungi.

The aim of this work was to study the conditions of the production of red soluble pigment which occurs only in some strains of **M. phaseoli**, and to improve a medium on which these strains will always produce pigment under the determined conditions. It was supposed that the pigmentation can be increased by adding $CaCO_3$ and yeast extract which contains abundant amino acids, vitamins and minerals to the PDA medium on which the colour takes place at times. In fact, the isolate from onion produced a dense soluble red pigment on PDA contained 1 g Yeast extract and 1 g $CaCO_3$. Subsequently, it was worked on this result obtained by chance.

MATERIALS and METHODS

A. Determining the formula of the most suitable medium for pigmentation:

1. The effects of some ingredients of medium: All the elements except agar of the medium given above were investigated one by one. The isolates SPF.1 from onion, SPF. 8 and SPF.12 from bean were used in the experiments.

For preparing the media, all of the dry materials were put into flasks. An extract obtained by boiling the potatoes in distilled water was filtered and poured upon the materials in the flasks. After sterilization for 45 minutes at 110°C, the media were poured into the Petri dishes. The media contained $CaCO_3$ were cooled down to 50-55°C after sterilization, and were agitated thoroughly to disperse the $CaCO_3$ into medium homogeneously, and then were poured into the dishes.

The inoculations were made with four replications. The results were observed after an incubation for 3-4 days at 30 (∓ 2) °C.

a. In order to determine the ef-

fect of $CaCO_3$, the 0.0, 0.5, 1.0 and 2.0 g $CaCO_3$ was added into the basal medium contained 1 g Yeast extract, 20 g Potato (peeled), 2 g Dextrose, 1.7 g Agar No. 3 and 100 cc Distilled water.

b. For determining the effect of dextrose, the 0 and 2 g of dextrose was added into the basal medium contained 1 g Yeast extract, 20 g Potato, 1.7 g Agar and 100 cc Distilled water.

c. The 0, 20, 40 and 60 g of the peeled potato (as extract) was added into the basal medium contained 1 g Yeast extract, 1.7 g Agar and 100 cc Distilled water in order to investigate the effect of potato

d. And also for determining the effect of yeast extract, the 0.0, 0.5, 1.0 and 2.0 g yeast extract was added into the basal medium contained 60 g Potato, 1.7 g Agar and 100 cc Distilled water.

2. The effects of some metallic salts: Only $CuSO_4.5H_2O$ and $FeSO_4.7H_2O$ were investigated. The salts were added into the basal medium contained 30 g Potato, 1.5 g Yeast extract, 1.5 g $CaCO_3$, 1.7 g Agar and 100 cc Distilled water, before sterilization. The final concentrations of salts were 10 ppm.

In this part of the work, the isolates SPF.1 from onion and SF.270 from cucumber were used. The inoculations were made with six replications. The results were observed after an incubation for 3 days, at 27 ($\mp$1) °C.

B. Determining the effects of temperature, visible light and hydrogen-ion concentration on pigmentation:

The isolate SF.270 from cucumber, which produced the red pigment more evidently than SPF.1 on the previous media, was used as the material of the work. The experiments were carried out by using the medium contained 30 g Potato, 1.5 g Yeast extract, 1.5 g $CaCO_3$, 1.7 g Agar and 100 cc Distilled water. The pH of media was adjusted to 6.5-7 in the experiments for determining the effects of temperature and light.

1. The experiment for the effect of temperature was carried out as five replications. The colonial growth, the formation of sclerotia, and the production of the soluble red pigment were determined at three different temperatures, 20 ($\mp$ 1) °C, 27 ($\mp$ 1) °C, 34 ($\mp$ 1) °C and, 3 and 5 days after inoculation.

2. The experiment for visible light was carried out as ten replications. The media were inoculated with the fungus. The dishes that would be kept in darkness were wrapped up in a black paper which does not transmit light. The others that would be exposed to light were not wrapped. All the dishes were placed into a irradiation cabinet where the temperature changed between 22-30°C for the duration of the experiment; and they were exposed to a visible white light emitted by two fluorescent

lamps above the 40 cm of the dishes, with 12 h intervals. The results were observed after 3 days.

3. The experiment to find out the effect of hydrogen-ion concentration was carried out as four replications. $CaCO_3$ was not used at the medium because it would be able to affect on the results. The pH of the medium was adjusted to 3, 5, 7, 9 and 11 by adding lactic acid or KOH after sterilization. The results were observed after an incubation for 3 and 5 days at 27°C.

C. Testing some isolates of M. phaseoli for the soluble red pigment productuon:

The 17 isolates from various plants were tested. The test was carried out as three replications, and the medium on which the effect of temperature had been experienced was used. The pH of medium was 6.5. The results were observed after an incubation for 4 days, at 27 ($\mp$1)°C, in darkness.

RESULTS

A. The most suitable medium for pigmentation:

1. The effects of some ingredients of medium:

a. $CaCO_3$: The pigment production occurred equally in all the agar plates on which the isolate SPF.1 had been planted, without in connection with amount of $CaCO_3$ in the medium, whereas no pigment production was observed in the plates on which SPF.8 and 12 had been planted as check.

b. Dextrose: There were not any difference between characters in point of the formation of the red pigment. The soluble red pigment could be observed better in the plates without dextrose because less sclerotia were produced.

c. Potato: The pigment was not observed in the plates on which SPF.8 and 12 had been planted. In the other plates on which SPF.1 had been planted, the red pigment was not produced at those without potato, but both red pigment and sclerotia were produced increasingly in correlation with the increasing amounts of potato at those contained potato (Table 1).

d. Yeast extract: SPF.1 didn't produce the soluble red pigment in the agar plates which did not contain yeast extract, whereas the same isolate produced pigment increasingly in correlation with the increasing amounts of yeast extract at the other plates contained yeast extract (Table 2).

2. The effects of $CuSO_4$ and $FeSO_4$:

There was not any difference from the point of the red pigment production between the plates with and without salts.

B. The effects of temperature, visible light and hydrogen-ion concentration:

1. Temperature: A typical red colour took place at 27°C while pig-

Table 1. The pigmentation of the isolates of **M. phaseoli** on the media contained potato in increasing amounts

		The amounts of potato in 100 cc medium			
	Isolates	0 g	20 g	40 g	60 g
Pigment production	SPF.1	—	+	+	++
	SPF.8	—	—	—	—
	SPF.12	—	—	—	—

— : No pigmentation
\+ : Moderate »
++ : Dense »

Table 2. The pigmentation of the isolate SPF.1 of **M. phaseoli** on the media contained yeast extract in increasing amounts

	The amounts of yeast extract in 100 cc medium			
	0 g	0.5 g	1 g	2 g
Pigment production	—	+	++	++

— : No pigmentation
\+ : Moderate »
++ : Dense »

ment was not produced at 20°C and 34°C, after an incubation for 3 days. But the colonial diameter of fungus expanded increasingly from 20°C towards 34°C. After an incubation for 5 days, same typical red colour took place in the plates at 20°C while the colour produced in the plates at 27°C became pale, but to pigmentation was observed at 34°C (Table 3).

2. Visible light: A pale red colour took place in the agar plates exposed to light, while a typical red colour took place normally at those in the darkness.

3. Hydrogen-ion concentration: The results have been given in Table 4.

As it will be clear from table, the initial pH of medium has been very

Table 3. The effect of temperature on the pigmentation, the colonial growth and the sclerotia production of the isolate SF.270 of **M. phaseoli**

	3 days after planting			4 days after planting		
	20°C	27°C	34°C	20°C	27°C	34°C
Pigmentation	—	++++	—	++++	++	—
Colonial growth	+	++	+++	++	+++	++++
Sclerotia produc.	—	+	+	+	++	—

— : No
\+ : Poor
++ : Moderate
+++ : Good
++++ : Abundant

The colonial growth and sclerotia production accompanied with the best pigmentation have been taken into the box.

Table 4. The pigmentation, the colonial growth and the sclerotia production of the isolate SF.270 at the five different pH degrees after an incubation for 3 days at 27°C

	pH DEGREES				
	3	5	7	9	11
Pigmentation	—	—	++++	—	—
Colonial growth	—	+++	++++	++	+
Sclerotia production	—	+	++	—	—

— : No
\+ : Poor
++ : Moderate
+++ : Good
++++ : Abundant

The colonial growth and sclerotia production accompanied with the best pigmentation have been taken into the box.

effective on the soluble red pigment production. The pigment has been produced densely only in the agar plates that pH were adjusted to 7, but no pigment production has occurred at the other pH degrees.

On the other hand, in consequence of an incubation for 5 days, a very poor red colour took place in the plates adjusted to pH 5, whereas the dense colour in the plates adjusted to pH 7 was becoming pale gradually.

If the medium determined befo-

rehand by starting from a supposition is reviewed again in the presence of these new knowledges obtained from the experiments, it will be clear easily that the soluble red pigment which is produced by some strains of **M. phaseoli** takes place best on the medium adjusted its pH to 7 and formulated:

Potato (peeled)	30.0 g
Yeast extract (Oxoid, L21)	1.5 g
Agar No. 3 (Oxoid, L13)	1.7 g
Distilled water	100.0 cc

at 27°C, in darkness, and after an incubation for 3 days. One should not be late for observation. This incubation period can change depending on temperature and the same results can be also taken after an incubation for 5 days at 20°C.

C. The cases of some isolates of **M. phaseoli** from the point of the soluble red pigment production:

The 10 out of 17 isolates which tested on the medium given above didn't produce the red pigment under the conditions mentioned above. The 4 isolates produced pigment moderately. The other 3 isolates produced a dense red pigment (Table 5).

Table 5. The cases of the 17 isolates of **M. phaseoli** from the point of view of the soluble red pigment production

NUMBER OF ISOLATE	THE PLANT FROM WHICH THE ISOLATE WAS OBTAINED	THE SOLUBLE RED PIGMENT PRODUC.
SPF.1	Onion (**Allium cepa L.**)	+
SPF.8	Bean (**Phaseolus vulgaris L.**)	—
SPF.12	» » »	—
SF.268	Pepper (**Capsicum annuum L.**)	—
SF.269	Potato (**Solanum tuberosum L.**)	—
SF.270	Cucumber (**Cucumis sativus L.**)	++
SF.271	Watermelon (**Citrullus vulgaris L.**)	—
SF.272	Chick-pea (**Cicer arietinum L.**)	—
SF.273	» » »	+
SF.275	Tobacco (**Nicotiana tabacum L.**)	—
SF.276	Cotton (**Gossypium hirsutum L.**)	—
SF.277	» » »	—
SF.278	Flax (**Linum usitatissimum L.**)	++
SF.279	Ground nut (**Arachis hypogaea L.**)	—
SF.280	Sesame (**Sesamum indicum L.**)	++
SF.281	Clove (**Dianthus caryophyllus L.**)	+
SF.282	Olive (**Olea europaea L**)	+

— : None
\+ : Moderately
++ : Densely

DISCUSSION

The initial pH of the medium and the incubation temperature have been found effective absolutely for the soluble red pigment production of **M. phaseoli.** These results are in conformity with those given at literature by Lilly and Barnett (1951). However, it seems as if temperature and pH effect indirectly, not directly, on the pigment production by influencing the sclerotia production. If the Table 3 and 4 is viewed, it will be understood easily that there is a relation between the pigmentation and the sclerotia production of the fungus; the pigmentation does not occur in absence of the sclerotia and the red pigment appears densely only when the first sclerotia have been seen, and then becomes paler gradually.

Leach (1971) has stated that the blue region of spectrum and the rays near UV are effective for stimulation of pigment production of some fungi. A result opposite to this was obtained in this work wherein the visible white light was used. This may be resulted probably from the breaking down of the red coloring matter by some rays in visible white light.

Cu and Fe given as their sulphate salts have not been effective remarkably on the red pigment production of **M. phaseoli,** though it has been stated that. Cu, Fe and some trace elements were effective on the pigmentation of some fungi according to some literature records (Lilly and Barnett, 1951; Bora, 1973).

It is not possible to say anything about whether the positive effectiveness of Potato and Yeast extract on the red pigment production is due to the features of C and N sources, or because of the vitamin and minerals in these materials. This problem can be solved only by an analytical work.

The importance of the medium determined, from the point of view of the taxonomy at subspecies level of **M. phaseoli** is that:

Reichert and Hellinger (1947) have reported that **Sclerotium bataticola (M. phaseoli)** is separated into three subspecies; ssp. **typica** is pathogenic on bean and comprises the isolated from bean, tomato, pepper, potato, egg-plant, squash and chick-pea; ssp. **sesamica** is non-pathogenic on bean and it is represented only by the isolate from sesame; ssp. **intermedia** is also pathogenic on bean and comprises the isolates from tobacco and cotton. Kulkarni and Patil (1966) have also reported that it was decided to classify in ssp. **intermedia** the isolates from castor bean, cotton and groundnut, and in ssp. **sesamica** an isolate from sesame. When the Table 5 is viewed carefully, it will be seen at once that the isolates from bean, pepper, potato, watermelon, chick-pea (one of isolates), tobac-

co, cotton and groundnut have not produced pigment, but the isolates from sesame, flax and cucumber have produced red pigment densely; and there is a similarity between the hosts of the isolates produced red pigment or not in Table 5 and the hosts of the isolates reported to belong to ssp. **sesamica**, ssp. **intermedia** and ssp. **typica**. By deciding from this, it can be said that the isolates belonged to ssp. **sesamica** produce the soluble red pigment and the others do not.

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ÖZET

Macrophomina phaseoli (Maubl.) Ashby'NİN BAZI IRKLARININ SUDA ERİR KIRMIZI PİGMENT OLUŞTURMASINA UYGUN BİR ORTAM

M. phaseoli'nin bazı ırklarının kırmızı pigment oluşumu üzerine bazı ortam unsurlarıyla, bazı metal tuzları, sıcaklık, ışık ve hidrojen iyon konsantrasyonunun etkileri araştırıldı ve en iyi renk oluşumunun 30 g Patates, 1.5 g Yeast extract ,1.2 g Agar, 100 ml Damıtık su ihtiva eden, pH'sı 7'ye ayarlı ortamda, 27°C sıcaklıkta, karanlıkta ve 3 günlük bir inkubasyon sonunda ortaya çıktığı belirlendi.

Verilen ortam üzerinde ve belirtilen şartlarda sınanan 17 **M. phaseoli** izolatından fasulye, biber, patates, karpuz, nohut, tütün, pamuk ve yerfıstığından elde edilen 10 tanesinin pigment oluşturmadığı; soğan, nohut, karanfil ve zeytinden elde edilen 4 tanesinin orta derecede; susam, keten ve hıyardan elde edilen 3 tanesinin ise oldukça yoğun renk oluşturduğu belirlendi. Son üç izolatın muhtemelen ssp. **sesamica** alttürüne ait olabilecekleri kanısına varıldı.

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Sensitivity to Benzimidazole Compounds in **Fusarium oxysporum** f.sp. **cucumerinum**[1]

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ABSTRACT

Totally 71 **Fusarium oxysporum** f.sp. **cucumerinum** isolates from Ege, Mediterranean and Marmara regions of Turkey were tested. According to these tests, resistance levels of the isolates to carbendazim, thiophanate-methyl and benomyl varied from 0,5 µg/ml to 4,0 µg/ml, 80,0 µg/ml to 1600 µg/ml and 2,0 µg/ml to 20,0 µg/ml a.i. respectively.

By the **in vivo** trainings it was demostrated that, sensitivity of the pathogen decreased gradually after the continious applications of the benzimidazole derivatives.

INTRODUCTION

The south and south-west coastal areas of Turkey have many vegetable greenhouses. Cucumber is one of the main crop of these places (13). But this economically important crop is subjected mainly to the wilt disease (24, 25). The disease causes great losses especially in the continiously cucumber growing greenhouses (25). According to an early study (25), the wilt pathogen was determined as **Fusarium oxysporum** f.sp. **cucumerinum** in Turkey.

Benomyl and carbendazim, from benzimidazoles, are the suggested chemicals for controlling **Fusarium** wilt in the different countries (2, 10). Results of the previous studies (7, 16) has revealed that benzimidazoles especially benomyl and carbendazim were also effective on the

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pathogen in Turkey. However, continuous applications of the benzimidazole derivatives could immerge the resistant isolates of some pathogens (3, 4, 22), and moreover resistance of **F. oxysporum** f.sp. **cucumerinum** to benzimidazoles **in vitro** conditions was also reported (7). Therefore in the present study it was attempted to find out the sensitivity in **F. oxysporum** f.sp. **cucumerinum** isolates to benzimidazoles, and to point out variations in sensitivity of the pathogen after continious applications of these chemicals **in vivo**.

MATERIALS and METHODS

Three benzimidazole derivatives carbendazim (Derosal, 57,6 % WP Türk-Hoechst), thiophanate-methyl (Enovit Super, 70 WP, Tarkim) and benomyl (Benlate WP 50 %, Timtas) were used in the tests. **Cucumis sativus** cv. «DERE» plants which were sensitive to **Fusarium** wilt (25) were used for **in vivo** studies.

In order to obtain the **F. oxysporum** f.sp. **cucumerinum** isolates wilted cucumber plants were collected from the cucumber growing greenhouses of Ege and Mediterranean regions, and also from the outdoor cucumber growing fields of Marmara region. Isolations were done according to MARTIN (14), and **F. oxysporum** isolates were selected among the isolates of **Fusarium** spp. (1, 9, 18, 25). To differentiate **F. oxysporum** f.sp. **cucumerinum** isolates their pathogenicities were tested in cucumber plants (9, 17, 18, 25).

The sensitivity of the isolates to benzimidazole derivatives were determined according to their colonial growths on the fungicide containing P.D.A. The measurements were done 6 days after the inoculations.

For testing the appearance possibility of resistance in the pathogen after continuous applications of benzimidazoles 12 **in vivo** trainings were done according to KOVACS and TÜSKE (11). The trainings were began from 500 µg/ml a.i. for each fungicide and concentrations were raised gradually up to 750 µg/ml a.i. and so 12 applications were done. By the end of the applications sensitivity of each isolate was tested on fungicide containing P.D.A.

In vitro tests were done at 26°C, in dark. But **in vivo** tests were conducted in the pots which were kept at 28°C and alternating light (15 h light - 9 h dark) conditions. To prevent the bacterial growth 50 µg/ml penicillin and 50 µg/ml streptomycin were added to PDA, following to sterilization (1). All the experiments were done according to the randomize plot design. Concentrations of the fungicides were arranged as to DELEN and YILDIZ (7).

RESULTS

Isolations made from the wilted cucumber plants which were collected 43 greenhouses, and 9 cucumber fields of outdoor, has yielded 204 fungal cultures, and 191 of those determined as **Fusarium** sp. Remaining 12 cultures were **Cylindrocarpon** sp., **Macrophomina** sp. and **Trichoderma** sp., but 13th culture was identified as **Verticillium dahliae.** Pathogenicity tests with the latter isolate resulted in causing severe wilt disease in cucumbers.

Ninety **F. oxysporum** isolates were determined from 191 **Fusarium** sp. After the pathogenicity tests it was concluded that 63 of them were **F. oxysporum** f.sp. **cucumerinum** Geographical distribution of **F. oxysporum** f.sp. **cucumerinum** isolates were given in the Table 1.

TABLE 1. Geographical distribution of **F. oxysporum** f.sp. **cucumerinum** isolates

Sampling Places		Number of obtained isolates	Total of Region
Region	Province		
Mediterranean	Mersin	5	13
	Antalya	8	
Ege	Muğla	9	48
	Aydın	1	
	İzmir	38	
Marmara	Bursa	2	2
Total			63

According to Table 1, the pathogen was very intensive in the greenhouses of İzmir.

The resistance levels of 8 **F. oxysporum** f.sp. **cucumerinum** isolates previously obtained by YILDIZ and DELEN (25) were tested together with these 63 isolates. Resistance levels of 71 isolates were summarized in the Table 2.

TABLE 2. Resistance levels of 71 F. oxysporum f.sp. cucumerinum isolates to three benzimidazole derivatives

Fungicide	Resistance level of the Isolates (μg/ml)	Number of the Isolates	Total
Carbendazim	0,5	13	71
	1,0	54	
	2,0	3	
	4,0	1	
Thiophanate-methyl	80,0	3	71
	100,0	6	
	200,0	11	
	400,0	18	
	800,0	27	
	1000,0	5	
	1600,0	1	
Benomyl	2,0	3	71
	4,0	53	
	20,0	15	

It can be seen from the Table 2, carbendazim and benomyl are more effective chemicals then thiophanate-methyl.

Distribution of the isolates according to their resistance levels were shown in the Fig. 1.

To find out the appearance possibility of the resistant isolates of the pathogen, 12 continious applications were done, and then sensitivity of the isolates were determined in the laboratory (Table 3).

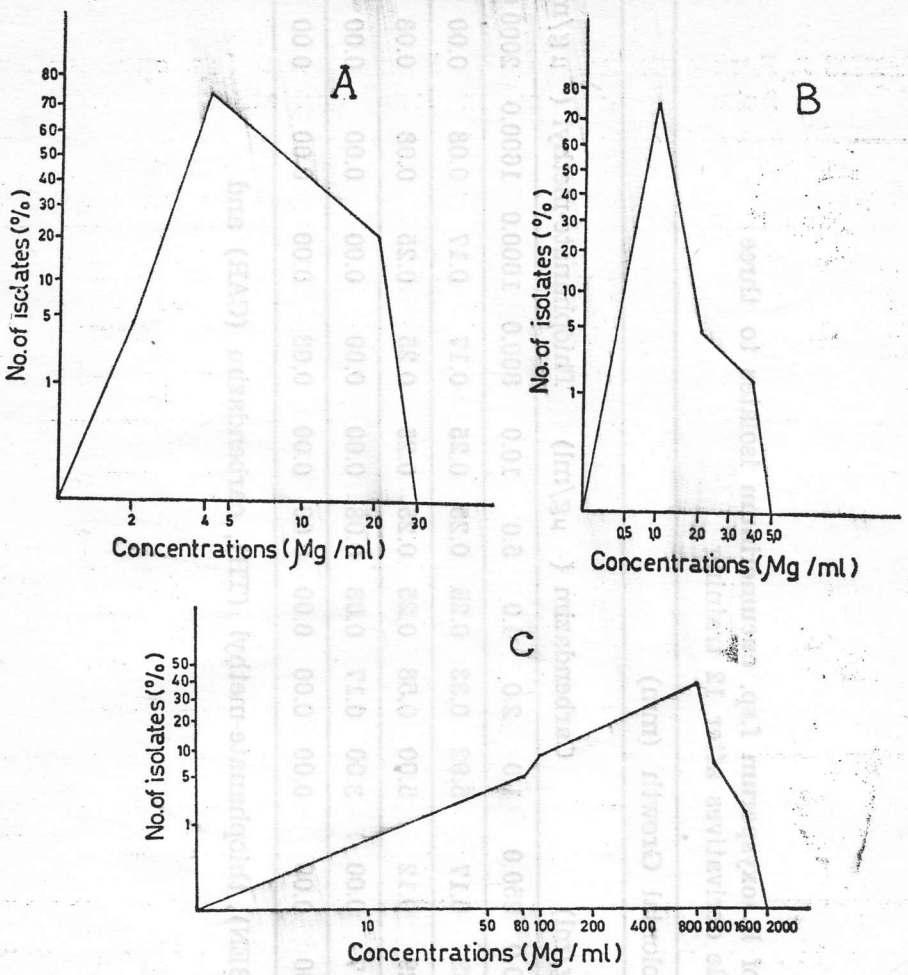

Fig. 1. Distribution of 71 **P. oxysporum** f.sp. **cucumerinum** isolates according to their resistance to benomyl (A), carbendazim (B) and thiophanatemethyl (C).

DISCUSSION

As the results of the samplings and isolations, incidence of **F .oxysporum** f.sp. **cucumerinum** was more often in İzmir as reported by YILDIZ and DELEN (25). Although the variations in the resistance levels of the isolates and existence of some highly resistant isolates (Table 2), it was considered that, a resistant population of the pathogen was not built yet, as reported by DEKKER (5), SAKURAI (19), WOLFE (23) (Fig 1). Moreover, benomyl and carbendazim was more effective fungicides to the pathogen than thiophanatemethyl, and this result agree with DELEN and YILDIZ (7).

According to **in vivo** trainings it can be said that, the pathogen can acquire resistance after continuous applications of the benzimidazole compounds. On the other hand, **in vitro** (7, 15, 20) and **in vivo** (12, 21) resistance of **F. oxysporum** isolates

TABLE 3. Sensitivity of **F. oxysporum** f.sp. **cucumerinum** isolates to three benzimidazole derivatives after 12 training

Isolate*	Colonial Growth (mm)														
	Control (μg/ml)	Benomyl (μg/ml)					Carbendazim (μg/ml)					Thiophanate-methyl (μg/ml)			
	0.0	20.0	100.0	150.0	200.0	250.0	1.0	2.0	4.0	5.0	10.0	800.0	1000.0	1600.0	2000.0
BEN	39.85	1.75	0.62	0.13	0.33	0.17	5.92	0.33	0.25	0.25	0.25	0.17	0.17	0.08	0.00
TH	40.65	1.00	0.58	0.37	0.29	0.12	5.00	0.58	0.25	0.25	0.25	0.25	0.25	0.08	0.08
CAR	36.45	1.17	0.33	0.17	0.17	0.00	3.00	0.17	0.08	0.08	0.00	0.00	0.00	0.00	0.00
ORIG	37.50	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00

* Isolate continuously treated by benomyl (BEN), thiophanate-methyl (TH), carbendazim (CAR) and only by water (ORIG).

to benzimidazole derivatives were reported. Because of this ability of the pathogen for preventing to buil dup a resistant population of F. **oxysporum** f.sp. **cucumerinum** in the country, benzimidazole compounds should not be used continiously, and the sensitivity of the isolates should be tested periodically (6, 8).

ÖZET

Fusarim oxysporum f.sp. cucumerinum'UN BENZİMİDAZOLE BİLEŞİKLERİNE DUYARLILIĞI

Ege ve Akdeniz Bölgelerinde hıyar yetiştirilen seralardan ve Marmara Bölgesinde açıkta hıyar tarımı yapılan bahçelerden elde edilen **F. oxysporum** f.sp. **cucumerinum** izolatlarının dayanıklılık düzeyleri, etkili maddelere göre, carbendazim'de 0,5 µg/ml ile 4,0 µg/ml, thiophanate-methyl'de 80,0 µg/ml ile 1600,0 µg/ml ve benomyl'de ise 2,0 µg/ml ile 20,0 µg/ml arasında bulunmuştur.

Yapılan **in vivo** alıştırmalar, benzimidazole grubu fungisidlerin sürekli kullanımıyla etmenin duyarlılığının yavaş yavaş azalacağını ortaya koymuştur.

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Monitoring Wheat Root and Foot Rots in Central Anatolian Region of Turkey

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ABSTRACT

Root and foot rots of wheat are often observed in high rainy and wet places, but in Central Anatolia the disease can cause considerable damage in spite of limited rainfall.

The results of «The National Winter Cereals Research Project» has revealed that **Helminthosporium** spp. and **Fusarium** spp. were most prevalent agents of rot diseases and followed by **Gaeumannomyces graminis** var. **tritici** and **Pseudocercosporella herpotrichoides**. Highly resistant varieties are not available yet. Most affected varieties were Bezostaya, Bolal 2973 and Kunduru.

Destruction of diseased plant debris, late fall sowing and rotation with legume crops are practical and economic control measures.

INTRODUCTION

Wheat diseases often encountered in the semi-arid regions such as Central Anatolia are closely associated with seed, soil, temperature and available moisture. The Central Plateau normally receives 200 to 450 millimeters of precipitation annually. In areas with less than 400 mm of precipitation, fallow system is practiced by farmers. In the areas with better than 400 mm rainfall, the practice of annual wheat cropping is also common. As expected, root and foot rots can cause severe damage in the higher rainfall areas with annual cropping, but more unexpected can also cause considerable damage in the lower rainfall area (1, 3, 6) where the environment maybe are thought to be unfavorable to these organisms.

Cultivars highly resistant to root and foot rots are not available, but

some degree of tolerance has been identified in certain cultivars. Land race varieties, old varieties, and local cultivars that have been grown in Central Plateau were tall with low yield potential, but with little year to year fluctuation in performance. The lodging in these cultivars has always been thought to be caused by their height and weak straw. Causes other than those factors such as root and foot rots, and losses due to those diseases has never been seriously considered.

After 1970, some high yielding wheat cultivars were released and their acreages significantly increased in last decade. Bezostaya is the one that spread over a wide areas of Thrace, Central Anatolia and its transitional zones (7).

In 1977-78 growing season a severe epidemic, caused by root and foot rot pathogens, was experienced in the Thrace region (2, 4) where 'Bezostaya' had become almost the only cultivar grown by farmers. In 1979, a winter wheat cultivar was released by Edirne Research Institute because of its tolerance to foot rots and named «Kırkpınar 79» (2). To prevent destructive epidemics and to minimize losses caused by disease, a reliable disease surveillance program would help make control measures effective and economic. «The National Winter Cereals Research Project» has a disease surveillance program which is concerned with all factors affecting wheat production (4).

Results of surveys and observations made on disease nurseries (5) and laboratory works, done during 1982-83 and 1983-84, are presented here.

MATERIALS and METHODS

Observation was made on the «Cereal Diseases Trap Nurseries», and on the «Cereal Diseases Observation Nurseries» planted nation and region-wide, respectively. Observations were also made on demonstration plots of the agricultural extension service, on commercial fields and on state farms in Central Anatolia. Plant collections which showed typical symptoms of lower stem and/or root rots made from province Ankara, Çankırı, Çorum, Eskişehir, Yozgat, Sivas, Konya and Kırşehir. All plant collections were brought to the Central Anatolian Regional Research Institute's plant disease diagnosis laboratory to verify. Collections were categorized according to the given name of cultivars and variety or given number of lines, and location of province. Each collection was divided into two parts. First part was placed in moist chambers overnight. Second part was plated in PDA for 4-7 days and verification was made under microscope on prepared samples from those two parts.

RESULTS and DISCUSSION

Observations made on material either on field or in laboratory indicated that common dryland foot and root rots, caused by **Helminthosporium** spp. and **Fusarium** spp. together, was predominant in Central Anatolian Region and its transitional zones. Take-all caused by **Gaeumannomyces graminis** var. **tritici** and eyespot caused by **Pseudocercosporella herpotrichoides** were often observed.

Common dryland foot and root rots were prevalent in the province Ankara, Yozgat, Çorum, Çankırı, Eskişehir, Kırşehir, Sivas and Konya respectively. Take-all was found in Ankara, Çankırı, Çorum, Eskişehir and Konya. Eyespot was seen in Ankara and Çankırı provinces.

Root and foot rots were destructive on the bread wheat cultivar Bezostaya, particularly where drought or moisture shortage were in evidence. Bolal 2973, and the durum wheat cultivar Kunduru 1149 were affected severely in many locations.

Our breeding efforts is concentrated on the development of cultivars which have stable resistance or at least some level of tolerance to root and/or foot diseases. Until some resistant or tolerant cultivars, with high yield and quality, are released to replace current susceptible ones, some cultural methods can be recommended. The disease level can be kept low and yield losses can be minimized with the destruction of diseased plant debris, late fall planting and rotation with a legume crop. Those control measures is believed to be most practical and economical in the problem areas of Central Anatolian region.

Ö Z E T

TÜRKİYE'DE ORTA ANADOLU BÖLGESİNDEKİ BUĞDAY KÖK VE DİP ÇÜRÜKLÜKLRİNİN GÖZLENMESİ

Daha çok, yağışı yüksek yerlerde görülen kök ve dip çürüklükleri Orta Anadolu gibi yağışı sınırlı bölgelerde de gözden uzak tutulamayacak zararlar meydana getirebilmektedir. Buğday kök ve dip çürüklüklerine yüksek derecede dayanıklı çeşitler henüz bulunmamaktadır. Zarar verici epidemilerin önüne geçebilmek ve verimde meydana gelecek düşmeleri azaltabilmek amacıyla uygulanacak kontrol metodlarının ekonomik ve etkili olması için Ülkesel Serin İklim Tahılları Araştırma Projesi içinde survey çalışmaları yapılmıştır. Çalışmalar sonucu **Helminthosporium** spp. ve **Fusarium** spp. etmenli çürüklüklerinin en yaygın olduğunu bunu **Gaeumannomyces graminis** var. **tritici** ve **Pseudocercosporella herpotrichoides** etmenli çürüklüklerin izlediği

bulunmuştur. Çürüklüklerden en fazla etkilenen ekmeklik buğdaylar Bezostaya ve Bolal 2973 olurken makarnalık buğdaylar içinde en fazla zarar gören Kundura 1149 olmuştur.

Dayanıklı çeşitler geliştirilene kadar, çürüklüklerle mücadelede bitki artıklarının yok edilmesi, geç ekim yapılması ve baklagillerle ekim nöbetine gidilmesi Orta Anadolu Bölgesinin bu probleme sahip bölgeleri için en pratik ve ekonomik yol olarak gözükmektedir.

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Preliminary Studies on the Distribution and Incidence of the Agents Causing Diseases in Soybean Growing Areas on the Blacksea Region of Turkey

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ABSTRACT

A survey of Soybean fields was carried out between June 20, 1983 and October 11, 1983 in the Black Sea Region of Turkey.

The results showed that 1.74 % of the soybean plants at seedling stage were infected with Alternaria Leaf Spot (**Alternaria** spp.) and 0.59 % of those were infected with the seedling diseases (**Sclerotium rolfsii, Fusarium** spp., **Pythium** spp. and **Rhizoctonia** spp.) in Samsun, while 0.71 % of the soybean plants were infected with the seedling diseases in Ordu.

The soybean plants at flowering stage were infected with Downy Mildew (**Peronospora manshurica**), Alternaria Leaf Spot, Southern Stem Blight (**Sclerotium rolfsii**), Charcoal Rot (**Macrophomina phaseoli**) and Soybean Mosaic Virus at the rate of 25.94 %, 2.54 %, 0.11 %, 0.02 % and 2.05 % respectively in Samsun, while those at this stage were infected with Downy Mildew and Soybean Mosaic Virus at the rate of 9.14 % and 0.28 % respectively in Ordu.

The soybean plants at mature pod stage were infected with Downy Mildew, Alternaria Leaf Spot, Southern Stem Blight, Stemphylium Leaf Spot (**Stempylium botryosum**), Soybean Mosaic Virus and Yellow Mosaic Virus at the rate of 38.91 %, 2.45 %, 0.05 %, 3.13 % and 0.22 % respectively in Samsun.

INTRODUCTION

In recent years, soybean (**Glycine max** (L.) Merr.) production increased in Turkey since it is an important crop because of its high vegetable oil and protein contents. Hence, while soybean growing areas in Samsun were 1761 ha in 1980, they increased 2742 ha in 1982 (ANONYMUS, 1982 a).

It is recorded that more than a hundred agents 35 ones of which are of economical significance, cause diseases on Soybean, (ANONYMUS, 1982 b).

In current years, a little work has been carried out on the agents causing diseases of soybean in Turkey because the cultivation of this crop gained importance in last few years in this country. There is only a record about **Peronospora manshurica** (AYAYDIN, 1972).

Therefore, this study has been conducted to determine the agents causing diseases of this crop.

MATERIALS and METHODS

A survey was carried out in Samsun and Ordu provinces where soybeans are being grown to a largest extent in the Black Sea Region of Turkey. The size of soybean growing area in each county of both province, the numbers of samples taken and the numbers of plants inspected are shown in Table 1.

Table 1. The soybean growing areas surveyed in the Black Sea Region.

Province	County	Soybean growing area (ha)	Number of samples	Number of soybean plants inspected
Samsun	Central	5	2	200
	Bafra	20	2	200
	Terme	800	10	1000
	Çarşamba	1900	21	2100
Ordu	Ünye	500	7	700

Each county was considered as an unit. Two fields were chosen for soybean growing areas up to 50 ha, 3 fields for those of 50-100 ha and one extra field was taken for each 100 ha in those of larger than 100 ha. Each field was 5-10 da and totally 100 soybean plants, as being 20 in each corner and 20 in the center of the field, were inspected. The plants infected with the diseases that could not be identified in the field brought to the laboratory for identification. Isolation studies were made using PDA medium and infected plants were sterilized with 0.5 % NaOCl. Percentage of infected soybean plants were found by the field inspections. The severity of the disease was determined for only Soybean Downy Mildew along with percentage of infected plants. The severity of the disease was detected on the basis of GRAINGER's 0.5 - 80 % scale (BORA and KARACA, 1970).

RESULTS and DISCUSSION

Surveys were carried out in three development stages of soybean, being at seedling stage between June 20, 1983 and June 30, 1983; at flowering stage between July 25, 1983 and August 5, 1983 and at mature pod stage between September 26, 1983 and October 11, 1983. As it is shown in Table 2 the rate of diseases (**Sclerotium rolfsii** Sacc., **Fusarium** spp., **Pythium** spp. and **Rhizoctonia** spp.) occurring at the seedling stage was 0.59 % in Samsun and 0.71 % in Ordu. It appears that the diseases occurring at the seedling stage were not at an important level. The results of a survey in U.S A. revealed that % 90 of soybean plants were infected with root rot caused by both **Fusarium** spp. and **Rhizoctonia** spp. (FRENCH and KENNEDY, 1964). Furthermore, it is recorded that **Pythium** spp. cause damping-off on soybeans (BROWN and KENNEDY, 1966; SINGLER, 1977), **Fusarium** spp. give rise to fusarium blight of soybean seedlings (DUNLEAVY, 1962). More over it was found that **Rhizoctonia solani** was responsible for root rots (WILLIE, 1962; TACHIBANA, 1968) and seedling root rots were caused by **S. rolfsii** (EPPS et all., 1951).

Alternaria leaf spot (**Alternaria** spp.) has been determined at three stages of soybean, namely seedling, flowering and mature pod stages, at the rate of 1.74 %; 2.54 % and 2.45 % respectively (Table 2).

Table 2. Soybean diseases and their rates that were determined at seedling, flowering and mature pod stages in soybean growing areas in counties of Samsun and Ordu in 1983.

Phenological Stage of Soybean	Names of the Diseases	Average incidence of the diseases (%)					
		SAMSUN					ORDU
		Central	Bafra	Terme	Çarşamba	Well-balanced mean	Ünye
Seedling Stage	Alternaria Leaf Spot	5.50	0.00	1.00	1.90	1.74	0.00
	Seedling Diseases	4.00	0.00	0.50	0.38	0.59	0.71
Flowering Stage	Downy Mildew	13.50	0.00	42.30	21.80	25.94	9.14
	Alternaria Leaf Spot	10.50	0.00	1.40	2 57	2.54	0.00
	Southern Stem Blight	2.00	0.00	0.01	0.00	0.11	0.00
	Charcoal Rot	0.50	0.00	0.00	0 00	0.02	0.00
	Soybean Mosaic Virus	11.50	0.00	1.70	1.52	2.05	0.28
Mature Pod Stage	Downy Mildew	25.50	0.00	44.70	41.14	38.91	8.85
	Alternaria Leaf Spot	12.50	0.00	0.90	2.47	2.45	0.00
	Southern Stem Blight	2.50	0.00	0.01	0.04	0.16	0.00
	Charcoal Rot	0.00	0 00	0.00	0.00	0.00	0.00
	Stemphylium Leaf Spot	1.00	0.00	0.00	0.00	0.05	0.00
	Soybean Mosaic Virus	16.50	0.00	2.50	2.47	3.13	0.71
	Yellow Mosaic Virus	1.50	0.00	0.10	0.19	0.22	0.00

On the other hand Southern Stem Blight (**S. rolfsii**), Charcoal Rot (**Macrophomina phaseoli** (Maubl.) Ashby) and Soybean Mosaic Virus were determined at the rate of 0.11 %, 0.02 % and 2.05 % at flowering stage and 0.16 %, 0.00 % and 3,13 % at the mature pod stage respectively in Samsun. On the contrary only the incidence of Soybean Mosaic Virus in Ordu was found as 0.28 % and 0.71 % respectively for the both stages of Soybean.

Moreover, Yellow Mosaic Virus and Stemphylium Leaf Spot (**Stemphylium botryosum** Wallr.) were determined at mature pod stage at the rate of 0.05 % and 0.22 % respectively in Samsun.

There are many published papers on soybean diseases which are caused by **S. rolfsii** (EPPS et all., 1951), **M. phaseoli** (THIRUMALACHAR et al., 1953; SINGLER, 1977; ACIMOVIC, 1964), Soya Mosaic Virus (IRWIN and SCHULTZ, 1981) and Yellow Mosaic Virus (SINGH et al., 1974). But no record as to presence of **S. botryosum** on soybeans is available.

The most important soybean disease in the region was Downy Mildew of Soybeans which was determined at flowering and mature pod stages at the rate of 25.94 % and 38.91 % respectively in Samsun, while at the rate of 9.14 % and 8.85 % respectively in Ordu. However, this disease was found to be at the rate of 100 % in Çangallı, Fatmagölü and Kırıklar villages in Terme county of Samsun province. The severity of the disease was 23.80 %, 30.26 % and 43.46 % in these villages respectively. Therefore, an extensive study on this disease has been initiated.

Ö Z E T

KARADENİZ BÖLGESİ SOYA EKİM ALANLARINDA GÖRÜLEN HASTALIKLAR

Türkiye'nin Karadeniz Bölgesinde 20.06.1983 ile 11.10.1983 tarihleri arasında soya fasulyesi tarlalarında hastalık sürveyleri yapıldı.

Fide döneminde Samsun'daki soya bitkilerinin % 1.74'ünde Alternaria Yaprak Leke Hastalığı (**Alternaria** spp.), % 0 59'unda ise Fide Hastalıkları (**Sclerotium rolfsii, Fusarium** spp., **Pythium** spp. ve **Rhizoctonia** spp.), Ordu'da ise % 0.71 oranında Fide Hastalıkları saptandı.

Çiçeklenme döneminde % 25.94 Soya Mildiyösü (**Peronospora manshurica**), % 2.54 Alternaria yaprak lekesi, % 0.11 Sclerotium gövde yanıklığı (**Sclerotium rolfsii**) ile % 0.02 Kara Çürüklük (**Macrophomina phaseoli**) ve % 2.05 oranında da Soya Mozayık Virüsü saptandı. Ordu'da ise bu dönemde sadece % 9.14 oranında Soya Mildiyösü ve % 0.28

oranında da Soya Mozayık Virüsü saptandı.

Olgun meyve döneminde Samsun'da % 38.91 Soya Mildiyösü, % 2.45 Alternaria Yaprak Lekesi, ğı, % 0.05 Stemphylium Yaprak Lekesi (**Stemphylium botryosum**), % 3.13 Soya Mozayık Virüsü ve % 0.22 oranında da Sarı Mozayık Virüsü saptandı.

Bu değerlere bakarak soya mildiyösünün bölgemiz için en önemli hastalık olduğu sonucu çıkarılabilir. Bu yüzden konu ile ilgili çalışmalara başlamış bulunmaktayız.

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Seed Treatment for Control of Cabbage Black Rot*

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ABSTRACT

Investigations for the effectiveness of the seed treatment of cabbage with several antibiotics, fungicides and hot water for control of black-rot disease revealed that soaking of seeds for 30 minutes in Plantomycin (100 ppm) gave best results in reducing the disease incidence and also increasing the yield. The per cent disease control and increase in yield in Plantomycin treated plots were 67.68 and 67.66 %, respectively. Next effective treatments in the order of merit were Agrimycin-100 and Paushamycin. Plantomycin was also found best at 7 different locations with disease incidence and yield of 10-50 - 16.19 % and 164.5 - 401.75 Q/ha, respectively.

INTRODUCTION

Cabbage (**Brassica oleracea** var. **capitata** Linn.) is an economically important vegetable crop of India and many other countries. This crop is afflicted by a number of diseases. Black rot caused by **Xanthomonas campestris** pv. **campestris** (Pam.) Dye is a very serious disease which affects all the above ground parts of the plant and causes heavy losses. There are only stray reports on control of this disease of cabbage (Huber and Gould, 1949; Rao and Das, 1981). In present investigations studies were undertaken for the control of cabbage black rot by seed treatment and results thus obtained are presented in this communication.

* Contribution No. 254/83 of Indian Institute of Horticultural Research, Bangalore-560080, India.

MATERIALS and METHODS

Experiments were conducted at Indian Institute of Horticultural Research Farm, Hessaraghatta, Bangalore, India during kharif (July-October) 1980 and subsequently repeated in the same season in 1981 and also during winter (November-February) of 1981-82. The treatments consisted of Agrimycin-100 (15 % Streptomycin Sulphate + 1.5 % Tetracycline), Paushamycin (15 % Streptomycin Sulphate + 1.5 % Oxytetracycline), Plantomycin (9 % Streptomycin Sulphate + 1 % Tetracycline + Vitamin B_{12}) (100 ppm), Bavistin SD (2-(Methoxy-carbomyl) benzamidazole), Ceresan dry (Phenyl mercury acetate) (2 gm/Kg seed) and hot water. Seeds of cabbage (cv. Pride of India) were inoculated by soaking for 30 minutes in bacterial suspension of 10^7 CFU/ml and were dried in shade. These inoculated dried seeds were again soaked for 30 minutes in aqueous solution of antibiotics, whereas Bavistin SD and Ceresan dry were used as dry seed dressers. Hot water treatment was given at 50°C for 30 minutes in water bath. Inoculated seeds without any treatment served as Control. All the treatments were replicated for 4 times in randomized block design. Antibiotics and Ceresan dry alongwith control were also tried on artificially inoculated seeds during kharif season at 7 different locations in Karnataka state. These locations were Anekal, Bangalore South, Kanakapura, Kolar, Malur, Nelamangala and Ramnagaram. Disease incidence was calculated on the basis of number of plants infected and for yield weight of heads was recorded.

RESULTS

Data presented in Table 1 shows that all the treatments were significantly superior over control in reducing black rot incidence in second and third seasons of testing but in first season only Plantomycin was significantly superior. Plantomycin seed treatment was most effective in all the seasons of testing. Next effective treatment was Agrimycin-100 but is was significantly superior over control only in second and third seasons. Seed treatment with Ceresan dry was least effective.

Per cent disease control (Table 1) was maximum (67.68 %) in Plantomycin treated plots followed by hot water (59.91 %) and Agrimycin-100 (59.68 %). Minimum control (22.91 %) was in Ceresan dry treated plots.

More yield was also obtained from the plots treated with Plantomycin during all the three seasons (Table 1). Plantomycin was at par to Paushamycin and Agrimycin-100 during first season only. Paushamycin and Agrimycin-100 did not differ significantly from each other in all the three seasons. Least effective treatment was Ceresan dry which

was at per with hot water treatment in second and third seasons and with Bavistin SD in all the three seasons.

Per cent increase in yield (Table 1) was maximum (67.66 %) in Plantomycin followed by Agrimycin-100 (43.40 %) and Raushamycin (41.51 %). Minimum increase (26 10 %) was in hot water treatment.

Results presented in Table-2 indicate, that out of three antibiotics and one fungicide, minimum disease (10.50 - 16.19 %) and maximum yield (164.5 - 401.75 Q/ha) of heads were recorded in Plantomycin treated plots at all the 7 locations. Next effective teatments were Paushamycin and Agrimycin-100 at all the locations as both the treatments gave almost equal yields. Maximum disease incidence (25.13-33.91 %) and minimum yield (112 5 - 255 Q/ha) was recorded in Ceresan dry treated plots. More yield was recorded in all the treatments at Nelamangala followed by Bangalore South. Lowest yield was recorded at Ramnagaram.

DISCUSSION

In present investigations cabbage seed treatment with Plantomycin (100 ppm) for 30 minutes was found most effective against black rot. This is in concurrence with those reported by earlier workers in cabbage (Huber and Gould, 1949) and in cabbage and other crops (Klisiewicze and Pound, 1961) but Plantomycin has not been tested by them. There is no report on the control of cabbage black rot by Plantomycin as a seed treatment.

Present studies also revealed that the next best treatments for cabbage black rot were Agrimycin-100 and Paushamycin. Agrimycin-100 has been reported effective against black rot by Rao and Das (1981) but they used it as foliar sprays. Similar to Plantomycin there is no report in literature on Paushamycin against black rot of cabbage.

Hot water treatment was not much effective in present trials, as per cent increase in yield was minimum. It may be due to reduced plant vigour. Similarly Huber and Gould (1949) found seed treatment with hot water reduced the plant growth which in turn gives less yield. In present studies antibiotics were found more effective as compared to hot water. Humaydem et al. (1980) also reported that seed treatment with antibiotics was better than hot water treatment which is in confirmity with the present results. Though there is not much work on seed treatment for control of black-rot but many workers have reported control of black-rot in Cauliflower (Shukla et al. 1979, Saini and Parashar, 1981), Brassica spp. (Humaydem et al. 1978) and raya (Gandhi and Parashar, 1978).

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ÖZET

LAHANA SİYAH ÇÜRÜKLÜĞÜ (**Xanthomonas campestris** pv. **campestris** «Pam.» Dye) HASTALIĞINA KARŞI TOHUM İLAÇLAMASI

Siyah Çürüklük (Black-rot) hastalığının kontrolunda, lahana tohumlarına çeşitli antibiyotiklerin, fungisidlerin ve sıcak su uygulamasının etkisi üzerinde yürütülen çalışmalar sonucunda, tohumların 100 ppm dozda Plantomycin'de 30 dakika süreyle bırakılması hastalık şiddetini düşürmede en iyi sonucu vermiş ve aynı zamanda verimi de arttırmıştır.

Plantomycin ile ilaçlı parsellerde hastalık kontrolu ve verimdeki artış sırasıyla % 67.68 ve % 67.66 olmuştur. Plantomycin'i Agrimycin-100 ve Paushamycin izlemiştir.

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Table 1 : Effect of seed treatment on black rot of Cabbage

Sl. No.	Treatments	Per cent disease incidence						Per cent disease control	Yield in Kg/15 m²			Per cent increare in yield over Control
		July to October 1980		July to October 1981		November to Feb. 1981-82			July to October 1980	July to October 1981	November to Feb. 1981-82	
1.	Agrimycin-100	22.63	(28.09)*	16.03	(23.94)	13.96	(22.32)	59.68	14.487	14.375	14.462	43.40
2.	Paushamycin	24.07	(28.31)	16.19	(24.09)	14.85	(23.02)	57.02	14.650	13.675	14.425	41.51
3.	Plantomycin	16.83	(23.49)	13.97	(20.21)	11.08	(19.86)	67.68	16.337	16.700	17.617	67.66
4.	Bavistin SD	27.40	(31.56)	19.30	(26.40)	22.89	(28.62)	46.70	13.925	12.000	13.412	30.21
5.	Ceresan dry	41.79	(40.55)	28.72	(32.68)	30.12	(33.57)	22.91	13.925	12.350	13.000	30.00
6.	Hot water	NT		17.61	(24.94)	17.27	(24.89)	59.91	NT	12.500	12.900	26.10
7.	Control (without any treatment)	44.17	(41.33)	42.58	(40.99)	43.78	(41.68)	—	10.262	9.650	10.300	—
	SEM		(5.24)		(0.742)		(0.932)		1.903	0.585	1.017	
	CD at 5 %		(15.79)		(2.205)		(2.775)		5.736	1.740	3.023	
	CD at 1 %		(21.83)		(3.021)		(3.802)		7.933	2.383	4.141	

*Figures in parenthesis are angular values
NT = Not tested

Table 2: Performance of different seed treating chemicals against Cabbage black rot at various locations in Karnataka State

Sl. No.	Locations	Seed treating chemicals									
		Plantomycin		Paushamycin		Agrimycin-100		Ceresan dry		Control	
		Per cent disease incidence	Calculated yield (Q/ha)	Per cent disease incidence	Calculated yield (Q/ha)	Per cent disease incidence	Calculated yield (Q/ha)	Per cent disease incidence	Calculated yield (Q/ha)	Per cent disease incidence	Calculated yield (Q/ha)
1.	Anekal	16.19	230.00	18.25	125.00	18.00	150.00	29.31	112.50	60.00	100.00
2.	Bangalore South	10.55	315.00	17.19	265.00	15.00	265.00	25.13	255.00	48.33	239.50
3.	Kanakapura	13.25	230.00	20.00	210.00	18.00	202.50	32.31	197.50	62.10	195.00
4.	Kolar	12.53	164.50	17.66	162.00	15.21	163.00	27.35	151 50	53.41	145.00
5.	Malur	14.15	175.50	23.35	173.00	20.00	168.00	33.91	164.50	63.55	159.00
6.	Nelamangala	10.50	401.75	17.13	327.10	16.23	254.10	29.30	251.25	60.00	216.20
7.	Ramnagaram	14.13	175.00	14.99	160.00	14.13	150.00	25.45	120.00	55.05	90.00

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Strains of Tomato Leaf Curl Virus and Its Perpetuation Under Field Conditions

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ABSTRACT

Strains of leaf curl virus of tomato, transmitted by the whitefly (**Bemisia tabaci** Genn.) were isolated and categorised into three groups based on symptomatology and transmission. Under field conditions, this virus was found to perpetuate on the weed hosts like **Acanthospermum hispidum** DC., **Ageratum conyzoides** L., **Parthenium hysterophorus** L., **Datura stramonium** L., **Euphorbia geniculata** Orteg and **Gynandropsis pentaphylla** DC and serves as source of inoculum to the tomato crop. The virus was also found to be naturally infecting the ornamental crops like **Zinnia elegans** Jacq., **Althaea rosea** L. and **Tagetes erecta** L.

INTRODUCTION

Among the virus and yellows type of diseases infecting tomato (**Lycopersicon esculentum** Mill) leaf curl is the most devastating and the extent of yield losses has been reported upto 92 % (Sastry and Singh, 1973). This disease is caused by tobacco leaf curl virus (**Nicotiana** virus-10) and is transmitted by the whitefly **B. tabaci** (Vasudeva and Samraj 1948). The infected plants exhibit curling, rolling, twisting and puckering of the leaves with occasional vein enations on the lower surface. Periodical surveys indicated that the disease incidence varies from 3 to 18 % during winter months and upto 92 % during summer months. The virus-vector relationship is reported to be semi-persistant type (Varma, 1959; Butter and Rataul, 1977). While the discease incidence was recorded lot of variations in symptoms were noticed, misleading the correct identification of the virus

involved. Hence attempts were made to study the cause for the variation of symptoms.

Even though the crop free period was followed, however, raising a healthy crop of tomato has become difficult due to the large population of whitefly vector and perpetuation of this virus on other alternate hosts. During the surveys, some of the weed hosts present in and around the tomato fields were found to exhibit various types of leaf curls and vein clearing symptoms and attempts were made to identify the viruses infecting these hosts. In this communication leaf curl virus strains of tomato and the alternate hosts in which the virus perpetuate under field condition is reported.

MATERIALS and METHODS

The cultures of the leaf curl virus isolates were maintained under insect proof glass house on the tomato var. «Pusa ruby». The colonies of the whitefly vector were maintained on cotton plants in the glass-micro cages. For transmitting the disease, the whiteflies were subjected to fasting for a period of one hr followed by 2 hrs each for acquisition and inoculation feeding periods. Initially, the virus isolates collected from the fields were maintained on tomato plants by grafting and further purified by transmitting through whitefly. Subsequently the isolates maintained in the glasshouse were grouped into three categaries based on symptom appearance and the host range studies were made by inoculating the test plants by the whitefly vector.

RESULTS

Three strains of leaf curl virus were identified, based on the differential type of symptoms on tomato. The strain-1 always exhibited curling on leaves and the enations were not very conspicuous. The strain-2 showed clear dark green enations in addition to the leaf curl symptoms. The strain-3 exhibited slight mottling symptoms followed by purpling of the veins. All the three strains were transmitted by the whitefly (**B. tabaci**) and there was no variation in the respective symptoms even after transferring the virus.

During the survey, it was noticed that some of the weeds such as **A. hispidum, A. conyzoides, D. stramonium, P. hysterophorus, E. geniculata** and **G. pentaphylla** showed leaf curl symptoms. Transmission tests to tomato and tobacco (**Nicotiana tabacum** var. Harrison's spl.) through **B. tabaci** indicated that all these six hosts are harboured by the same tomato leaf

curl virus and they serve as alternate hosts for this virus. Even the same virus was isolated from the ornamental plants like **Z. elegans, A. rosea** and **T. erecta** which were showing leaf curl and vein enation symptoms. The original symptoms were reproduced on these hosts by back inoculation from the infected tomato, through the whiteflies.

DISCUSSION

Variations in the symptom appearance caused by tomato leaf curl virus were generally noticed both in the glass house and field conditions. Very little work has been done on this subject and in earlier investigation from Sudan, Yassin and Nour (1965) reported only two strains of leaf curl virus. In the present studies three distinct leaf curl virus strains were reported based on symptomatology. Among the three strains, strain-1 is most commonly prevalent and of late strain-3 is also appearing sparingly.

Further it was found that tomato leaf curl virus perpetuates on atleast six weed hosts (**A. hispidum, A. conyzoides, D. stramonium, E. geniculata, P. hysterophorus** and **G. pentaphylla**) and three ornamental plants (**Z. elegens, A. rosea** and **T. erecta**). It was also observed that the whiteflies were found to multiply on these hosts. In earlier study from India, **Vernonia cinerea, Sida rhombifolia, Solanum nigrum, Schizanthus** sps, **Launaea hudicaulis, Euphorbia hirta, Flaveria australasica** and **Blainvillea rhomboidea** were found to be alternate hosts for leaf curl virus of tobacco and tomato, both of which are caused by **Nicotiana** virus-10 (Pruthi and Samuel, 1941; Mariappan and Narayanaswamy, 1977). In the present communication some more additional alternate hosts for tomato leaf curl virus were reported.

Generally, the chances for the outbreak of this disease epidemics will be more in an area where the abundant infected weeds exhist. Successful elimination of the weeds by the application of weedicides or by any other means, will help to minimise the vector population and inoculum source, so that there will increase the chance for raising healthy tomato crop.

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ÖZET

DOMATES YAPRAK KIVIRCIKLIĞI VİRUSUNUN IRKLARI VE TARLA KOŞULLARNDAKİ DEVAMLILIĞI

Beyaz sinek (**Bemisia tabaci** Genn.) tarafından taşınan domates yaprak kıvırcıklığı virusunun ırkları izole edilmiş ve simptomatolojik ve taşınma esaslarına göre üç kategoride guruplandırılmıştır. Tarla şartlarında virusun **Acanthospermum hispidum** DC., **Ageratum conyzoides** L., **Parthenium hysterophorus** L., **Datura stramonium** L., **Euphorbia geniculata** Ortag. ve **Gynandropsis pentaphylla** DC. gibi yabancı ot konukçularında bulunduğu ve domates için inokulum kaynağı oluşturduğu bulunmuştur. Virusun doğal olarak **Zinnia elegans** Jacq., **Altheae rosea** L. ve **Tagetes erecta** L. gibi süs bitkilerini de infecte ettiği bulunmuştur.

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Die Ausbreitung von Tabakvirosen im Ägäischen Gebiet, biologische, serologische und elektronenmikroskopische Untersuchungen mit isolierten Viren

Ülkü YORGANCI* und Seval SEKİN**

ZUSAMMENFASSUNG

Nach Untersuchungen im Freiland und mit Hilfe von Testpflanzen wurde der Verbreitungsgrad der Viruskrankheiten an Tabak als 16 03 % ermittelt. Das meisgefundene Virus war das Tabakmosaikvirus und drei Isolate von diesem Virus verursachten verschiedene Symptome an Tabak. Ausserdem wurden Kartoffel-X-Virus, Kartoffel-X-Virus und Tabakringfleckenvirus identifiziert. Diese Isolate wurden serologisch untersucht und gegen eigene typische Isolate wurden auch Antiseren produziert. Bei den elektronmikroskopischen Untersuchungen wurden die Dimensionen der Isolate bestimmt.

EINLEITUNG

Tabak ist eines der wichtigsten Exportprodukte der Türkei und sein Verkauf bringt erhebliche Devisen. Ein grosser Teil davon wird im Inland verbraucht. 55 % der gesamten Tabakproduktion kommt aus der Ägäis, die 50 % der Gesamtanbaufläche umfasst (2). Obwohl es von dem ökonomischen Blickpunkt her sehr bedeutend ist, wird aber auf die Verluste durch Tabakvirosen keinen Wert gelegt (13). Eiglentlich werden die Verluste durch viruskrankheiten nicht erkannt, weil sie die Pflanzen nicht gleich vernichten. Im Ausland wurden viele Versuche über die Einflüsse von Tabakviren auf Ertrag, Ausbeute, Qualität und chemische Zusammensetzung durchgeführt und

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die Ergebnisse zeigen, dass sie diese Eigenschaften beeinflüssen können (14, 19, 20, 22, 23). Bis heute wurde dieses Thema in der Türkei nicht von diesem Blickpunkt aus betrachtet.

Mit dieser Arbeit wurden die Verbreitungsgrade von viruskrankheiten an Tabak im Ägäischen Gebiet festgestellt. Darum wurden die Testpflanzen nach den Ergebnissen von Gemeinschaftsversuchen von CORESTA ausgewählt und die entstehenden Symptome ausgewertet. Und die Arbeiten von Vogel und Bode über dieses Thema wurden ebenfalls verwendet (5, 6, 7, 8, 10, 11).

Die gewonnenen typischen Virusisolate wurden elektronenmikroskopisch untersucht. Die Isolate wurden auch mit den originalen Antiseren, die vom Ausland geschickt wurden, serologisch identifiziert. Es wurden Kaninchen mit gereinigten Virusisolaten gespritzt, um auf diese Weise auch Antiseren in beachtenswerten Mengen gegen einheimischen Virusisolaten zu gewinnen. In dem zweiten Teil dieser Arbeit wurde es aus den Feldversuchen mit den typischen Virusisolaten und vier Tabaksorten, von denen drei heimisch sind, bestimmt, dass die Viren den Ertrag und die Ausbeute beeinflüssen können. In den gewonnenen Material wurden die chemische Veränderungen durch Virusinfektionen mit Hilfe von neuesten Methoden analysiert. Dieser Teil wird getrennt publiziert werden.

MATERIAL und METHODE

Testpflanzen: Die bei den Virusisolationen und Identifikationen benutzten Testpflanzen wurden mit Hilfe der Literatur ausgewählt, damit sie alle Tabakviren erfassen können, und selbst gezüchtet.

Die benutzten Testpflanzen sind:

Nicotiana tabacum cv. «Maden»
Nicotiana tabacum cv. «Xanthinc»
Nicotiana glutinosa L.
Chenopodium amaranticolor (Coste et Reyn)
Chenopodium quinoa Willd.
Vigna sinensis L. (Sorte Black eye)
Cucumis sativus L.
Capsicum annuum L.
Gomphrena globasa L.
Datura stramonium L.
Physalis floridana Rydb.
Petunia hybrida Hort.
Solanum demissum «Y»

Virusisolate: In den Untersuchungen wurden die Proben benutzt, die während Befallsaufnahme gesammelt wurden. Die folgenden Virusisolate wurden sorgfältig undersucht:

1. Akhisar (TMV-1): Ein typisches TMV-Isolat. Es wurde aus einer Probe von Akhisar, die typi-

sche Mosaiksymptome zeigt, isoliert. Es wurde auch serologisch und elektronenmikroskopisch identifizielrt.

2. Soma 1 (TMV-2): Dieses Isolat verursacht Deformationen und Anschwellungen an den Tabakblättern. Es wurde serologisch und elektronenmikroskopisch als TMV identifiziert.

3. Soma 2.: Es wurde Punkt-und Kommaförmigen weisse Nekrosen beobachtet. An den Testpflanzen wurde eine Mischinfektion von TMV und Kartoffel-Y-Virus festgestellt und dies auch serologisch und elektronenmikroskopisch bestätigt.

4. Kartoffel-X-Virus + TMV: Mit Hilfe ven Testpflanzen, serologisch und elektronenmikroskopisch wurden die Viren, die diese Mischinfektionen verursachten, identifiziert.

5. Tabakringfleckenvirus: Dieses Virus wurde auch an Testpflanzen, serologisch und elektronmikroskopisch identifiziert.

6. Muğla (TMV-3): Dieses Isolat wurde aus im Wuchs zurückgebliebenden und vergilbten Tabakpflanzen isoliert und ebenfalls mit Testpflanzen, serologisch und elektronmikroskopisch identifiziert.

Methode

Survey-Methode: Um den Verbreitungsgrad der Viruskrankheiten an Tabak im Ägäischen Gebiet zu bestimmen und um die Proben zu sammeln, wurden Untersuchungen (Survey) im Freiland in den Provinzen İzmir, Manisa, Muğla, Aydın, Denizli und Balıkesir im Juli 1977 durchgeführt. Bei der Feststellung der Probenzahl wurde der Jahrresbericht 1976 der Vereinigung der Tabakexporteure verwendet (1). Für je 10.000 Ballen Tabakproduktion wurde eine Probe vorgesehen. Danach wurden aus İzmir 53, Manisa 92, Muğla 25, Aydın 8, Denizli 14 und Balıkesir 12 und insgesamt 204 Proben entnommen. Auf einem Feld wurden an fünf Stellen insgesamt 100 Pflanzen kontrolliert und die Zahl der Krankheitssymptome zeigenden Pflanzen notiert. Von einem Feld wurde eine Mischprobe entnommen. Die entnommenen Proben wurden getrennt in Polyetilenbeutel in einer Eisbox transportiert und in einer Kühltruhe bei −35°C aufbewahrt.

Isolierung und Identifizierung von Viren mit Hilfe von Testpflanzen: Die für die Versuche benutzten Testpflanzen wurden unter kontrollierten Bedingungen im Gewächshaus oder in Klimaräumen gezüchtet und im geeigneten Alter wurden sie mit virusinfizierten Pflanzensaft mechanisch inokuliert.

Die Reinigung von Viren: Um die Antiseren zu produzieren und vorallem die serologischen Beziehun-

gen zwischen TMV-Isolaten zu bestimmen wurden die Virusisolate gereinigt. Für die Reinigung von TMV-Isolaten, Kartoffel-Y-Virus Methode 1, für Kartoffel-X-Virus Methode 2 bei Yorgancı (27) und für Tabakringfleckenvirus die Methode von Steere (25) und Schade (21) benutzt.

Spektrophotometrische Messungen: UV-Absorbtionspektren von Virusisolaten wurden mit Hilfe eines PYE Unicam SP-8-100 UV-VIS Spektrophotometer zwischen 200-350 nm Wellenlänge gemessen und danach wurden die Konzentrationen von Viruspräparaten errechnet.

Serologische Methoden: Als Versuchstiere benutzte Kaninchen wurden mit den konzentrierten Viruslösungen intravenös injiziert (26). Die Injektionen erfolgten bis man den gewünschten Titer erzielt hatte. Danach wurde Blut entnommen und durch Zentrifugation die Antiseren hergestellt. Die serologische Teste wurden nach dem Agargeldiffusionstest und Micropresipitintest durchgeführt (3, 18, 27).

Methoden für die elektronenmikroskopischen Untersuchungen: Um die isolierten Viren elektronenmikroskopisch zu untersuchen wurden die Säfte von infizierten Blättern durch niedrigtourige Zentrifugation grob gereinigt. Für die isometrische Viren wurden gereinigte Präparate benutzt. Aus diesen Lösungen wurden auf Formvar beschiechteten Objektträgern kleine Tröpfchen abpipetiert. Nach der Trocknung wurden sie mit 1 % igen Na-Phosphotungstat pH 6.5 negativ kontrastiert (15). Bei diesen Untersuchungen wurde ein JEOL JEM 100 C Elektronenmikıoskop benutzt. Mit Hilfe von Vergrösserungen wurden die originalen durchschnittlichen Dimensionen berechnet (27).

ERGEBNISSE

Die Ergebnisse von Befallserhebungen und Isolationen: Von allen gesammelten 204 Tabakproben wurden ein oder mehrere Viren isoliert und der Prozentsatz der virusinfizierten Pflanzen wurde als gewogener Durchschnitt errechnet. Der Verbreitungsgrad von Viruskrankheiten an Tabak im Ägäischen Gebiet wurde in Tabelle 1 dargestellt.

Tabelle 1. Der Verbreitungsgrad der Viruskrankheiten auf Tabakfeldern im Ägäischen Gebiet im Jahre 1977

Die Namen der Provinz	Die Zahl der kontrollierten Felder	Fläche (Dekar)	Virusinfizierten Pflanzen %
İzmir	53	336,5	17,38
Manisa	92	738,5	13,41
Muğla	25	192,0	21,73
Aydın	8	39,5	10,40
Denizli	14	102,0	5,46
Balıkesir	12	58,0	45,14
Gebietsdurchschnitt	204	Gesamtfläche 1466,5	16,03

Obwohl die Wachstumsstadien von Tabakpflanzen eine gewisse Wirkung haben, wurden höchsten Verbreitungsgrade in den Provinzen Balıkesir (45,14 %) und Muğla (21,87 %) festgestellt. Der Niedrigste war in Denizli mit 5,46 %. Der Verbreitungsgrad der Viruskrankheiten an Tabak in Ägäischen Gebiet wurde durchschnittlich mit 16,03 % berechnet.

Wenn man die Prozentsätze der Viruskrankheiten beobachtet, kann man leicht feststellen, dass das Verbreitetste Tabakmosaikvirus (TMV) ist. Im Ägäischen Gebiet gibt es drei verschiedene TMV-Isolate, die an Tabak unterschiedliche Symptome verursachen. Das erste Isolat ruft an den jungen Blättern normale Mosaikerscheiningen und Blattdeformationen hervor. Es kommt kein ausserordentliches Zurückbleiben des Wachstums der Pflanze vor. Beim zweiten Typ beobachtet man Verkräuselung, Grobheit und Mosaikerscheinungen an den Blättern. Die Blätter werden ziemlich dick und stärker. Das dritte Isolat verhindert das Wachstum an Tabakpflanzen, die Pflanzen verkümmern und verfärben sich nach gelb. Diese Symptome kommen besondern unter ungeigneten Boden-und Klimabedingungen vor. Aber die Inokulationen bewiesen dass die TMV-Infektionen zugleich vorhanden waren. Ausser TMV konnte man Kartoffel-X-Virus (PVX), Kartoffel-Y-Virus (PVY) und Tabakringfleckenvirus (TRSV) isolieren und diese Viren waren meistens in Mischinfektionen mit TMV auffindbar (Tabelle 2).

Tabelle 2. Der Prozentsatz der isolierten Viren.

Viren	Der Prozentsatz (%)
TMV (1)	90,2
TMV (2)	0,98
TMV (3)	4,41
Kartoffel-X-Virus	2,94
Kartoffel-Y-Virus	0,49
Tabakringfleckenvirus	0,98

Die Ergebnisse von Inokulationen der Testpflanzen: Der Vergleich der Symptome an den geeigneten Testpflanzen, die durch verschiedenen Virusisolate verursacht werden, ermöglicht eine grobe Identifizierung dieser Viren. In dieser Arbeit wurden die Symptome an den Testpflanzen, deren Namen in Material angegeben sind, beschrieben und die Ergebnisse in der Tabelle 3. dargestellt.

Die Ergebnisse der Reinigungsversuche: Die gewählten Reinigngsmethoden waren meistens erfolgreich. Infolgedessen konnte man in genügenden Konzentrationen, keine Pflanzenproteine enthaltende, gereinigte Präparate gewinnen, um Antiseren zu produzieren und zu testen. In den gereinigten Präparaten erreichte die reine Virusmenge für TMV bis 30 mg pro milliliter. Bei den anderen Viren konnte man in geringeren aber dennoch reichlichen Mengen Viren gewinnen.

Die Ergebnisse der serologischen Tests: Die Virusisolate des Tabaks wurden serologisch mit den originalen Antiseren von TMV, Kartoffel-X-Virus, Kartoffel-Y-Virus und Tabakringfleckenvirus, die aus Dänemark, Deutschland, Holland geschickt worden sind, identifiziert. Selbst wurden besonders gegen TMV Isolaten in grossen Mengen mit hohen Titern und gegen anderen Isolaten in genügenden Mengen Antiseren produziert. Die Ergebnisse der serologischen Tests, die mit diesen Antiseren durchgeführt wurden, um die serologischen Beziehungen zwischen den TMV-Isolaten zu bestimmen, wurden in der Tabelle 4 gezeigt. In dieser Tabelle wurden die Ergebnisse der Tests zusammengefasst, dies sind bei den drei TMV-Isolaten möglichen 3 homologen und 6 heterologen Kombinationen.

Tabelle 3. Die durch Virusisolaten des Tabaks verursachten Symptome an den verschiedenen Testpflanzen

Testpflanzen	Symptome TMV Lokale	TMV Systemisch	PVX Lokale	PVX Systemisch	PVY Lokale	PVY Systemisch	TRSV Lokale	TRSV Systemisch
Nicotiana tabacum cv. «Maden»	O	M	LSp	M	O	Vo,Sp	Ri	?
Nicotiana tabacum cv. «Xanthi»	NLL	O	LSp	M	O	Vo	LL	?
Nicotiana glutinosa	NLL	O	LSp	M	O	Sp(?)	NLSp	O
Chenopodium amaranticolor	NLL	O	NLL	O	LL	O	NLSp	O
Chenopodium quinoa	LL	O	LL	O	LL	O	NLSp	O
Datura stramonium	NLL	O	O	M	O	O	LSp(?)	O
Gomphrena globosa	NLL	O	NLL	O	O	O	NLSp	O
Petunia hybrida	NLL	M	O	O	O	O	LSp(?)	O
Capsicum annuum	LSp	Y,M,LeAb	LL	Vo,Wi,LeAb	O	M	O	O
Cucumis sativus	O	O	O	O	O	O	LSp	Stu
Physalis floridana	O	?	O	O	L	Sp	O	O
Solanum demissum «Y»	O	O	O	O	LL	O	O	O

LL : Lokalläsionen
NLL : Nekrotische Lokalläsionen
CLL : Chlorotische Lokalläsionen
M : Mosaik, Scheckung
LSp : Lokale Flecke
Vo : Adernaufhellung
Wi : Welke
LeAb : Blattabfall
C : Kräuselung
Y : Vergilbung

Ri : Ringflecke
Stu : Stauchung
O : Reaktion blieb aus
— : Angabe fehlt
? : Nicht sicher

Wie man aus der Tabelle ersehen kann, ergaben sich gegen drei TMV-Isolate Antiseren mit zeimlich hohen Titern. Die Ergebnisse der serologischen Tests bewiesen, dass es zwischen den Proteinzusammensetzungen dieser Isolaten keine erhebliche Unterschiede gibt.

Tabelle 4. Die Ergebnise der mit den Antiseren von der drei Isolaten des TMV durchgeführten homologen und heterologen Teste*

Antiseren	Virusisolaten		
	TMV (1)	TMV (2)	TMV (3)
TMV (1)	2048 / 1,25	128 / 1,25	2048 / 1,25
TMV (2)	2048 / 1,25	128 / 1,25	2048 / 1,25
TMV (3)	2048 / 2,5	32 / 2,5	2048 / 1,25

* Der Zähler des Quotienten stellt den reziproken Wert der letzten Antiserum-Verdünnung dar, bei der noch eine Präzipitationslinie im Agar-Geldiffüsionstest auftrat.
Der Nenner gibt die jeweils entsprechende Antigenkonzentration in mg Virus pro. ml an.

Die Ergebnisse der Elektronenmikroskopischen Untersuchungen:
Für die elektronenmikroskopisch untersuchten Virusisolate wurden folgende Dimensionen ermittelt:

Kartoffel-Y-Virus 730 nm Länge
Kartoffel-X-Virus 515-520 nm Länge
TMV-Isolate 285-320 nm Länge
Tabakringfleckenvirus etwa 30 nm Durchmesser.

Die Beobachtungen der Vektor-Insekten: Während der Freilanduntersuchungen wurden auch Vektornsekten beobachtet und besonders die beim Saugen gesehenden Blattläuse gesammelt. Alle Proben wurden als **Myzus persicae** identifiziert.

DISKUSSION

1. Die Verbreitungsgrade der Viruskrankheiten an Tabak und die Ergebnisse der Isolationen.

Aus insgesamt 204 Proben wurden ein oder mehrere Viren isoliert. Wenn die Provinzen verglichen wer-

den, bekommen Balıkesir und İzmir die vorderen Stellen (Tabelle 1). Der Verbreitungsgrad in Balıkesir wird deswegen höher sein, weil zu dieser Provinz verhältnismässig spät gefahren wurde. Demgegenüber trat der niedrigste Verbreitungsgrad mit 5,46 % in Denizli auf. Dies könnte durch die Höhenlage der Probennahme verursacht worden sein, wo infolgedessen die Populationen der Blattläuse verhältmismässig niedrig waren. Dazu waren die Tabakpflanzen in dieser Zeit noch jung und das Pflücken hatte noch nicht begonnen. Mit dem Beginn des Pflückens werden die Verbreitungsgrade noch höher. Der Provinz Denizli folgten die Provinzen Aydın und Manisa.

Die Isolierten Viren: Bei der Identifizierung der Viruskrankheiten an Tabak wurden Lucas (17), Berger (5, 6, 7, 8), Bode und Klinkowski (9), Bode und Vogel (10, 11) und Description of plant Viruses (4, 12, 16, 24, 28) benutzt. Diese Identifizierungen wurden mit serologischen und elektronmikroskopischen Ergebnissen befestigt. Unter den isolierten Viren aus den 204 Proben ist TMV das Verbreiteste und dieses Virus hat verschiedene bzw. drei Stämme. Disce Stämme wurden unter Abschnitt Ergebnisse näher beschriben. Für unser Gebiet kann TMV (3) der wirksamste Stamm sein und dieser Stamm kann besonders für Burley-Tabaksorten gefährlich sein, deren Anbau für die Zukunft in unserem Land geplant wird. Es wurde in den Feldversuchen beobachtet, dass die mit TMV (3) inokulierten Tabakpflanzen in Burley S_3 Perzellen zu Grunde gegangen oder ziemlich im Wuchs zurückgeblieben waren. Ausser TMV-Stämmen wurden auch Kartoffel-X-Virus, Kartoffel-Y-Virus und Tabakringfleckenvirus in Mischinfektionen mit TMV angetroffen. Mit Hilfe der verschiedenen Testpflanzen isolierten Viren wurden auch vom Ausland geschickten Antiseren getestet und positive serologische Reaktionen festgestellt. Die für die elektronenmikroskopisch untersuchten TMV-Stämme, Kartoffel-X-Virus, Kartoffel-Y-Virus und Tabakringfleckenvirus ermittelten Werte stimmten mit der Literatur in Form und Grösse dieser Viren überein (4, 12, 16, 24, 28). Gegen diesen Viren wurden originale Antiseren produziert und damit wurden insbesondere die serologischen Beziehungen zwischen den TMV-Stämmen näher untersucht. Obwohl sie ziemlich unter S. Schiedliche Symptome an Tabak hervorriefen, zeigten sie keine besonderen serologischen Differenzen.

ÖZET

EGE BÖLGESİNDE TÜTÜNLERDE VİRUS HASTALIKLARININ YAYILIŞLARI, ELDE EDİLEN İZOLATLARLA BİYOLOJİK, SEROLOJİK VE ELEKTRONMİKROSKOPİK ARAŞTIRMALAR

Ege Bölgesinde tütünlerde virus hastalıklarının yayılışlarını saptamak amacıyla survey yapılmış ve toplanan 204 örnekten test bitkileri kullanılarak bir veya birkaç virus izole edilmiştir. Virusla enfekteli bitkilerin yüzdesi alana orantılı olarak ifade edilmiş ve yayılış oranı % 16,03 olarak bulunmuştur. TMV başat virus olup bu virusun üç izolatı, tütünde farklı belirtiler oluşturmaktadır. Bundan başka Patates X-Virusu, Patates Y-Virusu ve Tütün Halkalı Leke Virusu saptanmıştır ve bu virusların serolojik olarak tanılamaları yapılmıştır. Arıtmalar sonucunda amaca yeterli miktarlarda virus elde edilmiş ve kendi tipik izolatlarımıza karşı antiserum üretilmiştir. Heterolog testlerde TMV-izolatları tütün üzerinde farklı belirtiler oluşturdukları halde birbirleriyle çok iyi reaksiyon vermişlerdir. Virus izolatlarının elektronmikroskopik incelenmeleri sonucu aşağıdaki boyutlar saptanmıştır:

TMV-Izolatları	285-320 nm	Uzunluk
Patates X-Virusu	515-520 nm	»
Patates Y-Virusu	730 nm	
Tütün Halkalı Leke Virusu	30 nm	çap.

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OUTBREAKS

Alternaria Spot Disease on Sesame

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Sesame is a plant which is used for seeds, oil and oil-cake. Growing land of this plant has gradually increased since it was grown as a second crop. According to the statistics in 1980, totaly 17501 hectars area was sown in Aegean Region (Anonymous, 1980).

During second crop survey in Aydın, disease symptoms were seen on stem, leaf and pod of first crop sesame. These diseased samples were taken and brought to the laboratory and transfered into the petri dishes. At the end of the isolation studies, **Alternaria sesami** (Kaw.) Mohant and Behera was found as the causal agent of this disease.

This pathogen was found first on sesame at Manisa by Göbelez (1960) in 1954. Then this fungus was found tobe carried by seed (Göbelez 1964).

Seed samples were taken from the store-house where the sesame seeds of field with 100 % disease incidence were stored. Of seed samples brought to the laboratory, 200 were used for agar plate method and 200 were used for blotter method.

In blotter method, seeds were not sterilized but in agar method sesame seeds were sterilized with sodium hypochloride (% 0.5) then 10 seeds were placed in each dish. PDA medium was used for agar plate method. These were incubated at 20 ∓ 2°C under alternating cycles of 12 hours light and 12 hours darkness. After 7 days incubation every seed was examined under a stereomicroscope with 10 magnification for the presence of **A. sesami** and it was found that **A. sesami** was carried by seed at the rate of 63,5 % and 16,5 % in blotter and agar plate method respectively.

The fact that A. sesami was at lower rate in agar plate method may be due to death of its spores on seed-surface during sterilization.

Owing to the fact that this pathogen is carried by seed at a high rate and gives rise to a serious damage in the fields where it occurs detailed investigations must be carried out on it.

ÖZET

SUSAM'DA Alternaria LEKE HASTALIĞI

Aydın'da ikinci ürün susam için yapılan survey sırasında birinci ürün susamlarda gövde, yaprak ve kapsül lekesi şeklinde % 100 oranında hastalık görülmüştür. Alınarak laboratuvara getirilen örneklerden yapılan izolasyon çalışmaları sonunda bu hastalığa neden olan etmenin **Alternaria sesami** (Kaw.) Mohant and Behera olduğu saptanmıştır.

Hastalığın % 100 oranında görüldüğü tarlanın ürününün kaldırıldığı depodan temin edilen tohumların 200 tanesi nemli hücre (Blotter), 200 taneside agar yöntemine göre kültüre alınmıştır.

Bir haftalık inkubasyondan sonra petri kabında gelişen funguslar mikroskopta incelenmiş ve A. sesami'nin tohumla nemli hücre yönteminde % 63,5, agar yönteminde ise % 16,5 oranında taşındığı saptanmıştır.

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FIRST REPORT

Verticillium Wilt of Plum in Turkey

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During a study in August 1984, the typical symptoms of Verticillium wilt were observed in four years old plums (**Prunus cerasifera** cv. papaz) in Menemen (İZMİR) for the first time. The symptoms were characterized by discolouration and wilting of leaves and defoliation of shoots, vascular browning of the twigs (Fig. 1, 2). Recently infected trees displayed partial drying, but severely infected trees showed die-back and sometimes the whole tree is killed by the disease. These are also the other typical symptoms of Verticillium wilt.

The results of the isolation studies which were carried out according to standart method, revealed that **Verticillium dahliae** Kleb. was the causal agent. This is the first record of Verticillium wilt on plums.

It is worthy to state that above mentioned plum orchard was situated on formerly cotton growing area.

Verticillium wilt symptoms seen on peach and apricot trees in the same vicinity where the plantations were established on the cotton growing area too (SAYDAM et al., 1971; SARIBAY et al., 1973). The disease was observed in a fruit-tree nursery which was also established on the cotton growing area in Saruhanlı (MANİSA), in 1982. Especially apricot seedlings were almost killed by Verticillium wilt.

Therefore, this problem must be taken into consideration before the establishing new orchard plantations.

Fig. 1. Symptoms of Verticillium wilt on plum tree.

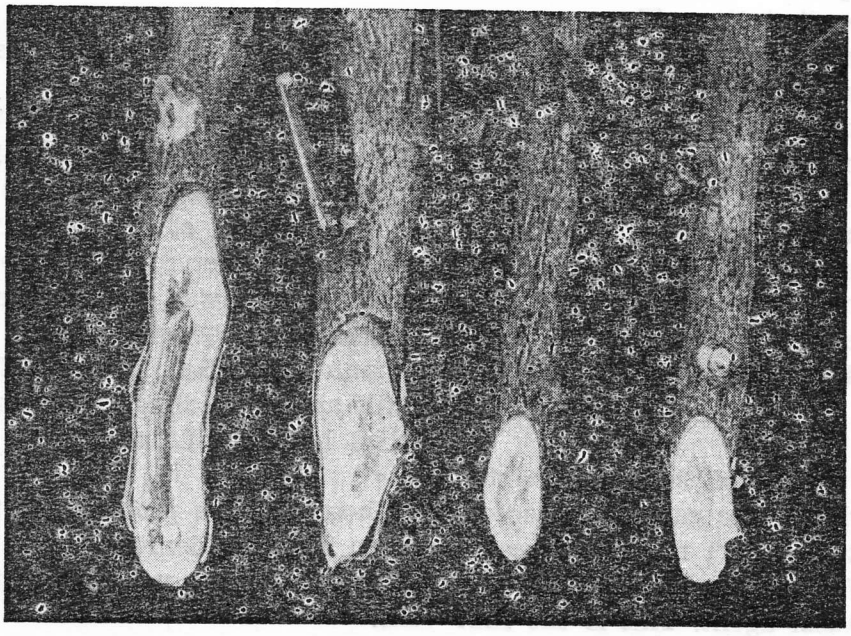

Fig. 2. Vascular browning of plum twigs.

ÖZET

ERİKLERDE VERTİSİLYUM SOLGUNLUĞU

1984 yılı Ağustos ayında üreticiden gelen şikâyetler üzerine yapılan survey çalışmalarında Menemen'de dört yaşındaki bir erik (papaz eriği) bahçesinde Vertisilyum solgunluğu belirtilerine rastlanmıştır. Enfekteli ağaçlarda yapraklarda renk değişimi, solgunluk, sürgünlerin çıplak kalması, iletim demetlerinde renk değişimi, tek taraflı, ileri hallerde tüm dal kurumaları dikkati çekmiş ve yapılan izolasyonlar sonucunda **Verticillium dahliae** Kleb. fungusu izole edilmiştir. Bu Türkiye için eriklerde ilk kayıttır.

Hastalığın görüldüğü erik bahçesinin pamuktan bozma bir arazi oluşu aynı yörelerde önceleri pamuk tarımı yapılan topraklarda kurulan şeftali ve kayısı bahçelerinde de Vertisilyum solgunluğunun sorun olması, 1982 yılında Manisa - Saruhanlı'da pamuktan bozma bir araziye kurulan meyve fidanlığında özellikle kayısı fidanlarının aynı hastalık yüzünden hemen tamamen elden çıkması, yeni kurulacak plantasyonlarda dikkatle üzerinde durulması gereken bir konuyu ortaya koymaktadır.

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FIRST REPORT

First Report on A New Virus Disease of Anise

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Anise (**Pimpinella anisum** L.) is a medicinal and spicy crop grown especially in the western part of Turkey. In recent years, the cultivation of this plant has been remarkably raised as it provides a good profit to the growers. In course of the last two growing seasons, it was observed that a virus disease had become a serious problem in anise plantings. The virus involved caused first yellow spotting (Fig. 1a) and, later vein clearing (Fig. 1b) and distortion on the anise leaves. At the further stage, the infected plants stunted and, in certain cases, dried.

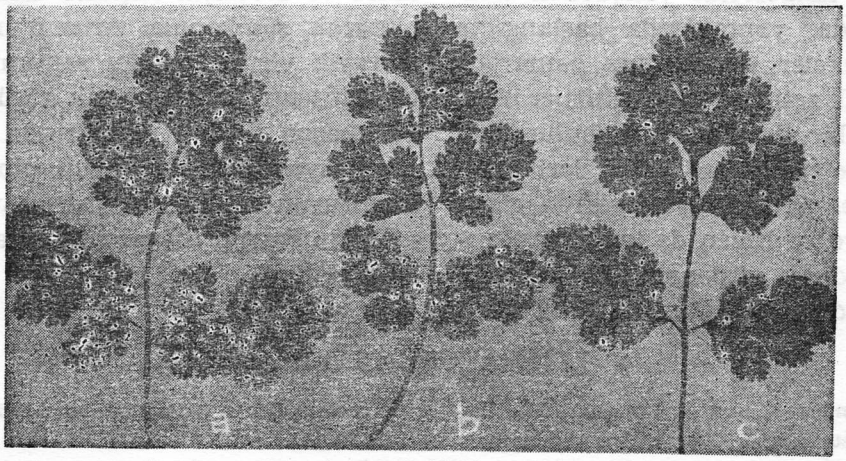

Fig. 1. Symptoms of CMV on anise plants.
(c: healthy leaf)

To determine the host range of the virus in question, mechanical inoculation trials were performed with sap obtained by homogenizing the infected leaves in 20mM phosphate buffer (pH 7,2) with thioglycollic acid.

The virus developed local lesions on **Chenopodium amaranticolor, C. quinoa** and **Vigna unguiculata. Da-**

tura stramonium and **Gomphrena globosa** showed local spots and then, systemic syptoms like mottling and distortion on young leaves. **Apium graveolens, Cucumis sativus, Nicotiana glutinosa** and **N. tabacum** cv. Samsun recated with a pronounced mosaic, crinkling and deformation on the leaves. These all symptoms are in aggrement with those of cucumber mosaic virus (CMV) compiled by Francki (2).

The physical properties (DEP 10^{-3} 10^{-4}, TIP 65 to 70°C and LIV «20°C» 5to 6 days) and the positive reaction with CMV-antiserum proved the identification as CMV, considering the data in earlier works (1, 2, 3, 4, 5).

CMV was the predominant virus and the disease incidence was at high levels in anise plantings. In our opinion, this state could be attributed to the intensity of vector (aphid) population in growing season. Moreover, due to the host range of this virus includes a number of weeds which may be exist throughout year, these hosts can serve as additional virus reservoirs. In order to partially prevent the spread of CMV in anise growing areas, therefore, all efforts should be directed to control vector and reservoirs of CMV.

ÖZET

YENİ BİR ANASON VİRUS HASTALIĞI'NIN İLK KAYDI

Ege Bölgesi'nde yetiştirilen anasonlarda, yapraklarda başlangıçta sarı lekeler, daha sonra damarlarda renk açılması gibi belirtiler oluşturan bir virus hastalığı bulunmuştur. Vejetasyonun daha ileri evrelerinde, virusun anasonlarda cüceleşmeye ve bazen de kurumaya neden olduğu gözlenmiştir. Test bitkilerindeki belirtiler, fiziksel özellikler ve serolojik ilişikler dikkate alınarak, söz konusu virusun hıyar mozayık virusu olduğu saptanmıştır. Virusun yaygın olarak görüldüğü anason üretim alanlarında afit populasyonunun da yoğun olması, bu virusun epidemi oluşturmasında afitlerin önemli rol oynadığını açıklamaktadır.

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NEW RECORD

Powdery Mildew on Calla Lily (**Zantedeschia** sp.)

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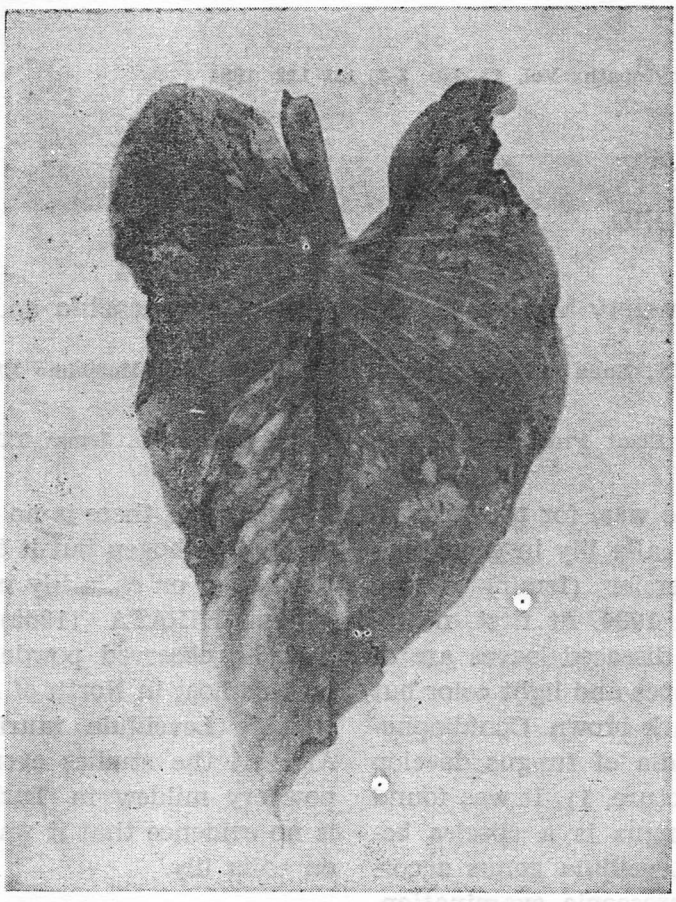
The disease was, for the first time, seen on calla lily in greenhouses at Bademler (Izmir) during November in 1984. At first, necrotic spots on diseased leaves are of irregular shapes and light color but soon turn dark brown. Conidiophores and conidia of fungus develop on them (Picture, 1). It was found that this fungus is a species belonging to **Leveillula** genus according to microscopic examination. In literature, there is no knowledge on this pathogen but it is recorded to be seen on calla lily in North of Africa. HIRATA (1966) reported that he observed powdery mildew on this host in North of Africa and that is **Leveillula taurica.** (Lev.) Arn. At the studies ever done on powdery mildew in Turkey, there is no evidence that it was observed on calla lily.

Ö Z E T

KALA (**Zantedeschia** sp.)'DA KÜLLEME HASTALIĞI

Hastalık ilk kez, 1984 yılı Kasım ayında Seferihisar - Bademler (İzmir) köyünde yetiştiriciye ait kala seralarında görülmüştür. Hastalıklı bitkilerin yapraklarında düzensiz şekillerde önceleri açık daha sonraları koyu kahverengine dönen nekrotik lekeler oluşmaktadır. Bu nekrotik lekelerin üzerinde fungusun konidiofor ve konidileri gelişmektedir (Resim, 1). Yapılan mikroskopik inceleme ile etmenin **Leveillula** genusuna ait bir tür olduğu saptanmıştır. Külleme hastalıkları ile ilgili yurdumuzda yapılan çalışmaların taranması sonucu kala üzerinde külleme hastalığının saptandığına dair hiçbir kayda rastlanmamıştır.



Picture 1. Powder mildew on calla lily.

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