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A preliminary survey on the occurrence of Fusarium wilt of watermelons in Ege region of Turkey

Tayyar BORA* Altekin ÖZKUT**

INTRODUCTION

In the Ege region of Turkey, a total area of 100.000 acres of land is under the cultivation of waterme lon (*Citrullus vulgaris*) and melon *(Cucumis melo).* In this region, three provinces: İzmir, Manisa and Aydın, an area of 40.000 acres, including irrigated and unirriga'ed land, is dispensed with the cultivation of watermelons.

In the recent years, there have been serious troubles to the gro wers due to the spread of wilt disease of watermelons in this region. This trouble has not been reported previously. The only measure adcpied by growers against the spread of disease is that to uproot the plants showing symptoms of wilt and replace by healthy seedlings. Akdoğan (1) reported Fusarium wilt of watermelons from other regions of Turkey. In the Aegean region, native varieties such as *Citrullus vulgaris* cv. Ödemiş Beyazı and *C. vulgaris* cv. Vaşinkton are cultivatad

In order to carry out investigations on breeding of resistant varieties, informations are required about the distribution of disease and the species of Fusarium which cause to the wilt of watermelons in the region. For this reason we made a survey on the different aspects of the topic, in the provinces mentioned above.

MATERIALS and METHODS

Tours were organised in May, June and July 1971. The survey area consisted 22 villages situated in the suburbs of three provinces: İzmir, Manisa and Aydın.

In each tour, 95 different fi-

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elds of watermelons were visited. At three random sites of each field succesive 10 hills were examined and the numbers of the wilted plants were recorded. At least three watermelon plants showing symtoms of wilt were uprooted for isolation.

The roots of wilted plants were washed with tap water and cut into small pieces of 0.5 cm in size. The root pieces were surface sterilized by dipping in 0.5 % NaOCl for 3 minutes. Later, rinsed in sterile water for three times and plated on PDA (pH 5.6) in petri dishes.

The species of Fusarium developing from root pieces were identified. In order to identify different species of Fusarium we consulted the following : MESSIAEN et CAS SINI (5), GILMAN (3) and DOMSCH und GAMS (2).

RESULTS and DISCUSSION

Table I summarized results of the Fusarium wilt ratios obtained from the surveyed provinces : İzmir, Manisa and Aydın, during the months of May, June and July 1971.

Table I. Percentage of Fusarium wilt of watermelons in the surveyed area

Place	Total watermelon area (acre)	Number of fields examined	Averages of wilt percentage May	June	July
İzmir					
Ödemiş	4576	21	0.20	1.73	0.66
Selçuk	4784	22	0.00	1 72	0.00
Tire	2288	13	0.43	0.00	0.00
Torbalı	2080	13	2.10	5.94	1.81
Dikili	644	4	—	0.00	0.00
Total	14372	73	0.82	2 86	0.71
Aydın					
Center	416	3	0 00	2.08	0.00
Kuşadası	728	3	0.00	0.00	0.00
Sultanhisar	145	3	0.00	0.00	0.00
Total	1289	9	0.00	0.61	0.00
Manisa					
Akhisar	1456	5	0.00	0·00	0.00
Salihli	1664	5	0.60	1.80	0.00
Alaşehir	52	3	28.57	11.71	0·00
Total	3172	13	3.23	1.80	0.00
Grand Total	18833	95	1·13	2.54	0-55

According to the Table I. June had highest degree of wilt It can be attributed to temperature which is most favorable for the development of Fusarium during this month in this region. Only one field in Manisa, out of 13, showed maximum wilt percentage (83 %). It lead to increase the average of this province. All the other fields percentage were coinciding with generalization. Since the farmers uprooted the wilted plants, examined in the first tour, were not observed in the second tour.

Table II shows the average wilt percentage with particular consideration of irrigation status and soil type. The results in Table II show the increasing effect of heavy and irrigated soil on watermelon wilt. Indeed, PALTI and JOFFE (6), in Israel, found that one variety of watermelon under artificial conditions showed high susceptibility to the wilt but it lost its susceptibilty in the fields which were unirrigated. Table II gives a generalization for the effect of irrigation and heavy soils

Table II. Average of wilt percentage in respect to irrigation and soil type of the fields.

Province	Tours	Average of wilt percentage			
		Irrigation		Soil Type	
		Irrigated	Unirrigated	Light	Heavy
İzmir	May	1.02	0.13	0.23	0.10
	June	3.82	0.41	0.87	3 76
	July	1.04	0.09	0.19	0.94
Manisa	May	0.00	1.15	0.00	0.98
	June	0.00	0.56	0.00	0.56
	July	0.00	0.00	0.00	0.00
Aydın	May	0.00	0.00	0.00	0.00
	June	0.08	0.00	0.08	0.00
	July	0.00	0.00	0.00	0.00

On the other hand, of the 95 fields examined, 22 fields showed Fusarium wilt. Seventy percent of 22 fields were irrigated and 30 % were unirrigated.

Six species of Fusarium were obtained from the isolations. These are below with the value of frequency in brackets: *Fusarium solani* (40), *F. oxysporum* (34), *F. equiseti* (17), *F. culmorum* (2), *F. moniliforme* (2), *F. roseum* (2).

JOFFE and PALTI 4) and PALTI and JOFFE (6) obtained *F. equiseti* in addition to *F. solani* and *F. oxysporum*. Isolation of *F. culmorum*, *F. moniliforme* and *F. roseum* from watermelon is new. But all these three need a pathogenicity test for establishing their relation with watermelon wilt

Only (8) *F. solani*, (8) *F. oxysporum*, (5) *F. equiseti*, (1) *F. culmorum*, (1) *F roseum*, were obtained as pure culture. In other cultures, the fungi were in associations, such as: *F. solani* + F. *oxysporum* (19), *F. solani* + *F. equiseti* (6), *F. soloni* + *F. oxysporun* + *F. equiseti* (4), *F. solani* + *F. moniliforme* (1), *F. solani* + *F. roseum* (1), F. *solani* + *F. oxysporum* + *F. culmorum* + *F. equiseti* (1), *F oxysporum* + *F. moniliforme* (*1*), *F oxysporum* + *F. equiseti (1)*.

We considered the frequency of Fusarium species in relation with soil type and irrigation status of the surveyed fields. The results are given in Table III.

Table III. Percentage of the most common Fusarium species isolated from watermelon, in respect to irrigation and soil type.

	F. solani		F. oxysporum		F. equiseti		Total
Fields	Number of isolates	%	Number of isolates	%	Number of isolates	%	number of isolates
Irrigated	28	43.0	27	41.5	10	15.3	65
Unirrigated	12	46.1	7	26.9	7	26.9	26
Light soil	20	43.4	17	36.9	9	19.5	46
Heavy soil	20	44.4	17	37.7	8	17.7	45

It can be conclueded from Table III that irrigation has an important effect only in case of *F. oxysporum*, in increasing the fungal frequency. F. *oxysporum* 15 % more frequent in the irrigated fields than that of the unirrigated. The soil type has no affect on the frequency of Fusarium species in the watermelon fields.

From Table II and Table III, it can be seen that irrigation has increasing effect on both of wilt percentage and the frequency of only *F. oxysporum*. This may be an interesting clue in consideration the role of *F. oxysporum* in watermelon wilt under natural conditions.

ÖZET

EGE BÖLGESİNDE KARPUZLARDA FUSARIUM SOLGUNLUĞU

Karpuz solgunluğu etmenlerini ve solgunluk oranını saptamak üzere Ege bölgesi İzmir, Manisa ve Aydın illerinde bir sürvey yapılmıştır. Sürvey; karpuzun yetişme durumu gözönüne alınarak, Mayıs - Haziran-Temmuz 1971 de olmak üzere üç tur olarak planlanmıştır.

Sürvey sonuçlarına göre solgunluk oranı İzmirde: birinci turda % 0,82, ikinci turda % 2,86, üçüncü turda % 0,71 ; Manisada : birinci turda % 3,23, ikinci turda % 1,80, üçüncü turda % 0,00 ve Aydında: birinci turda % 0.00, ikinçi turda % 0.61, üçüncü turda % 0.00 olarak bulunmuştur.

Solgunluk belirtisi gösteren bitkilerden yapılan izolasyonlarda: *Fusarium solani*, F. *oxysporum*, F *equiseti*, F *culmorum*, F. *moniliforme* ve F. *roseum* türleri saf ve müşterek olarak elde edilmişlerdir.

Kontrol edilen tarlaların sulama durumları ve toprak yapıları elde edilen isolatların sayılarını etkilemiştir. Sulanan topraklardan elde edilen isolatların % 43 ü F. *solani*, % 41,5 i F. *oxysporum*, 15,3 ü *F. equiseti*; Sulanmıyan topraklarda % 46,1 i *F. solani*, % 26,9 u F. *oxysporum*, % 26,9 u F. *equiseti*; Hafif topraklardan elde edilen isolatların % 43,4 ü F. *solani*, % 36,9 u F. *oxysporum*, % 19,5 i F. *equiseti*;; Ağır topraklarda ise % 44,4 ü F. *solani*, % 37,7 F. *oxysporum* ve % 17,7 i F. *equiseti* olarak bulunmuştur.

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Gleosporium olivarum Alm In Ege Region (Turkey)

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ABSTRACT

The main purpose of this study is to establish the occurrence of *Gleosporium olivarum* Alm. in Turkey scientifically. The fungus was isolated from the lesion and collapsed areas on olive fruits and from mummised fruits of *Olea europea* var. *Yerli yağlık*. Here the possibility of the effect of pathogen on the acidity of olive oil was also studied.

INTRODUCTION

During the observations of the diseases of olive trees in Ege Region some lesions and collapsed areas on olive fruits together with mummised fruits were noticed. Culture studies in the laboratory on diseased material showed that the causal agent of the disease is *Gleosporium olivarum* Alm.

Although there is a record about the occurrence of this disease in olive plantations of Turkey (İyriboz, 1968) there is not any research on this subject until today. The pathogen was observed in some other countries where the olive production is important but it was reported that only in Portugal and Greece (Pontis and Hansen, 1942) and in Italy (Marcelli, 1960) it causes an economical damage.

The main purpose of this study is to establish the occurrence of *G. olivarum* in Turkey scientifically. According to the statistics there are 802.000 tons olive fruits and 159.000 tons olive oil production in Turkey (State Institute of Statistics, 1968). Although a survey was not carried out, it is thought that the disease is especially important on the oil varieties, because the pathogen was first observed on an oil variety. The possibilitiy of the effect of pathogen on the acidity of olive oil was also studied here.

Thanks are extended to Tamay Işıklı and Ayten Aydoğan of the Institute of Olive Research for their kind help to analyse the olive oil samples.

MATERIALS and METHODS

Pathogen was isolated from the diseased olive fruits of ***Olea europaea*** var. ***Yerli yağlık*** from Güzelbahce (İZMİR). Diseased fruits which were showed distinctive symptoms and used for isolations were shown at fig. 1 and 2.

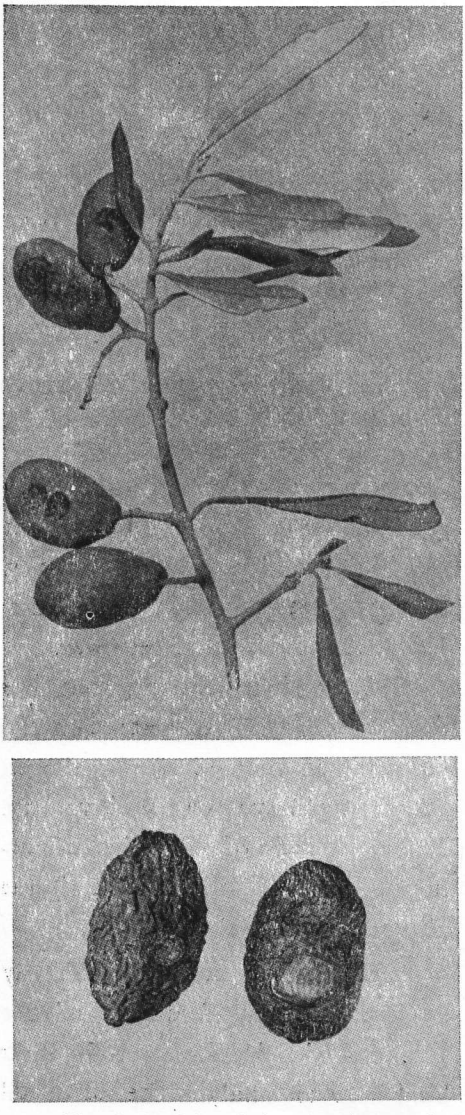

Fig. 1 - 2. Olive fruits showing typical symptoms of **G. olivarum.**

Pure cultures of the fungus were grown on Richard solution (Démétriades, 1959). Inoculum was prepared from these pure cultures with steril water and olive fruits were inoculated ***in vivo*** and ***in vitro*** conditions.

For the inoculations which were made ***in vitro*** some of the olive fruits were pricked by a needle and some of them were left as they were. These two lots of fruits were placed in separate Petri - dishes then the inoculum was given dropwise using a pippette. In the other Petri - dishes pricked and unpricked fruits were kept as controls.

For the studies ***in vivo*** five young shoots bearing fruits were chosen from the same variety in the garden of Institute. Fruits on two breanches were pricked and the fruits on the other two branches were left unpricked, the fifth branch was kept as a control. Inoculum was applied by pulverising the olive fruits when they were gain the susceptibility at the begining of the browning (Zachos and Markis, 1959). Inoculation was repeated two days in succession. The branches were covered with plastic bags after each inoculation.

First obervation was done 10 days after inoculations and continued

everyday until the symptoms occured on fruits. At the same time the leaves also checked.

In order to confirm the pathogen, reisolations were done and to find the dimentions of the spores a hundred measurment was taken. The effect of *G. olivarum* on the oil acidity was investigated by analysing the oil from infected and healthy fruits of the same olive variety (Yerli Yağlık) and same trees. The acidity of the oil was determined according to the "Methods for controlling the Cooking olive oil" (Turkish standarts, 1966).

RESULTS and DISCUSSION

In the laboratory tests, only the pricked fruits were covered with a dark coloured fungal growth. On the other hand the colour of the the unpricked and control fruits were turned to violet and they were shrivelled but no fungal growth was observed on them (Fig. 3).

As the results of the experiments in the garden of the Institute pricked and unpricked fruits showed symptoms but there was no symptoms on control fruits. Viennot-Bourgin (1949) reported that the pathogen can penetrate easily to the living tissue. At the end of the inoculation fruits showing the typical symptoms given in Fig. 4.

As a result of re-isolations from the diseased fruits *in vivo and in vitro* conditions the cup-shaped acervuli which contained the conidia of fungus were obtained. Spores liberating from the acervuli where ovoid, hyaline and granular and average dimention was 22.41 x 5.52. μ The dimentions were varied between

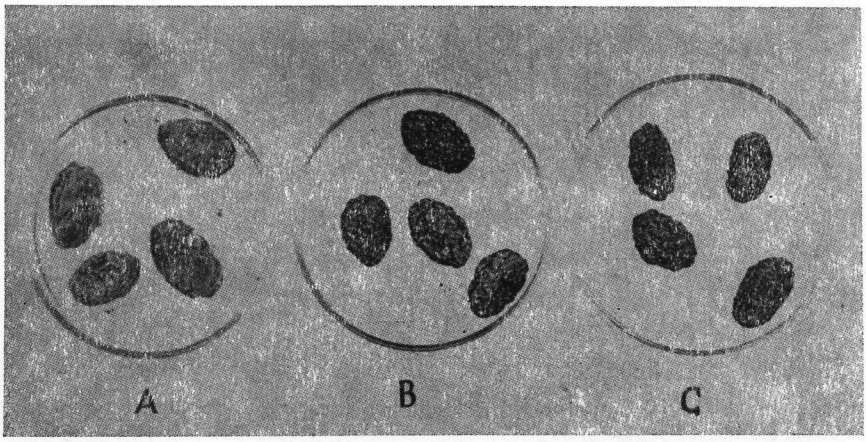

Fig. 3. Olive fruits in petri dishes inoculated under the laboratory conditions.
A) Pricked fruits showing the fungal growth B) Unpricked fruits C) Control fruits

17.59 — 28.98 × 4.14 — 7.24 μ. These results were supported the findings of Pontos (1967) and Zachos and Markis (1963). Fig. 5 shows the spores obtained from reisolations.

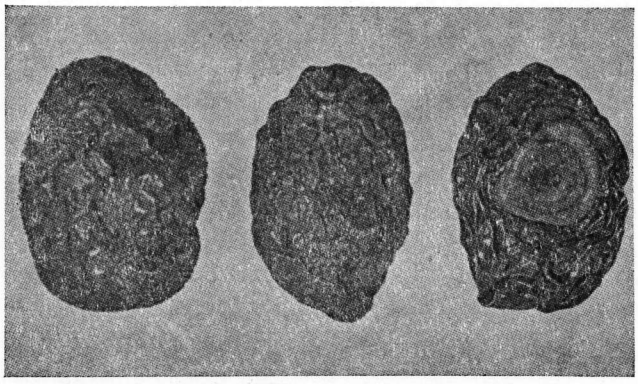

Fig 4. Olive fruits showing symptoms as a result of out - door inoculation.

Fig. 5. Spores of **G. Olivarum** (x 130)

During this study any symptoms were not observed on the leaves. Also Zachos and Markis (1963) recorded that the leaves were more tolerant to pathogen and only the pricked leaves were invaded slightly.

The results of the analyses of the oil from healthy fruits gave an acidity of 0.61 whereas the oil acidity from diseased fruits was 4.05.

So, there is a need of a more detailed study on the effect of *G. olivarum* to the acidity of the olive oil in our country where the olive oil production has an economical importance.

ÖZET

EGE BÖLGESİNDE GLEOSPORIUM OLIVARUM ALM.

Ege Bölgesinde yapılan tetkikler sonunda, zeytin meyveleri üzerindeki leke ve çökmeler ile meyvaların mumyalaşması dikkati çekmiş, materyal üzerindeki kültürel çalışmalar sonucu etmenin *Gleosporium olivarum* Alm. olduğu tesbit edilmiştir. Bu patojenin yurdumuz zeytinliklerinde bulunuşu literatürde kayıtlı olmasına rağmen, bu çalışmanın asıl amacı *G. olivarum*'un Türkiyede bulunuşunun bilimsel olarak saptanmasıdır

Gerek laboratuvar şartlarında üzeri delinen zeytin meyvelerine yapılan inokulasyonlar ve gerekse doğa şartlarında bir zeytin ağacının meyvaları delinen ve delinmeden bırakılan dallarına yapılan inokulasyonlarda hastalığın tipik simptomları görülmüş ve reisolasyonlar sonucu fungusun conidi taşıyan kadeh şeklindeki *acervuli*'si ve conidileri gözlenmiştir. Yapraklarda ise hiç bir enfeksiyon elde edilememiştir.

Her hangi bir sörvey yapılmamasına rağmen yağlık çeşitlerimizde yüksek nisbette hastalığa rastlamamız sebebiyle patogenin yağ kalitesine etkisi araştırılmıştır. Yapılan yağ analizleri sonucu, sağlam meyvalara ait yağın asiditesi 0,61,*G. olivarum* enfeksiyonlarına uğramış meyvalara ait yağın asiditesi ise 4,05 olarak saptanmıştır. Böylece zeytin yağcılığının önemli olduğu ülkemizde *G. olivarum*'un yağ asiditesine etkisi ile ilgili çalışmalara daha detaylı olarak devam edilmesinin gerektiği ortaya çıkmıştır.

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Verticillium wilt of olives in Turkey

Coşkun SAYDAM and Mustafa COPCU

INTRODUCTION

Olive is a crop of great importance and there are 72 280.000 olive trees in Turkey. Olive Oil production is 159 000 tons per year (7) The total olive tree in the Ege region is approximately over 60.000 000 trees and representing the 82 % of the total olive trees. In the Spring of 1970, some olive trees were first observed at Milas showing symptoms typical of Verticillium wilt. Later the disease was also found in Ayvalık. Verticillum wilt in Turkey has so far been recorded only on cotton and some vegetables and peach (1, 2, 3, 5, 6). Therefore, this constitutes is the first report of the fungus causing damage to olive trees.

SYMPTOMS

The symptoms observed on the olive trees caused by Verticillium wilt were essentially the some as those described by other workers (9, 11). The symptoms were loosing the natural colour of the leaves which are the deep green, and becoming dull gray and brown. Defoliation and dieback of twig may follow discoloration of the leaves. These symptoms may be observed only one side or more sides of the trees (Fig. 1 A). The branches of the affected trees showed no vascular discoloration.

Isolations were made from twig pieces of diseased trees on 0.8 % water agar. The bank of the diseased twigs was peeled off and surface sterilized with absolute alcohol, washed in sterile water and flamed. Chips cut from the wood of the twigs or entire cross sections cut with a hack knife were embedded in sterile petri-dishes and water agar at a temparature of 50°C was poured on them (9). The dishes were incubated at 22°C for a week and the fungal colonies were identified as *Verticillium* sp. Mycelium from several colonies was transferred to sucrose-nitrate agar medium (4) upon which the fungus formed-determined to be *Verticillium dahliae* Kleb. (Fig. 1 B, C.)

Capacity of the fungus to cause infection in other hostplants:

The experiment was conducted, using *V. dahliae* isolated from olive trees again to see if it can

cause infection in some other host plants commonly grown in this region. Capacity of the fungus the cause infection in other host plants were made with cotton, Chili Plants, tomato and eggplant seedlings in the 3 - to 4 leaf stage, by using the method based on Wiles (10) technique for inoculations.

Two mycelial mats obtained after 10 days growth each on 50 ml. sucrose - nitrate medium in 300 cc. flasks, were blended in 200 ml. distilled water at high speed in warring blender for 1 minute. Roots of the experimental seedlings were dipped in this solution for 10 minutes and the seedlings transplanted in pots. Roots of seedlings of check plants were dipped in distelled water alone.

Observations were made on the number of plants showing wilt (Fig. 1 D) and the intensity of disease was given adopting the scale of Staffeldt and Fryxell (7) in table I and fungus were re - isolated from all of the inoculated plants.

Table 1 Infection of other host plants by *V. dahliae* isolated from olive trees (VO_1 isolate from Milas, VO_2 isolate from Ayvalık).

Host plant	Variety	Number of inoculated plants		Number of diseased plants		Disease intensity after 1 month	
		VO_1	VO_2	VO_1	VO_2	VO_1	VO_2
COTTON	Coker $100/A_2$	12	12	12	12	4	4
CHECK	»	12	12	0	0	0	0
EGGPLANT	Halkapınar	12	12	12	12	4	4
CHECK	»	12	12	0	0	0	0
TOMATO	Karabağlar	12	12	12	12	3	3
CHECK	»	12	12	0	0	0	0
CHILI PLANT	Dolmalık	12	12	12	12	2	3
CHECK	»	12	12	0	0	0	0

We are grateful to Mr N. Kaşkaloğlu and Mr. D. Toplu for providing facilites in connection with this work.

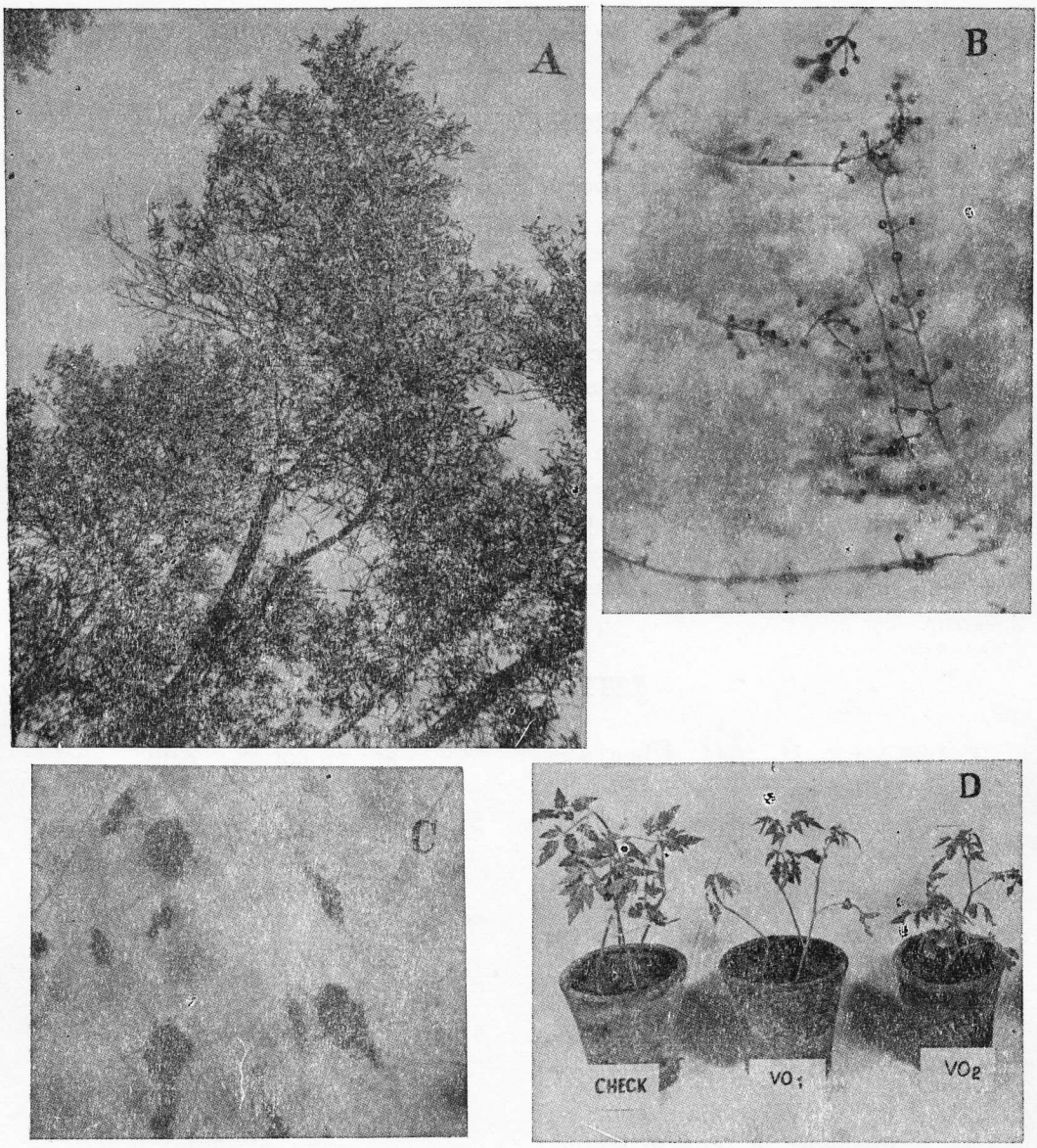

FIGURE 1.

A ——————— Symptoms of Verticillium wilt on olive tree

B,C ——————— Verticils and sclerotia of the pathogen (Approx. X 150)

D ——————— Artificialy inoculated with the V. dahliae isolated from olive trees (VO_1 isolated from Milas, VO_2 isolated from Ayvalık) and check tomato seedlings 1 month after inoculation.

ÖZET

TÜRKİYE'DE ZEYTİNLERDE VERTİCİLLİUM SOLGUNLUĞU

Zeytin Türkiyenin en önemli tarım ürünlerinden biridir ve Ege bölgesi yaklaşık olarak tüm zeytin plântasyonumuzun % 82 sine sahiptir. 1970 yılı ilkbaharında ki surveyler sırasında Milas'taki bazı zeytin ağaçlarında *Verticillium* solgunluğu dikkati çekmiş, daha sonra hastalığın Ayvalık zeytinlerinde de bulunduğu anlaşılmıştır. Türkiye'de pamuk, bazı sebzeler ve şeftalide *Verticillium* solgunluğu bildirilmişsede zeytinlerde ilk defa tesbit edilmektedir.

Hastalık belirtileri, zeytin yapraklarının normal yeşil renginin kaybolması ve koyu gri kahverengimsi renk alması, dökülme ve dallarda tepeden aşağı doğru kuruma şeklinde özetlenebilir. Bu kurumalar ağacın sadece bir veya bir iki kısmında görülür.

Hastalıklı ağaçlardan alınan örneklerden yapılan izolâsyon çalışmaları hastalık etmeni fungusun *Verticillium dahliae* Kleb. olduğunu ortaya koymuştur.

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Prevalence of some incitants causing dieback and timber decay of hardwoods

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ABSTRACT

An investigation was carried out to record different fungal pathogens responsible for dieback and timber decay to ***Morus alba***, ***Dalbergia sissoo***, and some other members of hardwoods, in the irrigated plantations of west Pakistan. Pathogens like ***Diaporthe mori*** ***(Phomopsis*** state ***)***, ***Eutypella russodes*** and ***Coniothyrium olivaceum*** caused characteristic dieback to ***Morus alba***. In the case of ***D. sissoo***, fungi such as ***Camarosporium quaternatum***, ***Coniothyrium fuckelli*** caused dieback. Timber decay was mostly by the members of Basidiomycetes. ***Ganoderma lucidum :*** an incitant of basal stem rot, was highly virulent in all the irrigated plantations.

INTRODUCTION

During an investigation on the survey of the diseases of hardwoods, some incitants were recorded causing dieback and timber decay in the plantations. The irrigated plantations of West Pakistan mainly consist of Toot ***(Morus alba)*** and Shishum ***(Dalbergia sissoo)***. The following: White poplar ***(Populus alba)***, Black poplar ***(P. nigra)***, Nim ***(Melia azedarach)***, Chinar ***(Platanus orientalis)*** and Kikkar (***Acacia spp.***) also occur amongst the plantings. Some of the plantations at Changa Manga, Kundian (Mianwali,) Pirawala (Khanewal) and Datter (Sarghoda) were visited to find out different pathogens causing twig blight and timber decay.

It is difficult to make an exact distinction of dieback but it is characterised by the progressive dieback of a stem from the tip (1). Commonly a fungus attacks at or near the tip and later moves down the stem accompanied with the death of tissue as it advances. Dieback is a common phenomenon in woody plants having an indefinite annual growth (1). Some fungi belonging to genera ***Coniothyrium***, ***Phoma***, ***Phomopsis*** and ***Sphaeropsis*** are reported to be associated with die-

cack of American Elms in Illinos (5,9). ***Diplodia pinia*** (Desm.) Kickx., causes dieback of American pines and in severe attack stunting of growth manifests (4). ***Deuterophoma tracheiphila*** Petri., is a serious twig blight or dieback pathogen of citrus tree in Turkey (8).

Sometimes dieback is resulted due to the potash deficiency in the soil (7). Moisture deficient soils also play a major role in interfering nutritive and physiological processes of a plant body. Eventually storage and production of food is ham pered. This shortage may not manifest in the same year but effect is delayed until the approach of next season when weak shoots are developed and the twigs, branches show dieback (6). When twigs die then pathogenic and nonpathogenic fungi attack. In such instances it is quite difficult to locate the reason of dieback.

Fungi are well adapted both as obligate and facultative parasites to cause decomposition of massive volumes of wood whether in standing tree or in the processed product (3). This is because the fungal hyphae posseses an intrusive growth habit and can easily penetrate the hardest and toughest wood. These hypha excrete wood dissolving enzymes and develop enzymatic cavities around the hyphae of wood decaying fungi (2).

RESULTS AND OBSERVATIONS

It was observed in most of the irrigated plantations that exposed tree stumps promote fungus multiplication. When the trees are felled in the forest plantations for thinning purposes such stumps are left unuprooted. These stumps undergo festering for a long time. During this time many species of fungi belonging to Ascomycetes, Deuteromycetes and Basidiomycetes occupy the woody debris and start establishing by producing mycelial mat from the food of the host. Fungi like ***Daldinia concentrica***, ***Lenzites*** sp. ***Schizophyllum commune*** and ***Ganoderma lucidum*** were commonly found growing on the stumps.

It was also observed in the Pirawal-Khanewal plantations that the unhealthy and death of many trees of ***Dalbergia sissoo*** and ***Morus alba*** was due to drought. The irrigation water is not properly supplied according to the need of the plant body subsequently the nutritive processes are affected. The storage reserve of carbohydrates become less and this shortage appears in the next season when new shoots develop and show dieback (6).

TABLE 1. Fungi causing dieback to *Morus alba*

Coniothyrium olivaceum Bon fuckel
Diaporthe mori Berb. *(Phompsis* state*)*.
Diplodia mori West
Eutypella russodes (Berk and Fr.) Berl.
Haplosporella mori West
Macrophoma pinea Lands
Nectria galligena Brcss.
Teichospora trabicola (Pers.) Rab.
Xylaria hypoxyla (L.) Grev.

TABLE 2. Fungi causing timber decay in *Morus alba*

Fomes applanatus (Pers. ex Fr.) Gill.
Ganoderma lucidum (Leys.) Karst
Lenzites betulina (L.) Fr.
Schizophyllum commune Fr.
Trametes hispida Bagl.

TABLE 3. Fungi causing dieback to *Dalbergia sissoo*

Camarosporium quaternatum Schulz
Coniothyrium fuckelii Sacc.
Diplodia dalbergiae Shani
Eutypella russodes (Berk and Fr.*)* Berl.
Hysterographium fraxini (Pers.) De N.
Microdiplodia conigena Allesch
Nectria cinnabarina (Tode) Fr.
Phomopsis dalbergiae shani.
Phoma herbarum West
Stagonospora subseriata (Desm.) Sacc.

TABLE 4. Fungi causing timber decay in *D. sissoo*

Ganoderma lucidum (Leys.) Karst
Irpex sp
Lenzites betulina (L.) Fr.
Poria sp.
Stereum hirsutum (Willd) Pers.

DISCUSSION

Diebaek caused by some fungi like *Coniothyrium olivaceum*, *Diaporthe mori*, and *Eutypella russodes* to *Morus alba* twigs was very characteristic and easily distinguishable. Sometimes twigs lowered in vitality and winter killed were attacked by saprophytic or weakly parasitic fungi and it became difficult to ascertain the reason of dieback. *Nectria cinnabarina* is a weak parasite and develops reddish brown cankers on the twigs of *D. sisso*. The twigs bearing such cankers manifested dieback.

Timber decay was mostly by members of Basidiomycetes. Infec tion started and the mycelium ramified in the tissues but identifi-

cation was possible only when conks developed on the trunk or branches. ***Ganoderma lucidum*** is a highly virulent pathogen of ***D. sissoo***. It at tacked the base of the tree causing basal stem rot disease, subsequently, the base of the tree becomes weakened and with wind storms or rains the tree collapses. This pathogen was found occurring in all the plantations of West Pakistan. ***Schizophyllum commune*** was very common on the fallen logs and on standing tree trunks of ***M. alba*** and ***D. sissoo.*** The exposed tree stumps of ***M. alba*** and ***D. sissoo*** were found attacked by ***Daldinia concentrica*** and ***Stereum hirsitum.*** Tree bearing conks and sporophores were rendered unmarketable and unfit for commercial exploitation.

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Effect of different nutrition sources and light on sporulation and mycelial growth of Fusarium oxysporum f. vasinfectum

Sevinç CEYLAN

ABSTRACT

In the present investigations it was found that for the sporulation of *Fusarium oxysporum* f. *vasinfectum* the suitable nitrogen, carbon and natural nutrition sources were urea, sucrose and ricemeal media, respectively. In case of mycelial growth, KNO_3 as nitrogen, maltose and starch as carbon, and oatmeal as natural nutrition sources were favourable. Light decreased the degree of sporulation of the fungus on all media. It caused positive effect on the size of spores and the radial growth of the fungus.

INTRODUCTION

Fusarium oxysporum f. *vasinfectum* (Atk.) Synder and Hansen, is one of the pathogens causing wilt disease of cotton (*Gossypium barbadense* L.)

Nitrogen and carbon are the most important nutrients responsible for the growth and sporulation of fungi. The best nitrogen source for the sporulation and mycelial growth of *Fusarium vasinfectum* Atk., is urea (14). Pai (11) found in his investigations on *F. moniliforme* Sheld and *F. vasinfectum* Atk., that both fungi had been grown on the nitrate media. *F. udum* Butler, exhibited rich sporulation on medium contaning urea, but there was no myeelial development and sporulation on the nitrite medium supplying nitrogen. In the medium containing ammonium tartarate the fungus could be grown only as mycelia (15).

An investigation carried out by Natarajan (9) to study development of fungi like *F vasinfectum* and *F. udum* arrayed the carbon source as following: Fructose > sucrose > maltose > glucose > mannose > lactose > mannitol > galactose >

sorbitol. Chi and Hansen (2) reported that the best carbon source for the sporulation of *F oxysporum* were starch and mannitol. According to Hendrix and Toussoun (6), the ratio in the medium affected sporulation. Keeping this idea Prasad and Choudhary (12), proposed that the production of macroconidia and microconidia of *F. udum* the C: N ratio should be 3.368 : 0.030 gr / 1., and for the production of chlamydospore it must be 3.368: 0.1220 gr / 1.

Light had definite effects on the sporulation of some species of *Fusarium*. The fungi produced larger conidia in light than in dark. Besides, the quantity of macroconidia and microconidia were abundant in light and in dark, respectively, (5). Fungi required intermittent light than the continous light. For example, in a phase of 12 hours light with 12 hours dark, the sporulation was rich as compared to continous light conditions (10).

In this paper, the effect of carbon, nitrogen and natural nutrition sources and light, on the sporulation and radial growth of *Fusarium oxysporum* f. *vasinfectum* was studied. The aim was to determine an inoculum which can be suitable and practicable for the pathogenicity and resistance experiments.

MATERIALS AND METHODS

Culture of *Fusarium oxysporum* f. *vasinfectum* (Atk.) Snyder and Hansen, was procured from Institute of Phytopathology, University of Justus Liebig, Germany, and used in the present study.

A semi-synthetic medium was used following Lilly and Barnett(7). To test the effect of nitrogen source, the carbon source was no changed (25 g glucose/1000 ml), but asparagine, urea and KNO_3 were added to the basic medium at different levels in order to get a nitrogen concentration as following: 0, 106.25, 212.50, 425 00, 37.50, and 850.00 ppm.

In case of carbon medium, the nitrogen content was fixed (8 g. asparagine/1000 ml). To get a carbon concentration of 0, 5,000, 10,000, 15,000, 20,000, and 25,000 ppm, glucose, maltose, sucrose, and starch were added to the basic medium.

In order to prepare the natural nutrition medium, Alexopoulos and Beneke (1) basic medium was adopted. Cornmeal, ricemeal, and oatmeal were used in the concentration of 0, 10,000, 20,000, 30,000, 40,000 and 50,000 ppm.

The seeding was carried with the pure cultures which were grown on PDA. The cultures were incubated in a humidity at 2000 luxes

light, 27° C, for 8 hours in light and 16 hours in dark, to record the effect of intermittent light on the sporulation.

Following the seeding, the cultures were examined during 10 days when the fungus spread in petri dishes. In these observations, the development, shape and the colour of colony, were recorded. At 19th day, the spores were counted and measured. For statistical analysis, spore counts per unit area were determined, and for each treatment 100 counts were made. Then spore numbers per mm^3 of the medium were computed.

RESULTS

1. The effect of nitrogon sources.

Urea was the best nitrogen source for the sporulation of fungus (Table 1). Next were asparagine and KNO_3. The highest sporulation was at urea level II (212 50 ppm N) and lowest sporulation was at KNO_3 level V (850.00 ppm N). For the radial growth of the fungus, the best nitrogen source was KNO_3. On these media, the fungus covered the petri dishes in 8 days. Although on urea and asparagine media this event was changing between 9 and 10 days

2. The effect of carbon sources.

The most suitable carbon source for the sporulation was sucrose followed by maltose and glucose as the second and third grade, respectively (Table 2). The least spore forming source was starch. For the mycelial growth maltose and starch media were favourable. Fungus could cover the petri dishes in 7 days on both media. On the media with sucrose, development of mycelium was slower (was in 11 - 12 days) as compared to that on other two carbon sources

3. Effect of natural nutrition sources.

As natural nutrition sources ricemeal medium was quite suitable for the sporulation (Table 3). But these media were favourable for the development of mycelium. On the media with ricemeal and cornmeal the mycelial growth was slower.

4. The effect of light.

Light decreased the degree of the fungus on all media which were tested, especially, it was not favourable for the development of microconidia (Table 1 - 3). Light also effected the size of spores Spores developed in light were bigger than those formed in dark. Light had positive effect on the radial growth of the fungus in some media containing asparagine (nitrogen), sucrose and maltose (carbon) But on urea and glucose media, light caused delay on the radial growth. On media

which contained natural nutrition sources there was no differences between two conditions.

DISCUSSION

Best nitrogen source for the sporulation of *Fusarium oxysporum f. vasinfectum* (Aks.) Snyder and Hansen, was urea, next were asparagine and KNO_3 (Table 1). For mycelial growth urea was unsuitable. Similar investigation carried out by Subramanian and Pai (14) reported that amongst all different nitrogen sources, urea was the best N source for the sporulation of *F. vasinfectum*. Subramanian (15) recorded same results with *F. udum* Butler. Nitrate media were more favourable as compared to the other nitrogen sources for the development of mycelium (11,14,8). In general, fungi make use of organic nitrogen sources for the sporulation, and inorganic nitrogen sources for the vegetative growth (3, 14).

In case of carbon sources for sporulation, the best was sucrose and maltose, glucose and starch were second, third and fourth grade better sources, respectiyely. At the sucrose level II the sporulation was maximum (Table 2). For the mycelial growth starch was favourable. According to Chi and Hanson (2), for the sporulation of *F. oxysporum* Shel., the best carbon sources were starch and mannitol It has been reported that lactic acid was favourable for the sporulation of *F udum*, and next were starch, inulin and sucrose (15). In general, for vegetative growth of microorganisms, starch was more suitable than glucose Since on starch media the acids collected more than on glucose media. Thus, fungus makes use of hydrolyzed source slowly but continously.

Light had no positive effect on the sporulation of *F. oxysporum f. vasinfectum*. It only caused increase in the amount of macroconidia and chlamydospore, and a decrease in the amount of microconidia (Table 1-3, Fig.1 2). Previously it has been recorded that light increased the sporulation of fungi (4, 10). Light had varied effects on the development of mycelium. It was unsuitable for the development of aerial mycelium, however, on some media (carbon, natural nutrition sources and asparagine) light caused positive effect on the radial growth of fungus It has been reported that fungi are susceptible to Ultroviole light (13).

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ÖZET

Çeşitli besin kaynakları ve ışığın **Fusarium oxysporum f. vasinfectum**'*un sporulasyonu ve mycelial gelişmesine etkisi.*

Bu çalışmada pamukta *(Gossypium barbadense L.)* solgunluk hastalığının etmeni *Fusarium oxysporum* f. *vasinfectum* (Atk.) Snyder and Hansen'un sporulasyonu ve mycelial gelişmesine azot, karbon ve doğal besin kaynakları ve ışığın etkisi incelenmiştir. Fungusun sporulasyonuna en uygun azot, karbon ve doğal besin kaynakları sırasıyla, üre, sakkaroz ve pirinç unu ortamlarıdır. Mycelium gelişimi bakımından KNO_3 (azot), maltoz ve nişasta (karbon) ve yulaf unu (doğal besin kaynağı) elverişli ortamlardı. Işık, denenen bütün ortamlarda, bu fungusun sporulasyonunu azaltmıştır. Yalnız ışık fungusun spor boyutları ve radial gelişmesi üzerine pozitif etkide bulunmuştur.

Table 1. The effect of different nitrogen sources on the sporulation of *F. oxysporum* f. *vasinfectum* in dark and light concitions

Nitrogen sources and levels[1]		The amount of spores in mm³					
		in dark			in light		
		micro-conidia	macro-conidia	chlamydo-spore	micro-conidia	macro-conidia	chlamydo-spore
Asparagine	I	194,700	—	2,500	58,750	—	—
»	II	420,300	—	—	118,100	300	1,900
»	III	292,000	—	—	104,000	1,700	500
»	IV	269,500	2,500	1,250	63,400	160	1,560
»	V	113,600	2,900	3,000	12,000	—	800
Urea	I	204,600	900	—	112,900	1,250	900
»	II	568,500	1,700	—	289,600	2,500	—
»	III	410,500	7,400	500	210,500	2,100	1,600
»	IV	251,600	5,300	2,200	100,200	1,100	600
»	V	192,400	1,300	1,300	48,400	900	300
KNO_3	I	238,400	—	2,900	46,400	1,600	—
»	II	351,700	4,340	—	73,500	2,500	1,000
»	III	368,400	900	—	66,400	2,000	800
»	IV	293,700	2,000	—	22,800	1,500	—
»	V	101,900	900	—	10,600	—	—
Control		23,700	2,200	—	16,800	—	—

1) 106.25 ppm N: Level I
212.50 » » : » II
425.00 » » : » III
637.50 » » : » IV
850.00 » » : » V

Table 2. The effect of different carbon sources on the sporulation of *F. oxysporum* f. *vasinfectum* in dark and light conditions

Carbon sources and levels[1)]		The amount of spores in 1 mm³					
		in dark			in light		
		micro-conidia	macro-conidia	chlamydo-spore	micro-conidia	macro-conidia	chlamydo-spore
Glucose	I	340,100	400	3,000	81,950	15,600	—
»	II	476,200	400	6,960	305,600	—	—
»	III	574,200	7,800	—	430,000	300	2,300
»	IV	645,300	2,200	2,170	296,100	1,250	200
»	V	334,000	900	1,300	203,750	—	—
Sucrose	I	545,100	85,900	10,000	142,950	5,470	500
»	II	1,329,300	14,200	10,000	438,000	5,150	600
»	III	423,000	5,200	6,900	378,000	—	—
»	IV	368,900	2,200	3,000	220,300	200	3,300
»	V	295,000	4,300	1,250	251,700	—	—
Maltose	I	344,200	7,000	1,700	115,000	600	—
»	II	795,900	1,300	—	208,000	2,300	300
»	III	706,400	2,200	1,300	280,000	1,100	800
»	IV	345,000	—	—	301,600	600	6,700
»	V	290,600	—	—	199,800	—	—
Starch	I	177,000	1,250	—	33,100	1,875	200
»	II	297,300	7,800	—	49,000	2,300	4,800
»	III	571,900	4,800	—	81,700	4,800	600
»	IV	532.000	6,500	—	135,000	5,500	—
»	V	469,200	13,000	—	112,500	2,800	200
Control		24,700	1,700	—	15,400	—	—

(1 5,000 ppm C: Level I
10,000 » »: » II
15,000 » »: » III
20,000 » »: » IV
25,000 » »: » V

Table 3. The effect of different natural nutrition sources on the sporulation of *F. oxysporum* f. *vaşinfectum* in dark and light conditions

Natural nutrition sources and revels[1]	The amount of spores in 1 mm³					
	in dark			in light		
	micro-conidia	macro-conidia	chlamydo-spore	micro-conidia	macro-conidia	chlamydo spore
Cornmeal I	187,300	1,700	3,900	24,200	200	—
» II	355,500	900	6,250	30,600	500	—
» III	315,000	3,000	5,600	53,600	3,400	—
» IV	175,000	400	14,000	32,800	4,500	—
» V	113,750	900	300	23,000	500	—
Ricemeal I	166,000	400	6,400	25,000	1,100	—
» II	407,900	3.900	1,700	43,000	200	—
» III	384,000	2,200	4,200	48,700	500	—
» IV	377,500	2,100	400	77,000	500	—
» V	281,250	6,100	6,600	62,600	1,250	—
Oatmeal I	262,900	3,900	—	22,200	500	—
» II	345,300	500	400	32,100	1,400	—
» III	267,300	1,300	—	57.200	300	—
» IV	203,500	400	—	22,900	200	—
» V	60,000	400	—	20,000	1,100	—
Control	133,100	400	17,200	20,500	600	200

1) 10,000 ppm natural nutrition: Level I
20,000 » » » : » II
30,000 » » » : » III
40,000 » » » : » IV
50,000 » » » : » V

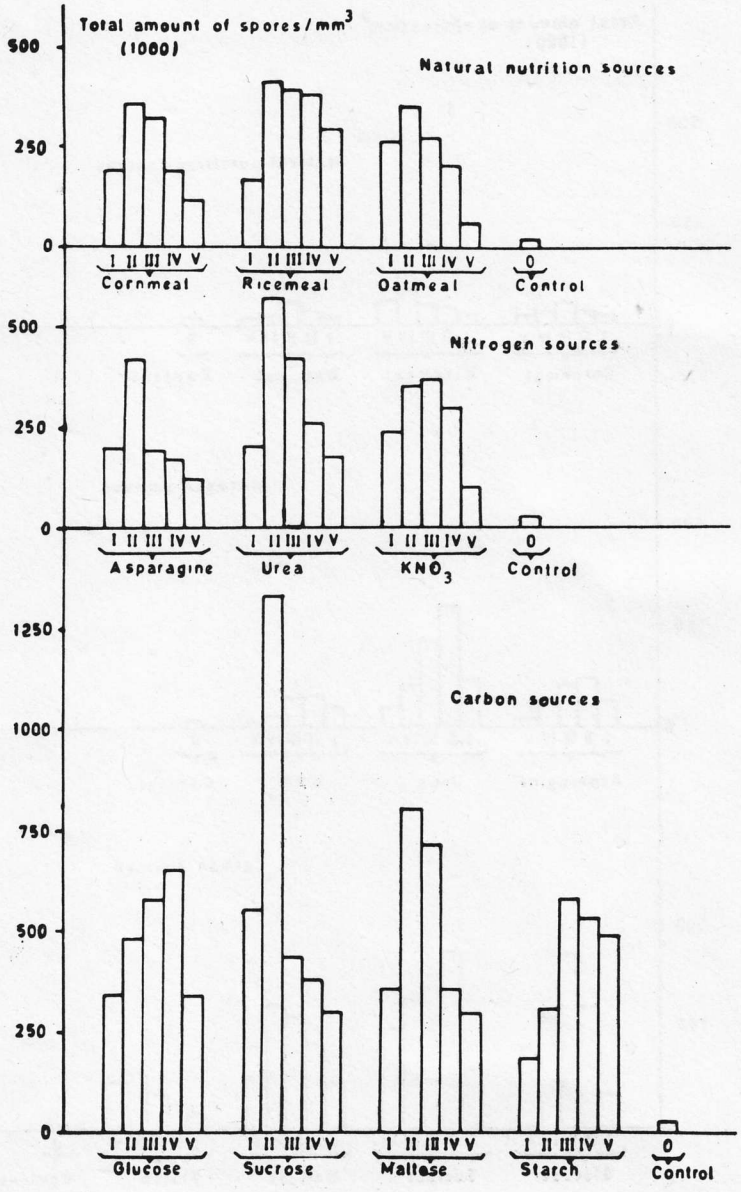

Fig. 1 The effect of different carbon, nitrogen and natural nutrition sources on the sporulation of **Fusarium oxysporum** f. **vasinfectum** in dark condition

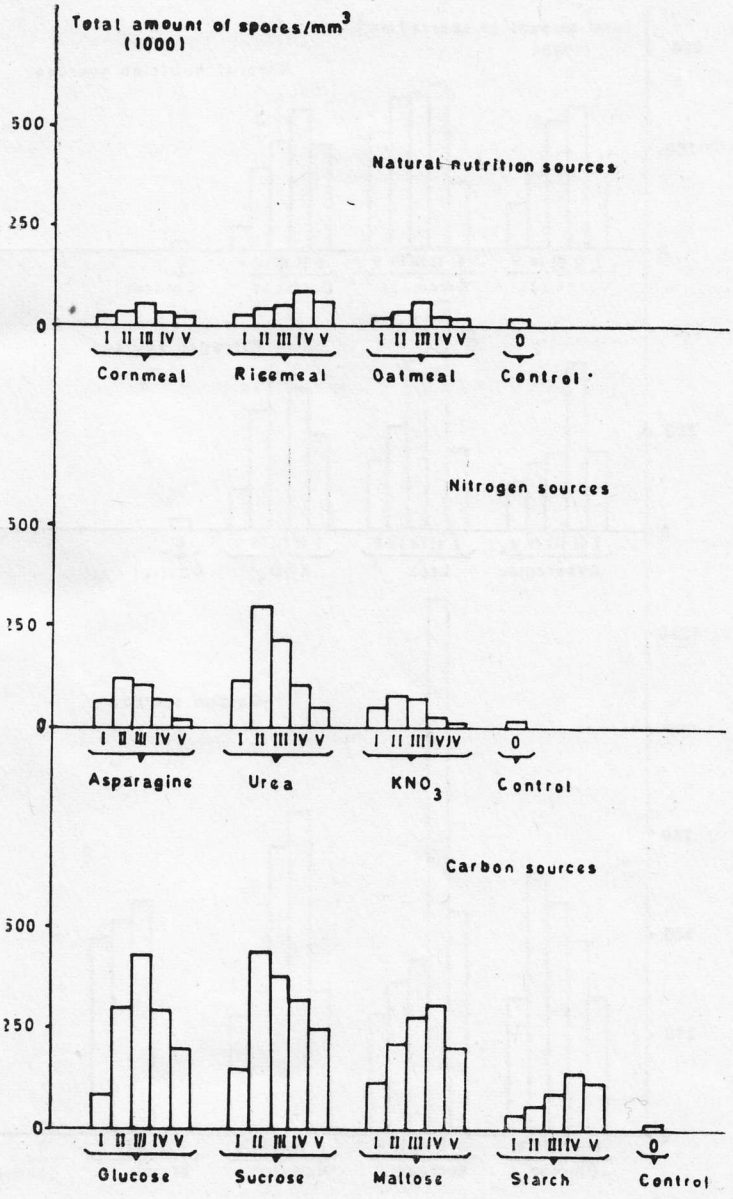

Fig. 2. The effect of different carbon, nitrogen and natural nutrition sources on the sporulation of **Fusarium oxysporum** f. **vasinfectum** in light condition.

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Biologische und serologische Untersuchungen an verschiedenen Isolaten des Gurkenmosaikvirus 1 [1]

Ülkü İZGİ [2]

ZUSAMMENFASSUNG

In dieser Arbeit wurde versucht, die Natur von zwei Virus-Isolaten aus der Türkei, eines von Gurke (GT) und eines von Paprika (PT) aufzuklären. Dafür wurden die biologischen, physikalischen und serologischen Eigenschaften dieser Isolate mit denen von vier verschiedenen Gurkenmosaikvirus - Isolaten (Y, Q, W_{14}, KD) verglichen, welche in der Literatur bereits beschrieben worden sind.

Auf Grund aller ermittelten biologischen, physikalischen und serologischen Eigenschaften ist das Isolat PT sehr wahrscheinlich ein Stamm des GMV, während dies für das Isolat GT mit Sicherheit festgestellt werden konnte

EINLEITUNG

In dieser Arbeit werden biologische und serologische Untersuchungen an vier verschiedenen Gurkenmosaikvirus-Isolaten und zwei weiteren Isolaten beschrieben.

In vielen Fällen ist die Identifizierung des Gurkenmosaikvirus mit Hilfe von Testpflanzen nicht ausreichend gelungen. Dagegen ist die serologische Methode eine schnelle und sichere Methode und in der Praxis gut andwendbar, wenn entsprechende Antiseren zur Verfügung stehen. Bis jetzt gab es nur wenige serologisehe Untersuchungen über das Gurkenmosaikvirus. Der Hauptgrund dafür dürfte in den Schwierigkeiten zu suchen sein, die früher bei der Reinigung des Gurkenmo

1) Auszug aus einer von der Landwirtschaftlichen Fakultät der Justus Liebig Universität Giessen genemigten gleichnamigen Dissertation.

2) Jetzige Anschrift : Ege Üniversitesi Ziraat Fakültesi, Fitopatoloji ve Ziraî ve Botanik Kürsüsü, Bornova-İzmir Türkei.

saikvirus (GMV) auftraten. Denn für die Herstellung von Antiseren sind hochgereinigte Präparate nötig und das ist in den letzten Jahren möglich geworden (SCOTT, 1963). So wird erst in jüngster Zeit das Gurkenmosaikvirus serologisch bearbeitet (KAHN and SCOTT, 1964; FRANCKI et al., 1966; VAN REGENMORTEL, 1966).

Das Ziel der eigenen Untersuchungen war es, verschiedene in der Literatur bereits beschriebene GMV-Isolate serologisch miteinander zu vergleichen und mit Hilfe serologischer Methoden die Natur der türkischen Virus-Isolaten festzustellen. Ausserdem wurde je ein Virus-Isolat von Paprika und Gurke mituntersucht, bei denen der Verdacht bestand, dass es sich um Isolate des GMV handeln könnte.

MATERIAL UND METHODE

Folgende Virus-Isolate wurden untersucht:

Virus Isolat	Wirtspflanze	Autoren
GMV-Y	Spinat	KUNTZ and WALKER (1947)
GMV-Q	Paprika	FRANCKI (1964)
GMV-W14	*Phaseolus vulgaris* L.	WHIPPLE and WALKER (1941)
GMV-KD	Ölkürbis	FORGHANI et al. (1966)
GT	Gurke	eigenes Isolat
PT	Paprika	» »

Testpflanzen: Als Testpflanzen wurden benutzt: *Nicotiana tabacum* L., Sorten «Xanthi nc» und «Samsun», *Nicotiana glutinosa* L., *Chenopodium amaranticolor* (Coste et Reyn.) *Chenopodium quinoa* Willd., *Vigna sinensis* Endl., *Cucumis sativus* L., *Capsicum annuum* L, *Lycopersicon esculentum* Mill.

Testverfahren: Alle Testplanzen wurden durch mechanische Inokulation mit virushaltigem Inokulum infiziert. Zur Inokulation wurden die Blätter vorher mit Karborund bestäubt

Bestimmung der physikalischen Eigenschaften:

Verdünnungsendpunkt: Zur Bestimmung des Verdünnunsgendpunktes wurden mit einer 10-faktöriellen Verdünnungsreihe mit 0.005 M Boratpuffer pH 9.0 hergestellt und die einzelnen Verdünnungsstufen getestet.

Thermale Inaktivierung: Die Inaktivierungstemperatur wurde mit virushaltigem Pflanzenrohsaft festgestellt. Für die einzelnen Temperaturstufen wurden je 10 ml des Inokulums 10 Minuten lang in ein

Wasserbad mit der entrprechenden Temperatur getaucht und nachher schnell im Eisbad gekühlt.

Bestimmung der Beständigkeit in vitro: Mit Blattmaterial und mit gereinigtem Virus.

Virusreinigung : Zur Reinigung des GMV wurde eine Reihe von Methoden angewendet, die bereits in der Literatur als für GMV geeignet beschrieben worden sind (TOMLINSON et al., 1959; SCOTT, 1963; GROGAN et al., 1963).

Differantial-Ultrazentrifugation:

Die differantial-Ultrazentrifugation wurde mit abwechselnd niedrigtourigen und hochtourigen Zentrifugationen durchgeführt.

Dichtengradienten Zentrifugationen : Nach Übereinschichtung der 40,30,20,10,% igen Saccharoselösung wurden die Gradienten mindestens 15 Stunden bei 4°C aufbewahrt. Dann wurde 0,5-1.0 ml Viruslösung hinzugegeben und zentrifugiert Nach dem Lauf wurden die Fraktionen aufgeteilt und serologische Aktivität gemessen.

Serologische Untersuchungen :

Die serologischen Teste wurden als Geldiffusionstest nach OUCHTERLONY (1949) durehgeführt. Zur Verhinderung der Austroeknung wurden die Agarplatten nach der Einfüllung mit Parafinöl überschichtet. Nach drei Tagen wurden die Präzipitionslinien bonitiert. Die Antiseren, die für die Teste nötig sind, wurden selbst hergestellt.

Zur Differenzierung wurden die Kreuzabsorptionsteste nach der Intra Gel methode von VAN REGENMORTEL (1967) durchgeführt.

Elektronenmikroskopie : Die hergestellten Präparate wurden zur Kontrastierung im Hochvakuum mit Platin - Iridium unter einem Winkel von 40° schrägbedampft und im Elektronenmikroskop EM 9a der Fa. Zeiss untersucht und aufgenommen.

ERGEBNISSE

Die Untersuchungen führten zu folgenden Ergebnissen :

1) Das türkische Isolat GT erzeugte an den entsprechenden Testpflanzen für das Gurkenmosaikvirus typische Symptome. Das Isolat PT unterscheidet sich in der Weise von allen anderen Iolaten, dass es sich nicht auf Gurke übertragbar ist und *Vigna sinensis* L. systemisch infiziert.

2) Zur Bestimmung der physikalischen Eigenschaften wurden die beiden Isolaten GT und PT mit dem Typ - Stamm Y des GMV verglichen. Es zeigt sich dabei, dass der Verdünnungsendpunkt für alle drei Isolate 1:1.000.000 beträgt und somit an der Grenze der in der Literatur angegebenen Werte liegt.

3) Die Inaktivierungstemperatur für die Isolate Y und PT liegt zwischen 70° und 75°C und für

das Isolat GT zwischen 75°-80°C. Diese Temperaturen liegen im oberen Bereich der Werte, die von verschiedenen Autoren ermittelt worden sind.

4) Für die Beständigkeit in vitro waren besonders zwei Fragen von Interesse :

a) Ob das virushaltige Blattmaterial direkt nach der Ernte im frischen Zustand verwendet werden muss, oder ob es möglich ist, die Blätter eingefroren zu lagern, um sie später aufgetaut zu verwenden.

b) Ob das gereinigte Virusmaterial sofort zur Immunisierung verwendet werden muss oder über längere Zeit im Kühlschrank oder im gefrorenem Zustand gelagert werden kann.

Nach den Versuchsergebnissen ist es nicht empfehlenswert für Virusisolation tiefgefrorenes Blattmaterial zu verwenden Die Infektiosität geht in eingefrorenen Blättern weitgehend verloren. Demgegenüber bleibt sie bei den gereinigten Präparaten nach dem Einfrieren fast vollständig erhalten.

5) Zur Feststellung der Verwandtschaftsbeziehungen zwischen Virus Isolaten sollte möglichst reines Antigen benutzt werden. Aus diesem Grund wurde versucht, für die untersuchenden Stämme die geeignetste Reinigungsmethode zu finden. Dafür wurden die einzelnen Methoden (TOMLINSON et al., 1963; GROGAN et al., 1963; SCOTT, 1963) bis zur zweiten hochtorigen Zentrifugation durchgeführt und auf ihre Infektiosität getestet. Die Reinigungsmethode nach SCOTT für GMV erwies sich für die türkischen Isolate GT und PT als die geeignetste Methode. Damit konnten pro Kg Blattmaterial zwischen 90-300 mg Virus isoliert werden.

6) Zur Feststellung der serologischen Verwandtschaftsbeziehungen zwischen den verschiedenen Virus Isolaten wurden homologe, heterologe und Kreuzabsorptionsteste durchgeführt.

Die Teste mit homologem System dienen zur Feststellung der Titer von Antigen und Antiserum. Die serologischen Teste wurden als Agar-Geldiffusionstest nach OUCHTERLONY (1949) durchgeführt. Bei den Testen wurde das Antiserum in 2-faktöriellen Verdünnung und das Antigen in 7 Konzentrationsstufen von 4-0 06 mg Virus/ml benutzt. Die Präzipitationslinien bei homologen Teste sind zusammen mit den heterologen Testen in Tab. 1 zusammengefasst. Aus diesen Resultaten kann man zusammenfassend folgern, dass die Gewinnung hochtitriger Antiseren trotz wiederholter Immunisierung mit konzentrierten Antigen-Lösungen nicht möglich zu sein scheint. Der höchste Antiserum-Titer, der erreicht werden konnte, war 1:128 für die Antiseren gegen die Isolate Y und Q. Die gegen die vier übrigen Isolate gerichteten Antiseren hatten lediglich einen

Titer von max. 1:32. Die Nachweisgrenze der Antigene liegt zwischen 0.125 und 0.0.6 mg Virus pro ml. Optimale Verhältnisse für den serologischen Nachweis des GMV dürften demnach etwa bei 0.25 bis 0.5 mg/ml Virus und bei einer Antiserum-Verdünnung von 1/4 liegen.

Hetcrologe Teste: Die heterologen serologischen Teste sollten dazu dienen, die serologischen Verwandtschaft zwischen den verschiedenen GMV-Isolaten festzustellen. Heterologe Teste führt man in der Weise, dass man ein bestimmtes Antiserum mit verschidenen heterelogen Antigenen reagieren lässt d h. mit solchen Virus-Isolaten, welche nicht zur Herstellung dieses Antiserums selbet benutzt worden waren. Die Ergebnisse der bei 6 Virus-Isolaten möglichen 30 heterologen Kombinationen sind in Tab.1 gemeinsam mit den Werten für die entsprechenden homologen Kombinationen zusammengefasst. Aus dieser Tabelle lassen sich folgende allgemeine Schlüsse ziehen :

a) Die besten Ergebnisse liefert das Antiserum Q, welches bei einem Titer von 1:128 mit den heterologen Antigenen bei relativ geringer Viruskonzentration reagiert.

b) In den Antiseren Y, Q, und GT müssen gemeinsame Antikörper vorhanden sein, da diese mit ihren jeweiligen heterologen Antigenen Y, Q, und GT bei relativ hoher Verdünnung des Antiserums und auch des Antigens unter Bildung eines Präzipitates reagieren.

c) Das Antiserum gegen Isolat PT reagiert bei einem Titer von 1:32 mit dem homologen Antigen und dem heterologen Antigenen Q, Y, und GT, allerdings sind mit Ausnahme von Q hohe Antigenkonzentrationen dafür Voraussetzung.

d) Das Antiserum gegen das Isolat KD reagiert mit allen heterologen Antigenen, aber hochkonzentriertes Antigen W_{14} ist für diese Reaktion nötig.

e) Das Antiserum gegen Isolat W_{14} reagiert mit den heterologen Antigenen nur bei geringer Antiserum-Verdünnung. Dieses Ergebnis deutet auf eine entfernte Verwandtschaft zwischen dem Isolat W_{14} und den anderen Isolaten hin.

Kreuzabsorptionsteste : Zur näheren Bestimmung der Verwandtschaftsverhältnisse wurden Kreuzabsorptionsteste nach der «Intra-Gel-Methode» von VAN REGENMORTEL (1967) durchgeführt. Dafür wurden die Virus-Antigene in einer Verdünnung von 1:8 benutzt. In einer Kontrollversuchs-Serie wurde zur Absättigung der Antiseren an Stelle von Virus-Antigen Tabakpflanzen-Antigen verwendet, welches aus gesunden Tabakpflanzen isoliert wurde.

Die Ergebnisse der serologischen Teste mit kreuzabsorbierten Antiseren sind folgende (s hierzu Tab. 2 und 3):

Tabelle 1

Ergebnise der homologen und heterologen Teste mit Antiseren gegen vier verschiedene GMV-Isolate und die Virus-Isolate GT und PT[a])

Antiserum gegen	Q	Y	KD	W_{14}	GT	PT
Q	128 / 0,125	128 / 0,125	128 / 0,125	128 / 0,5	128 / 0,125	128 / 1,0
Y	64 / 0,125	128 / 0,06	32 / 0,125	32 / 1,0	64 / 0,06	4 / 0,25
KD	32 / 0,5	32 / 0,125	32 / 0,125	32 / 2,0	32 / 0,125	32 / 0,5
W_{14}	2 / 0,06	4 / 2,0	4 / 0,06	32 / 2,0	4 / 0,06	2 / 2,0
GT	128 / 0,25	128 / 2,0	8 / 2,0	32 / 2,0	32 / 2,0	32 / 0,25
PT	32 / 0,125	32 / 1,0	4 / 2,0	16 / 2,0	32 / 4,0	32 / 0,5

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

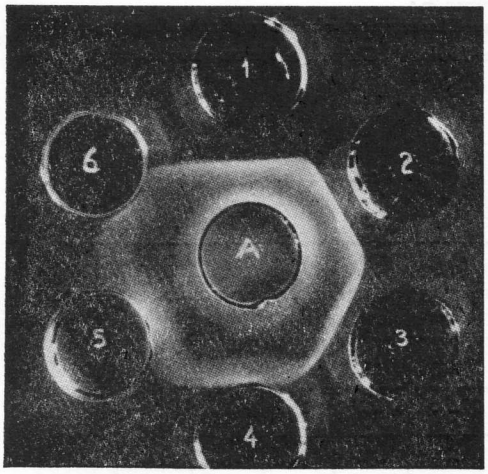

Abbildung 1
Serologische Reaktion im Geldiffusionstest zwischen Isolat GT und Antiserum gegen GMV-Q;A=Isolat GT (2mg/ml); in der peripheren Löchern Antiserum gegen GMV—Q; 1=u.v., 2=1/2, 3=1/4, 4=1/8,5=1/16,6—1/32.

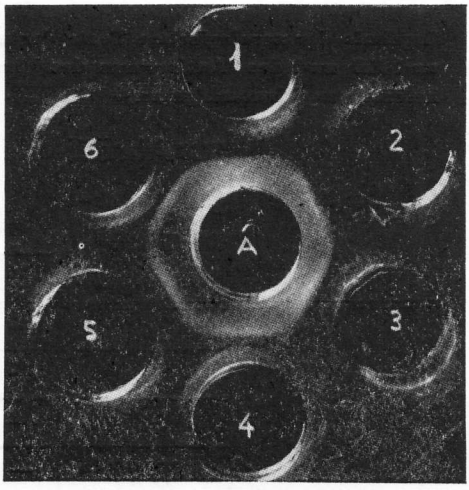

Abbildung 2
Serologische Reaktion im Geldiffusionstest zwischen Isolat GT und Antiseum gegen GMV—Y; A—Isolat GT (0,125 mg/ml); in den peripheren Löchern Antjserum gegen GMV—Y, 1=u.v., 2=1/2, 3=1/4, 4=1/8, 5=1/16, 6=1/32.

Isolat W_{14} besitzt mit allen anderen Isolaten gemeinsame serologisch aktive Gruppen. Darüberhinaus aber müssen im Antiserum noch W_{14}-spezifische Determinanten vorkommen, die bei keinem der heterologen Isolate vorhanden sind und daher auch durch die Kreuzabsorption nicht gebunden werden.

Beim Isolat KD sind mit allen Isolaten gemeinsame, serologisch aktive Gruppen vorhanden. Aber die Determinanten, die durch Kreuzabsorption nicht gebunden werden, reagieren noch mit den Virus-Antigenen Y, Q, und W_{14}.

Das türkische Isolat von Gurke (GT) ist ebenfalls mit allen anderen Isolaten serologisch verwandt, Darüberhinaus muss es aber mit den Isolaten Y, Q, spezifische Gruppen haben, die den anderen Isolaten fehlen.

Isolat Y teilt mit allen heterologen Isolaten serologisch aktive Gruppen; es besteht daher eine Verwandtschaft mit diesen Isolaten. Das zeigt sich auch im reziproken Test mit dem Antiserum Q, welches nach der Absorption vor allem noch mit dem Antigen Y reagiert. Daneben besitzt das Q natürlich mit

Tabelle 2:

Ergebnis der serologischen Teste der zwei kreuzabsorbierten GMV-Antiseren W14 und KD und des Antiserums des Virus-Isolats GT mit ihren homologen und heterologen Virusantigenen a)

Antiserum	zur Absättigung benutztes Antigen	Antigene						
		Y	Q	PT	W14	KD	GT	TP
W 14	Y	−	−	−	+	−	−	−
	Q	−	−	−	+	−	−	−
	PT	−	−	−	+	−	−	−
	W 14	−	−	−	−	−	−	−
	KD	−	−	−	+	−	−	−
	GT	−	−	−	+	−	−	−
	TP	−	−	−	+	−	−	−
KD	Y	−	−	−	−	−	−	−
	Q	−	−	−	+	−	−	−
	PT	−	+	−	−	−	−	−
	W14	+	+	−	−	−	−	−
	KD	−	−	−	−	−	−	−
	GT	−	−	−	−	−	−	−
	TP	−	−	−	−	−	−	−
GT	Y	−	+	−	−	−	−	−
	Q	−	−	−	−	−	−	−
	PT	+	+	−	−	−	−	−
	W14	+	+	−	−	−	−	−
	KD	+	+	−	−	−	−	−
	GT	−	−	−	−	−	−	−
	TP	−	−	−	−	−	−	−

a) Der Test wurde als Intra-Gel-Test nach VAN REGENMORTEL (1967) durchgeführt.
+= positive serologische Reaktion (Präzipitationslinie)
−= keine serologische Reaktion

Tabelle 3

Ergebnis der serologischen Teste der zwei kreuzabsorbierten GMV-Antiseren Y und Q und des Antiserums des Virus-Isolats PT mit ihren homologen und heterogen Virusantigenen)

Antiserum	zur Absättigung benutztes Antigen	Antigene						
		Y	Q	PT	W_{14}	KD	GT	TP
Y	Y	−	−	−	−	−	−	−
	Q	−	−	+	+	−	−	−
	PT	+	+	−	−	−	−	−
	W_{14}	+	+	−	−	−	−	−
	KD	+	+	−	−	−	−	−
	GT	+	+	−	−	−	−	−
	TP	−	+	−	−	−	−	
Q	Y	+	+	+	−	+	−	−
	Q	−	−	−	−	−	−	−
	PT	+	+	+	−	+	−	−
	W_{14}	+	+	+	−	+	−	−
	KD	+	+	+	−	−	−	−
	GT	+	+	−	−	+	−	−
	TP	−	+	−	−	+	−	
PT	Y	−	−	−	−	−	−	−
	Q	−	−	−	−	−	−	−
	PT	−	−	−	−	−	−	−
	W_{14}	−	−	−	−	−	−	−
	KD	−	−	−	−	−	−	−
	GT	−	−	−	−	−	−	−
	TP	−	−	−	−	−	−	

a) Der Test wurde als Intra-Gel-Test nach VAN REGENMORTEL (1967) durchgeführt.
+= positive serologische Reaktion (Präzipitationslinie);
−= keine serologische Reaktion.

allen anderen Isolaten gemeinsame serologisch aktive Gruppen.

Isolat PT fällt völlig aus dem Rahmen, denn das erschöpfte Antiserum gegen Isolat PT reagiert beim Rücktest mit keinem Virus - Antigen mehr.

Serologischer Nacgweis des GMV in Pflanzensäften : Alle untersuchten GMV - Isolate lassen sich in geklärten Rohsäften von Tabak und Paprika mit ausreichender Sicherheit schnell und einfach serologisch nachweisen, so dass dieses Verfahren auch in der Praxis gut anwendbar ist.

7) Im Saccharose - Dichtegradienten sedimentieren fünf der untersuchten Isolate als deutlische Bande, wobei die Infektiosität, die serologische Aktivität und die optische Dichte gut miteinander übereenstimmen. Isolat PT verhält sich völlig abweichend. Wegen Zerfalls der Virus-Partikel findet man bei diesem Isolat an Stelle der deutlichen Viruszone eine diffuse und sehr breite Bande. Danaben tritt noch eine sehr schwache und undeutlische Zone ca. 4 mm tiefer als bei den übrigen 5 Isolaten auf. Die Infektisität des Isolates PT liess sich nur in den unteren schwachen Zone nachweisen.

8) Die elektronenmikroskopischen Aufnahmen der beiden Isolaten PT und GT zeigen sphärische Partikel mit einem Durchmesser von 35 bis 38 mμ (Abb. 3 und 4). Sie entsprechen in Form und Grösse den eharakterischen Viruspartikeln aller beschriebenen GMV-Isolate.

Diskussion

Im Rahmen dieser Unterschungen sollten die Eigenschaften und die Natur der zwei türkischen Virus Isolaten bestimmt werden. Zu diesem Zweck wurden diese Isolate im Hinblick auf ihre biologischen und physikalischen Eigenschaften zusammen mit vier bereits in der Literatur beschriebenen Isolaten untersucht und verglichen. Ihre physikalischen Eigenschaften zeigten nämlich keinen grossen Unterschied zu denen der vier Vergleichsstämme und die Symptome auf Tabak-und Chenopodium Arten wichen ebenfals nicht von denen der Vergleichsstämme ab. Besondere Erwähnung verdienen allerdings die Reaktionen welche die türkischen Isolaten auf *Vigna sinensis* hervorrufen. Isolat GT produziert die üblichen punktförmigen Lokalläsionen auf den Primärblättern ohne systemische Erkrankung, wie es nach SILL and WALKER (1952a) für die meisten GMV-Stämme charakteristisch ist. Dagegen erzeugt Isolat PT keine Lokalläsionen auf den Primärblättern, sondern eine systemische Erkrankung der neu zuwachsenden Folgeblätter. Dieses Verhalten ist keineswegs ungewöhnlich für das GMV, denn SILL und WALKER (1952b) fanden bei dem GMV-W_{14} ein sehr ähnliches Verhalten und auch PAULUS et al (1962) isolierten von

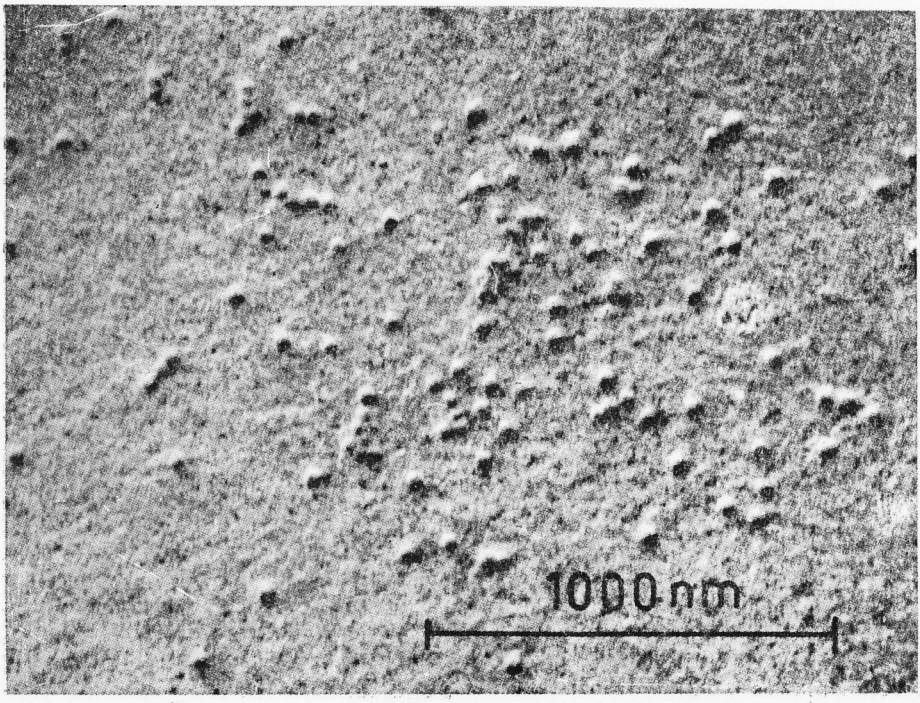

Abbildung 3
Elektronenoptische Aufnahme von charakteristischen Partikeln des Isolates GT
(Vergrösserung etwa 54.000-fach).

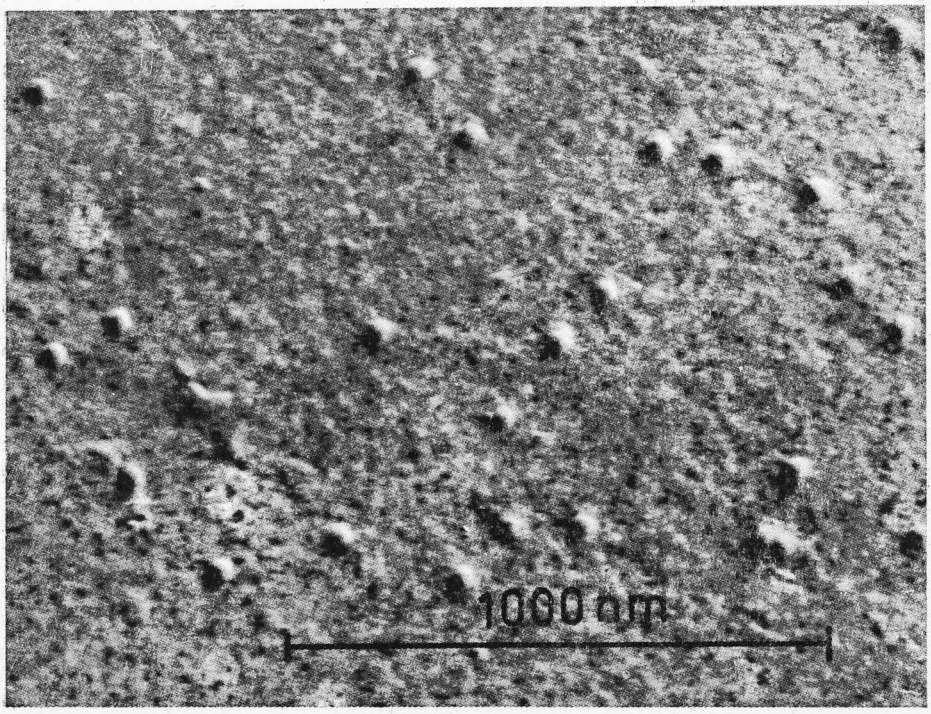

Abbildung 4
Elektronenoptische Aufnahme von charakteristischen Partikeln des Isolates PT
(Vergrösserung etwa 72.000-fach).

Paprika ein Isolat, welches Cowpea systemisch infiziert. Interessanterweise gelingt es nicht, das Isolat PT auf Gurke zu übertragen. Aber diese Abweichung ist bereits bei einigen Virus-Isolaten beobachtet worden, welche von Nicht-Cucurbitaceen isoliert wurden und sich als GMV-Stämme erwiesen Das gilt vor allem für bestimmte Isolate von Chrysanthemen (NOORDAM, 1952; GROGAN et al., 1963) und Tomaten (BLENCOWE and CALDWELL, 1949).

Das Isolat PT wurde ursprünglich von Paprika isoliert, bei dem es die sog. «Reisigkrankheit» hervorruft. Es ist auch sehr interessant, dass die fünf Isolate bei Paprika die gleichen Symptome der «Reisig krankheit» hervorrufen, wie das Isolat PT selbst.

Eine grössere Einheitlichkeit des Verhaltens der GMV-Isolate ergab sich beim Vergleich von drei verschiedenen Autoren zur Reinigung des GMV entwickelt worden waren (TOMLINSON et al. 1959 ; GROGAN et al., 1963; SCOTT, 1963). Es stellte sich nämlich dabei heraus, dass für alle Isolate eine einzige Reinigungsmethode nämlich die Methode nach SCOTT benutzt werden konnte. Weiterhin zeigte es, dass alle Isolate am besten aus frischem Blattmaterial aufgearbeitet werden, weil durch Einfrieren der infizierten Blätter ein erheblicher Teil der Infektiosität verloren geht

Als sehr kritische Faktoren für die Resuspension des gereinigten GMV haben sich die Art und Molarität des Puffermediums erwiesen (SCOTT, 1963). Bei der Verwendung eines ungeeigneten Puffers kann das GMV unlöslich bleiben oder sogar ausgefällt und denaturiert werden Bei der Klärung dieser Abhängigkeit stellte sich heraus, dass für alle Isolate der gleiche Puffer, nämlich 0.005 M Boratpuffer pH9.0 verwendet werden konnte. SCOTT (1963) hat diesen Puffer bei seinen Untersuchungen mit Isolat Y entwickelt, der sich auch anschliessend für das Isolat Q als am besten geeignet erwies (FRANCKI et al., 1966).

Alle bisher diskutierten Ergebnisse kann als eine Art Vorklärung der Zugehörigkeit der untersuchten Isolate zum GMV ansehen. Die entscheidenden Kriterien zur Klärung der Verwandtschaft liefern jedoch die serologischen Untersuchungen. In Übereinstimmung mit einigen Arbeiten allerjüngsten Datums (FRANCKI et al , 1966; SCOTT, 1963; VAN REGENNMORTEL, 1967) ergaben sich bei der Herstellung von Antiseren gegen verschiedenen GMV - Isolate keine grundsätzlichen Probleme. Aber trotz Injektion höchster Antigenkonzentrationen werden im Kaninchen Antiseren produziert, deren Titer 1:128 nicht übersteigt. Über ähnliche Resultate berichten auch SCOTT (1963) und FRANCKI et al. (1966). Diese Ergebnisse zeigen,

dass die verschiedenen GMV Isolate alle in gleicher Weise sehr schlechte Immunogene sind.

Trotz dieser Schwierigkeiten war es möglich, die zur Feststellung der serologischen Verwandtschaft notwendigen homologen und heterologen Teste und Absättigungsversuche erfolgreich durchzuführen. Alle sechs untersuchten Isolate zeigten miteinander Kreuzreaktion. In den Absättigungsversuchen stelle sich heraus, dass die Isolate Y, Q, und GT neben serologisch gemeinsamen Determinanten auch spezifisch aktive Gruppen besitzen. Das bereits symptomatologisch abweichende Isolat W_{14} reagierte allerdings nur bei höheren Antigenkonzentrationen und stets mit niedrigem Titer mit den übrigen Antiseren und zeichnete sich dementsprechend auch durch spezifische immunologisch aktive Gruppen aus. Das Isolat KD besitzt sowohl mit den Isolaten Y und Q als auch mit dem Isolat W_{14} gemeinsame serologische Gruppen In Übereinstimmung mit den Ergebnissen von FRANCKI et al. (1966) konnte schliesslich eine nähere Verwandtschaft des GMV-Q mit dem GMV-Y festgestellt werden.

Die Versuche mit dem Antiserum gegen das Isolat PT erwiesen sich durchweg als unbefriedigend, da es bereits im homologem Test nur einen Titer von 1:32 ergab und weil dann in den heterologen Kombinationen mit den übrigen Virus-Antigenen nur sehr schwache Reaktionen, selbst bei hohen Antigen-Konzentrationen auftraten. Auch bei den Absättigungsversuchen konnten mit dem Antigen bzw. dem Antiserum PT keine klaren Ergebnisse erzielt werden, weil höchstwahrscheinlich die im Serum vorhandenen wenigen PT-spezifischen Gruppen bei der Absättigung blokkiert wurden. Der unerwartete Befund, dass das Isolat PT sehr instabil ist und zerfällt, liefert eine plausible Erklärung für das serologisch stark abweichende Verhalten des Isolates PT im Vergleich zu den übrigen Isolaten

Die Bedeutung serologischer Untersuchungen beruht vor allem auf ihre Anwendbarkeit unter praxisnahen Bedingungen. Die unter diesem Gesichtspunkt durchgeführten Versuche zeigen, dass es möglich ist mit den entsprechenden Antiseren die verschiedenen GMV-Stämme mit grosser Sicherheit in Rohsäften infizierter Tabak und Paprikapflanzen serologisch nachzuweisen.

Die elektronenmikroskopische Untersuchung schrägbedampfter Präparate ergab, dass die Isolate GT und PT durch sphärische Partikel mit einem Durchmesser von 35 bis 38 mμ charakterisiert sind. Die bisher für das Gurkenmosaikvirus publizierten Durchmesser für bedampfte Partikel betragen 35 mμ (SILL et al., 1952), 36,2 mμ (BETTO et al., 1964), 40 mμ (GÜNTÜRK et al., 1957) und 42 mμ (FRANCKI et al., 1966).

Dementsprechend kann man die Isolate GT und PT auch auf Grund der Form und Grösse ihrer Partikel als Stämme des GMV ansehen.

Zum Schluss darf noch einmal betont werden, dass auf Grund aller untersuchten Eigenschaften das Isolat PT sehr wahrscheinlich ein Stamm des GMV ist, während dies für das Isolat GT mit Sicherheit festgestellt werden kann.

Herrn Prof Dr. F. Grossmann sei für die Überlassung und Förderung dieser Arbeit und Herrn Dr. H. L. Sänger für die Durchführung der elektronenmikroskopischen Untersuchungen herzlich gedankt.

ÖZET

Hıyar mozayık virusunun çeşitli izolatlarında biyolojik ve serolojik araştırmalar.

Bu çalışmada birisi hıyar ve birisi biberden olmak üzere Türkiye'den iki virus izolatının nitelikleri tesbit edilmeye çalışılmıştır. Bunun için bu izolatların biyolojik, fiziksel ve serolojik özellikleri literatürde tanımlanmış olan dört hıyar mozayık virusu izolatıyla (Y, Q, W 14 ve KD) karşılaştırılmıştır.

Araştırmalardan aşağıdaki sonuçlar elde edilmiştir:

1) GT izolatı test bitkilerinde hıyar mozayık virusu için karakteristik olan simptomlar oluşturmuştur.

2) PT izolatı hıyara taşınmamakla ve *Vigna sinensis* L. 'i sistemik olarak infekte ederek diğer izolatlardan ayrılmaktadır.

3) Sıcaklıkla inaktifleşme GT izolatında 75-80°C de ve PT izolatında 70-75° C de olmaktadır. Son sulandırma noktası her ikisi için 1: 1000.000 dur. Her iki izolatın infeksiyon meydana getirme gücü dondurulmuş yapraklarda önemli derecede zarar gördüğü halde, arıtılmış preparatlar dondurulduğu takdirde hemen tamamen saklanabilmektedir.

SCOTT (1963) tarafından hıyar mozayık virusu için geliştirilmiş olan arıtma yöntemi her iki Türk virus izolatı için de en uygun preparasyon metodu olarak bulunmuştur. 1 Kg yaprak materyalinden 90-300 mg virus izole edilmiştir.

4) Density gradient centrifugation' da araştırılan virusların beşi belirgin bandlar halinde çökelmişlerdir ve bulaşıcılık, serolojik aktiflik ve optik geçirgenlik çok iyi uygunluk göstermektedir. PT izolatı ise çok labil olup, virus partiküllerinin parçalanması sonucu çok miktarda denature olmuş virus-kılıf proteinine rastlanmıştır.

5) Elektronmikroskobunda her iki izolatın partikülleri 35 - 38 milimikron çapında sferik partiküller olarak görülmüşlerdir ve böylelikle şimdiye kadar tanımlanmış olan bütün hıyar mozayık virusu izolatlarının karekteristik partiküllerine şekil ve büyüklük yönünden uymaktadırlar.

6) Serolojik araştırmalarda Y, Q, ve GT izolatlarının yakın akraba oldukları tesbit edilmiştir. Buna mukabil W_{14} ve KD izolatları diğer izolatlarla ortak serolojik grupların yanısıra çok iyi tesbit edilebilen kendilerine has serolojik determinantlara sahiptirler. İzolat PT' nin stabil olmaması yüzünden çeşitli serolojik testlere rağmen çok kesin serolojik akrabalık ilişkileri tesbit edilememiştir.

7) Bütün Hıyar mozayık virusu izolatları berraklaştırılmış tütün ve biber ham özsularında yeterli derecede kesin, çabuk ve basit şekilde serolojik olarak tesbit edilebilmiştir ve bu yöntem pratikte de kullanılabilir.

8) Elde edilen bütün biyolojik, fiziksel ve serolojik özelliklere dayanılarak izolat PT büyük bir ihtimalle bir hıyar mozayık virusu ırkıdır, bu na karşılık GT izolatının hıyar mozayık virusunun bir ırkı olduğu katiyetle tesbit edilmiştir.

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