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Studies on two natural inhibitors of virus infection

Tomris NART *

ABSTRACT

The inhibitory effect of French bean (*Phaseolus vulgaris*) sap was studied together with the inhibitory effect of carnation (*Dianthus caryophyllus*) sap it was found that the sap from both species inhibited the number of local lesions induced by three different viruses, on five different local lesion hosts, when applied along with a virus extract. In most cases the inhibition of infection was significant at the level of 1 % .

Investigations of the mechanism of inhibition tended to support the idea that the inhibitors have their effect through the host plants, independent of the identity of the viruses. The inhibitory sap from French bean was effective when it applied to the host plants simultaneously with virus or before the virus inoculations.

An attempt was made to isolate and identify the inhibitory substance using different methods. Although a rough separation was obtained no specific identification could be made. The indications were that the inhibitory substance is a proteinaceous material.

The inhibitory effect of French bean sap was destroyed by heating the sap at 50°C for 10 min. While the inhibitor from carnation sap was destroyed only on heating at 90°C for 10 min.

After attachment to the leaf surface the inhibitor from French bean was not removed by washing with weak acid, weak alkali or water, but the reducing effect was modified when it was applied in an inoculum to which a weak acid was added.

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1. The studies reported in this paper were taken from'a thesis submitted by the author to the University of Exeter in part fulfilment of the requirements for the degree of Doctor of Philosophy.

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I. INTRODUCTION

It is known that some of the sap transmissible viruses are nevertheless non - transmissible from certain host plants to other hosts. This is usually due to the presence of a substance or substances in the sap of the virus infected plants which prevents infection by the virus when rubbed on the leaves of a susceptible plant. These substances are known as "inhibitors".

The existence of an inhibitor in plant sap was first noticed in 1914 when it was noted that the mosaic disease of pokeweed (*Phytolacca decandra*) was not mechanically transmitted from pokeweed to tobacco plants Allard (1918), came to the conclusion that the sap of pokeweed plants possessed a specific substance which prevented the virus from infecting hosts other than pokeweed itself.

Since then this subject has not been extensively studied. A certain amount of work has been carried out using inhibitors from sap of several herbaceous plants. Grant (1934), tested 8 species viz. *Nicotiana glutinosa*, tobacco (*Nicotiana tabacum*), French bean (*Phaseolus vulgaris*)snapdragon (*Antirrhinum majus*), spinach(*Spinacia oleracea*), beet (*Beta vulgaris*) and pokeweed (*Phytolacca decandra*). It was found that sap from all of these plants decreased the number of local lesions produced by tobacco mosaic virus (TMV) in *Nicotiana glutinosa* The average number of lesions obtained in the presence of extracts of bean was somewhat lower than control, but extracts from three other plants viz. sugar-beet (*Beta vulgaris*) Swiss chard (*Beta vulgaris* var. *cicla*) and spinach (*Spinacia oleracea*) greatly reduced the infectivity. Investigations which were concerned with the nature of the inhibitive property of the plant extracts were carried out later by Kuntz and Walker (1947), who used spinach sap as the inhibitory source, and studied some properties of the spinach extract They found that sap of spinach plants was effective against 6 viruses, and that the inhibition was immediate and did not increase with time. Although the extract did not lose its inhibitory power after 15 months at room-temperature, it was not inhibitive on TMV when heated at 70°C for 10 minutes, but inhibited cabbage mosaic virus after heating at 125°C for 15 minutes.

These results suggested that there were two inhibitory substances in the sap of spinach. The inhibitive property was destroyed in extremely acid or alkaline solutions.

Benda (1956) had also found two substances in the sap of New Zealand spinach (*Tetragonia expansa*). One was an inhibitor,a relatively stable protein which decreased the number of local lesion caused by tobacco ringspot virus in cowpea

(*Vigna sinensis*), and the other was an augmenter, which increased the number of local lesion and was identified as a soluble oxalate salt.

Kassanis and Kleczkowski (1948) described a method for concentrating and purifying an inhibitor from *Phytolacca esculenta* by differential precipitation with ethanol. Purified preparations contained 14-15 % nitrogen and 8 12 % carbohydrate They suggested that the inhibitor was probably a glycoprotein. Recently, Wyatt and Shepherd (1969) obtained highly purified preparation of the virus inhibitor from *Phytolacca americana* by column chromatography and found that the active material was not a glycoprotein but a protein which contains about 12 % lysine by wight.

Brierley and Smith (1950) noted that dahlia mosaic virus was saptransmissible from infected to healthy *Zinnia elegans*, but not from this species to dahlia. Other examples of this kind of non-transmissibility had been reported by Van der Want (1951) for a carnation mosaic virus which is transmissible by sap inoculation from carnation (*Dianthus caryophyllus*) to carnation but only rarely from carnation to French bean (*Phaseolus vulgaris*) or tobacco (*Nicotiana tabacum*) ; also tobacco ringspot virus is easily transmissible from infected to healthy Sweet william (*Dianthus barbatus*), but not from Sweet william to tobacco, cucumber, *Datura stramonium* or *Antirrhinum majus* (*Weintraub and Gilpatrick*, 1952). Similarly, sugar-beet mosaic virus (*Hoggan*, 1933) and cucumber mosaic virus (*Bhargava*, 1951) are not transmissible from sugar beet or spinach to tobacco plants by sap inoculation. Extracts from almost all parts of healthy or infected cucumber plants proved to be highly inhibitory to cucumber mosaic virus (*Sill and Walker*, 1952).

On the other hand,a considerable amount of work has been done on the role of certain chemicals as inhibitors of virus infection. The first report of inhibitors of virus infection from sources other than flowering plants concerned fresh, normal rabbit serum (*Mulvania, 1926*).

It was concluded that the blood from the rabbit which was previously infected with TMV inhibited the virus when mixed with the sap of TMV -infected tobacco plants. Later, in 1934 Chester studied this subject in detail and described a method which differentiates the effect of inhibitor whether it is acting through the host plant or inactivating the virus. According to his study normal rabbit serum inhibits the infection of virus by altering the susceptibility of the host plant.

Lindner *et al.* (1959) listed the chemicals which inhibit the replication of tobacco mosaic virus in cucumber cotyledons by killing the host cell

before the establishment of virus infection. Other work on this subject has been reviewed by Bawden (*1954*).

During investigations concerning the host range of barley stripe mosaic virus (*BSMV*)), it was not possible to transfer the disease mechanically, through back inoculations from French bean (*Phaseolus vulgaris cv. Masterpiece*) to barley (*Islam, 1968*) The BSMV infected French bean leaves were extracted with 0.1 M phos phate buffer, pH 7.0 then tested on barley but the extract was shown not to be infective.

Further investigations were needed in order to establish the inhibitory power of French bean sap. In preliminary experiments, some other species of plants were tested for their inhibitory effect on the initiation of virus infection using different hosts and different viruses. For more detailed studies only two species, viz. French bean (*Phaseolus vulgaris*) and carnation (*Dianthus caryophyllus*) were chosen as inhibitory sources.

Most of the experiments were done with the inhibitor occurring in French bean, because of its suitability. The plant requires only a short period of time to grow to reasonable size and more sap is obtainable per unit of fresh weight of leaves compared with carnation. However, almost all the experimental work was carried out using both French bean and carnation leaves as inhibitory sources and attempts were made to purify the inhibitor.

II. MATERIALS AND METHODS

The viruses used in this work were tobacco mosaic virus (TMV) alfalfa mosaic virus (AMV), and bar ley stripe mosaic virus (BSMV). A strain of TMV commonly used in the laboratory was maintained in *Nicotiana tabacum* cv. White Burley A strain of AMV originally isolated from naturally - infected alfalfa was maintained in *Nicotiana glutinosa*; and finally BSMV was maintained in barley.

Regular inoculations to young stock plants at frequent intervals were made to maintain the viruses in high concentration and inocula were prepared from systemically infected young leaves 3-4 weeks after inoculation by macerating leaves in a mortar, with the addition of a little 0.1 M phosphate buffer pH 7.0, then squeezing the pulp through muslin.

All test plants were grown in the glass houses in 7.0 cm "Fablo" pots in No.1 potting soil consisting of:

3 parts sterilized loam
2 Parts moss-peat
1 part sand

And to each 35 dm^3 of compost 113,4 g John Innes base fertilizer and 21,2g ground lime-stone were added.

The plants were transported to another glass house when they were ready for inoculation. The temperature in the first glass house was I8°C 30°C all the year around, but in the second, was maintained at 16°-20°C

except during summer, when the temperature fluctuated widely. When plants had developed sufficient numbers of fully expanded leaves, suitable for experiments, the growing tips were removed, usually 1-2 days before the inoculations.

French bean and carnation leaves were used as inhibitory sources In the case of French bean, 2 - 4 weeks old primary leaves and sometimes the first trifoliate leaves, and in the case of carnation mosty young leaves and shoots, were stored in deep freezer at-20°C until used. The inhibitory sap was obtained by macerating the frozen material in a mortar with addition of a little 0,1 M phosphate buffer pH 7.0, then the pulp squeezed through double muslin.

METHOD OF ASSAY

In each assay the concentration of virus was uniform throughout the experiment. Each inoculum consisted of a 1 : 1 mixture of virus-contaning sap and the extract under test, or buffer in control inocula ; 600 mesh "celite" was added to the inocula before inoculation of test plants. Inoculations were made by dipping the forefinger in inoculum and rubbing once over the leaf surface. Each leaf was supported with the hand. Inoculated leaves were rinsed with a jet of water from a wash-bottle and every possible care was taken to prevent spillage ; hands were washed thoroughly between inoculations. The re was practically no macroscopically visible mechanical injury after inoculations.

In each experiment, the different treatments were distributed over the test leaves according to the complete Latin Square design, usually employing at least 16 leaves. i e., 4 plants each with 4 leaves ; this was replicated whenever possible. This met hod was used throughout the present study to eliminate by Latin Square design, not only the differences betwen plants, but also the differentces due to leaf position on a plant. Local lesions were counted 4-6 days after inoculation and the statistical significance of the inhibitory effect was calculated by applying the Analysis of Variance test at the level of 5 % and I %. Later, when only two treatments were being compared, using half-leaves, the difference between con trol and test treament was analysed statistically using the "-t test" *(Snedecor, 1956)*.

III. RANGE OF ACTION OF INHIBITORS

It is generally believed that natural inhibitors are not effective or are less effective on the plants which contain them. With refference to the work of Ragetli (*1957*) the inhibitor from carnation sap was less effective on plant species belong to the order Centrospermae. This piece of information led to a preliminary study,

to investigate the inhibitory effect of sap from those species which belong to order Centrospermae viz. ***Celosia cristata, Mesembryanthemum criniflorum, Basella rubra and Amaranthus caudatus.*** The plants were raised from seed in the glass house and the experiments were carried out according to the general plan, using different virus and host plant combinations.

Celosia cristata was one of the species tested for its inhibitory effect on infection of barley stripe mosaic virus (*BSMV*) in ***Chenopodium amaranticolor.*** Four uniformly - grown plants of *C. amaranticolor.* were selected. The growing tips and the unwanted lower leaves were cut off leaving 4 fully expanded leaves. The plants were kept in the dark for 24 hours before the inoculations. The inhibitory sap was prepared by macerating fresh leaves of *C. cristata* plants. This inhibitory sap was diluted with 0.1 M phosphate buffer pH 7.0 in the proportions of 1/10 and 1/100, then these dilutions were mixed the virus containing sap to make the following inocula :

a- Undiluted sap + BSMV (1 :1)

b- 1/10 dilution of the sap + BSMV (1 : 1)

c- 1/100 dilution of the sap + BSMV (1 : 1)

d- Phosphate buffer pH 7 0 + BSMV (1 : 1)

BSMV - containing sap was prepared by macerating systemically infected barley leaves with the addition of 0.1 M phosphate buffer pH 7.0 The inocula were distributed over the leaves according to a complete 4 x 4 Latin square design. Local lesions were counted 6 days after inoculation and the following average local lesion counts were obtained for each treatment:

a- 152 b- 186- c 147 d- 282

There was no significant difference between the treatments when the results were analysed according to Analysis of Variance.

Mesembryanthemum criniflorum Dianthus barbatus, Basella rubra and ***Amaranthus caudatus*** plants were also tested in the same manner, for their inhibitory effect on different virus infections in different host plants but the results gave no significant decrease in local lesion numbers except in the case of ***Dianthus barbatus.***

As a conclusion from the above results, it appeared that not all the members of the Centrospermae tested caused significant inhibition ; only in the case of ***Dianthus*** spp. was there a constant decrease in the number of local lesion with a high degree of significance. At this time, it was known that French bean sap contained an inhibitor which inhibited barley stripe mosaic virus *(BSMV)* infecting barley (*Islam,1968*). In the light of this knowledge, it was decided

to concentrate on the inhibitors which occur in carnation and in French bean. These two plant species were chosen as inhibitory sources because of their suitability for the work, the former being very powerful and the latter being easy to work with.

I. INHIBITIVE EFFECT OF CARNATION SAP :

The inhibitory effect of carnation sap has been extensively studied by Ragetli *(1957)* and found to be active against various viruses in test on many different host plants. He studied 14 viruses and 20 plant species.

In this work, most of the inhibition tests were made with TMV on *Nicotiana glutinosa*. However, other viruses and host plant combinations were employed for the experiments whenever possible.

A series of experiments was carried out to determine the inhibitory effect of carnation sap on the establishment of infection of TMV in *Nicotiana glutinosa* using the following inocula :

a- Undiluted carnation sap + TMV (1 : 1)

b- 1/10 dilution of carnation sap + TMV (1 : 1)

c- 1/100 dilution of carnation sap + TMV (1 : 1)

d- Phosphate buffer pH 7,0 + TMV (1 : 1)

Four uniformly grown plants of *N. glutinosa* were selected. The growing tips of the plants were removed and the unwanted lower leaves were cut off, thus retaining the 4 middle fully-expanded leaves. These leaves were inoculated with the above-mentioned inocula according to the 4 x 4 Latin square design. The leaves were numbered 1-4, 1 being the youngest of the 4 leaves and 4 being the oldest.

The local lesion had formed distinctly and clearly 4 days after the inoculations and the number of local lesion was recorded with the aid of a mechanical counter. The mean number of local lesion per leaf for each treatment was as follows:

a- 7 b- 33 c- 159 d 331

Analysis of Variance Table for Local Lesion Production with: Inhibitor source: Carnation sap; Host plant: *N. glutinosa;* Virus: TMV

Sources	Sums of Squares	Degrees of Freedom	Mean Squares	F
Plants	73766	3	24588	4,71 N,S
Leaves	50042	3	16680	3,I9 N.S
Treatments	262466	3	87488	16,7 xx
Error	3I283	6	5213	
Total	417557	15		

$F_{0.05}$ 4.76
F = 0.78
0.01

N.S - Not Significant
xx - Significant at 1 % level

Later, the experiment was repeated in the same manner, but on a bigger scale. using *8 N. glutinosa* plants, each with 4 leaves The same highly significant result was obtained.

Experiments were carried out according the same plan to test the inhibitory effect of carnation sap on TMV infection in *Chenopodium amaranticolor* and on *Datura stramonium ;* also alfalfa mosaic virus (AMV) on *Phaseolus vulgaris,* on *Vigna sinensis* and on *D. stramonium ;*
finaly, barley stripe mosaic virus (BSMV) on *C.amaranticolor*. In all these experiments carnation sap has proved to be highly inhibitory on virus infections in these host plants and the inhibiton was significant at 1 % level.

2. INHIBITIVE EFFECT OF FRENCH BEAN SAP

The inhibitive effect of French bean sap was first noted during the host range studies of TMV *(Grant, 1934)*. It was found that the average number of local lesion obtained from extracts of bean was somewhat lower than control inoculum. Preliminary experiments in the present work agreed with this report, as the number of local lesion produced by TMV on the leaves of *N. glutinosa* were reduced by leaf extracts of French bean *(Phaseolus vulgaris)* in comparison with the control treament.

These results led to more detai led experiments to generalize the inhibitory effect of French bean sap. The work described below was carried out to determine the inhibition of virus infection by French bean sap

The inhibitory effect of French bean sap was tested for its effect on the establishment of virus infection in different host plants. Several experiments were carried out at different times of the year and in all of them the difference between the treaments and control inoculum was significant.

For one set of experiment 4 uniformly and vigorously growing host plants, each with 4 leaves, were inoculated with the following inocula according to the 4 x 4 Latin square design, and repeated whenever possible.

a- Undiluted French bean sap + Virus inoculum (1 : 1)
b- 1/10 dilution of French bean sap + Virus inoculum (1 : 1)
c- 1/100 dilution of French bean sap + Virus inoculum (I : 1)
d- Phosphate buffer pH 7.0 + Virus inoculum (1 : 1)

When the experiment was carried out to test the inhibirory effect on TMV infection in *N. glutinosa* using 8 plants, each with 4 leaves a highly significant result was obtained with the following mean number :

a- 31 b- 111 c-141 d- 221

Analysis of Variance Table for Local Lesion Production with : Inhibitor source : French Bean ; Host plant : *N. glutinosa* ; Virus TMV

Sources	Sums of Squares	Degrees of Freedom	Mean Şquares	F
Plants	3576	7	510,8	0.47 N.S
Leaves	2898	3	966	0 88 N.S
Treaments	14800)	3	49333	45. 42 xx
Error	19548	18	1086	
Total	174022	13		

For 7 - 18 d.f
F = 2.58
0.05
F = 3.85
0.01

For 3 - 18 d.f
F = 3.16
0.05
F = 5.09
0.01

N.S. - Not significant
xx - Significant at 1 % level

These experiments were repeated in the same manner using different viruses and host plants, thus the inhibitory effect of French bean sap was tested on TMV infection in *C. amaranticolor* and in *D. stramonium* ; on AMV infection in *Vigna sinensis*, in *D. stramonium* and in *Phaseolus vulgaris* and also on BSMV infection in *C. amaranticolor*. As the results of these experiments it was found that the inhibitor from French bean was very inhibitory.

IV- DETERMINATION OF THE MECHANISM OF INHIBITION

It is now virtually established that when a virus infects a plant, it first becomes attached to a "receptor site". Those receptor sites are believed to be created or exposed by wounding or abrasion of host plant leaves. After exposure, these receptor sites are available for the attachment of virus particals for only a limited period. Siegel (*1966*) reported that if the infectible sites are not converted to infective centres during their finite life time they disappear.

It is likely that on any one host plant there are different receptor sites for different viruses, presumably dependent upon the virus protein and the nature of the receptor sites. This possibly also explains why certain plants do not become infected by certain viruses. This was demonstrated in the work of Jedlinski (1964) that tobacco mosaic virus and tobacco nesrosis virus require different infectible sites on each of two host plants, *Nicotiana sylvestris* and *Phaseolus vulgaris*.

Many inhibitors of plant virus infection have their effect on the host plants and the inhibitors act by destroying or by blocking the infectible sites. The work of Rageili (1957) suggested that the inhibitor would act by blocking virus receptors on the surface of the le af. The inhibitory effect was not changed with the len gth of time during which virus and inhibitor remained in contact *in vitro;* and also by ultracentrifugation of sap of mosaic-infected carnation plants the inhibitor and active virus were separated.

Van Kammen *et al.* (1961) sup ported this hypothesis by examining the relationship between the number of local lesions on leaves of *Nicotiana glutinosa* and TMV concentration, and the effect of the concentration of the inhibitor on this. The results showed that the action of the inhibitor was on the receptor sites on the leaf surface.

In the present work the effect of French bean sap was studied for its inhibitory action on the initiation of virus infection. It was also thought to have its effect through its content of inhibitory protein, which attaches itself to relevant receptor sites, thus preventing virus particles from becoming attached. This was demonstrated on host plants which developed local lesions round the infective centres. It has been pointed out previously that French bean and carnation sap both decreased the numbers of local lesion when applied together with virus inoculum.

1 - Application of inhibitor before virus inoculation

Four plants of *N. glutinosa* of the same age, each with 4 fully expanded leaves, were used for the experi ment. The inhibitory sap of French bean was prepared by macerating frozen tissue with the addition of a little phosphate buffer, pH 7 0. The French bean sap thus obtained was rubbed on alternate leaves of the plants, while control leaves were rubbed with phosphate buffer pH 7.0. In both cases "celite" was added to the solutions before rubbing the leaves.

Five minutes later the leaves were washed with a jet of water from a wash-bottle, then they were inoculated with TMV and washed again immediately after inoculation in the same way. There was an interval of 10 minutes between the application of inhibitor and virus.

When local lesions had formed clearly and distinctly, an average of 139.1 local lesions were counted on the control leaves against an avarage of 64.5 local lesions on the leaves with inhibitor. Analysis of the results according to the Analysis of Variance and the "t" test gave a 1 % significant difference between the control and the treatment in both cases.

The experiments were continued using progressively longer intervals between application of the inhibitor and inoculation of the virus. The inhibitor proved to be still effective in reducing the infection of TMV in *N. glutinosa* when it was left on the leaves for 48 hours before virus inoculations.

2 - Application of inhibitor after virus inoculation

Since application of inhibitor before virus infection proved to be effective, another set of experiments was carried out to determine the effect of applying the inhibitor from French bean after TMV inoculation on the leaves of *N. glutinosa*. The experiment was carried out in the same manner but first inoculating the leaves with TMV then applying the inhibitory sap at intervals. When the results were analysed statistically there was no significance between the number of local lesions formed on control and treated leaves.

3 - Distance effect

As the application of the inhibitor from French bean sap proved to be active in reducing local lesion numbers when applied before virus inoculation the following experiments were carried out in order to determine whether the inhibitory agent was active or preventive when applied to the host plants in different ways. The ways used were :

a- Introducing the inhibitory sap into leaf veins

b- Application of the inhibitory sap on the lower surface.

It was thought that perhaps the inhibitory agent altered the susceptibility of the cell for virus attachment or created conditions within the cell unsuitable for the replication of the virus when it was introduced into the cell.

It was also desirable to find out whether the inhibitor would move along the vascular system to neighbouring tissue. Attempts were unsuccesful and the results were non-significant.

Another experiment was set up to determine whether the inhibitor had any effect on the upper surface of the leaves when it was applied on the lower surface. Although there was an interval of 24 hours between the inhibitor application and virus inoculation the same non-significant result was obtained.

5 - Isolation of the inhibitor from French bean:

Several attempts were made to isolate and purify the inhibitor which occurred in French bean sap. The methods used previously by other workers were modified in accordance with the equipment available The three following methods of isolation were applied to the sap :

1- Low speed centrifugation of sap

2- Phenol extraction of sap

3- Ethanol precipitation of sap

All fractions isolated during the isolation procedures were tested for the presence of the inhibitor on *N. glutinosa* leaves against TMV infection. When the actual local lesion counts for each fraction were analysed according to the "t" test the results were shown to be significantly different at the 1 % level between the controls and the fractions.

6 - Properties of the inhibitors

During the isolation procedures it was shown that all the fractions tested were inhibitory. These results led to another series of experiments to determine the characteristics of the inhibitors isolated from French bean and carnation.

A fraction of partly clarified French bean sap was investigated using thin-layer chromotography. The solution was shown to contain ninhydrin-positive material.

Another experiment was carried out with electrophoresis on a cellulase-acetate-membrane using carnation sap clarified by ethonal precipitation. At the end of this procedure there was no staning of proteinaceous material. Therefore further characterization experiments with the inhibitory solution were carried out using crude sap. Isolation methods were either not sensitive enough to provide more detailed characterization or owing to the instability of the inhibitors, the active principle was largely lost during certain treatments.

The inhibitory power was destroyed by heating the sap from French bean at 50°C for 10 minutes. On the other hand carnation sap was lost its inhibitory power when heated at 90°C for 10 minutes, and also to dialyse the inhibitor for longer than for 24 hours caused loss of inhibitory power.

The effect of different pH values on the inhibitory power of French bean sap was tested by

a- Washing the leaf surface with acid or alkaline solution after the application of ihe inhibitory sap

b- Adding acid or alkaline solutions to the inocula.

After attachment to the leaf surface the inhibitor from French bean was not removed by washing with acid or alkaline solutions or water, but the reducing effect was modified when it was applied in an inoculum to which a weak acid was added.

DISCUSSION

Few reports have been published on the virus inhibitors naturally occuring in plant sap. The inhibitors from spinach (Kuntz and Walker, 1947), from *Phytolacca esculenta* (Kassanis and Kleczkowski. 1948)

from *Tetragonia expansa* (Benda, 1956) and from carnation (Ragetli, 1957), however, have been studied in some detail. The work on these sources of inhibitors was mainly concerned with the nature and the iso lation of the inhibitory materials. These authors concluded that the inhibition was not through the host but due to the presence of certain chemicals in the extract which rendered the virus non infective. The sap from *Tetragonia expansa* contained an inhibitor of virus infection. Its mode of action has been investigated and it has been concluded that it has its effect through the host plants by making cells unsuitable for virus multiplication (Benda, 1956).

Little or nothing is reported about an inhibitor from French bean sap, nor there is any study on it. It was first noticed by Grant (1934) during host range studies on tobacco mosaic virus where the extracts from French bean produced somewhat fewer local lesions. The presence of an inhibitor of virus infection in French bean sap was confirmed and more detailed studies on its mode of action were carried in comparison with the inhibitor from carnation sap. The presence of these inhibitors and their action were demons trated on the local lesion host of different viruses. The evidence showed that the inhibitory substances in both French bean and carnation sap reduced the number of local lesions produced by these viruses when inoculated simultaneously.

In the work of Ragetli (1957) it was suggested that the inhibitor from carnation would act through the host plant. Later, it was proved that the inhibitor from carnation has its effect not on the virus itself but on the host plant (Van Kammen *et al.* 1961). The same theory was supported in the present study when it was establish that the action of the inhibitor from French bean was on the host plants independent of the virus. This was shown by applying the inhibitory sap before and after the virus inoculations. It was also believed that the inhibition took place as a result of competition for the receptor sites between the virus and the inhibitor.

There are two schools of thought regarding the initiation of virus infection. One is that the inhibition may occur by adsorption of plant virus particles on to infectible sites (Jedlinski, 1956); the other is that infection results from the introduction of the virus particle into a slightly wounded cell (Furumoto and man, 1963). Although the knowled ge about receptor sites in higher plants is not enough to demonstrate their actual existence, it may be postulated that in whatever manner the virus particle is infecting a host cell, the inhibitory substance is cap-

able of preventing the infection at the point of entry.

This was clearly demonstrated in the experiments where the inhibitory sap from French bean was applied to the host plants before inoculation with TMV.

In the present work, observations were based upon quantitative analysis and throughout the study it has been noticed that the inhibitor from carnation was much more powertul than the inhibitor from French bean.

Determination of the mechanism of inhibition was established by applying the inhibitor before and after virus-containing sap produced fewer lesions in the presence of inhibitory sap when compared with the number of lesions produced by control inocula The inhibition was still high even the inhibitiory sap from French bean was applied to the leaf surface 48 hours before virus inoculations. This indicates that the action of the inhibitor was through the host plant rather than on virus ; that is to say, once the inhibitor was introduced to the leaf surface it blocked the entry points. This could happen because the inhibitor actively prevented the virus from infecting or because the infectible sites were destroyed by the application of the inhibitor.

On washing leaf surfaces, to which the present inhibitor had been applied, with water, acid or alkaline solutions, there was no reduction in inhibitory effect. This suggests that the attachment of the inhibitor on the leaf surface is quite irreversible in a very short time.

Isolation of the inhibitory substance from French bean sap was attempted Like previous workers who isolated and identified other inhibitors of virus infection from plant sap, it is thought that the inhibitor from French bean sap is also a proteinaceous, material since several protein precipitating or separating methods applied to crudè sap extract resulted in precipitation of the inhibi tory substance. The end product was inhibitory in all cases, and severely reduced the number of local lesions of TMV in *N. glutinosa*.

The difference between the inhibitory substances from carnation and French bean sap was clear and most pronounced in the experiments where the effect of heating on the inhibitory power was tested. French bean sap lost its reducing effect by heating at 50°C for 10 min. but the inhibitor from carnation sap was affected only by heating at 90°C for 10 min. This would suggest that the inhibitor from carnation is more stable than the inhibitor from French bean and is different from it.

Ö Z E T

VİRUS İNFEKSİYONUN DOĞAL İKİ İNHİBİTORÜ ÜZERİNDE ÇALIŞMALAR

Fasulya *(Phaseolus vulgaris)* ve karanfil *(Dianthus caryaphyllus)* yaprak sularındaki inhibitör madde üzerinde çalışılmış ve her iki türden de elde edilen inhibitör değişik konukçular üzerinde değişik viruslar tarafından oluşturulan lokal lezyonların sayısını azaltmıştır.

İnhibisyon mekanizması üzerindeki çalışmalar daha önceki düşünüşü kuvvetlendirerek inhibisyonun virusa bağlı olmadan doğrudan doğruya konukçu bitki üzerinde meydana geldiği dolayısıyla infeksiyonu önlediğini göstermiştir.

İnhibitör maddenin ham yaprak suyundan izolasyonu için çalışılmış ve sonuçlar bu maddenin protein karakterinde olduğunu göstermiştir.

İnhibitör etkisi ısıtmayla azalmış ve Fasulya yaprak suyundaki inhibitör 50°C de 10 Dakika, karanfil yaprak suyundaki inhibitör de 90°C de 10 dakika ısıtma ile aktivitelerini kaybetmiştir.

İnhibitör maddenin yaprak yüzeyinde adsorbe olması irreversible olup asit alkali veya su ile yıkanma bu bağlantıyı çözememiştir.

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Psorosis Concave-Gum Virus Disease Of Satsuma Mandarins And Two Desirable indicator Plants

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ABSTRACT

Concavities and some irregular wood growth symptoms were found on the trunk of the psorosis concave-gum infected Satsuma mandarin *(C. unshiu Marcovîtch)* trees during the investigations of Citrus virus diseases.

Sexton tangelo *(C, reticulata* x *C. paradisi)* and Dweet tangor *(C. reticulata* Blanco X *C. sinensis* (L.) Osb.) were found very efficient and desirable indicator plants for psorosis concave-gum virus strains in glasshouse conditions at 18°C to 26°C.

INTRODUCTION

During the investigation of Citrus virus diseases on Satsuma mandarins, psorosis concave-gum infected Satsuma trees displayed strong oak-leaf pattern (4) and vein flecking on the spring growth flush. Dweet tangor and Sexton tangelo seedlings were used in this experiment as the test plants for identification of their susceptibility to concave-gum disease in the cool glasshouse conditions at 18°C to 26°C. The effect of Concave gum disease on the Satsuma mandarin woods and barks also were observed in the field investigations.

MATERIALS AND METHODS

In tehe spring of 1972, 8 year-old and older Satsuma mandarin trees buded on *P. trifoliata* (*L.*) Raf and Sour orange (*C. aurantium L.*) rootstocks were examined for symptoms of psorosis concave-gum virus disease in the Satsuma growing area near İzmir Province. The indexing test vas carried out in the cool glasshouse conditions at 18 to 26°C. Sexton tangelo (*C. reticulata* x *C. Paradisi*) and Dweet tangor (*C. reticulata* Blanco x *C. sinensis* (L.) Osb.) were used as the indicator plants for psorosis concave-gum virus trains. Indicator seedlings were grown in 25 cm. pots containing soil, sand and peat (2:1:1). Bud-wood from concave-gum infected Satsuma trees was collected during the field observation for inoculations (1).

Two pencil size seedlings of Sexton tangelo and Dweet tangor were used in each test. One uninoculated indicator seedling was left as a check. Two chip-bud grafts and one

T- bud graft were applied in each inoculation test (1, 10). At inoculation time, the seedlings were cut back at a point two or three buds above the inoculum (10). Plastic bands were used for wrapping the grafted buds. The temparature varied from 18°C to 26°C during the early spring and spring period in the glasshouse. Symptom examinations were made at 3-4 day intervals.

RESULTS and DISCUSSION

FIELD OBSERVATIONS: During the field observations in spring 1972, 150 out of the 1000 examined Satsuma trees from 20 orchards showed typical oak-leaf pattern and vein-flecking on the spring growth flush (Fig 1 A-B). Oak-leaf pattern was very pronounced on young leaves of some concave-gum infected Satsuma trees. Wallace (10) reported that, in California, concave gum infected Citrus trees display strong oak-leaf patterns on the spring growth. Most of our Concave gum infected Satsuma mandarins showed also psorosis A type leaf flecking during the spring months in April and May as described by wallace

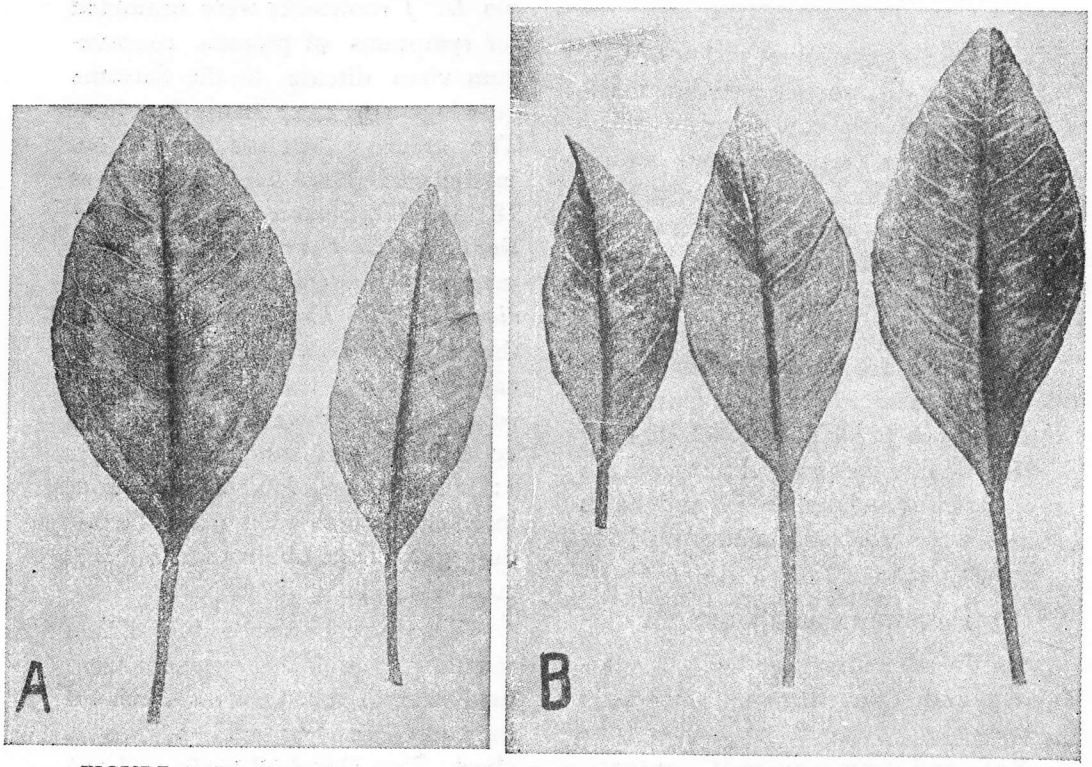


FIGURE 1. Young-leaf symptoms of Psorosis Concave-Gum Virus on Satsuma mandarin leaves. A, typical zonate or oak-leaf pattern. B, Oak-leaf pattern with flecking.

(10). The young-leaf symptoms dis appeared as the leaves matured and harden in the late June. Roistacher (5) informed that, mild strains of concave-gum virus caused some flecking without oak-leaf pattern on sweet orange. Our field observation showed that, most of our psorosis infected Satsuma trees carry the mild strains af concave-gum virus. A few concavities with gum layers an some irregular wood growth symptoms were found only on the trunk of some concave gum infected Satsuma trees (Fig.2) as described by Wallace (7,8,9,10) and Klotz et al. (2).

FIGURE 2. Concavity Symptom with gum layer on the trunk of concave - gum infected Satsuma mandarin.

Finger marks symptom was also observed on the main branches of some coneave-gum infected old Satsuma mandarin trees (Fig.3). So-far, this disease has not been reported on Satsuma mandarins. Rossetti and Salibe (6) described the presence of this disorder on sweet orange and mandarin trees in Brazil. They reported that, In Florida this malformation apparently is considered to be related to psorosis virus. Madaluhi (3) also reported the presence of finger marks symptoms on 15-year -old Avana-mandarin (*C. reticulata* Blanco) trees, that showed also clear symptoms of concave-gum psorosis. He attempted to transmit the cousal agent of finger marks. In his transmission experiment, indicator seedlings showed only flecking and oak-leaf patterns of concave-gum psorosis without finger marks symptoms. According to his transmission experiment, finger marks is a physiologic disorder and not transmissible.

We are unable to conclude whether finger marks disorder is related to psorosis virus or not. But, Further transmission experiments are necessary to show whether finger marks is transmissible disorder or not.

INDEXING TESTS: Six weeks after the inoculations, Oak-leaf patern symptoms of psorosis Concave-gum virus were appeared on the yo-

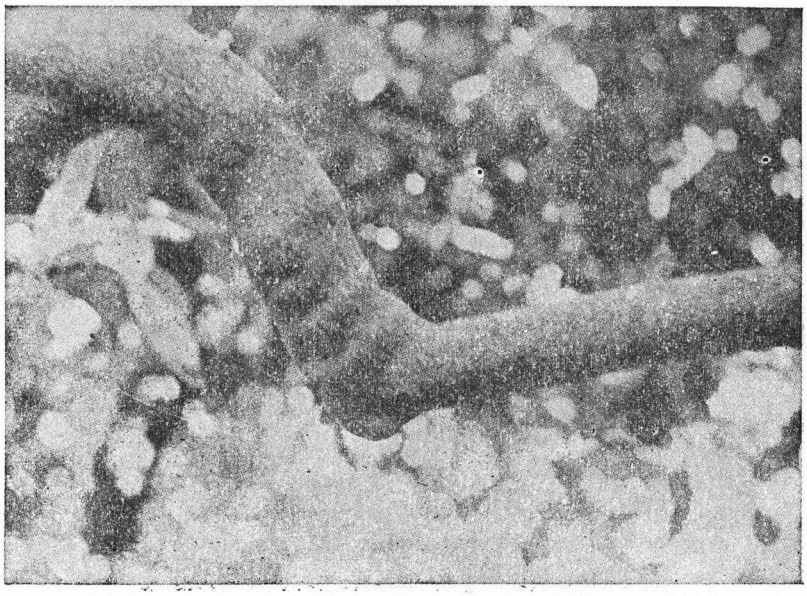
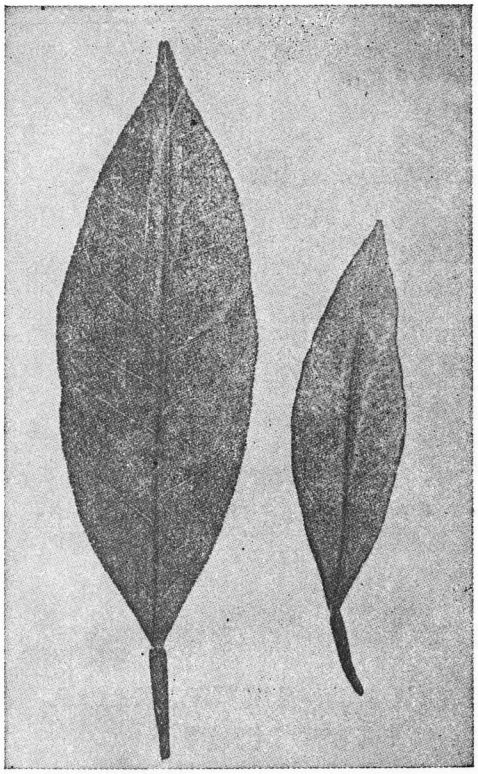


FIGURE 8. Finger mark symtom on a branch of a Psorosis concave - gum infected Satsuma mandarin tree.

ung leaves of Sexton tangelo and Dweet tangor test plants in the glasshouse conditions at 18°C to 26°C. Zonate Oak-leaf pattern was very strong on Dweet tangor young leaves as described by Roistacher and Blue (5) (Fig. 4). The same authors reported that, Dweet tangor is a very efficient indicator for a mild strain of the concave gum and is also sensitive to psorosis-A and crinkly-leaf viruses. Oak leaf symptom was not persistent on the young leaves of Dweet tangor. This symptom disappeared as the leaves matured and hardened. Sexton tangelo also showed the same symptoms (Fig. 5). After the oak-leaf symptom diseappeared, some vein flecking was visible on the indicator leaves for very short period, When the tem perature could not be controlled in glasshouse in the late spring, all the symptoms diseappeared on the leaves of test plants



FICURE 4. Zonate Oak-leaf pattern, on Dweet tangor young leaves 6 weeks after the inoculations.

Our experiment showed that, Dweet tangor and Sexton tangelo are useful indicator plants in the glasshouse Conditions during the spring indexing if the glasshouse temperature are not well controlled.

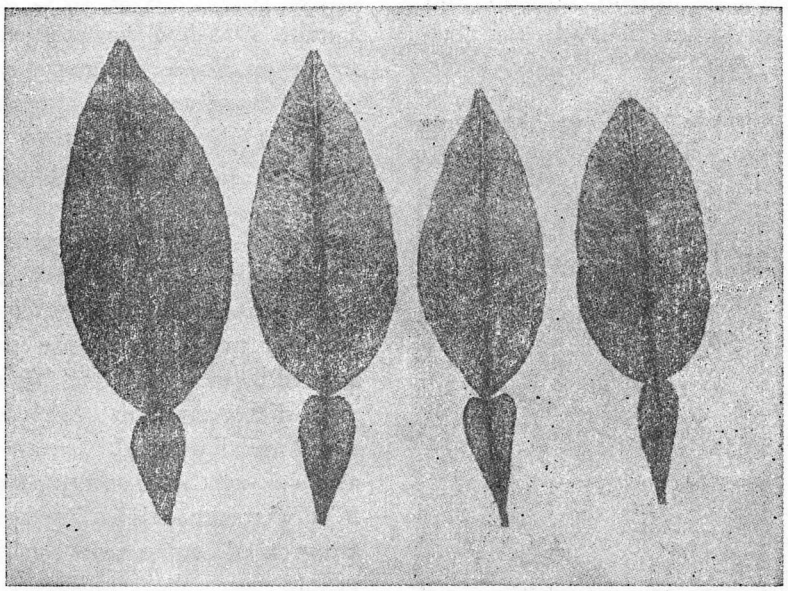


FIGURE 5. Zonate Oak-leaf pattern on Sexton tangelo young leaves. Control leaf, left.

ÖZET

SATSUMA MANDARİNLERİNDEKİ ÇUKUR-ZAMKLI KAVLAMA VİRÜSÜ VE UYGUN İKİ TEST BİTKİSİ

Satsuma mandarinlerimizdeki virus hastalıkları üzerinde yapılan araştırmalarda, Çukur-Zamklı Kavlama Virusü ile enfekteli Satsuma mandarinlerinde bahar sürgünlerinin körpe yapraklarında virüsün tipik yaprak belirtisi olan orta-ana damar etrafında meşe yaprağı şeklinde renk açılmaları (Oak-leaf pattern) ile birlikte damar aralarında 1-5 mm uzunluğunda beyaz-sarı renkli çizgi halinde lekeler (flecking) görülmüştür. Çukur zamklı kavlama virüsünün aynı yaprak belirtileri sera koşullarında test bitkisi olarak kullanılan Dweet tangor ve Sexton tangelo bitkilerinin körpe sürgün yapraklarında, inokülasyonlardan 6 hafta sonra belirmiştir. Bu iki test bitkisinin ilkbahar ve kış aylarında sera sıcaklığının iyice ayarlanamadığı durumlarda ve 18°C -26°C derecelerde çukur-zamklı kavlama virüsü için iyi birer test bitkisi olduğu kanaatine varılmıştır. Tar

la koşullarında yapılan gözlemlerde çukur-zamklı kavlama virüsü ile en fekteli bazı satsuma mandarinlerinin ana gövdeleri üzerinde virüsün tipik belirtisi olan uzunlamasına çukurluk lar izlenmiştir.

Kavlama virüsü belirtilerine ilâ ve olarak, çukur zamklı kavlama virüsü belirtileri gösteren bazı satsuma mandarinlerinin birinci ana dalları üzerinde yurdumuzda ve dünyada üretilen Satsuma mandarinlerinde şimdiye kadar bildirilmemiş olan par mak izleri (finger marks) görülmüştür. Bu hastalığın çukur-zamklı kavlama virüsü ile herhangi bir ilgisi bulunup bulunmadığı ve aşı gözleri ile geçip geçmediği hakkında kesin bir kanaatimiz yoktur. Ancak ilerdeki çalışmalarda önemli görüldüğü takdirde, hastalığın aşı yoluyla bulaşma durumunun araştırılmasının gerekli olduğu kanısındayız.

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Resistance Of The Principal Turkish Tobacco Varieties To Downy Mildew

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ABSTRACT

In the present investigation, the resistance behaviour of 22 Turkish tobacco varieties to downy mildew, ***Peronospora tabacina*** Adam, was determined by cotyledon test. The percentage of the diseased plants in the varieties ranged from 92,29 to 100 .00 % which indicated that the varieties were highly susceptible to downy mildew.

INTRODUCTION

The downy mildew of tobacco occurred for the first time around Edirne, a part of Trakya, in June 1961 and it spread all over the tobacco growing areas of Trakya, in a very short time, including Marmara and Ege regions in the same year. Then, it became a common disease in all tobacco growing areas of Turkey in 1963 (2,3).

Though there are several fungicides to be used to control the disease, the most efficient control measure is to grow improved resistant varieties. For this reason, studies on the improvement of the resistant varieties, as has been done in the other countries, were started in Turkey in 1962.

Turkish tobacco vatieties, particularly in the seedling stage, were highly susceptible to downy mildew (4, 5, 6,). Özbaş (4), reported that all of the 320 pure lines of these tobacco varieties were infected by the causal agent in the seed-bed. Özbaş *et al.* (7), working on the 14 varieties, found out that the percentage of diseased plants ranged 7.0-50.4 % when tested by the cotyledon test and 57.44-99.06 % in the seedling stage.

This paper reports the results of testing the principal Turkish tobacco varieties for resistance to downy mildew in order to establish a broader base for existing program in tobacco breeding

MATERIALS AND METHODS

In the experiments, 22 tobacco

varieties brought from the Monopoly Institutes in Istanbul were used. "Bel 61-10" was used in the tests as a resistant variety.

The Cotyledon test method developed by Schiltz and Izard (1, 9), was used in determining the reaction of each variety to downy mildew.

All experiments were conducted in a microphytotron maintained at 27°C with 4000 lux-light during the germination and at 24°C with 3500 lux for 10 hours and at 16°C for 14 hours darkness for the day and night times respectively, after inoculation.

Trials were set up in five replications for each variety. One ml. of spore suspension, at 50 000 spore/ml. concentration (8), was sprayed on the six days old seedlings in each petri dish.

Subsequent data on the diseased plants of each variety was obtained by counting the seedlings on the 7 th, and 11 th days after inoculation.

RESULTS AND DISCUSSION

Table 1 shows the results of cotyledon test with the 22 Turkish tobacco varieties. It is seen that the disease percentage of the varieties tested ranged from 92.29 to 100.00 %. It means that, none of the principal Turkish tobacco varieties were resistant to downy mildew in the cotyledon stage.

Results of our cotyledon tests are very much fitting to the results of the seedling stage tests made by Özbaş *et al*. (7) with the exception of Bursa and İzmir varieties. For example, for Malatya variety, we found 98.98 % diseased plants in the cotyledon test, they have recorded 93.98 % in the seedling stage. But they have obtained very low percentages in the cotyledon tests in contrasts with our very high records for the same varieties. As an example for the same variety of Malatya, they have found 7 0 % diseased plants, we observed 98.98 %. In our opinion, this low percentage might be due to lack of proper cotyledon test conditions during tests.

ACKNOWLEDGEMENT

Authors wish to express their appreciation to Mehmet Özyolcular, from İstanbul Monopoly Institutes, for his kind help in providing seeds.

Table 1. Susceptibilities of the pricincipal Turkish tobacco varieties to downy mildew. (Results of the cotyledon tests).

NO.	VARIETIES	INFECTION (%)					
		REPLICATIONS					AVERAGE
		I	II	III	IV	V	
1	ARTVİN 21243	95.93	98.38	97.11	100.00	97.11	97.11
2	BAFRA 6391	100.00	100.00	100.00	100.00	100.00	100.00
3	BALIKESİR 16880	77.61	100.00	100.00	100.00	92.52	94.83
4	BASMA 438	100.00	97.80	77.77	92.64	100.00	93.64
5	BURSA 18000	95.80	88.20	98.57	98.31	100.00	96.18
6	DÜZCE 985	100.00	100.00	100.00	100.00	100.00	100.00
7	TRAKYA 20292	96.69	100.00	98.92	95.00	98.13	97.74
8	EGE (İzmir) 64	99.19	100.00	99.07	99.09	100.00	99.47
9	İSKENDERUN 19696	100.00	100.00	100.00	100.00	100.00	100.00
10	İZMİT 13109	83.33	100.00	97.67	95.55	93.93	94.09
11	KARABAĞLAR 6042	100.00	100.00	100.00	100.00	100.00	100.00
12	MALATYA 676	97.78	99.00	100.00	98.85	98.75	98.88
13	SAMSUN - Canik 10821	95.00	97.33	93.15	91.76	84.21	92.29
14	SAMSUN - Dere 255	97.14	96.51	97.22	96.66	98.73	97.25
15	SAMSUN - Evkaf 454	100.00	94.17	100.00	98.76	95.91	97.77
16	SAMSUN - Maden 2421	100.00	96.42	89.33	92.94	93.57	94.45
17	PURO 21131	96.05	94.38	100.00	96.07	100.00	97.30
18	TAŞOVA 10670	91.54	92.72	97.82	97.05	97 29	95.28
19	TOKAT 9884	96.00	100.00	100.00	96.20	100.00	98.44
20	TÖMBEKİ 7703	98.11	98.31	97.18	94.33	97.64	97.31
21	TRABZON 18362	100.00	100.00	100.00	92.77	100.00	98.55
22	YAYLADAĞ 18205	100.00	98.75	100.00	100.00	96.92	99.13
23	BEL - 61-10	–	–	–	–	–	–

ÖZET

BAŞLICA TÜRK TÜTÜN ÇEŞİTLERİNİN MİLDİYÖYE KARŞI DAYANIKLILIK DURUMLARI

1963 yılından itibaren Türkiyenin bütün tütün bölgelerinde görülen bir hastalık haline gelen Tütün Mildiyösü ile bir taraftan çeşitli fungisitlerle mücadele edilirken, diğer taraftan bu hastalığa dayanaklı Türk tütün çeşitlerini elde etme çalışmalarına 1962 yılından itibaren başlanmıştır.

Dayanaklı çeşitlerin seçiminde, bir test metodu olarak Schiltz ve Izard tarafından geliştirilen, çenek yaprakları test metodunun "Cotyledon test" sür'atli ve kesin sonuçlar verdiği ve mevcut dayanaklılığın tarla sonuçları ile güçlü bir uygunluk gösterdiği bilinmektedir.

Bu çalışmada, bu metod uygulanarak 22 Türk tütün çeşidinin mildiyöye karşı durumları tesbit edilmiştir. Tablo 1 de, çeşitlerin % 92. 29 ile % 100,00 arasında hastalandıkları görülmektedir. Bu duruma göre, denemeye alınan çeşitlerden hepsinin mildiyöye karşı yüksek derecede duyarlı oldukları ortaya çıkmaktadır.

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Effects Of Some Chemicals Against Tilletia foetida (Wall.) Liro. In Vitro

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ABSTRACT

Effects of some chemicals against *Tilletia foetida* (Wall.) Liro. were investigated in vitro by using the technique of GASSNER (1943) and Ca $(NO_3)_2$ added water agar medium.

The results of Trimangol 80, Ceresan, and Hekmazin obtained from Ca $(NO_3)_2$ added water agar medium were agreed with the results of previous years field experiments. However the $Ca(NO_3)_2$ added water agar medium seems as a practical method for obtaining the effectiveness of chemicals by counting germinated and ungerminated chlamydospores in laboratory. The relations between the results obtained from the $Ca(NO_3)_2$ added water agar medium and results of the field experiments are in progress.

INTRODUCTION

Bunt is one of the serious disease of wheat in Turkey and it is prevalent throughout the country (3,5). During studies in 1956-1962, 70-95 % infection were observed in some fields sown without seed treatment and the average infection was assessed 10-12 % for the same years(6). Mercury products are used largely for seed treatment against the disease supplied by Goverment in Turkey. There fore it is possible to say that the large parts of wheat seeds are sown after treatment recently. Although the toxic characters of treated seeds with mercury products are main problem.

In this study the effects of some chemicals were discussed and compared at different levels in laboratory before field experiments.

MATERIALS and METHODS

The variety of Lerma-rojo-64 was used in these studies found as a susceptible variety previously (1,4). The bunt chlamydospores for artificial inoculations were obtained from diseased heads from different parts of Ege region.

The microscobic examination of chlamydospores showed characteristics of typical *Tilletia foetida* (Wall.) Liro. and their germination ratio

were 76.2 % in 2 % glucose soluti on after 72 hours.

Forty five groups from L. rojo 64 variety were weighted used in these studies, that each group was 50 grams. These groups were artificially inoculated in (0.3 %) ratio inoculum in a jar for five minutes separately. The seeds for control groups were seperated and the others were mixed with the chemicals in small containers for five minutes separately which their names and levels were given on table 1.

Table 1. The Chemicals And Levels

CHEMICALS			Dosage (g /100 kg. seed)
Name	Active Ingredient	Formulation	Product
Dithane M-45	% 46.5 Ethylene bisdithiocarbamate ion-% 12 manganez ion % 1. zinc ion	Dust	200 150
Trimangol 80	% 80 Maneb	Dust	200 150
Hekmazin	% 5.5 Zineb % 55 Maneb	Dust	200 150
Benlate	Benamyl Methyl 1-(Butylcarbomoyl) - benzimidazolecarbamate	W.P	100 50
Vitavax	2,3 - Dihydro-5 carboxanilido-6 methyl 1,4-Oxathiin	W.P	200 150
Brassicol 75 dust	PCNB	Dust	200
Brassicol 75 W.P	PCNB	W.P	200
Ceresan truck UT. (x)	% 1.5 Mercury	Dust	200 150

(×) as a comparative chemical

Two different laboratory techniques were used for obtaining the effectiviness of the chemicals against the T. *foetida*. One of the two techniques was based on GASSNER (1943) and the following method we re used for the second test.

0.8 % percent water agar was prepared and after sterilization 0.3 % $Ca(NO_3)_2$ was added to each litre and the medium was coloured with 20 mg. Rose-Bengale. Twenty cc medium was poured into 10 cm diameter petri-dishes. Twenty seeds were placed into each petri dishes putting the embryo sides down and five replications were made. The petri - dishes were incubated at 12°C for 7 days and the seeds were removed from the petri dishes and at the end of 7 days the surroundings of the seed-wells were examined under microscope germinated and ungerminated chlamydospores we re counted. The germination average of chlamydospores were found and the results were adopted the Abbott formula and the differences between the chemicals were obtained by Anal ysis of variance and Duncan test.

RESULTS

The experiment based on GASSNER (1943) was not satisfactory for these studies. The germination ratio of chlamydospores and the effectiveness of chemicals on second medium [water agar + $Ca(No_3)_2$] were given at table 2.

DISCUSSION

The effectiveness of chemicals were obtained from the germination ratio of chlamydospores localized around the seed-wells. Therefore it is necessary to count the germinated and ungerminated chlamydospores cle arly; but in the studies based on GASSNER (1943) it was not possible to count the chlamydospores because, the seed-wells are on mud and the chlamydospores are seemed as a white mycelium mat. Therefore this method in not suitable for sensitive laboratory tests

The percentage effects of the both levels of Trimangol 80, and Vitavax and high levels of Hekmazin, Dithane M-45, Brassicol 75 dust, Benlate, Brassicol 75 W.P. were 100.00 %, 98 84 %, 98.75 %, 99. 96 %, 99 42 %, 99.05 %, 98. 90 % at the Ca $(NO_3)_2$ added water agar medium respectively. On the other hand; when the comparision chemical Ce resan 200 g. was compared with these effectiveness they gave the same protection effect as Ceresan 200 g. which is shown table 2.

The results of Trimangol 80, Ceresan and Hekmazin obtained from Ca $(NO_3)_2$ added water agar medium were agreed with results of previous years field experiments (7) However, the Ca $(NO_3)_2$ added water agar medium seems as a practical method of obtaining the effectiveness of chemicals by counting

Table 2. The germination ratio of chlamydospores and the effectiveness of chemicals according to Abbott formula and the differences of chemicals on $Ca(NO_3)_2$ added water agar medium.

The names of chemicals	Dosage (g. Product/100 kg seed)	Germination ratio of chlamydspores (%) Replications				Effectiveness (%) Replications				The average of the effectivenes (%)	Tests
		I	II	III	IV	1	II	III	IV		
Trimangol 80	200	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00	100 00	A
Trimangol 80	150	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00	100.00	A
Hekmazin	200	0.00	0.00	0.10	0.00	100.00	100.00	99.83	100.00	99.96	A
Ceresan	200	0.00	0.00	0.65	0 00	100 00	100.00	98.89	100 00	99 72	A
Dithane M-45	200	0.36	0.00	0.58	0.32	99.46	100.00	99.01	99.21	99.42	AB
Brassicol dust	200	0 00	0.00	1.25	0.40	100 00	100.00	97.86	99 01	99.22	AB
Benlate	100	0.44	1.80	0 07	0.00	99 34	96.99	99.88	100.00	99 05	AB
Brassicol W.P.	200	2.27	0.60	0.00	0 00	96 60	99 00	100.00	100 00	98.90	AB
Vitavax	200	0.62	0.00	0.00	1.50	99 07	100.00	100.00	96.30	98.84	AB
Vitavax	150	1 80	0.20	0.00	0.80	97.30	99.67	100.00	98.02	98.75	AB
Dithane M-45	150	2.85	2.00	0.80	2.06	95.73	96.65	98.63	94.91	96.48	BC
Hekmazin	150	3.83	1.86	4.83	2.63	94.26	96.89	91.73	93.51	94.10	C
Ceresan	150	10.24	10.73	0.63	0.24	84 65	82.03	98.92	99.41	91.25	C
Benlate	50	3.34	8.41	13.39	0 29	94.99	85.92	77.09	99.20	89.83	C
Kontrol	-	66.73	59.72	58.43	40.50	-	-	-	-	-	

(Fig 1. A, B, C, D, E,) germinated and ungerminated clamydospores in laboratory.

The relations between the results obtained from the $Ca(NO_3)_2$ added water agar medium and the results of the field experiments are in progress.

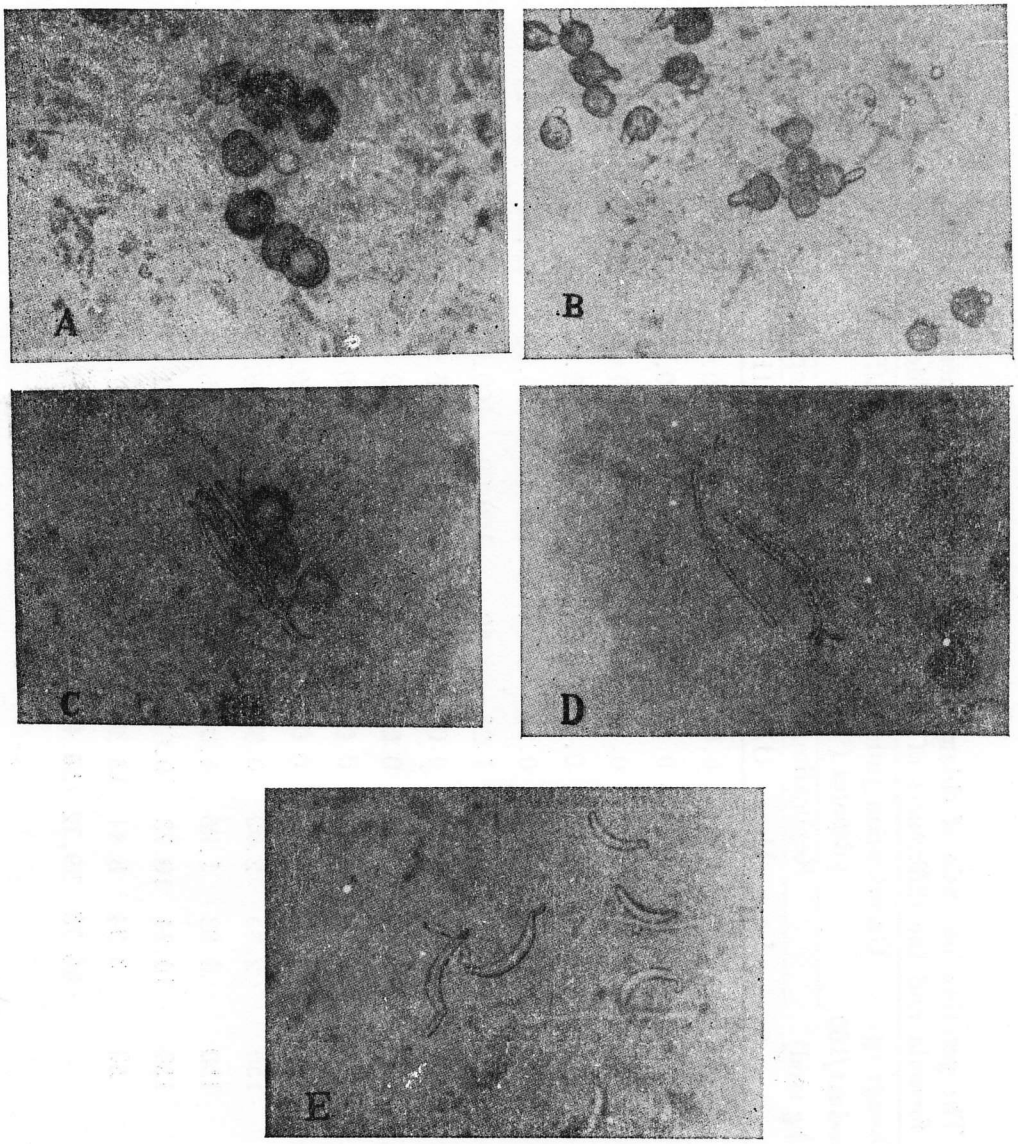

FİGURE 1.

A. The chlamydospores at the seed-wells of treated seeds on the $Ca(No_3)_2$ addet Water agar (appr. × 400)

B. The germinated chlamydospores at the seed-wells untreated seeds on $Ca(No_3)_2$ added water agar (appr. × 400)

C, D. E. basidium, plasmogamy and germinated conidia of **T. foetida** on the same medium (appr. × 400)

Ö Z E T

BAZI İLAÇLARIN in-vitro KOŞULLARDA BUĞDAY ADİ SÜRMESİNE (Tilletia foetida "Wall." Liro.) ETKİLERİ

Buğdayda adi sürme (*Tilletia foetida* " Wall. " Liro) hastalığına karşı Cetvel I de verilen ilâçların yine ayni cetvelde gösterilen dozları üzerinden etkileri, it-vitro koşullarda araştırılmıştır. Bu amaçla Gassner (1943) yöntemi ve laboratuvarımızda geliştirilen $Ca(NO_3)_2$ ilave edilmiş su agarı ortamı kullanılmıştır.

Gassner (1943) yöntemine göre yapılan çalışmada bu yöntemde; çim lenen ve çimlenmeyen sürme chlamydosporlarının sayılmasının ve buna bağlı olarakta önerilen skalanın sıhhatli olarak uygulanmasının mümkün olmadığı görülmüş ve bu yöntemle elde edilecek sonuçların güvenilir olmayacağı kanısına varılmıştır.

%o.8 lik su agarına sterilizasyonu takiben %.o,3 oranında $Ca(NO_3)_2$ ilavesiyle hazırlanan ortam da yapılan çalışmada ise bu ortamın şekil I A, B, C, D, ve E de de gösterildiği gibi sürme chlamydosporlarının gelişmesi için çok uygun olduğu görülmüş, bu ortamdaki çimlenen ve çimlenmeyen chlamydosporlar üzerinde yapılan sayımlarla denenen ilâçların yüzde etkileri saptanmıştır.

Buna göre Trimangol 80 ve Vitavax'ın her iki dozu başta olmak üzere Hekmazin, Dithane M-45, Bras sicol 75 toz, Benlate, Brassicol 75 W. P. in yüksek dozlarındaki etkiler sırasıyla % 100.00 % 98,84 % 98, 75, % 99,96, % 99,42, % 99,05, % 98,90 olmuştur. Diğer bir deyimle denemeye karşılaştırma ilâcı olarak alınan ceresan'ın 200 gramlık dozu esas alındığında yukarıda gösterilen yüzde etkilere sahip ilâçlar istenilen koruyuculukta bulunmuşlardır.

Trimangol 80, Ceresan ve Hekmazinin önceki yıllarda yapılan tarla denemelerinde elde edilen sonuçlarıyla ayni preparatların çimlenen ve çimlenmeyen chlamydosporlar üzerinden $Ca(NO_3)_2$ ilave edilmiş su agarındaki sonuçları karşılaştırıldığın da birbirini doğruladığı dikkati çekmiş, bu nedenlede söz konusu yöntemin güvenilir olabileceği kanısı doğmuştur. Bununla beraber laboratuvar denemelerine parelel yürütülecek tarla denemeleri ile, yöntemin güvenirliğinin araştırılması plânlanmıştır.

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The Diseases Of Economic Importance In Apple Stores In Central Anatolia

AND

INVESTIGATIONS ON ESTABLISHING A METHOD FOR DIMINISHING THEIR DAMAGE TO MINIMUM

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ABSTRACT

The types of the apple stores in Central Anatolia and the diseases of economic importance found in these stores were investigated.

During the studies the main diseases in these stores were determined and an attemp was made to provide certain control measurements and to determinine the best way of protection of the stored crop.

A brief description of the types and qualifications of the stores in Central Anatolia were given and the quality of the apples preserved in these stores were discussed.

INTRODUCTION

In Central Anatolia according to the 1963 statistics the quantitiy of apples preserved in stores is 160.000 tons, 100.000 tons of which being kept in unsuitable stores.

The object of this study is to attain the possibility to make the necessary recommendations to the farmer who always suffers from the extend of their damage and the measures to diminish the damage of these diseases to minimum are determined.

STUDIES

1. Table 1 indicates the extend of damage caused by the diseases.

2 - The Description of the diseases encountered with in the apple stores :

Common Green Mould

There is a kind of soft brown, black rot of various sizes around the stalk of the apple. On the rotted part the green fructifacations of *Penicilium sp.* which is the pathogen of the disease can be seen as spots.

Table 1. extend of damage in apple stores

Observation site	The approximate amount of the apples in the store	The amount of examined samples	The rate of infected apples. %
Ereğli-Centre	6.000 Kg.	600	20
Ereğli-Durlaz	5.000 "	500	16
Niğde-Centre	8.000 "	800	20
Niğde-Eskigümüş	20.000 "	1.500	21
Niğde-Yenigümüş	15·000 "	1.000	22
Nevşehir-Kırkpınar	8.000 "	800	8
Nevşehir-Bahçeli	50.000 "	3.500	10
Nevşehir-Ürgüp	30.000 "	2.000	5
Nevşehir-Ortahisar	60.000 "	3.000	11

Brown rot :

There are chestnut coloured spots a round the stalk, sepal and sometimes at the sides. These are somewhad hard, sunken and of various sizes. During our studies we have observed that the disease usually starts from the sepal and proceeds to the core. when the fruit is cut across there is a layer pink rot. Apples are bitter. The pathogen of the disease is *Trichothecium roseum* (Bull) Lk.

Black rot :

There are blach spots of various sizes around the stalk or on the sides the apple. These spots are rather hard. The pathogen of the disease is *Alternaria tenuis var. mali*. Marchal.

Apple Scab:

There are approximately 1—10 spots with diameters of 2 cm. on the apple. These are brown in the centre surrounded with a chestnut coloured line. The splits on the spots serve as an inlet for secondary fungi. The flesh under the spot is hard and greenish in colour. The pathogen of the disease is *Fusicladium dentriticum* (Wallr) Fck. The fruit shrivels up because of excessive loss of water.

Other disorders :

1- These are somewhat sunken brown spots of 5-6 cm. deep. They are as large as a lentil in size and scattered at random all over the apple. At the end of our studies no

pathogen of parasitic nature has been found. All these symptoms resemble the BITTER PIT disease described by Marcellin (1963).

2- There is a transparent hardness around the sepal and on the upper parts of the apple. Later on the parts soften and rot. No pathogen of parasitic nature has been found. There is a resemblence to the "mealy breakdown" disease described by Marcellin (1963).

3- There are black starlike splits around the stalk base and the sepal of the apple.

4- The skin of the fruit is speckled with epidermic dark brown spots. The flesh under the spots in healthy and has a normal colour. There is no pathogen of parasitic nature. It resembles the disease described by Marcellin (1963) as "Echaudure" or hot water blight The percentage of the disease specified according to the infected fruit shown in the table **2.**

Table 2. Conclusions of the studies carried out to specify the percentage of the apple storage diseases.

Name of the disease	Pathogen	The ratio of infected apples %
Common green mould	Penicillium Sp.	27
Brown rot	Trichothecium roseum	21
Black rot	Alternaria tenuis	18
Other disorders	Physlogical (1)	9
	" (2)	9
	" (3)	7
	" (4)	5
Scab.	Fusicladium dendriticum	4

During the inoculation studies out to control the above mentioned rots, reinoculations with pure cultures of ***Trichothecium roseum***, ***Alternaria tenuis***, ***Penicillium sp.*** are applied to uninfested apples in the early November in Ereğli Niğde and, Nevşehir.

During the investigations and trials in the last week of December it is specified that 100 % infection occurred and developed with Penicillium and Alternaria as for Trichothecium this rate is 94.6 %.

3- Store types used by the grow ers in Central Anatolia and their qualifications:

a. Store type: These consist of the carved volcanic tuff rocks in Nevşehir. They have an opening facing the north and two air holes one at the top. There is a groove all around the floor for dripping water. Collacted here keeps the relative humudity at a high degree. Temparature varies between - 2.5°C and 8°C and the relative humudity is between 85-98 %.

Second Type-Stores in the two villages of Niğde, Kırkpınar and Uluağaç. They are usually made of stone walls, 3/4 which are buried under the ground. The hight wall above the ground is arched style. There are two openings for ventilation facing each other. Temparature varies about —2.2°C to 12°C and the relative humudity is 37-92 %.

Third Type-This is the classical store type of the region and consists of the stores of various sizes in the cellars of the houses or in the gardens within the city. Usually the walls are made of stone whereas the floor and the ceiling is covered with cement. The hight is about 4 6 metters and the windows serve as openings for ventilation. Temparature varies between -2,2°C and 12°C. The relative humudity is 50 - 90 %.

b. The quality of apples preserved in stores.

The quality of apples that had been preserved in stores and considered uninfested has also been checked. Conslusions regarding the quality of the fruit, appearance of spots on the flesh etc. are indicated in the following table.

Table 3. The quality standards of the uninfested apples preserved in the stores in Ereğli, Niğde and Nevşehir.

Site	Percentage of the apples dried and tarnishet with brown spots and lines	Percentage of the *uninfested* apples
Ereğli	68 %	32 %
Niğde	29 %	71 %
Nevşehir	11 %	89 %

As it is indicated in the Table 3 the percentage of the uninfested apples in Nevşehir stores is 89 % where as the infested apples are 11 %, 71 % of the apples in Kırkpınar, Niğde is uninfested and 29 % is ungualified. In Ereğli 68 % of the apples considered infested gets unqualified subsequently. In Nevşehir stores the per centage of the unqualified apples is considerably low.

c. Cosequently, in case the farmer decides to built a new store within his own capacity the first and the second store types are recommended. The thrid type is not recommendable.

4 - Treatment Test

a - Before the apples were picked from trees. i.e. 10 days before the harvest 0.15 % and 0.2 % of Captan 50, Maneb (65), Zineb (65) preparations were applied. Coclusions regarding the experiment are shown in the table 4.

Table 4. Conclusions the investigations concerning the apples exposed to treatment 15 days before the Harvest in the second week of March.

Active ingredient of the chemicals	Dosages used %	The ratio of uninfested apples %
Captan (50) WP	0.15	75.0
Maneb (65) WP	0.15	72.3
Zineb (65) WP	0.15	70 9
Check plot	—	64.0
Captan (50) WP	0.2	73.6
Maneb (65) WP	0.2	72.9
Zineb (95) WP	0.2	70.9
Check plot	—	64.0

There is no difference between the 0 15 % and 0.2 % dosages as far is the effectiveness is concerned.

Among the chemicals Captan is found to be the best There is a positive low result of 6 11 % between the treated and the check plots. The fact that the sources of secondary parasitic pathogens play a great role in the achievement of this result.

b Results of the treatments applied to the apples during the storage are indicated as follows :

Ereğli	23 %
Niğde	12 %
Kırkpınar	6 %
Ürgüplü	6 %

According to the check plot Captan that gives the best results such an effectivenes.

This situation proves that it produces better results in case the apples to be stored in Ereğli and Niğde types of stores are treated before the storage.

If Captan 50 is applied at a rate of 0.2 % its residue at the end of the storage period is 0.82 ppm. captan according to the residue is cosiderably below the tolerance. There is no inconvenience in the recommendation of the above mentioned chemical.

c- Disinfectation of the empty stores.

One week before the storage of the apples the walls, celling and the floor of the store are treated with 5 % solution of 40 % formalin. Apart from this spraying is also applied inside of the store.

The conclusions derived from the examination of the apples preserved in such stores after the first half of March is stated in table 6.

Table 6. Results of the experiment carried out to specify the effect of the treatment of the empty stores on diseases.

(In Ereğli, Konya)	Uninfested apples %
In the store treated with 40 % formalin	78
In the untreated Check store	70

It is clear that treatment of stores has an 8 % effect on the prevention of apple rot. It is recommended that the stores should be exposed to such treatment and they should be cleared off the residues of the year before.

ÖZET

ORTA ANADOLU'DAKİ ELMA DEPOLARINDA EKONOMİK ÖNEMİ OLAN HASTALIKLAR VE BU HASTALIKLARIN ZARARLARINI MİNUMUMA İNDİRMEK İÇİN BİR METOD TESBİTİ ÜZERİNDE ARAŞTIRMALAR

Orta Anadolu'daki elma depoları ve bu depolarda rastlanan, ekonomik önemi olan hastalıklar üzerinde araştırmalar yapılmıştır. Çalışmalar sırasında bölgedeki elma depolarında rastlanan âdi yeşil küf (*Penicillium sp.*), kahverengi çürüklük [*Trichothecium roseum* (*Bull*) Lk.] siyah çürüklük (*Alternaria tenuis* var. *mali* Marchal), Kara leke [*Fusicladium dentriticum* (Wallr. Fck.] ve fizyolojik bozukluklardan ileri gelen hastalıklar kısaca tanımlanmıştır.

Orta Anadolu'daki elma depoları üç ayrı grup altında toplanmıştır. Birinci grubu Nevşehir'deki organik kayaların içinde oyulmuş olan depolar oluşturmuştur ki bu depolarda sıcaklık 2.5°C ve 8°C arasında orantılı nem ise % 96 98 arasında değişir. İkinci tip depolar 3/4 kısmı toprağa kömülmüş taş duvarlı depolardır, buralarda sıcaklık 2 2 °C ve -12°C arasında orantılı nem % 73 92 arasında değişir. Üçüncü tip depolar ise şehir içinde, ev bah çelerinde inşa edilen 4 6 metre yükseklikte taş duvarlı, tabanı ve tavanı beton olan depolardır. Burada sıcak lık -2,2°C ve 12°C arasında orantılı nem ise % 50 90 arasında değişir. Her üç depo tipinde infekteli ve infektesiz elma yüzdesi bulunarak birinci ve ikinci tip depoların şartlarının uygun olduğu ve bu iki tip depolama için öğütlenebileceği saptanmıştır.

İlâç denemelerinde hasattan 15 gün önce % 0.15 ve % 0.2 dozlarında Captan 50, Maneb 65 ve Zineb 65 etkili maddelerine sahip ilâçlarla yapılan ilâçlamanın iyi sonuçlar verdiği saptanmış ve Captan'lı preparatın en iyi etkiyi gösterdiği kanısına varılmıştır Ayrıca depolama zamanından bir hafta önce deponun tavan-taban ve duvarlarının % 40 Formalin'in % 5 lik eriyiği ile ilâçlanmasının hastalık çıkışışını önleme yönünden iyi sonuçlar verdiği görülmüştür.

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Untersuchungen über die antagonistischen Wirkungen der Bodenmikroorganismen gegen pflanzenpathogenen Pilze

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ZUSAMMENFASSUNG

Die Bodenmikroorganismen haben oft eine antagonistische Wirkung über pathogene Pilze. Die Saatbeizmitteln und Bodenentseuchungmitteln wirken tötend über pathogene Pilze und auch Bodenmikroorganismen. Wegen der tötenden Wirkung von Saatgutbeizmitteln über antagonistisch wirkenden Mikroorganismen (besonders Bakterien) gibt es nach angewendeten Dosis in der Umgebung von Saatgut ein steriles Medium. Infolgedessen können die bodenbürtigen Wurzelfäuleerregern gut wachsen und an den Pflanzen Schaden verursachen. In dieser Arbeit wurde mit einfachen Testen Zusammenhang zwischen antagonistisch wirkenden Bodenorganismen und der Wirkungsdauer von Präparaten untersucht. Die Wurzelfäule erreger, die untersucht worden, sind: ***Rhizoctonia solani*** Kühn., ***Pythium*** spp. und ***Fusarium*** spp. Die Gemüsearten sind Tomate, Paprika, Salat, Spinat, Kohl, Rübe und Bohne

Zuerst wurde die Erde mit der 2 % igen Lösung von 3.5 % igen Ethyl Mercury-Chloride (berechnet je qm 4 l), bei der zweiten Versuchsserie als 28 ppm behandelt. Tetracyclin Wirkstoff verwendete man von 500 ppm.

Bei der ersten Aussaat gerade nach der Behandlung zeigten die Präparate sowohl auf die verschiedenen Mikroorganismen als auch auf die Erreger der Wurzelfäule eine gute Wirkung. Danach folgende Aussaaten verringerte sich die Wirkungsgrad des Präparates auf die Erreger der Wurzelfäule, wenn sich der Zeitabstand zwischen der Behandlung und der Kontrolle verlängert.

EINLEITUNG

In einer früheren Arbeit stellte KARAHAN (1963) fest, dass bei den Isolationen aus der verfaulten Wurzeln der Keimlingen, doch auch bei den jungen Kulturen wo *Pythium debaryanum* und *Actinomyces spp.* zusammen gewachsen waren, die Mycelien von *Pythium* zerstört wur den. Dagagen wurde es beobachtet, dass die Mycelien von *P. debaryanum* und *P. ultimum* Kulturen aus Holland auf den gleichen Nährböden normales Aussehen und Wachstum haben.

KARAHAN (1968) stellte bei seinen Untersuchungen über Braunfleckenkrankheit der Kichererbse nach der Keimung der Samen, die mit Pimafucin (Streptomycin) gebeizt waren, einen höheren Prozentsatz der Wurzelfäule fest Nach seinen Ergebnissen haben die Mikroorganismen eine antagonistischen Wirkung über pathogene Pilze, und die Saatgut-und Bodenentseuchungsmitteln haben eine bestimmte tötende Wirkung über pathogene Pilze und über Bodenmikroorganismen. Wegen ihrer tötenden Wirkung der Beizmitteln über antagonistisch wirkenden Mikroorganismen (besonders über Bakterien) wird es nach angewendeten Dosis in der Nähe von Samen eine sterile Umgebung gebildet. Infolgedessen können Wurzelfäuleerreger gut wachsen und an den Pflanzen Schaden verursachen.

In dieser Arbeit wurde mit ein fachen Testen Zusammenhang zwischen antagonistischen Wirkung der Bodenmikroorganismen und der Wir kungsdauer von Päparaten untersucht.

MATERIAL UND METHODE

Die Versuche wurden in Holzkasten und in den Töpfen, die mit der gleichen Menge Erde gefüllt worden waren, durchgeführt. Die Töpfe und Kasten wurden mit der Erde und Pflanzenrückständen ausgefüllt, die aus den durch die Pilze *Rhizoctonia solani* Kühn., *Pythium* spp. und *Fusarium* spp. befallen und Wurzelfäule (Damping off) vorhandenen Saatbeeten entnommen wurden. Um die Schädigungsgrad und die Intensität der Krankheit festzustellen, wurdevor dem Versuchs beginn eine Samenmischung von Tomate, Paprika, Spinat, Salat, Kohl, Rübe, Bohne in die Kasten und auch in die Töpfen eingesät.

Nachdem die Keimlinge, die zu den verschiedenen Gemüsearten gehören, Blätter bekommen haben, wurden sie in kleinen Stücken geschnitten und mit der Erde gemischt. So waren wir davon überzeugt, dass die Versuchserde durch die pathogenen Pilze vollkommen verseucht wurde.

Zuerst wurde die Erde bei der ersten Versuchsserie mit der 2 % igen Lösung von 3.5 % igen Ethyl Mercury Chloride (berechnet je ρm 4 l), bei der zweiten Versuchsserie mit 28 ppm behandelt Tetracycline

hydro Chloride und Tetracycline-Alkali (Tetracyclin-Wirkstoff) verwentede man von 500 ppm.

Bei der Aussaat benutzte man die Samen von Tomate und Paprika. In die behandelten Kasten und Töpfe sowie in die Unbehandelten wurden die Samen in gleicher Zahl und Menge gesät. Als die Pflanzlinge zu dem phänologischen Stadium erreicht hatten, wurden sie herausgezogen, gewaschen und dann die gesunden und durch Wurzelfäule gestorbenen Pflanzen festgestellt.

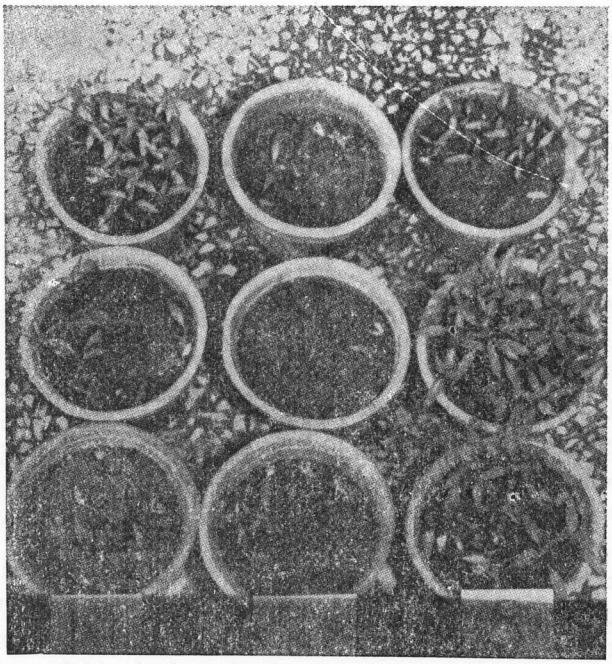

Abb. 1 Die befallene Keimlinge Bei der Tetracyclin Alkali - Behandlung
1-1 1-2 Bei der Tetracyclin Hydrochloride - Behanlung
Ş- Bei der Kontrolle

ERGEBNISSE UND DISKUSSION

Nach den Versuchsergebnissen verringert sich die Wirkung des Mittels auf die Erreger der Wurzelfäule, wenn sich de Zeitabstand zwischen der Behandlung und der Kontrolle verlängert. Wenn die Aussaat nach der Beendigung der Wirkung des Mittels vorgenommen wird, ist die Schädigung durch Wurzelfäule in der behandelten Erde grösser als die in der Unbehandelten. Es wurde festgestellt, dass 98.46 % des Bestandes bei der mit der Tetracyclin-Hydrochloride, 82.05 % mit Tetracyclin-Alkali und 67.17 % bei

der unbehandelten Erde durch Wurzelfäule gestorben sind (Abbildung 1 und Tabelle 1).

Bei der ersten Aussaat gerade nach der Behandlung hatten die Präparate sowohl auf die verschiedenen Mikroorganismen als auch auf die Erreger der Wurzelfäule eine gute Wirkung und verminderte sich die Schädigung der Krankheit daher im Vergleich mit Unbehandelten Bei den folgenden Aussaaten verringerte sich die Wirkungsgrad des Präparates mit der Zeit und verschwand dann ganz und gar.

Die tötende Wirkung des Präparates auf Bodenmikroorganismen, welche auf die pathogenen Pilze antagonistisch wirken, verursachte die Reduktion der Mikroorganismen-Population und die Störung der biologischen Stabilität.

Da es sich in diesem Zustand keine Konkurrenz mehr gab, fanden die Pilze günstige Entwicklungsmöglichkeit. Auch die Versuchsergebnisse mit Tetracyclin bestätigten dieses Urteil.

Tabelle: 1 Die Wirkung von Tetracyclin - Wirkstoff auf die Wurzelfäule

Behandlung	Wiederholungen						Zahl der befallenen Keimlinge	Prozentsatz der befallenen Keimlinge (%)
	1		2		3			
	gesund	beffallen	gesund	befallen	gesund	befallen		
Tetracyclin hydrochloride	1	64	2	63	0	65	192	98,46
Tetracyclin alkali	5	60	10	55	20	45	160	82,05
Kontrolle	9	56	37	28	18	47	131	67,17

ÖZET

TOPPAK MİKROORGANİZMALARININ BİTKİ PATOJENİ FUNGUSLARINA ANTAGONİSTİK ETKİLERİ ÜZERİNDE ARAŞTIRMALAR

Toprakta bulunan mikroorganizmalar patojen funguslar üzerinde antagonistik bir etkiye sahiptirler. Tohum ve toprak dezenfeksiyonu için kullanılan ilaçlar, hem patojen funguslar ve hem de topraktaki mikroorganizmalar üzerinde öldürücü etki yapar. Bu yüzden uygulanan doza göre tohum etrafında steril bir ortam oluşur. Dolayısıyla kök çürüklüğü etmenleri iyi gelişirler ve bitkilerde zararlı olurlar.

Bu çalışmada basit testlerle antagonistik etki ve kullanılan preparatların etki süreleri arasındaki ilişki araştırılmıştır.

Denemelerdeki Kök Çürüklüğü (Damping-off) etmenleri *Rhizoctonia solani* Kühn., *Pythium* spp. ve *Fusarium* spp. dir. Sebze çeşitleri ise domates, biber, ıspanak, marul, lâhana, havuç ve fasulyedir. % 3.5 lik Ethyl-Mercury Chloride'nin % 2 lik eriyiği, ikinci denemede 28 ppm. oranında; Tetracylin-Hydrochloride ve Terracyclin alkali 500 ppm oranlarında kullanılmışlardır.

Deneme sonuçlarına göre, ilaçlı muameleden sonraki ekimde preparatlar hem mikroorganizmalar ve hem de kök çürüklüğü etmenleri üzerine iyi bir etki yapmaktadır. Muamele ile kontrol arasındaki zaman uzadığı takdirde ilâcın etkisi azalmakta ve sonradan tamamen kaybolmaktadır.

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NEW RECORD

The First Report of Verticillium wilt on Sesame and Okra in Turkey

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Verticillium wilt was found on cotton, various vegetables peach and olive trees until now in Turkey (2, 3, 4, 5, 6, 7).

During a survey in September 1971 typical *Verticillium* wilt symptoms were first observed on sesame (*Sesamun indicum* L.) and okra (*Hibiscut esculentus* L.) in Menemen. Infection was about 25 % on sesame, and 60 % on okra in these fields

Okra is one of the most seriously affected vegetable by *Verticillium* (1). The young plants usually appe ar normal; but as the infected plants grow older, they show marked dwar fing.

Seriously affected plants, however, take on a yellow unhealty colour, while the lower leaves wilt and drop off. The symptoms observed on the plants caused by *Verticillium* wilt (Fig.1) also distinguis hed by vascular browning.

Isolations were made from stem pieces showing vascular browning on 08 % alcohol-water agar medium. The bark of diseased stem was peeled off and stem were cut into small pieces with a sterilized scalpel. Their surface sterilized with 01 % $HgCl_2$ and washed with distilled water twice and then was dried between sterile bloting papers.

The pieces were placed in sterile petri dishes which contained alcohol water agar. The dishes were incubated at 22°C for a week. The resulting fungus determined as to be *Verticillium dahliae* Kleb.

Pathogenicity tests were made by using the method based on Wiles (8) technique for inoculations. Observations were made on wilted plants and fungus was reisolated from the inoculated plants.

Therefore this constitute is the first report on *Verticillium* wilt of sesame and okra in Turkey.

Figure 1. Typical symptoms of Verticillium wilt on okra plants.

TÜRKİYE'DE SUSAM VE BAMYA'DA VERTİCİLLİUM SOLGUNLUĞUNA AİT İLK RAPOR

Verticillium solgunluğu Türkiyede şimdiye kadar pamuk, çeşitli sebze, şeftali ve zeytinlerde saptanmıştır.

1971 yılı Eylül ayında bir sürvey sırasında *Vertcillium* solgunluğu belirtileri susam ve bamya'da müşahade edilmiştir. İnfeksiyon bu tarlalarda susam'da % 25, bamya'da % 60 idi.

Enfekteli bitkilerin yaprakları normal yeşil rengini kaybederek sararır, daha ileri hallerde kahverengileşir ve kuruyarak dökülür. Hastalıklı bitkilerden alınan örneklerden yapılan izolâsyon çalışmaları sonunda hastalık etmeninin ***Verticillium dahliae*** Kleb. olduğu anlaşılmıştır. Böylece *Verticillium dahliae* Türkiye'de susam ve bamya üzerinde ilk kez saptanmış olmaktadır.

Etmen tesbitinden sonra patojenisite denemeleri yapılmış ve inokule edilen bitkilerden tekrar aynı fungus izole edilmiştir.

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