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Studies on the Control Possibilities of Chestnut Blight [Endothia parasitica (Murr.) A. and A.] in Turkey [1)]

I. POSSIBLE USES OF SOME SYSTEMIC FUNGICIDES AGAINST THE PATHOGEN

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ABSTRACT

Different systemic fungicides were used **in vitro** and **in vivo** condition with the purpose of controlling chestnut blight. At the end of the **in vitro** studies Bavistin, Benlate and Enovit Super were found out to be effective fungicides respectively. But **in vivo** conditions Enovit Super was the most effective, and it was followed by Bavistin and Benlate.

INTRODUCTION

In Turkey, chestnut blight [**Endothia parasitica** (Murr.) A. and A.] was first recorded in 1967, and was found to be wide spread in Marmara and Black Sea Region (1,22,23,58). Since the appearance of the disease in U.S.A. (67), nearly 75 years ago, various studies on the resistan varieties (5,8,9,25,26,27,34,35,36,37,38,39,40 41,42,49,57,74), inhibitive effect of

(1) Supported by the Faculty of Agriculture, University of Ege.

tannin (2,18,22,43,46,68), natural antagonists (61), quarantina (7,33,66) and on eradication measures (7) had been performed, but no effective disease control measures could be evolted so far. However, it was being shown that, virulent race can be controlled by the hypovirulent race of the pathogen (3,4,21,44,45,47,48,52, 82). For the chemical control of the disease, bordeaux mixture (64), lithium salts (69), Thorman 80 (60), D.N.O.C. (29), ethylene oxide (10) and an antibiotic which was named as AE 56 (76) were being used, but no practical significant result could be obtained. After the discovery of systemic fungicides, benomyl when tested as a drenching application, was found to influence the growth of the pathogen (53). After this report, it was showed that, carbendazim (= M.B.C.) injections were more effective than benomyl (54,55). According to the results of another study, mercury oxide was more effective than dimethylsulfoxide and benomyl when applied on the cankers (75).

The first part of the study reported here concerns with the various tests under laboratory and greenhouse conditions with different groups of systemic fungicides in order to find out a chemical control for the chestnut blight disease.

MATERIALS AND METHODS

Materials

The fungicides included in the experiments along with their characteristics are shown in Table 1.

For **in vivo** studies European Chestnut (**Castanea sativa** Mill.= **C. vesca** Gaertn) seedlings were used. This species, as reported by Woodroof (85), susceptible to the pathogen and have all the other characteristics of **C. sativa.**

Throughout, the study, a single-spor cultures of **E. parasitica** was used. The identity of this pathogen was earlier canfirmed by Dr. Grente of France and Dr.Von Arx of Holland and this culture was also used in one of the earlier study (22).

Methods

In the first **in vitro** test, all the eleven fungicides named in Table 1 were used in order to test their effectiveness against growth of the fungus. The concentration levels of fungicides were 500, 1000, 1500 and 2000 ppm. as a.i. For this method which is generally known as drop method (11,71), the fungus was inoculated in the centre of the P.D.A. medium in every petri dish, and respective fungicide was applied to the three edges of petri dish as a single

Table 1. Some characteristics of the tested systemic fungicides

Fungicide's				
Group	Trade Name	Company	Active Ingradient (a.i)	Formulation Type
Benzimidazole	Benlate	E.1. du Pont de Numerous and Co. Inc.	50 %, Methyl 1-(butyl-carbamoyl)-2-benzimidazolecarbamate (Benomyl)	W.P.
Benzimidazole	Enovit Super	Sipcam S.p.A	70 %,1,2,-bis(3-methoxy carbonyl-2-thioureido) benzene, (Thiophanate-methyl)	W.P.
Benzimidazole	Bavistin	BASF A.G.	50 %,Methyl-2-benzimidazolecarbamate, (Carbendazim=MBC)	W.P.
Benzimidazole	Derosol	Farbwerke Hoechst A.G.	60 % Carbendazim	W.P.
Anilide	Vitavax	Uniroyal Chimica S.p.A	37,5 %, 5,6-dihydro-2-methyl-1,4-oxathiin 3-carboxanilide, (Carboxin)	W.P.
Anilide	Plantvax	Uniroyal Chemica S.p.A	75 %, 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide-4, 4-dioxide, (Oxycarboxin)	W.P.
Pyrimidine	Milstem	1.C.1.Plant Protection Ltd.	50 %, 5-n-butyl-2-ethylamino-4-hydroxy -6-methylprimidine, (Ethirimol)	Em.

Table 1 (Continuing). Some characteristics of the tested systemic fungicides

Pyrimidine	Milcurb	1.C.1.Plant Protection Ltd.	12,5 %, 5-n-butyl-2-dimethylamino-4-hydroxy-6-methylprimidine, (Dimethirimol)	Em.
Morpholin	Calixin	BASF A.G.	85 %, N-Tridecyl-2,6-dimethyl-morpholine, (Tridemorph)	Em.
Piperazine	Saprol	Cela merck Gmb H and Co. KG.	20 %, N.N.-[1,4-Piperazindiyl-bis-(2,2,2,-trichlorethyliden)]-bis-(formadid), (Triforine)	Em.
Organophosphorious	Afugan	Fabwerke Hoechst A.G.	30 %, Diethyl 6-carbethoxy-5-methyl-pyrazolo-(1,5-a)-pyrimidine-2-yl phosphorothionate, (Pyrazophos)	

drop at equal distances from the centre. No fungicide was applied on the fourth edge which acted a control in the petri dish.

The second test was performed **in vivo** conditions. The fungicides which were found effective in the retarding the colonial growth of the pathogen, were applied to the chestnut seedlings as following combination for determining the most effective dose and application time.

1. Combination: One week befor inoculation, 3000 ppm. a.i.
2. Combination: With inoculation, 1500 ppm a.i.
3. Combination: With inoculation, 3000 ppm a.i.
4. Combination: With inoculation, 1500 ppm a.i. and one week after 1500 ppm a.i. again
5. Combination: One week after 3000 ppm. a.i.

Chestnut seedlings were inoculated by making wounds on the stems (6,54). But inoculations were done in August, due to the late procurement of the seedlings. Fungicides were applied to the pots by drenching method (70,78).

After these tests, minimum inhibition doses of the effective fungicides were studied by the molten P.D.A. method (12).

Control possibility of the pathogen by the selected fungicides were determined through the pot cultur test in the greenhouse. For this purpose, two doses of every fungicides were tested, first dose was that dose which was found most effective in previous **in vivo** test and the second dose was one level higher to the first. Time of application which gave maxi mum disease control in the previous test was also used in this test. The seedlings were inoculated in may through the three wounds made on the stem one above the other at equal distances. Chemicals were applied by drenching method.

The effectiveness period of those fungicides which emerged as most promising from the above mentioned test, were determined by the bio-assay method (22,28,50,51,73). In this test, chemicals were applied to the pots only once by the drenching method. During the period of thirteen weeks begining one week after the fungicides application, every week 5 seedling (replication) from every treatment were brought to the laboratory for bio-assaies. For this purpose, 3 stem pieces of 4,5 cm. long begining from the collar region were taken from with 25 cm. interval between every piece, every plant. So, the first sample were taken between 0,0-4,5 cm, the second 29,5-34,0 cm and the third at 59,0-63,5 cm. heights. The pieces were cut longitudinally and then put into the petri dishes which contained a sheet of cotton and filter paper on the base. The stem pieces were surface inoculated by drop 0,1 ml/cm^2 inoculum containing 8x10^5 picnidiospore/ml. Moreover, 10 ml. of steril water was poured into the base of every petri

dish. Fungal growth on the samples were measured 6 days after the inoculation, and calculations were done according to the percentages of the fungal growth. The logarithmic curves were drown on the basis of the statistical analysis in order to determine effectiveness of every fungicide concentration.

In all the experiments randomized plot design was applied. For **in vitro** tests 8 replications and **in vivo** studies 5 replications were used. The experiment performed for obtaining the most effective fungicides **in vitro** conditions, each replication contained 3 plants; while in other experiments replications contained one plant as one petri dish.

RESULTS

The results of the first **in vitro** test using different groups of systemic fungicides by drop method is given in Table 2.

Table 2. Effectiveness of some systemic fungicides against **E. parasitica** as shown by drop method

Fungicide's Trade Name	Dose (ppm., a.i.)	Average diameter of the inhibition zone (mm.)	Avarage of the colonial half diameter of non-fungicide applied zone (mm.)
Benlate	500	21,89	41,66
	1000	22,94	38,20
	1500	22,94	37,00
	2000	25,78	28,00
Bavistin	500	22,22	37,50
	1000	25,67	33,15
	1500	25,78	33,16
	2000	26,10	31,00
Enovit Super	500	17,39	42,00
	1000	19,78	40,83
	1500	20,50	38,60
	2000	22,95	37,16

Table 2 (Continuing). Effectiveness of some systemic fungicides against **E. parasitica** as shown by drop method

Derosal	500	22,33	34,00
	1000	26,21	30,80
	1500	26,34	29,80
	2000	26,72	28,83
Vitavax	500	18,78	34,66
	1000	20,22	36,50
	1500	20,89	36,00
	2000	22,67	35,66
Plantvax	500	0,00	41,83
	1000	0,00	41,50
	1500	0,00	42,50
	2000	0,00	40,00
Milstem	500	0,00	41,50
	1000	0,00	43,00
	1500	0,00	42,00
	2000	0,00	43,00
Milcurb	500	0,00	41,50
	1000	0,00	39,98
	1500	0,00	42,80
	2000	0,00	42,50
Calixin	500	23,55	17,16
	1000	24,67	16,00
	1500	24,72	16,83
	2000	24,83	15,50
Saprol	500	8,28	40,83
	1000	9,39	40,80
	1500	10,00	40,00
	2000	20,28	29,00
Afugan	500	0,00	40,50
	1000	0,00	42,20
	1500	0,00	41,66
	2000	0,00	40,00
Control (non applied)	—	—	43,60

As obvious from Table 2, Plantvax, Milstem, Milcurb and Afugan could not inhibit the colonial growth of the pathogen. The effectiveness of Saprol appeared after 1500 ppm. On the other hand, all the doses of the remaining fungicides inhibited the colonial growth of the pathogen (Fig. 1).

On the basis of these results, Plantvax, Milstem, Milcurb and Afugan were excluded from the subsequent experiments. The remaining fungicides were taken for the second study with the purpose of obtaining the most effective dose and application time **in vivo** conditions. The result of this **in vivo** test are summerized in Table 3.

From the results represented in Table 3, it is evident that, Bavistin and Enovit Super which were applied one week before inoculation at 3000 ppm. concentration are most effective fungicides. These two fungicides were followed by the Benlate, Derosal and Vitavax, Calixin.

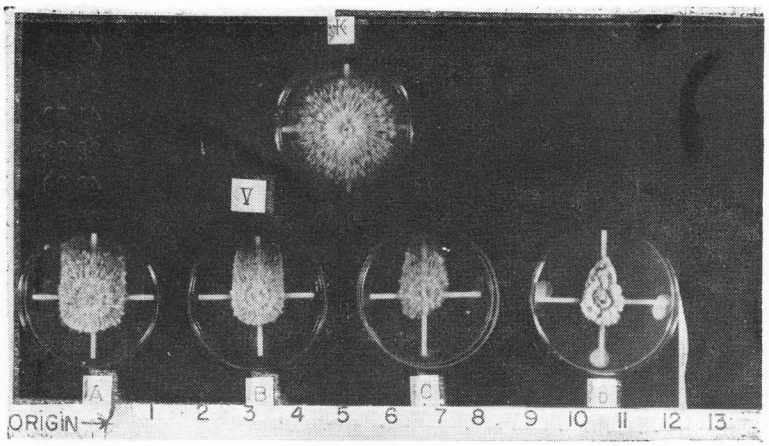

Fig. 1. Effectiveness of Enovit Super against the **E. paras tica** by drop method at 500 ppm (A). 1000 ppm (B), 1500 ppm (C) and 2000 ppm (D). The upper sides of the dishes no fungicide applied. K:Control (no fungicide applied on the all four corners).

Table 3. In the first pot culture test the effectiveness of the chemicals with combination of different doses and application times

Trade Name of the fungicide	Recorded average period when the seedlings dried up (days)					
	One week before inoculation, 3000 ppm, a.i.	With inoculation, 1500 ppm, a.i.	With inoculation, 3000 ppm. a.i.	With inoculation 1500 ppm. a.i., one week after 1500 ppm. a.i.	One week after inoculation 3000 ppm. a.i.	Inocluated, no fungicide applied (control)
Benlate	58,80 (BC)	33,00 (EF)	37,00 (EF)	36,60 (EF)	33,00 (EF)	—
Bavistin	77,40 (A)	60,80 (AB)	38,40 (EF)	34,40 (EF)	37,80 (EF)	—
Enovit Super	74,40 (A)	55,20 (BCD)	38,80 (DEF)	46,80 (BCDE)	33,00 (EF)	—
Derosal	31,80 (EF)	46,20 (BCDEF)	46,00 (BCDEF)	33,00 (EF)	42,60 (CDEF)	—
Vitavax	33,00 (EF)	34,40 (EF)	37,00 (EF)	33,00 (EF)	39,20 (CDEF)	—
Saprol	34,40 (EF)	30,00 (F)	42,40 (CDEF)	33,00 (EF)	34,40 (EF)	—
Calixin	33,80 (EF)	33,00 (EF)	33,00 (EF)	34,80 (EF)	33,00 (EF)	—
Control	—	—	—	—	—	33,00 (E)

( ): Statistical groups

In addition the statistically most effective fungicides Bavistin and Enovit Super, representatives of other statistical groups Benlate, Derosol and Saprol were included for the "t" test and minimum inhibition doses of these 5 fungicides were determined. For this purpose chemicals were applied to the pathogen at 1, 10 and 100 ppm a.i. concentrations **in vitro** conditions. But, no fungal growth was observed. For this reason fungicides were applied at 0.2, 0.4, 0.6, 0.8 and 1.0 ppm a.i. doses (Table 4).

Table 4. Minimum inhibition doses of the selected systemic fungicides against **E. parasitica**

Fungicide's Trade Name	Concentration (ppm.a.i.)	Measurement of colonial half diameter (mm.) days after application			
		2nd day	4th day	6th day	8th day
Benlate	0,2	0,16	1,44	2,58	3,93
	0,4	0,00	0,01	0,22	1,59
	0,6	0,00	0,00	0,00	0,00
	0,8	0,00	0,00	0,00	0,00
	1,0	0,00	0,00	0,00	0,00
Bavistin	0,2	0,09	0,32	1,12	1,70
	0,4	0,00	0,00	0,00	0,00
	0,6	0,00	0,00	0,00	0,00
	0,8	0,00	0,00	0,00	0,00
	1,0	0,00	0,00	0,00	0,00
Enovit Super	0,2	1,02	8,38	16,02	22,37
	0,4	0,00	0,06	0,24	0,89
	0,6	0,00	0,00	0,00	0,00
	0,8	0,00	0,00	0,00	0,00
	1,0	0,00	0,00	0,00	0,00
Derosal	0,2	0,79	5,82	8,55	10,75
	0,4	0,21	1,05	2,65	4,06
	0,6	0,00	0,00	0,24	0,48
	0,8	0,00	0,00	0,00	0,00
	1,0	0,00	0,00	0,00	0,00
Saprol	0,2	0,09	0,81	2,15	3,09
	0,4	0,00	0,46	0,68	0,95
	0,6	0,00	0,00	0,23	0,82
	0,8	0,00	0,00	0,06	0,49
	1,0	0,00	0,00	0,00	0,00
Control	—	1,32	9,93	25,03	38,50

According to Table 4, colonial growth of the pathogen was inhibited by Bavistin at 0,4 ppm, Benlate and Enovit Super at 0,6 ppm., Derosol at 0,8 ppm, and Saprol at 1,0 ppm. In the statistical analysis interaction between the fungicide and concentration were found significant ($P<0.01$)

As already explained in methods section, further tests under greenhouse conditions were conducted with the selected fungicides using only their two concentration levels. The results of this study are summerizied in Table 5.

Table 5. Effectiveness of the selected systemic fungicides against the **E. parasitica** in the greenhouse conditions

Fungicide's		Recorded average	Number		
Trade Name	Concentration (ppm.,a.i.)	period when the seedlings became dry	of inoculated plants	Number of dryed plants	Number of healty plants
Benlate	3000	127.13	15	15	0
	4500	138,73	15	15	0
Bavistin	3000	133,00	15	15	0
	4500	136,58	15	12	3
Enovit Super	3000	140,46	15	15	0
	4500	136,50	15	4	11
Derosal	3000	82,46	15	15	0
	4500	112,80	15	15	0
Saprol	3000	42,66	15	15	0
	4500	43,33	15	15	0
Control	—	36,26	15	15	0

Table 6. Growth of the E. parasitica on the chestnut

Fungicide's			Precantages of the				
Teade Name	Concen-teation (ppm.,a.i)	Heighst of the samples from the soil (cm.)	1 th. week	2 nd. week	3 th. week	4 th. week	5 th. week
Benlate	3000	0.0— 4.5	11.54	21.37	0.00	0.26	0.00
		29.5—34.0	54.17	33.10	3.97	2.04	3.63
		59.0—63.5	52.85	24.44	8.53	4.50	1.77
	4500	0.0— 4.5	8.55	7.74	0.00	0.00	3.32
		29.5—34.0	62.86	46.20	2.70	2.01	4.97
		59.0—63.5	68.18	40.26	2.39	0.00	2.64
Bavistin	3000	0.0— 4.5	1.68	11.11	10.23	4.03	2.30
		29.5—34.0	43.98	25.84	7.56	10.00	6.11
		59.0—63.5	31.31	43.99	6.16	20.00	2.59
	4500	0.0— 4.5	3.59	4.08	4.38	0.00	0.00
		29.5—34.0	57.76	33.75	3.30	0.00	0.00
		59.0—63.5	66.84	33.47	1.98	0.00	0.54
Enovit	3000	0.0— 4.5	19.73	15.73	18.13	1.50	3.88
Super		29.5—34.0	77.00	37.78	66.13	4.44	0.32
		59.0—63.5	86.59	21.94	58.50	0.00	0.00
	4500	0.0— 4.5	19.35	0.00	0.00	0.00	0.00
		29.5—34.0	27.38	0.74	0.00	0.00	0.00
		59.0—63.5	36.66	2.46	0.00	0.00	0.00
Control	—	0.0— 4.5	100.00	100.00	100.00	100.00	100.00
		29.5—34.0	100.00	100.00	100.00	100.00	100.00
		59.0—63.5	100.00	100.00	100.00	100.00	100.00

em samples applied with Benlate, Bavistin and Enovit Super.

fungal growth

6 th. week	7 th. week	8 th. week	9 th. week	10 th. week	11 th. week	12 th. week	13 th. week
2.34	0.00	0.00	0.00	0.00	28.31	26.04	48.56
0.78	0.00	2.64	2.95	6.62	81.95	80.73	66.27
0.00	0.00	12.61	3.71	8.76	70.46	75.59	60.54
0.15	0.16	6.22	2.54	1.27	46.39	15.69	2.00
4.50	6.75	26.28	22.76	16.28	62.80	50.23	70.82
3.48	36.56	30.50	0.00	23.92	59.84	37.50	84.92
0.00	0.00	0.00	0.00	0.00	19.82	9.44	34.67
0.00	0.00	0.00	0.00	0.19	45.53	97.42	82.72
0.00	2.23	0.00	0.00	5.70	69.18	89.54	86.78
0.00	0.05	0.00	0.00	0.00	3.78	49.67	9.33
3.14	0.00	0.00	0.00	6.96	20.90	74.03	38.50
0.00	0.00	0.00	0.00	0.00	65.51	75.43	26.46
0.00	0.00	0.00	2.48	3.14	14.47	1.23	31.01
19.32	0.00	0.00	13.60	2.13	44.10	36.59	50.00
19.32	0.00	0.00	0.35	12.35	81.53	38.35	72.90
0.00	0.00	0.00	0.00	0.00	0.00	1.12	0.00
0.00	0.00	0.00	0.00	0.00	0.00	30.74	23.32
0.00	0.00	0.00	0.00	0.00	0.06	25.58	41.53
100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

The results showed that, Enovit Super at 4500 ppm. concentration was the most effective fungicide against the disease. Bavistin at 4500 ppm, Enovit Super at 3000 ppm and Benlate at 4500 ppm, Bavistin at 3000 ppm, Benlate at 3000 ppm concentration were found effective against the disease in decreasing order. Derosal and Saprol were found to be least effective.

Enovit Super, Bavistin and Benlate gave promising results to control the pathogen, therefor only these three fungicides were used for determining their effectiveness in the plant tissue by bio-assay. The result of this study is summerized in Table 6.

The figures in Table 6 show that all fungicides were effective against the pathogen when compared with the controls, during the 13 weeks period (Fig. 2,3,4,5,6,7). But the most effective chemical was Enovit Super at 4500 ppm. concentration (Fig. 7). The results of the statistically, all the fungicides were significantly effective against the fungus than the controls. The most effective fungicide was Enovit Super 4500 ppm., and this was followed by Bavistin 4500 ppm. It was also statistically significant that, the effectiveness of the fungicides in influenced by the seedlings height.

During the 13 weeks period temperature records of the experimental site are given in Table 7.

DISCUSSION

Eleven systemic fungicides were used in order to evaluate their fungicidal potential against **E. parasitica**. From benzimidazole group Enovit Super (thiophanate-methyl), Bavistin (carbendazim) and Benlate (benomyl) gave the promising results **in vitro** and **in vivo** conditions. These results are agreeable with Jaynes and Anognostakis (53), Jaynes and Van Alfen (54,55) who used Benlate and MBC (=carbendazim) against the pathogen **in vivo** conditions. On the other hand, according to some investigations, this group of systemic fungicides were found to be effective against some important wilt disease fungi, e.g. **Ceratocystis ulmi, Fusarium** spp. and **Verticillium** spp. (15,24,73,79), so it can be said that, benzimidazole derivatives are the effective fungicides against the tracheomycoses.

Although Bavistin and Derosal contain the same active ingradients, the effectiveness of these two fungicides was found to be different. According to Evans (30) quoting Pitblado and Edgington, this difference is comming from some fungicidal chemicals which are added into the formulations. These kinds of differences were also being shown (17,24).

Table 7. Temperature records of greenhouse cabin during the 13 weeks of the experiment

	Week's		
Weeks	Mean temp.(°C)	Mean temp. of the hotest day (°C)	Mean temp. of the coolest day (°C
1 th. week: 22-28 June	26,9	28,4	25,6
2 nd week: 29 June-5 July	26,2	27,4	24,4
3 th. week: 6 -12 July	29,1	30,6	27,6
4 th. week: 13-19 July	27,9	29,9	26,9
5 th. week: 20-26 July	27,6	28,5	26,7
6 th. week: 27 July-2 August	30,3	33,3	27,9
7 th. week: 3-9 August	29,0	30,4	28,4
8 th. week: 10-16 August	27,3	29,2	25,3
9 th. week: 17-23 August	27,4	29,4	25,2
10th. week: 24-30 August	26,9	30,2	23,8
11th. week: 31 August-6 Sept.	25,2	28,1	23,8
12th. week: 7-13 September	24,5	26,8	22,4
13th. week: 14-20 September	22,0	23,7	19,2

This study indicated that, application time of the fungicides influence the effectiveness. For this reason, effectiveness of the chemicals were less when they were used in august, than used in may (Table 3 and 7). According to Crowdy (19), uptake and transportation of systemic fungicides are influenced by the climatic conditions and vegetation period.

It was found that, Enovit Super is the most effective fungicide **in vivo** tests, but Bavistin is most effective **in vitro** tests. According to Kaars Sijpesteijn (56) with reference to Mantha and Gentil, thiophanates are more effective in the plant tissue than the **in vitro** conditions. In another report it was statet that, quinons in the plant tissues has an important role on the activity of thiophanates (83).

Although Enovit Super and Benlate become hydrolised to canbendazim (16,32,59,63), but Bavistin (carbendazim) and these two fungicides had different effectiveness **in vivo** conditions. It is being considered that, this different effectivenesses between these fungicides is due to transformation rate of Enovit Super and Benlate to carbendazim, and uptake and transportation of these chemicals in the plants. For example, benomyl can be hydrolised to carbendazim rapidly than thiophanate-methyl (13,14,16,20). According to Lerox and Gredit (62), after thiophanate-methyl applications from the roots of bean plants, carbendazim and thiophanate methyl were obtained together in the leaves and stems, but after benomyl applications only carbendazim was obtained in the foliage However the results of Jaynes and Van Alfen (55) on the chestnut blight indicate that, carbendazim is translocated first to the crown, then redistributed downward.

Carbendazim is transported to the leaves in a short period after its absorption through the roots (70,72). But, transportation of thiophanate-methyl to the leaves is more slowly (13). Thats why, Enovit Super was found to be more effective **in vivo** conditions in this study. Higher effectiveness of benomyl than thiophanate - methyl against the powdery mildew on the cucumber leaves (31, 32) is also indicating the slow transportation of thiophanate - methyl to the foliage. On the other hand, thiophanate - methyl can accumulate in the roots and due to this accumulation the chemical can be taken up continiously (20,84) also agree with the results of this study.

In the bio-assay tests, because of low solubility of benomyl, 4500 ppm concentration was found less effective than the 3000 ppm. Transportation of high Benomyl doses is more difficult due to its precipitation into the vassels (80,81). Two doses of benomyl could not also to be found as statistically significant against the chestnut blight (53). On the other hand, the effectiveness of Bavistin and Enovit Super began to fall down after 9. and 10. weeks respectively, Beside some other discussed effects, this effect is also connected with the temperature, because passive transportation of the chemicals between the roots and foliage is also influenced by the temperature (65).

Although the possitive results are being obtained with some fungicides, but for practical purposes, some other considerations must be taken into accound. Firsty, can the pathogen aquire resistance to mentioned fungicides after continious applications. The second part of this study will deal in more detail about

this subject. Secondly, in the drenching method very high doses are needed. For this reason application of chemicals as trank injections must be studied. But, the low solubility of benzimidazole derivaties and less effectiveness of trank injected benomyl than drenched (53,54,55) must be remembered. Moreover, interaction between the hypovirulent races and chemicals, and residue levels of the fungicides is the fruits must also be known. Only after the through study of these problems we can advice the chemical control of chestnut blight by the mentioned fungicides.

ACKNOWLEDGMENT

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ÖZET

KESTANE KANSERİ [**Endothia parasitica** (Murr.) A. and A.] İLE SAVAŞ OLANAKLARI ÜZERİNDE ARAŞTIRMALAR

I. Bazı Sistemik Fungisidlerin Patojeni Önlemede Kullanılabilmesi

Kestane kanserini önlemek amacıyla 11 sistemik fungisid önce laboratuvar koşullarında, sonra da başarılı bulunanlar sera koşullarında denemelere alındılar. Laboratuvar koşullarında en etkililer, sırasıyla, Bavistin, Benlate ve Enovit Super olmasına karşın, sera koşullarında Enovit Super en başarılı fungisid olmuş ve onu Bavistin ile Benomyl izlemiştir. Özellikle Enovit Super'in 4500 ppm aktif madde içeren dozuyla toprağa içirme biçiminde ilâçlanan kestane fidanlarında 9 hafta süreyle patojenin gelişimini tamamen engelleyebilecek fungisid yoğunluğunun bulunduğu da saptanmıştır. Ancak, tüm olumlu sonuçlara karşın, patojenin etkili fungisidlere dayanıklılık kazanma olasılığı, daha düşük fungisid yoğunluklarının kullanılabileceği gövde enjeksiyonlarının durumu patojenin hypovirulent ırklarıyla etkili fungisidler arasındaki ilişkiler ve meyvalardaki fungisid kalıntılarının düzeyleri ortaya konmadan pratiğe dönük bir öneride bulunabilmek şimdilik olanaksızdır.

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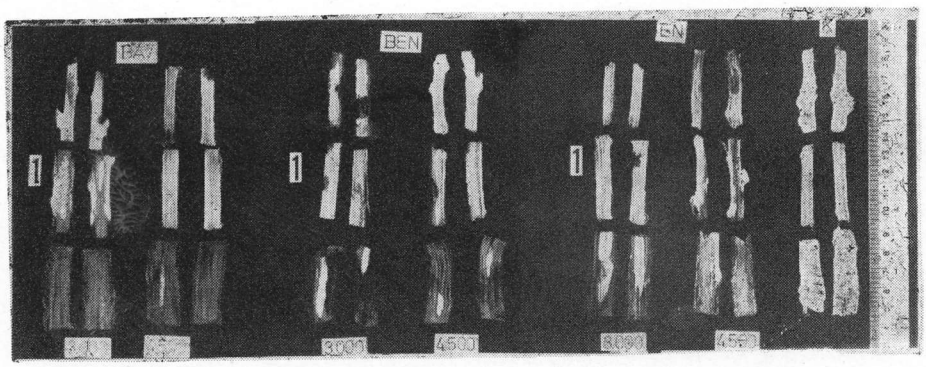

Fig. 2. E. parasitica inoculated wooden samples which were treated one week before with 3000 and 4500 ppm Bav'stin (BAV), Benlate (BEN) and Enovit Super (EN). (The rows of the samples are indicating each he'ght. K: Control)

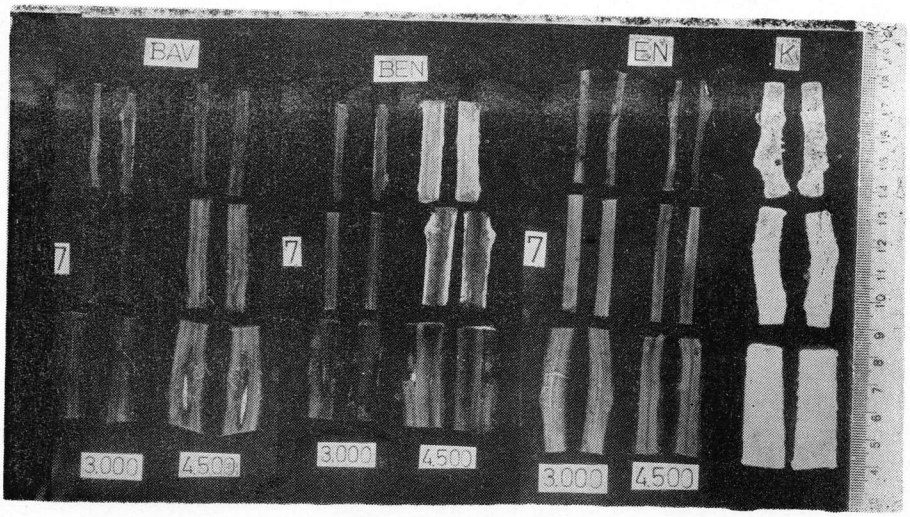

Fig. 3. **E. Parasitica** inoculated wooden samples which were treated seven weeks before with 3000 and 4500 ppm Bavistin (BAV), Benlate (BEN) and Enovit Super (EN). (The rows of the samples are indicating each heigh. K: Control)

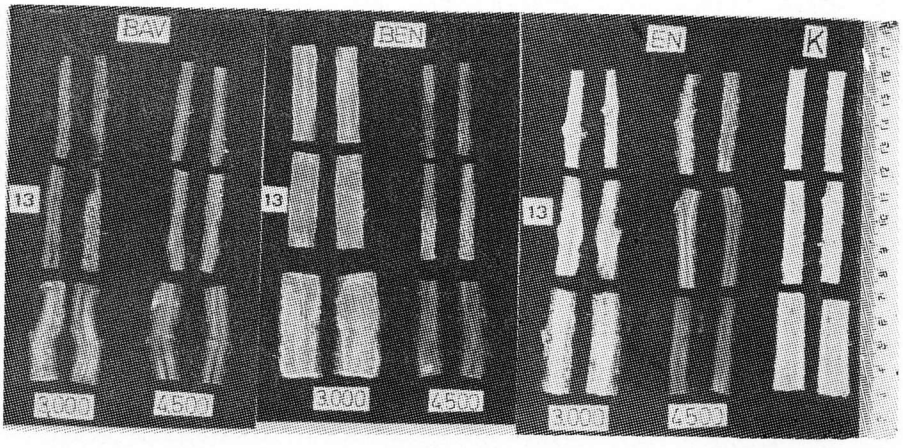

Fig. 4. **E. parasitica** inoculated wooden samples which were treated thirteen weeks before with 3000 and 4500 ppm Bavistin (BAV), Benlate (BEN) and Enovit Super (EN). (The rows of the samples are indicating each he'ght. K: Control)

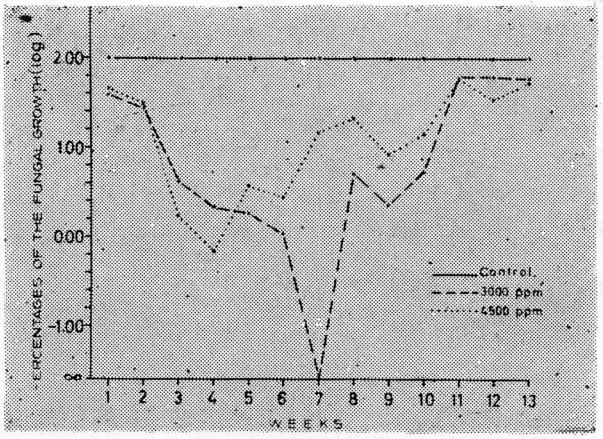

Fig. 5. During the 13 weeks period effectiveness of Benlate at 3000 and 4500 ppm concentrations against the pathogen as compared with the control in the form of logarithmic curve.

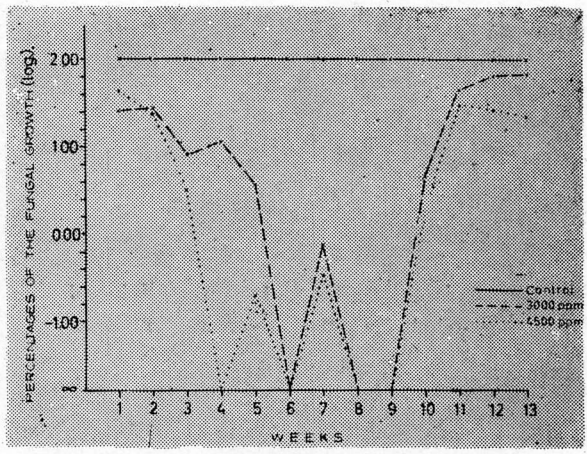

Fig. 6. During the 13 weeks period effectiveness of Bavistin at 3000 and 4500 ppm concentrations against the pathogen as compared with the control in the form of logarithmic curve.

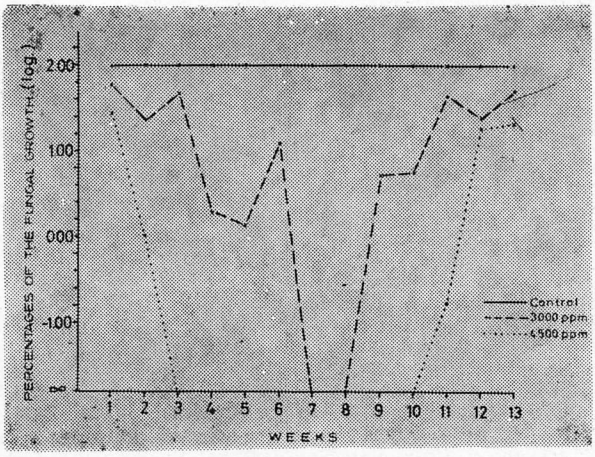

Fig. 7. During the 13 weeks period effectiveness of Enovit Super at 3000 and 4500 ppm concentrations against the pathogen as compared with the control in the form of logarithmic curve.

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Mosaic Virus of Opium Poppy in Turkey

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ABSTRACT

The leaf symptoms, seen on opium popy (**Papaver somniferum** L.) were chlorotic areas and distortion. Plants were generally stunted and produced little seed. The virus was transmitted mechanically from poppy to tomato and tobacco. Back inoculations from these hosts to poppy resulted in a pronounced mosaic symptoms and stunting.

INTRODUCTION

Opium popy has grown in restricted areas under the supervision of the Government and it is an important industrial crop in the region. During the growing season yellowing of plants and poor seed production was observed in vicinity of Uşak province. In order to find out the cause of the disease a series of experiments were carried out under the laboratory conditions and the results has revealed that a virus was responsible.

MATERIALS AND METHODS

The materials used in this work were naturally infected opium poppy plants as inoculum sources and tomato (**Lycopersicon esculentum**), tobacco (**Nicotiana tabacum**, Samsun), **Chenopodium quinoa** and **C. amaranticolor** as indicator plants. Inoculations were carried out according to

the "sap-inoculation" method using 0,1 M phosphate buffer pH 7.0 and "Celite" was added to the inoculum, as an abrasive, before inoculations of test plants. Back inoculations were made according to same method from tobacco to opium poppy.

RESULTS and DISCUSSIONS

The mechanical inoculations on **C.quinoa** and **C.amaranticolor** resulted in local lesions on the inoculated leaves only. The local lesions on these hosts were chlorotic at first and becoming necrotic spots with a light green halo later, systemic spread does not occurred. Infections on tomato plants first gave very diffuse chlorotic mottle on the inoculated leaves, then necrotic patches appeared and whole inoculated leaves withered. Systemic infections of tomato plants showed severe mosaic symptoms and turning out and down ward of the leaf margins of the newly growing leaves. Infections of **N. tabacum** plants gave very conspicious light and dark green mosaic pattern on actively growing young leaves only.

Back inoculations from this host to opium popy revealed in mosaic symptoms. These symptoms on poppy were very similar of those described by Klinskowski (1958). Therefore the disease called as "opium poppy mosaic" for the time being and more detailed works are under study towards the identification of virus more accurately.

This is the first record of "mosaic virus" on opium poppy for Turkey.

There are very few reports on mosaic virus occurring on opium poppy. Kovachevski (1966) has been reported that the virus isolated from naturally infected poppies was "Bean yellow mosaic virus" in Bulgaria and Hovarth ve Besada (1975) isolated a virus from naturally infected opium poppies and was identified the virus as the ordinary strain of "turnip mosaic virus" in Hungary.

The economical importance of the opium poppy in the region urges the more detailed works and the disease should be studied further on the basis of other aspects.

ÖZET

TÜRKİYE'DE HAŞHAŞTA MOZAYİK VİRUSU

Uşak haşhaş (**Papaver somniferum** L.) ekim alanlarından alınan hastalıklı bitki örneklerinden bir virus izole edilmiştir. Bu çalışma Türkiye'de haşhaşlardan virus izolasyonuna ait ek kayıttır.

Hasta haşhaş bitkilerinden öz suyu inokulâsyon metoduna göre test bitkilerine yapılan inokulasyonlar (**Chenopodium quinoa** ve **C. amaranticolor** üzerinde sadece lokal lezyonlar, domateste önce lokal daha sonra sistemik ve tütün üzerinde de yalnız sistemik infeksiyon belirtileri ile sonuçlanmıştır.

Tütün bitkisinden haşhaş üzerine yapılan geri inokulasyonlar sonucu yapraklardan moyazik lekesi ve bitkilerde bodurlaşma görülmüştür. Haşhaşlarda gözlenen bu belirtiler Klinkowski (1968) tarafından kaydedilen "haşhaş mozayik virusu" belirtileri ile uygunluk gösterdiğinden bu çalışmalarda da izole edilen virus şimdilik kaydıyla "mozayik virusu" olarak isimlendirilmiştir.

Bulgaristan'da Kovachevski (1966) tarafından yapılan bir çalışma sonucu, doğal olarak infekteli haşhaşlardan izole edilen virusun "fasulya sarı mozayık virusu" olduğu saptanmıştır. Hovarth ve Besada (1975) doğal olarak infekteli haşhaşlardan izole ettiği virusun "Şalgam mozayık virusu" olduğunu kaydetmektedir.

Haşhaşın gerek yurt ekonomisinde, gerekse bölgedeki önemine dayanarak izole edilen virusun kesin tanısı için gerekli çalışmalara devam edilmektedir.

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Behaviour of the Chlamydospores of Fusarium solani var. coeruleum (Sacc.) Booth in Soil

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ABSTRACT

The behaviour of chlamydospores of **Fusarium solani** var. **coeruleum** (Sacc.) Booth was examined in soil alone and in soil near potato roots. Some chlamydospores germinated in soil alone but progressively more germinated near potato roots. A similar stimulation of chlamydospore germination was observed near the roots of the non-hosts, barley and broad bean but measurements of the fungal population using a medium selective for **F. solani** var. **coeruleum** showed that while the numbers of propagules rose initially they soon declined to levels similar to that of soil alone. In contrast, the population of the fungus continued to increase in soil near young potato roots, stolons and new tubers. Generally, the populations were greatest near the developing tubers and they were higher near stolons than near roots. There appeared to be a levelling off of the number of propagules near roots as these matured. This could be related to the decrease of exudates from these structures. Even after mixing, the potato soil contained significantly more propagules of the fungus than the control soil, after harvest.

The results suggest that exudates from the underground parts of the potato specifically favour the growth of **F. solani** var. **coeruleum** and that this accounts for the relatively high levels of the fungus on tubers noted by other workers.

INTRODUCTION

Dry rot of potatoes is caused principally by the fungus, **Fusarium solani** var. **coeruleum** (Sacc.) Booth, and is essentially a disease of stored tubers. Much is now known about this disease the ways in which the fungus enters the tubers, the conditions which affect this process and the methods of control.

Experiments indicate that the fungus is often in soil on the potato tubers at lifting and it is generally believed that it is present as resting spores or chlamydospores. Very little is known, however, about the behaviour of this fungus in soil particularly during the growth of the potato or indeed, during the growth of other crops.

Work with other forms of **F. solani** suggests that host roots and those of non-hosts might well have substantial effects on the fungal population. The aim of this work was to examine this possibility in relation to the dry-rot pathogen, **F.solani** var. **coeruleum.**

MATERIALS AND METHODS

Preparation of chlamydospore suspension - For production of chlamydospore suspension, soil extract was prepared as described by Alexander et al. (1966). The conidial suspension was adjusted to 5 x 10^6 spores/ml and added to sterile soil extract at the rate of 1 ml suspension/10 ml extract in 250 ml flasks which were incubated at 23 C for 20 days. The resulting cultures were homogenized for 5 min. in micro attachment of a Sorwall omnimixer to produce a suspension of single chlamydospores.

Preparation of dried films of chlamydospores on slides. - Dried films of chlamydospores were prepared on chemically-clean slides, as follows; Chlamydospores produced in soil extract were washed by centri fuging in sterile distilled water and the concentration was adjusted to 2 x 10^5 spores/ml. A drop (0.02 ml) of this suspension was placed on each slide using an "Agla" micrometer syringe and this was evenly spread with a sterile loop over a 10 cm^2 area at the bottom of each slide. In all three hundred such slides were pre-

pared and each was marked with an adhesive label on the side on which the chlamydospore film had dried. The viability of chlamydospores just after drying the film on the slides was 45.7 % and 48.3 % in 0.5 % glucose and Papavizas agar respectively after 24h incubation at 20 C.

Preparation of infested soil.- The soil used in these experiments was a sandy soil obtained from agricultural land in Silwoodpark, Ascot, on which potatoes had been grown previously. The physical structure of the soil was as shown below :

Organic matter	10.2 %
Sand	93.1 %
Silt	2.2 %
Clay	4.7 %

The mineral content, per 100 g soil, was found by Sayles (1973) to be Fe^{+++} 910 mg, Mg^{++} 45.9 mg, Ca^{++} 65 mg, Na^{+} 21.5 mg K^{+} 81.5 mg, PO_4^{---} 16 mg. The soil was left to dry for one and a half months with several mixings. Then it was passed through a mesh of 0.5 x 0.5 cm pores and just before potting it was sieved with a No. 6 mesh (2800 μ).

About 288 kg. of this soil was treated with 34.61 of chlamydospore suspension in order to obtain an infested soil with about 12 % moisture content containing 3.6 x 10^4 spores/g. fresh soil. A hand-held "ASL Spraymist" sprayer was used for infestation the soil and the infested soil was then thoroughly mixed and passed three times through a riddle of 0.5 x 0.5 cm mesh. Five 1 kg. samples were taken at random from the pile, these were bulked, mixed thoroughly and divided into five sub-samples. Three 100 g portions were taken from one of the sub-samples. One was used to determine the moisture content and the other two to estimate the population of **F.solani** var. **coeruleum** Portions of the other samples were used to determine the soil pH. It was found to be 5.06 ∓ 0.03 and the moisture holding capacity to be 36.6 %.

Plant material. - Seed tubers **(Solanum tuberosum)** of the variety "Magestic" were used. They were thoroughly washed, surface -sterilized by immersing them in 10 % chloros (1 % available chlorine) for one hour, then rinsed in distilled water. They were kept in the dark until small sprouts developed. Undressed barley seed **(Hordeum sativum)** of the variety "Midas" and broad bean seed **(Vicia faba** var. **major)** of the variety "Meteor" were used as non-hosts. Germination capability of the seeds were assessed on wet blotting papers at 25°C before sowing and found to be 89 % and 88 % respectively.

A. Effect of potato roots on germination of the chlamydospores of F. saloni **var.** coeruleum .- Sieved and air-dried field soil was moistened by spraying with distilled water to a moisture content (c . 12 %), suitable for plant growth. Thirty 28 cm diameter plastic pots were filled with this soil and they were divided into two groups. By drawing lots, one group of pots was sown with potatoes and the other used as control. Ten microscope slides on which chlamydospore films had dried, were buried to a depth of 7 cm in a circle of 5 cm radius around the tuber in each pot. Slides with chlamydospores were placed in the other fifteen pots containing soil only (control) in a similar way. The pots were stood in trays of water and kept out of doors.

Microscope slides with dried films were examined at 3, and then at weekly intervals until day 35 after burying them in the soil. On each occasion two pots were removed from each treatment. Germination of chlamydospores was examined by staining five slides from one pot in 0.1 % acid-fuchsin in lactic acid. Germinated and non-germinated chlamydospores were counted under the low power of the microscope (X 150). About fifty spores were counted per slide.

B. Estimation of the population of F. solani **var.** coeruleum **near host and non-host plants** .- Thirty-two 28 cm diameter plastic pots were filled with chlamydospore-infested soil, 9 kg in each. They were divided into four groups of eight pots. One sprouted potato tuber was planted to depth of 5 cm in each of the pots of one group. In the second group, thirty-nine barley seeds were sown, 4 cm apart from each other and 1 cm deep, in each pot. In a third group, seven unsoaked broad bean seeds were sown, 10 cm apart and 2 cm deep in each. The remaining eight pots with infested soil were left as controls. The pots were then placed in trays of water in an unheated glasshouse in the completely random manner.

Immediately after infesting soil, the population of the fungus in soil was estimated. Then three week intervals, one pot was sampled from each treatment. Portions of soil near roots and stolons were separated with a small spatula and collected. The soil which was in immediate contact with young tubers was collected both by scraping the tubers and the cavities when the tubers were removed. If the seeds and old tubers decayed and completely rotted no sample was taken.

Each soil sample taken was mixed throughly, then 20 g portions were

taken from each sample for soil dilution plates and other sub - samples (100 g) were taken to determine soil moisture content. As soon as the soil samples were taken, dilution series were prepared and plated out. After several tests it was established that the best dilution for these pot soils was 10^{-3} and so this was then used throughout the experiment. The med ium chosen was a selective one developed by Boyd to detect the propagules of this fungus in soil. This contained 20 g sucrose, 2 g KNO_3, 70 ppm dodine-acetate, 20 g agar, 1 g KH_2PO_4, 0.5 g $MgSO_4$. 7 H_2O, 1 g PCNB 75 % W.P., 300 ppm streptomycin (added on cooling) per litre. About 18 ml of medium was used per petri-dish, plates were then kept to harden in a cool, dry, dark place for 12-13 days before use.

In preparing the soil plates, a 1 ml portion of a freshly agitated 10^{-3} dilution was pipetted onto the surface of the agar and was then spread out with a flame-sterilized loop. Fifteen such plates were prepared for each example. Inoculated plates were kept at 20°C for 18 days in an incubator containing a plastic box with water to create a high humidity so that the cultures would not dry out. The deep colour which distinguishes **F.solani** var. **coeruleum** from other fusaria started to appear after 14 days and colonies were counted after 18 days incubation. The numbers obtained were converted to numbers of propagules per gram oven-dry soil. The figures obtained in each time interval statistically analysed.

A similar experiment was set up in the field, Silwoodpark, to assess the population of the fungus under natural conditions in a soil with a much lower inoculum. The trial was sited in an area in which potatoes had been grown in the previous season, and was a completely randomized design with four treatments and eight sampling times. The treatments provided, soils near potato, barley and broad bean roots and soil in an unplanted (control) plot. All necessary cultural practices were taken during growth. Throughout the experiment soils near host and non-host plants were sampled at 3 week intervals from sowing (30 April) until potato harvest (4 September) in the pre-determined order. The dilutions of 10^{-2} were used. The methods were otherwise similar to those described for the pot experiment.

C. Microscopic examination of chlamydospores on roots and in soil adjacent to roots .- Six weeks after sowing seeds in the pots (5 June), plant roots and soil adjacent to host and non-host plant roots were examined microscopically.

1. Examining of chlamydospores in soil adjacent to plant roots .- For direct assay of germinated and non-germinated chlamydospores near potato, barley and broad bean, the roots were removed from soil with care and the adhering soil was washed with a wash-bottle on to hardened Boyd agar. The plate was swirled a few seconds until the suspension was spread uniformly and then left to allow the soil particules and fungal propagules to settle. The excess water was removed with a blotting paper and the plate was left in order to allow the suspension to be absorbed by the hardened agar. Then three drops of lacto-fuchsin were added on the agar surface and two No. 1 coverglasses (22 x 40 mm) were placed on them. The coverglasses were pressed gently onto the agar surface with a pencil eraser. Then they were examined immediately under the microscope (Papavizas, 1967). Three soil samples adjacent to roots were examined in this way for each treatment.

2. Examining of chlamydospores on roots.- The roots of potato, barley and broad bean were removed from the pot soil with care and the adhering soil particles were washed out. Then the wet roots were dried between sheets of sterile blotting paper for a few seconds. Small pieces of epidermis from roots were peeled off with the aid of a sterile scalpel and they were placed onto a slide. They were stained with lacto-fuchsin (0.1 % acid-fuchsin in lactic asid) to aid counting and examined under microscope. As much tissue as possiple was examined for germinated and non-germinated chlamydospores. Three preparations of roots were examined for each plant.

RESULTS

A. Effect of potato roots on germination of chlamydospores .- One week after burying the slides in soil, most of the potato root tips reached the slides and soon after, a mass of root tips came in contact with the chlamydospores.

The germination of chlamydospores in soil and near potato roots are summarized in Table 1.

Table 1. Germination of chlamydospores of **F. solani** var **coeruleum** in soil and near potato roots

			Mean % germination+ after		
Site	3	7	14	21	28 days
Soil only	26.6	26.3	31.6	30.8	38.4
Near Potato roots	35.0	39.3	53.2	55.1	60.3
Probability (P) that means differ ++	n.s.	n.s.	0.05	0.05	0.01

+ based on five samples from each site

++ based on t - tests using angular transforms of the data

Some chlamydospores (c . 27 %) were found to have germinated after 3 days in soil but not many more spores appeared to germinate after that because the percentage germination in soil after 28 days was still only c . 38 % . In contrast, the numbers of germinated chlamydospores near potato roots increased from 35 % to 60 % during the same period and were significantly more than in soil alone on days 14, 21 and 28. This suggested that the potato roots induced chlamydospores to germinate.

Just before day 35, there were heavy rains and the soil in the remaining pots were covered with 4 - 5 cm of water. When slides were examined on day 35, it was found that many germ - tubes and spores had lysed. Germination in the control soil apparently dropped from 38.4 % to 26.1 % and in soil near potato roots from 60.3 % to 33.4 %.

B. **Estimation of the population of F. solani var. coeruleum near host and non-host plants .-**

1. Glasshouse experiment

a) **During growth of the plants.-** The results of microscopic examination of chlamydospores in soil near

roots and on the roots themselves are given in following: the mean percentages of germinated chlamydospores in the soil adjacent to roots of broad bean, barley and potato were 15.6; 19.5 and 36.1 respectively. These values of mean percentages of germinated chlamydospores were 8.1; 5.3 and 24.9 on the roots of the same plants. These indicated no significant differences in the germination of the chlamydospores in the different situations. The soil isolations using Boyd's agar, however, revealed marked changes in the population of **F.solani** var.**coeruleum.** The numbers of propagules in the control pots and in soil near potato, barley and broad bean plants, from sowing (25 April) until potato harvest (18 September) are summarized in Figure 1. and appendix table 1.

Immediately after infesting the soil the number of propagules was **c.** 14,500 per gram dry soil. In all treatments, the numbers of propagules declined rapidly the first 6 weeks, possibly because some chlamydospores germinated and the germ-tubes were then lysed. The numbers of propagules in the control and also near the roots of the non-hosts, barley and broad bean continued to decline until the twelfth week. Then they remained steady at a low level of about 500 - 1000 propagules per gram dry soil. Old tubers and broad bean seeds also did not appear to affect the population of the fungus at all throughout the experiment. In contrast, from the ninth week onwards the population of the fungus in soil near potato roots, stolons and young tubers increased consistently. At week 9 there were significantly more propagules near stolons and young tubers than near potato roots, near those of the non-hosts or in the control. By week 12 the numbers of propagules near potato roots, stolons and developing tubers were all substantially greater than in the control or near roots of non-hosts. In this and later samplings there were also marked differences in the numbers of propagules associated with the underground parts of the potato. There were always most propagules near the newly-formed tubers. There were somewhat fewer near stolons and least near the roots.

After week 18 the numbers of propagules near these potato structures declined slightly. This was associated with the death of the shoots.

During isolations, it was noted that in soils with barley, broad bean and potato and also (to some extend) in the control soil, the number of other **Fusarium** species isolated increased towards the end of the experiment, whereas at the beginning most colonies isolated were **F.solani** var. **coeruleum.** Possibly drying the

soil before setting-up the experiment favoured the fungus (**F.solani** var. **coeruleum**) but later more competitive fusaria, like **F.solani, F.roseum, F.oxysporum** increased in soil.

b) **After harvest** (potatoes only).- A further count of the propagules in both potato soils and in control soils was made after harvest (18 September). The results are given in Table 4. Even after mixing, the "potato soil" contained significantly more propagules of the fungus than the control soil both on 18 September and on 9 October (as determined by t-tests). However, for each soil there was no significant difference in the numbers detected on these dates (Table 3). That is, population of the fungus did not change significantly in either soil within 3 weeks of harvest.

Table 2. Numbers of propagules per gram soil of **Fusarium solani** var. **coeruleum** after harvest

Week 21 (18 Sep)		Week 24 (3 Oct)	
Control soil	Potato soil	Control soil	Potato soil
1319.2	3412.4	1399.7	2760.8
t - value 3.60**		t - value 2.69*	

Significance at $P<0.05$ and 0.01 denoted by * and ** respectively

Table 3. Difference between numbers of propagules in soils after harvest in relation to time

Time interval	In "potato soil"	In control soil
21 Week	3412.4	1319.0
24 Week	2760.8	1399.7
t - value	0.4 N.S.	0.15 N.S.

+ Means based on 15 samples for each soil

2. **Field Experiment**

Potatoes produced stolons by the sixth week (12 june) and by the ninth week they had produced young tubers. The plants rather stunted because of the dry periods throughout the season but they produced a good yield. Broad beans started to produce pods on 10 July and completely dried on 28 August. The barley reached maturity by August by which time the broad beans had started to yellow. By the beginning of September the plants were all dried. The soil pH was found to be 5.5 (∓ 0.06) and the moisture holding capacity 31.92.

The numbers of propagules isolated from the various plots throughout the growing season are given in Figure 2. and appendix table 2. Before sowing, the mean numbers were 139.6 and 128 per gram dry soil in two samples with 20 replicates each. The soil moisture content was 14.5 and 14.3 respectively on that time.

The population of the fungus remained low throughout the experiment in the unplanted (control) plots. In soil near barley and broad bean roots there were slight increases in numbers of propagules 3-6 weeks after planting but then the numbers decreased again and by week 15, were not different to those of the control. In plots with potatoes the number of propagules of the fungus rapidly increased within the first three weeks and stayed stable for another three weeks. These increases were associated with the presence of potato roots. When stolons and young tubers began to form, after week 6, there were marked increases in the numbers of **F.solani** var. **coeruleum** in soil near them and these continued to week 18. During this period the numbers associated with roots declined. The overall result was that there were significant differences between the populations around stolons and new tubers and those around potato roots. Similarly there were significantly more propagules in soil taken around potato roots than in soil around either barley or broad bean roots or in soil from the unplanted control. These results were similar to those found in glass-house experiment.

The old potato (mother) tuber had some effect on the population up to week 6, whereas the broad bean seeds had no effect at all.

DISCUSSION

On slides (with dried films of chlamydospores) placed in soil only, the amount of germination did not increase much over one month. In contrast, germination of chlamydospores on similar slides placed near potato roots continued to rise, a clear indication that the chlamydospores of **F.solani** var. **coeruleum** are stimulated to germinate near potato roots It is particularly interesting that in this experiment, the final waterlogging (on day 35) of the soil resulted in lysis of many germ tubes and spores. Obviously under these excessively wet conditions which probably result in lack of oxygen, the fungus is unable to produce new chlamydospores. This has been noted with other soil fusaria (Newcombe, 1960).

Further evidence for a stimulation of germination by potato roots was obtained from the experiment in which potato, barley and broad beans were grown in pots of soil with a high population of chlamydospores. This experiment indicated, however, that chlamydospores germinated equally well near the roots of the non-host barley and broad bean. In this respect the fungus behaved similarly to **F.solani** f. **phaseoli,** the chlamydospores of which were found by Schroth and Hendrix (1962) to germinate near the roots of sixteen non-susceptible plants. These authors found that later the germ-tubes lysed. It would seem likely that a similar sequence of events occurred with **F. solani** var. **coeruleum** because counts of propagules near roots of barley and broad bean using Boyd's agar showed an initial rise in numbers followed by a decline to levels similar to those in soil alone. In cotrast, the fungal population increased near potato roots, stolons and young tubers (Figures 1 and 2) The extent of these increases varied. The population near young tubers was always higher than that near stolons and this in turn was higher than that near young roots. The reasons for this are not known but possibly the exudates from these plant parts differ (Frenzel, 1960; Rovira, 1965; Schroth and Snyder, 1961). This could be related to differences in the quality and quantity of the amino-acids and sugars exuded (Garrett, 1970). Equally, the greater number of propagules near young tubers could be due to the large surface area which these present to the surrounding soil. This line of research needs further investigation. It is especially interesting because whereas the association of **F.solani f.phaseoli** with

the bean host leads to a pathogenic relationship in the growing plant, with **F.solani** var. **coeruleum** it does not. There thus appears to be a host specific effect distinct from a parasitic or pathogenic relationship. The results suggest that **F.solani** var. **coeruleum** continues to grow near or on the underground parts of the potato following chlamydospore germination and that this growth is particularly favoured by exudates from young tissue. Thus there appeared to be a levelling-off of the number of propagules near roots as these matured. Support for the present result comes from the work of Schippers (1962) and of Boyd (1971). Both investigators found a higher level of **F.solani** var. **coeruleum** in soils from potato riddles than in the field soil in which the potatoes had been grown. This itself suggests that the tubers influence the population of the fungus.

In the pot experiment described, the number of propagules after harvesting the potatoes was still much higher than in the control soil, even after the soil had been mixed. The population changes after harvest in these soils would be worth investigating further, especially in relation to the field situation. Presumably in the field, the population of **F.solani** var. coeruleum will also be high in the areas previously occupied by potatoes. These levels will inevitably be decreased by the mixing of the soil through cultivation, but it is not known how well these levels are then maintained or indeed how they are affected by further crops.

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ÖZET

FUSARIUM SOLANI VAR. COERULEUM (SACC.) BOOTH KLAMİDOSPORLARININ TOPRAKTA DAVRANIŞI

Fusarium solani var. **coeruleum** (Sacc.) Booth klamidosporları patates kökleri yanında ve kontrol toprakta incelendiğinde, kontrolda bazı klamidosporlar çimlenmekle beraber patates kökleri çevresindeki toprakta artan bir şekilde çok daha fazla klamidospor çimlendiği saptandı. Klamidosporların benzer şekilde çimlenmeye teşviki, konukçu olmayan arpa ve bakla kökleri yanında da gözlendi fakat seçici ortam ile yapılan **F.solani** var. **coeruleum** populasyon ölçümleri arpa ve bakla rizosferinde, başlangıçta propagul sayısı artmakla birlikte kısa sürede kontroldaki düzeye düştüğünü gösterdi. Buna karşılık fungusun populasyonu patates kökü, stolon ve yeni teşekkül eden yumru çevresindeki toprakta artmasına devam etti. Genel olarak populasyon, stolon etrafında kök çevresindekinden daha fazla ve yeni teşekkül eden yumru çevresinde ise en yüksekti. Kökler yaşlandığında propagul sayısındaki artış durdu. Bu, kök salgılarının azalması ile ilgili olabilir. Patates ekilen toprağın, hasattan sonra iyice karıştırıldığında bile kontroldan daha fazla propagul içerdiği saptandı.

Sonuçlar, patatesin toprak altı salgılarının **F.solani** var. **coeruleum** gelişimini toprakta parazitik olmayan bir şekilde teşvik ettiğini göstermekte ve yumru üzerinde bulunan yüksek inokulum potansiyelini açıklamaktadır.

APPENDIX

Table 1. Changes in the number of propagules of **F. solani** var. **coeruleum** (per g. dry soil) during the growth of potato, barley and broad bean. (Glasshouse Experiment)+

Soil from pots planted with	0	Weeks after planting 3	6	9	12	15	18	21
No crop (Control)	+	10426	5068	1882	1310	1098	1240	1319
Barley-near roots	+	9138	4179	3043	1464	1146	991	—
Broad bean - near roots		11359	4449	1732	789	296	434	—
Potato -near roots		11793	4102	2838	2996	3352	3163	2375
-near stolons		—	5043	5111	5790	6129	6196	4633
-near new tubers		—	5189	5201	6511	7631	7364	6413
S.E. Diff. ±		835.2	592.3	541.3	453.0	395.7	413.4	429.6

+ Summary of the results for each sampling date

+ Means based on 15 replicates for each sample

Table 2. Changes in the number of propagules of **F. solani** var. **coeruleum** (per g. dry soil) during the growth of potato, barley and broad bean. (Field experiment)+

Soil from pots planted with:	0	Weeks after planting 3	6	9	12	15	18
No crop (Control)+		119	101	111	83	90	84
Barley -near roots		183	216	161	118	99	100
Broad bean - near roots		201	130	70	34	27	28
Potato -near		356	357	282	256	237	224
-near stolons		—	378	382	395	439	447
-near new tubers		—	—	418	486	523	517
S.E. Diff. ±		41.4	34.9	32.5	36.9	32.5	39.6

+ Summary of the results for each sampling date

+ Means based on 15 replicates for each sample

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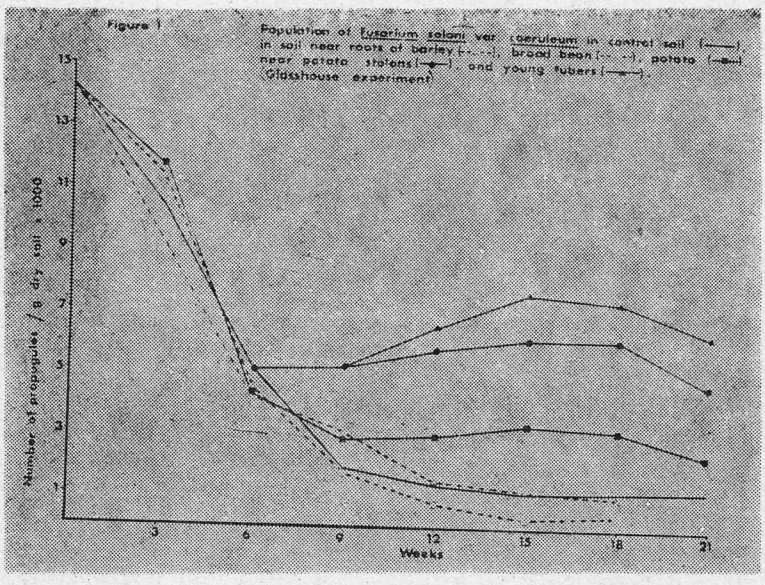
Figure 1
Population of fusarium solani var. coeruleum in control soil (——), in soil near roots of barley (- - -), broad bean (— - —), potato (—•—) near potato stolons (—•—) and young tubers (——).
(Glasshouse experiment)
Number of propagules / g dry soil x 1000
Weeks
3
6
9
12
15
18
21

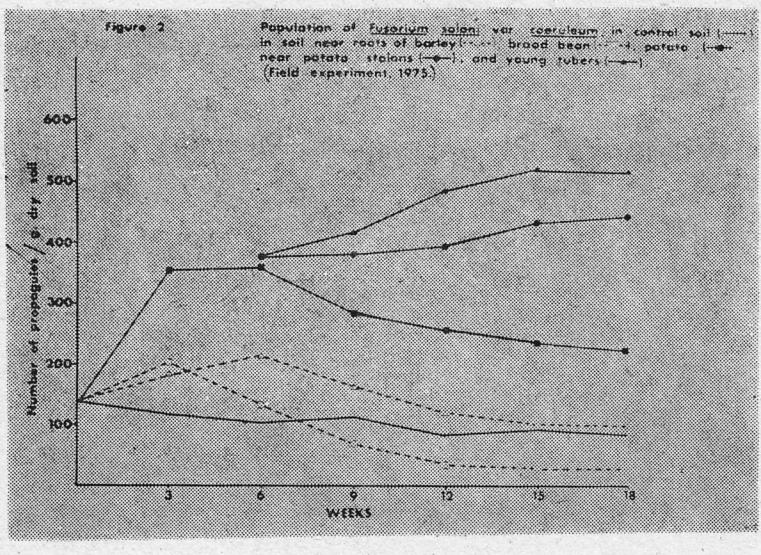
Figure 2
Population of fusarium solani var. coeruleum in control soil (——), in soil near roots of barley (- - -), broad bean (— - —), potato (—•—) near potato stolons (—•—), and young tubers (——).
(Field experiment, 1975.)
Number of propagules / g dry soil
600
500
400
300
200
100
WEEKS
3
6
9
12
15
18

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Determination of Moleculer Weight of Proteins for Bean Yellow Mosaic Virus by Polyacrylamide Gel Electrophoresis

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ABSTRACT

The Moleculer weight of Bean Yellow Mosaic virus from broadbean plants in Çukurova Region was determined by using polyacrylamide gel electrophoresis. The moleculer weight of virus was found as a 33.350-33.820 daltons.

INTRODUCTION

The Moleculer weight of protein subunits of the viruses belonging to potyvirus groups is about 33.000. Perhaps this is one of the important characters in distinguising this grup from others and is very helpful in identification of Potyvirus (Moghal and Franchi, 1976). Bean Mosaic is an important virus diseases in Turkey. The earliest studies with regard to morphology, host range, serology, sedimentation rate etc. revealed that it is a common Yellow Bean Mosaic Virus on broadbean (Yılmaz Unpublished). On the present investigation the Moleculer weight of protein subunits of three isolates of this virus was studied by electrophoresis.

MATERIALS AND METHODS

Polypeptide analysis was conducted by using 50 ml 8 % polyacrylamide SDS Gel. The gel contains:

a. 10 ml of 39.6 % acrylamide, 0.4% bisacrylamide
b. 25 ml 0.2 m phosphate buffer, 0.02 m EDTA pH 7.2
c. 5 ml 1 % SDS
d. 5 ml % TEMED
e. 5 ml 1.5 ammonium persulphate

All solutions were mixed together except for the ammonium persulphate, and the mixture was degassed (Gas is evacuated from solution). And then ammonium persulphate was added to the solution.

The gel was poured immediately into the slab gel apparatus and gel was allowed to set overnight. 10 μg samples of virus preparations, isolat 1, isolat 2 and isolat 3, were taken in each case.

The protein markers used were Bovine Serum Albumine (mol. wt. 68.000), Carbonic anhydrase (mol wt. 29.500). Hemoglobin (mol. wt. 64.500) and Ovalbumine (mol. wt. 43.000).

Solutions of 1 mg/ml were made and 7 μl of each of the solutions was added to each virus sample. Samples of virus, virus and markers and markers only were made 1 % with respect to SDS and 2-Mercaptaoethanol, and were boiled for 3 minutes. The samples were cooled by ice for a few seconds. Samples were made 20 % with respect to sucrose and bromophenol blue was added as tracker dye. Samples were loaded on to the gel under running buffer which is 0.1 M phosphate buffer, 0.01 m EDTA and 0.1 % SDS and the gel was run 9 hours at 50 volts 60 mA. The gel was removed from the slab gel appatus and was stained overnight in Commassie Blue, Methanol, Acetic Acid and H_2O (1.98 g; 450 ml; 90 ml and 450 ml) and was destained in the same solvent mixture without Coomassie Blue.

RESULTS and DISCISSION

Mobility and molecular weight of proteins of Bean Yellow Mosaic isolates were found by using standard protein markers in gel electrophoresis (Fig. 1.). It was observed that there is a linear relationship between the log of mol. wt. of protein and mobility from which the mole-

culer weight of Bean Yellow Mosaic Virus protein are determined (Fig. 2). The protein component have molecular weight ranging from 33.350 to 33.820.

Table 1. Molecular weight of BYMV isolates

Isolat Number	Molecular weight
1	33.350 — 3.5 %
2	33.820 — 4 %
3	33.500 — 2.8 %

It seems that there is a close agreement with the reports of Huttinga and Mosch 1974; Huttinga; 1975 and Moghal and Franch 1976 who reported the molecular weight of coat protein of virus group is 33.000 and 34.000 dalton respectively. The second polypeptide was visualised for the isolat 2 but mol. wt. of this polypeptide could not be determined with the markers available. From the mobility the gel. mol. wt. can be approximately determined to be 26.000 daltons by extrapolation from the graph in Fig. 1.

It could be possible that this polypeptide is the result of heavy protein degradation as reported by Huttinga and Mosch (1974).

ÖZET

FASULYE SARI MOZAYIK VİRÜSÜNÜN PROTEİN MOLEKÜLER AĞIRLIĞININ POLYACRYLAMIDE GEL ELEKTROFOREZIZ YÖNTEMİYLE SAPTANMASI

Fasulye Sarı Mozayık Virüsünün Protein Moleküler ağırlığı, Polacrylamide Gel electroforesis yöntemiyle 33.350-33.820. Dalton olarak saptanmıştır.

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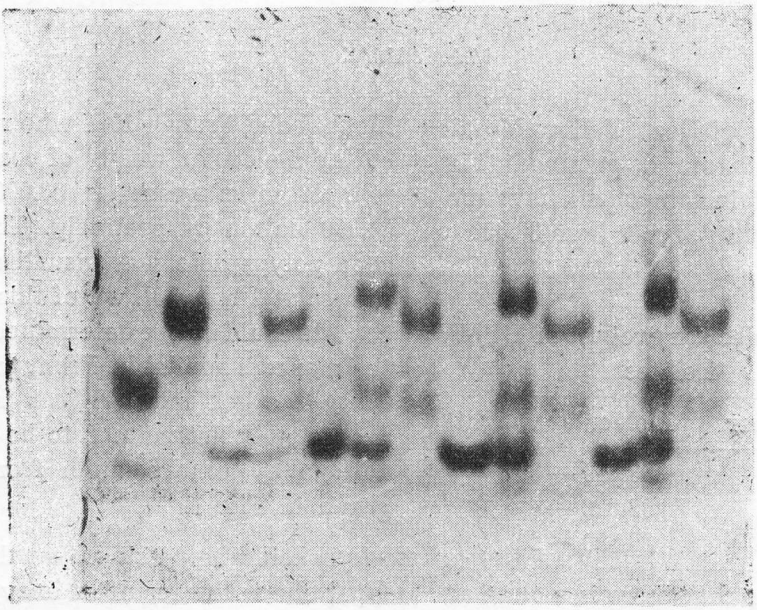

Fig. 1. Electrophoresis of Bean Yellow Mosaic Virus isolates in 8 % Polyacrylamide/SDS gels for polypeptide analysing. Column from left to right: 1: carbonic anhydrase (CA) and ovalbumin (Ov.). 2: Bovin serum albumin (BSA) and haemoglobin (HB). 3. 4: Ca + Ov + BSA. 5: Isolat 1. 6: Isolat 2, CA, Ov + BSA. 7: Ca, Ov, BSA + Hb. 8: Isolat 2. 9: Isolat 2, CA, Ov + BSA. 10: Ca, Ov, BSA + Hb. 11: Isolat 1. 12: Isolat 1, BSA, Ov + Hb. 13: Ca + Ov + BSA.

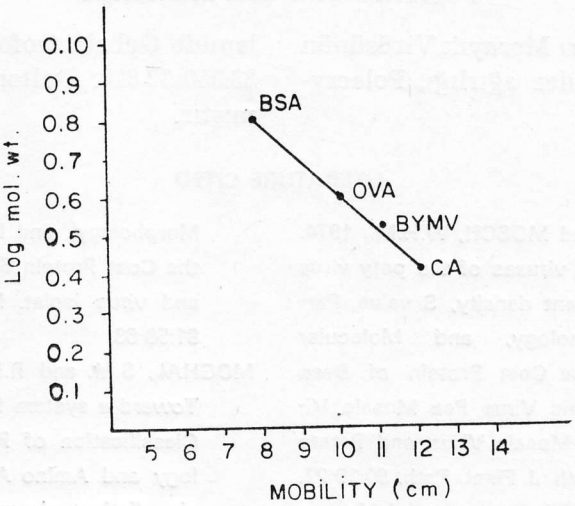

Fig. 2. Relationship between Leg. Mol. wt. and electrophoretic mobility to determine molecular weight of Bean Yellow Mosaic virus

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The Effect of Nutrition and Inoculum Density of Rhizoctonia solani Khun. on Damping-off of Cotton Seedlings

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ABSTRACT

The effects of the different carbon and nitrogen sources on the dry mycelial weight and the virulence of **Rhizoctonia solani** Khun. were tested **in-vitro** and **in-vivo** conditions.

The data presented in this study resulted that dry weights of the fungus grown in different carbon and nitrogen sources correspond directly with the nature of nitrogen sources.

The pathogenicity tests have shown that **R. solani** is highly pathogenic especially as a pre-emergence killer of cotton seedlings. Hundered percent mortality reached at the end of the experiment irrespective of nutrients available for fungus. Situation is interesting enough when amount of inoculum as low as 33 mg and as high as 752 mg are found to be almost equally responsible for 100 % disease incidence.

INTRODUCTION

It is now well known that disease producing capability of various plant pathogens is influenced by the quality and quantity of nutrients available to them. For **Rhizoctonia solani** Kuhn. a causal agent of damping-off in cotton, this phenomenon was demonstrated by Kamal and Weinhold (1967) when they observed that after a short period in soil, the pathogen became less effective in attacking cotton seedlings; thereby suggesting

that the decrease in virulence is mainly due to the loss of nutrients reguired to support pathogenic activity.

Weinhold et al (1969) provided on evidence that 20 g/l glucose as carbon source and 1 g/l asparagine as nitrogen source are optimal for normal growth of the fungus in vitro. They also demonstrated that the disease incidence is minimum when the pathogen was provided with 0-5 g/l asparagine. The subseguent study of the authors showed that an exogenous source of glucose considerably reduces the virulence of **R. solani** on cotton seedlings. As the influence of some other carbon and nitrogen sources on disease development is not known, so this forms one of the objectives of present study.

Rhizoctonia solani is a major cause of seed rot, pre and post emergence damping-off of cotton throughout much of the cotton growing areas of Turkey.

The recent study from Aegean region also revealed the importance of the disease. So the present study is an attempt to evaluate the effect of different carbon and nitrogen sources on pathogen development and subsequently the effect of inoculum density on the severity of the disease under local conditions.

MATERIALS and METHODS

An isolate of **R. solani** used in the study was recently isolated from heavily diseased cotton seedlings.

The nutritional status of the inoculum was varied by growing the fungus on liquid medium containing respective carbon and nitrogen sources. The basic medium was composed of 1.75 g. KH_2PO_4, 0,75 g $MgSO_47H_2O$, 37mg. $CaCl_2.2H_2O$, 0,8mg $CuSO_45H_2O$ 1,0 mg $FeCl_36H_2O$, 0,9 mg $ZnSO_47H_2O$ and 0,3 mg $MnSO_4H_2O$ in one litre of distilled water. Carbon sources name ly glucose, lactose, and starch were used at the rate of 20 g/l and that nitrogen sources were $NaNO_3$, $(NH_4)_2SO_4$ and Asparagine at the rate of 2 g/l.

The fungus grown in still culture in 250 ml erlenmayer flasks containing 50 ml medium. Each flask was inoculated by disc of mycelium (2 mm in diameter) growing on PDA. The cultures were incubated at 27 C° for 10 days. A completely randomized block desingn with 5 replications for each treatment was used.

Mycelial dry weights were obtained by filtering the cultures on Whatmann filter paper NO. 40 and

after drying the mycelial mass in an incubator, the average weights, were calculated for each treatment. The date was statistically analyzed.

In order to observe the pathogenic potential of the fungus feeded with different carbon and nitrogen sources an experiment was set up in which the amount of inoculum used was the same obtained from respective treatments. For this purpose all the dried mycelial mass in each was thoroughly homogenized with 207 ml sterilized distilled water and 50 ml of this suspension was poured to each 5 inch diameter plastic pots containing steam sterilized soil. Inoculum was mixed in the soils and pots left over in the green house. After one week, each pot was seeded with surface sterilized seeds of **Gossypium hirsutum** var. Coker 100/Az.

Seedlings survival was counted 14 and 21 days after planting. Germination of the seeds in the control series was 90 %, so the percent emergence in the inoculated series was calculated accordingly.

RESULTS and DISCUSSION

a) Effect of nutrients on fungal growth:

Addition of 20 g/l glucose as carbon source and 1 g/l asparagine as nitrogen source are considered to be essential for maximum growth of the fungus; therefore this treatment served as control in our case. The amounts of other two sources each of carbon and nitrogen were equivalent to the amounts of glucose and asparagine. Table 1 shows the dry mycelial weights obtained by the fungus in each medium provided with different carbon and nitrogen sources. As it is evident the highest three figures of dry mycelial weights are obtained when the fungus was grown on asparagine irrespective of carbon sources added. The fungal growth was at its peak when grown in lactose with asparagine (152 mgdmw) and was at its lowest ebb when cultured on lactose with ammonium sulphate (33 mg dmw.) Amongst the nitrogen sources stimulating the growth of the fungus, sodium nitrate emerged as second to asparagine. The dry weights of the mycelium were 617,557 and 401 mg when nitrate form of nitrogen was used with glucose, lactose and starch respectively. Ammonium sulphate was found out to be least effective in stimulating the growth of the fungus. This source of nitrogen when used with lactose gave the minimum figure (33 mg) and when used with

Table 1: The effect of different carbon and nitrogen sources on dry mycelial weight and on disease incidence

C/N Sources	Dry mycelial weights in mg	Mean of the group	No. of seedlings Pre-emergence damping-off	Affected by Post-emergence damping-off
Glucose + $NaNO_3$	617		40	0
Glucose + $(NH_4)_2SO_4$	160	502.7	23	17
Glucose + Asparagine	731		38	2
Laktose + $NaNO_3$	557		39	1
Laktose + $(NH_4)_2SO_4$	33.0	447.3	23	17
Laktose + Asparagine	752		35	5
Starch + $NaNO_3$	401		14	26
Starch + $(NH_4)_2SO_4$	162	428.7	30	10
Starch + Asparagine	723		39	1

glucose and starch, the dry weights of the mycelia were 160 and 162 mg respectively.

As it is clear from the table, the carbon sources used did not have effect of their own on fungal growth. Mean dry weights of the fungus containing glucose, lactose and starch along with respective nitrogen source are calculated. Although difference amongst them is insignificant, but glucose is comparatively more effective than lactose-which in turn gave more mycelial growth and starch.

b) **Disease Incidence**:

The fungus grown on all there nine media containing different carbon and nitrogen sources served as inoculum the disease inducing capability of these inocula was determined the date shown in the table indicates, the number of seeds unemerged (due to pre emergence damping - off) and number of emerged seedlings died due to post emergence damping-off, twenty one days after planting the seeds.

It was found out that isolate of

R. solani under study is highly pathogenic. Where the amount of inoculum is high, the seed rot or preemergence damping-off is also high. At the time of final score is 21 day after sowing the seed, 100 % mortality is found to occur.

All the 40 plants tested died due to pre emergence damping-off when the amount of inoculum was 617 mg in contrast to only 23 plants died when the amount of inoculum was 33 mg and 160 mg. The only exception to this is in case of inoculum grown on starch + sodium nitrate, having dry mycelial weight of 401 mg but the incidence of pre emergence damping off was minimum amongst all the treatments.

The date presented in the study gives us on evidence that dry weights of the fungus grown in different carbon and nitrogen sources correspond directly with the nature of nitrogen source. As in the present study and from the previous investigations of Weinhold et al (1969, 1974), it is found out that an asparagine an organic form of nitrogen is the best fungal growth promator, However, the present study also revealed that sodium nitrate as a nitrogen source is almost equally important. It is also being found that ammonium form of nitrogen rather retards the fungal growth.

The pathogenicity tests have shown that **R. solani** is highly pathogenic especially as a preemergence killer of cotton seedlings. Hundered percent mortality reached at the end of the experiment irrespective of nutrients available to fungus. Situation is interesting enough when amount of inoculum as low as 33 mg and as high as. 752 mg are found to be almost equally responsible for 100 % disease incidence. It is suggested that nutrients available to the pathogen to play a role in pathogenesis of **R. solani** Thirty milligrams mycelia used to inoculate forty plants is considerably low to cause heavy damage to the host. It is presumed that inspite of very low amount of inoculum, ammonium form of nitrogen enhanced the disease incidence. It is also being known that only 10-20 ppm of nitrogen preferably the ammonium form, it available to **Fusarium oxysporum** f. **phaseoli,** the pathogen can cause severe damage in pea seedlings (Toussoun, 1970). This also gives support to our view that pathogenicity of **R. solani** was influenced by ammonium form of nitrogen.

The amounts of glucose and asparagine used in our experiment, seemed to have no effect on disease development, inpite of the fact that lower amounts of these substances have been reported to suppress the disease it is thought that the viru-

lence of the pathogen may further be decreased by using lower amounts of glucose with ammonium form of nitrogen. To sum up it can be safely said that to prevent or rather to minimize the disease damage cotton, excessive nitrogenous fertilizers should be avaided.

ÖZET

FARKLI KARBON VE AZOT KAYNAKLARININ PAMUK ÇÖKERTEN HASTALIĞI ETMENİ **Rhizoctonia solani** Khun.'ün İNOKULUM YOĞUNLUĞU ÜZERİNE ETKİSİ

Değişik karbon ve azot kaynaklarının **Rhizoctonia solani** Khun.'ün kuru kitle ağırlığı ile virülensine etkileri in-vitro ve in-vivo koşullarda incelenmiştir.

Glikoz, Laktoz, Nişasta gibi karbon ve sodyum nitrat, amonyum fosfat, asparagin gibi azot kaynaklarının kullanıldığı çalışmada temel ortam olarak litrede 1,75 g, KH_2PO_4; 0,75 g $MgSO_4$-$7H_2O$; 37 mg $CaCl_2.2H_2O$; 0,8 mg $CuSO_4.5H_2O$; 1,0 mg $FeCl_3.6H_2O$; 0,9 mg $ZnSO_4.7H_2O$; 0,3 mg $MnSO_4.H_2O$; 20 g Glukoz ve 2 g asparaginin yer aldığı bir yapay besi ortamı kullanılmıştır. Karşılaştırmaya alınan ve yukarıda isimleri verilen karbon ve azot kaynakları bu ortamdaki glikoz ve asparaginin karşıtı ve eşit miktarlarında glikoz ve asparaginin yerini almıştır.

Fungusun saptanan kuru kitle ağırlıkları ile ilgili bulgular, gelişme üzerine değişik azot kaynaklarının doğrudan etkili olduğunu göstermiş ve azot kaynaklarına bağlı olarak etmenin kuru kitle ağırlığı 50 cc lik ortamdaki kültürlerinde 33-752 mg arasında değişmiştir. Buna karşın bu yoğunluktaki inokulumların patojenin virulensi üzerinde önemli bir farklılık göstermediği, diğer bir deyişle fungusun düşük inokulum yoğunluklarında da çıkış öncesi ve çıkış sonrası çökerten yönünden % 100 e ulaşan zararlara yol açtığı saptanmıştır.

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Utilization of Carbon, Nitrogen and Vitamins by Colletotrichum graminicolum Isolates

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ABSTRACT

The paper deals with the utilization of carbon, nitrogen and vitamins by three isolates (I_1, I_2 and I_3) of **Colletotrichum graminicolum** (Ces.) Wils., the incitant of anthracnose of sorghum. The studies were carried out in Richard's medium adjusted to pH 6.0 and incubated at 30+ 1C. Fructose was best utilized by I_1 and I_2 while I_3 utilized more of maltose. Among nitrogen sources peptone supported maximum growth of isolate I_2 and I_3. Isolate I_1 grew best on DL-Threonine. Ammonium nitrate and potasium nitrate favoured abundant sporulation of isolate I_1. Thiamine increased the dry weight of the three isolates and riboflavine supported abundant sporulation of isolate I_1 only. These results are an evidence that variability exist in nutritional requirement of **C. graminicolum** isolates.

INTRODUCTION

Knowledge of nutritioanl requirement of the pathogen helps in better understanding of host parasite relationship and variability in the isolates. **Colletotrichum graminicolum** (Ces.) Wils. attacks many graminaceous plants besides sorghum (**Sorghum bicolor** (L.) Moench). Pathogenic and cultural variability among the isolates of this pathogen from different hosts has been reported (Chowdhury 1936 and Chohan 1967). Therefore, three isolates from sorghum were taken to study their nutritional requirement.

MATERIALS and METHODS

Isolates I_1, I_2 and I_3 from sorghum were taken from different localities of Rajasthan (India). Cultures were maintained on potato dextrose agar. Richard's medium was used as basal, medium in this study. Carbon and nitrogen compounds were incorporated separately at the same carbon/ nitrogen level of the basal medium. The amount of vitamins added has been indicated in Table 3. Twenty ml of the medium was poured in 100 ml Erlenmeyer flasks. The medium was buffered to pH 6.0 before autoclaving at 15 psi for 15 minutes. In case 4 vitamins steam sterilization was done for 30 minutes for three consecutive days. The flasks were inoculated with 2 mm mycelial disc from one week old culture. The contents of flasks were filtered through previously dried and weighed Whatman (42) filter paper, after 10 days of inoculation. The filter papers with mycelial mat were dried in an electric oven at 60C for 24 hr and then cooled in a desiccator and weighed. Average of 24 replications was worked out and spores were counted in drops from a flask under microscope (10 x) and graded as follows: 0 = no spores; poor = 1-7 spores; moderate = 8 - 15 spores; good = 16 - 23 spores; abundant = above 24 spore.

RESULTS

Effect of different carbon sources on growth and sporulation:

The isolates did not grow in the absence of carbon source. Growth of the three isolates differed significantly from each other except the growth of isolate I_1 which was not significantly different on xylose, galactose and mannose. Maximum growth of isolate I_1 was on maltose, cellobiose and fructose and minimum on sorbose. Fructose was the best source for isolate I_2 and I_3 whereas sorbose for I_2 and mannitol for I_3 were the poorest source of carbon. Galactose, fructose, maltose and sucrose were excellent for sporulation of I_1 and lactose for I_2. Maltose and fructose supported good sporulation of I_3. Carbon sources for good sporulation of isolate I_1 were xylose, raffinose and mannitol; for isolate I_2, mannose, raffinose and sorbose and for isolate I_3, sorbose, cellobiose, raffinose, xylose and glucose (Table 1).

Effect of nitrogen sources on growth and sporulation:

Results show that there were significant difference among the isolates in the utilization of nitrogen sources. Peptone was best of nitrogen followed by threonine and aspartic acid for isolates I_2 and I_3. For isolate I_1 best sources of nitrogen were threonine, methionine and peptone with no significant difference in these sources. Urea for isolate I_3, ammonium nitrate for isolates I_1 and I_2, and sodium nitrate for isolate I_1 were poor sources of nitrogen. In general sporulation was good on potassium nitrate followed by ammonium nitrate and asparagine. Isolate I_1 on sodium nitrate and I_3 on glycine and phenylalanine did not sporulate (Table 2).

Effect of vitamins on growth and sporulation: Thiamine and inositol were significant for supporting growth over control. For all the isolates thiamine was good followed by inositol, pyridoxine and nicotinic acid. Mean dry mycelial weight of three isolates were non-significant. Ascorbic acid was inhibitory to growth.

Thiamine and riboflavine increased the sporulation of isolate I_1 but not of I_2 and I_3. Nicotinic acid and ascorbic acid completely checked the sporulation of isolate I_3 (Table 3).

DISCUSSION

Among the various carbon sources tested fructose was best for the isolates in general for growth and sporulation. However, isolates differed in their utilization of different sources of carbon. Maltose was reported to be most nutritious for **Colletotrichum lini** (Tochinai, 1926). Difference in the carbon utilization by different fungi have been reported by the various workers (Durairaj, 1956; Mathur **et al.**, 1950; Sahni **et al.**, 1975).

There were differences in the utilization of nitrogen sources by different isolates of **C. graminicolum.** Peptone, a complex mixture of peptides and amino acids is reported to be a good source of nitrogen for **Colletotrichum** sp by Ramakrishnan (1946), Mathur **et al.** (1950), and Sahni **et al.** (1975) is further corroborated by our results. Isolates of **C. graminicolum** utilized some of the vitamins supplied in the medium. Thiamine was utilized more by all the

isolates followed by inositol. Tandon (1961) noted that thiamine supported good growth of many fungi. Mathur **et al.** (1949) observed that different strains of **C. lindemuthianum** exhibited partial deficiencies for vitamins. Our results are in conformity with those of Misra and Mahmood (1961) and Ghouse and Khan (1963).

The results reveal that variability in the nutritional requirements exist among the isolates of **C. graminicolum** and perhaps may be an explaination for variation in virulence of the isolates.

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ÖZET

Colletotrichum graminicolum İZOLATLARININ AZOT, KARBON ve VİTAMİN BESLENMESİ

Sorgumda Antraknoz hastalığı etmeni olan fungusun üç izolatı Richard ortamında ve 30∓1C° ve 6 Plt derecesinde incelenmiştir. I_1 ve I_2 izolatları için en iyi karbon kaynağı Fruktoz, I_3 izolatı için ise Maltoz olmuştur. Azot kaynaklarından Pepton I_2 ve I_3 izolatlarında maksimum büyümeye neden olmuştur. I_1 izolatı ise en iyi DL-Threonine'de gelişmiştir. Amonyum nitrat ve Potasyum nitrat I_3 izolatında bol spor oluşturmuştur. Thiamin üç izolatda da kuru ağırlığı arttırırken, riboflavin sadece I_1 izolatında bol sporulasyon ortaya koymuştur. Bu sonuçlar **C. graminicolum** izolatlarının besin isteklerindeki değişkenliğin bir kanıtıdır.

Table 1. Effect of various carbon sources on growth and sporulation of **Colletotrichum graminicolum** isolates

Carbon sources	Dry mycelial weight mg			Mean for carbon source	Sporulation		
	I_1	I_2	I_3		I_1	I_2	I_3
D-Glucose	325	320	280	308.3	+++	++	+
D-Xylose	235	196	262	231.0	++	++	+
Mannose	246	253	230	248.0	+++	+	++
D-Galactose	239	180	270	229.7	++++	++	++
D-Fructose	345	373	391	369.7	++++	+++	+++
Lactose	243	203	289	245.0	+++	++++	++
Maltose	353	351	304	336.0	++++	+++	+++
Sucrose	297	313	346	318.7	++++	++	++
Raffinose	285	268	190	247.7	++	+	+
Mannitol	204	215	178	199.0	++	++	++
Sorbose	192	166	208	188.7	++	+	+
Cellobiose	351	365	330	348.7	+++	++	+
Control	60	41	33	44.7	+	+	+

	Isolate	Carbon	Interaction
SEm	1.582	3.294	5.705
C.D. at 5%	4.45	9.27	16.06

+ = poor
++ = moderate
+++ = good
++++ = abundant

Table 2. Effect of various nitrogen sources on growth and sporulation of **Colletotrichum graminicolum** isolates

Nitrogen sources	Dry mycelial weight mg			Mean for nitrogen source	Sporulation		
	I_1	I_2	I_3		I_1	I_2	I_3
Amonium nitrate	222	171	240	211.0	++++	++	+++
Amonium sulphate	254	207	231	230.7	+	+	+
Sodium nitrate	222	206	248	225.3	++	+	++
Sodium nitrite	253	213	192	219.8	—	+	+
Potasium nitrate	274	291	308	291.0	++++	+++	+++
Urea	259	209	115	194.3	+	+	+
Glycine	271	286	190	249.0	+	+	—
Peptone	333	370	388	363.7	+	++	+
L-Asparagine	312	327	285	308.0	+++	++	++
Aspartic acid	323	345	319	329.0	++	++	++
L-Histidine	317	323	298	311.6	++	++	+
DL-Tryptophan	232	234	251	239.0	+	++	+
DL-Threonine	339	351	337	342.3	++	+	+
L-Cysteine	291	312	305	302.7	++	++	+
Phenylalanine	318	331	301	316.7	+++	++	—
DL-Methionine	336	298	313	315.7	++	+++	++
Control	223	201	190	204.7	+	+	—

+ = poor
++ = moderate
+++ = good
++++ = abundant
— = no sporulation

	Isolate	Nitrogen	Interaction
SEm	1.032	2.530	4.381
C.D. at 5 %	2.89	7.08	12.27

Table 3. Effect of vitamins on growth and sporulation of **C. graminicolum** isolates

Vitamin sources	Concentration per litre in/μg	Dry mycelial wt. (mg.) of isolates			Mean for vitamins	Sporulation		
		I_1	I_2	I_3		I_1	I_2	I_3
Thiamine	100	392	370	402	388.0	+++	+++	++
Riboflavine	100	275	270	290	278.3	++++	++	+
Folic acid	100	261	250	242	251.0	+	+	—
Nicotinic acid	100	291	280	248	273.0	+	+	—
Pyridoxine	50	293	260	253	268.7	++	+	+
Ascorbic acid	50	255	230	224	236.3	+	+	—
Biotin	5	258	252	240	250.0	+	+	+
Inositol	5 mg	300	305	325	310.0	++	++	+
Control (No vitamin)			290	302	292.0	++	+++	++

		Isolate	Vitamin	Interaction
— = No sporulation	S.Em.	3.288	5.696	9.866
+ = poor	C.D. at 5 %	9.33	16.16	27.99
++ = moderate				
+++ = good				
++++ = abundant				

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