EFFECT OF VIRIDIN ON THE GROWTH AND INFECTIVITY OF SCLEROTIUM CEPIVORUM BERK †

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Sclerotium cepivorum causes serious losses in England by producing white rot disease on onion (*Allium cepa*), garlic (*A. sativum*), shallot (*A. ascalonicum*) and leek (*A. porrum*). Studies made by Ghaffar[1] indicated that *S. cepivorum* was strongly antagonised in plate culture by a number of soil micro-organisms. Of these *Trichoderma viride* (in the wide sense of Bisby[2]) a common soil inhabitant, inhibited the growth of the fungus and produced a characteristic coiling around of the hyphae of *S. cepivorum*. *T. viride* is known to produce an antibiotic, viridin, which has great fungistatic properties (Brian et al.[3]). Experiments were therefore made to study this effect of viridin on the growth and infectivity of *S. cepivorum*.

Methods for the assay of antibiotics are well summarised by Florey et al.[4] Weindling[5] used a visual killing effect of *Trichoderma* filtrates on the hyphae of *Rhizoctonia solani* as his criterion for antibiotic activity. In the present investigation, a 'disk immersion technique' was developed which also shows loss of infectivity by *S. cepivorum* after treatments with the antibiotic.

A sample of viridin, in pure form, supplied by Dr. Brian was used in this test. Followng the technique of Brian et al.[3], 2 mg. of viridin was dissolved in 4 ml. ethanol and made upto 200 ml. in McIlvaine's buffer at pH 3.4 at which the antibiotic substance is stable (Dr. Brian's personal communication). This was further diluted in buffer solution to give a series of concentrations of 1-100 μg. of viridin per ml. Controls were kept in which no antibiotic was used.

The isolate of *S. cepivorum* used was No. 388 in the culture collection of the Botany Department, University of Birmingham. Several groups of 5 mm. diam. disks from an actively growing edge of *S. cepivorum* colony on Czapek-Dox yeast agar*

Fig. 1.—Onion root tip, infected with *S. cepivorum* from disks treated with non-toxic solutions. (Photomicrograph × 100).

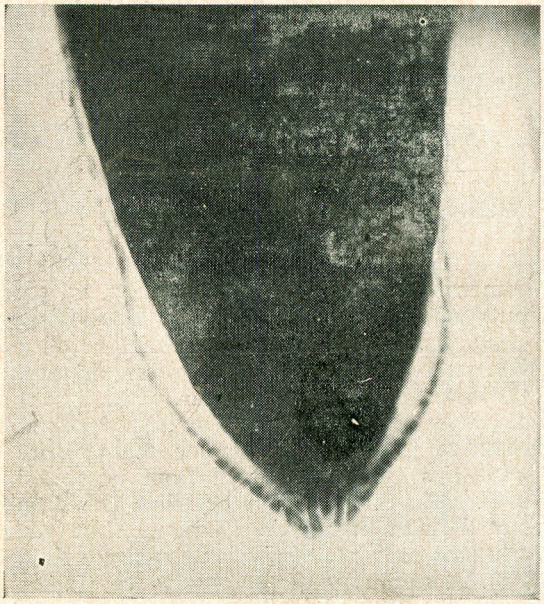

Fig. 2.—Onion root tip, healthy/uninfected from disks treated with toxic solutions. (Photomicograph × 100).

†This work was carried out at the Department of Botany, The University, Birmingham, England.

*$NaNO_3$, 2.0 g.; KH_2PO_4, 1.0 g.; KCl, 0.5 g.; $MgSO_4.7H_2O$, 0.5 g.; $FeSO_4$, 0.01 g.; sucrose, 30.0 g.; yeast extract, 2.0 g.; agar, 20.0 g.; distilled water, 1000.0 ml.

TABLE 1.—ANTIBIOTIC ACTIVITY OF AN AQUEOUS SOLUTION OF VIRIDIN TOWARDS *S. cepivorum* AS ASSAYED BY 'DISK IMMERSION TECHNIQUE.'

Treatment	Concentration of viridin (µg./ml.) 0	1	5	10	25	50	75	100
	No. of disks giving *S. cepivorum* infection*							
1. 5 mm. diam. disks of *S. cepivorum* inoculum immersed in test solutions for (minutes)								
0	10	.	.	.	.	.	.	.
5	10	10	10	4	4	0	0	0
15	10	10	10	0	0	0	0	0
30	10	10	10	0	0	0	0	0
60	10	10	10	0	0	0	0	0
Overnight, 10 hours	10	10	10	0	0	0	0	0
2. As in 1, but washed in distilled water after immersion of the disks.								
0	10	.	.	.	.	.	.	.
5	10	10	10	10	10	10	8	8
15	10	10	10	10	10	2	2	2
30	10	10	10	10	4	0	0	0
60	10	10	10	0	0	0	0	0
Overnight, 10 hours	10	10	10	0	0	0	0	0

*Observations based on 10 disks per treatment.

were immersed in the test solutions for 0, 5, 15, 30, 60 minutes and overnight (10 hours). These disks were then transferred to a moist chamber of a Petri dish and on them were placed pieces, 6-8 mm. long, of roots of onion seedlings (var. white lisbon) germinated on filter paper. When left overnight for about 10 hours at laboratory temperature these root tips become infected from untreated disks of *S. cepivorum* inoculum or when the disks are treated with non-toxic solutions, but the toxic solutions prevented infection (compare Figs. 1 and 2). A summary of the results from these treatments is presented in Table 1. A period of immersion of the disks from 30-60 minutes enables one to determine the antibiotic effects. Washing of the disks does not remove the effects especially after treatments in higher concentrations of viridin.

TABLE 2.—STABILITY IN ANTIBIOTIC EFFECTS OF VIRIDIN AGAINST *S. cepivorum*.

Concentration of viridin µg./ml.	No. of disks giving *S. cepivorum* infection* 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Storage in days														
0-5	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
10	0	0	0	0	0	0	0	0	0	0	10	10	10	10	10
25	0	0	0	0	0	0	0	0	0	0	6	10	10	10	10
50-100	No infection														

*Observations based on 10 disks per treatment.

An experiment was then conducted to study the stability of aqueous solutions of viridin during storage. Different concentrations of viridin prepared above were kept at laboratory temperature in flasks plugged with cotton wool and similarly assayed over a period of 14 days. The time for immersion of the disks was one hour. The results are presented in Table 2. Fresh aqueous solutions of viridin were active from a concentration of 10 µg. upto 100 µg. /ml. in preventing infection of onion root tips by *S. cepivorum*. However, storage beyond 10 days resulted in a gradual loss of antibiotic activity in the weaker solutions of viridin, while at concentrations of 50 to 100 µg. of viridin per ml. the solutions remained toxic after 14 days of storage at laboratory temperature.

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