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ANTIBIOTIC AND MYCOPARASITIC EFFECTS OF SEVERAL FUNGI AGAINST SEED- AND SOIL-BORNE PATHOGENS ASSOCIATED WITH WHEAT AND OATS[1]

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Abstract

Aspergillus clavatus Desm. produced toxic substances which reduced the growth of *Drechslera sorokiniana* (Sacc.) Subrain & Jain, *Fusarium culmorum* (W.G. Smith) Sacc. and *Rhizoctonia solani* Kühn in vitro. Hyphae of *Trichoderma aureoviride* Rifai and *T. harzianum* Rifai were found to penetrate those of *F. culmorum*. Hyphae of *Epicoccum nigrum* Link were observed to coil around and penetrate the hyphae of *F. culmorum*. Similarly, hyphae of both *Trichoderma* species were observed entering those of *R. solani*.

Introduction

Most research on biological methods of seed treatment has involved the introduction of antagonists on seeds. For instance, soaking seeds of oats and wheat in a suspension of *Bacillus subtilis* or *S. griseus* resulted in increased grain yield, dry matter and the number of tillers (Merriman *et al.* 1974). Similarly, Wu (1976) indicated that pelleted wheat or oat seed with either *Epicoccum nigrum*, *Trichoderma aureoviride* or *T. harzianum* had higher emergence and better growth vigor when these seeds were sown in the soil infested with *Drechslera sorokiniana*, *Fusarium culmorum* and *Rhizoctonia solani*. Antagonism is one mechanism applied in biolobical control. Antagonism consists of three types of activity, i.e. antibiosis, competition and parasitism (Baker & Cook, 1974). *T. viride*, actively parasitic on *R. solani*, also secretes gliotoxin which is highly toxic to *R. solani* and other organisms (Wood & Tveit, 1955). Similarly, toxic substances produced by *Cephalosporium roseum* have been

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claimed to suppress pathogenicity of *Helminthosporium* spp. and have been found to invade mycelium of *H. sativum* (Kenneth & Isaac, 1964) while hyphae of *Trichoderma* sp. and *Penicillium vermiculatum* have been shown to parasitize those of *R. solani* (Boosalis, 1956). Conversely, *R. solani* can be a parasite of *Pythium debaryanum* and *Rhizopus nigricans* by penetrating their aerial hyphae and establishing an internal parasitic mycelium, by coiling around their aerial hyphae, or by a combination of these mechanism (Butler, 1957).

This study tries to understand whether these interactions among the pelleted beneficial fungi and soil-borne pathogens are similar to any type of the mechanism of antagonism.

Materials and Methods

Antibiosis

Aspergillus clavatus, *E. nigrum*, *T. aureoviride*, and *T. harzianum* were cultured on potato dextrose broth for 2 weeks at 24°C. Culture filtrates were sterilized by passage through a millipore filter (0.02 μ). The pathogens *D. sorokiniana*, *F. culmorum*, and *R. solani* were cultured separately in 50 ml of liquid medium (4 flasks/fungus) consisting of 10 ml of each fungal filtrate and 40 ml potato dextrose broth. After incubation at 24°C for 7 days, mycelium of each pathogen was collected on filter paper with a Büchner funnel, dried (60°C) and weighed. Pathogens cultured in 50 ml of potato dextrose broth served as controls.

Mycoparasitism

Simultaneous dual culturing was used to determine parasitism. Mycelial plugs of three cereal pathogens, *D. sorokiniana*, *F. culmorum* and *R. solani* were separately transferred to one side of a PDA plate, and the other side was inoculated with mycelial plugs of the suspected mycoparasited *A. clavatus*, *E. nigrum*, *T. aureoviride* or *T. harzianum*. In addition, sterilized glass slides were dipped into sterilized melted PDA (50°C) and removed. After solidification, the layer of agar on one side of the slide was removed. Mycelial fragments of a pair of fungi, consisting of a pathogen and a potential parasite, were transferred separately to the ends of the agar-coated glass slides, and then placed in a petri dish with moistened filter paper for 5 days at 24°C. After the two fungi had grown together, transfers were made from the area of contacting hyphae to a clean slide. Cotton blue in lactophenol was added to facilitate microscopic observation.

Results

Antibiosis

The dry weights of the three pathogens cultured in different fungal filtrate-supplemented broths are indicated in Table 1. Filtrate from *A. clavatus* cultures significantly inhibited the growth of *D. sorokiniana* and *R. solani*, and retarded the growth of *F. culmorum* slightly (Fig. 1-A). The filtrates of the other fungi had no influence on the growth of the three tested pathogens.

In addition to showing antibiotic activity, culture filtrate of *A. clavatus* reduced the germination and retarded the growth of oats and wheat when added to nutrient broth contained in plastic pouches (Fig. 1 B-C).

Table 1. *Influence of fungal culture filtrates on growth of three cereal pathogens*(1)

Source of filtrate	Dry Weight of Fungus(2)		
	Drechslera sorokiniana (grams)	*Fusarium culmorum* (grams)	*Rhizoctonia solani* (grams)
Aspergillus clavatus	0.0871 b	0.3802	0.1105 b
Epicoccum nigrum	0.3079 a	0.4946	0.3225 a
Trichoderma aureoviride	0.2798 a	0.4668	0.3171 a
T. harzianum	0.3405 a	0.4897	0.2551 a
Control	0.2727 a	0.4975	0.3723 a

(1) Cereal pathogens were grown in 40 ml potato dextrose broth to which 10 ml fungal culture filtrate was added.

(2) Values, which are averages of 4 replicates, followed by common letter in the same column, do not differ significantly at the 5% level by the hsd test.

Mycoparasitism

By means of simultaneous dual culturing on slides and PDA plates, the phenomenon of hyperparasitism was observed under the microscope. Hyphae of *E. nigrum* were observed twisted around the hyphae of *F. culmorum* and penetration of *F. culmorum* hyphae by *E. nigrum* hyphae also occurred (Fig. 2 A-B). Hyphae of *T. aureoviride* were able to penetrate the hyphae of *F. culmorum* (Fig. 2C) and *R. solani* (Fig. 2D). Hyphae of *T. harzianum* also penetrated *F. culmorum* (Fig. 2E) and *R. solani* (Fig. 2 F-G). Hyphae of *T. harzianum* were sometimes branched within the hypae of *R. solani* (Fig. 2H).

Hyphae of the hyperparasite in the fungal host were constricted and finer than those outside the host. There were apparently no specific infection sites. The hyphae of infected fungi were always either devoid of, or deficient in cytoplasm, but the hyperparasite contained very dense cytoplasm in its

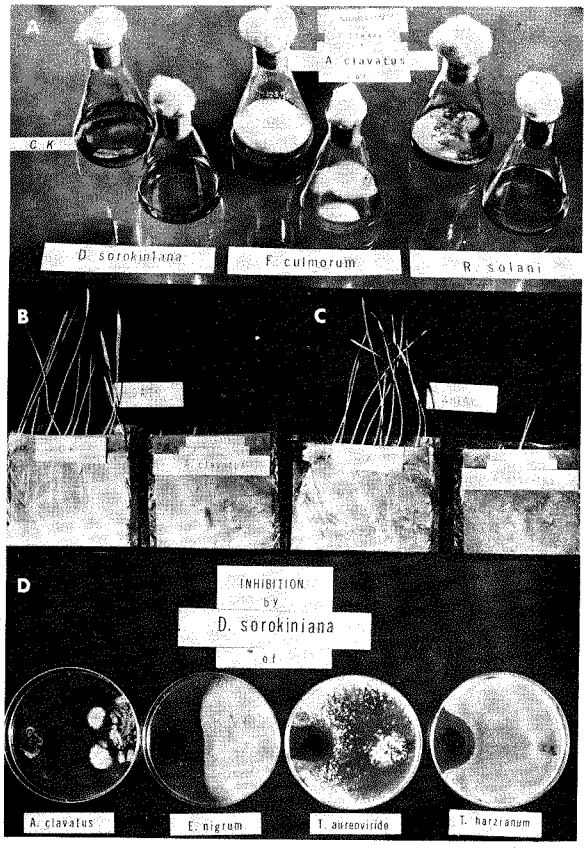


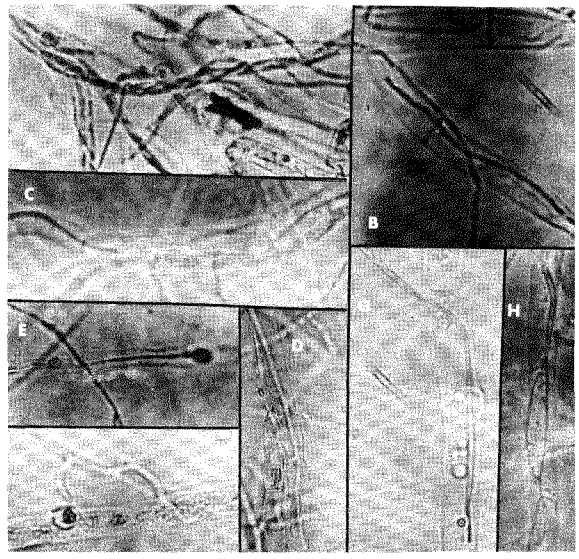
Fig. 1. Effect of *A. clavatus* culture filtrate on the growth of oat and wheat pathogens and on oat and wheat seedling emergence, and zone of inhibition between *D. sorokiniana* and selected seed- or soil-borne fungi.

A) *D. sorokiniana, F. culmorum* and *R. solani* inhibited by the filtrate of *A. clavatus* cultured in potato dextrose broth 14 days at 24°C.

B-C) Inhibition of germination and seedling development of wheat in the pouch test. Twenty ml of filtrate and 10 seeds were placed in each pouch.

D) Inhibition zone caused by *D. sorokiniana* on agar plate.

Fig. 2. Parasitism of *F. culmorum* and *R. solani* by *E. nigrum*, *T. aureoviride* and *T. harzianum*.



A) Hyphae of *E. nigrum* twisting around the hypha of *F. culmorum*.

B) Hypha of *E. nigrum* penetrating the hypha of *F. culmorum*.

C) Hypha of *T. aureoviride* penetrating the hypha of *F. culmorum*.

D) Hypha of *E. nigrum* penetrating the hypha of *R. solani*.

E) Hypha of *T. harzianum* penetrating the hypha of *F. culmorum*.

F) Hypha of *T. harzianum* beginning to penetrate the hypha of *R. solani*.

G) Hypha of *T. harzianum* within the hypha of *R. solani*.

H) Branching of *T. harzianum* hypha within the hypha of *R. solani*.

hyphae.

D. sorokiniana produced antibiotics that inhibited *A. clavatus*, *E. nigrum*, *T. aureoviride* and *T. harzianum* (Fig. 1D).

Discussion

The results of this study suggest that *Aspergillus clavatus* produced some fungistatic substance(s) in potato dextrose broth. This substance(s) reduced the growth of *Drechslera sorokiniana* and *Rhizoctonia solani* considerably, and *Fusarium culmorum* slightly. *A. clavatus* was found by Waksman *et al.* (1942) and Wiesner (1942) to produce clavacin which has both bacteriostatic and bactericidal properties. No attempt has been made to determine whether or not the fungistatic substance found in this study is related to clavacin. In addition to being fungistatic, the crude culture filtrate of *A. clavatus* also produced a phytotoxic effect in that it reduced the rate of growth of wheat and oats in the pouch test. Metabolites of other fungi tested such as *Epicoccum nigrum* and *Trichoderma* spp. had no adverse effects on growth of the three abovementioned pathogens although *T. aureoviride* and *T. harzianum* have been shown by other workers to produce ethanol-extractable antibiotics (peptide antibiotics) (Dennis and Webster, 1971). This suggests that the three pathogens are able to tolerate these antibiotics, or else the quantities present in the filtrates used in this study were insufficient to cause any inhibition.

In the present investigation, mycelium of *E. nigrum* was found to entwine with the hyphae of *F. culmorum* when the two fungi were in contact. Also, *E. nigrum* "attacked" *F. culmorum* by penetrating aerial hyphae and establishing itself as an internal parasitic mycelium. Hyphae of *Pythium debaryanum* and *Rhizopus nigricans* have been shown to be penetrated by coiling hyphae of *Rhizoctonia solani* (Butler, 1957). The results of this investigation indicate that *T. aureoviride* and *T. harzianum* can penetrate the hyphae of *F. culmorum* and *R. solani* by establishing an internal penetrating mycelium. According to Wells *et al.* (1972), *T. harzianum* has been applied successfully to tomato transplants in natural field conditions to reduce damage from *Sclerotium rolfsii*. In this instance, *T. harzianum* may have a pathogenic effect on *S. rolfsii*. Some isolates of *T. harzianum* and *T. aureoviride* have also been shown to inhibit *Fomes annosus* growth (Dennis & Webster, 1971).

Helminthosporium sativum (=*Cochliobolus sativus*) is regarded as a synonym of *H. sorokinianum* (Luttrell, 1955). Many species of *Helminthosporium* have been transferred to the genus *Drechslera* (Shoemaker, 1959; Subramanian and Jain, 1966). *H. sorokinianum* has been determined as being synonymous with *D. sorokiniana*. *Drechslera sorkiniana* has been found capable of forming potent phytotoxins and fungitoxins in culture media (Tyner, 1966). This may have

been the reason that no fungi attacked *D. sorokiniana* on agar in this test. However, Fokkema (1973) found that there are some dominant fungi in the phyllosphere with inhibitory activity against *D. sorokiniana*.

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真菌對與小麥及燕麥種子及相關土壤病原菌之抗生及寄生作用

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Aspergillus clavatus Desm 在培養基中所產生的一些毒素可阻止 *Drechslera sorokiniana* (Sacc.) Subrain & Jain, *Fusarium culmorum* (W.G. Smith) Sacc. 及 *Rhizoctonia solani* Kühn 的生長。*Epicoccum nigrum* Link, *Trichoderma aureoviride* 及 *Trichoderma harzianum* Rifai 的菌絲可以穿進 *F. culmorum* 的菌絲，同時 *E. nigrum* 的菌絲會纏繞 *F. culmorum* 的菌絲。同樣地，*T. aureoviride* 及 *T. harzianum* 的菌絲可以穿進 *R. solani* 的菌絲。