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BIOLOGICAL CONTROL OF SEED- AND SOIL-BORNE FUNGI ASSOCIATED WITH WHEAT AND OATS[1]

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Abstract

Introduction of ***Epicoccum nigrum*** Link and ***Trichoderma harzianum*** Rifai into soil infested with ***Drechslera sorokiniana*** (Sacc.) Subrain & Jain, ***Fusarium culmorum*** (W. G. Smith) Sacc. and ***Rhizoctonia solani*** Kühn improved emergence and vigor of wheat and oat seedlings. Pelleting seed with a mixture of either ***Aspergillus clavatus*** Desm., ***Trichoderma aureoviride*** Rifai, or ***T. harzianum*** conidia and binder likewise improved seedling emergence and vigor in soil infested with ***D. sorokiniana, F. culmorum*** and ***R. solani.***

Introduction

Although the application of chemicals has obvious benefits in pest control, it possesses an important limitation that appears to be basic to all environmental pollution problems. Often, extensive disruption of normal seed and soil microflora causes an ecological "vacuum" around the spermosphere, the microhabitat around the seed in soil. In this part of the soil a few surviving plant pathogens can build up rapidly in the absence of normal competitors. Once a soil-or seed-borne pathogen has been introduced into a cropping ecosystem, it must be considered at least a potential threat in all future uses of that ecosystem. However, a biological control does not upset the biological balance because it is gentler and uses more subtle methods to achieve its goal. Furthermore, effective biological control usually is more enduring. Therefore, a method of biological control for controlling disease without the disruption of the environment is the reason for this investigation.

Materials and Methods

Foundation seed of Orbit oats and Arrow wheat, used in this study, was obtained from the New York Seed Improvement Cooperative, Inc., Ithaca, New

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York.

A. *Addition of common seed- and soil-borne fungi to soil*

Fungi were cultured for one month at 24°C in 125 ml flasks containing 40 g of sterile wheat kernels and 35 ml distilled water. For evaluation of biological control, the fungi for infesting soil were *Drechslera sorokiniana*, *Fusarium culmorum*, and *Rhizoctonia solani*. Soil contained in each 5 inch diameter clay pot was infested with 27±2 g of fungal culture (8.5–9.5 g of fungal culture of each of the abovementioned pathogens). Thirty grams of these potentially benefical fungi, i. e. *Aspergillus clavatus*, *A. niger*, *Epicoccum nigrum*, *Penicillium spinulosum*, *Trichoderma aureoviride*, *T. harzianum*, or *Zygorrhynchous heterogamus*, were then mixed in the top 1 inch of soil which layer had been pre--viously infested with the pathogens *D. sorokiniana*, *F. culmorum* and *R. solani*. The seeds were then sown in this top 1 inch layer and covered with another layer of field soil.

Evaluations of this method were conducted under both soil tank and greenhouse conditions. Soil tank temperatures were 8°C for oats and 15°C for wheat, whereas the temperature in the greenhouse was 22°C during the day, and 15°C during the night. Twent-five seeds were planted in each pot. Four replicates were used to study the effectiveness of each fungus tested under both greenhouse and soil tank conditions. After one month, treatments were evaluated by calculating the percent seed germination and determining seedling dry weight.

B. *Pelleting seeds with beneficial fungi*

A. clavatus, *T. aureoviride* and *T. harzianum* were grown on PDA at 24°C for 7 days. Conidia were suspended in 50 ml and 70 ml of binder (Polyvinyl alcohol) for pelleting 100 g of wheat and oats, respectively. Pelleted seed was sown in infested soil in pots described previously. Pots of both oats and wheat were placed in a greenhouse with day and night temperature of 18°C and 15°C, respectively. A soil tank was used to maintain constant soil temperatures of 8°C for oats and 15°C for wheat. Evaluation of results were the same as abovementioned.

The concentration of conidial inoculum of each fungus per seed, as measured by the dilution plate method, was as follows:

Fungus	Concentration of inoculum (conidia/seed) Osts	Wheat
A. clavatus	1.04×10^3	1.84×10^3
T. aureoviride	1.60×10^3	1.10×103
T. harzianum	6.00×10^2	2.00×10^2

Results

A. *Addition of common seed-and soil-borne fungi to soil*

Addition of common seed-and soil-borne fungi to soil along with inoculum did not affect the emergence of oats significantly under either greenhouse or soil tank conditions (Table 1). *E. nigrum* increased root and total dry weight of oat seedlings which were sown under soil tank conditions. The other fungi had no effect on the root, shoot and total dry weights under either greenhouse or soil tank conditions.

A. clavatus, E. nigrum and *T. harzianum* enhanced both the emergence and dry shoot weight of wheat under greenhouse conditions (Table 2). *A. clavatus* and *E. nigrum* also increased emergence, dry shoot weight and total dry weight under soil tank conditions. *A. niger* and *T. aureoviride* had no effect on emergence of wheat in the greenhouse, but it did increase emergence at a soil temperature of 15°C.

B. *Pelleting seeds with selected soil-borne fungi*

Under both soil tank and greenhouse conditions, oat seeds pelleted with

Table 1. *Effects of addition of specific fungi to soil infested with **D. sorokiniana, F. culmorum** and **R. solani** on emergence and growth of oat seedling*[1]

Fungus	Greenhouse				Soil tank			
	Emergence (%)	Dry root weight (g)	Dry shoot weight (g)	Total dry weight (g)	Emergence (%)	Dry root weight (g)	Dry shoot weight (g)	Total dry weight (g)
Aspergillus clavatus	71	0.22	0.93	1.15	68	0.07ab	0.53ab	0.60ab
A. niger	48	0.15	0.65	0.80	56	0.03b	0.38b	0.41c
Epicoccum nigrum	66	0.16	0.77	0.93	74	0.14a	0.65a	0.79a
Penicillium spinulosum	62	0.19	0.87	1.07	59	0.07ab	0.51ab	0.58ab
Trichoderma aureoviride	73	0.13	0.85	0.98	68	0.06b	0.65a	0.71ab
T. harzianum	72	0.13	0.85	0.98	68	0.09ab	0.69a	0.78ab
Zygorrhynchous heterogamus	68	0.14	0.66	0.80	—	—	—	—
Control	50	0.13	0.63	0.76	62	0.02b	0.51ab	0.53bc

(1) Values, which are averages of 4 replictes, followed by common letters in the same column do not differ significantly at the 5% level by the hsd test. Total number of treated seeds=25/replicate, 4 replicates.

Table 2. *Effects of addition of specific fungi to soil infested with* ***D. sorokiniana, F. culmorum*** *and* ***R. solani*** *on emergence and growth of wheat seedlings*(1)

Fungus	Greenhouse				Soil tank			
	Emergence (%)	Dry root weight (g)	Dry shoot weight (g)	Total dry weight (g)	Emergence (%)	Dry root weight (g)	Dry shoot weight (g)	Total dry weight (g)
Aspergillus clavatus	50ab	0.09	0.46ab	0.55ab	75a	0.06	0.45ab	0.51ab
Aspergillus niger	31c	0.03	0.22c	0.25b	60a	0.07	0.34cd	0.41bc
Epicoccum nigrum	58ab	0.06	0.45ab	0.51ab	70a	0.16	0.55a	0.71a
Penicillium spinulosum	43bc	0.07	0.45ab	0.52ab	32b	0.02	0.17d	0.19d
Trichoderma aureoviride	45bc	0.07	0.58a	0.65a	61a	0.05	0.38bc	0.43bc
Trichoderma harzianum	64a	0.06	0.48ab	0.54ab	47b	0.23	0.36bc	0.59ab
Zygorrhynchous heterogamus	41bc	0.06	0.38bc	0.44b	—	—	—	—
Control	36c	0.08	0.21c	0.29b	54b	0.06	0.31cd	0.37cd

(1) Values, which are averages of 4 replicates, followed by common letters in the same column do not differ significantly at the 5% level by the hsd test. Total number of treated seeds=25/replicate, 4 replicates.

Table 3. *Effects of pelleting oat seeds with selected fungi on emergence and seedling growth*(1)

Fungus	Greenhouse				Soil tank			
	Emergence (%)	Dry root weight (g)	Dry shoot weight (g)	Total dry weight (g)	Emergence (%)	Dry root weight (g)	Dry shoot weight (g)	Total dry weight (g)
Aspergillus clavatus	44b	0.10bc	0.38b	0.48bc	91a	0.16a	0.81ab	0.97a
Trichoderma aureoviride	66a	0.17ab	0.68ab	0.85ab	88a	0.16a	0.84a	1.00a
Trichoderma harzianum	79a	0.23a	1.00a	1.23a	89a	0.21a	0.91a	1.12a
Pelleting only	39b	0.03c	0.39b	0.42c	50b	0.04b	0.60b	0.64b
Untreated control	38b	0.02c	0.30b	0.32c	70b	0.03b	0.54b	0.57b

(1) Values, which are averages of 4 replicates, followed by common letters in the same column do not differ significantly at the 5% level by the hsd test. Total number of treated seeds=25/replicate, 4 replicates.

T. aureoviride or *T. harzianum* significantly improved emergence compared to oat seeds pelleted without fungus or non-pelleted seeds (Table 3). *A. clavatus*-pelleted oat seeds yields superior emergence only at soil temperature of 8°C. Pelleting of oat seeds with *T. aureoviride* and *T. harzianum* increased the root, shoot and total dry weight under both growing conditions, except *T. aureoviride* failed to increase oat shoot dry weight under greenhouse conditions. *T. harzianum*-pelleted oat seeds produced a greater dry weight of plant tissues than did *A. clavatus*-pelleted seed in the greenhouse, but all three fungi, i.e., *A. clavatus, T. aureoviride,* and *T. harzianum* caused a similar increase in root, shoot and total dry weights in the soil tank except *A. clavatus* which failed to increase shoot dry weights at 8°C.

Wheat seeds pelleted with the selected fungi had a higher percentage of emergence compared to untreated seeds under both growing conditions (Table 4). Pelleting of wheat seeds with *A. clavatus, T. aureoviride, T. harzianum* and the combination of these three fungi increased the root and total dry weight under both greenhouse and soil tank conditions compared to pelleting only and untreated controls.

Table 4. *Effects of pelleting wheat seeds with selected fungi on emergence and seedling growth*[1]

Fungus	Greenhouse				Soil tank			
	Emergence (%)	Dry root weight (g)	Dry shoot weight (g)	Total dry weight (g)	Emergence (%)	Dry root weight (g)	Dry shoot weight (g)	Total dry weight (g)
Aspergillus clavatus	64a	0.48a	0.83a	1.31a	64a	0.21a	0.44b	0.65b
Trichoderma aureoviride	65a	0.35b	0.75ab	1.10a	57a	0.30a	0.60a	0.90a
Trichoderma harzianum	52ab	0.41ab	0.79ab	1.20a	68a	0.26a	0.63a	0.89ab
Mix of *A.c., T.a. T.h.*	58a	0.44ab	0.81a	1.25a	73a	0.31a	0.69a	1.00a
Pelleting only	46b	0.21c	0.50b	0.71b	31b	0.18b	0.27c	0.45c
Untreated control	20c	0.02c	0.12c	0.14c	25b	0.17b	0.20c	0.37c

(1) Values, which are averages of 4 replicates, followed by common letters in the same column do not differ significautly at the 5% level by the hsd test. Total number of treated seeds=25/replicate, 4 replicates.

Discussion

In this research, the emergence and total dry weight of seedlings grown from seeds pelleted with fungi (beneficial fungi) were greater than those obtained from seeds pelleted without fungal spores or not pelleted. Similarly, Chang and Kommedahl (1968) reported that coating corn kernels with *Bacillus subtilis* or *Chaetomium globosum* and sowing these seeds in soil infested with *Fusarium roseum* f. sp. 'Graminearum' resulted in improved plant development. In this study, the fungi on pelleted seeds may have produced an unfavorable environment in the soil for the development of fungal pathogens so that pathogenic activities of these fungi in infested soil were reduced. Also, these fungi might produce compounds that increase seedling vigor. Once seedlings are growing vigorously, they may be more resistant to attack by pathogens in soil. Possible mechanisms by which pathogens are inhibited by beneficial fungi include: inability to compete with beneficial fungi for nutrients, development of a fungal mycelial barrier between plant tissue and soil organisms (mycorrhizae), the production of antifungal substances from plants induced by the beneficial fungus, mycoparastism, or antibiosis.

Baker and Cook (1974) have suggested that there are no other factors of greater significance in root disease initiation than root exudation and the resultant rhizosphere effect. The fungi coated on the surface by pelleting probably grow profusely when the seed is sown because they have priority over other soil-borne fungi in utilizing the nutrients diffusing from the seed and in the pellet itself. In addition, the fungi pelleted on seed are probably able to grow abundantly in the rhizosphere during the initial development of the seedling because the zone between the spermosphere and the rhizosphere is continuous. Jackson (1957) indicated that the nuturients released into the spermosphere and the rhizosphere are responsible for the relative abundance of fungi in the rhizosphere and on the seed surface as compared with soil away from the seed and roots. Similarly, Duke and Apple (1961) observed the attraction of zoospores of *Phytophthora parasitica* var. *nicotianae* to tobacco roots when the root epidermis was broken by girdling with a scalpel or punctured by a needle and immersed in a zoospore suspension. Furthermore, nutrients secreted from either seed or root could be depleted by the fungi which had been applied to the seed surface by pelleting. Therefore, seed pelleted with fungi might cause autolysis of mycelia and fungistasis of pathogens in soil due to nutrient deprivation. In other words, soil-borne pathogens might be inhibited by the seed-surface and rhizoosphere fungi living on the exudate nutrients from seed or roots. Apparently, a soil-borne pathogen must be able to compete successfully with soil saprophytes if it is to germinate and cause root infection. Balis (1970) found that both *Phialophora radicicola* and *Ophiobolus graminis* required thiamine and biotin for growth on inorganic nitrogen. The saprophyte *P. radicicola* growing on wheat roots prior to colonization by *O. graminis* competed successfully with *O. graminis* for nutrients

(Balis, 1970; Deacon, 1973). The large number of fungi on the roots of wheat or oats may be beneficial because they have the ability to produce antibiotic substances which are detrimental to plant pathogens but are non-phytotoxic to the plant. Ferguson (1958) suggested that addition of cellulose to the soil increased the population of *Myrothecium verrucaria* which produces toxins that suppress *Rhizoctonia solani*, the causal fungus of damping-off of pepper seedlings. The beneficial fungi on roots of wheat or oats may form a mycelial mantle to hinder infection by soil-borne pathogens. Theodoron (1971) inoculated pine seeds with spores of *Rhizopogon luteolus*, which established mycorrhizae and subsequently increased plant growth 36 percent. Similarly, shortleaf pine seedlings with ectomycorrhizae formed by *Pisolithus tinctorius* or *Cenococcum graniforme* grew as well in nonsterile soil with *Phytophthora cinnamoni* as did untreated seedlings in nonsterile without the pathogen (Marx, 1973). Beneficial fungi may also produce growth promoting substances. Brown (1972) found that species of *Nocardia* and *Arthrobacter* on roots of wheat seedlings produced indole-3-acetic acid (IAA) and gibberellic acid (GA_3). From the above discussions, it is evident that fungi pelleted on seeds produces a more direct and also gives a faster beneficial effect than any other method known. Two other possible mechanisms mentioned above, antibiosis and mycopastitism, will be discussed in the next study.

The optimal concentration of fungal spores for seed coating, the ability of a fungus to survive in storage and the most suitable binder are all parameters which need to be more carefully defined in order to develop the most effectively pelleted seeds.

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與小麥及燕麥種子和土壤有關的病原性真菌之生物防治

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將 *Epicoccum nigrum* Link 或 *Trichoderma harzianum* Rifai 引入染有 *Drechslera sorokiniana* (Sacc.) Subrain & Jain, *Fusarium culmorum* (W. G. Smith) Sacc. 及 *Rhizoctonia solani* Kühn 的土壤中，可以增進小麥及燕麥幼苗的出土及生長勢。以 *Aspergillus clavatus* Desm., *Trichoderma aureoviride* Rifai 或 *T. harzianum* 的分生孢子摻在連結劑（binder）中，然後所製成的丸衣種子也具有相同的效果。