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Effect of Antibiotic Antagonists on Control of Basal Stem Rot of Chrysanthemum Caused by *Rhizoctonia solani*

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ABSTRACT

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The antagonists *Aspergillus, Gliocladium, Paecilomyces, Trichoderma* and *Bacillus* spp. were used for the control of basal stem rot of chrysanthemum caused by *Rhizoctonia solani*. All the antagonists protected chrysanthemums from infection by *R. solani.* Solid media for the culture of the antagonists were more useful than culture broths for application as soil additives or as coating materials. When chrysanthemum cuttings were treated with culture extracts of the antagonists, basal rot disease was also inhibited. The degree of the control of *Rhizoctonia* disease in chrysanthemums differed according to the species of antagonists used and the application method.

(Key words: biological control, chrysanthemum basal stem rot, antagonist, *Rhizoctonia solani, Aspergillus, Gliocladium, Paecilomyces, Trichoderma, Bacillus*)

INTRODUCTION

The basal stem rot disease of chrysanthemum caused by *Rhizoctonia solani* is an important soilborne disease in Taiwan. Its incidence during the seedling stage is high and damping-off occurs. As with other *Rhizoctonia* diseases, fungicides have been used to control this disease in Taiwan[10]. In recent years, the use of antagonistic microorganisms to control soilborne plant diseases has also been discussed[2,4]. The control of *Rhizoctonia* diseases on seedlings of rice, mungbean and potato by antagonist, *Bacillus subtilis* F29-3, which produces fengy(my)cin, was first demonstrated by members of our laboratory[5,7,9].

Recently antibiotic-producing antagonists against *R. solani,* including species of the genera *Aspergillus, Gliocladium, Paecilomyces, Penicillium, Trichoderma* and *Bacillus,* have been isolated from Taiwanese soils and their antagonistic actions to *R. solani* studied [8,12]. We present here results on the control of basal stem rot in chrysanthemums by antagonists which have been grown in different culture media and applied with different methods.

Infection of Chrysanthemums by *R. solani*

R. solani anastomosis group 4 (AG-4) was isolated from chrysanthemums showing basal stem rot symptom. The fungus grew rapidly on yeast extract agar and produced mycelia in abundance. The fungus grew to maturity in a 8 cm-diameter petri dish four days after inoculation. Its pathogenicity was also established when chrysanthemum cuttings were planted in plastic pots (20 × 8 cm) containing soils with a soil-potato inoculum in the middle of each pot. The cuttings began to rot from their basal parts after on week. Disease incidence increased in proportion to the weight of inoculum (Table 1).

Antibiotic Activities of Antagonists of *R. solani*

Antagonists were grown in liquid media to obtain their antibiotic metabolites. The substances produced by the nine antagonists were tested on diffusion agar plates against different indicator microorganisms, *B. subtilis, Saccharomyces cerevisiae, Paecilomyces marquandii* and *Rhizomucor miehei.* Results indicated that all antagonists produced antibiotics and that most of the antagonists inhibited growth of the test fungi (Table 2). The antibiotic activities were related to the growth media used to culture the antagonists and the sensitivity of the test microorganisms. Because antibiotic inhibition of *B. subtilis* and *P. variotii* by metabolites of the antagonists were clearly visible, the two microorganisms were considered to be suitable as test microoranisms for checking the production of antibiotics by the antagonists.

Control of *R. solani* by Coating Chrysanthemum Stems with Antagonists

When the basal portions of chrysanthemum stems were coated with antagonists and the cuttings planted in sands infested with *R. solani*, basal stem rot was inhibited (Table 3). Infection of *R. solani* was reduced by application with different antagonists. The disease incidence was reduced 30% by *B. Subtilis* F29-3 and 78% by *P. marquandii* CF407 relative to the control (100%). The control efficacies by *P. marquandii* (CF407) and *Gliocladium deliquescens* (F-92) were greater than that of *B. subtilis* and was statistically significant. Both fungi are known as hyperparasites of *R. solani*[8,12]. The control effects of mycoparasites appear to be associated with the in-

Table 1. Effect of amount of *R. solani* inoculum on the incidence of basal stem rot in chrysanthemums

Inoculum of *R. solani*[1] (g/pot)	Disease incidence (%)
1.5	66.7
1.0	41.7
0.5	16.7
Control	0.0

1) The inoculum was prepared in soil-potato medium.

Table 2. Antibiotic activities of antagonists of *R. solani* tested on several microorganisms

Antagonist	Test microoranism			
	Bacill. subtilis	*Saccharom. cerevisiae*	*Paecilom. variotii*	*Rhizom. miehei*
Penicillium sp. CF111	+[1)]	+	+	+
Paecilomyces marquandii CF302	+	+	+	+
Paecilomyces marquandii CF407	+	+	+	+
Gliocladium deliquescens F-92	+	+	+	+
Trichoderma harzianum TVCN1	+	+	−	+
Trichoderma harzianum TVCN2	+	−	−	−
Trichoderma koningii T12	+	+	+	+
Bacillus cereus CB22	−	+	+	+
Bacillus subtilis F29-3	+	+	+	+

1) + : positive; − : no reaction tested with >100 mg/ml crude antibiotic.

Table 3. Control of *R. solani* by coating chrysanthemum stems with antagonists

Antagonist	Disease incidence (%)
Penicillium sp. CF111	46.3 a*
Paecilomyces marquandii CF302	46.3 a
Paecilomyces marquandii CF407	15.6 b
Gliocladium deliquescens F-92	18.8 b
Bacillus sp. CB9	51.9 a
Bacillus sp. CB20	48.1 a
Bacillus cereus CB22	43.8 a
Bacillus subtilis F29-3	48.1 a
Control	68.8 a

* Values not followed by the same letter are significantly different at 5% level (according to Duncan's multiple range test, DMRT).

hibitory activities of their antibiotic metabolites.

Control of *R. solani* by Sawdust Compost Containing Antagonists

In Taiwan, sawdust has been used for cultivation of mushrooms, after which the sawdust waste is used as compost for gardening. We used sawdust compost to cultivate antagonists and samples of it were used as the antagonist inocula that were added in sand beds to study the control of *R. solani.* By experiment 0.5 to 4% of antagonist inoculum was used. The results indicated that any of these concentrations of the inoculum controlled basal stem rot in chrysanthemum and that the degree of control increased in proportion to the amount of antagonist used. Of the 16 antagonists investigated, TVCN1, TVCN2, CF302, CF111 and F29-3 provided the highest control effects followed by T12 and CB22 (Table 4). Generally speaking, 1

% of antagonist inoculum was useful; a higher amount of inoculum not only wasted more antagonists but was also toxic to plants. The phytotoxic phenomenon was observed in applications with CF111, F-92 and F29-3. Because sawdust compost is rich in nutrients, a high concentration of sawdust may promote the growth of *R. solani* and increase the incidence of disease in the no antagonist control.

Control of *R. solani* by Various Organic Substances Containing Antagonists

Three different delivery systems were used for application of antagonists to control of *R. solani*. Sawdusts, rice husks and sawdust composts were inoculated with different antagonists; the control effects by these three preparations on basal stem rot are shown in Figure 1. The results indicated that fresh sawdust is not suitable for the application of antagonists, as only T12 showed some control efficacy. In the other two systems all antagonists inhibited *R. solani* to different extents. The rice husk and sawdust compost systems were more effective than with sawdust. Similar results were observed when these substrates were used for commercial cultivations of chrysanthemums. We speculate that the different degress of inhibiton of *R. solani* shown by the antagonists depended on the application method used[1,6]. Although rice husks are not easy to compost because of their high silica content, they could be used without prior treatment for preparation the inocula of antagonists.

Table 4. Control of *R. solani* in chrysanthemum by sawdust compost containing antagonists

Antagonist	Disease incidence (%)
Penicillium sp. CF111	9.2 efgh[1)]
Paecilomyces marquandii CF302	8.0 fgh
Paecilomyces marquandii CF407	15.6 cd
Gliocladium deliquescens F-92	14.1 def
Penicillium vermiculatum F-60	23.3 bc
Trichoderma viride TD	23.3 bc
Trichoderma koningii T2	25.2 b
Trichoderma viride T20	13.8 def
Trichoderma pseudokoningii T33	25.4 b
Trichoderma harzianum TVCN1	6.8 h
Trichoderma harzianum TVCN2	7.9 gh
Trichoderma koningii T12	12.3 efg
Bacillus sp. CB9	22.4 bc
Bacillus sp. CB20	20.6 bcd
Bacillus cereus CB22	13.5 defg
Bacillus subtilis F29-3	9.7 efgh
Control	42.3 a

1) Values within the column not followed by the same letter are significantly different at 5% level (based on DMRT).

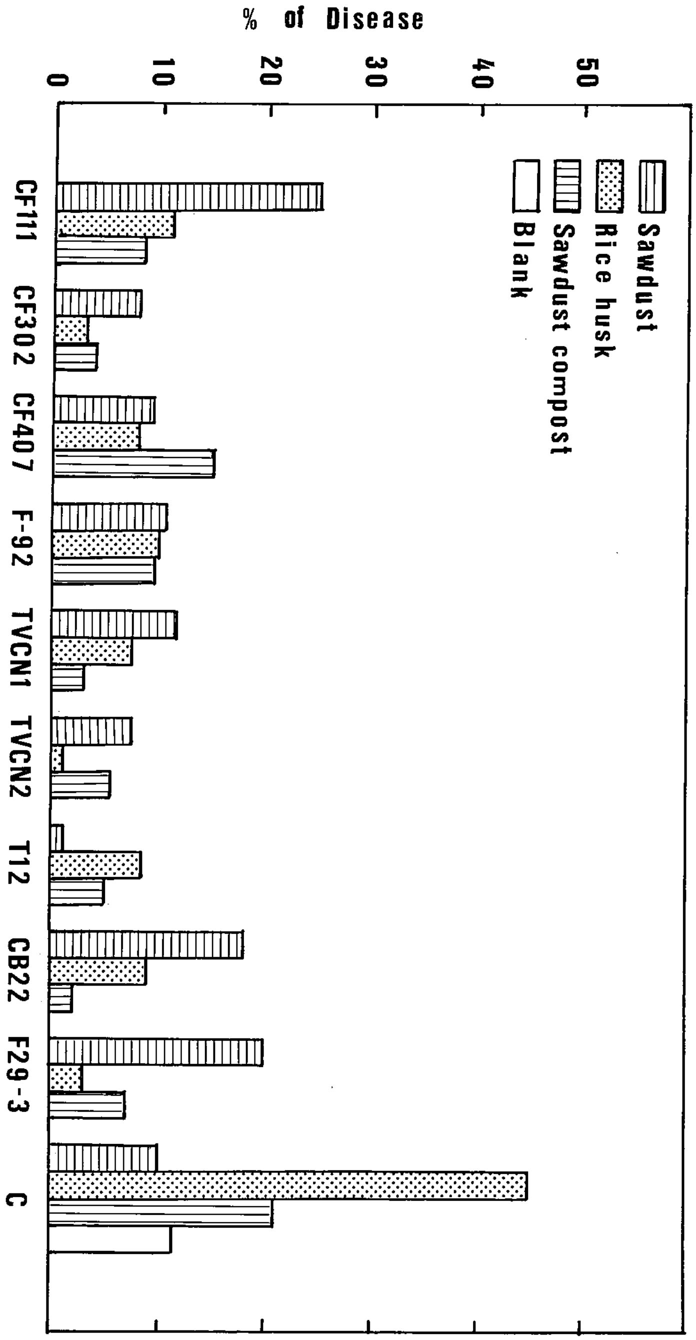

Fig 1. Control of *Rhizoctonia solani* in chrysanthemums by different vehicles containing antagonists.

The disease incidence (Y axis) was reduced by application of different antagonist strains (X axis).

Control of *R. solani* by Antagonist Extracts

Chrysanthemum cuttings were treated with culture extracts of the various antagonists before planting. The result indicated that the extracts protected the chrysanthemums from infection by *R. solani* (Table 5). The control effects of F-92 and CF302 extracts were greater than those of the other antagonists. Inhibiton of the growth of *R. solani* was tested with extracts of some antagonists. Such crude extracts contain antibiotics which inhibited growth of *R. solani.* The antibiotic effects from fungal antagonists were greater than those of the bacterial antagonists. The inhibition mechanisms of the antibiotics that function against *R. solani* must be explained at the physiological level in relation to the mode of action of the antibiotics[3,11]. Our experiments were made in a garden system, and therefore could not give an explanation.

CONCLUSION

The basal stem rot disease in chrysanthemum caused by *R. solani* can be controlled by several antagonistic microorganisms in three ways: (1) by coating chrysanthemum cuttings with the antagonists; (2) by application of solid additives of antagonists to soil when chrysanthemum cuttings are planted and (3) by treatment of chrysanthemum cuttings with extracts from antagonists. Control results differed with the application method used. Our experiments indicated that all the antagonists tested inhibited infection by *R. solani* and that the efficacy of prevention depended on the application method used. A solid additive that contained the antagonist was the best means for applying the antagonist to control *R. solani.* The advantages of the solid inocula are ease of handling and high reproducibility, though its preparation takes a long time. Coating chrysanthemum cuttings with antagonists was better than treating them with extracts of antagonists.

Control of *Rhizoctonia* disease by antibiotic producing antagonists has been evidenced. The disease may be controlled more effectively by improving the efficiency of antagonists with genetic manipulation in the future.

Table 5. Control of *R. solani* in chrysanthemums by crude extracts of various antagonists

Antagonist	Disease incidence (%)
Penicillium sp. CF111	65.5 bc[1)]
Paecilomyces marquandii CF302	52.3 c
Paecilomyces marquandii CF407	63.6 bc
Gliocladium deliquescens F-92	51.9 c
Trichoderma harzianum TVCN1	62.9 bc
Trichoderma harzianum TVCN2	69.5 ab
Trichoderma koningii T12	59.7 bc
Bacillus cereus CB22	66.4 bc
Bacillus subtilis F29-3	81.2 a
Control	71.3 ab

1) Values not followed by the same letter are significantly different at 5% level (based on DMRT).

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摘　要

陳昇明 1991 抗生拮抗菌對菊莖腐病菌之生物防治　植保會刊 33:56～62。（國立中興大學植物學研究所）

以麴菌（*Aspergillus*）、黏帚黴菌（*Gliocladium*）、擬青黴菌（*Paecilomyces*）、木黴菌（*Trichoderma*）和芽胞桿菌（*Bacillus*）等抗生拮抗菌，對立枯絲核菌（*Rhizoctonia solani*）感染之菊莖腐病，從事防治試驗。實驗結果顯示全部拮抗菌均具有防治菊莖腐病之效果。用於土壤添加物或包埋材料之拮抗菌，以固體培養基繁殖者優於液體培養基。菊苗如果以拮抗菌萃取液處理，則可防止其感染莖腐病。拮抗菌對菊莖腐病之防治效果，則因使用拮抗菌種類和施用拮抗菌方法之不同，而有差異。

（關鍵字：生物防治、菊莖腐病、立枯絲核菌、拮抗菌、麴菌、黏帚黴菌、擬青黴菌、木黴菌、芽胞桿菌）