Plant Protection Bulletin 32 : 77−90, 1990

Integrated Conrol of Chrysanthemum Stem Rot

Wu, Wen−Shi[1], Kuo, Mei−Huey[2], Tschen, J[3]., and Liu, S. D.[4]

1. Professor of Department of Plant Pathology & Entomology, National Taiwan University, Taipei, Taiwan, R. O. C.; 2. Research Associate, Development Center of Biotechnology, Taipei; 3. Professor of Department of Botany, National Chung-Hsing University, Taichung, Taiwan; 4. Professor of Department of Plant Protection, National Ping-Tung Institute of Agriculature, Ping-Tung, Taiwan.

(Accepted for publication: March 31, 1990)

ABSTRACT

Wu, W. S. Kuo, M. H., Tschen, J., and Liu, S. D. 1990. **Integrated control of Chrysanthemum stem rot.** Plant Prot Bull. 32 : 77−90.

Chrysanthemum stem rot caused by *Rhizoctonia solani* AG4 was a very common and severe diease in almost all chrysanthemum nurseries. For developing a lasting effective control method, integrated control was considered in this study. In order to devise a suitable combination of integrated control, 122 antagonists, six chemicals and different temperature treatments had been screened. Seven bacterial antagonists, *Trichoderma harzianum and T. viride* were the most effective microorganisms to inhibit the hyphal growth of *R. solani*. Bavistin, Benlate and Monceren at 10 ppm inhibited the growth of *R. solani* significantly. The population density of the pathogen was decreased in the sand when treated with Basamid and calcium cyanamide at concentrations above 74 mg / kg sand and 370mg / kg sand, respectively. Although the lethal temperature of *R. solani* was 50°C / 10 min, the population of *R. solani* was decreased from 67 to 10 propagules / 10 g sand when the sand was heated to about 65°C / 30 min. In field, bioagent, heat or Monceren was able to control stem rot significantly. *B. cereus* 501 and Monceren was the best treatment combination to significantly reduce the disease incidence and the survival of *R. solani*. This integrated control method is very worth to be recommended to the extension people as well as growers.

(key words : *Rhizoctonia solani*, chrysanthemum, disease control, integrated control).

INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) was the most important

cutting flower in Taiwan. This flower plant was mostly grown in Chang-hua County (彰化) and the rests were planted in Nan tou (南投), Chia-Yi (嘉義) and Tainan (台南) Counties. Chrysanthemum used for exporting was mainly blossom in the winter. For this purpose, chrysanthemum cuttings must be rooted in the nurseries in the summer and the beginning of autumn when the weather was hot and humid. Consequently, chrysanthemum seedling were readily infected by *Rhizoctonia solani* Kühn and became stem rot. This disease was the most important problem among all the nurseries and caused more than 30% losses every year.

Although chrysanthemum stem rot has been reported in Taiwan[3], the disease still existed and continued to threathen the growers even they followed the recommendations by the government. Effective disease control methods remain to be developed. The main purpose of this paper was to propose a successful method to control chrysanthemum stem rot.

MATERIALS AND METHODS

Isolation and identification of the pathogen

Infected chrysanthemum seedlings and the associated infested soil were collected from Chang-hua County. Routine tissue isolation and soil plate methods were used to isolate the related pathogen. The isolated fungi were cultured in both potato dextrose agar and potato-sand medium (PSM, potato 50g, dextrose 5g, sand 200g, distilled water 20 ml). Mixture of one part of PSM with profuse growth of cultural fungi with 9 parts of sand (v/v) was filled in pots in which healthy chrysanthemum cuttings were planted. The control was water washed sand only. Anastomosis group (AG) of *R. solani* was determined by dual culture with the standard cultures of five different AG isolates.

Isolation and screening of antagonists

Dilution plate method was applied for the following three preparations. Beef peptone agar, glycerol asparaginate medium, soil extract agar, water agar and PDA－streptomycin medium (PDA＋1000mg streptomycin, 1 ℓ basis) were prepared to isolate various kind of microorganisms.

1). Soils were collected from chrysanthemum, rice, sorghum and maize fields in the fall of 1983. Soil was kept in cool (5℃) condition when it was possible and was used for isolation of microorganisms within 48 hours.

2). Agar disc with *R. solani* was contacted firmly with collected field soil and incubated for three days. The lysed hyphae were picked up under microscope and shaked in sterile distilled water.

3). Healthy intact and infected tissues of chrysanthemum were collected and seperately stirred in sterilized distilled water.

All isolated microorganisms were dual cultured with *R. solani* AG4 on PDA. The size of inhibition zone was measured and the change of morphology of *R. solani was* observed under light and scanning electron microscope (Hitachi S－550). *R. solani*－agar disc (0.6 cm in diams.) was immersed in the suspension (10^{10} colony forming unit, cfu / ml) of isolated antagonistic bacteria for two minutes and then transferred to PDA and incubated under 25℃ for seven days before rcording the influence of those antagonists on *R. solani*.

Screening of chemicals

Each of six different fungicides (Table 1) was prepared at four concentrations, i.e. 1, 10, 100 and 1,000 ppm (active ingredients, a.i.) with PDA or potato dextrose borth (PDB). Thirty milliliters of those different preparations were poured into

petri dish(10 cm in diam., Pyrex). Disc (0.6 cm in diam.) of *R. solani* which has been grown on water agar plate for five days was transferred on to the center of the agar plate and incubated under 25℃ for specific durations. Dry weight of *R. solani* was also measured when the inoculum (0.4 cm in diam.) was transferred to 50 ml of PDB containing the tested chemical at either 1, 10, 100 or 1,000 ppm (a.i.) and incubated under 25℃ for 30 days statically.

Either 74, 180, 370, or 740 mg of Basamid(dazomet, BASF)and either 74, 370, 740 or 1110 mg of calcium cyanamide (Perlka, SKW Trostberg) were incorporated into 1 kg of sands and mixed with 1,000 ml of water and then covered by a sheet of PVC (0.2 mm in thickness). The initial ammount of *R. solani* in the sand was 89 and 92 propagules / 10 g sand determined by soil plate method on water agar for the treatment of Basamid and calcium cyanamide, respectively. The amount of *R. solani* in the treated sand was determined after a certain period of incubation.

Effectiveness of heat on the survival of *Rhizoctonia solani*

Discs (0.6 cm in diam.) of *R. solani* which has been grown on water agar for five days were placed into different test tubes in which 10 ml of sterile distilled water were added. Those test tubes were separately immersed into water bath with the temperature from 35℃ to 70℃, 5℃ difference among every treatments, for different period of time. After treatment with hot water, disc of inoculum was transferred to the center of PDA plate. The activity of *R. solani* was recorded after three days of incubation.

Sand was infested with *R. solani* which was cultured in PSM. The amount of *R. solani* in the sand was 67 propagules / 10 g sand. Ten grams of this *R. solani*−infested sand were heated from 35℃ to 65℃, with about 10℃ interval for each treatment, on hot plate. The survivability was measured on water agar by direct transferring the treated sand onto it.

Disease control test in the greenhouse

1). Biological control

1,000 g *R. solani*-infested sand (1 part of inoculum of *R. solani* mixed with 9 parts of washed sand) were filled in plastic box(15×15×7cm, L×W×H). Thirty ml of either suspension of bactrial cells of B101, B501(identified as *Bacillus cereus*[2])(1×10^{10} cfu / ml)or conidia of *Trichoderma viride* (TD)(4.6×10^{8} spores / ml) were added to the sand in the plastic box and mixed thoroughly. Twenty cuttings were planted in each treatment. Each treatment had five replicates and repeated once. Percent of infected cuttings was recorded after 20 days of planting.

2). Chemical control

The preparation of *R. solani*−infested sand was as aforementioned. Besides, 100, 250, and 750 ppm (a.i.) of Monceren were prepared. Two ways of applying this chemical were used in this study : (1) immersing cuttings in the chemical for 2 hr before planting, and (2) 30 ml of these preparation were separately mixed with infested sand before planting. Twenty cuttings were planted for each treatment. Each treatment had three replicates and repeated once. Disease index was recorded after 14 days of planting.

3). Integrated control by bioagents and fungicides

Suspension containing 500 ppm (a.i.) Monceren plus either *B. cereus* 501 (10^{10} cfu / ml) or *T. viride* (4.6×10^{8} spore / ml) was prepared. Chrysanthemum cuttings were immersed into this suspension for 2 hours before planting. Another parts of cuttings were planted in the *R. solani*−infe-

sted sand which was mixed with 30 ml of this suspension. 500 ppm (a.i.) benomyl and untreated were used as control. Each treatment had 10 cuttings with three replicates. Results were recorded after 10 days of planting.

One kilogram of *R. solani*—infested sand was mixed with either 180 or 370 mg of Basamid, or 740 or 1110 mg of calcium cyanamid. Afterwards, the sand was covered with a sheet of PVC (0.2 mm in thickness) for 10 days. The amount of *R. solani* was then measured by soil plate method. Ten ml of suspension of either antagonistic bacteria (B101, B501) or *T. viride* (2.2×10^{5} spore / ml) added and mixed in the sand before 10 cuttings were planted. Each treatment had three replicates and remained in the greenhouse for 14 days.

4) Integrated control by heat and bioagent

One kilogram of *R. solani*—infested sand was treated with heat (45 and 55 C / 2 hr) in dry oven. After cooling, the survivability of *R. solani* in the sand was measured. Ten ml suspension of either antagonistic bacteria (B101, B501) or *T. viride* were added and mixed with the heat-treated sand. Bioassay was tested as aforementioned.

Field experiment

Six sand beds of 140×280×20 cm and another six sand beds of 150×250×20 cm were used. All beds were infested with *R. solani*. Six treatments (Table 7) were tested and based on randomized complete block design. Each treatment consisted of 400 cuttings with two replicates. After planting for 25 days, percent of live stands, fresh weight and disease index were recorded.

The way of application of bioagents was the same as the test in greenhouse. The only difference was the concentration of *T. viride* which was reduced to 3.9×10^{7} spores / ml. The concentration of antagonistic bacteria (B101 and B501) remained unchanged i.e. 1×10^{10} cfu / ml. Five hundred ml of either suspension of bioagent and Monceren were mixed in sands. The concentration of Monceren was 1,000 ppm. The temperature of sand was raised by burning charcoal on the surface of seedling bed till the temperature of sand was around 60 C at the top layer of 5 cm.

RESULTS

Isolation and identification of the pathogen

Rhizoctonia solani was always isolated from diseased tissues and sand around the infected seedlings. Actually, mycelium of this fungus was readily detected on infected tissues and even on the surface of sand bed. This fungus was proven to be pathogenic and able to cause the typical brown necrotic lesion and made the seedlings tending to damping—off within three days. Chrysanthemum cuttings produced normal root system within 15 days in sterilized sand.

R. solani produced brown colony on potato dextrose agar with few aerial mycelium and almost no sclerotia. The test isolate was identified to belong to AG4.

Isolation and screening of antagonists

One hundred and twenty two isolates demonstrated their antagonistic effect against *R. solani* AG4. Among them, there were 33 fungal isolates, 69 bacterial isolates and 20 isolates of Actinomycetes. Seven bacterial isolates (B101, B102, B201, B301, B401, *Bacillus cereus* (B501), B601) and *Trichoderma viride* (TD) and *T. harzianum* (TVCN 2) were selected from dual culture for further study. Different isolates showed their different capability to inhibit the growth of *R. solani*. The hyphae adjacent to the antagonistic bacteria showed irregular short branchings, bulging at the hyphal tip, crinkle along the hyphae or lysed. When *R.*

solani grew with these selected antagonistic bacteria, the growth rate was almost stopped by all these bacterial isolates, except B 301. The hyphae of *R. solani* shrunk and lysed when grew with either B101, B201, B301, B401, or *B. cereus* 501(Fig. 1). Except B201 and B301, the other bacterial isolates were able to multiply in the sterilized sand, e.g. *B. cereus* 501 increased from 5.5×10^5 to 3.2×10^6cfu / g sand when incubated for three weeks. Suspension of either bacterial or fungal isolates caused no adverse effect for the development of chrysanthemum cuttings, whereas cultural filtrate whcih was obtained by culturing B401, B601 and *Trichoderma* spp. in PDB for 7 days and sterilized by passing through sterile millipore filter(0.2μ pore size)turned the young leaves blackened, necrosis and wilting within two days when cuttings were immersed into these metabolites.

Screening of chemicals

One ppm of either Bavistin, Benlate, Campogran or Monceren was able to significantly inhibit the growth of *R. solani* on PDA (Table 2), but only 1 ppm Monceren could significantly reduce the dry weight of *R. solani* grown in PDB (Table 3). One hundred ppm of Demosan or polycaptan also showed the ability to significantly inhibit the growth of *R. solani* on PDA. 100 ppm of Bavistin stopped the growth of *R. solani* completely in PDB, whereas higher concentration (i.e. 1,000 ppm) was needed for Benlate to achieve the same effect.

Each concentration of Basamid or calcium cyanamide tested in this study was able to significantly reduce the propagules of *R. solani.* For example, 180 and 740 mg Basamid / kg sand could reduce the propagules of *R. solani* from 89 to 10 and 0 per 10 g sand, respectively. *R. solani* in low concentration(i.e. 74mg / kg sand)treatment was reduced when the sand was treated within 7 days, but the propagules started to increase gradually from the 7th day on.

Effectiveness of heat on the survival of *Rhizoctonia solani*

R. solani started to declone when it was immersed in hot water (45℃) for 5 min and stopped to grow when the temperature increased to 50℃ for 10 min. *R. solani* in sand decreased from 67 (untreated control) to 47, 30, 23, and 10 propagules / 10g sand when the sand was treated with 35, 45, 55 and 65℃, respectively, for 30 minutes.

Disease control test in the greenhouse

1). Biological control

All three selected antagonists effectively controlled chrysanthemum stem rot. The percent disease incidence was reduced from 41 (untreated control) to 13 for *B. cereus* 501, to 16 for B101, and to 25 for *T. viride*.

2). Chemical control

Either dipping or soil treated with Monceren reduced the disease incidence more than 50% (Table 4). The effectiveness of controlling the tested disease increased with the increasing concentration of Monceren. Dipping improved significantly the survivability of chrysanthemum cuttings as compared with direct soil treatment, except treating the soil with 750 ppm Monceren.

3). Integrated control by bioagent and fungicides

Soil treated with either bioagents(B101, B501, *T. viride*)or chemicals (Monceren, Benlate) significantly increased the survivability of chrysanthemum and reduced the disease rate (Table 5). Soil treated with the combination of Monceren with either B101, B501 or *T. viride* had significantly higher survivability and lower disease rate than soil treated with either bioagent alone.

Either Basamid alone or combined with B101 increased the survivability significantly

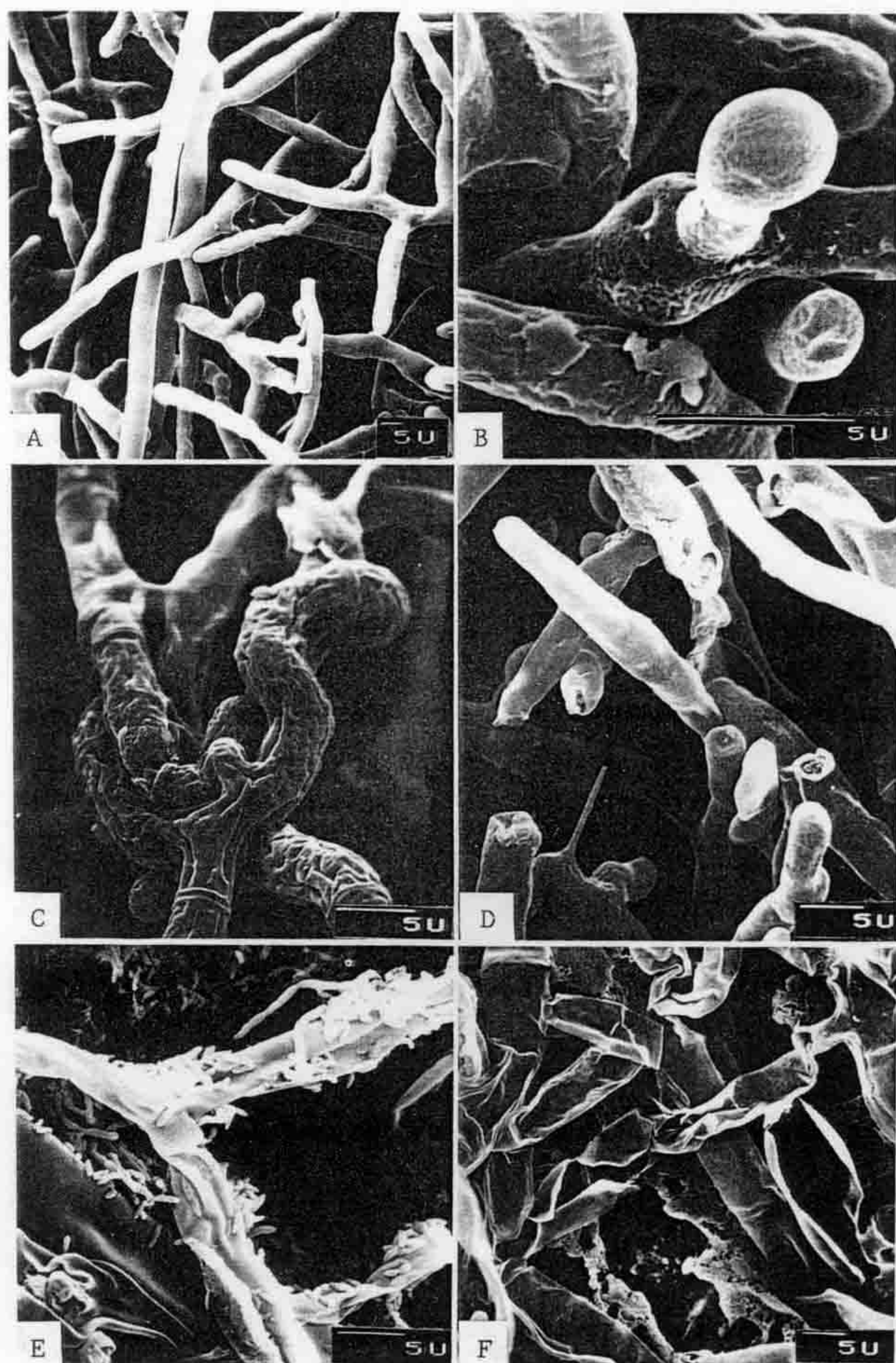

Fig. 1. The different antagonistic effect on *Rhizoctonia solani* produced by different antagonists. (A) healthy *R. solani*, (B) bulging hyphae caused by B201, (C) hyphal wrinkle caused by B401, (D) B501 cleaved the hyphae, (E) B301 adhered on the collapsed hyphae when it grew with *R. solani*, and (F) B501 lysed and crushed *R. solani* when they grew together.

Table 1 Fungicides used in this study

Commercial name	Common name	Chemical name
Bavistin 50% W.P.	carbendazim	Methyl – 2 – benzimidazole carbamate or 2 – (Methoxy – carbamyl) – benzimidazole
Benlate 50% W.P.	benomy1	1 – (Butyl carbamoyl) – 2 – benzimidazo1e Carbamic Acid, Methyl Ester or Methyl – 1 – (buty1 carbamoy1) – 2 – benzimidazole Carbamate
Campogran 40% W.P.	furmecyclox	N-cyclohexyl-N-methoxy – 2, 5 – dimethyl – 3 – furancarboxamide
Demosan 35.5% Flowable	chloroneb	1,4 – Dichloro – 2,5 – dimethoxybenzene
Monceren 25% W.P.	penaguran	N – { (4 – chlorophenyl) – methyl } – N – cyclopenthyl – N¹ – phenylurea
Polycaptan 55%W.P.	polyoxin – B + captan	(1)1 – { 5′ – n – (5″ – O – carbamoyl – 2″ – amino – 2″ – deoxy – 2 – xylonyl – 5′ – amino – 5′ – deoxy – B – D – allfulan-osyluzonic acid) } – 5 – hydroxy methyl – uracil (2)N – Trichloromethylthio – 4 – cyclohexene – 1, 2 – dicarboximide

Table 2 Effect of different fungicides on the growth of *Rhizoctonia solani*

Fungicides	Diameter of colony (cm)[1]							
	Concentration of fungicide (ppm)							
	1		10		100		1000	
	WA	PDA	WA	PDA	WA	PDA	WA	PDA
Bavistin	1.10 E[2]	2.18 E	0	0	0	0	0	0
Benlate	8.84 A	2.38 E	0	0	0	0	0	0
Demosan	9.00 A	6.66 A	9.0 A	4.92 C	0	1.54 GH	0	1.36 H
Campogran	4.08 B	1.74 FG	1.22 E	*[3]	0	*	0	0
Monceren	2.14 C	1.90 FG	1.48 D	*	*	*	*	*
Polycaptan	9.00 A	6.18 B	9.00 A	3.98 D	0	1.33 H	0	0
CK	9.00 A	6.80 A	9.00 A	6.80 A	9.00 A	6.80 A	9.00 A	6.80 A

1) The size of colony was measured on water agar (WA) and potato dextrose agar (PDA) after 5 and 2 days of incubation, respectively.

2) Each data was the mean value of five replicates. Data followed by the same letter expressed no significant (P=0.05) difference measured by Duncan's multiple range test.

3) No colony was formed, but some aerial mycelia were produced on the inoculum.

Table 3 Effect of different fungicides on the growth of *Rhizoctonia solani* in potato dextrose broth

Fungicides	Dry weight of mycelia (mg)[1]			
	Concentration of fungicide (ppm)			
	1	10	100	1000
Bavistin	143.3^{C}[2]	3.3^{D}	0.0	0.0
Benlate	547.0^{A}	1.6^{E}	0.3^{E}	0.0
Campogran	439.3^{B}	34.3^{D}	3.3^{D}	1.0^{E}
Monceren	66.0^{D}	3.3^{D}	2.6^{D}	2.3^{D}
CK	575.3^{A}	575.3^{A}	575.3^{A}	575.3^{A}

1) Dry weight was measured when the tested fungus had been cultured for 30 days. The fungus was dried in 80℃ dry oven for 24 hours and then weighted.

2) Each data was the mean value of five replicates. Data followed by the same letter expressed no significant (P=0.05) difference measured by Duncan's multiple range test.

Table 4 The effectiveness of different concentrations of and different method applying Monceren to control chrysanthemum stem rot

Concentration (ppm)	Soil treatment[1]		Dipping treatment[1]	
	% survival	Disease incidence[2]	% survival	Disease incidence[2]
100	95.0^{B}[3]	30.8^{B}	100^{D}	13.3^{D}
250	98.3^{C}	13.3^{D}	100^{D}	16.7^{C}
500	98.3^{C}	11.3^{E}	100^{D}	11.3^{E}
750	100^{D}	6.7^{F}	100^{D}	7.1^{F}
CK	51.7^{A}	82.9^{A}	51.7^{A}	82.9^{A}

1) Date were collected after 14 days of planting.

2) $$\text{Disease incidence} = \frac{\text{disease index} \times \text{number of specific category of cuttings}}{4 \times \text{total no. of cuttings}} \times 100\%$$

Disease index was expressed as 0 : healthy, 1 : 1−3 leaf lesions or 1 crater lesion on stem , 2 : 2 lower leaves soft rotted and brown lesion appeared on stem, 3 : only top 2−3 leaves remain alived , the rests were rotted , 4 : damping−off or dead.

3) Data, mean of 60 cuttings, followed by the same letter in the same category of both treatments expressed no significant (P=0.05) difference by Duncan's multiple range test.

than untreated control. Combination of soil treatment with low concentration (i.e. 740 mg / kg sand) of calcium cyanamide and B501 increased the survivability significantly as compared with either untreated control or calcium cyanamide or bioagent alone, but was not significantly different from soil treated with high concentration of calcium cyanamide (i.e. 1,110 mg / kg sand). There was no different effectiveness of increasing survivability among all the different combinations of disease control when the concentration of calcium cyanamide was 1, 110 mg / kg sand.

4). Integrated control by means of heat and bioagent

Adding B501 to the soil which had been treated with 45C for 2 hr could increase the survivability of chrysanthemum and had the same effectiveness of disease control as the soil treated with 55 C for 2 hr. (Fig. 2). Actually, there was no *R. solani* survived in the sand when it had been treated with 55℃ for 2 hr. Consequently, the survival of chrysanthemum cuttings was 100%.

Field experiments

Either heat treatment was able to reduce the amount of inoculum in soil significantly (Table 6).

Among different control methods, 1,000 ppm (a.i.) Monceren was the most effective treatment to control the disease or increase the survivability of chrysanthemum cuttings (Table 7). B501 alone was also able to reduce the amount of disease significantly. B501 did not provide synergistic effect to control the studied disease with heat treatment or Monceren.

DISCUSSION

In vitro, those tested antagonistic bacteria formed a clear inhibition zone adjacent to the colony of *R. solani*. This might be due to the antibiotic effect initiated by those antagonists. It has been reported that *Streptomyces* spp.[25] and *Bacillus subtilis*[30] were able to stop the growth of *R. solani* and made its hyphal tip swollen. Besides antibiotic action, antagonistic fungi, mainly *Trichoderma* spp., expressed hyperparasitic and enzymatic activity toward *R. solani* AG 4 in this study. *Trichoderma* spp. were well illustrated to be able to coagulate the protoplasm of *R. solani* and lyse the hyphae into fragments[1,8,13,15,20,34,36]. However, the metabolites of *Trichoderma* spp. which were directly applyed into the soil or to the cuttings caused some distinguished harmful effects onto the seedlings in this study. Although both *T. viride* and *T. harzianum* controlled the studied disease in greenhouse, they were not tested in the field trial. Monceren was the most effective fungicide tested in this study to inhibit the growth and developoment of *R. solani*. Even 1 ppm of Monceren was able to malform and inhibit the development of *R. solani* in cultural medium. This fungicide was then selected for field test. Fumigant was commonly used in nurseries. When the dosage of fumigant was sublethal to the certain target organism, the population of the target organism might be overwhelmed in the treated soil due to sufficient available nutrients or substrate[23]. Basamid and calcium cyanamide treated at the concentration of 740 mg / kg sand and 74 mg / kg sand, respectively, were able to eliminate *R. solani* from sand.

Although *R. solani* was decreased under 50 C / 10 min in laboratory, this pathogen could not be eliminated from field soil when it treated with dry heat at 65C for 30 min. This might be due to the substrates colonized by *R. solani* provided a protective effect[17]. since colonized plant tissues were used as inoculum to apply into the field in this study.

Table 5 Effectiveness of integrated bioagent and Monceren to control chrysanthemum stem rot

Treatment	Soil treatment[1]		Dipping treatment[1]	
	% survival	Disease incidence[2]	% survival	Disease incidence[2]
B101	46.7^{C}[3]	85.0^{C}	26.7^{B}	90.0^{B}
B501	56.7^{F}	78.3^{D}	50.0^{D}	74.2^{E}
T. viride	53.3^{E}	80.0^{D}	0^{A}	100^{A}
Monceren	100^{H}	0.8^{L}	100^{H}	14.2^{I}
Monceren + B101	100^{H}	5.0^{K}	100^{H}	15.8^{H}
Monceren + B501	100^{H}	0^{L}	100^{H}	10.8^{J}
Monceren + *T. viride*	100^{H}	0^{L}	100^{H}	27.5^{G}
Benlate CK	76.7^{G}	49.2^{F}	100^{F}	17.5^{H}
Untreated CK	26.7^{B}	91.7^{B}	26.7^{B}	91.7^{B}

1) Data were collected after 14 days of planting.

2) Disease incidence was calculated as Table 4.

3) Data, mean of 30 cuttings, followed by the same letter in the same category of both treatments expressed no significant ($P=0.05$) difference by Duncan's multiple range test.

Table 6 Effect of heat on the survival of *Rhizoctonia solani* in soil

Replications		Soil temperature (℃)[1] Soil depth (cm)			Propagules of *R. solani* (10g soil)[2]	
		5	10	15	Before treatment	After treatment
A	1	66	45	36	89^{A}[3]	28^{B}
	2	54	38	31	92^{A}	34^{B}
B	1	62	40	30	34.1^{A}	9.8^{B}
	2	65	35	28	30.6^{A}	3.92^{B}

1) Soil temperature was raised by burning charcoal on the surface of soil.

2) Propagules of *R. solani* were measured from 5 soil samples randomly collected from each replication.

3) Data, followed by the same letter, were not significantly($P=0.05$)different by Duncan's multiple range test.

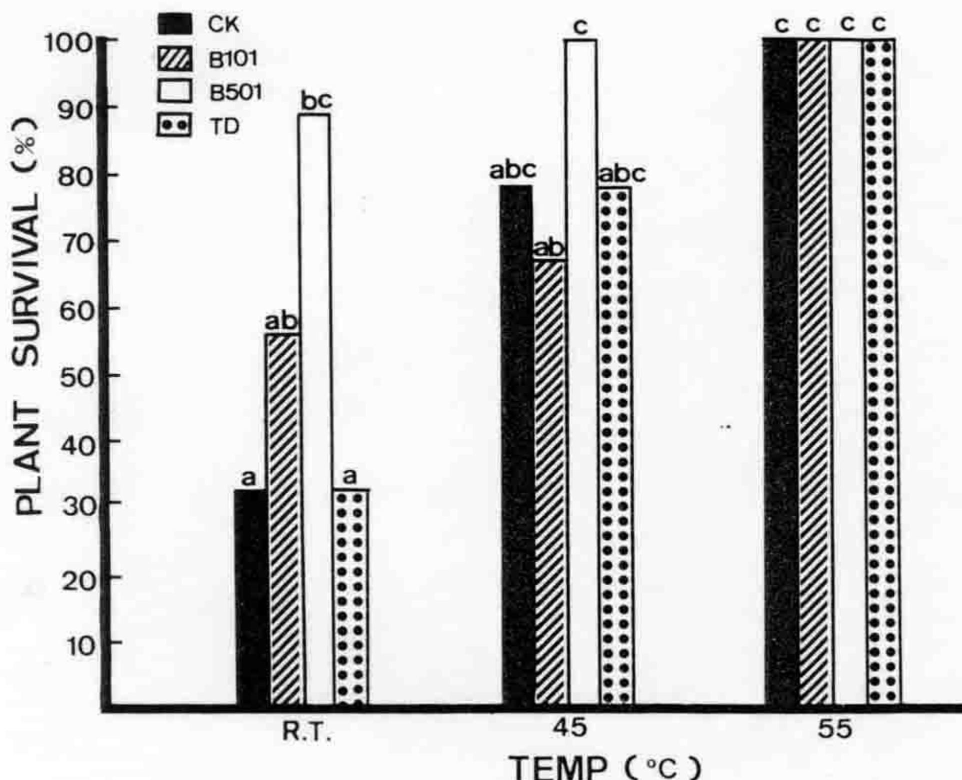

Fig. 2. The effectiveness of integrated control of chrysanthemum stem rot by means of heat treatment and bioagents. The same letter on each bar means no significant (p=0.05) difference estimated by Duncan's multiple range test. R. T. represented room temperature. B101 and B501 were two antagonistic bacteria. TD was *T. viride*. Each bar represented the % survival of chrysanthemum cuttings whcih were planted in the sand treated with either bioagents and heat alone or their combinations.

Table 7 Effectiveness of different measurements to control chrysanthemum stem rot in the chrysanthemum production field

Treatment[1]	% diseased plants	
	Replication	
	I	II
B. cereus 501	58.5[B2)]	29.5[B]
heat	10.8[D]	20.5[C]
heat + B.C.501	18.5[C]	20.5[C]
Monceren	0[F]	2[D]
Monceren + B.C.501	5.6[E]	1.5[D]
CK	90.3[A]	33.5[A]

1) Each treatment consisted of 400 seedlings for each replication. Data were recorded after 20 days of planting.

2) Data, followed by the same letter in each column, were significantly different (P=0.05) by means of Duncan's multiple rang test.

Either antagonistic bacteria or fungi could control the studied disease in greenhouse. B501 was the most effective antagonist in this study and had been identified to be *Bacillus cereus*[2]. *B. cereus* is a common soil inhabitant and could survive well in a wide range of environment. *B. cereus* has been reported to control *Gaeumannomyces graminis* var. *tritici*[7]. *Bacillus* sp. was effective to control the disease caused by *R. solani*[5,14,32,33], by *Fusarium roseum*, *Nectria galligena*, *Sclerotium cepivorum* or *Streptomyces scabies*[4,10,29,31,35]. *B. megaterium* may control *Colletotrichum corchori*[28]. Treatment combined with Monceren and B501 reduced the amount of disease significantly in greenhouse but not in the field. This difference might be due to the amount of Monceren applied in greenhouse was much lower than in field (i.e. 500 vs 1,000 ppm). Reducing the amount of applying chemicals may reduce the risk of pollution and the formation of chemical-resistant mutants, and may not provide the adverse effect to the non-target organism. For example, applying PCNB to control *R. solani* resulted in increasing the damage caused by *Pythium* spp[18]. Applying *T. harzianum* with PCNB provided better disease control than they were alone[12,19,21]. Besides reducing disease, 500 ppm Monceren plus B501 improved the root development of chrysanthemum cuttings in this study. In addition to controlling disease, B501 might produce some plant growth factor to improve the root development. Different antagonists expressed the similar effect when they were either treated directly with seeds or roots[6,9,16,22,24,26,27]. Conclusively, applying the combination of low rate of recommended dosage of Monceren and B501 (*B. cereus* 501)was the most effective method to control this studied disease.

ACKNOWLEGEMENT

This study, 75-0409-B002-61, was financially supported by National Science Council of Republic of China.

LITERATURE CITED

1. 朱芬芬、吳文希.1980. 馬鈴薯黑痣病之生物及化學防治。植保會刊。22：269－286。
2. 謝明璟.1987. 拮抗細菌B501之鑑定。國立台灣大學學士論文。
3. 謝式坪鉦、羅靜儀.1976. 菊花莖腐病之研究（1）病徵及病菌之生理性質。植保會刊18：338－345。
4. Aldrich, J., and Baker, R. 1970. Biological control of *Fusarium roseum* f. sp. *dianthi* by *Bacillus subtilis*. Plant Dis. Reptr. 54：446－448.
5. Broadbent, P., Baker, K. F., and Waterworth, Y. 1971. Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soil.Aust. J. Biol. Sci. 24：925－944.
6. Brown, M. E. 1974. Seed and root bacterization Annu. Rev. Phytopathol. 12：181－197.
7. Cambell, R., and Faull, J. L. 1979. Biological control of *Gaeumannomyus graminis*：Field trials and the ultrastructure of the interaction between the fungus and a successful antagonistic bacterium. In Soil－borne plant pathogens (B, Schippers and W. Gams, eds.), p.603－610. Academic Press, New York.
8. Camporota, P. 1985. Antagonism in vitro of *Trichoderma* spp. to *Rizoctonia solani* Kuhn. Agronomie 5：613－620. (Ref. Plant Pathol. 65 (2) ：62, 1986).
9. Chang, Y. C., Chang, Y. C., Baker, R., Kleifeld, O., and Chet, I. 1986. Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. Plant Dis. 70：145－148.
10. Chang, I., and Kommedah1, T. 1968.

Biological control of seedling blight of corn by coating kernel with antagonist organisms. Phytopathology 58 : 1395–1401.

11. Corke, A. T. K., and Hunter, T. 1979. Biocontrol of *Nectria galligena* infection of pruning wounds on apple shoots. Jour. Hortic. Sci. 54 : 47–55.
12. Curl, E. A., Wiggins, E. A., and Anders, J. C. 1976. Interactions of *Rhizoctonia solani* and *Trichoderma* with PCNB and herbicides affecting cotton seedlings disease. Proc. Am. Phytopathol. Soc. 3 : 221.
13. Dennis, C., and Webster, J. 1971. Antagoistic properties of species-groups of *Trichoderma*. Ⅲ. Hyphal interaction Trans. Br. Mycol. Soc. 57 : 363–369.
14. Dunleavy, J. 1954. Controll of damping-off of sugar beet by *Bacillus subtilis*. Phytopathology 44 : 252–258.
15. Elad, Y., Chet, I., Boyle, P., and Henis, Y. 1983. Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclecrotium rolfsii*–Scanning electron microscopy and fluorescences microscopy. Phytopathology 73 : 85–88.
16. Elad, Y., Chet, I., and Katan, J. 1980. *Trichoderma harzianum* : a biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology 70 : 119–121.
17. Garrett, S. D. 1970. Pathogenic root-infecting fungi. Cambridge Univ. Press, 294 pp.
18. Gibson, I. A. S., Ledger, M., and Boehm, E. 1961. An anomalous effect of pentachloronitrobenezens on the incidence of damping-off caused by a *Pythium* sp. Phytopathology 51 : 531–533.
19. Hadar, Y., Chet, I., and Henis,Y. 1979. Biological control of *Rhizoctionia solani* damping-off with wheat bran culture *Trichoderma harzianum*. Phytopathology 69 : 64–68.
20. Harman, G. E., Chet, I., and Baker R. 1981. Factors affecting *Trichoderma hamatum* applied to seed as a biocontrol agent. Phytopathology 71 : 569–572.
21. Henis, Y., Ghaffar, A., and Baker, R. 1978. Integrated control of *Rhizoctonia solani* damping-off of radish : Effect of successive plantings, PCNB, and *Trichoderma harzianum* on pathogen and disease. Phytopathology 68 : 900–907.
22. Kommedah1, T., and Windels, C. E. 1978. Evaluation of biological seed treatment for controlling root disease of peas. Phytopathology 68 : 1087–1095.
23. Krentzea, W. A. 1960. Soil treatment. in Plant Pathology, An Advance Treatise (J. G. Horsfall and A. E. Dimon, eds.), Vol. 3, P.431–436. Academic Press, New York. 1675.
24. Kundu, P. K., and Nandi, B. 1984. Control of cauliflower damping-off by using antagonist coated seeds. Pedobiologia 27 : 43–48. (Rev. Plant Pathol. 65 (1) : 44, 1986).
25. Links, J., Rombouhts,J. E., and Keulen, P. 1957. The bulging factor, a fungistatic antibiotic produced by a *Streptomyces* strain, with evidence of an active water-excreting mechanism in fungi. J. Gen. Microbiol. 17 : 596–601.
26. Merriman, P. R., Price, R. D., and Baker, K. F. 1974. The effect of inoculation of seed with antagonists of *Rhizoctonia solani* on the growth of wheat. Aust. J. Agric. Res. 25 : 213–218.
27. Merriman, P. R., Price, R. P., Kollmorgen, J. F., Piggott, T., and Ridge, E. H. 1974. Effect of seed inoculation with *Bacillus subtilis* and *Streptomyces griseus* on the growth of cereals and carrots. Aust. J. Agri. Res. 25: 219–226.
28. Purkayastha, B. P., and Bhattacharyya, B. 1982. Antagonism of micro-orga-

nisms from jute phyllosphere towards *Colletotrichum corchori*. Trans. Brit. Mycol. Soc. 78：509−513.

29. Swinburne, R. 1973. Microflora of apple leaf scars in relation to infection by *Nectria galligena*. Trans. Brit. Mycol. Soc. 63：389−403.
30. Tschen, J., Kuo, W. L., and Liu. J. M. 1982. Inhibition of *Rhizoctonia solani* by *Bacillus* antagonist. Journal of Science and Engineering 19： 61−68.
31. Utkhede, R. S., and Rahe, J. E.1980. Biological control of onion white rot. Soil Biol. Biochem. 12：101−104.
32. Vargas, R., and Ramirez, C. 1983. Biological control of damping-off caused by *Rhizoctonia solani* on cotton. Agronomia Costarricense 7：73−75 （Rev. Plant Pathol. 64(5)：204, 1985).
33. Venkatasubbaiah, P. 1985. Efficacy of *Bacillus subtilis* as a biocontrol for collar rot of coffee pathogen. Geobios, India 12：101−104（Rev. Plant Pathol. 65（1）：24, 1986）.
34. Weindling, R. 1932. *Trichoderma lignorum* as a parasite of other soil fungi. Phytopathology 22：837−845.
35. Weinhold, A. R., Dswald, J. W., Bowman, T., Bishop, J., and Wright, D.1964. Influence of green manures and crop rotation on common scab of potato. Am. Potato Jour. 41：265−273.
36. Wu, W. S., Liu, S. D., Chang, Y.C., and Tschen, J. 1986. Hyperparasitic relationship between antagonists and *Rhizoctonia solani*. Plant Prot. Bull., Taiwan.28：91−100.

摘　要

吳文希[1]、郭美慧[2]、陳昇明[3]、劉顯達[4] 1989 **菊花莖腐病之綜合防治** 植保會刊31：77−90。（1.台北市國立台灣大學植物病蟲害學系；2.台北市長興街，生物技術開發中心；3.台中市國立中興大學植物學系：4.屏東縣國立屏東農業專科學校植物保護科）

立枯絲核菌AG4 所引起的菊花莖腐病，是本省菊花苗圃中最常見，也是最嚴重的一種病害，爲求長期有效地防治此一病害，本報告乃介紹一可行之綜合防治方法。爲組成有效的綜合防治方法，所以篩選了122種拮抗菌，6種農藥及各種溫度；其中有7種細菌，*Trichoderma harzianum*及*T. viride*可有效抑制立枯絲核菌之生長；10 ppm的Bavistin, Benlate及Monceren即可明顯地抑制此病原之生長；另每公斤沙土中分別添加74及370毫克的Basamid及烏肥，也可明顯降低病原之族群數量；當沙土的溫度提昇至65℃，並維持30分鐘，可使立枯絲核菌的族群數由每克沙中之67個繁殖體降爲10個；如將以上三類防治措施妥善配合，可有效明顯地防治病害，如利用*Bacillus cereus* 501及Monceren即可明顯降低病害程度及病原之存活率，其效果穩定，故可提供花農直接採用。

（關鍵字：立枯絲核菌，菊花，病害防治，綜合防治）。