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Control of Sclerotinia Rot of Sunflower and Chrysanthemum

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ABSTRACT

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Benomyl and DCNA were able to inhibit the growth of *Sclerotinia sclerotiorum* in cultural medium and able to control sclerotinia rot of sunflower in field. Vinclozolin expressed better effectiveness of disease control on sunflower than DCNA and iprodione. Vinclozolin inhibited the germination of ascospore completely and caused the ascospore losing its cytoplasm. When soil treated with calcium cyanamide, the survival of sclerotia declined and the disease on chrysanthemum was reduced significantly due to the amount of apothecium was reduced. *Trichoderma viride* isolated from decayed sclerotium was able to infect *S. sclerotiorum* and produced some inhibitory substance(s) to inhibit the growth of the studied pathogen. Spraying the spore suspension of *T. viride* onto sunflower was able to reduce the disease rate significantly, but was unable to increase the yield of sunflower. Soil solarization by mulching the field soil with black polyethylene sheet was an effective method to reduce the amount of apothecium significantly. Consequently, the percent of disease was significantly reduced in 1986 but not in 1987. The temperature on the top soil at solar energy-treated field was 10℃ higher than untreated soil, but 5℃ higher when the soil temperature was measured at the depth of 5 cm from the soil surface. Sclerotia lost their vitality when the soil was wet and the soil temperature was raised to 45℃ for four hours a day for four days.

(Key words: *Sclerotinia sclerotiorum,* chemical control, biocontrol, integrated control)

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is an important plant pathogen due to the fact that it has a wide spectrum of host ranges and ability of survival for long time. This pathogen was able to infect about 400 different species of plant in 72 families[1,3,19,30,31,33,40].

Generally, *S. sclerotiorum* produced black, irregular-shaped slerotia under various stress conditions. Sclerotium is the fundamental structure of this pathogen to survive for several years under natural field conditions[4,10,34]. Although sclerotium of this pathogen can be the primary inoculum[6,14,39], ascospore, usually, plays much more important role as primary inoculum to cause plant to be infected. Ascospore was produced in apothecium which was initiated from sclerotium. Each apothecium contained approximately 1.3-2.3 $\times 10^6$ ascospores[34,41]. The duration of each apothecium to release ascospore can last for 11-12 days, once the apothecium started to discharge ascospore[41]. Since ascospores were produced only once from one specific sclerotium and infected host plant once in one growing season, the disease caused by *S. sclerotiorum* should be regarded as monocyclic or simple interest disease[38].

S. sclerotiorum is world-wide spread and existed in Taiwan, especially in notrhern Taiwan. This pathogen caused evidently losses on vegetables and flower plants when the temperature was below 25℃ and weather was wet in Taiwan. In order to reduce the incidence of this pathogen, chemical control was the most popular practice. However, the adverse effect of applying chemical was mentioned frequently[7,11]. For minimizing the drawbacks of using chemicals, the most promised chemical should be screened. Besides, other control measurements should be considered solely or applied integratly with less amount of fungicides. Biological control has endurable effect to inhibit pathogens without polluting or disturbing the environment dramatically[7]. Since 1976, solar energy has been applied to control soil-borne pathogens[18], solarization has been identified as an effective measurement to improve plant growth, increase yield and reduce the amount and severity of disease[12,13,28,29,35,37]. The main purpose of this study is try to find out some effective and available methods to control this pathogen in Taiwan.

MATERIALS AND METHODS

Tested plants

Sunflower and chrysanthemum were used in this study. Sunflower seeds and chrysanthemum seedling were sown and transplanted, respectively, in field when bioassay was needed.

Chemical control

1. laboratory experiment

Benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate, Benlate 50%, du Pont), DCNA (2, 6-dichloro-4-nitroaniline, Dicloran 50%, The Boot's Co.), PCNB (pentachloronitrobenzene, Terraclor 75%, Olin) and zineb (zinc ethylene bisdithiocarbamate, Zineb 75%, Luxan) were prepared in potato dextrose agar (PDA) and broth (PDB) at either 250, 500 or 1000 ppm (active ingredients, a.i.). The same size and age of inoculum was transferred either on the center of PDA plate or in PDB. All the cultures were incubated at 25℃ for certain period of times. Each treatment consisted of four replicates and repeated twice.

Compared to DCNA, iprodione (3-(3, 5-dichlorophenyl)-N-isopropyl-2, 4-dioxoimidazolidine-1-carboximide, Rovral 50%; Rhone-Pouienc Inc.) and vinclozolin (3-(3, 5-dichlorophenyl)-5-methyl-5-vinyl-1, 3-oxazoladine-2, 4-dione, Ronilan 50%, BASF) were also prepared at either 0.01, 0.1, 1, 10, 100 or 1000 ppm (a.i.) in PDA. When the ED_{50} was less than 1 ppm, further tests were made at concentrations of 0.05, 0.1, 0.5, 1, 5 and 10 ppm. The mycelial growth was measured after incubating this pathogen under 22℃ for

certain period of time.

DCNA and vinclozolin (1000 ppm, a.i.) were sprayed onto sunflower 5, 2 or 0 days before and after inoculation of ascospores. After inoculation for two days, the inoculated tissues were treated and observed under scanning electron microscope (Hitachi S-550).

Either 0.5, 0.25 or 0.05 g of calcium cyanamide (SKW Trostbery) was mixed with 100 ml of field soil (40% moisture content). Ten sclerotia were immersed in the soil and incubated in 40℃ for 4 hr and in 30℃ for the rest of 20 hr. everyday. After incubating for either 2, 4, 8 or 12 days, the survivability of sclerotia was determined. Each treatment consisted of three replicates.

2. field experiments

Sunflower was planted in 0.05 ha. field which was divided into four blocks. Each treatment consisted of four replicates. Each replicate consisted of either 40 or 50 sunflower or 200 chrysanthemums. Randomized completely block design was used in this study. The field soil was clay loam.

For the first field experiment, each treatment consisted of 40 plants which were planted in adjacent two rows. Every row was 25 cm apart from each other. Each row was 600 cm long. Benomyl, DCNA, PCNB, and zineb were sprayed at the concentration of 5.25 g (a.i.)/6 l when sunflower had grown for one month. Afterwards spraying was once every week. In the second field experiment, DCNA, vinclozolin, mancozeb, PCNB were used. Each chemical consisted of two treatments. One was applying 50 g (a.i.) of chemical along the seeding sites plus spraying with the same chemical 14 days after planting. The other was spraying only which was started 14 days after planting. The concentration of each sprayed chemical was 1000 ppm (a.i.). DCNA, iprodione and vinclozolin were used in the third field trial. After 30 days of planting, 1500 ppm (a.i.) of each chemical was sprayed and continued to be sprayed for eight times at 7-day interval.

Chrysanthemum was planted in the *S. sclerotiorum*-infested field for determing the effective of soil treatment with different chemicals. The size of each block was $2.2 \times 3.9\ M^2$. Two hundred chrysanthemum seedlings were planted in each blocks. 250 g of either urea or calcium cyanamide and 1000 ppm vinclozolin were treated with and sprayed on, respectively, the field. The disease rate and survival of sclerotia were recorded and analysed by Duncan's multiple range test.

Biological control

1. isolation of antagonists

The sclerotia of *S. sclerotiorum* were buried in field at the depth of 5-10 cm. After 30 days, microflora on the sclerotia were isolated by dilution plate on PDA. Soil microflora were also isolated by dilution method on sclerotia-powder medium. The sclerotia-powder medium consisted of 2 g powder of sclerotia, 8 g agar and 400 ml dist. water.

2. screening the antagonists

Capability of either hyperparasitism, antibiotic activity or competition[22] were used as the criterion to determine which isolated soil microorganisms were good for further study.

3. field experiment

The spore suspensions of *Trichoderma viride* 8I, *T. viride* 8IR, *Gliocladium virens* and *Bacillus* sp. were prepared in glycerol-sodium nitrate nutrient solution[22]. Spraying began 30 days after planting sunflower and continued to spray for eight times at 7-day interval. All treatments in the field experiment had four replicates and followed by randomized

completely block design.

Physical control

1. soil solarization

Black polyethylene sheet (0.2 mm in thickness) was covered on field plots for 50 days (Sept. 5-Oct. 25, 1986) in the first experiment, and for 35 days (Aug. 29-Oct 2, 1987) in the second experiment. Afterwards, 200 chrysanthemum seedlings was transplanted to each of these treated plots. Control was seedling growing in the untreated plots.

2. determining the effect of temperature on the survival of *S. sclerotiorum*

Every 10 sclerotia (0.4-0.6 cm in diam.) were buried in field soil which was filled in 100 ml beaker. Those beakers were separately incubated under 45, 40, 35 and 30℃ for 1, 2, 4, 8 and 14 days. When these beakers were incubated under 45, 40 and 35℃ , the incubation time was 4 hr a day and they were transferred to 30℃ for the rest of 20 hr everyday. Survivability was determined by counting whether those scleotia was able to germinate on PDA.

Every 10 sclerotia was also buried at the depth of 5 cm in each field plot which was treated with solarization. After 50 days, sclerotia were removed from field and cleaned with running tap water. Then the survivability of sclerotia was also determined as aforementioned.

RESULTS

Chemical control

1. laboratory experiment

Benomyl and DCNA stopped the growth of *S. sclerotiorum* after three days of incubation, this inhibitory effects remained even till 14 days after incubation (Table 1). PCNB and zineb slowed down the growth rate of *S. sclerotiorum* on PDA. Besides, PCNB at all tested concentration altered the mycelial color from white to brown. Small sclerotia were formed on PDA containing either 250 of 500 ppm zineb, but irregular colonies were formed when the concentration of zineb was 1000 ppm.

This studied pathogen was unable to grow in PDB which contained 1000 ppm benomyl (Table 1). All the other chemicals reduced significantly the dry weight of *S. sclerotiorum* compared to control. *S. sclerotiorum* was unable to grow in PDB which contained 1000 ppm of DCNA in the first two weeks, but started to form a dense layer of mycelia and irregular sclerotia after three weeks of

Table 1. The effects of four different fungicides on the growth of *Sclerotinia sclerotiorum*

parameter	concentr. (ppm)	diam. of colonies (cm)[1]				
		benomyl	DCNA	PCNB	zineb	CK
diameter of colonies[3]	250	nil	nil	2.8 a[2]	3.4 a	8.4 b
	500	nil	nil	3.0 a	3.4 a	8.4 b
	1000	nil	nil	3.0 a	2.8 a	8.4 b
dry wt. (g.)[4]	1000	0 d	0.46 b	0.16 cd	0.26 bc	0.69 a

1) Each treatment consisted of four replicates and was repeated twice.
2) Data, the average of every treatment, followed by common letter in the same row did not differ significantly ($p = 0.05$) by the Duncan's new multiple range test.
3) Data were obtained after three days of incubation under 25℃ .
4) Data were obtained after 21 days of incubation under 25℃ .

incubation.

Compared to DCNA and iprodione, vinclozolin was the most effective in inhibiting the mycelial growth of *S. sclerotiorum* on PDA. The concentration of ED_{50} of vinclozolin, iprodione, DCNA to inhibit the growth of *S. sclerotiorum* was 0.25, 0.27 and 1.47 ppm (a.i.), respectively. The concentration of ED_{90} of vinclozolin, iprodione, and DCNA to inhibit the growth of *S. sclerotiorum* was 1.24, 1.37 and 7.37 ppm, respectively.

2. field experiment

Benomyl, DCNA and PCNB were able to reduce the percent of disease when sunflower grew for 70 days, but were unable to continue to control this disease when sunflower was ready for harvest (Table 2). Among the four fungicides, DCNA was the most effective fungicide to control *S. sclerotiorum* in field.

Among DCNA, vinclozolin, mancozeb and PCNB, DCNA and vinclozolin were able to reduce disease rate and increase the percentage of surival of sunflower significantly (Table 3). However, only vinclozolin provided the capability to increase the yield significantly than the other three chemicals. There was no significant difference between two methods of applying either chemical.

DCNA, iprodione and vinclozolin were able to control sclerotinia rot of sunflower (Table 4). Besides, DCNA and vinclozolin were able to increase yield, but not for iprodione.

Ascospores germinated readily on the untreated sunflower, but failed to germinate on vinclozolin-treated sunflower after ten days of inoculation. Although DCNA allowed some ascospores to germinate, the germination was limited. Besides inhibiting the germination of ascospore, DCNA, iprodione and vinclozolin were able to cause the affected ascospore to leak their cytoplasm.

Chrysanthemum sclerotinia rot was significantly reduced when the field soil was sprayed with vinclozolin or mixed with calcium cyanamide (Table 5). Besides, calcium cyanamide reduced the survivability of sclerotia of *S. sclerotiorum* significantly in field soil. Chrysanthemum grew significantly higher than other treatments when field soil was mixed with urea.

Biological control

One hundred and eleven isolates of microorganism were isolated from sclerotia which were incubated in field soil for 30 days. Among them, *Trichoderma viride* 8I and 8IR, as well as *Gliocladium virens* were identified as hyperparasites of *S. sclerotiorum*. They produced short hyphal branches which attached to the susceptible host hyphae and caused them to be evacuated or disintegrated.

Table 2. The effects of four different fungicides on controlling sclerotinia rot of sunflower

days after planting[1)]	percent of disease[2)]				
	benomyl	DCNA	PCNB	zineb	CK
70	10.6[3)]	9.8	12.5	22.5	20.6
120	51.3	46.1	58.3	57.7	55.2

1) Spraying chemical (5.25 g/6 l, a.i.) was once a week after 30 days of planting.

2) Percent of disease = disease rating × no. of plant of the same category of disease rating/5 × 160 (total tested plants).

3) Data were the average of four replicates.

Table 3. The effects of four different fungicides on controlling sclerotinia rot of sunflower

parameter	DCNA		mancozeb		PCNB		vinclozolin		CK
	I[1]	II[1]	I	II	I	II	I	II	
disease rate[2]	2.16 b[3]	2.50 b	3.50 a	3.75 a	3.75 a	3.28 a	0.75 c	1.19 c	3.66 a
% survival	21.00 b	19.25 b	4.75 c	3.00 c	4.75 c	7.25 c	32.50 a	32.75 a	7.5 c
yield (kg)	220.13 b	176.9 b	91.25 b	110.6 b	130.0 b	163.1 b	546.3 a	576.3 a	163.8 b

1) I: soil incorporated and plant sprayed with a specific tested fungicide;
II: spray tested fungicides only.

2) O: healthy, 1: leaves infected only, ——, 4: wilting.

3) Data, average of four replicates and recorded at harvest time, followed by the same letter in the same row were not significantly ($p = 0.05$) different.

Table 4. The effects of three fungicides on controlling sclerotinia rot of sunflower

parameter	treatment[1]			
	DCNA	inprodione	vinclozolin	CK
disease rate after 79 days of planting	8.3 bc[2]	8.8 bc	3.8 c	22.4 a
disease rate after 92 days of planting	38.9 b	39.6 b	27.1 c	60.4 a
average yield (kg.)	10.7 ab	5.7 c	13.7 a	3.8 c

1) Each treatment consisted of four replicates. Each replicate had 50 tested plants. The concentration of all tested fungicides was 1500 ppm (a.i.)

2) Data, average of four replicates, followed by the same letter in the same row differed significantly ($p = 0.05$) by Duncan's multiple range test.

Table 5. The effects of different soil treatment on controlling sclerotinia rot of chrysanthemum

parameter	treatment[1]			
	vinclozolin	urea	calcium cyanamide	CK
percent of disease	3.36 b[2]	7.26 ab	3.30 b	12.88 a
height of plant (cm)	39.39 bc	49.70 a	46.71 ab	39.96 bc
% survival of sclerotia	—	100.00 a	10.00 b	100.00 a

1) Each treatment consisted of four replicates. Each replicate had 200 seedlings. 250 g of urea and calcium cyanamide was mixed thoroughly with soil of each block ($2.2 \times 3.9 m^2$). 1000 ppm vinclozolin was sprayed on the soil before planting.

2) Data, average of four replicates, followed by the same letter in the same row differed significantly by Duncan's multiple range test.

G. virens was able to penetrate into and grew within the hyphae of *S. sclerotiorum*. *T. viride* produced appressorium-like structure to press against the hyphae of *S. sclerotiorum*. Besides, they produced some inhibitory metabolites which caused swelling and plasmolysis of affected cells of *S. sclerotiorum*. The growth of *S. sclerotiorum* was inhibited eventually.

T. viride 8IR, the DCNA-tolerant strain, had the same capability to reduce the disease rate significantly as *T. viride* 8I (Table 6), but was unable to increase the yield of sunflower significantly. *G. virens* and *Bacillus* sp. were also able to reduce some degree of disease rate.

Physical control

Solarization was able to reduce the amount of apothecium as effective as soil treated with calcium cyanamide (Table 7). Spraying vinclozolin and soil treated with calcium cyanamide reduced disease rate significantly in 1986 and 1987, but solarization reduced disease rate significantly only in 1986 and not in 1987. Spraying vinclozolin was not able to reduce the amount of aprothecium significantly in field.

The temperature of solarization-treated top field soil can be reached to 45-50℃ and

Table 6. The effects of different antagonists on controlling sclerotinia rot of sunflower

parameter	treatment[1]				
	T. viride 8I	*T. viride* 8IR	*G. virens*	*Bacillus* sp.	CK
disease rate after 79 days of planting	11.4 b[2]	10.5 b	13.3 ab	13.9 ab	22.4 a
disease rate after 92 days of planting	46.8 b	45.8 b	48.9 ab	51.9 ab	60.4 a
yield (kg)	4.8	4.3	3.9	3.3	3.8

1) Each treatment consisted of four replicates. Each replicate had 50 tested plants.
2) Data, average of four replicates, followed by the same letter in the asme row differed significantly ($p = 0.05$) by Duncan's multiple range test.

Table 7. The effects of solarization on controlling of sclerotinia rot of chrysanthemum compared to spraying vinclozolin and soil treated with calcium cyanamide

treatment	no. of apothecium		% disease	
	1986	1987	1986	1987
solarization[1]	2.00 b[3]	4.50 b	2.61 b	46.57 a
vinclozolin[2]	22.00 a	20.75 a	3.36 b	24.55 b
calcium cyanamide[2]	1.50 b	5.75 b	3.30 b	24.87 b
CK	22.00 a	20.75 a	12.88 a	56.25 a

1) Black polyethylene sheet was covered on wetted field for 50 days in 1986 and 35 days in 1987.
2) The method of application was the same as in Table 5.
3) Data, average of four replicates, followed by the same letter in the same column did not differ significantly ($p = 0.05$) by Duncan's multiple range test.

the untreated field soil was 35-40℃ . The temperature at the depth of 5 cm in solarization-treated soil and untreated aoil was 35-40 ℃ and 30-35℃, respectively.

The survivability of sclerotia was reduced to 26.67% when sclerotia were buried in wet field soil (40% water content) and incubated under 45℃ for 4 hr only. Sclerotia incubated in the same type of soil at other temperatures remained 100% alive. After incubation for four days under 45℃ , sclerotia were dead completely. The survivability of sclerotia which were kept under 40, 35 and 30℃ for 14 days was 96.67%, 46.67% and 70%, respectively. The survivability of sclerotia was reduced to 50% when they were buried in solarization-treated soil in field after 35 days of treatment.

DISCUSSIONS

Although benomyl was found to be able to control white rot of cucumber[8] and sclerotinia blight of peanuts[27] effectively, benomyl failed to control sclerotinia rot of sunflower in the first field trial regardless of the fact that benomyl was able to inhibit this studied pathogen to grow in cultural medium within 21 days of incubation. This was probably due to the timing of first spray was late, high inoculum potential and suitable environment for disease development were existed in the field. DCNA reduced the amount of disease of sunflower the most compared to benomyl, PCNB and zineb in this study. DCNA was regarded better than benomyl and PCNB to control lettuce drop[23]. However, vinclozolin provided the best effectiveness to control sclerotinia rot of sunflower than DCNA and iprodione in this study. 5 ppm of vinclozolin was able to reduce the production of sclerotia and controlled lettuce drop very effectively[24]. Ascospore of this studied pathogen failed to germinate on vinclozolin-treated sunflower. Besides, vinclozolin caused the ascospore to lose its cytoplasm. This may be due to vinclozolin was able to inhibit the bio-synthesis of triglyceride and cell wall[26].

Urea was reported to be able to reduce the inoculum density of *Pythium ultimum, Thielaviopsis basicola* and *Macrophomina phaseolina* by producing ammonia in soil[9]. Sclerotinia rot of chrysanthemum was not reduced when soil treated with urea in this study. This was probably due to the amount of urea used to treat the field soil was low and there was no coverage to stop the ammonia to release to the air from soil. Calcium cyanamide was hydrolyzed and released cyanamide in soil. The production of cyanamide was regarded as the major factor of calcium cyanamide which can be used as a soil fungicide to inhibit soil microorganism[15]. Calcium cyanamide was proven to be able to control sclerotinia rot of chrysanthemum effectively in this study. This chemical reduced the production of apothecium from sclerotium of *S. sclerotiorum,* especially significantly when the soil was wet with high temperature. Consequently, the disease was reduced significantly in this study.

Coniothyrium minitans[16], *Gliocladium virens*[36] and *Sporidesmium sclerotivorum*[5] could penetrate in and destroy the sclerotia of *S. sclerotiorum* and reduced the diseases of sunflower and lettuce, etc. *T. viride* was able to control sclerotinia rot of sunflower when spore suspension of *T. viride* was sprayed in this study. This was due to the capability of *T. viride* to infect the hyphae and sclerotia directly and to produce some inhibitory metabolite(s) to inhibit the growth of *S. sclerotiorum.* Besides *T. viride*, the other species of *Trichoderma,* e.g. *T. koningli, T. harzianum,*

had the capability to destroy all the sclerotia in field within 60 days[32]. *T. viride* and *T. polysporum* were proven to be able to produce trichodermin to inhibit the growth and production of apothecium of *S. sclerotiorum*[25]. Whether the inhibitory substance produced by *T. viride* in this study was trichodermin had not been investigated yet.

Soil-borne *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *lycopersici* were reduced significantly by soil solarization[17]. Transparent thin polyethylene sheet had better effect to raise the soil temperature than black polyethylene sheet[17]. Usmani and Ghaffar [37] regarded that there was no different effect on raising soil temperature by either black or transparent polyethylene sheet when the soil was wet. Black polyethylene sheet was used for soil solarization in this study and was efficiently to reduce the amount of apothecium in the field. This treatment was able to raise the soil temperature to 45-50℃. Sclerotia of this studied pathogen were killed when they were incubated under 45℃ four hours a day for four days. This treatment reduced the initial inoculum significantly. Consequently, the disease on chrysanthemum was reduced in 1986, but not in 1987. This inconsistent result was probably due to that this treatment can not impede the arriving of inoculum (air-borne ascospore) from surroundings or other sources.

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摘　要

吳文希 1991 **向日葵及菊花菌核病的防治**　植保會刊 33:45～55。（台北市國立台灣大學植物病蟲害學系）

Benomyl及DCNA在培養基中可以抑制菌核病菌的生長，同時也可在田間防治向日葵的菌核病；但vinclozolin防治向日葵菌核病的效果比DCNA及iprodione還好，vinclozolin在低濃度下即可完全抑制菌核病菌子囊孢子的發芽，並導致子囊孢子的細胞質流失。當土壤以烏肥處理，存活在土壤中的菌核會因而降低其活性，於是菊花菌核病相隨的就因子囊盤數量的減少而減少。由分解的菌核上所分離到的 *Trichoderma viride*，可以感染菌核病菌，並可以所產生的抑制性物質抑制菌核病菌之生長；將 *T. viride* 的分生孢子噴灑在向日葵上，可以顯著地降低病害嚴重度，但是卻未明顯地增產。利用黑色塑膠布覆蓋土面的太陽能處理，可以顯著地降低子囊盤的數目；因太陽能處理的土面溫度比未處理者高10℃，而土面下5公分處的溫度，處理者也比未處理者高5℃；菌核在潮濕的土壤時，當溫度每天有4小時高達45℃時，連續4天，於是就會喪失活力；所以種在以太陽能處理過之土壤中的菊花，其罹患菌核病的數量顯著地降低。

（關鍵字：菌核病菌、化學防治、生物防治、綜合防治）