

EFFECT OF SOME PHYSIOLOGICAL AND ENVIRONMENTAL FACTORS ON WHITE MOLD OF CUCUMBER CAUSED BY *Sclerotinia sclerotiorum* AND ITS CONTROL BY SOME BIOAGENTS

Khalil, M.N. and Nour-Jehan, M.M. Eisa
Plant Pathology Research Institute, ARC, Giza Egypt

ABSTRACT

Physiological and environmental factors have the most important effect on cucumber white mold development. The disease increased with increasing sclerotial number in soil. The best method for artificial infection with the pathogen was adding sclerotia to the soil followed by sclerotia + mycelia, but rotted plant wastes caused low infection. Disease incidence was not affected by soil type. Sclerotial inoculation of the pathogen two weeks before planting caused more infected plants than the inoculation at the same time of planting. More apothecia were produced in pots with sclerotia placed at 0-3 cm than those buried deeper.

Moreover, using *T. harzianum* at all tested rates highly reduced mycelial growth and sclerotial formation followed by *Bacillus* spp. No 2 and No 1 at the rate 100%. The commercial bioagent Rizo-N (3 g/L) was the best bioagent preparation followed by Plant-Guard at the same rate.

Keywords: White mold, cucumber, *Sclerotinia sclerotiorum*, physiological, environmental and bioagents.

INTRODUCTION

White mold caused by *Sclerotinia sclerotiorum* is a serious and wide spread disease on cucumber in Egypt. Under some physiological factors and favorable environmental conditions the causal organism may be caused serious damage.

Present control include the application of fungicides are not always beneficial and cause environmental pollution. Moreover, some fungicides tolerant or resistant isolates of *S. sclerotiorum* were detected (Bychenko and Simmul 1974, Panda 1985, Sharma and Sharma 1986, Singh and Tripathi 1997). Many researchers reported the effect of some fungi or bacteria as biocontrol agents against cucumber white mold (Baodi *et al.* 1995, Hannusch and Boland 1996, Zizzerini *et al.* 1987).

Using of *Trichoderma harzianum* for biological control of cucumber white mold was recorded by Baodi *et al.* 1995, Cassiolato *et al.* 1996, Gracia Garza *et al.* 1997. Also *Bacillus subtilis* was applied as biocontrol agent by Zizzerini *et al.* 1987.

This research was carried out to determine the effect of some environmental and physiological factors on cucumber white mold incidence, and to study the effect of some biocontrol agents against the disease.

MATERIALS AND METHODS

1. Isolation and identification of the causal pathogen:

Samples of cucumber showing white mold symptoms collected from different localities in Giza, Kalyobia, Ismailia, Menofia and Behera Governorates were cut into small pieces (1 cm), and washed thoroughly with tap water then surface sterilized with sodium hypochlorite solution (5%) for 2 minutes, and washed several times with sterilized water. After drying with sterilized filter papers, the pieces were placed on PDA medium in Petri dishes and incubated at $20^{\circ}\text{C} \pm 1$ for 3-5 days. The fungal growth was microscopically examined and purified by hyphal tip technique (Hensen and Valleu, 1940). The pure isolates were identified according to the morphological characteristics of mycelia, microconidia and germinated sclerotia with apothecial heads.

After pathogenicity test, isolate Kaha (the highly pathogenic isolate) was used for all experiments.

2. Effect of some physiological and environmental factors on white mold incidence:

a. Inoculum potential:

Sterilized Petri dishes contained PDA medium were inoculated by agar disks colonized by the pathogen from 5 days old colony, and incubated at 20°C for one month. Sclerotia were harvested, then sandy peatmoss soil (1:1 w/w) was infested with equal sclerotia at 7 different rates i.e. 5, 10, 15, 20, 25, 30 and 35 sclerotia per 100g of soil. Pots (15 cm in diameter) were filled with the infested soil and planted with cucumber seeds at the rate of 10 ones for each pot. Four replicates were used for each treatment. Four uninoculated pots were served as control. After one month, all plants were uprooted, and data were recorded as percentage of disease infection.

b. Inoculum type:

Four types of *S. sclerotiorum* inoculum were used: mycelia, sclerotia, mycelia + sclerotia and plant wastes rotted by the pathogen.

Sandy clay soil (1:1 w/w) was sterilized and packed in 25 cm formaldehyde sterilized pots. Four pots were infested, before planting with mycelia at the rate of (5 mm) agar dishes, or sclerotia at the rate of 50 equal sclerotia 1kg of soil, or mycelia + sclerotia at the same rate mentioned before, or plant wastes rotted by the pathogen at rate 5% of soil weight. Ten seeds of cucumber were planted in each pot. Four disinfested pots were served as control.

The pots were kept under a moist chamber (85-95% R.H. and 18-22°C) in a greenhouse for the first 24 hrs. before being uncovered. The percentage of disease infection was recorded after 30 days from planting.

c. Soil type:

Sandy, clay and sandy clay soil (1:1 w/w) were sterilized using 5% formalin solution and left 3 weeks before infesting. The soil was packed in formalin sterilized pots. Four replicates containing one of the soil types soil types were inoculated with the pathogen sclerotia at rate of 5% grown on

corn sand medium for 20 days. Each pot was planted with 10 seeds of cucumber Beit Alpha variety. Four pots filled with sterilized soil were used as control. The pots were regularly watered once for 4 days. Percentages of disease incidence were recorded after one, two and three months of planting.

d. Inoculation date:

Sandy clay soil (1:1 w/w) and pots (25 cm diameter) were sterilized as mentioned before. The pathogen was grown on moistened sterilized carrot medium for 20 days at 20°C to heavy sclerotial production. Soil infestation was carried out using 50 equal sclerotia/kg of soil at different dates, i.e. 2 and 1 weeks before planting and at the same time of planting. Inoculum was mixed thoroughly with the soil in each pot. Four pots were filled with the same amount of sterilized carrot medium and used as control. Ten healthy seeds of cucumber, Beit Alpha var. were planted in each pot at the depth of 2cm and watered as needed under greenhouse conditions (20-25 °C and 90% R.H.). Four replicates were used for each treatment. The percentage of infection was recorded after 30 and 60 days from planting.

e. Inoculum burial depth:

Sterilized sandy peatmoss soil (1:1 w/w) was packed in pots (10 cm diameter). Ten sclerotia, 6 months old and equal in weight, were placed on soil surface in each pot or buried at different depths from soil surface, i.e. 3, 5, 7, 9 and 11 cm. Four replicates were used for each treatment. Pots were kept under greenhouse conditions (15-22 °C) and irrigated regularly to keep soil moisture at 40% of water holding capacity during the experimental period (Keep and Kowski 1921). Percentages of carpogenic sclerotial germination were recorded after 7 weeks from soil infestation.

3. Biological control:

a. The antagonistic effect of *T. harzianum* and *Bacillus* spp. (N. 1 and 2) against *S. sclerotiorum* under lab. conditions:

1. Interaction between the pathogen and the biocontrol agents on PDA medium:

Trichoderma harzianum and *S. sclerotiorum* were grown in sterilized Petri dishes contained PDA medium, but *Bacillus* spp. (No 1 and 2) were grown in sterilized Petri dishes contained soil extract agar medium (Lochhead, 1940). Dishes were incubated at 20°C for 4-7 days. PDA medium was poured in sterilized Petri dishes and seeded on one side with 4mm, in diameter, discs of *T. harzianum* or *Bacillus* spp. (No. 1 or 2) culture. Similar discs of the pathogen were placed on the opposite side of the Petri dishes. Four replicates were used for each treatment. Cultures were incubated at 20°C for five days, then the type of interactions were classified as suggested by Sabet and Khan (1969).

2. Effect of biocontrol agents culture filtrate on mycelial growth and sclerotial formation of *S. sclerotiorum*:

The biocontrol agents, *T. harzianum* and *Bacillus* spp. (No. 1 and 2) were grown in conical flasks (500 ml) contained 200 ml potato dextrose liquid medium. Flasks were incubated at 20°C. After 9 days, cultures were centrifuged for 10 minutes at 3000 rpm to separate fungal and bacterial growth. Filtrates were then sterilized using Seitz's filter.

Sterilized filtrates were poured in sterilized PDA medium in Petri dishes at four rates i.e. 25, 50, 75 and 100% in five Petri dishes at four rates, i.e. 25, 50, 75 and 100%. Different dishes were inoculated with 4 mm diameter discs obtained from 4 days old colony of *S. sclerotiorum*. Five dishes, without any culture filtrate, were inoculated with the same discs of the pathogen and used as control. Inoculated and non-inoculated dishes were incubated at 20°C for 10 days. When mycelial growth of the pathogen covered control dishes, the percentages of mycelial growth of different treatments were recorded. The percentage of sclerotial formation were also, reported after two weeks from inoculation.

b. The antagonistic effect of some commercial biocontrol agents against cucumber white mold under greenhouse conditions:

Three commercial bioagents were used: Plant Guard (*T. harzianum*) 30×10^6 cells/ml at different rates (3, 2, 1 ml/L). Promot (*T. harzianum* and *T. konigii*, 50×10^6 cells/g) at rate 10 g/L, and Rizo-N (*Bacillus subtilis*, 30×10^6 cells/g) at rates (4, 3, 2 g/L). In addition, Ronilan 50% WP (150 g/100L) was used as a check.

Light clay soil was inoculated with *S. sclerotiorum* as mentioned before. Cucumber seeds (Beit Alpha var.) were treated with bioagents and Ronilan, then planted in infested soil. Each treatments contained 4 replicates. Four replicates contained infested soil planted with untreated seeds were served as control. Survival plants percentage were recorded after 90 days for planting.

RESULTS

A. Effect of some physiological and environmental factors on white mold incidence:

1. Inoculum potential:

Data obtained in Table (1) indicate that the white mold infection on cucumber plants gradually increased by increasing sclerotial numbers in soil. It reached its maximum at 35 sclerotia/100g soil (62.50% infection) and its minimum by using 5 sclerotia/100g soil (6.25% infection).

Table (1): Effect of different sclerotial inoculum levels of *S. sclerotiorum* on cucumber white mold under greenhouse conditions.

Sclerotial number/100 g soil	Infection %
0.0	0.00
5	6.25
10	12.50
15	18.75
20	25.00
25	37.50
30	50.00
35	62.50
L.S.D. at 5%	5.06

2. Inoculum type:

Data presented in Table (2) clear that the best method for artificial infection with *S. sclerotiorum* was sclerotia (40% infection) after 60 days from planting, followed by mixture of sclerotia and mycelia (35% infection), while using the fungus mycelium gave 25% infection. On the other hand, inoculation with the rotted plant wastes gave only 10% infection. While, no significant differences were noticed between all inoculum type treatments, after 90 days from planting.

Table (2): Effect of inoculum type of *S. sclerotiorum* on cucumber disease infection under greenhouse conditions.

Inoculum type	Infection %	
	After 60 days	After 90 days
Sclerotia	40.0	50.0
Mycelia	25.0	55.0
Sclerotia + Mycelia	35.0	65.0
Rotted plants waste	10.0	50.0
Check	0.0	0.0
L.S.D. at 5%	9.4	17.2

3. Soil type:

No significant differences were noticed between disease infection in different soil types after 60 days or 90 days from planting Table (3). The infection in all treatments increased with increasing plant staying in greenhouse.

Table (3): Effect of soil type on disease infection of *S. sclerotiorum* on cucumber under greenhouse conditions.

Soil type	Disease infection % after		
	1 month	2 months	3 months
Sandy	32.5	37.5	65.0
Sandy + Clay	37.5	42.5	67.5
Clay	42.5	45.0	75.0
L.S.D. at 5%	1.24	1.8	2.4

4. Inoculation date:

As mentioned in Table (4), no significant differences between all inoculation dates were noticed after 30 days or 60 days from planting, although there were clearly differences between sclerotial inoculation 2 weeks before planting treatment, after 30 days or 60 days from planting (25% and 45% infection respectively), and at the same time of planting treatment (7.5% and 20% infection after 30 days or 60 days from planting, respectively).

Table (4): Effect of inoculation date with *S. sclerotiorum* sclerotia on cucumber white mold infection under greenhouse conditions.

Sclerotial inoculation date from planting	Infection %	
	After 30 days	After 60 days
Before 2 weeks	25.0	45.0
Before 1 week	17.5	40.0
At the same time	7.5	20.0
Control	0.0	0.0
L.S.D. at 5%	2.1	3.08

5. Inoculum burial depth:

Data presented in Table (5) clear the significant differences between burial depth of the sclerotia and its percentage of germination. The sclerotial carpogenic germination decreased by increasing the burial depth (100% germination at the soil surface and 2.5% only at 11 cm under surface).

Table (5): Relation between burial depth of sclerotia in soil and sclerotial carpogenic germination under greenhouse conditions.

Burial depth (cm)	Carpogenic sclerotial gemination %
0.0	100.0
3.0	85.0
5.0	62.5
7.0	35.0
9.0	17.5
11.0	2.5
L.S.D. at 5%	15.5

B. Effect of some biocontrol agents on *S. sclerotiorum* development and white mold incidence:

1. The antagonistic effect of *T. harzianum* and *Bacillus* spp. (No. 1 and 2) against *S. sclerotiorum* under lab conditions:

a. Interaction between the pathogen and the biocontrol agents mycelial growth on PDA medium:

The interaction types were: Growth around the contender organism (G.A.C.O) between the pathogen and *Trichoderma harzianum*. Mutual inhibition at a considerable distance (M.I.) between pathogen and *Bacillus* spp. isolates.

b. Effect of biocontrol agents culture filtrate on mycelial growth of *S. sclerotiorum*.

Data in Table 6 show that using *T. harzianum* at the tested rates highly reduced the mycelial growth of the pathogen. It was followed by *Bacillus* spp. No. 2 and 1 at the rate of 100%, respectively.

Table (6): Effect of biocontrol agent culture filtrates on mycelial growth of *S. sclerotiorum*.

Treatment	Mycelial growth % at different rates of culture filtrate of biocontrol agent			
	25	50	75	100
<i>Bacillus</i> sp. No. 1	9.0	7.0	5.0	3.0
<i>Bacillus</i> sp. No. 2	8.0	6.0	4.9	3.0
<i>T. harzianum</i>	4.0	2.0	0.0	0.0
Control	9.0	9.0	9.0	9.0
L.S.D. at 5%	2.7			

c. Effect of biocontrol agents culture filtrate on sclerotial formation of *S. sclerotiorum*.

Data in Table 7 show that *T. harzianum* culture filtrate highly decreased sclerotial formation at all tested rates and followed by *Bacillus* spp. No. 2 and No. 1 at the rate of 100% and 75% respectively.

Table (7): Effect of biocontrol agent culture filtrates on sclerotial formation of *S. sclerotiorum*.

Treatment	Sclerotial formation % at different rates of culture filtrate of biocontrol agent			
	25	50	75	100
<i>Bacillus</i> sp. No. 1	32.3	30.0	27.0	22.5
<i>Bacillus</i> sp. No. 2	31.5	28.3	22.5	18.0
<i>T. harzianum</i>	18.0	12.0	0.25	0.0
Control	35.3	35.3	35.3	35.3
L.S.D. at 5%	8.9			

2. The antagonistic effect of some commercial biocontrol agents against cucumber white mold under greenhouse conditions

Data obtained in Table (8) reveal that using Rizo-N at the rate 3g/100 L gave survival plants 75%, while the fungicide Ronilan (1.5 g/L) gave 65%. At the same time all other tested bioagents gave less survival plants: Plant Guard (3 ml/L) 62.5% and Promot (10 g/L) 57.5%.

Table (8): Effect of some commercial biocontrol agents against cucumber white mold under greenhouse conditions.

Treatment	Survival plants%
Promot (10 g/L)	57.5
Rizo N (4 g/L)	70.0
Rizo N (3 g/L)	75.0
Rizo N (2 g/L)	57.5
Plant Guard (3 ml/L)	62.5
Plant Guard (2 ml/L)	60.0
Plant Guard (1 ml/L)	42.5
Ronilan (1.5 g/L)	65.0
Control	20.0
L.S.D. at 5%	9.3

DISCUSSION

White mold caused by *S. sclerotiorum* is considered as one of the most serious diseases affecting cucumber plants in Egypt. The effect of some environmental factors on *S. sclerotiorum* development and disease incidence were studied.

As for inoculum potential the results indicated the correlation between white mold incidence and density of *S. sclerotiorum* sclerotia. The infection gradually increased by increasing sclerotial numbers in soil. This was agreed with Akazawa and Ono (1984); and Ghanim (1993). Also, Nelson *et al.* (1989) reported that inoculum density of the pathogen is one of the most important factors affecting progress of Sclerotinia wilt of sunflower. This may be due to increase oxalic acid production from sclerotia. Lumsdon (1979), Noyes and Hancock (1981) reported that oxalic acid is a toxin produced by *S. sclerotiorum* in sunflower and other crops.

At the same time, the data revealed that the best method for inoculation with the pathogen was adding the sclerotia to soil followed by inoculation with a mixture of sclerotia and mycelia. This was agreed with Newton and Sêqueira (1972) who reported that *S. sclerotiorum* mycelium had very low competitive saprophytic ability in soil. Also, Steadman (1975) recorded that infection with *S. sclerotiorum* mycelium was unimportant in the initiation of white mold in the field. But, Chaves *et al.* (1996) showed that dried mycelium of *S. sclerotiorum* was infective when deposited near the stem collar of young plants of soybean. The same data recorded that wastes gave low percentage of infection. This may be due to long stay of *S. sclerotiorum* growth on the rotted plant wastes before extension to any new plant tissues. Also, sclerotis in the inner part rotted wastes may be under unfavourable germination conditions, as a result in nutrient depletion and rupture of the outer layers of the sclerotium, which may allow entry of soil-borne microorganisms reduce the ability of sclerotia to survive, Merriman (1979).

Effect of soil types on white mold disease incidence was also tested. Sandy soil was the best in reducing disease infection than sandy clay or clay soil after 1, 2 or 3 months from inoculation. In this respect, Abarvi and Gorgan (1975), reported that apothecia of *S. sclerotiorum* were produced only in moisture saturated or near saturated soil than in sandy loam one (a mixture of sand and clay). Mitchell, *et al.* (1990) suggested that the low number of apothecia produced by sclerotia in sandy soil may be due to nutrient depletion to a level which cannot sustain the development of the apothecium. Also, no significant differences were noticed between the percentage of infection in all treatments of inoculation data after 30 or 60 days from planting. But, mean of infection clear a difference between sclerotial inoculation 2 weeks before planting (25%) and sclerotial inoculation at the same time of planting (7.5%). This was agreed with the results obtained by Honda and Yunoki (1977) who reported a few weeks after the irrigation to *S. sclerotiorum* sclerotia germinate and produce the sexual stage.

On the other hand, the relation between sclerotial germination and burial depth of *S. sclerotiorum* sclerotia in soil was highly significant. Most carpogenic sclerotial germination were produced from sclerotia buried 0 or 3 cm deep (100% and 85%). This result may be due to formation of a long stipe to reach the soil surface [the maximum stipe length recorded by Cook *et al.* (1975) was 6 cm], also possibly depletion of food reserves by apothecia and the damage caused by soil-borne microorganisms reduce the ability of sclerotia to germinate.

The effect of some biocontrol agents were also studied. Data cleared that a mutual inhibition was observed when *Bacillus* spp. No. 1 or 2 was placed with *S. sclerotiorum*, while growth around the contender organism was noticed when *T. harzianum* was placed with the studied fungus. In this respect, Cassiolate *et al.* (1996) concluded that the mutants of *T. harzianum* on regments of celery were able to reduce the mycelial growth of *S. sclerotiorum*.

Also, the effect of *T. harzianum* or *Bacillus* spp. No. 1 or 2 culture filtrate on mycelial growth and sclerotial formation of the pathogen were tested.

The obtained results indicated that *T. harzianum* culture filtrate at all the tested rates highly decreased *S. sclerotiorum* mycelial growth, followed by *Bacillus* spp. No. 2 or 1 at the rate of 100%. This agrees with Gracia-Graza *et al.* (1973) who reported that *T. harzianum* TMCS₃ was capable to inhibit mycelial growth of *S. sclerotiorum* in lab and pot tests. Yuen *et al.* (1997) showed that *Bacillus polymyxa* strain B8 inhibited mycelial growth of the same pathogen. Many authors recorded that the antagonistic fungus *T. harzianum* was the most effective in controlling many pathogenic fungi. This may be due to the production of polypeptide which act as inhibitor to plant pathogenic fungi (Kao and Hsieh, 1989), or directly attached fungal mycelium either by hyphal coils, hooks or appressoria (Elad *et al.*, 1983).

Moreover, using culture filtrates of *T. harzianum* at all the tested rates gained more reduction on sclerotial formation, followed by *Bacillus* spp isolate No. 2 at the rate of 100% and 75% respectively. In this respect, Jones *et al.* (1974) found B-1, 3-glucanase in culture filtrate of *Trichoderma* spp., which degrades and lyses sclerotial tissue.

The results also clear that using the commercial bio-preparation, Rizo-N (*Bacillus subtilis*) for the disease control gave higher average percentage of survival plants (75%), while the fungicide Ronilan gave 65% only. Pusey and Wilson (1984) recorded the post harvest control of stone fruit brown rot by *B. subtilis*. They suggested that a heat antibiotic was interfering with spore germination or early germ tube development Karyala *et al.* (1993) reported that a chitinolytic strain of *Bacillus* (Au 192) was identified that inhibited all fungi tested. The antifungal inhibition was correlated with chitinase activity of the bacterium.

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تأثير بعض العوامل الفسيولوجية والبيئية على العفن الأبيض في الخيار المتسبب
عن الفطر أسكليروتينيا سكليروتورم وتأثير بعض العوامل الحيوية في مقاومته
محمد نجيب خليل، نورجيهان محمد عيسى
معهد بحوث أمراض النباتات - مركز البحوث الزراعية - الجزيرة - مصر

بعض العوامل الفسيولوجية والبيئية لها تأثير مهم على مرض العفن الأبيض في الخيار ، فقد وجد أن زيادة عدد الأجسام الحجرية في التربة تؤدي إلى زيادة حدوث المرض وأن أفضل طريقة للمدوى الصناعية بالمرض هي استخدام اسكلوروشيات الفطر ويليها استخدام الاسكلوروشيات + الميسيليوم. أما استخدام بقايا النباتات المصابة تؤدي إلى قلة حدوث الإصابة. أما اختلاف نوع التربة فليس له تأثير على حدوث الإصابة. ولكن حقن الاسكلوروشيات في التربة قبل الزراعة بأسبوعين أدت إلى زيادة نسبة النباتات المصابة عن تلك المحقونة في نفس يوم الزراعة.

عمق وجود الإسكليروشيات على مستويات مختلفة من سطح التربة له تأثير كبير في تكوين الأجسام الثمرية للفطر. فالإسكليروشيات الموجودة على سطح التربة إلى عمق 3 سم تعطي كثيراً من الأجسام الحجرية عن تلك المنقولة في عمق أكثر.

أما المقاومة الحيوية فأنبتت أن ترايكوندوما هيرزبانوم أدت إلى تثبيط نمو ميسيليوم الطفيل وكذلك تكوين إسكليروشيات في جميع التركيزات المستخدمة ، يليها الباسيليوس رقم 2 ورقم 1 عند تركيز 100%. أما استخدام عامل المقاومة الحيوي التجاري رايزون-بمعدل 3جم/لتر كان أفضل التجهيزات التجارية يليه بلانت جارد في نفس التركيز.