BIOLOGICAL CONTROL OF WHITE ROT OF CUCUMBER CAUSED BY *SCLEROTINIA SCLEROTIORUM* UNDER GREENHOUSE CONDITIONS

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Abstract

Single isolates of each of four species of *Trichoderma* and two species of *Gliocladium* and two isolates of each of *Bacillus subtilis* and *Pseudomonas fluorescens* were tested for the control cucumber stem rot disease caused by *Sclerotinia sclerotiorum*. All tested isolates significantly inhibited the radial growth of the fungus. The maximum inhibition was induced by *T. hamatum* (80.34%), *G. virens* (79.54%) and *T.viride* (79.31%) respectively, while *G. deliquescens* was the least effective (73.83%). The two isolates of. *P. fluorescens* (1,2) reduced the radial growth of the pathogen more than *B. subtilis* did .Culture filtrate of each species of *Tichoderma, Gliocladium* and bacteria significantly reduced the mycelial growth and sclerotia formation . Soil treatment with *T.hamatum, G. virens or B.subtilis* gave the maximum protection against the fungal infection followed by *T.viride* and *P. fluorescens.* Moreover, this treatment improved plant height and increased number of flowers as well as fruit yield. The biocontrol agents tested were nearly as effective as the fungicide Topsin M70 .The seedling treatment with the bioagents was less effective than soil treatment in two seasons.

INTRODUCTION

White rot caused by *Sclerotinia sclerotiorum* is one of the most serious diseases attacking cucumber under protected agriculture and field conditions in Egypt It is a widespread disease in many crops and may lead to great yield losses . Biocontrol agents and their effects in reducing disease incidence and severity have opened promising avenues for practical application in agriculture and environmental safety(Boland, 1990). Dhiman (1997) evaluated 10 strains of *T. harzianum, T. viride, T. aureoviride, T. hamatum and G. virens* against *S. sclerotiorum* on lettuce. All the tested antagonists, however, inhibited the pathogen, with *T. harzianum*, and *T. viride* followed by *G. virens* being the most effective .Menendez and Godeas (1998) showed a significant reduction in the number of germinated sclerotia of *S. sclerotiorum* in *Trichoderma harzianum* treated soybean roots. Gerlagh *et al.* (1999) found that *Trichoderma* spp. suppress *S. sclerotiorum* on potato, bean ,carrot and cucumber and that *Coniothyrium minitas* infected at least 90% of the fungus sclerotia on treated crops by the end of the season. Chang *et al.* (2002) found that 36 isolates of

Trichoderma showed a strong inhibitory effect on *S. sclerotiorun in vitro.* Phookan and Chaliha (1997) reported that mycelium and sclerotia of *S. sclerotiorum* were significantly suppressed by *B.subitilis, G. virens* and *T. viride in vitro.* Duncan *et al.*(2002) found that *Pseudomonos* spp. reduced disease severity of Sclerotinia head rot in sunflower . Savchuk and Fernando (2002) tested 4 antagonistic bacterial strains against *S.sclerotiorum* on canola and found that the treatments gave complete disease suppression.

The present work aimed to evaluate the efficacy of some isolates of *Trichoderma* and Gliocladium sp. as well as *Bacillus subtilis and Pseudomonas fluorescens* in controlling white rot disease caused by *S.sclerotiorum* on cucumber under greenhouse conditions.The effect of these bioagents on plant growth and fruit yield was also considered.

MATERIALS AND METHODS

Bioagents used and the causal pathogen :

The bioagents tested included 4 isolates belonging to four species of *Trichoderma (T.hamatum, T.harzianum, T.koningii and T.viride),* two isolates of *Gliocladium* sp. (*G.virens & G.deliquescens)* and 4 isolates of bacterial bioagents namely *Bacillus subtilis* (1&2) and *Pseudomonas fluorescens* (1&2). These isolates were previously identified by Plant Pathology Department Staff, Fac. Agriculture, Minufiya University. The causal pathogen was isolated from diseased cucumber plants showing typical symptoms of sclerotinia rot. Samples were taken from greenhouses at Tokh, where the disease usually causes severe damage, limiting the production of cucumber. The Isolated fungal cultures were identified as *S. sclerotiorum* at the Department of Mycology, Plant Pathology Research Institute, Agricultural Research Center, Giza , Egypt.

Antaganistic effect *in vitro*

The antagonistic effect of the used antagonists against an isolate of *S. sclerotiorum in vitro* was examined on Petri plates (9cm) containing PDA medium. A disc (6mm), from three day-old culture of each antagonist, was transferred to one side of the Petri plates containing solidified PDA medium and the other side was inoculated with a mycelium disc (6mm) taken from the edge of a three day-old culture of *S. scelrotiorum.* Four plates were used for each particular antagonist and four inoculated with the pathogen served as control. The inoculated plates were incubated at 25°c for 7 days. The antagonistic effects of *B.subtilis* (1&2) and *P.fluorescens* (1&2) were tested by streaking-2cm long streak on the other side of the pathogen disc. Four plates inoculated with the pathogen only served as control and four replicates were used for each treatment. Percentage of inhibition of the pathogen was calculated according to the formula of Kucuk and Kivanc (2003).

Effect of biocontrol agent culture filtrates on mycelial growth and sclerotial formation of *S.sclerotiorum* :

The antagonistic fungi, *T. hamatum, T. harzianum, T. koningii, T. viride, G.virens and G. deliquescens* and the antagonistic bacteria, *i.e. B.subtilis(1)* and *P. fluorescens* were grown in Potato Dextrose Broth (PDB) or King's medium in 250ml flasks each containing 100ml medium for 7 days at 25ºC. Culture filtrate was prepared by double filtration through filter paper under sterilized conditions. Then, the filtrate was centrifuged at 3000 rpm for 20 min The clear supernatant mixed with PDA medium at different concentrations i.e [0,10,25,50,75% of the medium ,v/v] was poured in five Petri plates and inculated with 9mm mycelial disc obtained from 7 days old colony of *S. sclerotiorum*. Five plates without any culture filtrate served as control.The average reduction in mycelial growth and number of sclerotia formed in different treatments was calculated .

Greenhouse experiment :-

Assessment of biocontrol potential of the bioagents tested was carried out in the greenhouse at Tokh, Khalubia governorate having a disease history of white rot employing either soil or seedling treatment.

1- Soil treatment:-

Inocula of the antagonistic fungi and bacteria were prepared on rice hulls-sand medium Each antagonist was mixed with the soil at the rate of 3%(w/w) before transplanting.

2- Seedling treatment :-

Culture filtrate of each biocontrol agents was prepared as mentioned before. Cucumber seedlings (15- days old) Delta Star cv. were soaked into culture filtrate of each biocontrol agent for 30 minutes. The fungicide Topsin M 70 was used to treat seedlings at the rate of 3gm/l for 15 minutes. Seedling were transplanted in rows on two ridges, the distance between transplants being 50 cm. Each treatment was replicated in 4 randomized experimental plots. Each plot measured 10 m^2. Data were recorded as percentage of wilted plants after transplanting, plant height(m), number of flowers and fruit yield (kg/plot) .

Disease assessment:

The percentange of disease incidence was determined according to the formula:

$$D.I = \frac{\text{No .of dead seedling /plot}}{\text{No. of transplants /plot}} X100$$

RESULTS AND DISCUSSION

1- Antagonitic effect on the mycelial growth of the causal pathogen:-

Data presented in Table (1) and Fig.(1,2 and 3) show that the ten isolates of antagonists tested significantly inhibited the radial growth of *S.sclerotiorum*, when compared with the control. *Trichoderma hamatum, T. viride* and *T. koningii* grew over the pathogen mycelium. The highest inhibition zones for the fungal radial growth were induced by *T. hamatum G. virens, T. viride T. harzianum, T. koningii and G. deliquescens.* They resulted in inhibitions of 80.34, 79.54, 79.31, 78.06, 77.71 and 73.83%, respectively. Bacteria recorded less effect in this respect, where the values of inhibition were 69.26 and 61.37% for *P.fluorescens* (1and 2), respectively, while the inhibition percentages caused by *B. subtilis* isolates were less than *P. fluorescens* recording 55.89 and 44.91 % for *B.subtilis 1and 2.* These findings were somewhat similar to those reported by Paulitz & Belanger(2001) and Chang *et al.* (2002) . They found that *Trichoderma* sp., *Gliocladium* spp. and *Bacteria* (*B.subtilis* and *P.fluorescens*) showed strong antagonistic activity against *S.sclerotiorum* growth.. The bioagents may inhibit the radial growth of the pathogen thuorgh different mechanisms such as mycoparasitism, antibiosis, lysis of the pathogen and competition for nutrients.

Table 1. Effect of biocontrol agents on the radial growth of *S. sclerotiorum in vitro.*

Biocontrol agents	Radial growth (cm)	Inhibation (%)
T.hamatum	1.72	80.34
T.harzianum	1.92	78.06
T.koningii	1.95	77.71
T.vride	1.81	79.31
G.virens	1.79	79.54
G.deliquescens	2.29	73.83
B.subtilis(1)	3.86	55.89
B.subtilis(2)	4.82	44.91
P.fluorescens(1)	2.69	69.26
P.fluorescens(2)	3.38	61.37
Control	8.75	00.00
L.S.D. at 5%	1.20	-----

T.=Trichoderma *G.=Gliocladium*

B.=Bacillus *P.=Pseudomonas*

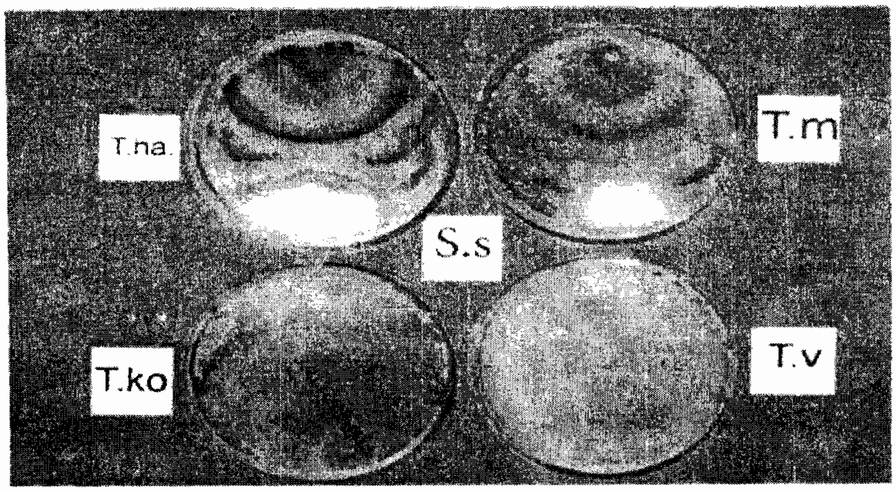

Fig 1. Antagonistic effect of *T. hamatum*(T.m), *T. harzianum(T.ha)* and *T. koningi* (T.ko) and *T. Viride(T.v)* on *S. sclerotiorum in vitro.*

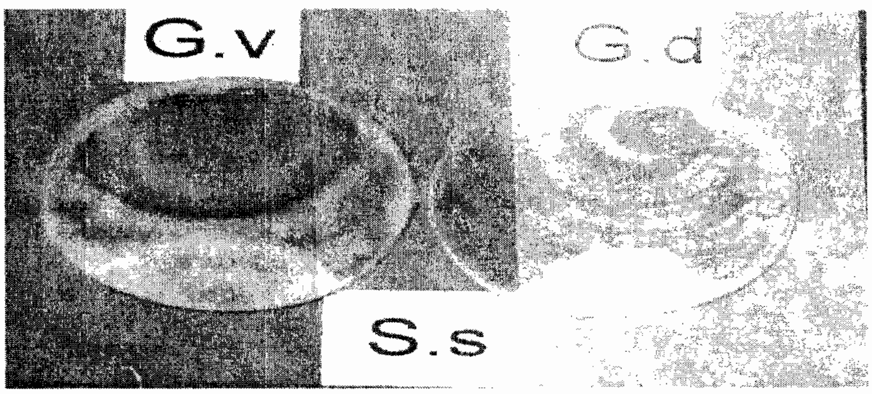

Fig 2. Antagonistic effect of *G. virens(G.* v) and *G. deliquscens(G.d)* on *S. sclerotiorum in vitro*

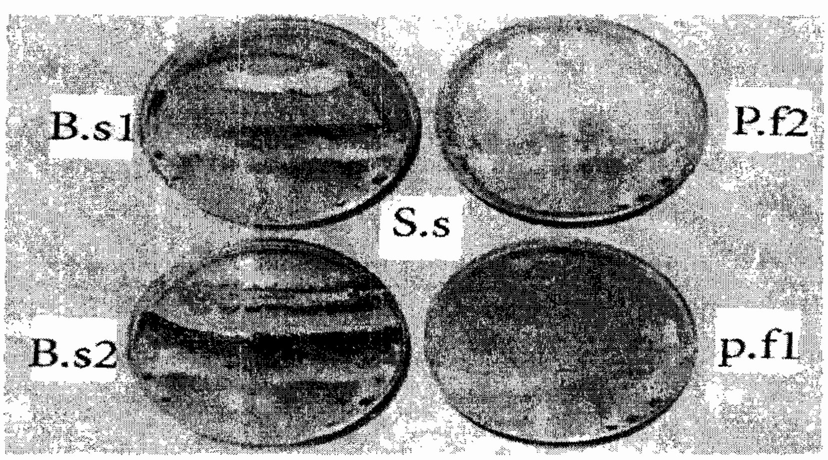

Fig. 3. Antagonistic effect of *B. subtilis* (1) (B.s1), *B. subtilis* (2) (B.s 2), *P. fluoresccens* (1) (P.f1)and *p.fluorescens* (2)(P.f2) on *S. sclerotiorum in vitro.*

2- Effect of bioagents culture filtrates on mycelial growth and sclerotia formation of the pathogen:

Culture filtrate of each of *Trichoderma* , *Gliocladium* and bacteria significantly reduced the mycelial growth and sclerotial formation. There was an inverse releationhip between the concentrations of culture filtrate and the mycelial growth or sclerotia formation.

Data presented in table (2) indicate that the most inhibitive bioagents on mycelial growth and sclerotia formation of the pathogen were *T.hamatum* and *G.virens* at 75% concentrations, where complete inhibition of the mycelial growth and sclerotia formation was observed. Culture filtrate of *T.viride, B.subtilis 1, T.harzianum and P.fluorescens 2* at 75% concentration followed in efficacy Chaliha (1997) and Savchuk &Fernando (2002) stated that mycelial and sclerotial growth of *S.sclerotiorum* were significantly suppressed by *B.subtilis*, *G.virens* and *T.viride in vitro*. They also found that culture filtrates of *Trichoderma, Gliocladium* and bacteria contained various toxic substances and antibiotics such as trichodermin, gliotoxin, subtilin, fluorescent pigments and phenolic compounds which were responsible for their toxicity against mycelial growth and sclerotia formation of *S.sclerotiorum* .

Greenhouse experiment :

1- Effect of some bioagents on white rot disease incidence:

Data presented in Table (3) indicate that soil treatment with the bioagents gave higher protection against the disease than did the seedling treatment in both seasons of the experiment. This may be attributed to the fact that the antagonist colonized larger volume of the soil, consequently reaching more propagules of the pathogen whose population is reduced. Moreover, adding *T.hamatum,G. virens and B. subtilis* to soil increase the percentages of survivals to 97.5 -100%, 97.5-100% and 95-100% respectively . Soil treatment with *T.viride* resulted in 87.5-90% survival while, *P.fluorescens* led to 85-90% survival compared to Topsin M at 90-95%. *G.deliquscens* was the less effective resulting 75% survival. These results are in accordance with those obtained by Sharma *et al.* (1999), who found that *T.harzianum* and *Absidia cylindrospora* were most effective in inhibiting mycelial growth of *S.sclerotiorum* causing chickpea stem rot. Application of *T.harzianum* to the soil, a week before sowing, reduced disease incidence in the field as compared to the application at sowing time. Duncan *et al.*(2002)and Savchuk & Fernando (2002) found that the antagonistic *Bacillus* spp. and *Pseudomonas* spp. reduced disease incidence of *S.sclerotiorum* stem rot of cucumber,pototo,carrot, chicory and bean.

2- Effect of the bioagents on the plant height, flowering and fruit yield:

The effect of antagonists on plant growth and fruit yield are presented in Table (4). Soil treatment with the antagonists increased the plant height, flowering and fruit yield more than the seedling treatment in both seasons 2004 and 2005. Data also revealed that treating the soil with *G.virens,B.subtilis* and *T.hamatum* was the most effective in improving the plant height from 1.75 to 2.95,2.95 and 2.90m, respectively. The number of flowers per plant increased from 50.30 to 87.73, 87.20 and 86.94 and the fruit yield increased from 79.80 to 145.37, 144.69 and 141.96 kg/plot, respectively . On the other hand, *G.deliquscens* was the least effective. *P.fluorescens, T.viride , T.harzianum and T.koningii* were intermediate. The improvement of these parameters could be due to the control of the pathogen, on one hand, and the possible change in metabolic behavior of the plant itself and to the effect of some growth promoting substances possibly produced by the antagonists (Paulitz and Belanger, 2001), on the other hand. The results obtained in 2004 are similar to those of 2005. Several possible mechanisms have been suggested to explain this phenomenon of increased plant growth. The biological control agents can produce plant hormone substances and vitamins that increase plant length and yield. Also, conversion of non available materials to available forms for plant uptake and translocation of minerals (Inbar *et al.* 1994). Many organisms produce Indole-3-acetic acid (IAA), phytohormons, gibberellic acid like substances (GA3) in culture media and caused significant increases in shoot, root and yield of many crops (Haggag, Wafaa, 1997 and Green *et al.*, 2001). These results are in agreement with those obtained by Marten *et al.* (1999) who found that treating cucumber in plant with *P.fluorescens* and *B.subtilis* cause significant increases plant growth and number of fruits per plant of different cultivated (sunflower, cucumber and cabbage) and ornamental plants. They suggested that, such isolates may be grouped as plant growth promoting rhizobacteria (PGPR). Mohamed (2005) found that soil and seedling treatments with the antagonists *Trichoderma* spp,*Gliocladium* spp. and bacteria significantly increased plant height, flowering, fruit setting and the yield of cucumber plants. This was attributed probably the better plant growth parameters as a results of disease control and the possible direct effect of antagonists metabolites. Furgo et al. (1997) found that *T.viride and T.harzianum* increased shoot and root length and fruit yield and decreased wilt incidence.

In conclusion, effective biocontrol agents could be used to reduce infection and disease severity caused by *Sclerotinia sclerotiorum* in cucumber under greenhouse condition.

Table 2. Effect of biocontrol agent culture filtrates on mycelial growth (cm) and number of sclerotia of *S. sclerotiorum* .

Treatments	Concentration of culture filtrate%									
	0		10		25		50		75	
	Mycelial growth (cm)	No of Sclerotia	Mycelial growth (cm)	No of Sclerotia	Mycelial growth (cm)	No of Sclerotia	Mycelial growth (cm)	No of Sclerotia	Mycelial growth (cm)	No of Sclerotia
T.hamatum	8.90	42.5	3.22	22.5	2.42	18.2	1.64	5.5	0.0	0.0
T.harizanum	8.90	42.5	4.15	28.0	3.28	25.0	2.19	14.0	0.92	4.5
T.koningii	8.90	42.5	4.50	30.5	3.70	28.5	2.48	12.0	1.18	7.0
T.viride	8.90	42.5	3.42	24.0	2.89	23.0	1.87	10.5	0.75	3.5
G.virens	8.90	42.5	3.89	26.5	2.73	21.0	1.69	7.0	0.0	0.0
G.deliquescens	8.90	42.5	4.78	34.0	3.96	30.5	2.98	19.0	1.62	8.0
B.subtilis(1)	8.90	42.5	4.65	32.0	3.78	27.0	2.12	16.0	0.86	5.5
P.fluorescens(2)	8.90	42.5	4.86	36.5	3.98	34.0	2.67	21.5	0.98	6.0
L.S.D. at 5%	N.S.	N.S.	0.42	3.40	0.32	4.14	0.25	3.87	0.25	2.45

T. = *Trichoderma*
G. = *Gliocladium*

B. = *Bacillus*
P. = *Pseudomonas*

Table 3. Effect of soil and seedling treatment with biocontrol agents and Topsin M on cucumber damping-off caused by *S.sclerotiorum* under greenhouse conditions.

Treatments	Soil treatment				Seedling treatment			
	2004		2005		2004		2005	
	Disease incidence (%)	Survival (%)	Disease incidence (%)	Survival (%)	Disease incidence (%)	Survival (%)	Disease incidence (%)	Survival (%)
T.hamatum	2.5	97.5	0.0	100.0	12.5	87.5	10.0	90.0
T.harzianum	15.0	85.0	12.5	87.5	20.0	80.0	12.5	87.5
T.koningii	17.5	82.5	12.5	87.5	20.0	80.0	17.5	82.5
T.viride	12.5	87.5	10.0	90.0	15.0	85.0	12.5	87.5
G.virens	2.5	97.5	0.0	100.0	12.5	87.5	7.5	92.5
G.deliquescens	22.5	77.5	17.5	82.5	30.0	70.0	25.0	75.0
B.subtilis	5.0	95.0	0.0	100.0	15.0	85.0	7.5	92.5
P.fluorescens	15.0	85.0	10.0	90.0	17.5	82.5	12.5	87.5
Topsin M 70	10.0	90.0	5.0	95.0	10.0	90.0	10.0	90.0
Control	62.5	37.5	68.5	31.5	68.5	31.5	70.0	30.0
L.S.D. at 5%	4.5	4.8	6.4	6.8	7.1	7.4	6.2	8.2

T. = *Trichoderma*
G. = *Gliocladium*
B. = *Bacillus*
P. = *Pseudomonas*

Table 4. Effect of soil and seedling treatment with biocontrol agents on plant height, flowering and yield of cucumber grown in soil infested with *S. sclerotiorum* under greenhouse conditions at Tokh,2004&2005 seasons.

Treatments	Soil treatment						Seedling treatment					
	2004			2005			2004			2005		
	Aver. Plant height (m)	Aver. No of Fowers /plant	Aver. Fruit Yield kg/plot	Aver. Plant height (m)	Aver. No of Flowers/ plant	Aver. Fruit Yield kg/ plot	Aver. Plant hight (m)	Aver. No of Fowers/ plant	Aver. Fruit Yield kg/ plot	Aver. Plant height (m)	Aver. No of Fowers/ plant	Aver. Fruit Yield kg/ plot
T.hamatum	2.90	86.94	141.96	3.00	89.26	151.26	2.70	85.42	136.94	2.80	87.8	139.62
T.harziannum	2.75	80.52	130.82	2.80	83.69	138.94	2.60	79.56	125.39	2.70	80.4	129.48
T.koningii	2.60	79.36	129.78	2.75	81.54	137.89	2.50	78.21	122.43	2.60	79.3	126.19
T.viride	2.80	82.69	136.45	2.90	84.95	148.62	2.65	80.34	132.18	2.75	82.5	136.75
G.virens	2.95	87.73	145.37	3.05	90.33	154.14	2.75	85.63	140.92	2.85	88.2	143.79
G.deliquescens	2.50	77.48	126.48	2.65	79.82	136.43	2.30	75.95	120.87	2.45	76.8	124.18
B.subtikis	2.95	87.20	144.69	3.05	89.86	153.92	2.70	85.52	140.15	2.80	88.1	142.87
P.flourescens	2.80	85.56	138.26	2.95	86.51	149.83	2.59	84.68	133.75	2.65	86.7	136.48
TopsinM70	2.60	84.47	143.98	2.70	87.62	150.67	2.52	84.77	140.05	2.65	86.9	140.98
Control	1.75	50.30	79.80	1.85	56.84	80.4	1.65	48.50	74.54	1.70	52.40	78.60
L.S.D. at 5%	0.13	5.52	8.12	0.20	5.93	9.72	0.10	4.93	9.65	0.18	6.18	9.49

T. = Trichoderma *G.=Gliocladiun* *B. = Bacillus* *P. = Pseudomonas*

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المقاومة الحيوية لمرض العفن الأبيض فى الخيار المتسبب عن سكليروتينيا سكليروشيورم تحت ظروف الصوبة

أحمدأبوريا الكفراوى

معهد بحوث أمراض النبات – مركز البحوث الزراعية – الجيزة

يعتبر مرض العفن الأبيض فى الخيار من الأمراض الخطيرة ولـذلك تـم أسـتخدام بعـض العزلات من فطريات التضاد مثل الترايكودرما والجلايوكلاديوم والبكتريا ،وقـد أدت بنجـاح إلـى خفض مستوى الاصابة بمرض العفن الأبيض .

وقد أوضحت النتائج فى المعمل والصوبة مايلي :-

١- تم تثبيط نمو فطر اسكليروتينيا أسكليروشيورم بواسطة عزلات من فطريات التضـاد وكانـت أعلى نسبة تثبيط بواسطة ترايكودرما هاماتم (٣٤و٨٠%) وجليوكلاديوم فيـرنس (٥٤و٧٩%) و ترايكودرما فيردى (٣١و٧٩%) بينما كان الفطر جليوكلاديوم دليكوينسس (٨٣و٧٣%) أقل تأثيرا .

٢- تم تثبيط نمو فطر أسكليروتينيا أسكليروشيورم بواسطة عـزلات مـن البكتريـا بسـيدوموناس فلوروسنس (١و٢)بينما كانت باسيليس ستيلس (١و٢)كانت أقل تأثيرا فى هذا الخصوص .

٣- وجد أن راشح فطر الترايكودرما والجليكوكلاديوم والبكتريا أدت إلى خفض معنوى فـي نمـو وعدد الأجسام الحجرية لفطر العفن الأبيض .

٤- ادت معاملة التربة بفطرى ترايكودرما هاماتم وجليوكلاديوم فيرنس والبكتريا باسيلس سـاتليس إلى خفض التأثير المرضى كما أدت إلى تحسين أطوال النباتات وزيادة عدد الأزهـار و محصـول الثمار من الخيار عن بقية المعاملات الأخرى

٥- كان تأثير عوامل المقاومة الحيوية المختبرة قريبا إلى حدا ما من تأثير المبيد الكيماوى (توبسين م ٧٠) فى هذا الخصوص حيث نتج عن الاخير خفض الاصابة الى ٥- ١٠ %وزيـادة النباتـات الباقية الى ٩٠ – ٩٥ % .

٦- كانت معاملة الشتلات بفطريات التضاد أقل تأثيرا من معاملة التربة فى هذا الخصوص فى كلا الموسمين .