STUDY ON *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY, THE CAUSAL OF WHITE ROT DISEASE IN GREEN BEANS AND ITS CONTROL UNDER LABORATORY CONDITIONS

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ABSTRACT

Sclerotinia white rot of bean, caused by *Sclerotinia sclerotiorum*, is a major disease in green beans in Egypt, mainly in ismailia region. It affects on the quantity and quality of the exportable yield. The purpose of this study is to quantify the effects of some factors affecting on growth of *S. sclerotiorum* under laboratory conditions i.e. culture media, pH, Relative humidity (RH), light hours, carbon, nitrogen source and C/N ratios. Also, we did some attempts to control the fungus *in vitro* such by means of antagonists (17 fungal antagonist) and also bacterial antagonists (3 bacteria). Results showed that the best solid medium was Potato dextrose agar (PDA) and the lowest suitable medium was the Yeast extract agar with average of 51.50 and 30.00 mm., respectively. While, Oatmeal extract was the best liquid medium giving 2.39 mycelial dry weight/m, while Leonian medium was not suitable for growth giving 1.21 mycelial dry weight/ml, respectively. The optimal pH was affected by both 5.0 and 5.5 with 2.40 and 2.41 mycelial dry weight/ml, respectively. At RH, the optimal RH was 100% which gave 90 mm mycelial growth. Under C, N and C/N ratio, it was noticed that D-glucose was the best carbon source, while L-alanin was the best N source and both 9:1 and 20:1 were the best C/N ratio for growth. Under control studies, among all tested fungal antagonists, we noticed that *Coniothyrium. minitans* and *Trichoderma. hamatum* were the best antagonistic fungi in growth inhibition and sclerotial damage with 91.11, 85.83% and 95.8, 391.67%, respectively. Among all tested bacterial antagonists, it is noticed that *Pseudomonas fluorescens and Streptomyces griseoviridis* giving 61.94 and 59.72% inhibition in radial growth, while *Bacillus subtilis* was the best bacteria in destroying the sclerotia with 70.83%.

INRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary, one of the most destructive soil borne pathogens has been reported to affect a wide range of wild and cultivated host plants infect over 400 species of plants (**Boland** and **Hall, 1994**) and causes considerable damage to the host under congenial environments. The pathogen has been reported to hamper the cauliflower cultivation by causing stalk rot in different cauliflower growing areas in the United States during 1942-1943 (**Snyder and Baker 1945**). The fungus is classified within the genus *Sclerotinia* of the Sclerotiniaceae, an important family of Discomycetes of the class

Ascomycetes (**Kora *et al*. 2003**). Important crops affected include arrange of vegetable such as beans, lettuce, cabbage, carrot, potato and field crops such as oilseed rape, sunflower, and tobacco as well as a number of flower crops. The epidemiology of *S. sclerotiorum* has been investigated for number of crops (**Abawi** and **Grogan, 1979**). Bean (*Phaseolus vulgaris*, L.) is one of the most important vegetable crops in Egypt and many other countries. On of the major limitation in bean (*Phaseolus vulgaris*, L.) production and export in Egypt is the infestation white rot disease cause by *S. sclerotiorum* which decreasing the quantity and quality of the exportable yield. There are a few studies on the effect of environmental factors on *S. sclerotiorum* growth and sclerotia formation, despite these being potentially important factors in life cycle of the pathogen. Traditionally, crop losses due to ascospore infection have been reduced prophylactic spraying with fungicides. If the disease builds up in glasshouse soils, sclerotia of the pathogen were effectively controlled by using methyl bromide (**Bell *et al*. 1996**). However, concern regarding the potential of methyl bromide to deplete ozone led to its inclusion among the substances controlled by the Montereal protocol whose use must be eliminated by the end of 2004 from industrialized countries **(Gullino *et al*. 2003**). Therefore, alternatives disease control friendly methods, such as biological control, essential oils and microelements resistance were studies. The mycoparasites are promising biocontrol agents of *S. sclerotiorum* and have been shown to control the pathogen in numerous greenhouses and field trails (**Budge** and **Whipps 2001**). The present study aimed to study the effect of some factors on morphological and physiological characters of *S. sclerotiorum*; evaluate the antifungal activity of some antagonistic fungi i.e. 5 isolates of *Trichoderma harzianum*, 4 isolates of *T. viride*, 2 isolates of *T. hamatum*, 2 isolates of *Glicoladium roseum*, 2 isolates of *Coniothyrium minitans* and one isolate from *G. virens* and *G. catenulatum* against *S. sclerotiorum*.

MATERIALS AND METHODS

Morphological studies

Solid media

Six solid media i.e., Potato dextrose agar(PDA), Czapek, Carrot dextrose agar(CDA), Oatmeal agar (OMA), Cornmeal agar (CMA) and Yeast extract agar(YEA) were prepared and autoclaved for twenty minutes. Uniform quantities (20 ml) of each medium were poured in 90 mm Petri plates. Each Petri plate was inoculated separately with uniform culture bits (5 mm) cut from young (5 days) vigorously growing culture of the tested pathogen and incubated at 20±2^{o}C. Each treatment was replicated four times. Observations on radial growth/mm of mycelium were recorded.

Liquid media

Four liquid media i.e., Oatmeal extract, Czapek sucrose nitrate solution, Leonian solution, Potato dextrose broth were prepared. Each medium (100 ml)

was poured separately in 150 ml Erlenmeyer flasks, and sterilized in an autoclave. Each flask was inoculated separately with uniform quantity of homogenous culture suspension (1 ml) prepared by triturating mycelial mat of one flask grown on Potato dextrose broth. The inoculated flasks were incubated at 20±2°C for 15 days. Thereafter, the mycelial contents were filtered out through already weighed Whatman filter papers No 1. Then the mycelial mat was dried at 75°C in a hot air oven for 24 hours. Each treatment was replicated four times and data were recorded on dry weight of mycelium.

Physiological studies

• Effect of pH on growth of *S. sclerotiorum*

Potato dextrose broth was used as a basal medium for finding out the pH requirement of the fungus. Different pH levels i.e., 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 were maintained. Each flask was inoculated separately with uniform quantity of homogenous culture suspension (1 ml) prepared by triturating mycelial mat of one flask grown on Potato dextrose broth. For inoculation, the same procedure was done as in case of liquid media. Data on and dry weight of mycelium were reordered.

• Effect of light hours on growth of *S. sclerotiorum*

Petri plates containing uniform quantities (20 ml) of sterilized PDA medium were inoculated with 5-day old uniform culture bits of the pathogen (*S. sclerotiorum*) and incubated at different light hours i.e., 0, 12, 16 and 24h. The pH of each medium was adjusted at pH 5.5 prior to autoclaving. Each treatment was replicated four times and data were recorded on radial growth.

• Effect of relative humidity on growth of *S. sclerotiorum*

The agar dish equilibration technique, described by **Harries, *et al*. (1970)** and modified by **Xu, *et al*. (2001)** was used to control the relative humidity (RH) inside the sealed agar plates, this being related to the NaCl morality according to the value given by **Lang (1967)**. The levels of RH tested were 100, 99, 97, 90, 95, 90, 80, 70, 60, and 50%. These were obtained by amending the agar with 0.0, 0.3, 0.9, 1.5, 3.0, 6.0, 9.0, 12.0 and 15 mole from NaCl, respectively. All plates were inoculated with 5-day old culture from *S. sclerotiorum* and incubated at 20±2°C. Each treatment was replicated four times and data were recorded on radial growth of mycelium.

• Effect of carbon, nitrogen source and C/N ratio on growth of *S. sclerotiorum*.

A defined glucose–alanine medium based on Czepek mineral salts medium (**Dhingro** and **Sinclair, 1986**) was used routinely and contained (L^{-1}distilled water; 1 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$; 0.5g KCl; 0.01g $ZnSO_4.7H_2O$; 0.005g $CuSO_4.5H_2O$; 40 g D-glucose; 2 g L-alanine. Batches of based medium (without D-glucose and L-alanine) were prepared by combining appropriate amounts of individual chemical solution after autoclaving (15 min. At 121°C)

separately. The pH of each medium was adjusted at pH 5.5 prior to autoclaving. The effect of different carbon sources (D-glucose, D- dextrose, Maltose, sucrose and Glycerol) was tested in basal medium with L-alanin (2 gl^{-1}) as the nitrogen source. The effect of different nitrogen sources (Ammonium chloride, L-alanin, L-agrinin and Glutamic acid) were tested in basal medium with D-glucose 40 g/L as carbon source. To study the effect of carbon / nitrogen (C/N ratio).The basal medium was supplemented with amount of D-glucose and L- alanin. The C/N ratio in medium was adjusted between 9:1and 200:1 by varying the concentration of D-glucose with L-alanin fixed at 2 gl^{-1}. Each flask was inoculated separately with uniform quantity of homogenous culture suspension (1 ml) prepared by triturating mycelial mat of one flask grown on Potato dextrose broth, and inoculated at 20±2 ºC. Data were recorded on dry weight.

Effect of certain fungal antagonists on radial growth of *S. sclerotiorum* causing white rot in green bean.

17 antagonistic fungi mentioned mentioned in Table (1) which some of them came from Hungary, while the other were isolated from soil collected from different governorates isolated from rhizosphere of healthy bean plants grown in Ismailia and Sharkia governerate. The inhibitory effects of these antagonistic fungi on radial growth of *S. sclerotiorum* were studied. Each of obtained fungal antagonist, and *S. sclerotiorum* grown on PDA for 5-7 days at 20±2^{o}C. The antagonistic effect of the used antagonists on the pathogens was done through using one disc (5 mm. in diameter) of the antagonist facing one disc of the pathogen carrying mycelia growth on the PDA surface and relatively closed to the periphery of the plates. The untreated control treatment was done on the same medium in Petri dishes by growing one disc of the pathogenic fungus in the same place where there was no antagonistic disc. Four replicates were used, all plates were incubated at 20±2^{o}C for 3 and 15 days. Data were recorded on the diameter average of zones of the pathogenic fungus.

Effect of a certain bacterial antagonists on the radial growth of *S. sclerotiorum* causing white rot in bean:

The inhibitory effects of *B. subtilis*, *P. fluorescens* and *S. griseoviridis* (Mycostop®) on radial growth of *S. sclerotiorum* were studied. *B. subtilis*, *P. fluorescens* were obtained from laboratory of organic agriculture, Agriculture Research Center (ARC) in Cairo, where the commercial product Mycostop® was supplied from the company Kemira OY of Finland. All pure cultures of *B. subtilis* and *P. fluorescens* were grown on Nutrient Agar medium (NA), while *S. griseoviridis* was used as spore suspension from the commercial product Mycostop®. The antagonistic effects of the used bacteria on the fungal pathogens were done through streaking the antagonistic bacteria facing one disc of the pathogen on the PDA surface and relatively closed to the periphery of the plates. The untreated control treatment was done on the same medium in Petri dishes by growing one disc of the pathogenic fungus in the same place of treatments without antagonistic bacteria. Incubation was done for 3 and 15 days at (20±2^{o}C).

Average of radial growth (mm.) was recorded and compared with the untreated control %.

Effect of a conidial suspension of antagonistic fungi on sclerotia of *S. Sclerotiorum*:

The effect of conidial suspension of bioagents used on sclerotia of *S. sclerotiorum*, sclerotia were incubation with conidial suspension (10^3 conidial ml^{-1}, 20 ml for 10 sclerotia), sclerotia were inoculated in a typical experiment. After 24 hours of inoculation at 20±2ºC in the dark, sclerotia were removed from the suspension and placed on the surface of water agar plate under the same conditions. After 2 week, sclerotium mortality was assessed as follows: each sclerotium was cut in halves with sterilized, sharp knife, which were transferred out to carrot slices, previously sterilized in H_2O_2 (28 %) for 30 min. After 3-5 days of incubation at 20ºC, mycelia growth of *S. sclerotiorum* was used to identity and count surviving sclerotia. Mortality was calculated from the difference between the number of inoculated and germinated sclerotia **(Grendene and Marciano, 1999).**

Effect of bacterial cells on sclerotia of *S. Sclerotiorum*.

The effect of bacterial cells of bacteria used on sclerotia of *S. sclerotiorum*, sclerotia was done through incubation with bacterial cells (10^3 cell ml^{-1}, 20 ml for each 10 sclerotia), sclerotia were inoculated in a typical experiment. After 24 h. of inoculation at 20±2 ºC in the darkness, sclerotia were removed from the suspension and placed on the surface of water agar plate under the same conditions. After 2 week, sclerotium mortality was assessed as follows: each sclerotium was cut in halves, which were transferred out to carrot slices, previously sterilized in H_2O_2 (28 %) for 30 min. After 3-5 days of incubation at 20 ºC, mycelia growth of *S. sclerotiorum* was used to identity and count surviving sclerotia. Mortality was calculated from the difference between the number of inoculated and germinated sclerotia **(Grendene and Marciano, 1999).**

Statistical analysis

Data collected from all experiments were statistically analyzed using the Statistic Analysis System package (SAS institute, Cary, NC, USA). Differences between treatments were studied using Fisher's least significant difference (LSD) test and Duncan's multiple range list **(Duncun,1955).** All analysis were performed at P 5%.

RESULTS AND DISCUSSION

Effect of Solid medium on growth of *S. sclerotiorum*.

Data in Table (1) show that PDA was the best medium for having the best

mycelial growth (51.50 mm.), this was followed by Czapek and CDA which gave radial growth averages of 49.25 and 47.50 mm, respectively. These results agree with **Nguyen and Dohroo (2006).**

Effect of Liquid medium on growth of *S. sclerotiorum*.

oatmeal extract was found to be the best for the mycelial growth giving 2.39 mg/ml, this was followed by Czapek sucrose nitrate solution, Potato dextrose broth and Leonian solution giving 2.20, 2.16 and 1.21 mg/ml, respectively Table (1). The result was in close agreement with finding of **Sharma (1979)** who also reported similar results. **Khan (1976)** reported that Czapek medium was not suitable for mycelium growth as well as sclerotial formation while **Kaith (1977)** did not found that sarson seed extract or oat seed extract suitable for the mycelium growth.

Physiological studies

• Effect of PH on growth of *S. sclerotiorum*

Results in Table (1) indicate that the best growth of the fungus occurred at PH 5.5 with dry weight of 2.41 mg/ml followed by pH 5.0 with mycelial dry weight of 2.40 mg/ml. Whereas, PH levels of 7.5 and 8.0 were not suitable for growth of the fungus. The results are also in close agreement with **Sharma (1979**) who found that the PH 5.0 was suitable for the vegetative growth of the fungus. However, **Willetts and Wong (1980)** reported that the PH below 5.0 was optimum, whereas, **Khan (1976)** confirmed that PH of 4.6 and 4.5 gave best growth of the fungus. Sclerotial formation was found to be directly correlated with vegetative growth of mycelium with optimum pH levels of 5.0 and 5.5. **Le Tourneau (1979)** explained that numerous sclerotia were formed by the fungus growing on a suitable medium and supported good growth of the fungus. There was no sclerotia formation at pH levels of 7.0 and 8.0**. Khan (1976), Kaith (1977) and Sharma (1979)** found that pH 9.0 was not suitable for vegetative growth as well as sclerotia formation of S. sclerotiorum.

Effect of light hours on growth of *S. sclerotiorum*

Data in Table (1) illustrate that, all numbers of light hours were appropriating good growth of *S. sclerotiorum*, ranging from 87.00 (zero hour) to 90.00 mm when using 12, 16 and 24 hours . This findings are in agreement with **Sun and Yang (2000.**

Effect of relative humidity on growth of *S. sclerotiorum*

Data in Table (1) point out that, the best growth of the fungus occurred at 100% relative humidity (RH) with radial growth of 90.00 mm., this was followed by 99% with radial growth of 75.75 mm. in diameter. On the other hand, RH levels ranging from 50-80 were not suitable for the growth of the *S. sclerotiorum*. This finding is in agreement with **Sun and Yang (2000).**

Effect of carbon, nitrogen source and C/N ratio on growth of *S. sclerotiorum*.

D-glucose was the best carbon source for mycelial dry weight with 1.34 mg/ml followed by D-dextrose with 1.29 mg/ml. while, Sucrose and Glycerol gave 0.80 and 0.37 mg/ml of mycelial dry weight, while Maltose did not opposite for development of the fungus Table (1). In case of Nitrogen source, between all nitrogen tested, it was noticed that L-Alanin was the best N source for mycelial

dry weight of *S. sclerotiorum* with 0.95 mg/ml, followed by L-Arginin with 0.66 mg/ml. On the other hand, the Methionin was not suitable for growth of *S. sclerotiorum* Table (1). Information in Table (1) indicate that the best C/N ratios were 9:1 and 20:1 with mycelium dry weight of 1.40 mg/ml in both, these were followed by 50:1 ratio giving 0.7 mg/ml mycelium dry weight. Nonetheless, the 100:1 and 200:1 were not suitable for growth when gave 0.1 mg/ml in two ratios.

Control studies

Effect of certain fungal antagonists on radial growth of *S. sclerotiorum* causing white rot in green bean.

Data in Table (2) show that there were significant differences between all antagonistic' fungi for decreasing the mycelial growth of *S. sclerotiorum*, it was noticed after 3 days *C. minitans* was the best antagonistic fungus in reducing the mycelial growth with 75.83% when compared with untreated control. This was followed by *T. hamatum* and *T. viride* with 52.50% inhibition ratio, while *G. virens*, *G. catenulatum* and *G. roseum* gave moderate inhibition rates of 43.06, 40.28 and 39.17% in the mycelial growth, respectively. After 15 days, it was found that *C. minitans* was the most effective on radial growth of *S. sclerotiorum* giving 91.11% decrease in mycelial growth, followed by *T. hamatum* and *T. viride* giving 85.83 and 74.71% reduction in mycelial growth of *S. sclerotiorum* when compared with untreated control. In a separate study using the treatment of sclerotia with a layer of antagonistic fungi it was noticed that *C. minitans* prevented sclerotia germination with ratio of 95.83% when compared with the untreated control followed by *T. hamatum* and *T. viride* which gave 91.67 and 87.50% . In the same time, *G. catenulatum* gave the lowest effect on sclerotial preventation with 54.17%. These results agree with **Dubey and Patel (2001)**; **Cheng, *et al.* (2003); Jones, *et al.* (2004)**; **Kulikov, *et al*. (2006) and Zhao, *et al*. (2006)**. About the high antagonistic effect of *C. minitans* against *S. sclerotiorum* may belongs to its enzymatic effect against hypha and sclerotia (**Campbell, 1947**and **Huang and Kokko, 1988**).It was supported by **Giczey *et al*., 2001** how mentioned that the extra-cellular enzyme ß-1,3-glucanase (EC 3.2.1.39) appears to be an important enzyme involved in the mycoparasitism of *S. sclerotiorum* by *C. minitans*, as the expression of the gene cmg1 encoding ß-1,3-glucanase increases during infection of sclerotia of *S*. *sclerotiorum* by *C*. *minitans*

In this study it was noticed that *Trichoderma* spp. were the second best antagonistic fungi against *S. sclerotiorum* (*T. hamatum, T. viride* and *T. harzianum*). This high antifungal activity of *Trichoderma* spp. is almost depends on some lytic enzymes, which response on fungal cell-well-degrading (**Elad** *et al*., **1995 Antal *et al***., **2000 and Elad *et al.,*1998**., **Kapat *et al.,* 1998 and Zimand *et al.,* 1996**. *Glicoladium* spp. gave moderate to slight inhibition effect on mycelial growth and sclerotia damage of *S. sclerotiorum. G. virens*, *G*. *catenulatum* and *G. roseum* reduced mycelial growth of *S. sclerotiorum* by 56.67, 50.28 and 49.72% and damaged sclerotia with 70.83, 54.17 and 79.17%rates,

respectively (Table 2). These result are in agreement with **Singh** and **Mukhopadhyay, 2000**, **Howell and Stipanovic, 1995** and **Jung Li *et al*., 2005**.

Effect of certain bacterial antagonists on radial growth of *S. sclerotiorum* causing white rot in bean.

Results in Table (3) demonstrate that all antagonistic bacteria gave significant reduction in mycelial growth of *S. sclerotiorum*. After 3 days, the high inhibition came from *P. fluorescens* by 38.04% when compared with untreated control. This was followed by *B. subtilis* and *S. griseoviridis* with 36.02 and 23.28% as reduction in mycelial growth of *S. sclerotiorum*, respectively. After 15 days, *P. fluorescens* was the most effective on radial growth of *S. sclerotiorum* giving 61.94% reduction followed by *S. griseoviridis* and *B. subtilis* giving 59.72 and 54.44% reduction in mycelial growth of *S. sclerotiorum*, respectively. These results are in agreement with those obtained by **Rong, *et al.* (2000); Andrea, *et al*. (2006); and Valerie, *et al.* (2007)**. This antifungal activity of *P. fluorescens* against *S. sclerotiorum* probably related to degradation of chitin in hyphal and sclerotia cell consist of several enzymes hydrolyzing (**Gooday, 1990**) such as effect coming from antagonistic bacteria may belongs to Chitinase or endochitinase and Exochitinase, or chitobiosidase **Tronsmo and Harman, 1993**. Additionally, *P. fluorescens* produced three antibiotics possibly involved in its biocontrol activity: 2, 4-diacetylphloroglucinol, pyrrolnitrin and pyoluteerin **(Sonia- Humphris *et al*., 2005).** On the other hand, this bacterial antifungal activity of *S. griseoviridis* probably related to some antibiotic as quinolone and coumorin antibiotic (novobiocin), which attach and deprives pathogen of living space and nourishment by colonization the plant roots in advance of the pathogen **(Andrea *et al*., 2005 and Tamechiro *et al*., 2002**).

Table (2): Effect of some factors on growth *S. sclerotiorum* the causal of white rot disease of green bean (*P. vulgaris*, L..)

										LSD at 5%
	Solid medium									
	PDA		Czapek		CDA		OMA	CMA	YEA	
Radial growth (mm.)	51.50a		49.25b		47.50c		44.00d	34.00e	30.00f	1.71
Liqiud medium										
	Richard solution		Czapek sucrose nitrate solution				Leonian		PDB	
Mycelium dry weight (mg/ml)	2.39a		2.20b				1.21d		2.16c	0.28
PH										
	5.0		5.5		6.0		6.5	7.0	7.5	8.0
Mycelium dry weight (mg/ml)	2.40a		2.41a		1.99b		1.48c	1.04d	0.61e	0.33f
Light hours										
	0		12		16		24			
Radial growth (mm.)	87.00b		90.00a		90.00a		90.00a			1.41
relative humidity (RH)										
	50	60	70	80	90	95	97	99	100	
Radial growth (mm.)	0.00f	0.00f	0.00f	0.00f	20.00e	34.25d	60.25c	75.75b	90.00a	7.56
Carbon sources										
	D-Glucose		D-Dextrose		Maltose		Sucrose		Glycerol	

Mycelium dry weight (mg/ml)	**1.34a**	**1.29a**	**0.00d**	**0.80b**	**0.37c**	**0.13**
Nitrogen sources						
	Ammonium chloride	**L-Alanin**	**L-Arginin**	**Methionin**	**L-Glutamic acid**	
Mycelium dry weight (mg/ml)	**0.49b**	**0.95a**	**0.66b**	**0.00c**	**0.53b**	**0.25**
C/N ratio						
	9:1	**20:1**	**50:1**	**100:1**	**200:1**	
Mycelium dry weight (mg/ml)	**1.40a**	**1.40a**	**0.71b**	**0.14c**	**0.13c**	**0.32**

Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05)

Table (2): Effect of certain fungal antagonists on radial growth of *S. sclerotiorum* causing white rot in bean under lab conditions.

Fungi	Radial growth				Sclerotia germination	
	After 3 days	Inh. %	After 15 days	Inh. %	Sclerotia germination	Sclerotia destructive %
***T. harzianum* (Hu)**	43.75hg	51.39	28.25hi	68.61	2.00def	83.33
***T. harzianum* (DK)**	52.00cde	42.22	30.75efg	65.83	3.00cde	75.00
***T. harzianum* (SH)**	42.75h	52.50	30.00gh	66.67	3.00cde	75.00
***T. harzianum* (IS)**	43.25hg	51.94	31.00efg	65.56	2.00def	83.33
***T. harzianum* (SO)**	46.00fgh	48.89	36.50def	59.44	3.50bcd	70.83
***T. viride* (Hu)**	44.00hg	51.11	32.25efgh	64.17	1.50def	87.50
***T. viride* (DK)**	48.50defg	46.11	22.75i	74.72	2.00def	83.33
***T. viride* (SH)**	46.75efgh	4800	37.25de	58.61	3.5bcd	70.83
***T. viride* (IS)**	42.75h	52.50	34.75defg	61.39	2.00def	83.33
***T. hamatum* (Hu)**	35.75i	60.28	15.25j	83.06	1.50def	87.50
***T. hamatum*(IS)**	33.00i	63.33	12.75jk	85.83	1.00ef	91.67
***G. roseum* (Hu)**	58.75b	34.72	45.25b	49.72	5.00bc	58.33
***G. roseum* (So)**	54.75bc	39.17	45.25b	49.72	2.50def	79.17
***G. virens* (Hu)**	51.25cdef	43.06	39.00cd	56.67	3.50bcd	70.83
***G. catenulatum* (Hu)**	53.75bcd	40.28	44.75bc	50.28	5.50b	54.17
***C. minitans* (Hu)**	23.00j	74.44	8.00k	91.11	0.50f	95.83
***C. minitans* (Ge)**	21.75j	75.83	11.25jk	87.50	0.50f	95.83
Control	90.00a	0.00	90.00a	0.00	12.00a	0.00
L.S.D	5.49		5.96		2.00	

Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05)

Table (3): Effect of certain bacterial antagonists on radial growth of *S. sclerotiorum* causing white rot in bean.

Fungi	Radial growth				Sclerotia germination	
	After 3 days	Inh. %	After 15 days	Inh. %	Sclerotia germination	Damage %
P. fluorescens	53.75c	38.04	34.25c	61.94	4.00b	66.67
B. subtilis	55.50bc	36.02	41.00b	54.44	3.50b	70.83
S. grisioviridis	58.75b	32.28	36.25c	59.72	5.00b	58.33
Control	86.75a	0.00	90.00	0.00	12.00a	0.00
L.S.D	4.09		4.02		3.29	

Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05).

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الملخص العربي

دراسات علي الفطر *S. sclerotiorum* المسبب لمرض العفن الأبيض قي الفاصوليا ومحاولات لمقاومته تحت الظروف المعملية.

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يعتبر مرض العفن الأبيض في فاصوليا التصدير الخضراء المتسبب عن الفطر *Sclerotinia sclerotiorum* مرض رئيسي يصيب الفاصوليا في مصر خاصة في محافظة الإسماعيلية ismailia . هذا المرض يؤثر علي كل من المحصول الكلي والمحصول القابل للتصدير . الغرض من هذه الدراسة كَانت دراسة تـأثيراتَ بعض العواملِ المؤثرة علي نمو هذا الفطر مثل تـأثير أنواع البيئات المعملية المستخدمة في انمائه (سواء صلبة أو سائله) و درجة الحموضة pH والرطوبة النسبية و عدد ساعاتَ الإضاءة ومصدر الكربون و مصدر النتروجينِ و نسبة الكربون إلي النتروجين في البئات المغذية . كذلك تمت دراسة بعض المحاولات علي مقاومة الفطر بيولوجياً عن طريق إستخدام الفطريات المضادة (17 فطر مضاد) و البكتريا المضادة (3 بكتيريا). أظهرت النتائجَ بـأن أفضل بيئة صلبة مناسبةٍ لنمو الفطر الممرض كانت بيئة البطاطس والدكستروز و كَانت بيئة مستخلص الخميرة و الأجار أقل البيئات المناسبة لنمو الفطر بمعدلِ 51.50 30.00 مليمتر ، على التوالي. بينما، كانت بيئة مستخلص

الشوفان أفضل بيئة سائلة مناسبة للنمو حيث اعطت وزن جاف قدره 2.39ملليجرام/مل بيئة بينما كانت بيئة Leonian غير مشجعة للنمو حيث اعطت 1.21ملليجرام وزن جاف لكل ملي بيئة . أما درجة الحموضة المثالية فكانت هي 5.0 و 5.5 بمعدل 2.40 2.41 ملليجرام وزن جاف، على التوالي. أما بالنسبة لتاثير درجة الرطوبة النسبية فان معدل 100 % كان أفضل المعدلات المستخدمة حيث اعطت نمو كامل غطي كل سطح البيئة . أما بالنسبة لمصدر الكربون فقد لوحظ أن سكر –الجلوكوز كان أفضل المصادر الكربونية و L- alanin كان أفضل مصادر النتروجين المستخدمة بمعدل 1.29للاول و 0.95 للثاني ملليجرام/مل بيئة , أما تاثير نسبة الكربون إلي النتروجين فكان 1:9 و1:200 تمثل أفضل النسب للنمو بمعدل 1.40 مليجرام وزن جاف. أما بالنسبة لمحاولات المقاومة فقد وجد أن كلا من الفطريين المضادين *C. minitans* و *T. hamatum* كانت أفضل الفطريات المضادة تأثيراً علي النمو الفطري أو انبات الأجسام الحجرية للفطر الممرض بنسبة 91.11, 85.83 % و95.8, 391.67 %، على التوالي. أما بالنسبة للبكتريا المضادة, فقد لوحظ أن البكتريا *P. fluorescens* كانت أفضل بكتريا اختبرت في تثبيط النمو الفطري بمعدل 61.34% أما بالنسبة لمنع انبات الأجسام الحجرية فقد وجد أن البكتريا *B. subtilis* هي الأفضل باعطائها نسبة منع انبات قدرها 70.83% من مجموع الاجسام الحجرية المعاملة.