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# Efficacy of Several Potential Biocontrol Agents against *Sclerotinia sclerotiorum*

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***In vitro* seventeen antagonistic microorganisms were evaluated against phytopathogen *Sclerotinia Sclerotiorum* by dual culture techniques. After 3 days from incubation, the maximum growth inhibition of the tested pathogen was noticed by *Coniothyrium minitans* (75.83%) followed by *Trichoderma hamatum* (63.33%). Also, after 15 days, the highest reduction of mycelial growth of *S. sclerotiorum* came from *C. minitans* (91.11%) followed by *T. hamatum* (85.83%). In case of sclerotia germination, *C. minitans* and *T. hamatum* significantly inhibited sclerotia germination with 95.83 and 91.67% reduction, respectively.**

**Key words:** *Sclerotinia sclerotiorum*, White rot, *Glicoladium catenulatum*, Biocontrol.

*Sclerotinia sclerotiorum* is a global pathogen on many economically important crops including bean (*Phaseolus vulgari*, L.)[1,2]. It occurs in all bean-growing in protected areas and open field in Egypt, especially in Ismailia governorate where the temperature and moisture are conducive to outbreaks of this disease[2,3]. Control of white rot disease in Egypt's bean is mainly by the use of synthetic fungicides and no resistant bean cultivars are available[3]. Growing evidence of fungicide resistance in populations of *S. sclerotiorum* and concerns about the reduction of environmental sustainability of agrochemicals use have formed a need to find other sustainable measures as alternatives to chemical control. Several of antagonistic microorganisms have been described for the management of *S. sclerotiorum* such as *Trichoderma harzianum*, *T. asperellum*, *Coniothyrium minitans* and *Bacillus subtilis*[4-8]. The fungus *C. minitans* produces a wide range of enzymes such as glucanases and chitinases as well as secondary metabolites that improve colonization and degradation of the sclerotia of *S. sclerotiorum*[9]. *Trichoderma* species have been generally used to inhibit many pathogens including *S. sclerotiorum*[6] and have several modes of action including nutrient competition, mycoparasitism, plant growth promotion and antibiosis[10].

The aims of the present study were to evaluate and compare the efficacy of several biological control agents on *S. sclerotiorum*

## MATERIALS AND METHODS

### Isolation and identification of microorganisms

### Pathogenic fungus

The isolate of *Sclerotinia sclerotiorum* used in this study was derived from sclerotia on diseased bean plants (*Phaseolus vulgaris*, L.) from Ismailia governorate. Original isolations were made by surface sterilizing the collected sclerotia in 50%

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(v/v) Na-hypochlorite and 70% ethanol for four minutes, with agitation followed by two washes in sterile distilled water (SDW) for 1 minute, sclerotia were then bisected, placed on PDA and incubated for 4 weeks at 10 °C and used as stock[11].

**Antagonistic fungi**

Pure culture of *Trichoderma harzianum, T. viride, T. hamatum, Glicoladium virens, G. roseum, G. catenulatum* and *Coniothyrium minitans* came from and identified by Plant Pathology Dept., Plant Protection Research, Budapest, Hungary. Isolate of *C. minitans* came from Prof. Dr. Katrin Hedke**,** Phytomedicine Dept., Faculty of Agriculture, Rostock University, Germany. While strains of *T. harzianum, T. viride, G. roseum* and *T. hamatum* were isolated from different locations of Egypt[12], and identified by Plant Pathology Dept., Faculty of Agriculture, Mansoura University.

**Inhibitory effect of certain fungal antagonists on the radial growth of *Sclerotinia sclerotiorum***

Seventeen antagonistic fungi which some of them came from Hungary and some of them isolated from soil collected from different governorates isolated from rhizosphere of healthy bean plants grown in Ismalia and Sharkia governerates, Egypt were used. 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°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 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 but there was not antagonistic disc. Four replicates were used; all plates were incubated at 20±2°C for 4 and 15 days.

**Data were recorded on the diameter average of zones of the pathogenic fungus**

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 incubated 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±2°C in the dark, sclerotia were removed from the suspension and placed on the surface of water agar plate under the same conditions. After 2 weeks, 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°C, 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[13].

**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 lest[14]. All analysis were performed at P 5 % level.

## RESULTS AND DISCUSSION

**Inhibitory effect fungal antagonists on radial growth of *Sclerotinia sclerotiorum***

Data in Table 1. showed that there were significant differences between all antagonistic fungi for decreasing the mycelial growth of *S. sclerotiorum*. After 3 days, *C. minitans* was the best antagonistic fungi in reducing the mycelial growth with 75.83% reduction when compared with untreated control. This was followed by *T. hamatum* and *T. viride* with 52.50% inhibition, while *G. virens, G. catenulatum* and *G. roseum* gave a moderate inhibition rate 43.06, 40.28 and 39.17% as reduction in the mycelial growth, respectively.

After 15 days, it noticed that there were differences between all antagonistic fungi for reducing the mycelia growth of *S. sclerotiorum*. *C. minitans* was the most effective on radial growth of *S. sclerotiorum* giving 91.11% decreasing in mycelial growth, followed by *T. hamatum* and *T. viride* giving 85.83 and 74.71% reducing. Nevertheless, *G. virens, G. catenulatum* and *G. roseum* gave a moderate inhibition with 56.67, 50.28 and 49.72% reduction in mycelial growth of *S. sclerotiorum* when compared with untreated control.

Under sclerotia studies, data reveal that there were significant differences between all

antagonists fungi tested and control treatment in sclerotia damage. *C. minitans* had the best effect against sclerotia giving 95.83% destructive in sclerotia followed by *T. hamatum* and *T. viride* with 91.67 and 87.50 decaying of sclerotia. At the same time as, *G. catenulatum* showed the lowest effect on sclerotia decomposing with 54.17%.

**Comparison between strains of antagonistic fungi tested**

*Trichoderma harzianum* and *T. viride*, it noticed that the best strain in the two categories was Hungarian strain in reducing the mycelial growth and sclerotia damage.

*Trichoderma hamatum* which was isolated from Ismailia governorate was the best

**Table 1.** Inhibitory effect of certain fungal antagonists on radial growth of *S. sclerotiorum* causing white rot in bean

| Fungi | Radial growth | | | | Sclerotia | |
|---|---|---|---|---|---|---|
| | After 3 days | Inh. % | After 15 days | Inh. % | S.G | S.D. % |
| *T. harzianum* (1) | $43.75^{hg}$ | 51.39 | $28.25^{hi}$ | 68.61 | $2.0d^{ef}$ | 83.33 |
| *T. harzianum* (2) | $52.00^{cde}$ | 42.22 | $30.75^{efg}$ | 65.83 | $3.0^{cde}$ | 75.00 |
| *T. harzianum* (3) | $42.75^{h}$ | 52.50 | $30.00^{gh}$ | 66.67 | $3.0^{cde}$ | 75.00 |
| *T. harzianum* (4) | $43.25^{hg}$ | 51.94 | $31.00^{efg}$ | 65.56 | $2.0^{def}$ | 83.33 |
| *T. harzianum* (5) | $46.00^{fgh}$ | 48.89 | $36.50^{def}$ | 59.44 | $3.5^{bcd}$ | 70.83 |
| *T. viride* (1) | $44.00^{hg}$ | 51.11 | $32.25^{efgh}$ | 64.17 | $1.5^{def}$ | 87.50 |
| *T. viride* (2) | $48.50^{defg}$ | 46.11 | $22.75^{i}$ | 74.72 | $2.0^{def}$ | 83.33 |
| *T. viride* (3) | $46.75^{efgh}$ | 4800 | $37.25^{de}$ | 58.61 | $3.5^{bcd}$ | 70.83 |
| *T. viride* (4) | $42.75^{h}$ | 52.50 | $34.75^{defg}$ | 61.39 | $2.0^{def}$ | 83.33 |
| *T. hamatum* (1) | $35.75^{i}$ | 60.28 | $15.25^{j}$ | 83.06 | $1.5^{def}$ | 87.50 |
| *T. hamatum*(2) | $33.00^{i}$ | 63.33 | $12.75^{jk}$ | 85.83 | $1.0^{ef}$ | 91.67 |
| *G. roseum* (1) | $58.75^{b}$ | 34.72 | $45.25^{b}$ | 49.72 | $5.0^{bc}$ | 58.33 |
| *G. roseum* (2) | $54.75^{bc}$ | 39.17 | $45.25^{b}$ | 49.72 | $2.5^{def}$ | 79.17 |
| *G. virens* | $51.25^{cdef}$ | 43.06 | $39.00^{cd}$ | 56.67 | $3.5^{bcd}$ | 70.83 |
| *G. catenulatum* | $53.75^{bcd}$ | 40.28 | $44.75^{bc}$ | 50.28 | $5.5^{b}$ | 54.17 |
| *C. minitans* (1) | $23.00^{j}$ | 74.44 | $8.00^{k}$ | 91.11 | $0.5^{f}$ | 95.83 |
| *C. minitans* (2) | $21.75^{j}$ | 75.83 | $11.25^{jk}$ | 87.50 | $0.5^{f}$ | 95.83 |
| Control | $90.00^{a}$ | 0.00 | $90.00^{a}$ | 0.00 | $12.0^{a}$ | 0.00 |
| L.S.D | 5.49 | | 5.96 | | 2.00 | |

Inh.% = inhibition %

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

strain by 85.85% reduction in mycelia growth and 91.67% sclerotia damage.

*G. roseum* which was isolated from Ismailia governorate was the best strain when compared with Hungarian strain by 49.72% reduction in mycelia growth and 79.17% sclerotia destructive. *C. mintans*, it noticed that two strain tested (Hungarian and German) gave the same results. Among all tested antagonistic fungi, it was noticed that *C. minitans* gave the best inhibitory effect on mycelial growth and sclerotia destructive of *S. sclerotiorum* with 91.11 and 95.83%, respectively, followed by *T. hamatum, T. viride, T. harzianum, G. virens, G. catenulatum* and *G. roseum* giving 85.83, 74.72, 66.67, 56.67, 50.28 and 45.25% inhibition in mycelial growth, and 91.67, 87.50, 75.00, 70.83, 54.17and 79.17% sclerotia damage, respectively. This highly antifungal activity of *C. minitans* Campbell possibly due to causing destruction of hyphae[15] and sclerotia[16] of *S. sclerotiorum*. The extra-cellular enzyme β-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 β-1,3-glucanase increases during infection of sclerotia of *S. sclerotiorum* by *C. minitans*[17]. Another extra-cellular enzyme chitinase (EC 3.2.1.14) may be less important than

β-1, 3-glucanase in infection of *S. sclerotiorum* by *C. minitans*, as the yield of this enzyme in cultures of *C. minitans* and in infected sclerotia of *S. sclerotiorum* was negligible[18]. McQuilken *et al.*,[19] observed that *C. minitans* produced an antifungal antibiotic, macrosphelide A, which seemed to demonstrate selective toxicity to *S. sclerotiorum* and *S. cepivorum* compared with microorganism's cell lines. Also, this antibiotic may play a role in the parasitism of *Sclerotinia* species by *C. minitans*. *C. minitans* requires a host to be in a vegetative stage[20]. Also, Ren, *et al.*[21] found that degradation of oxalic acid which produced by *S. sclerotiorum* and plays a determinant role in pathogenesis of this pathogen by *C. minitans* might also be a mechanism by which *C. minitans* from secreting β-1, 3-glucanase can protect plants from infection by *S. sclerotiorum*.

In this study it noticed that *Trichoderma* spp. was the second best antagonistic fungi against (*T. hamatum, T. viride* and *T. harzianum*). Results are in agreement with those Melo and Faull[22], who found that the *T. harzianum* and *T. koningi* were effective in inhibiting the mycelial growth of *R. solani*. Also, Ramezani[23] demonstrated that *T. harzianum* significantly suppressed the radial growth of *M. phaseolina*. Our study showed *T. hamatum* had a greater inhibition on *S. sclerotiorum* than *T. harzianum* and *T. viride* is known to act through numerous mechanisms such as antibiosis hyperparasitism and inhibition. *Trichoderma* spp. is almost certainly depends on some lytic enzymes, which response on fungal cell-well-degrading[24] such as *N*-acetyl- β-*D*-glucosedaminidase, chitinase, β–1,3 gluconase, chitobiosidase and protease[25,26]. *T. harzianum* was found to restrain enzymes of *B. cinerea*, the activities of exo-endo polygalacturonase, pectin methyl esterase, pectate lyase, chitinase and cutinase, which are thought to be involved in mycoparasite process in bean leaves infested with *B. cinerea*[27,28]. Additionally, *Trichoderma* spp. could manufacture cynamide hydratase, rhodanese and β–cyanoalnine syntheses, which has play an important function in reducing the growth of pathogenic fungi[29].

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