# *In-vitro* efficacy of some fungicides, bioagents and culture filtrates of selected saprophytic fungi against *Sclerotium rolfsii*

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**Abstract:** The inhibitory effect of the fungicides, different formulations of bioagents and culture filtrate of some saprophytic fungal isolates against the growth of *Sclerotium rolfsii* was evaluated *in-vitro*. The obtained results indicate that all evaluated materials significantly reduced the linear growth of *Sclerotium rolfsii.* Rhizolex fungicide was found to highly effective and gave 100% reduction in growth when used at lower concentration (12.5ppm). The same effect was recorded at 100, 200, and 400 ppm of Top zin and at 400ppm of Score fungicide. Tazolen showed also 100% inhibition at concentrations of 100 to 400ppm.The bioagent Bio-arc (*T. albium*) was the most effective against *S. rolfsii* growth responsible for 44.66 mean % inhibition, followed by Biocure-F (*T.viride*) responsible for 29.08 mean % inhibition with significant difference. Bio-arc (*B. megatherium*), Biocure-Z (*T. harzianum*) and Plant guard (*T. harzianum*) bioagents were fare less effective, as the mean percentage inhibition were 2.81, 2.97 and 3.49% respectively. Maximum inhibition was observed in the culture filtrate of *T. harzianum* drawn from potato dextrose broth with mean reduction of 52.33%. Among the tested saprophytic fungal isolates, *Aspergillus ochraceus* and *Rhizopus nigricans* showed high mean reduction of 47.18 and 46.71 % respectively.The treatments with culture filtrates of all tested fungal isolates were effective in reducing mycelia growth of *S. rolfsii.*
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## 1. Introduction:

Now, scientists have to look for methods which are ecologically friendly safe and specific for controlling pathogens. Root-rot disease of vegetables in general and of tomatoes in particular is a widespread and serious soil-borne disease in Saudi Arabia (Ismat *et al*., 2012). Root-rot disease caused by *Sclerotium rolfsii* being one of the most important diseases of crops ( Aycock, 1966 and Punja,1985). It has very wide host range and not easily controlled by chemical means (Sharma, *et al*., 2002). Pathogen will produce mustard seed like sclerotia, which are very resistant to degradation in soil and serve as inoculums for the next season and also, help in spreading of the disease to other plant (Rekha *et al*., 2012).

Sclerotial diseases caused by *Sclerotium rolfsii* occur primarily in warm climates, especially at high moistures and high temperatures (Al-Askar, *et al.*, 2013). The pathogens of sclerotial diseases cause damping-off of seedlings, stem canker, crown blight, root- rot, crown rot, bulb, tuber and fruit rots. Sclerotial diseases frequently affect a wide variety of plants, including most vegetables, flowers, legumes, cereals, forage plants and weeds (Agrios, 1997 and Farr, *et al*., 1995).

As control measures, chemical fungicides are successfully reported to control *Sclerotium rolfsii* by calixin (Siddaramaiah, *et al*., 1979); propiconazole (Hagans *et al*., 1991); plantavax and vitavax (Kulkarni, *et al*., 1986). Also, Fahim *et. al*. (1984) found that the growth of *S. rolfsii* on PDA medium was completely inhibited by Homi, Orthocide, Vitavax-Captan and Vitavax-Thiram at 25, 10, 0.5 and 1 ppm respectively.

Synthetic fungicides are helpful to sustain crop production by protecting plant from fungal diseases, but resistance to fungicides is one of the critical causes of poor disease control (Arafat, 2011), there are potential harmefull effects on human health and the environment (Demoz and Korsten, 2006).There is then need to examine possible non-synthetic biotic agents approaches for disease management ( Widyastuti *et al*., 2003, Adandonon *et. al*., 2006 and Aguin *et al*., 2006). Research during the last two decades has led to the possibility of biological control as an increasingly realistic option for management of plant pathogens. Also, earlier studies indicated that strains of *Trichoderma* and *Bacillus* spp. were effective against *S. rolfsii* (Elad *et al.*, 1983, Sariah, 1991, Adandonon *et al.,* 2006 and Bosah *et al.*, 2010). Narasimha (2000) stated that biological control is an eco-friendly and effective means of reducing the growth of *S. rolfsii* through potential antagonistic microorganisms. *Penicillium* sp. showed an antagonistic effect toward *S. rolfsii* (Agrawal *et al.,* 1978 and Prabhu *et al*., 2003). Study by Omorusi

*et al.,* (2007) suggested that filtrate of *Penicillium* sp. isolate as control agent was effective than other organisms. The main mechanism of action of these antagonistic microorganisms consist of mycoparasitization of pathogen, secretion of bioactive molecules, competition for space and nutrients and stimulation of the plant defensive capacity.

The inhibitory effect of culture filtrates of *Trichoderma* spp. on the growth of root-rot fungi has been reported by several workers (Agrawal *et al*., 1978; Pande, 1985 and Prasad and Kumar,2011). D'Ambra and Ferrata (1984) showed that the culture filtrate of *T. harzianum* checked the growth of *S. rolfsii* on agar medium and the effectiveness of the filtrates reduced with increase in dilution. However, Bosah *et al*. (2010) showed that of the three antagonists evaluated for inhibitory efficacy, *Trichoderma* sp. proved to be the most effective to *S. rolfsii* followed by *Aspergillus* sp. and *Penicillium* sp.

The present study was undertaken to evaluate new fungicides, different formulations of bioagents and culture filtrate of some saprophytic isolated fungi to know efficacy against *Sclerotium rolfsii* for further application in the field.

## 2.Materials and Methods

**Isolation and maintenance of *S. rolfsii* pathogen:**

Tomato *(Lycopersicum esculentum*) plants showing stem and foot rot symptoms were collected from Huda Al-Sham agricultural farm located at 110 km north-east Jeddah district, Saudi Arabia. The isolation of the causal pathogen from diseased plants was performed on potato dextrose agar (PDA) medium adopting the standard issue of isolation and identified according to their morphology and colony characteristics. Stock culture of *S. rolfsii* was maintained on PDA slants and stored at 4 ºC.

**Isolation and identification of native fungal isolates:**

Rhizospheric soil from different healthy plants such as tomato, cowpea, green bean and *Moringa oleifera* were collected in poly-ethylene bags and brought to the research laboratory. Serial dilution technique (Jonson and Curl, 1972) was used to isolate fungal antagonist from rhizosphere soil samples of healthy plants and shade dried. Antagonistic mycoflora were isolated on rose bengal agar medium by using a dilutions of $10^{-3}$and $10^{-4}$. One ml of soil suspension was poured into sterilized petriplates, and the melted and cooled medium was poured and then rotated gently to get uniform distribution of soil into the medium. Then, the plates were incubated at 27±2ºC and observed intervals for the development of colonies. The developed colonies were picked and identified based on mycological keys described and identified to the generic or species level according to Gilman (1957); Nelson *et al*. (1983) and Barnett and Hunter (1986).

**Evaluation of fungicides:**

The efficacy of ten fungicides viz., Ranger(50%), Oxidase(50%), Porto(30%), Score(75%), Benlate(50%), Tazolen (64%), Coprozin(35%), Topzin-m(70%), Rhizolex (50%), Top zin(100%) synthetic fungicides were assayed *in-vitro* against *S. rolfsii* at concentration of 0. 12.5, 25, 50, 100, 200 and 400ppm based on their active ingredient. These fungicides were evaluated under laboratory conditions by poison food technique.

Required quantity of individual fungicide was added separately into sterilized molten and cooled potato dextrose agar, so as to get the desired concentration of the fungicide. Later 15ml of the poisoned medium was poured into sterilized Petri plates. Mycelium discs of 5 mm size from seven days old culture was cut and one such disc was placed at the centre of each agar plate. Three replications were used for each concentration. The plates were incubated at 27±2ºC and the radial growth was measured when fungus attained maximum growth in control plates. The efficacy of the fungicides was expressed as percent inhibition of mycelial growth over control, calculated by using the formula given by Vincent (1947).

$$\text{Percent inhibition} = \frac{\text{Growth in control} - \text{growth in treatment}}{\text{Growth in control}} \times 100$$

**Evaluation of bio- agents:**

*In-vitro* evaluation was carried out with eight commercial biocides, e.g., Bio-Arc (*Trichoderma albium*) 25 x$10^6$ spores/ml; Bio-Arc (*B. megatherium*) 10x$10^6$ spores/ml; Plant guard (*T. harzianum*) 30 x$10^6$ spores/ml; Biocure-Z (*T. harzianum*) 30 x$10^6$ spores/ml; Biocure –F ( *T.viride*) 20 x$10^6$ spores/ml; Bio-Dewcon *(Amplomyces quisqualis*) 10 x$10^7$ spores/ml ; Bio-Mit (*Hirsatella thompsoni*) 3 x$10^6$ spores/ml and Bio-catch (*Verticillium lecanii*) 20 x$10^6$ spores/ml by dual culture technique for their antagonistic effect against *S. rolfsii*. Different volumes of each bioagent were added to conical flasks containing 100ml of sterilized PDA medium before its solidification, to obtain seven dilutions, i.e., 0, 125, 250, 500, 1000, 2000 and 4000 ppm based on recommended dose.The control treatment was bioagent–free medium. The percent inhibition of

growth of the pathogen was calculated as abovementioned procedures.

**Assaying of culture filtrate of selected saprophytic fungi to *S. rolfsii***

The effect of culture filtrates of the eleven selected saprophytic fungal isolates on the growth of *S. rolfsii* was studied as pre method given by Dennis and Webester (1971). 100 ml of sterilized potato dextrose broth taken in 250 ml flask was inoculated with a 5mm mycelial disc of the biocontrol agents cut from the edge of 7 day old culture. Inoculated flasks were incubated at 27±2ºC for 14 days with constant shaking at 150rpm. The culture filtrate was filtered through Whatman no.1 filter paper and the filtrate was fractioned at high centrifuge to get red of spores. The culture filtrate of bioagent and molten PDA of different strength were mixed together in different concentrations of 0.0, 12.5, 25 and 50%. For 50% dilution, 50ml of culture filtrate and 50ml double strength PDA were mixed together in equal portion (Prasad and Kumar, 2011). Control treatment (0, concentration) was simultaneously without using culture filtrate. The medium was then poured into the petriplate at 15ml/ plate. After solidification the plates were carefully inoculated with 5mm a disc of *S. rolfsii* pathogen cut from the 7 day old culture. Three replicates were used for each concentration. Plates were then incubated in an incubator at 27±2ºC and the radial growth was measured when fungus attained maximum growth in control plates. The growth inhibition percent of the pathogen was calculated as abovementioned before.

**Statistical analysis:**

All experiments were replicated three times and the data were statistically analyzed using the General Linear Model Procedure of the Statistical Analysis System. The significance of the differences among treatment groups was determined by Waller-Duncan k-ratio. All statements of significance were based on probability of $P \leq 0.05$.

## 3.Results and Discussion

**Effect of fungicidal products on growth of *S. rolfsii*:**

Data presented in Table (1) show that increasing all fungicides concentration decreased the mycelia linear growth of *S. rolfsii* under *in-vitro* conditions. Rhizolex was found to highly effective and gave 100% reduction in growth when used at lower concentration (12.5ppm). While, the same effect was recorded at 100, 200, and 400 ppm of Top zin and at 400ppm of Score fungicides. Tazolen showed also 100% inhibition at concentrations of 100 to 400ppm. Similar inhibitory effects of Provax-200 fungicide on *S. rolfsii* were recorded by Bhuiyan *et al*. (2012). Data also showed that Benlate and Coprozin were the least effective fungicides on mycelial growth at all concentrations, as the percentage reduction of 16.85 and 26.47 in mycelial growth were achieved at 400ppm respectively. Opposite trend was reported by Yaqube (Fouzia) and Saleem (2006) they reported that at low concentration of Benomy, Sancozeb, Thiovit, Dithan M-45, Carbandazin and Topsin-M showed no effect on the growth of *S. rolfsii*. Ranger and Oxidase fungicides resulted in ≤ 50% reduction on the growth of *S. rolfsii* when used at 400ppm without significant difference. Topsin-m significantly inhibited the radial growth of *S. rolfsii* by 21.47 and 31.50% at 200 and 400ppm concentrations respectively. The inhibitory effect of Topsin-M70 against many plant soil-borne pathogenic fungi, viz. *R. solani, F. oxusporum* and *S. rolfsii* has been reported by many researches (Vyas and Joshi, 1977, Fahim, *et al.,* 1984; Sujatha 1991; Ammar, 2003; Korra, 2005 and Arafat, 2011).

It seems from the results obtained that the concentration required to inhibit the vital process in the cells of the hyphae is differed for different fungicides. The majority of antifungal components act within the cell by the inhibition of vital processes (Fahim *et al*., 1984).It seems that the uptake of fungicide is not a normal diffusion process, but rather transport mechanism (Richmond and Somers, 1962). The fungicides used in this investigation may also differ in the ability of penetration and uptake via cell wall of the hypha of the *S. rolfsii* pathogen.

**Effect of bioagent products on growth of *S. rolfsii*:**

Eight commercial bioagents were tested against the growth of *S. rolfsii* on PDA medium and the results are presented in Table (2). Results show that all tested bioagents statistically reduced the mycelia linear growth of *S. rolfsii* compared to control. The maximum inhibition in growth was recorded at the higher concentration of 4000ppm (40.63% inhibition). The results suggest that an exposure to high concentration of bioagents was high toxic to fungal growth as compared to control. The bioagent Bio-arc (*T. albium*) was the most effective against *S. rolfsii* growth responsible for 44.66 mean % inhibition, followed by Biocure-F responsible for 29.08 mean % inhibition with significant difference. Elad (1996) stated that mechanisms of the antagonism of *Trichoderma* spp. against different pathogens may be due to mycoparasitism, competition and antibiosis. On the other hand, Bio-arc (*B. megatherium*), Biocure-Z (*T. harzianum*) and Plant guard (*T. harzianum*) bioagents were fare less effective, as the mean percentage inhibition were 2.81, 2.97 and 3.49% respectively. Moreover, all other bioagents, i.e., Bio-Dewcon, Bio-Catch and Bio-Mit were also moderately capable to reduce the radial growth of *S. rolfsii* by 18.44, 19.21 and 16.22 mean% inhibition respectively.

**Table 1: Effect of Fungicides on Percent inhibition of mycelia growth of *Sclerotium rolfsii***

| Fungicide | Percent inhibition of mycelia growth (ppm) | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | 12.5 | 25 | 50 | 100 | 200 | 400 | |
| **Ranger (50%)** | 0.00 | 23.33 | 30.93 | 31.87 | 36.100 | 37.70 | 42.03 | **28.85E** |
| **Oxidase (50%)** | 0.00 | 15.57 | 28.70 | 29.60 | 29.40 | 34.10 | 37.97 | **25.05E** |
| **Porto (30%)** | 0.00 | 46.70 | 51.67 | 56.10 | 52.03 | 70.73 | 71.13 | **49.77D** |
| **Score (75%)** | 0.00 | 74.37 | 80.57 | 86.70 | 87.07 | 87.07 | 100.0 | **73.68B** |
| **Benlate 50%** | 0.00 | 0.00 | 6.30 | 8.90 | 11.50 | 13.70 | 16.87 | **8.18G** |
| **Tazolen (64%)** | 0.00 | 15.73 | 52.00 | 74.27 | 100.0 | 100.0 | 100.0 | **63.14C** |
| **Coprozin (35%)** | 0.00 | 0.93 | 3.17 | 10.33 | 10.20 | 19.63 | 26.47 | **10.11G** |
| **Topzin-m(70%)** | 0.00 | 10.77 | 12.97 | 26.87 | 23.33 | 21.47 | 31.50 | **18.13F** |
| **Rhizolex (50%)** | 0.00 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | **85.71A** |
| **Top zin (100%)** | 0.00 | 32.60 | 81.30 | 85.20 | 100.0 | 100.0 | 100.0 | **71.30B** |
| **Mean** | **0.00F** | **32.00E** | **44.76D** | **50.98C** | **54.96B** | **58.44B** | **62.59A** | |

*Value within a column with the same letter do not differ significantly (p= 0.05%)
LSD at 5% for Fungicides = 5.572
LSD at 5% for concentration = 3.594
LSD at 5% for interaction = 9.311

**Table 2: Effect of different bioagents on Percent inhibition of mycelia growth of *Sclerotium rolfsii***

| Bioagents | Percent inhibition of mycelia growth (ppm) | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | 125 | 250 | 500 | 1000 | 2000 | 4000 | |
| **1-Bio-arc (*T. albium*)** | 0.00 | 19.83 | 44.23 | 44.83 | 53.87 | 65.20 | 84.67 | **44.66A** |
| **2-Bio-arc (*B.megatherium*)** | 0.00 | 0.00 | 0.00 | 0.00 | 2.97 | 7.20 | 9.47 | **2.81E** |
| **3-Plant guard (*T. harzianum*)** | 0.00 | 0.00 | 0.00 | 0.00 | 1.67 | 10.00 | 12.77 | **3.49E** |
| **4-Biocure- Z (*T. harzianum* )** | 0.00 | 0.00 | 0.00 | 1.87 | 4.83 | 6.87 | 7.23 | **2.97E** |
| **5-Biocure- F (*T. viride*)** | 0.00 | 0.00 | 0.00 | 0.00 | 65.93 | 68.00 | 69.63 | **29.08B** |
| **Bio-Dewcon (*Amplomyces quisqualis*)** | 0.00 | 0.0 | 0.00 | 0.00 | 37.60 | 44.47 | 47.03 | **18.44C** |
| **Bio-Mit (*Hirsatella thompsoni)*** | 0.00 | 0.00 | 0.00 | 0.00 | 61.70 | 37.57 | 44.27 | **16.22D** |
| **Bio-Catch (*Verticillium lecanii*)** | 0.00 | 0.00 | 0.00 | 0.00 | 41.10 | 43.33 | 50.00 | **19.21C** |
| **Mean** | **0.00F** | **2.48E** | **5.53D** | **5.83D** | **29.96D** | **35.33B** | **40.63A** | |

*Value within a column with the same letter do not differ significantly (p= 0.05%)
LSD at 5% for Bioagents = 2.049
LSD at 5% for concentration = 1.891
LSD at 5% for interaction = 8.286

It is evident that as the concentration of any bioagent increase the percentages inhibition of radial growth were also increased reached maximum at the higher concentration (4000ppm). Data also showed that Bio-arc (*B. megatherium*), Plant guard, Biocure F, Bio-Dewcon, Bio-Mit and Bio-Catch showed no effect (0% inhibition) on *S. rolfsii* at 125, 250 and 500ppm,while at the recommended dose (4000ppm) the percentage inhibition in the radial growth were 9.47, 12.77, 69.63, 47.03, 44.27 and 50.00% respectively. These results were agree in general with the findings of Bari *et al.* (2000),) Arafat (2011) and Manu *et al.* (2012).

**Effect of culture filtrates of saprophytic fungi on *S. rolfsii* growth:**

Isolation from rhizosphere soil of different healthy plants revealed the presence of several fungi, i.e. *Fusarium graminearum*, *Fusarium moniliforme, Fusarium oxysporum, Aspergillus ochraceus*, *Aspergillus niger*, *Alternaria tenues, Alternaria alternate, Penicillium sp., Pacilomyces sp., Rhizopus nigricans* and *Trichoderma harzianum* (un tabulated data). These fungi were previously reported to be isolated from the rhizosphere of different plants (Paul, and Clark, 1996).

Bioefficacy of culture filtrates of the eleven fungal isolates previously identified was studied against *S. rolfsii* and the obtained results on the inhibition of radial growth in dual culture plate technique was presented in Table (3). The data showed that the tested culture filtrate of all saprophytic fungal isolates were effective in reducing mycelia growth of *S. rolfsii*. It is clear that maximum inhibition was observed in the culture filtrate of *T. harzianum* drawn from potato dextrose broth with mean reduction of 52.33%. Among the tested fungal isolates, *Aspergillus ochraceus* and *Rhizopus nigricans* showed high mean reduction of 47.18 and 46.71 % respectively. This is followed by culture filtrates of *F. moniliforme* (44.35% inhibition) and *Aspergillus niger* (44.22% inhibition). Similar observations were reported by Pandy *et al.,* (1993) that culture filtrate of *A. niger* caused more than 50% inhibition of *Colletotrichum gleosporioides*.

Data showed that with the increase in culture filtrate concentration of the saprophytic fungi, the radial growth of the pathogen was proportionally decreased. The same trend was observed by Reshu and Khan (2012) who reported that *T. viride* caused maximum inhibition (80.17%) against *Alternaria* spp. growth at highest concentration of 3:1. Data also showed that maximum inhibition of the mycelia growth of the pathogen was observed with culture filtrate of *T. harzianum* used at concentration of 50%. These results are in agreement with those obtained by Raju *et al*.(2008)and Prasad and kumar (2011).who found that the cultures of cell free filtrates of all *Trichoderma* suppressed the radial growth of *R. solani*.

**Table 3: Effect of culture filterates of some saprophytic fungal isolates on Percent inhibition of mycelia growth of *Sclerotium rolfsii.***

| Culture filtrate of | Concentration of culture filtrate ( %) | | | | Mean |
|---|---|---|---|---|---|
| | 0 | 12.5 | 25 | 50 | |
| ***Fusarium graminearum*** | 0.00 | 52.43 | 57.57 | 58.53 | **42.13CDE** |
| ***Fusarium moniliforme*** | 0.00 | 52.80 | 53.47 | 71.13 | **44.35BCD** |
| ***Fusarium oxysporum*** | 0.00 | 39.07 | 42.60 | 57.97 | **34.91G** |
| ***Aspergillus ochraceus*** | 0.00 | 58.53 | 56.10 | 74.07 | **47.18B** |
| ***Aspergillus niger*** | 0.00 | 46.83 | 50.03 | 80.00 | **44.22BCD** |
| ***Alternaria tenues*** | 0.00 | 42.00 | 48.50 | 64.80 | **38.98EFG** |
| ***Alternaria alternate*** | 0.00 | 39.07 | 46.50 | 61.27 | **36.71FG** |
| ***Penicillium sp.*** | 0.00 | 44.27 | 56.27 | 62.03 | **40.64 DEF** |
| ***Pacilomyces sp.*** | 0.00 | 41.30 | 51.30 | 58.87 | **37.87EFG** |
| ***Rhizopus nigricans*** | 0.00 | 51.47 | 56.67 | 78.70 | **46.71BC** |
| ***Trichoderma harzianum*** | 0.00 | 61.87 | 67.30 | 80.17 | **52.33A** |
| **Mean** | **0.00 D** | **48.2 C** | **53.30 B** | **67.96 A** | |

*Value within a column with the same letter do not differ significantly (p= 0.05%)
LSD at 5% for C. filtrate = 4.695
LSD at 5% for concentration = 3.895
LSD at 5% for interaction = 8.286

Minimum inhibition was exhibited by culture filtrates of *F. oxysporum*, *Alternaria tenuis, Pacilomyces* and *Alternaria alternata* responsible for a 34.91, 38.98, 37.87 and 36.71 % reduction in pathogen growth respectively. As mentioned before, the culture filtrate of *T. harzianum* isolate was found to be more effective due to its more percentage inhibition.

**4. Conclusions:**

Systemic fungicides like Rhizolex, fungicide showed complete inhibition of the pathogen at low concentration. Whereas, Top zin, Score and Tazolen fungicides were found inhibitive at higher concentration. Among the bioagents, Bio-arc (*T album*) showed maximum inhibition of *S.rolfsii*. Results revealed that culture filtrates of *T. harzianum*, *Aspergillus ochraceus* and *Rhizopus nigricans* were

potentially effective against *S.rolfsii* known to be destructive to most economic crops.

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