Factors affecting on *Sclerotinia sclerotiorum* isolated from beans growing in Ismailia, Egypt

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Abstract: *Sclerotinia sclerotiorum*, causal agent of white rot in bean (*Phaseolus vulgaris*, L.) are highly variable pathogens. This study was conducted on cultural and physiological characters. The data revealed that maximum mycelial growth, sclerotia and sclerotia dry weight was obtained in Potato dextrose agar as semi-solid media i.e. 78 mm, 29 sclerotium and 232 mg, respectively. While Potato dextrose broth as liquid broth media was the best suitable medium to growth of the fungus with 2.29 mg dry weight per flask. The optimum pH for growth of both *S. sclerotiorum* was 5.5 by 2.41 mg/flask, however the 5 degree was the best for sclerotia formation and sclerotia dry weight which produced 17 sclerotium/flask and 134 mg/flask, respectively. Alternating light and darkness and continuous light were found to be the most suitable for maximum growth of the fungus that giving 90 mm while alternating light and darkness (16 hours light+8 hours darkness) was the best for sclerotia formation and dry weight of sclerotia by 16 sclerotium/plate and 128 mg/plate, respectively. This study also revealed that a relative humidity of 98.6% was most suitable for the growth, sclerotia formation and sclerotia dry weight of the pathogen.
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1. Introduction

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, 1987) and causes considerable damage to the host under greenhouses and protected agricultural areas. The pathogen has been reported to hamper the cauliflower cultivation by causing stalk rot in different cauliflower growing areas such as 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 crops (Newton and Sequeira, 1972; Abawi and Grogan, 1979; Clarkson *et al*., 2001), where ascospores are the primary infection source. Ascospores are released by apothecia which develop following carpogenic germination of sclerotia at or near the soil surface. Bean (*Phaseolus vulgaris*, L.) is one of the most important vegetable crop in Egypt and many countries of the world. In Egypt, the cultivated area was 69921 feedan in 2011 season yielded approximately 305561 tons with average about 4.37 ton/feddan (FAO Stat. database, 2011). One of the major limitation in bean (*Phaseolus vulgaris*, L.) production and export in Egypt is the infestation by *S. sclerotiorum* which cause losses in the total yield and decreasing the 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. The present study aimed to study the effect of some factors on morphological and physiological characters of *S. sclerotiorum*.

2. Material and Methods

Isolation of the pathogen

Plant samples with blighted stems and branches, and displaying sclerotia and mycelium, were arbitrarily sampled from the affected field. The plants were placed in a plastic bag over ice in a cooler and transported to the laboratory for processing. Four sclerotia were collected from each of sample, wrapped in a piece of sterile cheesecloth and submerged in 1% NaOCl solution for 1 minute. Sclerotia were then removed from the cheesecloth and rinsed in sterilize distilled water, and blotted dry between two layers of sterilize paper towel. Each of the selected sclerotia was individually plated on acidified PDA (APDA; 40 g/liter of Difco PDA amended with 1 ml of 85% lactic acid after

autoclaving and prior to dispensing into petri plates) in a 9-cm-diameterplastic petri plate. Plates were mentioned daily and emerging mycelia colonies were transferred to PDA. The purified fungal isolates were identified by Dept. of Plant Path., Fac. of Agric., Mans. Univ. (Kora *et al*., 2005).

Radial growth, sclerotia formation and dry weight *S. sclerotiorum* growing in semi-solid medium

In this study, five solid media viz. Potato Dextrose Agar (PDA), Czapek's Dox Agar (CD), Carrot Dextrose Agar (CDA), Cornmeal Agar (CMA), Oatmeal Agar (OA) and Yeast Extract Agar (YEA) were used. All the media were prepared according to the manufacturer instructions. Each Petri plate was poured with 20 ml sterilized medium for solidification. Discs of a 5 mm in diameter of *S. sclerotiorum* grown from the 7-day-old pre-cultured Petri plate on PDA were taken out with the help of a cork borer and placed at the center of each set of Petri Plates containing different medium. After inoculation, all Petri plates were incubated at 20±2°C. The diameter of the fungus was recorded in millimeters. Also, Number and dry weight of sclerotia per petri plates were recorded.

Mycelial growth, sclerotia formation and dry weight *S. sclerotiorum* growing in liquid broth medium

For proceeding this study, four broth media viz. Czapek's Dox Broth (CDB), Czapek Sucrose Nitrate Solution (CSN), Leonian and Potato Dextrose Broth (PDB) were used. All the media were prepared using the standard method. Sterilized 250 ml conical flasks were taken. Each flask had 100 ml sterilized medium poured into it. Equal discs measuring 5 mm in diameter of the test pathogen grown from the 7-day-old pre-cultured Petri dishes on PDA, were taken out with the help of a cork borer and placed in each set conical flask containing different medium. After inoculation, flasks were incubated at 20±2°C for 7 days and were shaken twice every day. Mycelial growth of each of the tested pathogen was harvested in pre-weighed moisture less Whatman filter paper No. 42, oven dried at 60°C and weighed again to record mycelial growth in milligrams. Studies of sclerotia formation and sclerotia dry weight on different liquid media used, was also undertaken.

Mycelial growth, sclerotia formation and dry weight *S. sclerotiorum* growing in different pH levels

Mycelial growth, sclerotia formation and sclerotia dry weight of *S. sclerotiorum* were studied at 7 pH level 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8. In all 250 ml conical flask (7 pH degrees, 4 replicates) were poured with 100 ml PDB. The desired pH levels of PDB medium were maintained by adding the required amount of buffer 0.1 N Citric acid or 0.1 N Sodium hydroxide with the help of a digital pH meter. Then medium was sterilized in autoclave at 120°C for 15 min. A five mm mycelial disc was transferred from the margin of the 7-day-old growing colony, to the flask with PDB. Mycelium harvested after seven days were measured according to the above mentioned method. Studies sclerotia formation and sclerotia dry weight were also undertaken according to the above mentioned method.

Radial growth, sclerotia formation and dry weight *S. sclerotiorum* growing in light and darkness

To study the effect of light and darkness on radial growth, sclerotia formation and sclerotia dry weight of *S. sclerotiorum*, 5 mm culture discs were cut with the sterilized cork borer from advancing margin of the colonies of the tested fungus and inoculated on PDA plates separately for 4 days. Carbon paper was used to wrap the Petri dishes for darkness. Fluorescent lamp was used for light exposure. All the Petri dishes were incubated at 20+2ºC in quadruplicates under following conditions.

(a) Complete darkness (0 hours).
(b) Alternating light and darkness (12 hours light + 12 hours darkness)
(c) Alternating light and darkness (16 hours light + 8 hours darkness)
(d) Continuous light (24 hours).

Radial growth, sclerotia formation and dry weight *S. sclerotiorum* growing in different relative humidity

The influence of humidity on mycelial growth, sclerotia formation, sclerotia dry weight of *S. sclerotiorum* was studied using PDA. To achieve various humidity levels, the osmotic potential of the agar was adjusted to -2,-4,-6,-8 and -10 MPa (corresponding 98.6, 97.1, 95.7, 94.4 and 93% RH, respectively) using NaCl concentration, based on data of Robinson and Stokes (1955). Plates were sealed with Parafilm and incubated at 20±2 ºC for 4 days (Alderman and Beute, 1986). mycelial growth, sclerotia formation, sclerotia dry weight of *S. sclerotiorum* were recorded.

3. Results and Discussion

Radial growth, sclerotia formation and dry weight *S. sclerotiorum* growing in semi-solid medium

The data in Table1 revealed that maximum mycelial growth was obtained in PDA by 51.5 mm. and produced the maximum number of sclerotia (29 sclerotiumr/plate). This was followed by CD and CDA which giving 49.25 and 47.50 mm in radial growth, while they giving 17.5 and 15.5 scleotium/plate, respectively. In contrast, OA was the

second medium for producing sclerotia (19.5 sclerotium/plate). While, YEA was not found suitable for growth of *S. sclerotiorum*, which required 7 days to fill the Petri plate, with average radial growth 30.0 mm and 1.75 sclerotium/plate. These results agree with those Steadman *et al*., (1994) who reported that PDA amended with pentachloronitrobenzene, penicillin, streptomycin was the best medium for growth of *S. sclerotiorum.* Also, Rai and Agnihotri (1971) found that Capek's medium supported good growth and sclerotia formation of *S. sclerotiorum.*

Table 1. Radial growth, sclerotia formation and dry weight *S. sclerotiorum* growing in semi-solid medium

Media	Radial growth after 3 days	Days to fill the Petri plate	No. of sclerotia/Petri	Dry weight of sclerotia/Petri (mg)
PDA	51.50[a]	4	29.00[a]	232.0[a]
CD	49.25[b]	4	17.50[bc]	147.0[b]
CDA	47.50[c]	4	15.50[c]	120.0[c]
CMA	34.00[e]	6	5.75[d]	46.0[d]
OA	44.00[d]	5	19.50[b]	136.0[bc]
YEA	30.00[f]	7	1.75[e]	10.0[e]
LSD	1.71		2.63	2.21

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

Mycelial growth, sclerotia formation and dry weight *S. sclerotiorum* growing in liquid broth medium

Among liquid media used, PD Broth was found the best for vegetative growth (2.39 mg/ml) as well as sclerotia formation (16 sclerotium/flask). This was followed by CSN, CDB and Leonian solution giving 2.2, 2.16 and 1.21 mg/ml dry weight and 14, 16 and 11.5 sclerotium/flask, respectively Table 2. Rai and Agnihotri (1971) also obtained the maximum growth of *S. sclerotiorum* on Capek's media.

Mycelial growth, sclerotia formation and dry weight *S. sclerotiorum* growing in different pH levels

Mycelia growth of the fungus was studied at 7 pH level 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8. The data revealed that the pH level significantly differentiates the mycelia growth. The maximum growth was recorded when the pH was at the level of 5.5 with dry weight of 2.41 mg/ml followed by pH 5.0 with mycelium dry weight of 2.40 mg/ml.

Table 2. Mycelial growth, sclerotia formation and dry weight *S. sclerotiorum* growing in liquid broth medium

Media	Mycelium dry weight (mg/ml)	No. of sclerotia/flask	Dry weight of sclerotia/flask (mg)
CDB	2.16[c]	16.0[a]	125.0[a]
CSN	2.20[b]	14.0[b]	94.0[b]
Leonian	1.21[d]	11.5[c]	69.0[c]
PDB	2.39[a]	16.0[a]	124.0[a]
LSD	0.28	2.18	6.22

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

Conversely, the pH level of 5 was the best for sclerotium production followed by level 5.5 giving 17 and 14 sclerotium/flask, respectively. Whereas, pH levels of 7.5 and 8.0 did not suitable for growth of the fungus, which gave 0.61 and 0.33 mg/ml mycelial dry weight, respectively and did not have any sclerotia. Hence, it is clearly indicated that the tested *Sclerotinia* favored acidic pH for its growth. Rai and Agnihotri (1971) reported that *S. sclerotiorum* could tolerate a wide range of pH, but good growth and sclerotia formation were favored at pH varying from 4 to 5.5.

Table 3. Mycelial growth, sclerotia formation and dry weight *S. sclerotiorum* growing in different pH levels

pH values	Mycelium dry weight (mg/ml)	No. of sclerotia/flask	Dry weight of sclerotia/flask (mg)
5.0	2.40[a]	17.00[a]	134.0[a]
5.5	2.41[a]	14.50[b]	131.0[b]
6.0	1.99[b]	13.50[b]	108.0[c]
6.5	1.48[c]	11.50[c]	64.0[d]
7.0	1.04[d]	5.25[d]	29.0[e]
7.5	0.61[e]	0.00[e]	0.0[f]
8.0	0.33[f]	0.00[e]	0.0[f]
LSD	0.05	1.13	1.17

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

Radial growth, sclerotia formation and dry weight *S. sclerotiorum* growing in lighting and darkness

Inside number of light hours, growth of the fungus was observed between 0-24 light hours. All number of lighting hours were appropriate good growth of *S. sclerotiorum*, ranging from 87 mm (ze0 hour) to 90 mm (12, 16 and 24 hour). In addition, results show that, 16 light hours was the best for sclerotia production with 16 sclerotium/plate, followed by 12 light hours by 13.75 sclerotium/plate. Nevertheless, zero light hours did not proper for producing sclerotia. Previous studies with *S. sclerotiorum* reveal that number of the lighting hours very important to the growth of *S. sclerotiorum* (Clarkson *et al*., 2003).

Table 4. Radial growth, sclerotia formation and dry weight *S. sclerotiorum* growing in light and darkness

Lighting hours	Radial growth after 3 days	No. of sclerotia/Petri	Dry weight of sclerotia/Petri (mg)
0	87.0[b]	0.00[d]	0.0[d]
12	90.0[a]	13.75[b]	103.0[b]
16	90.0[a]	16.00[a]	128.0[a]
24	90.0[a]	7.25[c]	58.0[c]
LSD	1.41	1.96	12.41

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

Radial growth, sclerotia formation and dry weight *S. sclerotiorum* growing in different relative humidity

Higher relative humidity significantly increased the growth of the *S. sclerotiorum*. The growth of the fungus was significantly increased by the increasing RH%. The best growth of the fungus occurred at 98.6% RH with radial growth of 90 mm. and 15 sclerotium/plate (Table 5). The growth shows a linear increase in all the treatments with time indicating that the *S. sclerotiorum* require high relative humidity for good growth. This is in agreement with the findings of Grogan and Abawi (1975) and Partyka and Mai (1962) who confirmed that low ambient relative humidity is often a factor that limits growth of the fungus and the growth of this fungus was closely correlated with RH.

Table 5. Radial growth, sclerotia formation and dry weight *S. sclerotiorum* growing in different relative humidity%

RH%	Radial growth after 3 days	No. of sclerotia/Petri	Dry weight of sclerotia/Petri
93	20.00[e]	13.00[a]	104.0[c]
94.4	34.25[d]	13.25[a]	106.0[c]
95.7	60.25[c]	13.25[a]	108.0[bc]
97.1	75.75[b]	13.75[a]	111.0[b]
98.6	90.00[a]	15.00[a]	120.0[a]
LSD	7.56	4.11	2.14

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

4. Conclusion

It is apparent from the study that low relative humidity and pH alkaloid do not support the growth of *S. sclerotiorum*. Therefore, it is highly recommended that fruits and vegetables should be stored at low relative humidity and alkaloid pH regimes to avoid infections due to *S. sclerotiorum* and this may contribute significantly in controlling the heavy losses our beans farmers incurred due to this fungus.

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