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MORPHOLOGICAL, CULTURAL AND PHYSIOLOGICAL STUDIES ON *Slerotinia sclerotiorum* CAUSING STALK ROT OF CAULIFLOWER

Nguyen Duc Cuong[(1)] and N. P. Dohroo[(2)]

(1) Cuu Long Delta Rice research Institute, Cantho, vietnam
(2) Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, H.P., India

ABSTRACT

Morphological, cultural and physiological of Sclerotinia sclerotiorum were studied on solid and liquid media at Vegetable Pathology Laboratory, Department of Mycology and Plant Pathology, Dr. Y.S. Parmar University of Horticulture and Forestry, Himachal Pradesh, India. The fungus produced aerial mycelium, which was hyaline consisting of closely septate hyphae and measured 2.0-9.4 µm. Micro-conidia were produced on conidiophores of the vegetative mycelium and measured 1.5-3.5 µm. Sclerotia were black in colour, round to irregular and measured 2-15 x 1.5-7 mm. Apothecia were round or globate type with diameter ranging from 2-9 mm. The asci were hyaline and cylindrical and measured 91-165 x 4.9-8.5 µm. Ascospores were elliptical and ranged from 6.5-13 x 4.2-6.6 µm. The fungus was found to grow best on potato dextrose agar medium and Richard solution. Optimum temperature and pH for the growth of the fungus were 20-25^{o}C and pH 5.0, respectively.

Key words: culture, morphology, physiology, *Sclerotinia sclerotiorum*.

INTRODUCTIONS

S. sclerotiorum, one of the most destructive soil borne pathogens has been reported to affect a wide range of wild and cultivated host plants 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 such as United States during 1942-1943 (Snyder and Baker 1945) and particularly in India in Himachal Pradesh in 1973 (Sharma *et al.*, 1983). The fungus is classified within the genus *Sclerotinia* of the *Sclerotiniaceae,* an important family of *Discomycetes* of the class *Ascomycetes* (Kora *et al.* 2003). The early taxonomy of the species was based on the size and general characteristics of the *sclerotiorum*, host range, and dimension of ascospores and asci (Willetts and Wong 1980). However, several studies (Purdy 1955; Grogan 1979) showed that this system was inadequate. The taxonomy of the *Sclerotiniaceae* has been a source of controversy for years and continues to be under investigation (Kora *et al.* 2003). Several lines of evidence, including genetic markers and nucleotide sequence homologies (Kohn et al. 1988), substantiate the taxonomic position of the genus Sclerotinia and characterization of *S. sclerotiorum* as a distinct species of genus Sclerotinia. However, *S. sclerotiorum* is a destructive soil borne pathogens yet the future of cauliflower seed industry in the hilly region has been threatened with the serious recurrence of Sclerotinia rot. The research was therefore undertaken to study on morphology, culture and physiology of *Sclerotinia sclerotiorum* with an aim to give more information for taxonomy as well as disease management strategy.

MATERIALS AND METHODS

The experiment was laid out at Vegetable Pathology Laboratory, Department of Mycology and Plant Pathology, Dr. Y.S. Parmar University of Horticulture and Forestry, Himachal Pradesh, India, during the time of October 2004 to November 2005.

a. Morphological study

- **Myceliogenic study**

Morphological characters of mycelium and conidia of the pathogen were studied while growing it on PDA medium. The PDA was prepared and sterilized at 1.05 kg cm^{-2} for 20 minutes in an autoclave, cooled and was poured in 90 mm Petri plates (30 ml for each plate) under aseptic conditions. The poured Petri plates were inoculated with a uniform disc of 5 days old culture of the pathogen at the centre of Petri plates. The inoculated Petri plates were incubated at 25±1°C and observed for morphological characters.

• Carpogenic study

To see the carpogenic germination of sclerotia in moist sand in Petri plates, clean sand was sterilized for one hour in autoclave at 1.05 kg /cm^{-2} for two consecutive days. Sixty gram of sterilized sand was spread in each Petri plate. Five sclerotia per Petri plate were buried in sand and moistened with sterilized distilled water regularly and kept for carpogenic germination at room temperature (10±5°C). Observations on mycelial growth from sclerotia and apothecia formation were made regularly.

b. Cultural studies

• Solid media

Six solid media i.e., potato dextrose agar, corn meal agar, French bean seed agar, oat meal agar, pea seed agar, lettuce leaf agar were prepared and autoclaved at 1.05 kg cm^{-2} pressure for twenty minutes. The pH of each medium was adjusted at pH 5.0 prior to autoclaving. Uniform quantities (30 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 and incubated at 25±1°C. Each treatment was replicated four times. Observations on radial growth of mycelium, number of days taken by mycelium to fill Petri plate and average number of sclerotia formed per Petri plate were recorded.

• Liquid media

Five liquid media i.e., Richard solution, Asthana and Hawker medium, Czapek sucrose nitrate solution, Leonian solution, Corn liquid medium were prepared. The pH in each case was also adjusted at 5.0 before autoclaving. Each medium (30 ml) was poured separately in 150 ml Erlemeyer flasks, plugged with non-absorbent cotton and sterilized in an autoclave at 1.05 kg cm^{-2} for 20 minutes. Each flask was inoculated separately with uniform quantity of homogenous culture suspension (1 ml) prepared by triturating mycelial mat of one flask grown on Richard solution. The inoculated flasks were incubated at 25±1°C for 15 days. Thereafter, the mycelial contents were filtered out through already weighed Whatman No 1 filter papers and constant dry weight of the mycelial mat from each flask was recorded after drying 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 and sclerotia as well as average number of sclerotia formed per flask.

c. Physiological Studies

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

Petri plates containing uniform quantities (30 ml) of sterilized PDA medium were inoculated with 5-day old uniform culture bits of the pathogen (*S. sclerotiorum*) and incubated at different temperatures *i.e.*, 5, 10, 15, 20, 25 and 30°C. Each treatment was replicated four times and data were recorded on radial growth of mycelium, number of days taken by mycelium to fill Petri plate and average number of sclerotia formed per Petri plate.

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

Richard medium was used as a basal medium for finding out the pH requirement of the fungus. Different pH levels *i.e.*, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 were maintained. For inoculation, the same procedure was done as in case of liquid media. The experiment was designed in a CRD with eight treatments, which were replicated four times. Data on dry weight of mycelium and sclerotia as well as average number of sclerotia formed per flask were recorded.

RESULTS AND DISCUSSIONS

a. Myceliogenic and sporogenic characters

The fungus produced aerial mycelium, which was hyaline, branched, well developed and

appeared cottony, consisting of closely septate hyphae which were both inter- and intracellular. In addition, Colotelo (1974) reported that, subsurface mycelial cells swelled, became pigmented and formed the dark bulbous rind cells; which darkened with age. The hyphae were 2.0-9.4 µm in width and contained dense granular protoplasm. The micro-conidia were produced on conidiophores of the vegetative mycelium and measured 1.5-3.5 µm in culture. This observation agrees to Sharma (1979) who reported micro-conidia to be 1.5-3.5 µm while Khan (1976) reported that hyphae were 6-16 µm in width. The sclerotia were round to irregular in shape in culture and measured 1.5-7 mm in width and 2-15 mm in length. Singh (1985) reported that, sclerotia varied in shape and size according to environment and location. Sclerotia formed on host surface were usually loaf shaped or globose while those formed in the pith of the stem were elongated, which could be due to the space available for growth. Sharma (1979) confirmed that, sclerotia produced in culture were similar to those produced on the host in all morphological characters. Sclerotia started germination and giving rise to several columnar structures (stipes). The stipes later developed funnel shaped cup (apothecium) at the tip. Apothecia were brown in colour and were round or globate type. The length of apothecia measured from 5-21 mm, whereas diameter of the discs ranged from 2-9 mm with number ranging from 1-9 per sclerotia. The asci were hyaline and cylindrical in shape, which measured 91-165 x 4.9-8.5 µm in size. Each asci contained eight ascospores which were found to be released in clouds. Ascospores were elliptical and ranged from 6.5-13 x 4.2-6.6 µm in size. The morphological characters of the fungus were in accordance with the taxonomic keys given by Willetts and Wong (1980) thus confirming the identity of the fungus as *Sclerotinia sclerotiorum* (Lib.) de Bary.

Table 1. Myceliogenic and carpogenic characters of *S. sclerotiorum*

Fungal part	Colour	Number per unit	Shape	Size	
				Width/ diameter	Length
Hyphae	Hyaline	--	Branched, cottony, closely septate	2.0-9.4 µm*	Indeterminate
Micro-conidia	Hyaline	--	Globose	1.5-3.5 µm**	--
Sclerotia	White to black	22-45 (Petri plate)	Round or irregular	1.5-7 mm*	2-15 mm
Apothecia	Brown	1-9 (Sclerotia)	Round or globate	2-9 mm**	5-21mm
Asci	Hyaline	Numerous (Apothecium)	Cylindrical	4.9-8.5 µm*	91-165 µm
Ascospore	Hyaline	8 (Ascus)	Elliptical	4.2-6.6 µm*	6.5-13 µm

* Width; ** Diameter; -- Nil/ Not recorded

b). Growth of *S. sclerotiorum* on solid and liquid media

Cultural studies with six solid media showed that potato dextrose agar medium was the best supporting media for mycelial growth (52.47 mm) of the fungus and produced maximum number (30.75) of sclerotia. These results are in accordance with the findings of Khan (1976) and Sharma (1979) who also found potato dextrose agar medium suitable for growth of the fungus and production of maximum number of sclerotia. While, lettuce leaf agar was not found suitable for growth of the fungus, which required 7 days for full growth in the Petri plates, with average radial growth of 30.65 mm and 1.5 average number of sclerotia per Petri plate. The medium was tried for the first time and there was no conformity with previous study though, the

fungus infected lettuce plants as per earlier reports. However, Khan (1976) and Kaith (1977) also reported poor growth on some other natural media such as sunflower seed extract and rapeseed extract, which might be due to presence of some growth inhibitory substances in their extract.

Table 2. Mycelial growth of *S. sclerotiorum* and sclerotial development on different solid media

Solid medium	Average radial growth of mycelium after 4 days (mm)	Days to fill the Petri plate	No. of sclerotia formed/ Petri plate (90 mm)
Potato dextrose agar	52.47	4	30.75
Pea seed agar	50.92	4	18.00
French bean seed agar	48.33	4	11.50
Oat meal agar	44.05	5	15.25
Corn meal agar	34.66	6	4.75
Lettuce leaf agar	30.65	7	1.50
CV (%)	4.50		14.50
LSD (5%)	2.91		2.93

Among liquid media studies, Richard solution was found the best for vegetative growth (242.85 mg) as well as sclerotial formation. The results are in agreement with those of Khan (1976) and Sharma (1979) who also found Richard solution suitable for the growth of the fungus and thus used this medium in their physiological studies. While, Asthana and Hawker medium was not found suitable for vegetative growth (71.90 mg) as well as sclerotial formation. The result was in close agreement with finding of Sharma (1979) who also reported similar results for Asthana and Hawker medium. Khan (1976) reported that Czapek medium was not suitable for mycelium growth as well as sclerotial formation while Kaith (1977) did not found sarson seed extract and oat seed extract suitable for the mycelium growth.

Table 3. Mycelial growth of *S. sclerotiorum* and sclerotial development on different liquid media

Liquid medium	Dry weight of mycelium (mg)	No. of sclerotia formed	Dry weight of sclerotia (mg)
Richard solution	242.85	15.00	122.63
Czapek sucrose nitrate solution	222.58	14.00	93.14
Leonian solution	122.15	11.25	76.40
Coon liquid medium	103.10	6.50	34.60
Asthana and Hawker medium	71.90	0.75	0.90
CV (%)	20.05	26.14	34.68
LSD (5%)	46.10	3.74	34.26

c). Effect of temperature and pH on growth of *S. sclerotiorum*

In temperature studies, growth of the fungus was observed between 5-30^{o}C. The result indicated that, the fungus tolerated low temperature much better for its growth than high temperature. The optimum temperature for the growth of the fungus ranged between 20-25^{o}C with average colony diameter of 43.99 and 50.28 mm after four days of inoculation. However, optimum temperature for production of sclerotia was 15-20^{o}C. Growth of the fungus was very poor at 30^{o}C with average colony diameter 3.17 mm after four days of inoculation with no sclerotia formation. Bedi (1962), Khan (1976) and

Sharma (1979) also reported similar results. Abawi and Grogan (1975) reported that mycelial growth and sclerotial production were optimum at 20-25°C. Kapoor (1994) multiplied *S. sclerotiorum* at 24±1°C on PDA medium. Coe (1944) reported best growth of *S. sclerotiorum* at 19-20°C on PDA medium while Purdy (1956) found that temperature effected size of sclerotia with the largest size occurring at 25°C. Willetts and Wong (1980) reported that growth of fungus occurred over a wide range of 0-35°C. While Bilgrami and Verma (1981) confirmed that optimum temperature ranged for many fungi between 15-30°C.

Table 4. Effect of temperature on mycelial growth of *S. sclerotiorum* and sclerotial development

Temperature (°C)	Radial growth of mycelium (mm)	Days to fill the Petri plate	No. of sclerotia formed
5	5.87	15	5.00
10	20.93	10	19.75
15	29.23	7	27.25
20	43.99	5	28.50
25	50.28	4	30.00
30	3.17	--	0.00
CV (%)	12.62		11.90
LSD (5%)	4.79		3.25

Under pH studies, suitable growth of *S. sclerotiorum* was obtained on Richard liquid medium at different pH levels ranging from 4.0-8.0. The results indicated that the best growth of the fungus occurred at pH 5.0 with mycelium dry weight of 241.65 mg followed by pH 5.5 with mycelium dry weight of 239.06 mg. While, pH levels of 7.5 and 8.0 did not favour good growth of the fungus. The results are also in close agreement with Sharma (1979) who found pH 5.0 suitable for vegetative growth of the fungus. However, Willetts and Wong (1980) reported that pH below 5.0 was optimum, whereas, Khan (1976) confirmed optimum pH of 4.6 and 4.5 for 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*.

Table 5. Growth of *S. sclerotiorum* at different pH levels

pH level	Dry weight of mycelium (mg)	No. of sclerotia formed	Dry weight of sclerotia (mg)
4.0	227.29	14.00	122.21
5.0	241.65	17.25	133.59
5.5	239.06	14.75	132.83
6.0	204.25	13.50	110.61
6.5	157.48	11.00	65.65
7.0	108.23	5.25	30.05
7.5	65.54	0.00	0.00
8.0	38.05	0.00	0.00
CV (%)	9.35	23.93	21.39
LSD (5%)	21.88	3.30	23.21

CONCLUSIONS

The fungus produced aerial mycelium, which was hyaline consisting of closely septate hyphae and measured 2.0-9.4 μm. Micro-conidia were produced on conidiophores of the vegetative mycelium and measured 1.5-3.5 μm. Sclerotia were black in colour, round to irregular and measured 2-15x1.5-7 mm. Apothecia were round or globate type with diameter ranging from 2-9 mm. The asci were hyaline and cylindrical and measured 91-165x4.9-8.5 μm. Ascospores were elliptical and ranged from 6.5-13 x 4.2-6.6 μm.

The fungus was found to grow best on potato dextrose agar medium, whereas, lettuce leaf agar gave the least growth of the fungus. In liquid media, the fungus gave maximum growth on Richard solution; followed by Czapek sucrose nitrate solution. The growth of fungus was very poor on Asthana and Hawker medium.

The fungus tolerated low temperature ranges much better for its growth than high temperatures. Growth was observed between temperature ranges of 5-30^{o}C. However, the optimum temperature for the growth of the fungus ranged between 20-25^{o}C. The fungus was found to grow at all the pH levels (4.0-8.0) with an optimum at 5.0. Sclerotial formation was found to be directly correlated with its temperature and pH level requirements.

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Nghiên cứu đặc điểm hình thái và sinh lý của nấm *Sclerotinia sclerotiorum* gây bệnh thối thân trên cải bông

Nghiên cứu đặc điểm hình thái và sinh lý của nấm *Sclerotinia sclerotiorum* được thực hiện trong môi trường lỏng cũng như môi trường đặc tại bộ môn bệnh cây, trường đại học lâm nghiệp và nghề làm vườn Dr. Y.S. Parmar, bang Himachal Pradesh, nước cộng hoà Ấn Độ. Trong điều kiện môi trường nuôi cấy, nấm bệnh hình thành sợi nấm khí sinh, trong suốt, chứa vách ngăn, đường kính sợi nấm từ 2.0-9.4 µm. Tiểu bào tử được hình thành trên cuống bào tử của sợi nấm sinh dưỡng với kích thước 1.5-3.5 µm. Hạch nấm màu đen, hình tròn đôi khi hình dạng không xác dịnh với kích thước 2-15 x 1.5-7 mm. Quả thể dạng hình tròn hoặc bầu dục với đường kính từ 2-9 mm. Nang trong suốt, hình trụ, kích thước từ 91-165 x 4.9-8.5 µm. Bào tử có hình elip với kích thuớc 6.5-13 x 4.2-6.6 µm. Nấm bệnh phát triển tốt nhất trên môi trường đặc PDA cũng như môi trường lỏng Richard. Nhiệt độ thích hợp cho nấm bệnh phát triển khoảng 20-25^{o}C với pH tối hảo pH 5.0.