Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-7: 1044-1052

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-7 pp. 1044-1052**
Journal homepage: http://www.ijcmas.com

Original Research Article

Morphological, Cultural and Physiological studies on *Slerotinia sclerotiorum* causing Stem rot of Oilseed brassica

Mohammad Akram Husain and C. S. Choudhary*

Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar – 848 125, India
**Corresponding author*

A B S T R A C T

Morphological, cultural and physiological of *Sclerotinia sclerotiorum* were studied on solid and liquid media at Laboratory of the Department of Plant Pathology, Tirhut College of Agriculture, Dholi, Muzaffarpur, Bihar, India. Isolation of the pathogen was made on PDA medium and identified as *Sclerotinia sclerotiorum* (Lib.) de Bary. The pathogen produce aerial mycelium, which was hyaline, branched well developed and appeared cottony, consisting of closely septate hyphae which were both inter and intra cellular. The hyphae were 2.0 to 11.5 µm in width and contained dense granular protoplasm. The sclerotia were round to irregular in shape in culture and measured 1.5 to 7 mm in width and 2 to 15 mm in length. Cup-shaped apothecia were developed on germination of sclerotia. Apothecia were brown in colour and were round to globate type. The length of apothecia measured from 5 to 21 mm, whereas diameter ranged from 0 to 7 mm with number ranged from 1 to 9 per sclerotium. The pathogenic behaviour of the fungus was also confirmed after test. Potato dextrose agar (PDA) medium among solid media and Richards' medium among liquid media were found most supportive for the growth and sclerotial formation of the *Sclerotinia sclerotiorum.* Under physiological studies, the result concluded that the temperature 20-25^{0}C and pH 4.5 to 5.5 was most suitable for the growth and sclerotial formation by the pathogen.

Keywords

Culture, morphology, physiology, *Sclerotinia sclerotiorum*

Introduction

The oil yielding brassica crops grown in India include rai or raya or mustard [*Brassica juncea* (L.) Czern. & Coss.], and rapeseed (*B. rapa* sp. *oleifera*). Rapeseed and mustard are supposed to be the second important oilseed crop of the country adopted to temperate and subtropical regions and contribute much to agricultural economy of the nation because of its extensive use as a staple cooking oil in daily diet of common people in northern and eastern part of India. India is one of the leading oilseeds producing country in the world accounting for 11.12 per cent of the world's rapeseed-mustard production, and ranks third in the world next to China and Canada. Because of its extensive use as a staple cooking oil in daily diet of common people in northern and eastern part of India there is a pressing need to increase the production and productivity of rapeseed-mustard to meet day to day demand.

The severe attack of many diseases not only deteriorates the quality of the seed, but also reduces the oil content considerably. Losses

due to disease vary between 10-20% in rapeseed-mustard depending upon the prevailing environment. Among the fungal diseases, Sclerotinia rot disease of oilseed brassica incited by *Sclerotinia sclerotiorum* (Lib.) de Bary, earlier considered to be a minor disease, is now becoming increasingly destructive and widely damaging in recent years. Significant increase in the sclerotial population in the soil due to monocropping and cultivation of rapeseed-mustard under irrigated condition, has made Sclerotinia rot very serious disease of oilseed brassica crops in states including Rajasthan, Haryana, Punjab, Assam, West Bengal, Madhya Pradesh, Uttar Pradesh, and Bihar (Aggarwal *et al*., 1997; Saharan and Mehta, 2002). In fact, the disease incidence upto 80% has been reported in Punjab and Haryana (Kang and Chahal, 2000; Sharma *et al*., 2001), and 72% in Uttar Pradesh (Chauhan *et al*., 1992). In Rajasthan, 60% seed yield loss has been reported in severely infected plants (Krishnia *et al*., 2000; Ghasolia *et al*., 2004).

However, *S. sclerotiorum* is a destructive soil borne pathogens yet the future of oilseed brassica production and ultimately the edible oil industry of India will be 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

During the course of present investigations all the experiment pertaining to the Sclerotinia rot disease of oilseed brassica occurring in the Bihar state were carried out in the laboratory of the Department of Plant Pathology and Research Farm of Tirhut College of Agriculture, Dholi. The trials were conducted during *Rabi*, 2016-17.

To confirm the identity, detailed studies on the morphological features of the pathogen was undertaken. The organism was cultured *in vitro*. Before the culture, glass wares, inoculation needle and other equipments used in laboratory during the course of studies were sterilized.

(A) Morphological studies

a. Myceliogenic study

Morphological characters of mycelium and conidia of the pathogen were studied while growing it on PDA medium. 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^{0}C and observed for morphological characters. Morphological studies of the pathogen were carried out by examining the slide through a research microscope. Characters of mycelium *viz.* branching patterns, width and colour, scleritia *viz.* texture, shape, size and colour and conidia and conidiospores, size, shape and colour were recorded with the help of ocular and stage micrometers calibrated on a research microscope.

b. 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^{0}C). Observations on mycelial growth from sclerotia and apothecia formation were made regularly.

(B) Cultural studies

a. Solid media

Six solid media *i.e.*, Potato dextrose agar, Asthana and Hawker's agar, Czapek's (dox) agar, Richards' agar, Mustard stem decoction agar and Mustard leaf decoction 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 (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 (7 days) vigorously growing culture and incubated at 25±1^{0}C. Each treatment was replicated three 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 plates were recorded.

Measurement of radial growth

Radial growth of mycelium in Petri plates on different solid media were measured by drawing two lines passing through centre of the cultured plates at right angles to each other on the back side of each Petri plates. The diameters of the fungal growth on these two lines were measured and the average of the two expressed as diameter of the developing colony in mm. thus, linear growth of the colony was measured into two directions. In case of wavy, irregular growth, colony average growth was measured by averaging the largest and shortest diameter of the fungal growth.

b. Liquid media

Six liquid media *i.e.*, Richards' medium, Asthana and Hawker's medium, Czapek's (dox) medium, Potato dextrose broth, Mustard stem decoction and Mustard leaf decoction were prepared. The pH in each case was also adjusted at 5.0 before autoclaving. Each medium (30 ml) was poured separately in 100 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 culture bits (5 mm) cut from young (7 days) vigorously growing culture and incubated at 25±1^{0}C for 14 days. Thereafter, the mycelial contents were filtered out through already weighed Whatman filter papers No. 1 and constant dry weight of the mycelial mat from each flask was recorded after drying at 80^{0}C in a hot air oven for 24 hours. Each treatment was replicated three times and data were recorded on dry weight of mycelium and sclerotia as well as average number of sclerotia formed per flask.

The dry weight of the mycelial mats of the fungus was calculated in mg as follows:

$$DW = W_2 - W_1$$

Where,
DW = Dry weight of fungal mass
W_1 = Weight of fungal mass along with filter paper
W_2 = Weight of filter paper alone.

(C) Physiological studies

Potato dextrose medium was taken as basal medium for the physiological studies of identified pathogen *i.e. Sclerotinia sclerotiorum* (Lib.) de Bary.

a. Effect of temperature

Effect of temperature on mycelial growth of the *Sclerotinia sclerotiorum* was studied *in vitro*. Twenty ml of sterilized PDA medium was poured in each sterilized Petri plates. Inoculation was made with 5 mm disc of 7

days old uniform culture of the pathogen (*S. sclerotiorum*) and incubated at 4 different levels of temperature *i.e.* 10, 15, 20, and 25^{0}C. Each treatment was replicated five 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**.**

b. Effect of pH

To study the effect of different pH on mycelial growth of the pathogen, 30 ml of potato dextrose medium was pipetted in 100 ml Erlenmeyer flasks and seven different pH levels *i.e.* 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 were adjusted with the help of pH meter by using N/10 HCl or N/10 NaOH solutions. For inoculation, the same procedure was done as in case of liquid media. The experiment was designed in CRD with seven treatments, which were replicated three times. Data on dry weight of mycelium and sclerotia as well as average number of sclerotia formed per flask were recorded.

Results and Discussion

The results so obtained during investigations have been described under following subheads:

Morphological studies

Myceliogenic study

To study the myceliogenic characters of the fungus, 7 days old culture obtained from PDA was examined under microscope.

The pathogen produce aerial mycelium, which was hyaline, branched well developed and appeared cottony, consisting of closely septate hyphae which were both inter and intra cellular. The hyphae were 2.0 to 11.5 µm in width and contained dense granular protoplasm. The sclerotia were round to irregular in shape in culture and measured 1.5 to 7 mm in width and 2-15 mm in length. Sclerotia formed on host surface were usually loaf shaped or globose while those formed in the pith of the stem were elongated. Sclerotia produced in culture were similar to those produced on the host in all morphological characters. *In vitro* studies reveals its distribution at the edge of Petri plates on PDA. They were mostly globose, elongated and irregular shaped.

Carpogenic study

After installation of the sclerotia in moist sand as described above at 10-15^{0}C for 15 to 20 days the sclerotia starts to germinate and give rise to several columnar structures (stipes). The stipes later developed funnel shaped cup (apothecium) at the tip. Cup-shaped apothecia were developed on germination of sclerotia. Apothecia were brown in colour and were round to globate type. The length of apothecia measured from 5 to 21 mm, whereas diameter ranged from 2 to 9 mm with number ranged from 0 to 7 per sclerotium (Table1).The morphological characters of *S. sclerotiorum* thus obtained were found in accordance with the studies of Cuong and Dohroo (2006), Goswami *et al.* (2012) and Gill *et al.* (2014).

Cultural studies

On solid media

The fungus capable of growing on different solid media, but variation in growth and number of sclerotia formed was observed on six solid media. It was found that the growth of the pathogen on Potato dextrose agar (PDA) was maximum (54.67 mm) and significantly superior over all other media tested which was followed by Mustard leaf decoction agar and Mustard stem decoction

agar. Minimum growth was observed in Asthana and Hawker's agar medium (13.33 mm) after 3 days of incubation. After 5 days of inoculation also maximum mycelial growth was recorded in Potato dextrose agar medium (90 mm) which was at par with mustard leaf decoction agar (87 mm) and mustard stem decoction agar (85 mm) which was followed by Czepek's (dox) agar (55.33 mm), Richards' agar (47.67 mm). Minimum mycelial growth was observed in Asthana and Hawker's agar (29 mm). (Table 2).

Number of sclerotia formed per plate was counted after 14 days of inoculation. Maximum number of sclerotia was formed on Potato dextrose agar medium (36.33) which was at par with Richards' agar (31.67) and followed by Czepek's (dox) agar (29.00). Least number of sclerotia was formed on Mustard stem decoction agar (13.33) after 14 days of incubation. Cuong and Dohroo (2006), Elgoban *et al.* (2012), Singh *et al.* (2013) also found PDA as best medium support maximum growth of *S. sclerotiorum*

On liquid media

Perusal of data presented in table 3, revealed that maximum dry mycelial weight (194.22 mg) was recorded in Richards' medium after 14 days of incubation at 25±1^{0}C temperature and was found significantly superior over all other media tested. This was followed by Potato dextrose broth (186.11 mg), Mustard leaf decoction (139.46 mg) and Mustard stem decoction (129.29 mg). The least dry mycelium weight was recorded in Asthana and Hawker's medium (88.74 mg).

In case of sclerotia formation, significantly maximum number of sclerotia was formed on Potato dextrose broth (23.33) after 14 days of incubation at 25±1^{0}C temperature. This was followed by Richards' medium, Mustard leaf decoction, Mustard stem decoction and Czepek's (dox) medium which produced 21.33, 16.67, 10.00 and 9.67 sclerotia, respectively. Minimum number of sclerotia was observed in Asthana and Hawker's medium (4.67).

Results showed that Richards' medium support maximum growth after 14 days of incubation followed by Potato dextrose broth while maximum number of sclerotia formed on potato dextrose broth which was at par with Richards' medium. Rai and Agnihotri (1971), Khan (1976), Sharma (1979), Cuong and Dohroo (2006) and Panchal *et al.* (2012) also found Richards' medium suitable for the growth of the fungus.

Table.1 Morphological characters of *Sclerotinia sclerotiorum*

Fungal part	Colour	Number per unit	Shape	Size	
				Width/diameter	Length
Hyphae	Hyaline	-	Branched, cottony, closely septate	2.0-11.5 µm*	Indeterminate
Sclerotia	Dark brown to black	27-51 (per plate)	Round to irregular	1.5-7 mm	2-15 mm
Apothecia	Brown to dark brown	0-7 (per sclerotia)	disc shaped	2-9 mm	5-21 mm

Table.2 Growth of *S. sclerotiorum* on different solid media

Sl. No.	Solid media	Radial growth of mycelium (in mm) after days*		No. of days to fill Petri-plate	No. of sclerotia formed per plate*
		3 days	5 days		
1.	Patato dextrose agar	54.67	90.00	5	36.33
2.	Mustard leaf decoction agar	46.33	87.00	5	18.33
3.	Mustard stem decoction agar	39.67	85.00	6	13.33
4.	Czepek's dox agar	25.67	55.33	7	29.00
5.	Richards' agar	23.67	47.67	8	31.67
6.	Asthana & Hawker's agar	13.33	29.00	10	15.67
SEm ±		1.732	2.293	-	1.821
C.D. at 5%		5.396	7.145	-	5.672

* Average of three replications

Table.3 Growth of *S. sclerotiorum* on different liquid media

Sl. No.	Liquid media	Dry weight of mycelium (in mg) after 14 days*	No.of sclerotia formed*
1.	Potato dextrose broth	186.11	23.33
2.	Mustard leaf decoction	139.46	16.67
3.	Mustard stem decoction	129.29	10.00
4.	Czepek's (dox) medium	88.74	9.67
5.	Richards' medium	194.22	21.33
6.	Asthana & Hawker's medium	54.28	4.667
SEm ±		3.775	1.759
C.D. at 5%		11.761	5.479

* Average of three replications

Table.4 Effect of temperature on growth of *S. sclerotiorum*

Sl. No.	Temperature	Average radial growth of mycelium after 4 days*	No. of days to fill petri plates	No. of sclerotia formed per plate*
1.	10 ^{0} C	38.4	8	16.80
2.	15 ^{0} C	55.8	6	31.20
3.	20 ^{0} C	77.0	5	44.20
4.	25 ^{0} C	72.6	5	40.40
SEm ±		2.300	-	1.404
C.D. at 5%		6.955	-	4.244

* Average of five replications

Table.5 Effect of pH on growth of *S. sclerotiorum*

Sl. No.	pH	Dry weight of mycelium (in mg) after 14 days*	No.of sclerotia formed/flask*
1.	4.5	182.6	21.0
2.	5.0	196.8	29.0
3.	5.5	159.3	32.7
4.	6.0	128.2	18.3
5.	6.5	113.9	11.3
6.	7.0	77.4	6.0
7	7.5	37.5	2.7
SEm ±		3.12	1.351
C.D. at 5%		9.54	4.138

* Average of three replications

Physiological studies

Effect of temperature on growth and sclerotial formation of *S. sclerotiorum*

The data shown in Table 4 revealed that the pathogen *S. sclerotiorum* grew at all the temperatures. Significantly maximum growth (77.00 mm) was recorded at 20^{0}C temperature which was found at par with 25^{0}C (72.6 mm) as was followed by 15^{0}C (55.80 mm) after 4 days of incubation. While minimum radial growth of mycelium was recorded at 10^{0}C (38.40 mm).

The data presented in table 4, also evident that the maximum number (44.20) of sclerotia formed per plate was recorded at 20^{0}C which followed by 25 and 15^{0}C with 40.40 and 31.20 sclerotia respectively. While minimum number (16.80) of sclerotia formed per plate were recorded at 10^{0}C.

Bedi (1962), Khan (1976), Sharma (1979) and Panchal *et al.* (2012) also reported similar results. Abawi and Grogan (1979) reported that mycelial growth and sclerotial production were optimum at 20-25^{0}C. Purdy (1956) found that temperature effected size of sclerotia with the largest size occurring at 25^{0}C. Kumar *et al.* (2004) observed 20^{0}C as optimum temperature for growth and 20^{0}C to 25^{0}C for sclerotia formation of *S. sclerotiorum* causing stem and root rot of broccoli.

Effect of pH on the growth and sclerotial development of *S. sclerotiorum*

It is evident from the data presented in table 5 that among all the pH levels, pH 5.0 was found to be ideal and produced the maximum dry mycelium weight (196.8 mg) followed by pH 4.5 and 5.5 which supported 182.6 mg and 159.3 mg dry mycelium weight per flask, respectively. At pH above and below 5.0, the dry mycelium weight was found to be declined. The least dry mycelium weight was recorded at pH 7.5 (37.5) indicated that it is unsupportive for the growth of the pathogen.

Significantly maximum number of sclerotia were formed at pH 5.5 (32.7) after 14 days of incubation at 25±1^{0}C temperature. This was followed by pH 5.0 (29.0) and 4.5 (21.0). The least number of sclerotia (2.7) were formed at pH 7.5. Sharma (1979) also found pH 5.0 suitable for vegetative growth of the fungus. However, Willetts and Wong (1980) reported pH below 5.0 was optimum. Jani (1990), Kumar *et al.* (2004) and

Panchal *et al.* (2012) also found same type of results obtained.

It can be concluded that the isolation of the pathogen was made on PDA medium and identified as *Sclerotinia sclerotiorum* (Lib.) de Bary. The fungus produced aerial mycelium, which was hyaline, branched well developed and appeared cottony with closely septate hyphae. The hyphae were 2.0-11.5 µm in width and contained dense granular protoplasm. The sclerotia were round to irregular in shape in culture and measured 1.5-7 mm in width and 2-15 mm in length. Apothecia were brown in colour and were round to globose type, measured 5-21 mm in length, 2-9 mm in diameter and 0-7 numbers arose from single sclerotium. The pathogenic behaviour of the fungus was also confirmed after test. Potato dextrose agar (PDA) medium among solid media and Richards' medium among liquid medium were found most supportive for the growth and sclerotial formation of the *Sclerotinia sclerotiorum*. Temperature 20-25^{0}C and pH 4.5 to 5.5 are most suitable for the growth and sclerotial formation of *Sclerotinia sclerotiorum*.

References

Abawi, G.S. and Grogan, R.G. (1979) Epidemiology of Diseases Caused by Sclerotinia Species. *Phytopathology*, 69: 899-904.

Aggarwal, R.A.K., Kumar, A. and Thakur, H.L. (1997). Effect of Sclerotinia rot on oil quality in low erucic acid cultivars of rapeseed. *Crucifereae Newsletter* 19: 103-104.

Bedi KS. 1962. Effect of temperature on the formation of Sclerotinia sclerotiorum (Lib.) de Bary. *Indian Phytopathology.* 11(1): 55-60.

Chauhan, L.S., Singh, J. and Chandra, D.R. (1992). Assessment of losses due to stem rot of yellow sarson Proc National Sym, Univ. of Allahabad, Allahabad, Nov.19-21, pp. 65-66.

Cuong, N.D. and Dohroo, N.P. (2006). Morphological, cultural and physiological studies on *Sclerotinia sclerotiorum*, causing stalk rot of cauliflower. *Omonrice.* 14: 71-77.

Elgorban, A.M., Elsheshtawi, M., Al-Sum, B.A. and Bahkali, A.H. (2012). Factors affecting on *Sclerotinia sclerotiorum* isolates from beans growing in Ismailia, Egypt. *Life Sci. J.* 10: 1278-1282.

Ghasolia, R.P., Shivpuri, A. and Bhargava, A.K. (2004). Sclerotinia rot of Indian mustard (*Brassica juncea*) in Rajasthan. *Indian Phytopathology.* 57: 76-79.

Jani, S.M. (1990). Studies on stem blight of mustard [*Brassica juncea* (L.) Czern and Coss] caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, Ph.D. thesis, *Gujrat Agric. Univ., Sardar Krushinagar.* p.78.

Kang, I.S. and Chahal, S.S. (2000). Prevalence and incidence of white rot of mustard incited by *Sclerotinia sclerotiorum* in Punjab. *Plant Dis. Res.* 15: 232-233.

Khan, M.A. 1976. Studies on stalk rot of cauliflower caused by Sclerotinia sclerotiorum. M.Sc. Thesis, H. P. University, College of Agriculture, Solan, 80p.

Krishnia, S.K., Meena, P.D. and Chattopadhyay, C. (2000). Seed-yield and yield attributes of Indian mustard affected by Sclerotinia rot. *J. Mycol. Pl. Pathol.* 30: 265.

Kumar, B., Pandey, R. and Verma, A.K. (2004). Effects of temperature and pH on growth and sclerotial formation of *Sclerotinia sclerotiorum* causing stem and root rot of broccoli. *Prog. Agric.* 4: 89-90.

Panchal, J.A., Patel, K.D.L., Jalman, R.K.

and Patel, N.R. (2012). Physiological studies of *Sclerotinia sclerotiorum* causing stem rot of fennel (*Foeniculum vulgare* Mill.) *Int. J. Seed Spi.* 2: 46-53.

Rai, R.A. and Agnihotri, J.P. (1971). Influence of nutrition and pH on growth of *Sclerotinia sclerotiorum* (Lib.) de Bary from *Gaillardia pulchella* foug. *Mycopathologia et Mycologia Applicata.* 43: 89-95.

Saharan, G.S. and Mehta, Naresh. (2002). *Fungal Diseases of Rapeseed-Mustard*. In: Diseases of Field Crops (Gupta, VK and Paul YS eds.). Indus Publishing Company, New Delhi. pp. 193-228.

Sharma, R.C. 1979. Stalk rot of cauliflower caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. Ph.D. Thesis, HPKVV, College of Agriculture, Solan, 146p.

Sharma, S., Yadav, J.L. and Sharma, G.R. (2001). Effect of various agronomic practices on the incidence of white rot of Indian mustard caused by *Sclerotinia sclerotiorum. J. Mycol. Pl. Pathol.* 31: 83-84.

Singh, M., Sharma, O.P., Bhagat, S. and Pandey, N. (2013). Effect of systemic fungicides, culture media, temperature and pH on growth of *Sclerotinia sclerotiorum* (Lib.) de Bary causing white mold of chickpea. *Ann. Plant Prot. Sci.*, 21: 136-139.

Willetts, H.J. and Wong, J.A.L. (1980). The biology of *Sclerotinia sclerotiorum, S. trifoliorum*, and *S. minor* with emphasis on specific nomenclature. *Bot. Rev.* 46: 100–165.