

Original Research Article

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Carpogenic Germination and Viability Studies of Sclerotia of *Sclerotinia sclerotiorum* Causing Lettuce Drop

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ABSTRACT

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Lettuce is one of the most widely consumed vegetable throughout the world. Amongst various diseases infecting this crop, lettuce drop caused by *Sclerotinia sclerotiorum* is one of the most prominent and destructive one. Lettuce drop pathogen, *S. sclerotiorum* produces black, hard survival structures known as sclerotia. In the present studies, carpogenic germination of sclerotia by producing apothecia and releasing ascospores as well as viability of sclerotia was studied. Sclerotia after germination formed funnel shaped cup i.e. apothecium at the tip which were brown in color and were round or globose type. The length of apothecia varied from 4-19 mm, whereas diameter of the apothecial discs ranged from 3-8 mm with number ranging from 1-10 per sclerotia. Viability studies revealed that with the increase in duration of water soaking from 4 days to 32 days, there was a gradual decrease in germination of sclerotia of *S. sclerotiorum*. 20°C temperature was found to be optimum resulting in maximum sclerotial germination (99.73%) followed by 25, 15, 10 and 30°C temperature. Soil depth (0 to 12cm) was inversely proportional to sclerotial germination and sclerotia placed at soil surface (0 cm soil depth) resulted in highest sclerotial germination (93.75 %) followed by 2, 4 and 6 cm soil depths.

Introduction

Lettuce (*Lactuca sativa* L.) is one of the most widely consumed vegetables around the world. It is often grown as a biennial leafy vegetable and is typically eaten raw in salads, sandwiches and in many other ethnic dishes. Its tender leaves and head are chopped and used as salad with salt and vinegar. In India, it is gaining popularity with the change in food habit and health consciousness among the people (Kaushal and Kumar, 2010). During its cultivation, lettuce drop caused by *Sclerotinia sclerotiorum* has been found to be predominant and destructive disease (Bharat et al., 2014). It produces hard, durable structures known as sclerotia, which function

as survival structures and also as disease inoculum in subsequent lettuce crops. Sclerotia of *S. sclerotiorum* usually germinate eruptively resulting in symptoms development; however, they can also germinate carpogenically producing numerous apothecia that forcibly eject thousands of ascospores to be wind-dispersed throughout the lettuce field and adjacent production areas (Subbarao, 1998). The result of ascospore-mediated lettuce drop is often complete loss of crop at the primary infection site and considerable damage to surrounding lettuce fields. However, much information is not available regarding its carpogenic

germination and viability studies, therefore, present investigations were undertaken.

Materials and Methods

Production of sclerotia

Wheat seeds (25g) were placed in 250 ml flasks containing 60 ml water and autoclaved. After cooling, each flask was inoculated with three mycelial discs (10 mm diameter) taken from the colony margin of 3-4 day old PDA cultures of *S. Sclerotiorum*, isolated from infected lettuce leaves. Flasks were incubated at 20°C for 3 weeks in darkness, with each flask being shaken after 1 week to facilitate mixing of inoculum (Plate 1a). After 3 weeks, the contents of each flask were washed with sterile distilled water in a sieve (1 mm diameter pore size) and aseptically air-dried overnight in a laminar flow hood (Plate 1b). Sclerotia were separated from the wheat grains, stored at room temperature in the dark (Plate 1c) and used within 2 weeks (Mylchreest and Wheeler, 1987).

Carpogenic germination of sclerotia

To record the carpogenic germination of sclerotia in moist sand in Petri plates, clean sand was sterilized for one hour in autoclave at 1.05 kg/cm² for two consecutive days. Sixty gram of sterilized sand was spread in each Petri plate (9 cm dia.). 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) and observations on formation of apothecia were recorded regularly.

Effect of different durations of water dipping on viability of sclerotia

Effect of different durations of water dipping i.e. 4, 8, 12, 16, 20, 24, 28, 32 days on viability of sclerotia was studied by soaking sclerotia in water for different durations (Plate

2) and then plating them on PDA plates and observation on sclerotial germination (%) was recorded up to five days.

Effect of different temperature regimes on viability of sclerotia

Germination of sclerotia was studied by maintaining different temperature levels i.e. 0, 5, 10, 15, 20, 25, 30, 35 and 40°C for four weeks in BOD incubator (Plate 3) and then plating them on PDA plates. Observations on sclerotial germination (%) were recorded up to thirty days at an interval of ten days each.

Effect of different soil depths on viability of sclerotia

Different soil depths i.e. 0, 2, 4, 6, 8, 10 and 12 cm on germination of sclerotia was studied by burying the sclerotia in nylon bags at different soil depths (Plate 4) for four weeks and then plating them on PDA plates. Observations on sclerotial germination (%) were recorded at ten days interval up to one month.

Results and Discussion

Formation of sclerotia and their carpogenic germination

Small, round or oval to irregular in shape, transparent bodies started developing after 8-9 days of incubation and these later turned black in color and became hardened sclerotia of the fungus (Plate 5a,5b and 5c). The sclerotia were round to irregular in shape in culture and measured 1.6-8 mm in width and 2.1-17 mm in length and their number varied from 20-30 per Petri plate.

Singh (1985) found that sclerotia varied in shape and size according to environment and location. Saharan and Mehta (2008) reported that sclerotium formation in *S. sclerotiorum* follows a general sequence but variations due

to isolates, substrates and conditions exist. Later, Bharat *et al.*, (2014) confirmed sclerotia to be black in colour, smooth, rounded or elongated and 1-6 x 1-20 mm in size.

The sclerotial germination gave rise to several columnar structures (stipes) which later formed funnel shaped cup (apothecium) at the tip (Plate 6). Apothecia were brown in color and were round or globose type. The length of apothecia measured from 4-19 mm, whereas diameter of the apothecial discs ranged from 3-8 mm with number ranging from 1-10 per sclerotia. Eddins (1937) reported that apothecial cups were 1.5-10 mm in diameter.

Apothecial production from stipes produced on sclerotia placed on a substrate low in nutrients under proper conditions have been reported by Saito (1973) and Steadman and Nickerson (1975).

Effect of different durations of water dipping on viability of sclerotia

It is evident from the data (Table 1) that with the increase in duration of water soaking from 4 days to 32 days, there was a gradual decrease in germination of sclerotia of *S. sclerotiorum* from 88.66 to 8.65 per cent, respectively, though all treatments were statistically different from each other. Thus, soaking of sclerotia in water reduced its viability.

Moore (1949) indicated that the survival of sclerotia of *S. sclerotiorum* under flooded conditions ranged between 23 and 45 days.

As a result, flooding has been used for control of diseases caused by *Sclerotinia* on a number of crops in Florida (Moore, 1949; Steadman, 1979) and in western Washington (Niem *et al.*, 2013). Increasing soil moisture accelerates sclerotium degradation (Coley-Smith and Cooke, 1971) and also increases

their susceptibility to damage from solar radiation (Willettts and Wong, 1980). Metha (2014) reported that moist sclerotia die rapidly than the dried ones.

It is concluded from the data in table 1 that soaking of sclerotia in water for about one month hampers its viability.

Effect of different temperature regimes on viability of sclerotia

The perusal of data (Table 2) revealed that sclerotia of *S. sclerotiorum* were able to germinate at all the temperatures ranging from 0 to 40°C, however, 20°C temperature was found to be optimum resulting in maximum sclerotial germination (99.73%) followed by 25, 15, 10 and 30°C temperature giving 96.95, 94.67, 81.70 and 76.13 per cent sclerotial germination, though statistically at par with each other. Minimum sclerotial germination was observed at 40°C temperature.

Data further suggested that a decreasing trend in sclerotial germination, irrespective of the temperature, was observed after 10 days to 30 days. It was highest after 10 days (68.33%) followed by 20 days (64.44%) and 30 days (59.56%), though statistically similar to each other.

Interaction studies showed that minimum sclerotial germination was found at 40°C temperature after 30 days of temperature exposure (4.59%), whereas, highest sclerotial germination was observed at 20°C temperature after 10 and 20 days of exposure.

Singh (1985) observed that sclerotia of *S. sclerotiorum* were able to germinate at 40, 50 and 60°C even after 24 hours of exposure, however, at 70°C, exposure for longer periods inhibited germination while the sclerotial germination occurred when exposed for 2 hours only.

Table.1 Effect of different durations of water dippings on sclerotial germination of *S. sclerotiorum*

Days of water dipping	Sclerotial germination (%)
4	88.66 (70.34)
8	62.04 (51.95)
12	56.67 (48.81)
16	44.48 (41.81)
20	31.88 (34.35)
24	22.32 (28.16)
28	12.45 (20.61)
32	8.65 (17.02)
CD _(0.05)	(2.61)

Figures in the parenthesis are arc sine transformed values

Table.2 Effect of different temperature regimes on sclerotial germination of *S. sclerotiorum*

Temperature (°C)	Sclerotial germination (%)			
	After 10 days	After 20 days	After 30 days	Mean
0	40.42 (39.25)	34.54 (35.99)	28.72 (32.83)	34.56 (36.02)
5	65.58 (53.78)	59.24 (50.20)	53.82 (47.08)	59.54 (50.35)
10	86.24 (68.37)	81.39 (64.73)	77.48 (61.71)	81.70 (64.94)
15	98.24 (82.15)	95.34 (79.66)	90.43 (72.43)	94.67 (78.08)
20	100.00 (90.00)	100.00 (90.00)	99.19 (85.59)	99.73 (88.53)
25	98.24 (83.86)	96.89 (79.25)	95.73 (79.96)	96.95 (81.02)
30	82.75 (65.27)	75.73 (60.40)	69.91 (56.54)	76.13 (60.74)
35	34.46 (35.94)	28.46 (32.45)	16.18 (23.91)	26.36 (30.74)
40	09.08 (17.77)	08.44 (16.29)	04.59 (12.17)	07.37 (15.41)
Mean	68.33 (59.60)	64.44 (56.55)	59.56 (52.47)	
CD _(0.05)				
Temp		33.21		
Days		19.18		
Temp x days		57.54		

Figures in the parenthesis are arc sine transformed values

Table.3 Effect of different soil depths on sclerotial germination of *S. sclerotiorum*

Soil depth (cm)	Sclerotial germination (%) after days			
	10	20	30	Mean
0	98.00 (83.42)	97.22 (63.42)	86.04 (39.21)	93.75(62.01)
2	97.52 (80.42)	90.85 (61.33)	83.87 (51.92)	90.74 (64.55)
4	87.63 (68.03)	80.06 (65.64)	77.63 (44.40)	81.77 (59.35)
6	83.07 (80.42)	71.22 (57.40)	51.35 (35.64)	68.54 (57.82)
8	73.85 (71.59)	63.54 (45.36)	40.34 (46.70)	59.24 (54.55)
10	62.04 (65.64)	49.04 (58.68)	34.19 (38.62)	48.42 (54.31)
12	53.10 (68.87)	39.09 (52.51)	27.28 (31.28)	39.82 (50.88)
Mean	79.31 (74.05)	70.14 (57.76)	57.24 (41.11)	
CD _(0.05)				
Soil depth		33.23		
days		21.75		
Soil depth x days		57.56		

Figures in the parenthesis are arc sine transformed values

Plate.1a Inoculated wheat seeds after incubating at 20°C for 3 weeks in darkness



Plate.1b Harvesting of sclerotia



Plate.1c Harvested sclerotia for further use

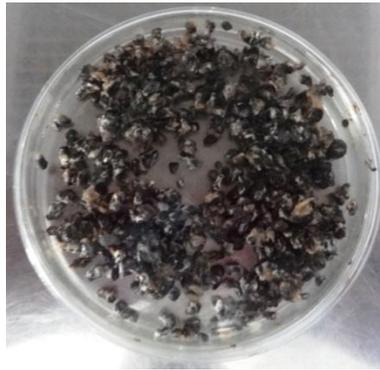


Plate.2 Water dipping of sclerotia



Plate.3 Sclerotia exposed to different temperatures

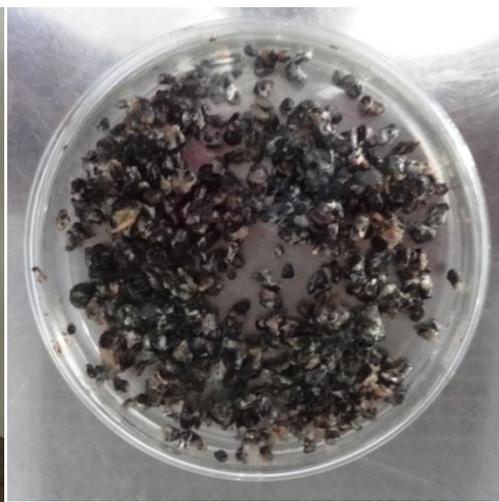


Plate.4 Burial of sclerotia at different soil depths

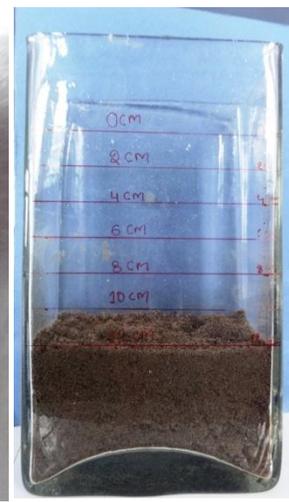


Plate.5a Sclerotia of *S. sclerotiorum* produced on PDA



Plate.5b Sclerotia of lettuce drop pathogen



Plate.5c Cross section of sclerotia of *S. sclerotiorum*

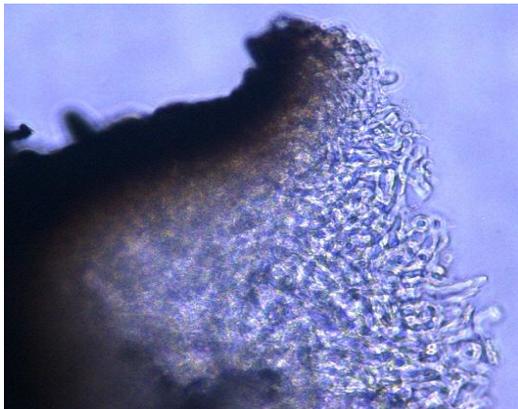


Plate.6 Apothecia of *S. sclerotiorum*



Cartia and Asero (1994) reported that 40 per cent mortality of sclerotia of *S. sclerotiorum* was obtained at a temperature of 35°C after three days while 100 per cent mortality was achieved after nine-ten days of exposure.

Abdullah *et al.*, (2008) found that sclerotia did not grow after 7 days at 30 and 35°C, however, when these sclerotia were incubated at 25°C, normal myceliogenic growth resumed. Sclerotia that were exposed to 40 and 45°C failed to grow after 21 days at 25°C. The upper thresholds temperature for

conditioning and germination of sclerotia were 20 and 25°C, respectively (Clarkson *et al.*, 2007). The sclerotial germination of *S. sclerotiorum* tended to decrease as soil temperature increased from 15 to 40°C, with no germination observed at 40°C after 1 and 2 weeks, respectively (Matheron and Porchas, 2005).

It can be concluded from the present experiment (Table 2) that a temperature range of 10 to 30°C supports higher sclerotial germination.

Effect of different soil depths on viability of sclerotia

Result presented in table 3 showed that with the increase in soil depth from 0 to 12cm, there was a corresponding decrease in sclerotial germination, irrespective of days of burial in soil. Sclerotia placed at soil surface (0 cm soil depth) resulted in highest number of sclerotial germination (93.75 %) followed by 2, 4 and 6 cm soil depth giving 90.74, 81.77 and 68.54 per cent germination of sclerotia, respectively. Data further showed that sclerotial burial in soil for 10, 20 and 30 days, irrespective of soil depth did not differ significantly among each other, however, 10 days of burial of sclerotia in soil gave highest percentage of sclerotial germination (79.31%).

It was found from interaction studies that highest percentage of sclerotial germination was observed at soil surface (0 cm soil depth) after 10 days of burial in soil (98.00 %). This was followed by burying the sclerotia at 2 cm soil depth up to 10 days giving 97.52 percent sclerotial germination, though statistically similar to each other.

Viability of sclerotia of *Sclerotinia* spp. in soil has been reported to be as short as a few weeks to as long as 8 years or more (Moore, 1949) and it is difficult to measure in the field even in the absence of susceptible hosts because the “primary” sclerotia produce “secondary” or “daughter” sclerotia in the absence of suitable substrates for colonization (Adams and Ayers, 1979; Coley-Smith and Cooke, 1971). This not only causes an increase in the number of sclerotia, but it also extends their viability (Coley-Smith and Cooke, 1971; Kruger, 1975; Willetts and Wong, 1980). Imolehin *et al.*, (1980) reported that the increased mortality of sclerotia at deeper soil is mainly due to the colonization of sclerotia by antagonistic fungi. Merriman

(1976) reported that burial of sclerotia at 4 cm for 35 weeks reduced recovery of sclerotia to zero in sandy clay loam and by 50 percent in sandy loam. Because survival of sclerotia decreases with time and depth of burial, movement of sclerotia to depths greater than 10 cm will prevent lettuce drop infections (Grogan *et al.*, 1980 and Imolehin and Grogan, 1980). Factors such as soil temperature, O₂, CO₂, and ethylene concentrations change with soil depth and may influence survival (Wu and Subbarao, 2008).

It can be inferred from the data in table 3 that sclerotial germination was inversely proportional to soil depth and duration of burial in soil and with the increase in soil depth as well as duration of burial, there was a decrease in sclerotial germination.

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