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Effect of Temperature and Salinity on the Growth of *Sclerotinia sclerotiorum* Causing Head Rot of Cabbage

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A B S T R A C T

Sclerotinia sclerotiorum is a temperate plant pathogen. It is soil borne and has a worldwide distribution. It affects cabbage leading to a diseased condition called head rot in which rotting of fully grown cabbage heads takes place. The rotted cabbage heads exhibit cottony white mycelial growth on their surface. With advancement of the disease the mycelial growth becomes dense and numerous carbon black coloured bodies called sclerotia are formed on the surface. Effect of temperature and salinity was studied to determine the optimum temperature for growth of the pathogen and the optimum salinity of soil tolerable for the pathogen growth respectively. Results indicated that temperature of 20^{0}C was the most optimum for the pathogen growth and formation of sclerotia. Growth of *S. sclerotiorum* was maximum at 1 per cent NaCl concentration. The growth of the pathogen decreased with increasing salinity levels. Thus the study demonstrated the optimum temperature and salinity for the pathogen growth.

Introduction

Sclerotinia sclerotiorum is a vigorous plant pathogen and affects temperate crops around the world (Bolton *et al.*, 2006). It infects cabbage leading to a diseased condition known as head rot. The disease leads to watery rot of fully grown cabbage heads in the field, during post harvest operations and storage (Hudyncia *et al.,* 2000). This pathogen is recognized as important due to its worldwide distribution (Adams and Ayers, 1979; Purdy, 1979), its wide host range (Schwartz *et al.*, 1977; Purdy, 1979; Boland and Hall, 1994) and the difficulties encountered in controlling the diseases it causes (Lumsden, 1979; Steadman, 1979). Sclerotia are the primary survival structures of the pathogen (Korf and Dumont, 1972). Sclerotia formed on or within the host tissue are dislodged on the soil surface by wind or during harvesting and threshing which are distributed in the vertical soil profile during land preparation (Cook *et al.,* 1975). Approximately 90 per cent of the life cycle of *Sclerotinia* species is spent in soil as sclerotia (Adams and Ayers, 1979). Sclerotia are hard asexual resting structures composed of vegetative hyphal cells which become interwoven and aggregate together. There is a huge variation in the morphology of sclerotia when it is produced on different substrates (Kamesh *et al.*, 2016). *S.sclerotiorum* attacks nearly all kinds of succulent plants including flowers, shrubs, weeds and almost all vegetables (Chupp and Sherf, 1960). The pathogen has a broad host range which includes high value crops like alfalfa, bean, cabbage, canola, lettuce, peanut, soybean, sugarbeet, sunflower, tobacco and tomato (Farr *et al*., 1989). Seed infection by this pathogen has been reported in dry beans (Blodgett *et al*., 1946; Hungerford *et al*., 1953; Steadman *et al*., 1975), soybean (Nicholson *et al*., 1972; Stovold and Priest, 1986; Hartman *et al*., 1998; Hoffman *et al*., 1998) and sunflower (Sackston *et al*., 1960).

In India head rot of cabbage was first reported during Febraury 1978 from Kodaikanal area in Dindigul district of Tamil Nadu (Alagianagalingam *et al*., 1978). Infection of cabbage can occur when infected materials such as neighbouring cabbage plants, fallen flower petals, or pollen contact with healthy tissue (Mc Lean, 1949; Mc Lean 1958; Dillard *et al*., 1986). Long distance transmission potential of *Sclerotinia* spp. is most likely by seed infected with mycelia, or sclerotia mixed with the seed (Blodgett *et al*., 1946; Adams and Ayers, 1979).

Kapoor (1994) multiplied *S.sclerotiorum* at 24±1°C on PDA medium. Nguyen and Dohroo (2006) reported that the optimum temperature range for the growth of the pathogen lied between 20 and 25^{0}C. Optimum temperature for production of sclerotia was 15-20^{0}C. Growth of the pathogen was found to be poor at 30^{0}C. Monika *et al*., (2013) found 25-30^{0}C as optimum temperature for growth of *S. sclerotiorum* in liquid media.

Syed and Saleem (2004) observed growth of *S. sclerotiorum* after 7 days in media amended with 1 per cent NaCl which progressed slowly and filled the plate after 15 days. Rapid growth of *S. sclerotiorum* was observed on unamended medium with a growth rate of 25.7mmd^{-1}. Growth rate decreased with decreasing solute potential. Maximum production of sclerotia was observed on medium amended with 2 per cent NaCl (Mansour *et al*., 2008).

Materials and Methods

Isolation of pathogen

Cabbage plants showing symptoms of *Sclerotinia* head rot were collected from Kothagiri area in Nilgiris district of Tamil Nadu. The age of the crop varied from 60 to 75 days. Sclerotia collected from infected cabbage heads were surface sterilized with 0.1 per cent mercuric chloride and rinsed with three changes of sterile water. The surface sterilized sclerotia were plated on PDA in sterile Petri plates and kept in an incubator at 19^{0}C for 5 days. The fungus was subcultured and maintained on Potato Dextrose agar (PDA). The stock cultures were maintained in PDA slants for long time storage under refrigerated condition at 4^{o}C.

Effect of temperature on growth of *S. sclerotiorum*

Petri plates containing uniform quantities of sterilized PDA medium were inoculated with 5 day old mycelial discs of the pathogen and incubated at different temperatures *viz*., 10, 15, 20,25,30,35 and 40^{0}C. Each treatment was replicated four times and data were recorded on radial growth of mycelium and number of sclerotia formed per plate.

Effect of salinity on the growth of *S. sclerotiorum*

Petri plates containing uniform quantity of sterilized PDA medium were amended with NaCl at different concentrations viz., 0,1,2,3,4,5,6,7,8,9 and 10 %. The plates were inoculated with 5-day-old mycelial disks of pathogen. Each treatment was replicated three times and data were recorded on radial growth of mycelium and number of sclerotia formed per plate.

Statistical analysis

The data recorded were analysed statistically using the IRRISTAT version 92 developed by the International Rice Research Institute (IRRI), the Philippines (Gomez and Gomez, 1984).Prior to statistical analysis of variance (ANOVA) the percentage values of the disease index were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels ($P < 0.05$ and $P < 0.01$) and means were separated by Duncan's Multiple Range Test (DMRT).

Results and Discussion

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

The effect of temperature on the growth of *S. sclerotiorum* was observed on 3 and 5 DAI (Table 1). Maximum mycelial growth was observed when the pathogen was incubated at temperature of 20°C (60.25 mm and 88.75 mm) followed by temperature of 15°C (32.5 mm and 74.50 mm). Least growth was observed at temperature of 30°C (3.75 mm and 7.25 mm). Growth of the pathogen was absent at temperature of 35°C. Sclerotial production was maximum at temperature of 20°C (15.5) and least at 15°C (7.25). There was no production of sclerotia at 10°C and beyond 30°C.

Effect of salinity on the growth of *S. sclerotiorum*

The mycelial growth of pathogen was highest in medium unamended with NaCl at 3DAI and 5DAI (50.0 mm and 89.0 mm) (Table 2). Growth rate decreased with increasing concentration of NaCl. NaCl concentration of 1 per cent favoured highest mycelial growth (38.7 mm and 76.3 mm) followed by 2 per cent NaCl concentration (33.3 mm and 61.3 mm) at 3 and 5 DAI. There was no growth of pathogen at 9 and 10 per cent NaCl concentration at both the time intervals. Unamended medium produced highest number of sclerotia (18.3) followed by medium with 3 per cent NaCl (18).

Table.1 Effect of different temperature on growth of *S. sclerotiorum* under *in vitro*

Sl. No.	Temperature (^{0}C)	Mycelial growth		No. of Sclerotia produced /plate
		3DAI*	5DAI	
1	10	3.25^{c} (1.90)	11.75^{c} 3.48)	0.0^{c} (0.70)
2	15	32.5^{b} (5.72)	74.50^{b} (8.65)	7.25^{b} (2.77)
3	20	60.25^{a} (7.78)	88.75^{a} (9.44)	15.5^{a} (3.98)
4	25	29.00^{b} (5.42)	74.00^{b} (8.63)	7.75^{b} (2.86)
5	30	3.75^{c} (2.03)	7.25^{d} (2.77)	0.0^{c} (0.70)
6	35	0.0^{d} (0.70)	0.0^{e} (0.70)	0.0^{c} (0.70)

*Days after inoculation

Figures in parantheses are square root transformed values

In a column means followed by the same letter are not significantly different at the 5% level of DMRT

Table.2 Effect of salinity on the growth of *S. sclerotiorum* under *in vitro*

Sl. No.	NaCl concentration (%)	Mycelial growth		No. of sclerotia/plate
		3DAI* (mm)	5DAI (mm)	
1	1	38.7^{b} (6.2)	76.3^{b} (8.76)	17.0^{a} (4.17)
2	2	33.3^{b} (5.8)	61.3^{c} (7.86)	16.0^{a} (4.05)
3	3	20.0^{c} (4.5)	52.6^{d} (7.28)	18.0^{a} (4.29)
4	4	18.7^{c} (4.3)	51.6^{de} (7.21)	7.0^{b} (2.75)
5	5	13.0^{d} (3.6)	46.6^{de} (6.85)	8.0^{b} (2.90)
6	6	11.7^{d} (3.4)	44.6^{e} (44.52)	7.3^{b} (2.79)
7	7	4.0^{e} (2.0)	25.2^{f} (25.33)	6.0^{b} (2.52)
8	8	2.7^{e} (1.7)	22.0^{f} (4.74)	3.0^{c} (1.85)
9	9	0.0^{f} (0.7)	0.0^{g} (0.70)	0.0^{d} (0.70)
10	10	0.0^{f} (0.7)	0.0^{g} (0.70)	0.0^{d} (0.70)
11	Control	50.0^{a} (7.0)	89.0^{a} (9.46)	18.3^{a} (4.32)

*Days after inoculation
Figures in parantheses are square root transformed values
In a column means followed by the same letter are not significantly different at the 5% level of DMRT

In the current study, maximum mycelial growth and sclerotial production by *S. sclerotiorum* was observed at temperature of 20°C followed by 15°C. Least growth of the pathogen was observed at temperature of 30°C. Coe (1944) reported best growth of the pathogen at temperature range of 19 to 20°C on PDA medium. Abawi and Grogan (1975) reported temperature of 20-25°C as optimum for growth and sclerotial production by *S. sclerotiorum*. Mycelial plugs of *S. sclerotiorum* inoculated on PDA had the highest growth rate at 25°C. No growth was observed at 35, 40 and 45°C (Mansour *et al*., 2008). The results of effect of salinity levels on the pathogen growth showed that mycelial growth of *S. sclerotiorum* was highest in medium unamended with NaCl and growth rate decreased with increasing concentration of NaCl. NaCl concentration of 1 per cent favoured highest growth. El-Abyad *et al.* (1992) reported that myceliogenic germination of sclerotia of *S. sclerotiorum* decreased when it was grown on Czapek's Dox agar medium amended with salts. Production of sclerotia following myceliogenic germination is decreased by over 50 per cent with increasing salt concentration.

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