Short Research Article

Effect of inoculum levels on disease development and survival potential of *Sclerotinia sclerotiorum* sclerotia

Abstract- Sclerotinia stem rot is constantly appears from moderate to severe form in major mustard growing tracts. Most of life *S. sclerotiorum* passes in form of sclerotia in the soil. Present study was carried out in order to determine effect of inoculum level on disease development and survival of *S. sclerotiorum*. Different inoculum levels were prepared from culture of *S. sclerotiorum* and 10 sclerotia/pot were buried at various depths ranging from 1 to 20 cm for 60 days. The maximum disease incidence (93.67%) was observed with 10 per cent inoculum level; however disease incidence was least (6.34%) in 1 per cent inoculum level. Disease incidence was increased with increasing in inoculum levels. In case of plant mortality, maximum plant mortality (43.00%) was documented at 10 per cent inoculum level followed by 32.67 and 29.34 per cent @ 9 and 8 per cent, respectively. No plant mortality was noticed at 1 and 2 per cent inoculum level. Maximum amount of sclerotia germination (83.33%) was observed at 1 cm soil depth followed by 75.65, 73.34, 70.34 per cent germination @ 2, 3 and 4 cm depth of soil, respectively. It was observed that sclerotial germination decreased in tune with increase in soil depth. Lowest sclerotial germination (10%) was observed in 20 cm vertical soil depth. Findings of this study can be harnessed in varietal screening and formulation of disease management modules.

Key Words: Survival, *S. sclerotiorum*, mustard and Sclerotinia stem rot

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Introduction- Among biotic stresses, diseases are major constraint in successful cultivation of Indian mustard. Sclerotinia stem rot, incited by *Sclerotinia sclerotiorum*, disease has been recognized as a challenge that causing yields losses of this oilseed crop. Pathogen has ability to reproduce asexually and

sexually where sclerotia germinate and brings about hyphae (asexually), apothecia is formed in case carpogenic germination (sexually) from sclerotia [1].Being a homothallic fungus, a single ascospore of *S. sclerotiorum* can complete the life cycle. Pathogen passes a part of its life cycle as sclerotia, a resting structure, in the soil. These sclerotia mixed into the soil during harvesting and threshing, and act as a source of inoculum for future years. Sclerotia are made of three layers namely rind, cortex and medulla. These layers are consisting of hyphal aggregates. Melanin is a principal compound of cell wall of outer rind and it made up of several phenolic or indolic substances, which protect sclerotia from adverse environmental threats [2]. Number of Sclerotia in the soil play significant role in disease epidemic incited by *S. sclerotiorum* [3]. A positive correlation was documented between density of sclerotia and disease incidence [4]. Sclerotia are resilient to chemical, physically and biological deterioration [5] and are capable of to survive in 5cm top layer of the soil for approximately 4 years and has ability to withstand against adverse conditions [6]. Experiments of [7] demonstrated that viability of sclerotia can be 100 per cent after three years, although [8] results exhibited that sclerotia had only 78 per cent viability for three years when buried in fallow land. [9] reported sclerotial survival 4-5 years. Sclerotia buried deeper in undisturbed soil flourish longer than those in upper soil profile [7] whereas [10] observed that the viability of sclerotia decreased with depth. Similarly, [11] also showed that sclerotia viability decreased with increasing depth, which may be due to increased parasitism. He also observed that sclerotia present within the top 5 cm soil will carpogenically germinate. Pathogen survival in form of apothecia has been recorded about four weeks and 12-21 days in form of ascospore [12, 13].Germination ability of sclerotia of *S. sclerotiorum* on the soil surface or placed at 5 cm depth in irrigated soil was comparatively least as compare to dry soil condition where sclerotia kept at the same depths. On the other hand, there was no significant difference in germination of sclerotia in irrigated and dry soil when sclerotia maintained at a depth of 10 cm [14]. The aim of present investigation was to determine effect of inoculum level on disease development and survival of *S. scletiorum* sclerotia at various vertical depths.

Materials and Methods: The sterilized soil is weighed and filled into a surface-sterilized clay pot. *S. sclerotiorum* culture (containing mycelium and sclerotium) grown in sorghum meal medium for 25 days was mixed separately in each pot to obtain different levels of inoculum, *i.e.* 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 per cent (w/w). A pot filled with sterile soil without any inoculum served as a control. Healthy seeds of the Bio-902 variety were sterilized in a solution of mercury chloride (0.1%) for 1 minute, washed three times under running tap water and sown in pots. Three replications were maintained for each treatment. Regularly irrigate the pot to keep the soil moist. Plant mortality and symptoms expression were recorded up to 20th days of sowing and were used to calculate the infection rate. Similarly an experiment was planned to know survivability of sclerotia at different depths of soil. The sclerotia of *S. sclerotiorum* were obtained from master culture. About 10 sclerotia were buried in surface disinfected clay pots (30 cm in diameter) containing field soil at various depths ranging from 1 to 20 cm for 60 days. The pots were kept

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in a glass house. After 60 days, the sclerotia were collected from pots, and subsequently immersed in 0.1% mercuric chloride solution for 1 minute, were washed 3 times with sterile distilled water. According to the standard procedure, the surface-sterilized sclerotia were aseptically inoculated into a Petri dish containing PDA medium. The per cent germination of sclerotial bodies was recorded.

Results- The maximum disease incidence (93.67%) was observed with 10 per cent inoculum level; however disease incidence was least (6.34%) in 1 per cent inoculum level (Table1, Figure1). At 5 per cent inoculum level 35 per cent and 24.67, 21.34 per cent disease incidence was noticed @ 4 and 3 per cent inoculum level, respectively. At 2 per cent inoculum level, 12.67 per cent disease incidence was observed. Disease incidence was increased with increasing in inoculum levels. Disease incidence was 83.34 per cent @9 per cent inoculum level followed by 63.00, 53.34 and 39.34 Per cent at 8, 7 and 6 per cent inoculum level, respectively. Similarly, 35.00 per cent disease was recorded at 5 per cent inoculums level. In case of plant mortality, maximum plant mortality (43.00%) was documented at 10 per cent inoculum level followed by 32.67 and 29.34 per cent @ 9 and 8 per cent, respectively. No plant mortality was noticed at 1 and 2 per cent inoculum level, whereas, 4.34 per cent plant mortality was observed @ 3 per cent inoculum level. At 7 per cent plant inoculum level, 19.67 percent mortality was observed than 17.34% mortality at 6 per cent inoculum level. Likewise, at 4 and 5 per cent inoculum level plants were showed 10.34 and 13 per cent plant mortality, respectively.

Table1. Effect of different inoculums levels of *S. sclerotiorum* on disease development

Inoculum Levels (%)	Per Cent disease incidence**	Mortality (%) **
1	6.34 (14.58)	00.00 (00.00)
2	12.67 (20.85)	00.00 (00.00)
3	21.34 (27.51)	4.34 (12.02)
4	24.67 (29.78)	10.34 (18.76)
5	35.00 (36.27)	13.00 (21.13)
6	39.34 (38.85)	17.34 (24.61)
7	52.34 (46.34)	19.67 (26.33)
8	63.00 (52.54)	29.34(32.80)
9	83.34 (65.91)	32.67(34.86)

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10	93.67 (75.43)	43.00(40.98)
Control	00.00 (00.00)	00.00(00.00)
SEm±	0.87	0.43
CD(5%)	2.54	1.25
CD(1%)	3.45	1.70
CV(%)	3.82	4.79

**Figures in parentheses are arcsine per cent angular transformed value

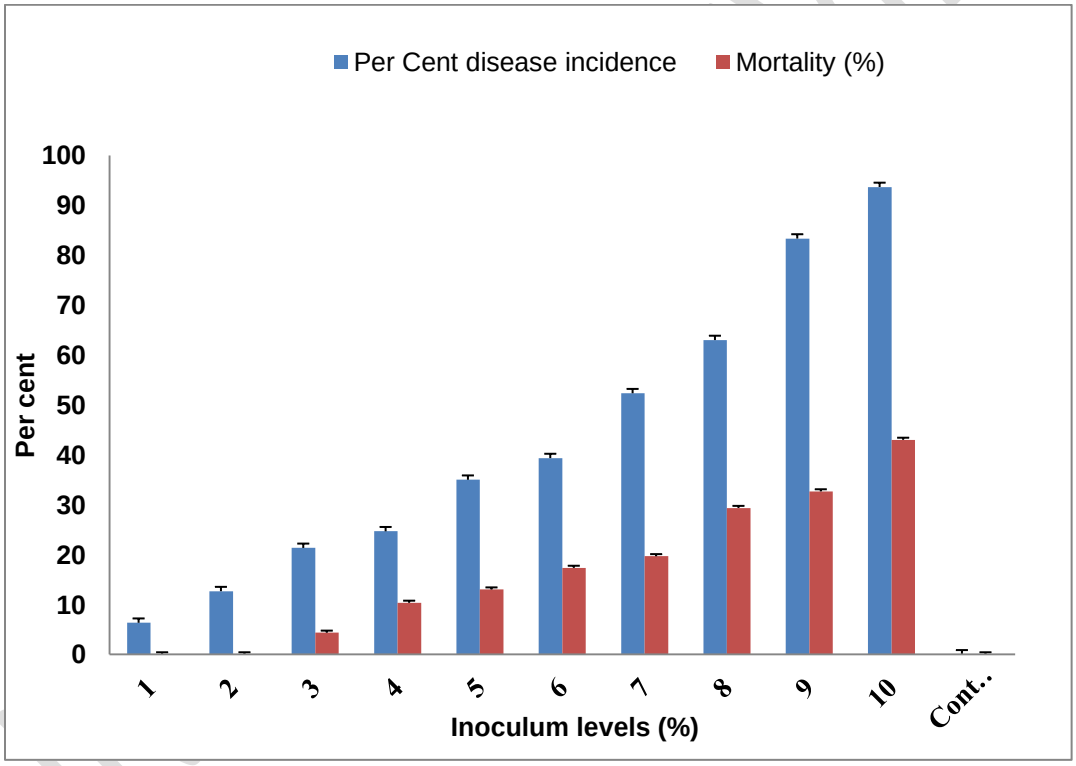

Fig1. Effect of different inoculum levels of *S. sclerotiorum* on disease development. Vertical error bar is showing standard error.

Maximum (83.33%) germination of sclerotia was observed at 1 cm soil depth followed by 75.65, 73.34, 70.34 per cent germination @ 2, 3 and 4 cm depth of soil, respectively (Table2 and Figure2). At 6cm soil depth, 64.67 per cent sclerotial germination was recorded that was at par with 66.34 per cent plant mortality @5 cm soil depth. It was observed that sclerotial germination decreased in tune with increase in soil depth. Lowest sclerotial germination (10%) was observed in 20 cm vertical soil depth.

Table2. Effect of different soil depths on sclerotial germination of *S. sclerotiorum*

Depth (cm)	Sclerotial germination (%)*
1	83.34 (65.91)
2	75.65 (60.43)
3	73.34 (58.91)
4	70.34(57.00)
5	66.34(54.32)
6	64.67(53.53)
7	60.00(50.70)
8	56.34(48.64)
9	53.34(46.92)
10	50.00(45.00)
11	45.34(42.53)
12	43.34(41.17)
13	40.00(39.23)
14	35.67(36.67)
15	32.34(34.66)
16	29.67(33.00)
17	25.34(30.22)
18	20.34(26.81)
19	12.67(20.85)
20	10.00(18.43)
Control (0cm)	100 (90.00)
SEm±	0.63
CD(5%)	1.81
CD(1%)	2.42
CV(%)	2.20

*Figures in parentheses are arcsine per cent angular transformed value

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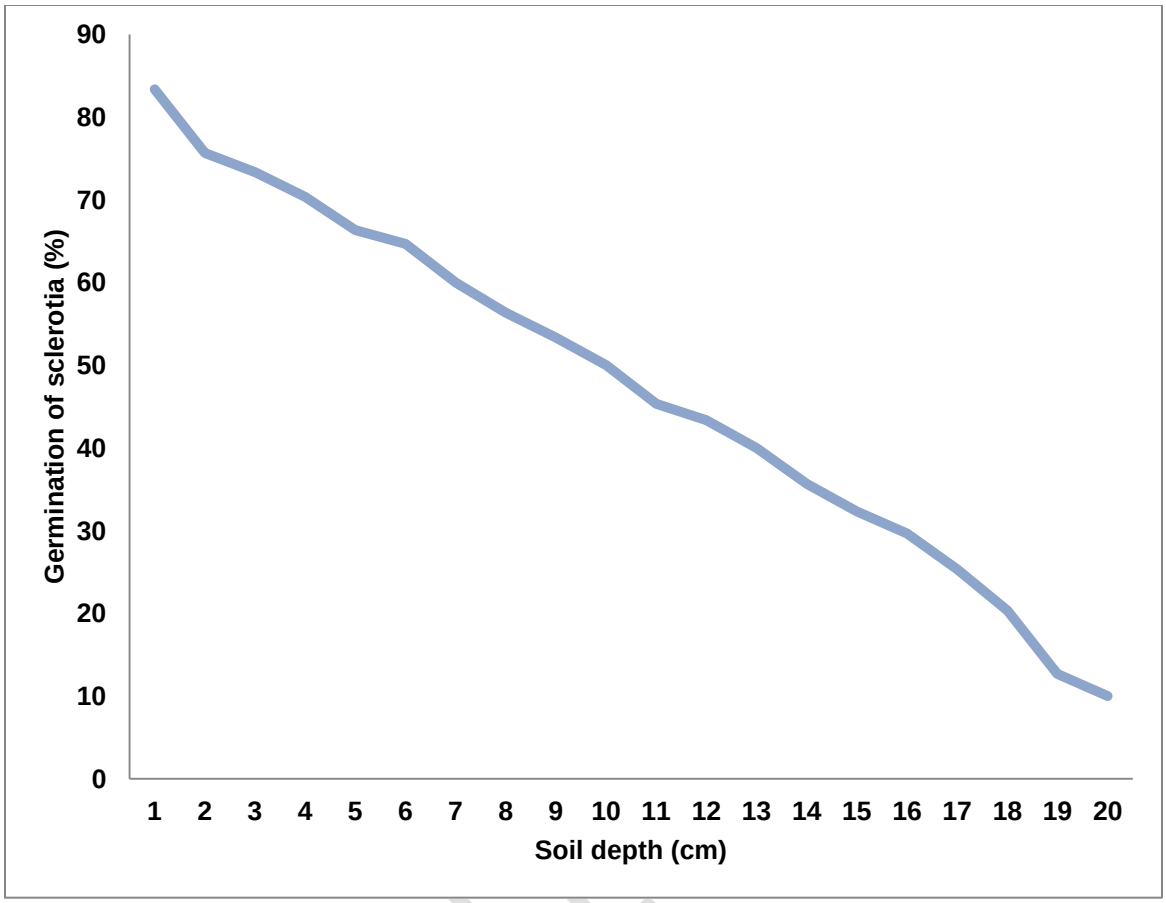

Fig2. Effect of different soil depths on germination of *S. sclerotiorum* sclerotia.

Discussion- The results of different inoculum level depicted that maximum disease incidence (93.67%) was in 10 per cent inoculum level; however disease incidence was least (6.34%) in 1 per cent inoculum level. Disease incidence was increased with increasing in inoculum levels. In case of plant mortality, maximum plant mortality (43.00%) was observed at 10 per cent inoculum level; however no plant mortality was noticed at 1 and 2 per cent inoculum levels. Results of [15] experiments described that low inoculum (less number of germinated sclerotia) resulted lower disease incidence while maximum disease outbreak combined with high level of inoculum and these findings were in conformance with our observation. Disease frequency was increased from 10 to 80 per cent with increasing inoculums density by 7 to 250 sclerotia/100gm soil [13]. Positive correlation had confirmed between infection of plant parts and inoculums density of *S. sclerotiorum* [16].

The results of sclerotial survival in soil depth depicted that maximum (83.33%) germination of sclerotia was observed at 1 cm soil depth, while minimum sclerotial germination (10%) at 20 cm soil depth. It was observed that sclerotial germination decreased as the soil depth increased. Similar to our

observation, [6] reported more sclerotia of *S. sclerotiorum* were remained alive at 5cm depth as compare to 15 cm depth. [10] determined maximum viability (57.5%) of sclerotia on soil surface than 12.5, 2.5 per cent at 5 and 10 cm, respectively and which was similar to our results. Germination ability of sclerotia of *S. sclerotiorum* ranged from 22 to 39 % on the soil surface or placed at 5 cm depth in dry soil [14]. Microbial activity of soil is the key factor for reduction of germination ability of sclerotia. Increase in vertical soil depth was negatively associated with viability of sclerotia that might be due to antagonistic action of soil dwelling microbes [10].

Conclusion: On the basis of outcomes, it has been evident that all the tested inoculum levels were responsible for causation of disease. Per cent disease incidence and plant mortality was raised with increasing in inoculum levels. However, sclerotial germination decreased as the vertical soil depth increased. It is concluded, from the results, that inoculum level studies can be exploited in screening of verities in resistance breeding programme. On the other hand, Findings of sclerotia survival in soil have the potential to aid in disease prediction and management strategy adoption.

References:

1. Aldrich-Wolfe L, Travers S, Nelson BDJ. Genetic Variation of *Sclerotinia sclerotiorum* from Multiple Crops in the North Central United States. PLoS ONE. 2015; 10(9): e0139188. doi:10.1371/journal.pone.0139188.
2. Butler MJ, Gardiner RB, Day A. Melanin synthesis by *Sclerotinia sclerotiorum*. Mycologia. 2009; 101:296-01.
3. Taylor A, Coventry E, Handy C, West JS, Young CS, Clarkson JP. Inoculum potential of *Sclerotinia sclerotiorum* sclerotia depends on isolate and host plant. Plant Pathol. 2018; 67, 1286-95.
4. Chitrampalam P, Turini TA, Matheron ME, Pryor BM. Effect of sclerotium density and irrigation on disease incidence and on efficacy of Coniothyrium minitans in suppressing lettuce drop caused by *Sclerotinia sclerotiorum*. Plant Dis. 2010; 94, 1118–24.
5. Khangura R, MacLeod WJ. Managing the risk of Sclerotinia stem rot in canola. Farm note vol 546. Department of Agriculture and Food, Western Australia; 2012.
6. Wu BM, Subbarao KV. Effects of soil temperature, moisture, and burial depths on carpogenic germination of *Sclerotinia sclerotiorum* and *S. minor*. Phytopathol, 2008; 98:1144-52.
7. Cosic J, Jurkowic D, Vrandecic K, Kaucic D. Survival of buried *Sclerotinia sclerotiorum* sclerotia in Undisturbed soil. Helia. 2012; 35:73-78.

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8. Cook GE, Steadman JR, Boosalis MG. Survival of *Whetzelinia sclerotiorum* and initial infection of dry edible beans. Phytopathol. 1975; 65:250-55.
9. Adams P, Ayers W. Ecology of Sclerotinia species. Phytopathol. 1979; 69(8):896-99.
10. Duncan RW, Fernando WGD, Rashid KY. Time and burial depth influencing the viability and bacterial colonization of sclerotia of *Sclerotinia sclerotiorum*. Soil Biol Biochem. 2006; 38:275-84.
11. Kurle JE, Grau CR, Oplinger ES, Mengistu A. Tillage, crop sequence, and cultivar effects of sclerotinia stem rot incidence and yield in soybean. Agron J. 2001; 93:973-82.
12. Stelfox D, Williams JR, Soehngen U, Topping RC. Transport of *Sclerotinia sclerotiorum* ascospores by Rapeseed pollen in Alberta. Plant Dis Rep. 1978; **62**:576-79.
13. Abawi GS, Grogan RG. Epidemiology of disease caused by sclerotinia species. Phytopathol. 1979; 69:899-04.
14. Matheron ME, Porchas M. Influence of soil temperature and moisture on eruptive germination and viability of sclerotia of *Sclerotinia minor* and *S. sclerotiorum*. Plant Dis. 2005; **89**:50-54.
15. Gugel RK, Morrall RAA. Inoculum-disease relationships in sclerotinia stem rot of rapeseed in Saskatchewan. Canadian J Plant Pathol.1986; 8:89-96.
16. Perveen K, Haseeb A, Shukla PK. Effect of *Sclerotinia sclerotiorum* on the disease development, growth, oil Yield and biochemical changes in plants of *Mentha arvensis*. Saudi J Biol Sci. 2010; 17:291-94.

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