Study on Response of Different pH Levels on Radial Growth and Sclerotial Development of Sclerotinia sclerotiorum Isolate Causing White Rot on Field Pea (Pisum sativum L.), In Vitro

Devesh Pathak and Rais Ullah Khan

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 13
Issue: 06

Res. Jr. of Agril. Sci. (2022) 13: 1702–1705

CARAS

Res. Jr. of Agril. Sci. (Nov-Dec 2022)
13(6): 1702–1705

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

www.rjas.org

Full Length Research Article

Study on Response of Different pH Levels on Radial Growth and Sclerotial Development of *Sclerotinia sclerotiorum* Isolate Causing White Rot on Field Pea (*Pisum sativum* L.), *In Vitro*

Devesh Pathak*[1] and Rais Ullah Khan[2]

Received: 11 Aug 2022 | Revised accepted: 15 Oct 2022 | Published online: 11 Nov 2022

A B S T R A C T

Sclerotinia sclerotiorum is recognized as the most deleterious polyphagous plant pathogen with worldwide distribution. The pathogen is reported to causes disease over 500 plant species belong to diverse group of plants. The symptoms produced by this pathogen can develop at all the above-ground parts of the plant i.e., stems, leaves and pods during the pre-maturity or at the post-harvest stage, thereby, resulted in causing severe losses in both quantity and quality of the crop. The yield losses due to this pathogen varied from 0-100 percent which is dependent on the severity of the disease and the plant parts affected severely. Several nutritional and non-nutritional factors such as temperature, moisture, photoperiod, nutrients, pH, oxygen concentration, type soil, depth and mechanical factors affected the growth and reproduction of the pathogen. Therefore, the present study was conducted to ascertain the effect of different pH levels i.e., 3.5 to 8.5 on growth and sclerotial development of this pathogen. The observations thus clearly ascertain that the growth and sclerotial production of *S. sclerotiorum* was significantly influenced by different pH levels, but fungus preferred pH 4.5 to 6.0 for their maximum radial growth and sclerotial development.

***Key words*:** *Sclerotinia sclerotiorum*, Sclerotia, Field pea, pH, Temperature

Field pea (*Pisum sativum* L.) is known as a popular winter pulse crop, cultivated throughout the world, including India. The crop requires cold and dry climate thus it is currently grown in temperate regions at high elevations or during cool seasons in warm regions throughout the world [1]. The crop is considered as a good source of dietary protein that complements the cereal-based diet, particularly for a vegetarian population of the country [2]. Pea is the fourth leading pulse crop in terms of consumption in the world and an important vegetable and field crop of India [3]. According to the recent statistics of India, the area and production of pea recorded 0.54 mha and 5.43 mt, respectively.

The crop is associated with multiple diseases i.e., fungal, bacterial and viral diseases, but significant yield losses are caused by fusarium wilt (*Fusarium oxysporum* f. sp. *pisi*), powdery mildew (*Erysiphe pisi*), ascochyta blight (*Ascochyta pisi)*, white rot (*Sclerotinia sclerotiorum*), downy mildew (*Peronospora viciae f. sp. pisi*), seed and root rot, and seedling damping-off caused *Rhizoctonia solani*, *Aphanomyces euteiches* and *Pythium spp.*, pea enation mosaic (PEMV), bacterial blight (*Pseudomonas pisi)*, pea seed-borne mosaic virus (PSbMV) and bean yellow mosaic (BYMV) [4-5].

Amongst the significant diseases, white rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is considered as the most significant, devastating fungal disease, distributed throughout the world [6-7]. The disease occurs more frequently in cool and moist regions of temperate, sub-temperate and subtropical zones of the world [8-9] and reported to inflict considerable yield reduction in economically important crops [10-11].

Sclerotinia sclerotiorum is an opportunistic and necrotrophic parasite that can effectively attack, kill and consume the pea plant. The sclerotia refer to the primary survival structure of the pathogen [12] that developed by aggregation of mycelium in culture as well as in plant [13]. The resting sclerotia can remain viable for an extended period as they are resistant to physiologically and chemically adverse conditions, as well as to degradation by another beneficial microorganism [14-15]. Several factors i.e., soil type, burial depth, soil moisture, pH and temperature significantly influenced the carpogenic germination of *Sclerotinia sclerotiorum* [16-18].

The role of pH in growth and sclerotia formation is also be recognized by several researchers in their work. It has been reported that the pathogen could tolerate a wide range of pH. However, the maximum growth and sclerotial formation were only recorded in the pH range of 4 to 5.5 [19-20].

* **Devesh Pathak**
✉ devpathak58@gmail.com

[1-2] Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh - 202 002, Uttar Pradesh, India

MATERIALS AND METHODS

Isolation and molecular characterization of pathogen

During the survey, the pea plants showing the characteristic symptoms of white rot disease were collected and procured to the laboratory for the isolation of causal pathogen. The affected plant parts washed thoroughly with running tap water to remove adhering soil and then placed between two folds of sterilized blotting sheets to remove the excess moisture. A small piece of infected plant parts or sclerotia were surface sterilized with 0.1 % mercuric chloride for 1-2 minutes, following 2-3 changes of immediate washing with distilled water to remove the traces of mercuric chloride [21]. Subsequently, such pieces and sclerotia were placed aseptically under laminar flow in previously sterilized Petri plates, containing solidified potato dextrose agar (PDA) medium and 0.001% chloramphenicol. These Petri plates were then kept for incubation in a B.O.D incubator at 20±2 °C temperature and observed regularly for the presence of mycelial growth, if any around the bits or sclerotia.

The isolate was diagnosed using the polymerase chain reaction (PCR), with the presence of the primer pair ITS1 and ITS4 at Macrogen, Inc., South Korea and registered in National Center for Biotechnology Information (NCBI)).

Effect of different pH levels on growth and sclerotial development of S. sclerotiorum

The inconsistency in radial growth and sclerotial formation of *S. sclerotiorum*, this study was conducted using eleven varying pH levels with an interval of 0.5 viz., 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 on solid and liquid Potato dextrose agar medium. The pH levels were maintained by pH meter using 0.1 N HCl or 0.1 N NaOH solutions.

(a) *On solid media*

After adjusting the desired pH level of the medium, the petri plates were poured with media and inoculated by placing a 5 mm bit of mycelium of the pathogen. Such inoculated petri plates were incubated at 20± 2 °C temperature for two weeks. Each treatment was replicated thrice and observations were recorded on radial growth and sclerotial formation, after 72 and 94 hours after incubation.

(b) *On liquid media*

A 250 ml flask of potato dextrose broth medium was served for conducting this experiment. The desired pH levels for each flask was adjusted as mentioned above. After adjusting the desired pH level of the liquid medium, each flask were inoculated with the mycelial growth of pathogen and incubated at 20 ± 2 °C temperature for two weeks. Each treatment was replicated thrice and observations were recorded on fresh and dry weight of mycelium and sclerotial formation, after 72 and 94 hours after incubation.

RESULTS AND DISCUSSION

Molecular characterization of pathogen

The analysis of the sequence of nucleotide sequences of the PCR products using BLAST proved that the isolates was diagnosed as *S. Sclerotiorum*, with accession number (OM319647) which showed a 100% nucleotide sequence homology with many isolates previously registered in NCBI.

Table 1 Effect of different pH levels on growth and sclerotial formation of *S. sclerotiorum*

pH levels	Radial growth (hrs after inoculation) *		Sclerotial formation (DAI)	No. of sclerotia/plate*	Weight of sclerotia g/plate*
	72 DAI	96 DAI			
3.5	50.66 (45.36)	72.33 (58.25)	6	21.00 (27.23)	0.91 (5.49)
4.0	63.00 (52.51)	82.66 (65.40)	5	27.66 (31.71)	1.30 (6.53)
4.5	86.00 (68.00)	90.00 (71.53)	4	34.00 (35.65)	1.64 (7.35)
5.0	90.00 (71.53)	90.00 (71.53)	4	33.66 (35.49)	1.55 (7.16)
5.5	90.00 (71.53)	90.00 (71.53)	4	30.33 (33.40)	1.43 (6.88)
6.0	90.00 (71.53)	90.00 (71.53)	5	24.66 (29.76)	1.02 (5.81)
6.5	62.33 (52.12)	80.33 (63.67)	6	22.33 (28.17)	0.91 (5.84)
7.0	52.66 (46.51)	75.66 (60.42)	6	22.33 (28.16)	0.71 (4.83)
7.5	44.00 (41.53)	70.00 (56.77)	7	7.33 (26.31)	0.50 (4.07)
8.0	40.66 (39.60)	63.00 (52.51)	9	0.33 (23.81)	0.10 (4.24)
8.5	34.66 (36.04)	43.00 (40.95)	0	0.00 (0.00)	0.00 (0.00)
CD (P=0.05)	1.80	1.95	-	2.24	0.04

Figures in parentheses are the arcsin√percentage transformed values
*Each value is an average of three replicates

Effect of different pH levels on growth and sclerotial development of S. sclerotiorum

a) *On solid medium*

The data presented in (Table 1) revealed that all tested pH levels significantly influenced the radial growth of *S. sclerotiorum* at both intervals of incubation (72 and 96 Hours After Incubation) when compared from each other. After 72 HAI, significantly maximum growth (90.00 mm) was measured at pH levels 5.0, 5.5 and 6.0 that are statically at par with each other followed by 86.00 and 62.33 mm at pH levels 4.5 and 6.0, respectively. The fungus obtained their minimum growth (34.66 mm) at higher level of pH i.e., 8.5 followed by pH 8.0 (40.66 mm) (Table 1, Plate 1). Similar to previous interval i.e., 96 HAI, significantly maximum growth (90.00 mm) was recorded at pH level i.e., 4.5, 5.0, 5.5 and 6.0, however, fungus produced minimum growth (43.00 mm) on pH level 8.5 (Table 1).

The sclerotial formation was also significantly influenced at varied pH levels. The initiation of sclerotia was started at 4-9 days after incubation at all levels of pH except 8.5. But significantly maximum sclerotia (34.00) were formed at pH 4.5 at 4 days after incubation that are non-significantly at par with pH 5.0 (33.66), followed by 33.33 at 4 DAI and 27.66 at 5 DAI at pH 5.5 and 4.0, respectively. (Table 1, Plate 1). However, the minimum sclerotia (0.33) was yielded from pH 8.0 followed by pH 7.5 (7.33) at 9 and 7 DAI, respectively (Plate 1). The weight of sclerotia was also influenced by the different levels of pH. However, the significantly maximum sclerotial weight (1.64 g/plate) was obtained at pH 4.5 [22]. It

is therefore, clear from the results that the growth and sclerotial production of *S. sclerotiorum* was significantly influenced by different pH levels, but fungus preferred pH 4.5 to 6.0 for its maximum radial growth and sclerotial development.

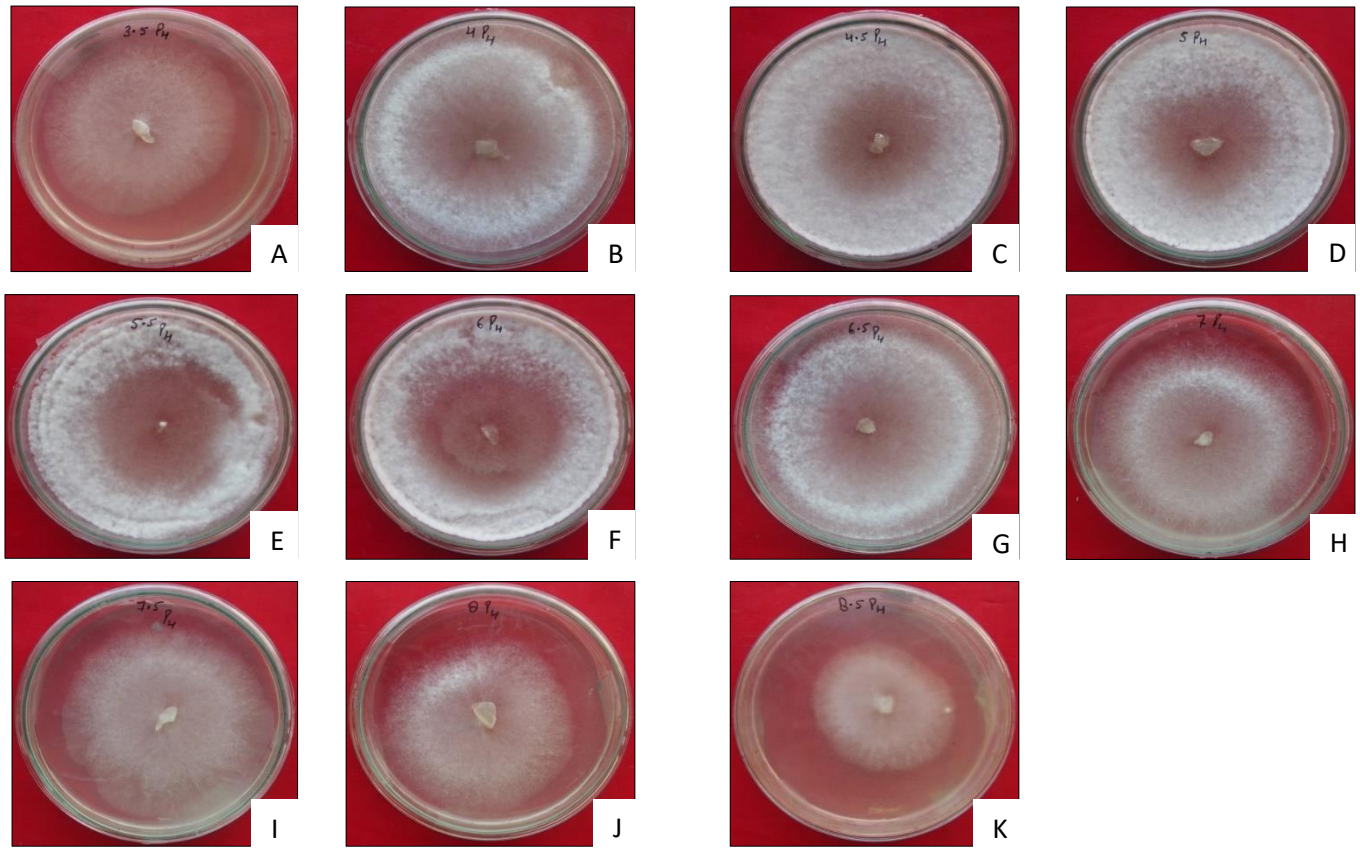

Plate 1 Effect of different pH on cultural and morphological characteristics of *S. sclerotiorum* 3.5 (A), 4.0 (B), 4.5 (C), 5.0 (D), 5.5 (E), 6.0 (F), 6.5 (G), 7.0 (H), 7.5 (I), 8.0 (J) and 8.5 (K)

Table 2 Effect of different pH levels on mycelial weight (Fresh and dry weight) and sclerotial formation of *S. sclerotiorum* on Potato Dextrose Broth

pH levels	Fresh mycelium wt. gm/flask	Dry mycelium wt. gm/flask	Initiation of sclerotia	Number of sclerotia/flask	Wt. of sclerotia gm/flask
3.5	2.17 (8.44)	0.43 (3.74)	5	9.33 (17.75)	1.23 (6.35)
4.0	2.83 (9.67)	0.47 (3.91)	5	10.00 (18.41)	1.33 (6.60)
4.5	6.90 (15.72)	1.10 (6.01)	4	21.33 (27.49)	7.67 (16.06)
5.0	7.33 (15.70)	1.73 (7.55)	4	23.00 (28.64)	8.60 (17.04)
5.5	7.23 (15.59)	1.70 (7.48)	4	22.67 (28.41)	8.17 (16.59)
6.0	7.20 (15.55)	1.13 (6.10)	5	21.00 (27.26)	7.80 (16.21)
6.5	5.17 (13.13)	0.93 (5.53)	6	16.00 (23.56)	5.60 (13.67)
7.0	4.33 (12.00)	0.83 (5.32)	6	13.33 (21.39)	3.53 (10.82)
7.5	3.60 (10.93)	0.47 (3.91)	6	5.67 (13.75)	2.27 (8.64)
8.0	1.43 (6.87)	0.27 (2.94)	7	1.00 (4.62)	0.43 (3.06)
8.5	0.00 (0.00)	0.00 (0.00)	-	0 (0.00)	0 (0.00)
CD (P=0.05)	0.60	0.58	-	2.48	1.55
SE (m)	0.20	0.19	-	0.84	0.52

Figures in parentheses are the arcsin√percentage transformed values
*Each value is an average of three replicates

b) *On liquid media*

The perusal of (Table 2) indicated that all tested pH levels significantly influenced the fresh and dry mycelial weight and sclerotial development of *S. sclerotiorum*. The significant maximum fresh mycelial weight (7.33 gm/flask) was yielded at the pH 5.0 and it was at par with pH 5.5 and 6.0 that yielded 7.23 and 7.20 gm/flask fresh mycelial weight of the fungus, respectively at seven days after incubation (Table 2, Plate 2). The fungus did not produced mycelium at highest levels of pH i.e., 8.5, however, minimum fresh mycelial weight was obtained at pH 8.0 followed by pH 4.0 (2.83 gm/flask) and pH 3.5 (2.17 6.90 gm/flask) (Plate 2). The dry mycelial weight also significantly influenced by different pH levels. However, maximum dry mycelial weight (1.73 gm/flask) was yielded at the pH 5.0 and it was at par with pH 5.5 and 6.0 that yielded 1.73 and 1.13 gm/flask dry mycelial weight of the fungus, respectively (Table 2, Plate 2). The minimum dry mycelial weight (0.27 gm/flask) was obtained at pH 8.0 followed by pH 3.5 (0.43 gm/flask), pH 4.0 (0.47 gm/flask) and pH 7.5 (0.47 gm/flask) that are non-significantly at par with each other (Plate 2). The fungus initiated sclerotia at 4-7 days after incubation at all pH levels except 8.5 (Table 2). However, significantly maximum sclerotia (23.00) and their weight (8.60 gm/flask) was recorded at pH 5.0 and it was at par with pH 5.5 (22.67 and

8.17 gm/flask), 4.5 (21.33 and 7.67 gm/flask) and 6.0 (21.00 and 7.80 gm/flask), respectively (Table 2). The minimum sclerotia and their weight (1.00 and 043 gm/flask) was recorded at pH 8.0 followed by pH 7.5 i.e., 5.67 and 2.27 gm/flask, respectively [23] (Table 2, Plate 2).

CONCLUSION

H^+ ion concentration is one among the environmental factors that govern the growth and sporulation of fungi. A little variation in pH can induced significant differences in the growth and reproduction of this pathogen. Most workers have found that *S. sclerotiorum* can grow and produce sclerotia on media with pH ranged 2.5–9.0, but moderate acidic pH ranged 4.5 to 6.0 produced maximum fresh and dry mycelial weight and sclerotia of this fungus. The pH of culture medium significantly influenced the sclerotial development. Thus, it is confirmed from the present study that fresh and dry mycelial weight and sclerotial production of *S. sclerotiorum* was significantly influenced by different pH levels, but fungus preferred pH 4.5 to 6.0 for its maximum fresh and dry mycelial weight and sclerotial development.

Plate 2 Effect of different pH on mycelial weight (fresh and dry) and sclerotial development of *S. sclerotiorum.* 3.5 (A), 4.0 (B), 4.5 (C), 5.0 (D), 5.5 (E), 6.0 (F), 6.5 (G), 7.0 (H), 7.5 (I), 8.0 (J) and 8.5 (K)

LITERATURE CITED

1. Elzebroek T, Wind K. 2008. *Guide to Cultivated Plants*. CAB International, Oxfordshire, UK.
2. Chaudhary RP, Pandey R, Chaturvedi AK, Prasad R. 2014. Enhancing yield and economics of field pea through Front line demonstration. *Agriculture Update* 9(4): 494-498.
3. Gurjar B, Sharma S, Kashyap Y, Sharma NP. 2018. Economic analysis of green pea production in Jabalpur district of Madhya Pradesh. *Journal of Pharmacognosy and Phytochemistry* SP1: 2858-2861.
4. Kraft J, Pfleger F. 2001. Compendium of pea diseases and pests. 2nd Edition. APS Press, The American Phytopathological Society, St. Paul, MN.
5. McGee R. 2012. USDA-ARS. Personal communication.
6. Willetts HJ. Wong JAL. 1980. The biology of *Sclerotinia sclerotiorum*, *S. trifoliorum*, and *S. minor* with emphasis on specific nomenclature. *Botanical Review* 46: 100-165.
7. Boland GJ, Hall R. 1994. Index of plant hosts of *Sclerotinia*. *Canadian Jr. Plant Pathology* 16: 93-108.
8. Purdy LH. 1979. *Sclerotinia sclerotiorum*: History, disease and symptomatology, host range, geographic distribution and impact. *Phytopathology* 69: 875-880
9. Saharan GS, Mehta N. 2008. *Sclerotinia* diseases of crop plants: Biology, ecology and disease management: Springer Press. *Sclerotinia sclerotiorum* Isolates in Northern Parts of Iran. *World Appl. Sci. Journal* 8(3): 326-333.
10. Malvarez M, Carbone I, Grunwald NJ, Subbarao KV, Schafer M, Kohn LM. 2007. New populations of *Sclerotinia sclerotiorum* from lettuce in California and peas and lentils in Washington. *Phytopathology* 97: 470-483.
11. Parveen K, Haseeb A, Shukla, PK. 2007. Management of *Sclerotinia sclerotiorum* on *Mentha arvensis* C.V. Gomti. *Journal of Mycol. Plant Pathology* 37(1): 33-36.
12. Coley-Smith JR, Cooke RC. 1971. Survival and germination of fungal sclerotia. *Annu. Rev. Phytopathology* 9: 65-92.
13. Ordonez-Valencia C, Ferrera-Cerrato R, Quintanar-Zuniga RE, Flores-Ortiz C.M, Guzman GJM, Alarcon A, Larsen J, Garcia-Barradas O. 2014. Morphological development of sclerotia by *Sclerotinia sclerotiorum*: A view from light and scanning electron microscopy. *Ann. Microbiology* 65: 765-770.
14. Merriman PR. 1976. Survival of sclerotia of *Sclerotinia sclerotiorum* in soil. *Soil Biol. Biochemistry* 8: 385-389.
15. Wu BM, Subbarao KV. 2008. Effects of soil temperature, moisture, and burial depths on carpogenic germination of *Sclerotinia sclerotiorum* and *S. minor*. *Phytopathology* 98: 1144-1152.
16. Clarkson JP, Phelps K, Whipps JM, Young CS, Smith JA, Watling M. 2004. Forecasting Sclerotinia disease on lettuce: toward developing a prediction model for carpogenic germination of sclerotia. *Phytopathology* 94(3): 268-279.
17. Sharma P, Meena PD, Verma PR, Saharan GS, Mehta N, Singh D, Kumar A. 2015. *Sclerotinia sclerotiorum* (Lib) de Bary causing Sclerotinia rot in oilseed Brassicas: A review. *Journal of Oilseed Brassica* 1(2): 1-44.
18. Smolinska U, Kowalska B. 2018. Biological control of the soil-borne fungal pathogen *Sclerotinia sclerotiorum* - A review. *Journal of Plant Pathology* 100: 1-12.
19. Townsend BB. 1957. Nutritional factors influrncing the production of sclerotia by certain fungi. *Ann. Botsny* 21: 153-166.
20. Rudolph ED. 1962. The effect of some physiological and environmental factors in *sclerotia aspergilli. American Journal of Botany* 49: 71-78.
21. Marukawa S, Funakawa S, Satomura Y. 1975. Some physical and chemical factors on formation of sclerotia in *Sclerotinia libertiana* Fuckel. *Agric. Biol. Chemistry* 39: 463-468.
22. Maxwell DP, Lumsden RD. 1970. Oxalic acid production by *Sclerotinia sclerotiorum* in infected bean and in culture. *Phytopathology* 60: 1395-1398.
23. Rollins JA, Dickman MB. 2001. pH signaling in *Sclerotinia sclerotiorum*: Identification of apacC/RIM1 homolog. *Applied Environ. Microbiology* l67: 75-81.