

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.907.074>

Nutritional and Epidemiological Requirements for Growth and Sclerotia Formation by *Sclerotium rolfisii* (Sacc.) Causing Collar Rot of Chickpea (*Cicer areitinum* L.)

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ABSTRACT

Keywords

Chickpea,
Sclerotium rolfisii,
Culture media, pH,
Temperature,
Colony Growth and
Sclerotia formation

Article Info

Accepted:
08 June 2020
Available Online:
10 July 2020

Sclerotium rolfisii was isolated from infected chickpea plant sample collected from the chickpea's field of Agriculture Research Station, Ummedganj, (Kota). In epidemiological studies, mycelium growth and sclerotia formation by *S. rolfisii* was significantly influenced with media, temperature and pH level. Maximum colony diameter (90.00 mm) and sclerotia count (691.00 per plate) were recorded on oat meal agar (Hi-media) at 96 hrs. and 20 days after inoculation respectively, followed by potato dextrose agar (natural) in radial mycelial growth 87.00 mm and potato dextrose agar (Hi-Media) in number of sclerotia count 665.00 per plate. Maximum colony diameter (90.00 mm) and sclerotia counts (538.50) of the pathogen were recorded at the 25 °C temperature was statistically superior over rest temperature level tried. While, in context to pH maximum colony diameter 89.00 mm of pathogen and sclerotia count 570.00 sclerotia per plate were recorded at the pH level 7.0.

Introduction

Chickpea is known in this country since ancient times. It is a widely grown major pulse crop in India, accounts for nearly 75 per cent of the total pulse production in the world. Chickpea crop is prone to many diseases viz., Fusarium wilt, dry root rot, collar rot, Ascochyta blight, Verticillium wilt, black root rot, Phytophthora root rot, wet root rot, foot rot, Pythium rot and seed rot etc. Among these, collar rot caused by *Sclerotium rolfisii*

which is gaining importance. Collar rot of chickpea is well known and wide spread disease in India. About 2-5% of losses are caused every year which may even reach up to 60% under severe conditions.

It was reported that 54.7 – 95% of mortality occurred in chickpea seedlings because of collar rot disease (Mathur and Sinha, 1968). *Sclerotium rolfisii* is an economically important pathogen on numerous crops worldwide.

It has an extensive host range; at least 500 species in 100 families are susceptible, the most common hosts are legumes, crucifers and cucurbits, and commonly occurs in the tropics, subtropics, and other warm temperate regions (Punja, 1985). Present experiment deals with study of *Sclerotium rolfsii* growth and sclerotia formation under different culture media, at various temperatures and pH level. To carry out the research, eight distinctive culture media viz., Potato dextrose agar (Natural), Potato dextrose agar (Hi-Media), V-8 juice agar (Hi-Media), C-zepak's agar (Hi-Media), Oat meal agar (Hi-Media), Corn meal agar, Chickpea seed meal extract agar and Host (Chickpea) stem extract agar were used to evaluate the growth and sclerotia formation of pathogen. Later suitable temperature and pH were also analyzed for the pathogen growth and sclerotia formation by incubating at different pH level viz., 5.0, 6.0, 7.0, 8.0 and 8.5 and temperatures level viz., 10 °C, 15 °C, 20 °C, 25 °C, 30 °C and 35 °C.

Materials and Methods

Collection

Infected plants which showing typical collar rot symptoms were collected during month of October to December, 2018 from the chickpea fields of Agriculture Research Station, Ummedganj (Kota). Samples were brought in to Department of Plant Pathology, College of Agriculture, Ummedganj, (Kota) for isolation and further studies.

Isolation of fungus

For isolation of the pathogen standard tissue isolation technique followed. The part of collar region showing typical symptoms of disease was cut into small pieces (3mm²). Then these pieces were surface sterilized with 0.1% HgCl₂ solution for one minute. Such pieces were washed thoroughly in sterile

distilled water three times to remove the traces of mercuric chloride solution, and then aseptically transferred to sterilized potato dextrose agar (PDA) plates. They were incubated at 25±1°C for three days for growth of fungus. Later, the bit of fungal growth was transferred to PDA plates. The pure culture of fungus was obtained by further growing the culture and following hyphal tip culture under aseptic conditions.

Identification of fungus

The pathogen *S. rolfsii* forms cottony white colonies on PDA. The colonies appeared as dull white to pure white mycelial growth and formed sclerotial bodies after 8-9 days of incubation. Sclerotia are brown in colour and mustard seed like in shape. On the basis of these characters pathogen was identified as *S. rolfsii*. Further, pathogen was identified from ITCC (Indian Type Culture Collection) Lab, IARI, Division of Plant Pathology, New Delhi (Ref. No. PP/3260; date- 25/03/2019).

Preparation of culture media

Different culture media viz., Potato dextrose agar (Natural), Potato dextrose agar (Hi-Media), V-8 juice agar (Hi-Media), C-zepak's agar (Hi-Media), Oat meal agar (Hi-Media), Corn meal agar, Chickpea seed meal extract agar and Host (Chickpea) stem extract agar were prepared to carry out the study. All the media were sterilized at 121.6 °C temperature and 1.05 kg/cm² (15lbs psi) pressure for 15 min. To carry out study, cool & molten media near about 20 ml of each of the medium was poured in each prior Sterilized petriplates. After solidification of media such petriplates were inoculated with 6 mm disc cut from the periphery of actively growing fungal culture grown in petri plates by using sterilized cork borer and incubated at 25±1 °C. Each treatment was replicated thrice. Radial growth

of the colony was recorded by measuring the colony diameter in millimetre at regular intervals of 48hrs and sclerotia count at 20th day. The data obtained were analysed statistically.

Effect of variable temperatures on growth and sclerotia formation of *S. rolfsii*, in-vitro

To determine the minimum, optimum and maximum range of temperature for mycelial growth and sclerotia formation of *Sclerotium rolfsii*, PDA was poured into the petriplates and a 6 mm mycelial disc from actively growing culture of the fungus placed on the surface of solidified medium (20 ml for each Petri plate) in a laminar air flow. The inoculated plates were incubated at different regimes of temperature viz., 10, 15, 20, 25 30 and 35±1 °C in different BOD incubator. Four replications were maintained for each temperature level. Radial growth of the colony was recorded by measuring colony diameter in millimetre at regular intervals of 48hrs. and sclerotia count at 20th day. The data obtained were analysed statistically.

Effect of variable pH on growth and sclerotia formation of *S. rolfsii*, in-vitro

For determining the optimum level of pH for mycelial growth and sclerotia formation of *Sclerotium rolfsii*, 100 ml of medium (PDA) was dispensed into the 5 Erlenmeyer flasks of 250ml capacity and different regimes of pH viz., 5.0, 6.0, 7.0, 8.0 and 8.5 were adjust by with the Digital pH meter by using N/10 HCl or N/10 NaOH solution prior to sterilization. The sterilized medium was poured into petriplates. After solidification of medium, a 6 mm mycelial disc from actively growing culture of fungus was cut with sterilized cork borer and placed on the surface of medium (20 ml for each Petri plate) under aseptic condition. The inoculated plates were incubated at 25±1°C. Four replications were

maintained for each treatment of pH level. Radial growth of the colony was recorded by measuring colony diameter in millimetre at regular intervals of 48hrs and sclerotia count at 20th day. The data obtained were analysed statistically.

Results and Discussion

Effect of different solid culture media on mycelial growth and sclerotia formation of *S. rolfsii*

Effect of different solid media, viz., Potato dextrose agar (Natural), Potato dextrose agar (Hi-Media), V-8 juice agar (Hi-Media), C-zepak'sagar (Hi-Media), Oat meal agar (Hi-Media), Corn meal agar, Chickpea seed meal extract agar and Host (Chickpea) stem extract agar on radial growth and sclerotia formation was studied and observation have been presented in Table- 1, illustrated in Fig.-1(a) or (b) and Plate-1(a) & (b). Maximum colony diameter (90.00 mm) and sclerotia count (691.00) were recorded on oat meal agar (Hi-media) at 96 hrs. and 20 days after inoculation respectively, which was statistically significant over rest of medium tried, Similar result were also reported by (Basamma2008; Sab, 2013). The next was potato dextrose agar (Natural) medium which yielded 87.00 mm, colony diameter followed by C-zapek's agar medium and chickpea seed meal extract agar both were similar and yielded 84.25 mm growth of fungus, potato dextrose agar (Hi-Media) yielded 83.25 mm radial growth which were also statistically at par each other. Least colony diameter (56.63 mm) of the pathogen was observed in host stem (chickpea) extract agar medium and it was statistically inferior than all other agar medium tried. In number of sclerotia count per plates oat meal agar (Hi-media) was followed by potato dextrose agar (Hi-Media), potato dextrose agar (natural), C-Zepak'sagar (Hi-Media) and V-8 juice agar (Hi-Media) in

numbers respectively 665.00, 628.00, 390.00 and 334.00. Very minute sizes sclerotia is appeared in V-8 juice agar (Hi-Media). Least sclerotia produced in chickpea seed meal extract agar 137.00 was statistically inferior than all other agar medium used, similar finding regarding potato dextrose agar (PDA) medium was found to be highly supportive for the pathogen growth resulting full growth (9cm) within 7 days (Bana Sravani and Ram Chandra, 2020).

Effect of different temperature level on radial growth and sclerotia formation of *S. rolfsii*

The temperature ranges for growth vary for all microorganisms as well as for host pathogen interactions. The fungus showed considerable variation in mycelial growth and sclerotia formation at different temperature level studies on PDA viz., 10, 15, 20, 25, 30 and 35°C. It's evident from data presented in Table- 2, illustrated in Fig.- 2(a) or (b) and Plate- 2(a) & (b) that *S. rolfsii* grew at temperature ranges 10 to 35°C under study. The fungus had showing considerable variation in radial growth and sclerotia formation at different temperature level studies viz., 10, 15, 20, 25, 30 and 35°C, maximum colony diameter(90.00 mm) of the pathogen was recorded at the 25°C temperature was statistically superior over rest temperature level tried and found ideal for the growth of pathogen followed by 30°C(85.00 mm) and least growth observed at 10°C (22.63 mm). Results indicate that both increase and decrease in level of the temperature significantly alter mycelial growth and growth rate of pathogen. In context to number of sclerotia formation per plates was maximum at 25°C temperature with sclerotia count (538.50), followed by 30°C (480.50). The less numbers of sclerotia were produced at 10°C (185.25) was statistically inferior than all other temperature

level tested. Similar finding regarding mycelial growth and number of sclerotia formation was reported by Dey *et al.*, (1992) found that most sclerotia were obtained in culture medium on P.D.A., the mycelial and sclerotia formation was best over the temperatures range of 25-30°C. Prasad *et al.*, (1986) reported that best mycelial growth of *S. rolfsii* at $30 \pm 0.5^\circ\text{C}$, whereas $25 \pm 0.5^\circ\text{C}$ for the sclerotial formation. Zape *et al.*, (2013) observed that the *Sclerotium rolfsii* showed rapid mycelia growth at 30°C and maximum sclerotial production was recorded at 25°C the pathogen was unable to grow and produce sclerotia at minimum & maximum temperatures of 10°C and 40°C studied. Muthukumar and Suthinraj (2019) revealed that the maximum mycelia growth and biomass produced by pathogen at 30°C (89.33mm; 240mg) which was significantly reduced below 20°C and above 35°C. Bana Sravani and Ram Chandra (2020) found that 30°C with maximum mycelial growth of 9.0 cm within 5 DAI followed by 25°C temperature with 9.0 cm at 7 DAI whereas other temperature ranges gradually reduced the growth of pathogen. Least growth was noticed when pathogen was allowed to grow at 15°C and 35°C with only 5.60 and 5.13 cm radial growth at 7 DAI.

Effect of different pH level on mycelial growth and sclerotia formation of *S. rolfsii*

The pH value of a medium has a marked effect on radial growth and sclerotia formation by *S. rolfsii*. The fungus had showing considerable variation in radial growth and sclerotia formation at different pH level viz., 5.0, 6.0, 7.0, 8.0 and 8.5 was studied and observation have been presented in Table- 3, illustrated in Fig.- 3(a) or (b) and Plate- 3(a) & (b). The pH value of medium has a noticeable effect on radial growth and sclerotia formation by *S. rolfsii*.

The fungus had showing substantial variation in radial growth and sclerotia formation at different pH level viz., 5.0, 6.0, 7.0, 8.0 and 8.5 was studied, maximum colony diameter 89.00 mm of pathogen was recorded at the pH level 7.0, This was followed by pH level 6.0, 5.0, 8.0 and 8.5 with colony diameter 82.75, 70.75, 62.75 mm and 56.75 mm of the *S. rolfsii* respectively. Result indicates that both increase and decrease in the level of pH significantly alter mycelial growth of Pathogen. Maximum number of sclerotia 570.00 recorded at pH level 7.0 per plate which was statistically at par pH level 8.0 with numbers of sclerotia 538.00 per plates.

Least sclerotia produced in medium having pH 5.0 (232.00) was statistically inferior than all other pH level tested. This shows that pathogen requires slightly acidic condition for its growth and sclerotial formation.

Similar finding reported by Kumar *et al.*, (2008) also reported that maximum growth obtained at pH 6.5 followed by pH 7.0. Zape *et al.*, (2013) reported maximum radial growth of *S. rolfsii* observed at pH 6.5 followed by pH 6.0 and 7.0 and whereas, maximum sclerotial formation at pH 7.0. optimum range of pH 5.5 to 7.5 was better for pathogen growth and sclerotial formation.

Table.1 Effect of different solid culture medium on mycelial growth and sclerotia formation by *Sclerotium rolfsii*, *in-vitro*

S. No.	Medium Name	Average colony diameter (48 hrs. after in mm) *	Average colony diameter (96 hrs. after in mm) *	Average Number of Sclerotia (20DAI) *
1.	Potato dextrose agar (Natural)	64.75 (53.58) **	87.00 (68.89)	628.00 (25.07) ***
2.	Potato dextrose agar (Hi-Media)	61.50 (51.65)	83.25 (65.85)	665.00 (25.80)
3.	V-8 juice Agar (Hi-Media)	39.50 (38.94)	57.75 (49.46)	334.00 (18.29)
4.	C-zepak's Agar (Hi-Media)	63.50 (52.83)	84.25 (66.63)	390.00 (19.74)
5.	Oat meal Agar (Hi-Media)	66.75 (54.79)	90.00 (71.57)	691.00 (26.30)
6.	Corn meal Agar	46.75 (43.14)	74.00 (59.35)	190.00 (13.80)
7.	Chickpea seed meal extract Agar	54.75 (47.73)	84.25 (66.62)	137.00 (11.72)
8.	Chickpea stem extract Agar	36.50 (37.17)	54.63 (47.65)	273.00 (16.54)
SEm.± =		0.77 (0.45)	0.81 (0.58)	14.14 (0.35)
C.D. at 0.05% =		1.59 (0.94)	1.66 (1.20)	29.19 (0.73)
C.V. (%) =		2.01(1.35)	1.48 (1.33)	4.84 (2.55)

*Mean of four replications; **Figures in parentheses are Arc sine transformed values;

***Figures in parentheses are Square root transformed values

Table.2 Effect of different temperature level on mycelial growth and sclerotia formation by *Sclerotium rolfsii*, *in-vitro*

S. No.	Temperature (°C)	Average colony diameter (48 hrs. after in mm) *	Average colony diameter (96 hrs. after in mm) *	Time taken in hrs. for cover whole plates by fungal growth	Average Number of Sclerotia (20DAI) *
1.	10°C	11.00 (19.35) **	22.63 (28.39)	182	185.25 (13.63) ***
2.	15°C	20.88 (27.18)	32.88 (34.98)	168	204.00 (14.29)
3.	20°C	41.50 (40.11)	67.63 (55.32)	130	360.00 (18.98)
4.	25°C	62.88 (52.46)	90.00 (71.57)	96	538.50 (23.21)
5.	30°C	59.75 (50.62)	85.00 (67.22)	120	480.50 (21.93)
6.	35°C	28.63 (32.34)	56.63 (48.81)	144	285.00 (16.89)
SEm.± =		0.68 (0.47)	0.86 (0.56)		13.37 (0.34)
C.D. at 0.05% =		1.44 (0.99)	1.81 (1.18)		28.08 (0.72)
C.V. (%) =		2.58 (1.80)	2.06 (1.55)		5.52 (2.67)

*Mean of four replications; **Figures in parentheses are Arc sine transformed values;

***Figures in parentheses are Square root transformed values

Table.3 Effect of different pH level on mycelial growth and sclerotia formation by *Sclerotium rolfsii*, *in-vitro*

S. No.	pH level	Average colony diameter (48 hrs. after in mm) *	Average colony diameter (96 hrs. after in mm) *	Average Number of Sclerotia (20DAI) *
1.	5.0	44.00 (41.55) **	70.75 (57.26)	232.00 (15.22) ***
2.	6.0	50.00 (45.00)	82.75 (65.47)	388.00 (19.71)
3.	7.0	56.50 (48.74)	89.00 (70.66)	570.00 (23.87)
4.	8.0	35.75 (36.72)	62.75 (52.39)	538.00 (23.20)
5.	8.5	27.75 (31.78)	56.75 (48.88)	335.00 (18.31)
SEm. ± =		1.02 (0.61)	1.12 (0.75)	19.78 (0.51)
C.D. at 0.05% =		2.18 (1.30)	2.38 (1.60)	42.17 (1.08)
C.V. (%) =		3.39 (2.12)	2.18 (1.80)	6.78 (3.56)

*Mean of four replications; **Figures in parentheses are Arc sine transformed values;

***Figures in parentheses are Square root transformed values

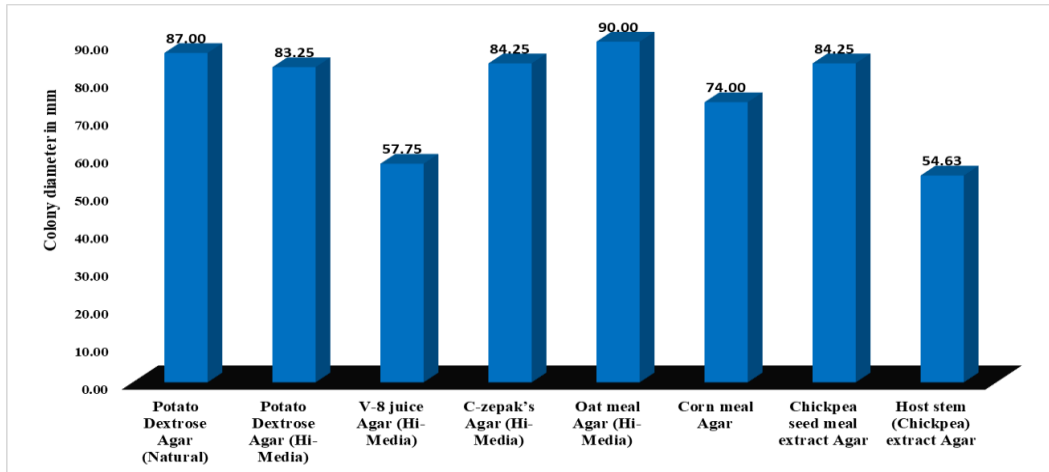


Fig.1(a) Effect of different solid media on mycelial growth of *S. rolfsii* (96 hrs. after inoculation)

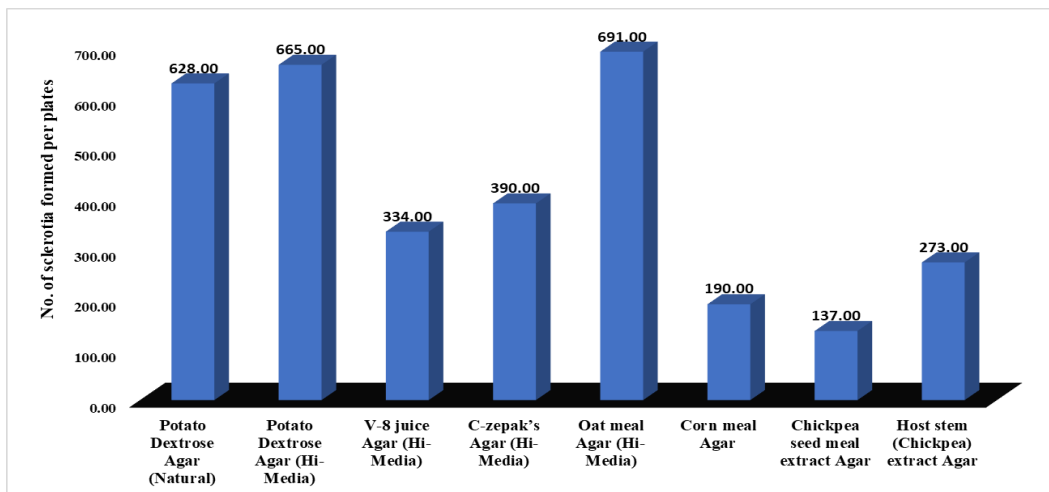


Fig.1(b) Effect of different solid media on sclerotia formation by *S. rolfsii* (20 days after inoculation)

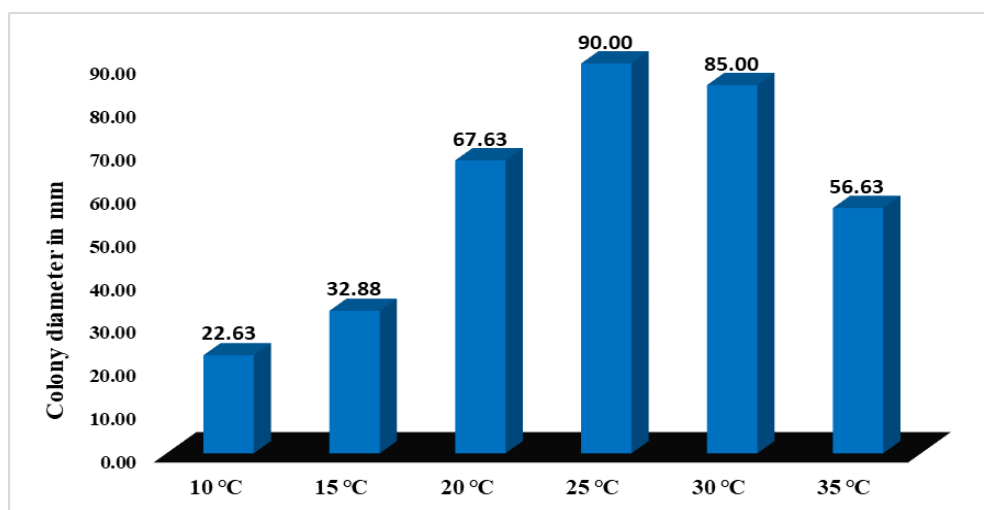


Fig.2(a) Effect of different temperature level on mycelial growth of *S. rolfsii* (96 hrs. after inoculation)

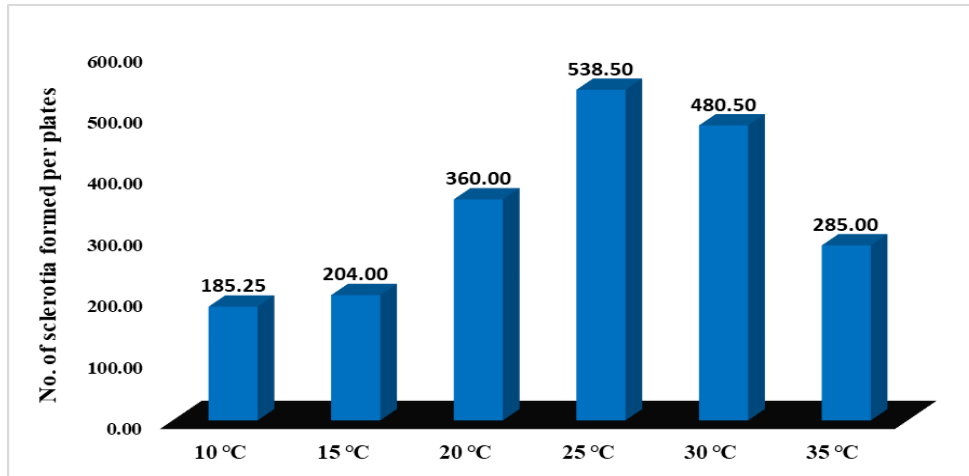


Fig.2(b) Effect of different temperature level on sclerotia formation by *S. rolfsii* (20 days after inoculation)

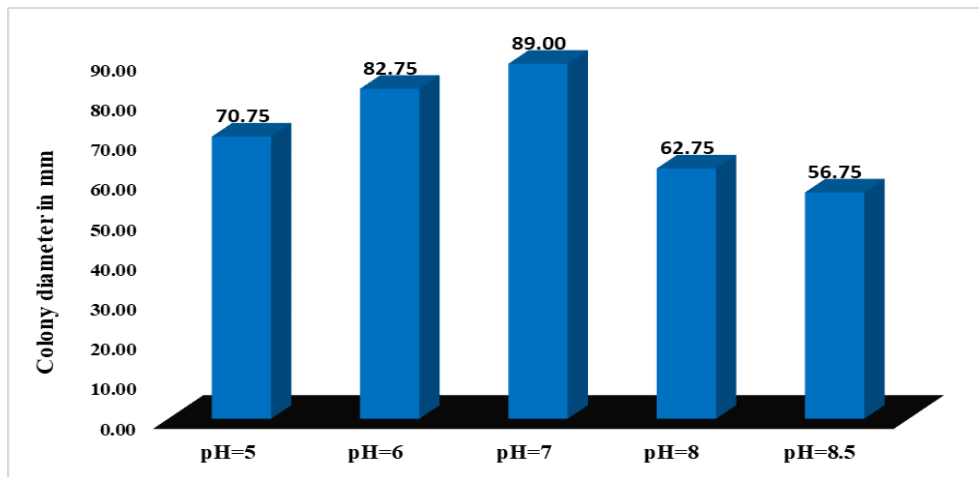


Fig.3(a) Effect of different pH level on mycelial growth of *S. rolfsii* (96 hrs. after inoculation)

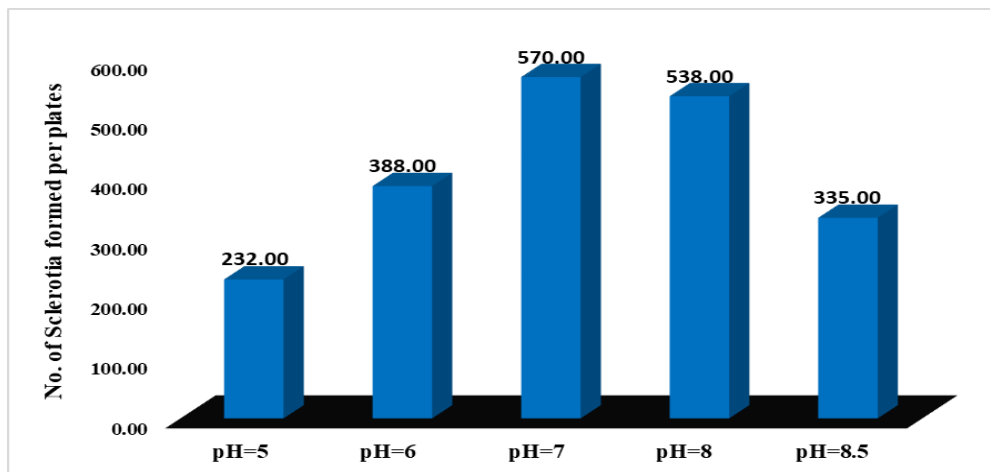


Fig.3(b) Effect of different pH level on sclerotia formation by *S. rolfsii* (20 days after inoculation)



T₁=Potato dextrose agar (Natural), T₂=Potato dextrose agar (Hi-Media), T₃=V-8 juice agar (Hi-Media), T₄=C-zepak's agar (Hi-Media), T₅=Oat meal agar (Hi-Media), T₆=Corn meal agar, T₇=Chickpea seed meal extract agar, T₈=Chickpea stem extract agar
Plate.1(a) Effect of different solid media on mycelial growth of *S. rolfsii* (96 hrs. after inoculation)



T₁=Potato dextrose agar (Natural), T₂=Potato dextrose agar (Hi-Media), T₃=V-8 juice agar (Hi-Media), T₄=C-zepak's agar (Hi-Media), T₅=Oat meal agar (Hi-Media), T₆=Corn meal agar, T₇=Chickpea seed meal extract agar, T₈=Chickpea stem extract agar
Plate.1(b) Effect of different solid media on sclerotia formation by *S. rolfsii* (20 days after inoculation)

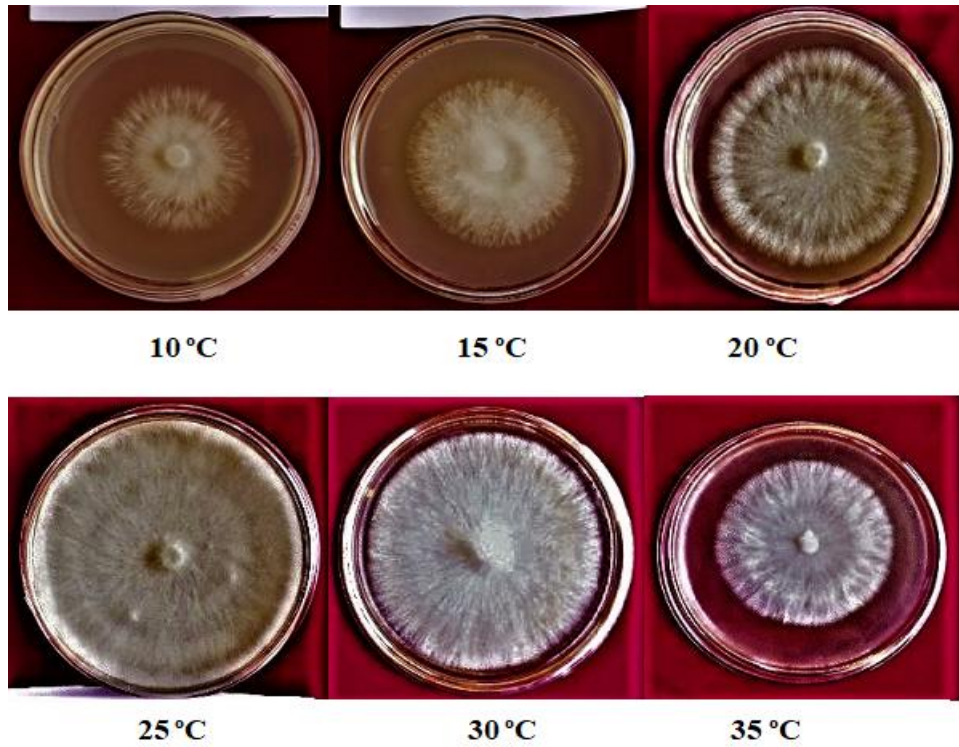


Plate.2(a) Effect of different temperature level on mycelial growth of *S. rolfsii* (96 hrs. after inoculation)

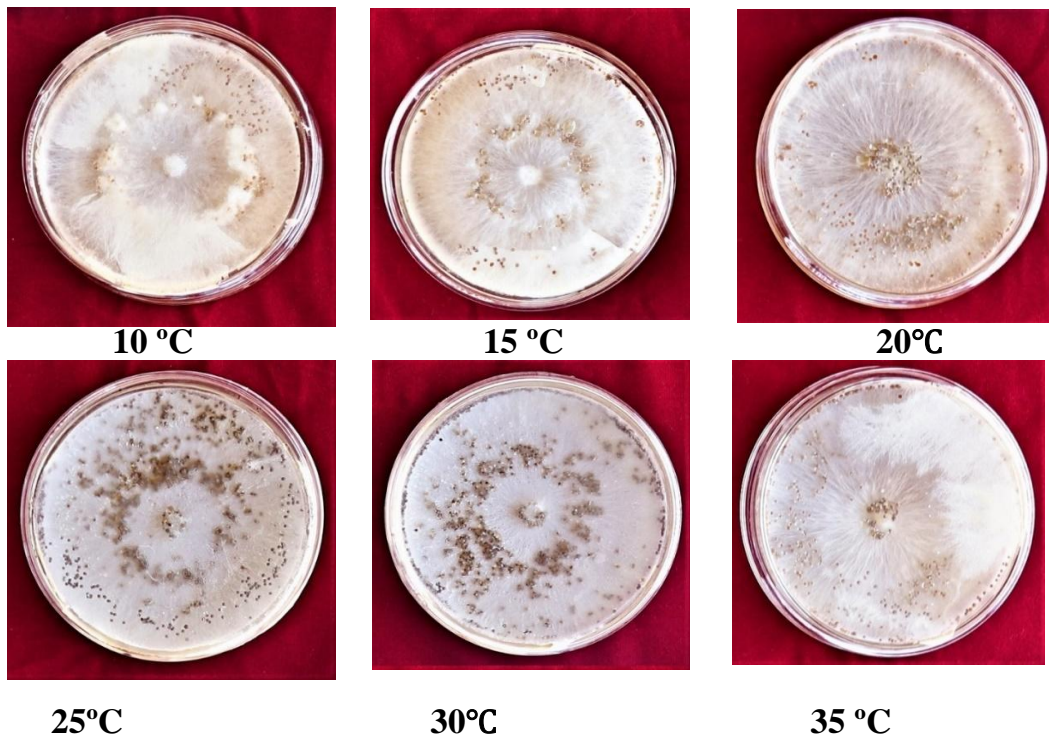


Plate.2(b) Effect of different temperature level on sclerotia formation by *S. rolfsii* (20 days after inoculation)



T₁ = pH 5.0, T₂ = pH 6.0, T₃ = pH 7.0, T₄ = pH 8.0, T₅ = pH 8.5

Plate.3(a) Effect of different pH level on mycelial growth of *S. rolfsii*(96 hrs. after inoculation)



T₁ = pH 5.0, T₂ = pH 6.0, T₃ = pH 7.0, T₄ = pH 8.0, T₅ = pH 8.5

Plate.3(b) Effect of different pH level on sclerotia formation by *S. Rolfsii* (20 days after inoculation)

Sclerotium rolfsii was grown better under pH 6.0 which depicts the preference of slightly acidic condition by fungus. Next best radial growth was observed under pH 7.0. All other pH has shown declined growth rate and it was clear that pH beyond 9.0 is detrimental to pathogen.

This concludes that alkaline condition is not suitable for pathogen development. (Bana Sravani and Ram Chandra, 2020). Current experiment was conducted to unveil the suitable pH, temperature and culture media required for the growth of pathogen. The

findings and conclusions resulted from the study are here as follows. Among the eight tested culture media, Maximum colony diameter (90.00 mm) and sclerotia count (691.00) were recorded on oat meal agar (Hi-media) at 96 hrs. and 20 days after inoculation respectively, followed by potato dextrose agar (natural) in radial mycelial growth 87.00 mm and potato dextrose agar (Hi-Media) in number of sclerotia count 665.00 per plates found to be best for providing better nutrients for pathogen growth and sclerotia formation. *Sclerotium rolfsii* was grown better under pH 7.0 which was followed by pH level 6.0,

depicts the preference of slightly acidic condition by fungus. All other pH has shown declined growth and sclerotia formation by pathogen. Vigorous fungal radial growth and sclerotia production were observed under the temperature of 25°C followed by 30°C. Temperatures above 35 °C and below 20 °C have been recorded to be unfavourable for the growth of *Sclerotium rolfsii*.

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How to cite this article:

Karan Singh, C. B. Meena, Chirag Gautam, B. K. Patidar and Koli. N. R. 2020. Nutritional and Epidemiological Requirements for Growth and Sclerotia Formation by *Sclerotium rolfsii* (Sacc.) Causing Collar Rot of Chickpea (*Cicer areitinum* L.). *Int.J.Curr.Microbiol.App.Sci*. 9(07): 645-656. doi: <https://doi.org/10.20546/ijcmas.2020.907.074>