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In vitro Effect of Physicochemical Properties on the Growth of *Sclerotium rolfsii* Sacc. Causing Collar Rot in Chickpea (*Cicer arietinum* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Author DS conceptualization and designing of the research work. Author Premlata execution of lab experiments and data collection. Author HS analysis of data and interpretation. Author ML preparation of manuscript. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Collar rot of chickpea caused by *Sclerotium rolfsii* Sacc. is an emerging threat to chickpea production particularly under stress conditions such as high temperature and low soil moisture. This study showed response of culture media, different temperatures and pH levels on the growth of *Sclerotium rolfsii* Sacc.causing collar rot in chickpea. The fungus was isolated from different locations of Satna, M.P. and was purified for further studies through tip and single sclerotium. Pure

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culture was with white cottony mycelium and brown macrosclerotia. Potato dextrose agar (PDA) was found best medium for culturing the fungus in laboratory. Optimum mycelial growth was achieved at temperature 30°C and pH 5.0-7.0 in laboratory conditions. The results showed that the fungus *Sclerotium rolfsii* Sacc. causing collar rot disease in chickpea favours thermophilic conditions in slightly acidic to neutral pH.

Keywords: Chickpea; Sclerotium rolfsii; collar rot; temperature; pH.

1. INTRODUCTION

Chickpea often referred to as the "King of Pulses" is one of the most important pulse crops in India. Chickpea (*Cicer arietinum* L.) is significant pulse crop, contributing about 27% of global production (ICAR - Indian Institute of Pulses Research) it provides 21.1% protein, 61.5% carbohydrates, 4.5% fat and also rich in calcium, iron niacin (Singh et al., 2015). In India, chickpea is extensively grown in Madhya Pradesh under rainfed conditions. However, the crop is increasingly threatened by many fungal diseases including: Ascochyta blight (*Ascochyta rabiei)*, Botrytis gray mold (*Botrytis cinerea),* Alternaria blight (*Alternaria alternata),* Colletotrichum blight (*Colletotrichum dematium),* Phoma blight (*Phoma medicaginis),* Rust (*Uromyces ciceris-arietini),* Powdery mildew (*Leveillula Taurica),* Sclerotinia stem rot (*Sclerotinia sclerotiorum),* Fusarium wilt (*Fusarium oxysporum* f. sp. *Ciceri,* Verticillium wilt (*Verticillium albo-atrum),* dry root rot (*Rhizoctonia bataticola*), Phytophthora root rot (*Phytophthora medicaginis),* Foot rot (*Operculella padwickii)* (Nene et al., 2012).

Sclerotium rolfsii Sacc. is a well-known plant pathogen having a wide host range (500 plant species) mostly comprised of dicotyledonous and few monocotyledonous plants and is one of the most destructive soil inhabiting plant pathogens which cause collar rot in different crops all over the world as well as in India (Aycock, 1966; Ciancio & Mukerji, 2007). It induces different types of symptoms like crown and root rot, collar rot, foot rot, stem rot, stem canker, damping off, southern wilt or blight or southern stem rot. The fungus was first detected by Rolfs (1893) as one of the reasons for tomato blight in Florida. Later, Saccardo (1911) named the fungus as *Sclerotium rolfsii*.

Collar rot is an important disease of chickpea causing quantitative and qualitative losses and farmers often misidentify it as Fusarium wilt, Verticillium wilt and Collar rot due to similar symptoms. The pathogen survives as macrosclerotia in soil and infects collar and roots when plants are under high soil moisture. Collar rot is prevalent in many sub-agroclimatic zones of M. P. viz., Central Narmada valley (8.08-17.20%), Kymore Plateau and Satpura hills (9.30-14.80%), Northern Hill region (8.10-11.76%), Satpura plateau (9.30-11.88%) and Vindhyan plateau (12.00-18.20%) of Madhya Pradesh (Singh et al., 2022). The objective of the present study was to find out effect of culture media, temperatures and pH levels against the growth of *Sclerotium rolfsii* causing collar rot of chickpea.

2. MATERIALS AND METHODS

2.1 Survey of the Disease

A robing survey was conducted in the Sohawal block of Satna district during the Rabi season 2024–25 to assess collar rot of chickpea. Ten fields (including field of AKS University) spaced 5–7 km apart were surveyed and disease incidence was calculated using:

Percent Disease Incidence =

$$\frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

2.2 Isolation, Identification and Purification of Fungus

Infected chickpea plants from different surveyed fields were collected and brought to the laboratory. The white fungal mycelium showing on the infected collar and roots of chickpea was inoculated separately on PDA medium in petri plate and the petri plates were incubated at 27 ± 2°C in BOD incubator for 5 days. Pure cultures were obtained by using the hyphal tip as well as single sclerotium methods on the same medium. The fungus was identified morphologically on the basis of following characters- a) color of hyphae, b) color and morphology of colony, c) septation and location of septa d) Type and structure of sclerotia and e) color of sclerotia.

2.3 Selection of Suitable Culture Medium

Three culture media, viz., corn meal agar, Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) were used to select one for fast growth of the fungus. These culture media were prepared and sterilized as per the standard protocol and poured in 90mm polyethylene petri plates in laminar air flow. Three petri plates (R_1, R_2 and R_3) with culture medium were inoculated with a single sclerotium each while two petri plates (R_4 and R_5) with culture medium were inoculated with discs of 5mm diameter cut from fresh culture of *Sclerotium rolfsii.* Total five replicates of each culture media were maintained. All inoculated petri plates were incubated in a BOD incubator at 27 ± 2°C for 7 days. Radial growth of the test fungus and sclerotial formation were observed at 3, 5 and 7 Days After Inoculation (DAI). The data were tabulated to determine the best medium for fungal growth.

2.4 Effect of Temperatures on Mycelial Growth

Three replicates (R_1, R_2 and R_3) with PDA culture medium were inoculated with a single sclerotium each from more than 7 days old culture while two replicates (R_4 and R_5) were inoculated with discs of 5mm diameter cut from 3-5 days old culture of *Sclerotium rolfsii.* Total five replicates of each temperature level were maintained. Inoculated petri plates (each five replicates) were incubated at six different temperatures: 10°C, 15°C, 20°C, 25°C, 30°C, and 35°C up to 7 DAI. Two-dimensional average radial growth was measured at 3, 5, and 7 days after inoculation (DAI) and tabulated for statistical analysis. When maximum radial fungal growth was found from any tested temperatures, two lower and two upper ranges of temperatures of maximum growth temperature were also tried to find out the optimum temperature. The experiment was arranged in a Completely Randomized Design (CRD) with five replications.

2.5 Effect of pH levels on Mycelial Growth

Suitable culture medium (Potato dextrose agar) was prepared and divided into eight separate parts (according to pH levels) in conical flasks. Each amount of PDA was adjusted to pH level of 5.0 to 8.5 using 0.1N NaOH or HCl before autoclaving. Cultures medium adjusted to different pH (5-8.5) was poured in petri plates (5 petri plates/pH level having 20 ml/petri plate). Single sclerotium/petri plates (replicates R_1, R_2 and R_3) were placed (inoculated) at the centre of petri plates (culture medium) and two petri plates (R_4 and R_5) were inoculated with discs of 0.5mm diameter cut from fresh culture. Inoculated petri plates were incubated at optimum temperature 30°C ± 2°C for 7 days and radial growth was measured at 3, 5, and 7 DAI. Fungal growth data were tabulated for statistical analysis.

3. RESULTS AND DISCUSSION

3.1 Survey of the Disease

Dried foliage of young seedlings with rotting of collar region and roots (Fig. 1A) of chickpea plants were found during survey. Young seedlings collapsed (Fig. 1B) due to weak stem but older seedlings dried up without collapsing. The whole plant appeared chlorotic (Fig. 1C) in early stage of infection A white mycelium was also seen on the tap root of completely dried seedlings. When infected plants were uprooted during robing survey in Rabi season of 2024–25 in AKS University fields and surrounding villages of Satna M.P., the entire taproots found with ruptured and dried epidermis (Fig. 1A).

Fig. 1. Symptoms of the disease; collar & roots of infected plants (A), dried and collapsed seedling (B), infected young plant (C)

3.2 Isolation and Identification of the Fungus (*Sclerotium rolfsii*)

Collar rot causing fungus (*Sclerotium rolfsii*) isolated from infected plants on PDA. The isolates grown on PDA medium exhibited the highest colony growth achieving a diameter of 90 mm, within 5 days. The colony colour of *Sclerotium rolfsii* appeared cottony white with dense radiating mycelial growth (Fig. 2A) and it developed numerous brown to dark brown macro-sclerotia dispersed throughout the mycelial mat (Fig. 2B). They were initially white turning brown to dark black upon maturity (Fig. 2C). The sclerotia of *Sclerotium rolfsii* were hard, round to oval in shaped and compact in texture. They were typically dry and brittle when matured and the surface of mature sclerotia was rough to slightly wrinkled. The hyphal cells of *Sclerotium rolfsii* were septate and hyaline. Further study for identification up to the group of the isolated fungus *(Sclerotium rolfsii)* is going on.

3.3 Selection of Suitable Culture Medium

The results presented in Table 1 and Fig. 3 clearly indicated that PDA and CMA both supported higher radial growth of the fungus as compared to SDA. The radial growth of fungus (*Sclerotium rolfsii*) was found very sparse on SDA and sclerotia were also sparsely scattered while on CMA radial growth was less sparce and sclerotia were more in number as compared to SDA (Fig. 3). At 7 DAI fungal colony growth (8.50 cm) was equal on PDA and CMA but at 5 DAI Fungal radial growth was found higher (5.23 cm) on CMA and lower (4.37 cm) PDA (Table 1). Since, fungus growth and sclerotia were very dense on PDA therefore, PDA was more suitable than other two (SDA & CMA) culture media.

3.4 Effect of Temperature on Growth

There was no fungal (*Sclerotium rolfsii*) growth at 10°C (Fig. 5A). Maximum fungal growth (9.00 cm at 7 DAI) was recorded at 30°C (Fig. 5E) followed by 25°C, 20°C, 15°C and 35°C at 5 and 7 DAI (Table 2). Significant differences in growth were observed across all tested temperatures (Table 2 & Fig. 4). When two lower temperatures (28 & 29°C) and two upper temperatures (31 & 32°C) of 30°C were tested, optimum temperature for collar rot causing fungus (*Sclerotium rolfsii*) in chickpea was found 30°C (Table 3 & Fig. 4). The temperature effect on the growth of *Sclerotium rolfsii* was found significant as shown in Table 2 and Table 3.

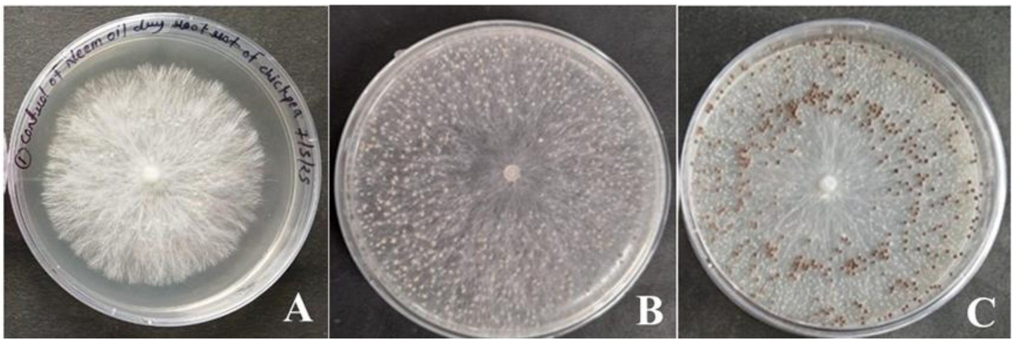

Fig. 2. *Sclerotium rolfsii* fungus; growth of mycelium (A), early stage of sclerotia (B), types of sclerotia (C)

Table 1. Growth of *Sclerotium rolfsii* on different culture media

Treatment	Mean Radial growth (cm)		
	3 DAI	5 DAI	7 DAI
PDA	0.21	4.37	8.50
SDA	0.00	1.07	3.48
Corn meal agar	0.23	5.23	8.50
SEm±	0.03	0.22	0.12
C.D. (P =.01)	0.12	0.95	0.51
C.V. (%)	46.15	14.10	4.17
F- Tab	(6.92)	(6.92)	(6.92)
F- Cal	17.709	95.844	517.992

SEm+ = Standard Error of Mean (plus/minus), C.D. (P =.01) = Critical Difference at Probability Level = 0.01, C.V. (%) = Coefficient of Variation (in percent), F – Tab = Fisher's Tabulated Value, F-Cal = Fisher's calculated value

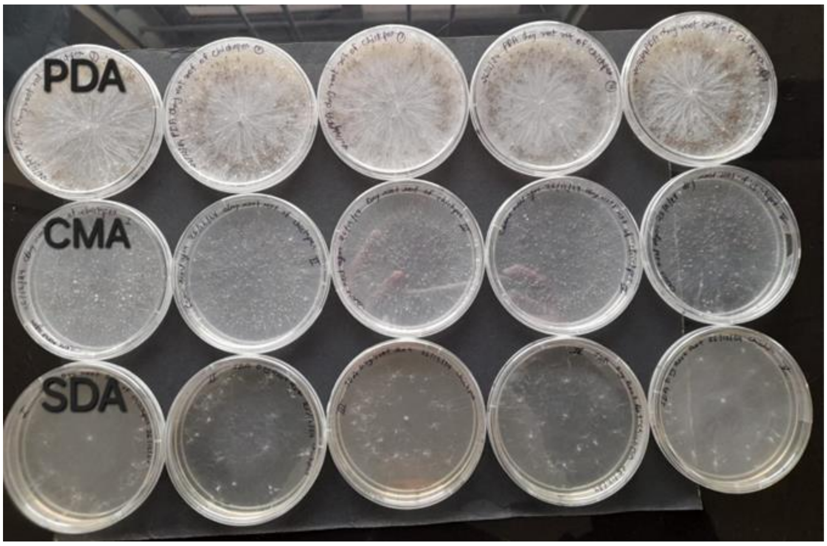

Fig. 3. Growth of *Sclerotium rolfsii* on different culture media at 7 DAI

Table 2. Effect of Different Temperatures on the radial growth of *Sclerotium rolfsii*

Treatment (Temperatures in °C)	Mean radial growth (cm)		
	3 DAI	**5 DAI**	**7 DAI**
10°C	0.00	0.00	0.00
15°C	0.00	1.47	4.31
20°C	1.00	4.47	8.32
25°C	1.28	6.82	8.53
30°C	5.18	8.52	9.00
35°C	0.23	0.80	3.63
SEm±	0.45	0.30	0.20
C.D (P =.01)	1.77	1.18	0.79
C.V. (%)	79.90	18.16	7.94
F- Tab	(3.89)	(3.89)	(3.89)
F- Cal	18.747	129.149	322.139

SEm+ = Standard Error of Mean (plus/minus), C.D. (P =.01) = Critical Difference at Probability Level = 0.01, C.V. (%) = Coefficient of Variation (in percent), F – Tab = Fisher's Tabulated Value, F-Cal = Fisher's calculated value

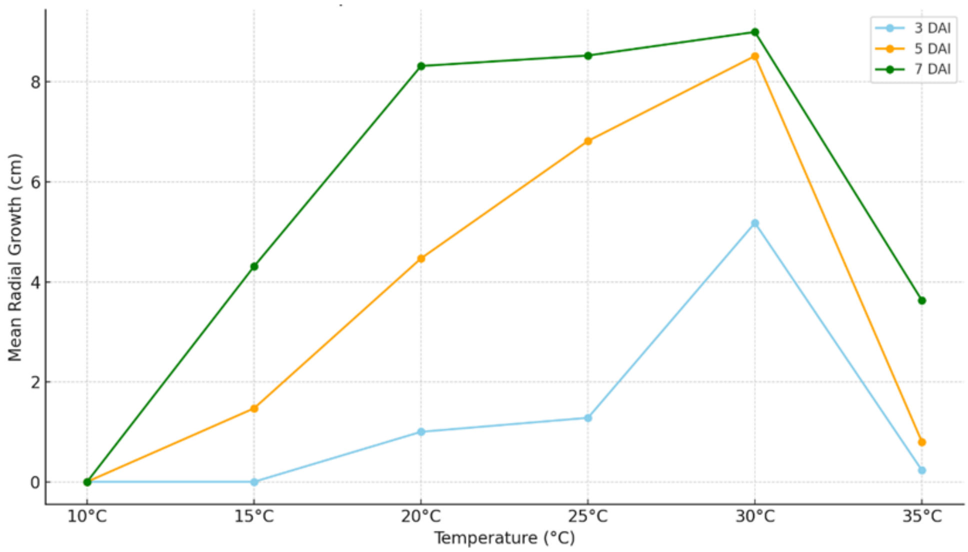

Fig. 4. Effect of different temperatures on radial growth of *Sclerotium rolfsii*

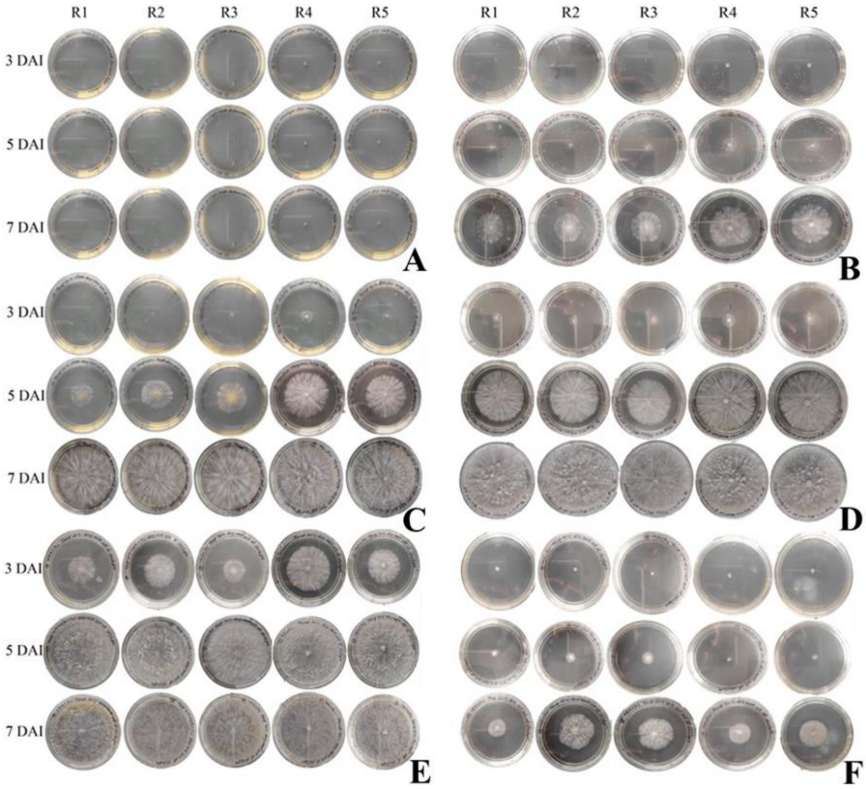

Fig. 5. Effect of temperature; effect of 10^0C (A), effect of 15^0C (B), effect of 20^0C (C), effect of 25^0C (D), effect of 30^0C (E) and effect of 35^0C (F) on radial growth of *Sclerotium rolfsii*

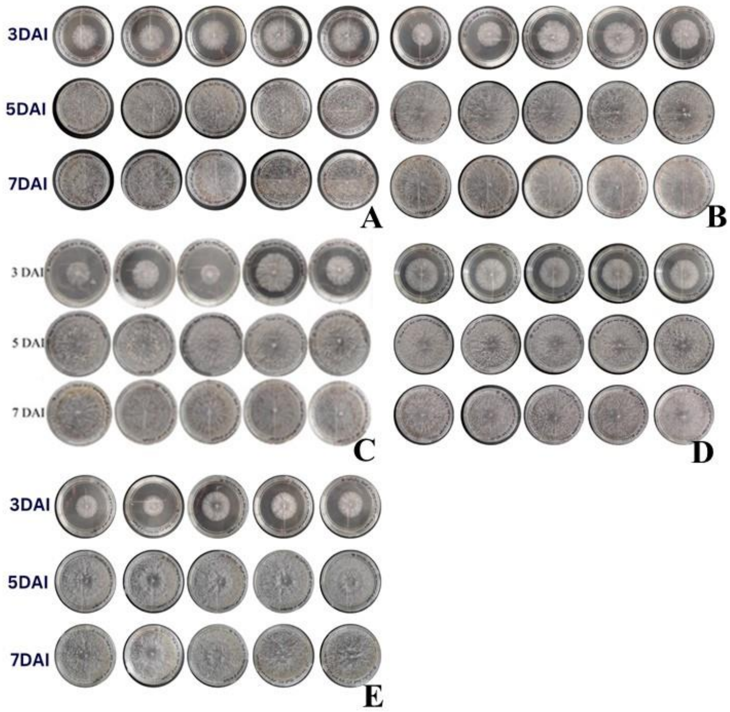

Fig. 6. Optimum temperature for the growth of *Sclerotium rolfsii*; at 28^0C (A), at 29^0C (B), at 30^0C (C), at 31^0C (D) and at 32^0C (E)

Table 3. Optimum temperature for growth of *Sclerotium rolfsii*

Treatment (Temperatures)	Mean radial growth (cm)		
	3 DAI	**5 DAI**	**7 DAI**
28°C	4.19	6.16	8.26
29°C	4.29	7.42	8.36
30°C	5.18	8.52	9.00
31°C	4.33	7.48	8.42
32°C	5.20	7.84	8.62
SEm±	0.47	0.27	0.04
C.D. (P =.01)	N/A	0.80	0.13
C.V. (%)	23.06	8.11	1.16
F-Tab.	(4.43)	(4.43)	(4.43)
F- Cal	1.121	10.278	42.394

SEm+ = Standard Error of Mean (plus/minus), C.D. (P =.01) = Critical Difference at Probability Level = 0.01, C.V. (%) = Coefficient of Variation (in percent), F – Tab = Fisher's Tabulated Value, F-Cal = Fisher's calculated value

Table 4. Effect of Different pH levels on the radial growth of *Sclerotium rolfsii*

Treatment (pH levels)	Mean radial growth (in cm)		
	3DAI	**5DAI**	**7DAI**
5.0	3.83	8.32	8.90
5.5	4.19	8.34	8.99
6.0	4.46	8.43	8.97
6.5	3.91	8.48	8.98
7.0	3.99	8.47	8.99
7.5	2.05	5.84	7.17
8.0	3.50	8.48	8.91
8.5	1.35	3.43	4.76
SEm±	0.56	0.66	0.78
C.D. (P =.01)	2.16	2.55	3.02
C.V. (%)	36.90	19.79	21.23
F-Tab	3.25	3.25	3.25
F- Cal	3.872	7.970	3.840

SEm+ = Standard Error of Mean (plus/minus), C.D. (P =.01) = Critical Difference at Probability Level = 0.01, C.V. (%) = Coefficient of Variation (in percent), F – Tab = Fisher's Tabulated Value, F-Cal = Fisher's calculated value

3.5 Effect of pH on Growth of *Sclerotium rolfsii*

The optimal pH for fungal (*Sclerotium rolfsii*) growth was found 5.0-7.0 with a minor difference in all tested pH levels except pH 7.5 (Table 4 & Fig. 7). Replications (R_1, R_2 and R_3) were inoculated with single sclerotium and replications (R_4 and R_5) were inoculated with mycelium disk therefore, in all pH levels radial growth through sclerotium was slow while radial growth through mycelium disk was fast (Fig. 7). In pH level 7.5 fungal growth in replication R_4 was normal (Fig. 7F) as in other lower pH levels (5.0-7.0) but when mean value of radial fungal growth of all replications in pH level 7.5 was calculated then it was found low (2.05, 5.84 and 7.17cm at 3, 5 and 7 DAI respectively) as compared to other pH levels (Table 4). Statistical analysis confirmed that there was no significant effect on radial growth of *Sclerotium rolfsii* causing collar rot of chickpea at all tested pH levels.

The robing survey conducted in some villages including AKS University field of Sohawal block, Satna, Madhya Pradesh during the Rabi season 2024–25 revealed the widespread occurrence of collar rot disease caused by *Sclerotium rolfsii*. During the survey, infected seedlings and young plants of chickpea exhibited dried leaves with rotten collar region and root epidermis (Fig. 1A, 1B & 1C). Lateral roots were also absent in most infected plants of chickpea. Such types of symptoms were also observed by Nene *et al*. (2012). Potato dextrose agar (PDA) and corn meal agar (CMA) media supported vigorous mycelial growth of *Sclerotium rolfsii* but PDA was found to be more suitable for routine growth and morphological studies during isolation and purification of the test fungus (Fig. 3). The rapid growth on PDA with a colony diameter of 90 mm (Table 1) within 5 days aligns with findings by Divyashree *et.al.* (2024) and Srividya *et.al.* (2018; 2022). Vigorous mycelial growth on PDA with colonies reaching full diameter (90mm) within 7 days Sravani and Chandra (2020). The high sclerotial density observed particularly in isolates from Sohawal block indicates a higher sporulation capacity and pathogenic potential. Srividya *et.al.* (2022) also reported a positive correlation between sclerotial density and growth.

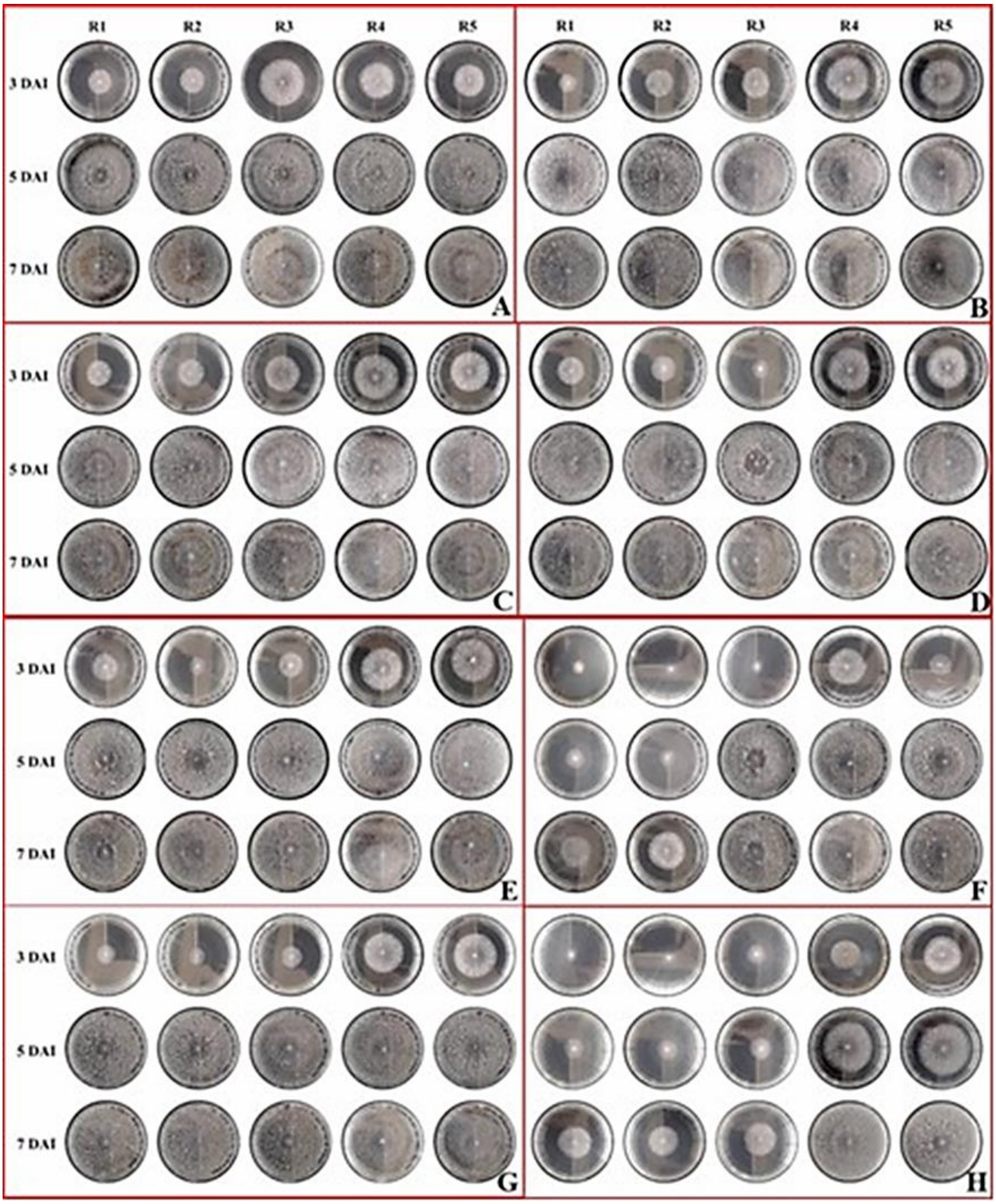

Fig. 7. Effect of pH levels on the radial growth of *Sclerotium rolfsii;* growth on pH 5.0 (A), growth on pH 5.5 (B), growth on pH 6.0 (C), growth on pH 6.5 (D), growth on pH 7.0 (E), growth on pH 7.5 (F), growth on pH 8.0 (G), and growth on pH 8.5 (H)

Temperature plays a critical role in influencing the mycelial growth of *Sclerotium rolfsii*, the causal agent of collar rot in chickpea. The results clearly showed that among tested temperatures (10°C to 35°C with 5°C difference) 30°C was the most favorable (Fig. 5E) temperature for mycelial development yielding the maximum radial growth (9.00 cm) at 7 DAI followed by 25°C, 20°C, 15°C and 35°C (Table 2). These findings emphasize the thermophilic nature of *Sclerotium rolfsii* and its preference for warm conditions. Again, when experiment was laid down to find out optimum temperature the fungus exhibited maximum mycelial growth at 30°C (Table 3). These findings strongly suggested that 30°C is the optimum temperature for *In vitro* growth of *Sclerotium rolfsii* under the conditions of the present study (Fig. 4). The present findings are supported by those of Sravani and Chandra (2020) who reported that *Sclerotium rolfsii* exhibits vigorous mycelial growth (9cm) within 5DAI at 30°C temperature followed by 25℃, 20℃, 15℃ and least at 35℃. The present study revealed that pH has not significant effect on the radial growth of *Sclerotium rolfsii*, the collar rot pathogen of chickpea. Among the tested treatments, the fungus exhibited maximum growth (with minor difference) at pH 5.0 to pH 7.0 (Table 4, Fig. 7). These results suggested that moderately acidic to neutral pH conditions (pH 5.0–7.0) are most favorable for the mycelial proliferation of *Sclerotium rolfsii* under *In vitro* conditions. This is suggested that *Sclerotium rolfsii* prefers a slightly acidic to neutral soil reaction and extreme alkaline conditions may suppress its metabolic activities.

Maximum mycelial growth of *S. rolfsii* was also found at pH 6.0 followed by 5.0, 7.0 and 8.0 pH (Sravani & Chandra, 2020). Maximum colony diameter (89 mm) of *S. rolfsii* causing collar rot in chickpea was also recorded at pH level 7.0 (Singh et al., 2020). Optimal mycelial growth of three Tunisian isolates (Sr1, Sr2 & Sr3) of *Sclerotium rolfsii* was also occurred at pH 6 -7 (Ayed et al., 2018).

4. CONCLUSION

Sclerotium rolfsii thrives best at 30°C and pH 5.5-7.0 under *In vitro* conditions. These findings suggested that warm and moderately acidic environments are conducive to the development of collar rot in chickpea. The virulence of collar rot of chickpea is correlated with soil pH and temperature (soil & atmospheric temperature) Because most of farmers know that wilt is the common disease of chickpea which has more or less same symptoms. Though collar rot and wilt of chickpea both produce identical symptoms which can be diagnosed by a experienced person/farmer but most of farmers are not able to diagnose both diseases exactly on the basis of symptoms. Therefore, the present study may be helpful for diagnosis and management of collar rot of chickpea. These findings can also assist in developing targeted management strategies against collar rot in chickpea.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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