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Influence of media, pH and temperature on the growth of *Sclerotium rolfsii* (Sacc.) causing collar rot of chickpea

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Abstract
Sclerotium rolfsii is the most devastating pathogen causing collar rot of chickpea especially in tropical and sub-tropical countries where high temperatures prevail during monsoons. An attempt was made to study the best suitable medium and physiological factors such as temperature and pH on the growth of pathogen. Among the six different media tested, Potato Dextrose Agar (PDA) and Yeast Potato Dextrose Agar (YPDA) medium were found to be highly supportive for the pathogen growth resulting full growth (9cm) within 7 days and least growth was observed with Paddy Husk medium (6.33cm). Among different pH studied, maximum growth was reported at pH 6 followed by 7, 8, 5 and 8.Fungal growth was found to be vigorous at 30 ℃ temperature where radial growth was observed to be maximum (9cm) within 5DAI followed by 25 ℃, 20 ℃, 15 ℃ and least growth at 35 ℃.

Keywords: *Sclerotium rolfsii*, collar rot, media, temperature, pH

Introduction
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) [6]. Present experiment deals with study of *Sclerotium rolfsii* growth under different culture media, at various temperatures and pH. To carry out the research, six distinctive culture media *viz*. Potato Dextrose Agar (PDA) Medium, Yeast Potato Dextrose Agar (YPDA) Medium, Malt Extract Agar Medium, Paddy Husk Medium, Garden Pea Extract Medium and Chickpea Pod Extract Medium were used to evaluate the growth of pathogen. Later suitable temperature and pH were also analyzed for the pathogen growth by incubating at different pH *viz*. 5.0, 6.0, 7.0, 8.0 and 9.0 and temperatures *viz*. 15 °C, 20 °C, 25 °C, 30 °C and 35 °C.

Materials and Methods
Isolation of pathogen
The root samples showing typical symptoms were collected from Agriculture Farm, BHU and packed in polythene bags and sealed. They were bought to laboratory for isolation of pathogen. Collected disease roots were first sterilized with ethyl alcohol using cotton swab. Later they were cut into small pieces of 3 mm^2 size by using sterile scalpel. They are surface sterilized by dipping in 0.1 per cent mercuric chloride solution for 30 sec. Then immediately rinse them in three changes of sterilized distilled water to remove the traces of mercuric chloride. Allow to air dry it by placing on sterilized filter paper and then transfer them on to PDA plated petri dishes using forceps. Inoculated plates were incubated in B.O.D incubator at 28 ± 2 °C by providing favorable conditions for growth of pathogen. Cultures were purified by using hyphal tip method. It was done by picking up pure hyphal structure by using low power of the microscope and carefully transferring to fresh PDA petri dish and maintained at 25 ± 2 °C for 10 days. After purifying the infected fungus, their morphological and cultural characters such as color, size, growth rate, type of mycelium were recorded under microscope for their identity. By comparing with available standard literature, pathogen was identified as *Sclerotium rolfsii* (Barnett and Hunter, 1972) [5].

Preparation of Culture Media
Different culture media namely Potato Dextrose Agar (PDA) Medium, Yeast Potato Dextrose Agar (YPDA) Medium, Malt Extract Agar Medium, Paddy Husk Medium, Garden Pea Extract Medium and Chickpea Pod Extract Medium were prepared to test the pathogen mycelial

growth and sclerotial production. Each sterilized petri plate is poured with twenty ml of sterilized medium. 5mm discs were cut from seven days old fungal culture grown in petri plates by using sterilized cork borer and incubated at 25 ± 2 ℃ for 7-10 days. Later observe the growth rate, colony color, mycelia growth after incubation. Three replications of each treatment were maintained.

Effect of pH on the growth of *Sclerotium rolfsii*

In vitro experiment was conducted to know the effect of different pH ranging from 4, 5, 6, 7, 8 and 9 on the growth rate of pathogen. Prepare the PDA media and measure its pH before sterilization by using digital pH meter. Adjust the pH by addition of 0.1N HCL and 0.1N NaOH. Later sterilize the medium and pour twenty ml of PDA on to each petri plate. Petri plates were inoculated with discs of *Sclerotium rolfsii* culture aseptically and were incubated at 27 ± 2 °C. Each treatment is maintained with three replications and observations of mean radial growth after 3, 5 and 7 days interval were recorded.

Effect of temperature on the growth of *Sclerotium rolfsii*

This experiment was conducted to find out the optimum temperature required for the growth of pathogen. Petri plates were poured with PDA (Potato Dextrose Agar) and inoculate them by placing 5 mm mycelial disc of four day old culture and allow for incubation at various temperatures of 15 °C, 20 °C, 25 °C, 30 °C and 35 °C. Maintain three replications for each treatment and measure mean radial growth after inoculation.

Results and discussion

Suitability of different culture media

In this investigation, six different culture media viz. Potato Dextrose Agar (PDA) Medium, Yeast Potato Dextrose Agar (YPDA) Medium, Malt Extract Agar Medium, Paddy Husk Medium, Garden Pea Extract Medium and Chickpea Pod Extract Medium were evaluated for the suitability of culture medium for fungal growth.

The final growth developed over different types of culture media is displayed in Table 1. It was observed that Potato Dextrose Agar (PDA) Medium and Yeast Potato Dextrose Agar (YPDA) Medium was greatly supported the development of pathogen by forming 9.0 cm radial growth within 7 DAI. Malt Extract Agar Medium was also proved to be best media after PDA and YPDA followed by Chickpea Pod Extract Medium. Least supported media was Paddy Husk Medium with only 6.33 cm radial growth.

These suitable culture media results were found to be appropriate with the results of Mishra *et al.* (1996) [7], Punja and Damini (1996) [8], Dey *et al.* (1992) [2] and many other scientists where they evaluated various culture media and found that PDA produced maximum dry weight along with maximum mycelial growth.

Table 1: Evaluation of different culture media on mycelial growth of *Sclerotium rolfsii*

S. No.	Culture Media	Radial growth (cm) at different interval			
		1DAI	3DAI	5DAI	7DAI
1	Potato Dextrose Agar Medium	2.00	4.73	7.90	9.00
2	YPDA (Yeast Potato Dextrose Agar Medium)	1.80	4.70	7.83	9.00
3	Malt Extract Agar Medium	1.63	4.43	7.33	8.73
4	Paddy Husk Medium	0.00	2.03	3.80	6.33
5	Garden pea Leaf Extract Medium	0.00	3.03	5.63	8.13
6	Chickpea Pod Extract Medium	0.00	3.80	6.63	8.47
SEm ±		0.31	0.92	1.03	0.54
CD at 5%		0.98	2.84	3.19	1.67

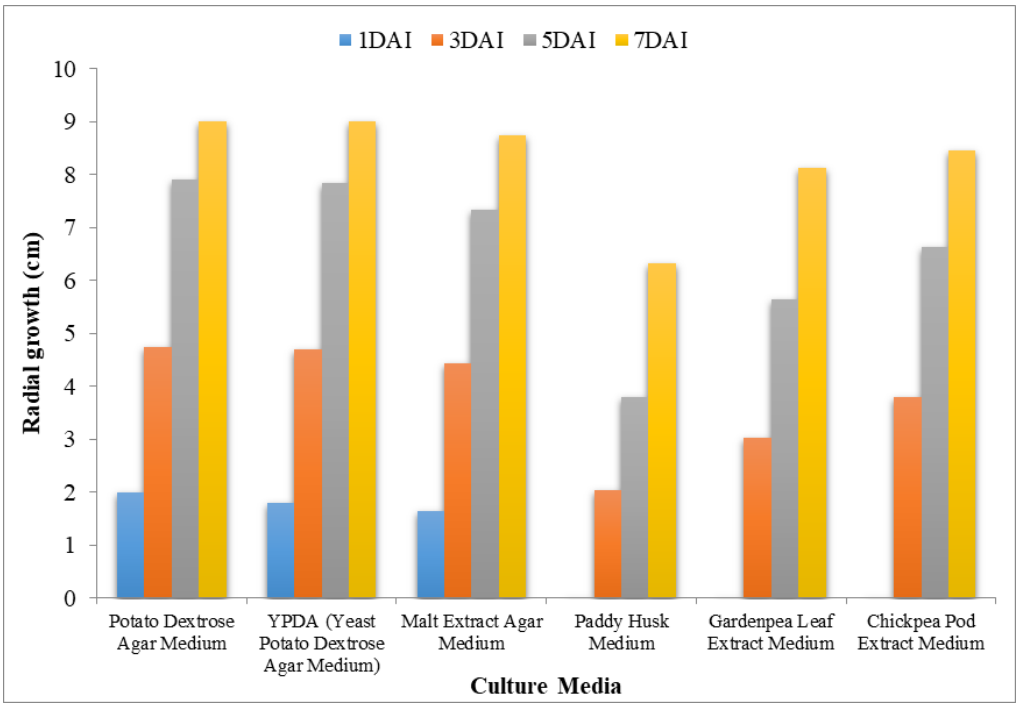

Fig 1: Evaluation of different culture media on mycelial growth of *Sclerotium rolfsii*

Mycelial growth at different pH

After evaluating suitable media for pathogen, study was conducted to find suitable pH for pathogen. Five different pH were analyzed to figure out the suitable pH for appropriate growth of the pathogen. *Sclerotium rolfsii* was allowed to grow under various pH viz. 5.0, 6.0, 7.0, 8.0 and 9.0 for its radial growth observation.

Table 2 depicts the variation in mycelial growth at various tested pH range. Maximum mycelial growth was recorded at pH 6.0 with 9.00 cm radial growth of mycelium developing within 7 days compared to other pH range. This result was followed by pH 7.0 and pH 8.0 with 8.70 and 8.37 cm radial growth respectively at 7 days after inoculation. Even pH 5.0 was less suitable when compared to above pH range and least growth was recorded at pH 9.0 with only 6.80 cm radial growth at 7 DAI. This shows that pathogen requires slightly acidic condition for their growth and sclerotial formation.

The obtained results were similar to the findings of Kulkarni and Kulkarni (1998) [4]. They have observed the growth of pathogen at various pH levels and obtained maximum dry weight of pathogen when cultured at pH 6.0. Zape *et al.* (2013) [10] and Kumar *et al.* (2008) [5] also reported that maximum growth obtained at pH 6.5 followed by pH 7.0.

Table 2: Effect of different pH on mycelial growth of *Sclerotium rolfsii*

S. No.	pH	Radial growth (cm) at different interval			
		1DAI	3DAI	5DAI	7DAI
1	5	1.90	3.97	6.43	8.27
2	6	2.00	4.73	7.90	9.00
3	7	2.07	4.47	7.20	8.70
4	8	1.90	4.37	7.00	8.37
5	9	1.87	3.87	5.87	6.80
SEm ±		0.84	0.91	0.71	1.14
CD at 5%		2.65	2.89	2.25	3.60

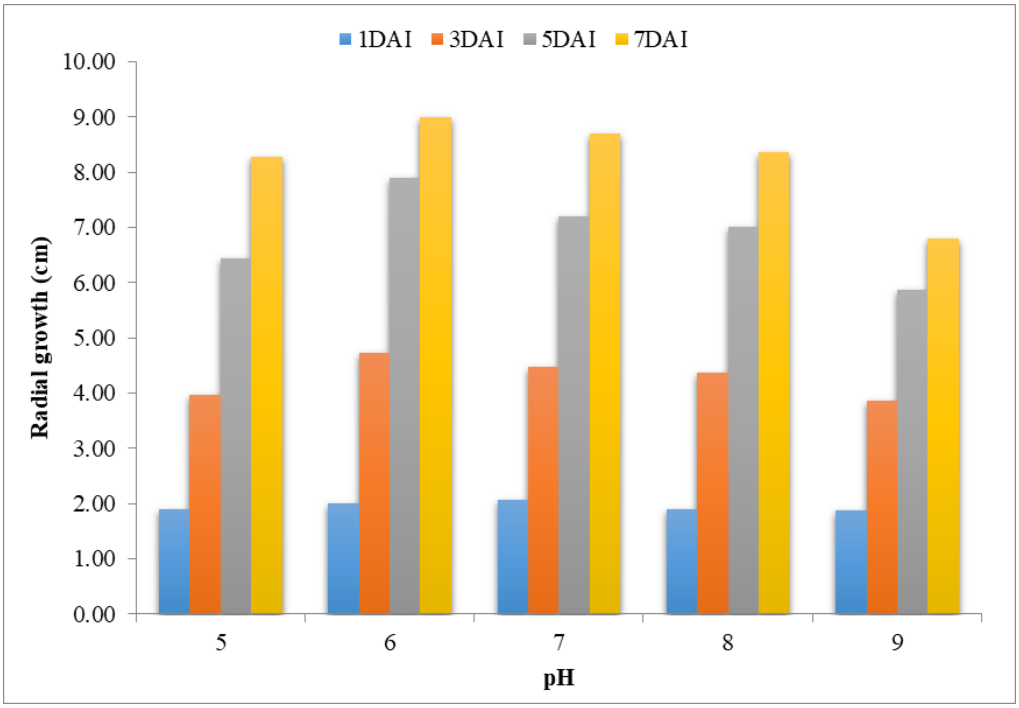

Fig 2: Effect of different pH on mycelial growth of *Sclerotium rolfsii*

Mycelial growth at different temperature

After observing pH 6.0 as best suited pH for maximum mycelial growth of *Sclerotium rolfsii*, suitable temperature was also analyzed for the pathogen growth. *Sclerotium rolfsii* was incubated at different temperatures viz. 15 °C, 20 °C, 25 °C, 30 °C and 35 °C for the observation of mycelial growth.

The effects of above temperatures are shown in Table 3. Among them, suitable temperature was found to be 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.

The results obtained clearly depicts that optimum temperature required for the pathogen growth is 30 °C. Above 35 °C and below 15 °C temperature was found to be detrimental to pathogen development. The findings of this experiment is found to be matched with reports of Mishra *et al.* (1996) [7] which concluded that maximum growth of pathogen occurred at 30 °C. Kanzaria and Patel (1995) [3] and Singh and Gandhi (1991) [9] also reported that optimum temperature for the growth of *Sclerotium rolfsii* is 30 ± 0.5 °C.

Table 3: Effect of different Temperature on mycelial growth of *Sclerotium rolfsii*

S. No.	Temperature	Radial growth (cm) at different interval			
		1DAI	3DAI	5DAI	7DAI
1	15 °C	0.00	2.40	4.10	5.60
2	20 °C	1.13	4.27	5.50	7.43
3	25 °C	1.63	6.23	8.50	9.00
4	30 °C	1.73	6.47	9.00	9.00
5	35 °C	1.27	4.30	4.77	5.13
SEm ±		0.65	1.00	0.47	0.63
CD at 5%		2.05	3.19	1.48	1.99

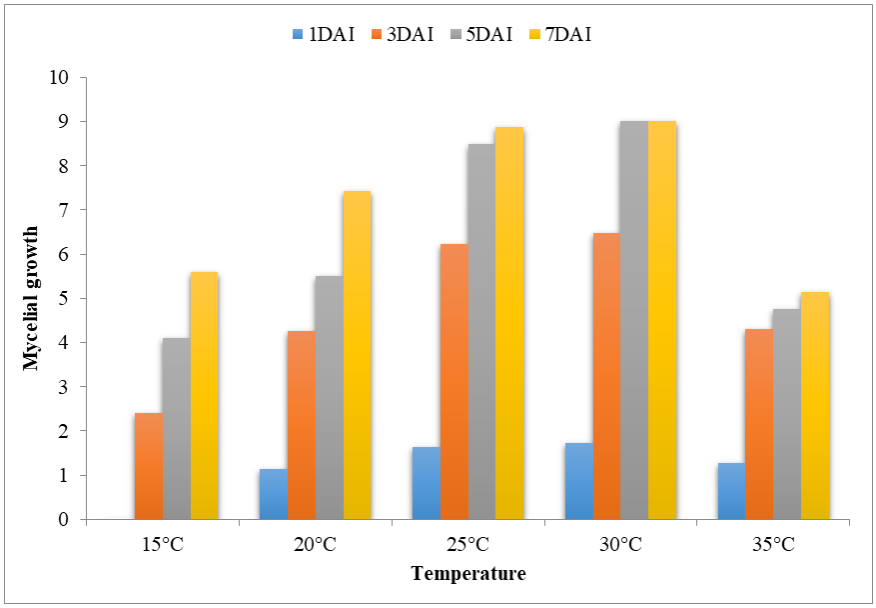

Fig 3: Effect of different Temperature on mycelial growth of *Sclerotium rolfsii*

Plate 1: Mycelial growth of *Sclerotium rolfsii* on different culture media

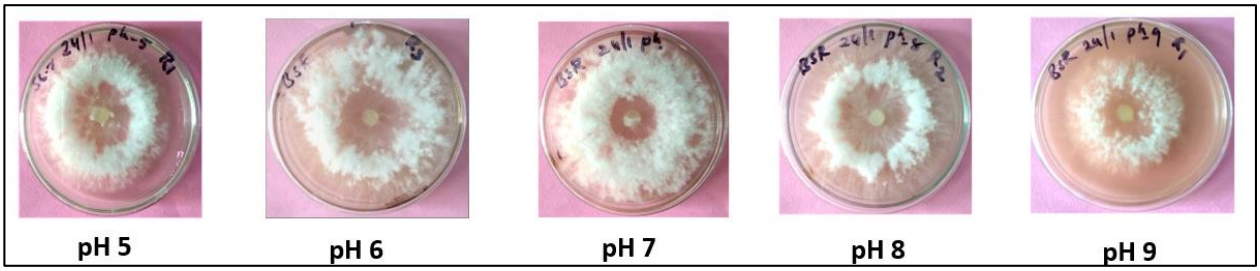

Plate 2: Mycelial growth of *Sclerotium rolfsii* at different pH

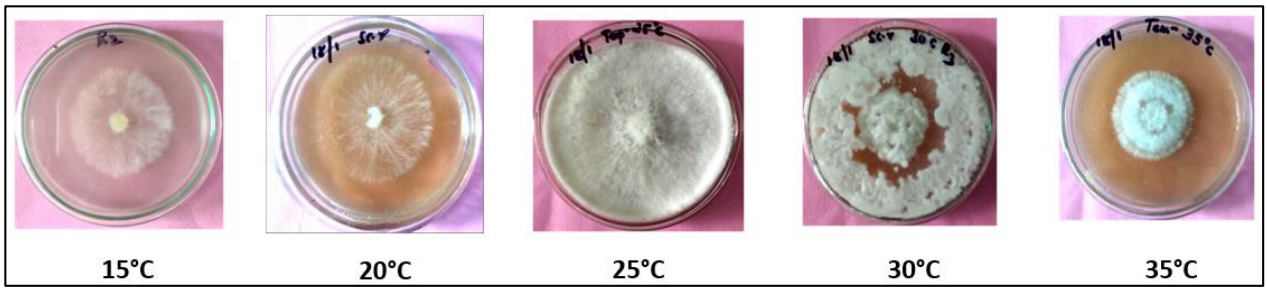

Plate 3: Mycelial growth of *Sclerotium rolfsii* at different temperature

Summary and conclusion

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 six tested culture media, Potato Dextrose Agar (PDA) medium and Yeast Potato Dextrose Agar (YPDA) medium was found to be best for providing better nutrients for pathogen growth. Malt Extract Agar Medium has also shown more or less similar results to PDA medium whereas Paddy Husk Medium was found to be least provider of suitable nutrients for pathogen growth. Garden pea Leaf Extract Medium and Chickpea Pod Extract Medium have also observed to be unsupportive for fungal growth. *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 have 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. Vigorous fungal radial growth was observed under the temperature of 30 °C followed by 25 °C where maximum growth rate was recorded. Temperatures above 35 °C and below 20 °C have been recorded to be unfavorable for the growth of *Sclerotium rolfsii*.

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