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Research Article

FACTORS AFFECTING THE GROWTH AND SCLEROTIAL PRODUCTION IN *SCLEROTIUM ROLFSII* CAUSING FOOT ROT OF BRINJAL

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ABSTRACT
Brinjal (*Solonum melongena* Linn.) crop has been observed to suffer due to serious disease foot rot of stem caused by *Sclerotium rolfsii* Sacc. The test pathogen was studied in comparison with influence of culture media, temperature and effect of different hydrogen ion concentrations on mycelial growth followed by its sclerotia production. Comparative results were recorded for most suitable culture medium, optimal temperature and the optimal hydrogen ion concentration for *Sclerotium rolfsii*. Potato-dextrose medium was found to be more suitable for mycelial growth and sclerotia production, 30^0C temperature was proved to be the best and optimum for maximum mycelial growth of *Sclerotium rolfsii*, while the excellent degree of sclerotia production was observed at 30 and 35^0C temperature. The pH 5.0 was optimum for mycelial growth while pH 4.0 to 7.0 were found to be most favourable for the production of sclerotia.

Key Words: *Sclerotium Rolfsii, Culture Media, Temperature, pH*

INTRODUCTION

Sclerotium rolfsii Sacc. is a soil born plant pathogen of world wide importance with a very extensive host range including more than 500 plants species. Most *Sclerotium rolfsii* diseases have been reported on dicotyledonous host, but with several monocotyledonous species also being infected. *Sclerotium rolfsii* is especially severe on legums, solanaceous crops, cucurbits and other vegetables grown in rotation with beans (Tu, 1978; Wydra, 1996; chaurasia, 2000). This pathogen is one of the most destructive and common pathogens of brinjal crop causing foot rot disease in fields of Tikamgarh district of madhya pradesh. Due to this disease, the local brinjal growers suffered a lot every year.

It is well known that the fungal pathogen attacks the host plant for their nutritional requirement. The nutritional requirement of various fungi differs and there is no one medium which can be universally suited for the growth of all fungi. There fore in laboratory for the experimental work first of all the pathogen is successfully grown on suitable medium and selection of suitable medium is very necessary.

The environmental factors such as incubation period, temperature, pH etc. not only influence the development and spread of fungal disease but also greatly influences the growth and sporulation of pathoges. (Mishra and Hoque, 1962; Mathur and Sorbhoy, 1976; Sharma and Kaushal, 1979; Singh and Sundhu, 1982; Khan and Quazi, 2010)

On this background the present work was undertaken to study the influence of culture media, temperature and hydrogen ion concentration on the mycelial growth and sclerotia production of *sclerotium rolfsii* Sacc.

MATERIALS AND METHODS

Sclerotium rolfsii Sacc. was isolated from diseased brinjal plant, identified and maintained as described earlier (Chaurasia, 2000).

Culture Media

The various media of composition (g/L) shown below were tested.

Asthana and Hawker's:

Glucose 5, KNO_3 3.5, KH_2PO_4 1.75, $MgSO_4$. $7H_2O$ 0.75, Distilled Water to 1 L.

Basal mucor:

Dextrose 10, Asparagine 2, KH_2PO_4 0.5, $MgSO_4.7H_2O$ 0.25, Thiamine chloride 0.5, Distilled Water to 1 L.

Brown's:

$MgSO_4.7H_2O$ 0.75, KH_2PO_4 1.25, Asparagine 2, Dextrose 20, Starch 10, Distilled Water to 1 L.

Czapek's:

$NaNO_3$ 2, KH_2PO_4 1, $MgSO_4.7H_2O$ 0.5, KCl 0.5, $FeSO_4.7H_2O$ 0.01, Sucrose 30, Distilled Water to 1 L.

Dextrose-asparagine phosphate:

Dextrose 30, $MgSO_4.7H_2O$ 0.5, Asparagine 1, KH_2PO_4 1.5, Distilled Water to 1 L.

Elliot's:

Dextrose 5, Asparagine 1, Sodium Carbonate 1.06, $MgSO_4.7H_2O$ 0.5, KH_2PO_4 1.36, Distilled Water to 1 L.

Fernando's:

$MgSO_4$ 5, KH_2PO_4 6.8, Asparagine 5, Glucose 15, Distilled Water to 1 L.

Glucose-dox:

$MgSO_4.7H_2O$ 0.5, KH_2PO_4 1, $FeSO_4.7H_2O$ 0.01, $NaNO_3$ 2, KCl 0.5, Glucose 15, Distilled Water to 1 L.

Glucose-nitrate:

Glucose 10, $NaNO_3$ 1, KH_2PO_4 1, Distilled Water to 1 L.

Potato dextrose:

Peeled potato slices 200, Dextrose 20, Distilled Water to 1 L.

(A) Measurement of Radial Growth

Radial growth and production of sclerotia of *Sclerotium rolfsii* were studied on solid medium. To solidify the medium 2% agar-agar was added in all the above referred media. 20 ml. of melted sterilized agar medium was poured asceptically into each petridish. For each tested medium, three petridishes were taken and inoculated by placing a 8.0 mm. diameter agar disc taken from margin of the freshlygrown colony of *Sclerotium rolfsii*. All the inoculated petridishes were incubated at 30^0C and radial growth of the pathogen was measured after every 12 hours upto 72 hours. The radial growth as mm/hr. was also calculated in each case with the help of the following formula (Singh and Sandhu, 1982) :

$$\textbf{Radial Growth (mm/hr.)} = \frac{\textbf{Radial Groth in m.m.}}{\textbf{Total Time period in hours}}$$

(B) Production of Sclerotia

For study the production of sclerotia of *Sclerotium rolfsii*, the all the petridishes, after measuring the radial growth, kept, in incubator for 15 days. After 15 days of incubation, number of sclerotia per petridish was counted and categorized as Nil (-), Poor (1^+), Fair (2^+), Good (3^+) and Excellent (4^+) according the table given below :

Number of Sclerotia per Petridish	Degree of Sclerotia Production	Symbol
0	Nil	-
1-100	Poor	1+
101-200	Fair	2+
201-300	Good	3+
More than 300	Excellent	4+

(C) Determination of Mycelial Weight

The growth of the pathogen was also measured in terms of mycelial dry weight.The *Sclerotium rolfsii* was allowed to grow in 150 ml Erlenmeyer flask containing 20 ml of sterilized broth medium (Excluding agar-agar). Each set of experiment runs in triplicate. Each flask was inoculated by transferring an agar disc (8.0 mm diameter) containing hyphal inoculum. Inoculated flasks were incubated at 30^0C for 3,6,9,12,15 and 18 days.

After the given incubation period, the mycilial mat of the pathogen was removed and collected in pre-weighed whatman's filter paper No. 42. Now the filter papers with mycelial mat were dried at 60^0 C for 6 days in electric oven. After drying, the filter papers with mycelium were re-weighed. The mycelial dry weight per culture was determined by substracting the weight of filter paper from the weight of filter paper + mycelial mat.

The mycelial dry weight as mg/day was also calculated in each case with the help of the following formula:

$$\text{Mycelial dry weight (mg/day)} = \frac{\text{Mycelial dry weight in mg}}{\text{Total Time period in days}}$$

Temperature

After selection of nutrient medium, the effect of seven different temperatures, i.e., 15, 20, 25, 30, 35, 40 and 45^0C was tried to study their influence on the growth and sclerotia production.

The *Sclerotium rolfsii* was cultured on potato dextrose medium. This medium was found to be a suitable one for the growth as well as sclerotia production of the pathogen.

After incubation at above said different temperatures, the radial growth, sclerotia production and mycelial dry weight were determined by the methods as described earlier.

pH

To study the effect of different pH, the selected medium i.e., potato dextrose medium was taken and different pH values, viz, pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 were adjusted by addition of 0.1N NaOH or 0.1N HCl.20 ml of potato dextrose medium, adjusted at desired pH was inoculated and incubated at 30^0C. The radial growth, sclerotia production and mycelial dry weight were determined by the methods as described earlier.

RESULTS AND DISCUSSION

Effect of Culture Media

The results recorded during the present investigation was presented in Table 1, 2 and represented graphically in Fig 1, photographically in plate 1 & 2.

From the results, it is clear that the radial growth of *sclerotium rolfsii* has not been recorded on Basal mucor, Brown's and Elliot's media within 12 hours of inoculation period. The radial growth on these three media has been recorded after 24 hours of inoculation period amongs the tested media. Potato-dextrose medium was found to be the best for radial growth. Dextrose-asparagine phosphate, Fernando's, Brown's and Basal mucor media were proved to be the next to Potato dextrose medium. Elliot's medium has been found to be very poor supporters of radial growth.

In all the six media, in which the radial growth of *sclerotium rolfsii* has been recorded, Petridishes kept in incubator for 15 days for the development of sclerotia. From the results, it is clear that the excellent degree (4^+) of sclerotia production takes place on Potato dextrose agar medium (Plate 1) and thus found to be the best for the development of sclerotia. Next to Potato dextrose agar medium, a fair degree of sclerotia production (2^+) has been recorded on Basal mucor, Brown's and Fernando's agar media. Comparatively poor degree of sclerotia production was observed on Dextrose asparagine phosphate and Elliot's agar media.

Table 1: Effect of different agar media on the growth and sclerotia oroduction of *Sclerotium Rolfsii*

Media	Radial Growth in mm* Hours after inoculation						Radial Growth (mm/hr.)	Sclerotia production (After 15 days)
	12	24	36	48	60	72		
Asthana and Hawker's agar	0.0	0.0	0.0	0.0	0.0	0.0	0.00	-
Basal mucor agar	0.0	8.2	19.5	29.6	38.2	44.5	0.45	2^+
Brown's agar	0.0	8.0	22.0	32.5	39.4	46.0	0.48	2^+
Czapek's agar	0.0	0.0	0.0	0.0	0.0	0.0	0.00	-
Dextrose-asparagine phosphate agar	4.0	18.5	26.0	33.0	40.1	47.5	0.63	1^+
Elliot's agar	0.0	14.0	17.0	19.0	20.5	22.0	0.34	1^+
Fernando's agar	2.0	16.7	23.0	28.2	33.1	37.3	0.52	2^+
Glucose-dox agar	0.0	0.0	0.0	0.0	0.0	0.0	0.00	-
Glucose-nitrate agar	0.0	0.0	0.0	0.0	0.0	0.0	0.00	-
Potato-dextrose agar	7.0	22.0	37.7	56.2	73.0	82	1.00	4^+

* After deducting the inoculum disc of 8.0 mm diameter

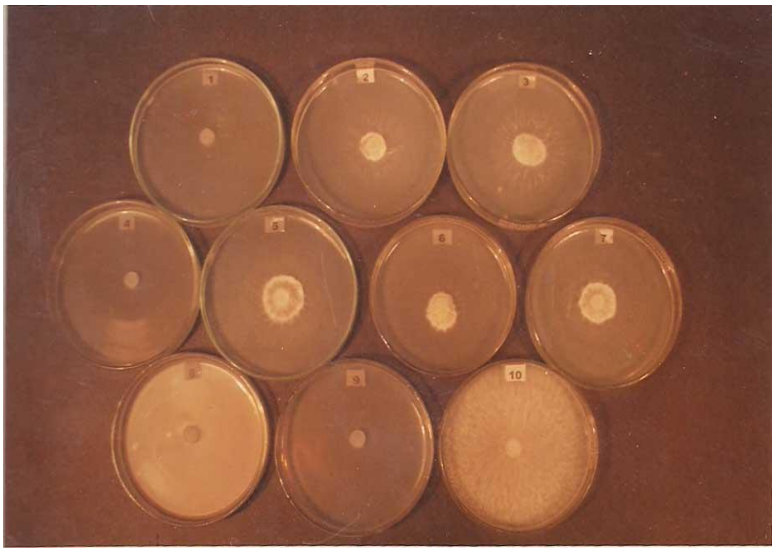

Figure 1: Showing the effect of following culture media on the radial growth of *Sclerotium Rolfsii* (After 72 hours of inoculation)

1. Asthana and Hawker's agar medium.
2. Basal mucor agar medium.
3. Brown's agar medium.
4. Czapek's agar medium.
5. Dextrose-asparagine phosphate agar medium
6. Elliot's agar medium.
7. Fernando's agar medium.
8. Glucose-dox agar medium.
9. Glucose-nitrate agar medium.
10. Potato-dextrose agar medium.

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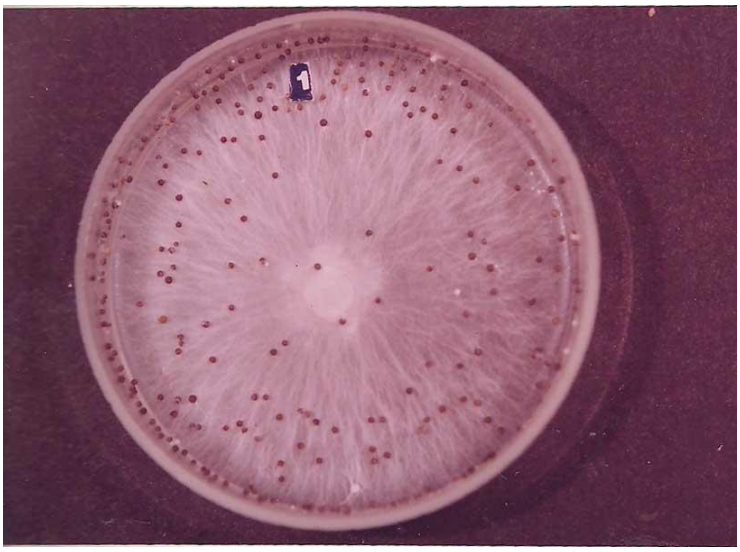

Figure 2: Showing sclerotia formation of *Sclerotium Rolfsii* on potato-dextrose agar medium at 30^0C (After 15 days of inoculation).

Growth of the pathogen in terms of dryweight of mycelium was also determined by growing the pathogen on ten different broth media. From the results it is clear that amongs all the tested media, Potato-dextrose broth media proved to be the best supporter of growth. Brown's, Basal mucor, Fernando's and Dextrose-asparagine phosphate broth media were proved to be the next, in all these four medium sufficient amount of mycelial growth have been recorded. Elliot's broth medium found to be poor supporter of growth. Asthana and Hawker's, Czapek's, Glucose-dox and glucose nitrate broth media did not support the growth, as in all these four media *sclerotium rolfsii* was unable to grow. Perhaps, nitrogen source and other ingradients of the medium may interfered with the growth of pathogen. Several workers like Ritter (1909), Bach (1927), Thornton (1956), Sarbhoy (1963, 1977), Baijal (1967) and Bilgrami (1975) having also similar opinion as they have reported that nitrate containing media are very toxic to several members of microorganisms. Chaurasia (1980) reported the inhibitory effect of inorganic nitrogen sources on the growth of *phytophthora parasitica var. piperina* and a decrease in the rate of oxygen uptake.

Table 2: Effect of different liquid media on the growth of *Sclerotium Rolfsii*

Media	Mycelial Dry Weight (in mg)*						Mycelial Dry Weight (mg/day)
	Days of incubation						
	3	6	9	12	15	18	
Asthana and Hawker's	0.0	0.0	0.0	0.0	0.0	0.0	0.00
Basal mucor	75.0	101.4	131.3	150.3	168.1	192.4	15.14
Brown's	75.5	95.2	135.4	180.2	217.6	237.3	16.45
Czapek's	0.0	0.0	0.0	0.0	0.0	0.0	0.00
Dextrose-asparagine phosphate	52.7	80.6	130.1	170.3	186.4	200.5	13.86
Elliot's	10.0	20.5	42.6	55.8	65.0	71.2	4.06
Fernando's	60.2	85.3	115.2	150.4	190.3	230.6	14.18
Glucose-dox	0.0	0.0	0.0	0.0	0.0	0.0	0.00
Glucose-nitrate	0.0	0.0	0.0	0.0	0.0	0.0	0.00
Potato-dextrose	217.3	237.5	267.3	312.6	352.2	382.5	35.41

* Average of three replicates

On the whole, it is concluded that Potato dextrose medium was found to be the best for excellent growth as well as sclerotia production. Asthana and Hawker'sl, Czapek's, Glucose-dox and Glucose-nitrate media have not suitable for the growth of *Sclerotium rolfsii*. In these four media, complete inhibition in growth as well as in sclerotial production of *Sclerotium rolfsii* may be due to the presence of nitrogen source and other ingradients of the medium. Several investigators like Chet et al (1966), Chet and Henis (1968), Bozarth and Tweedy (1971), Melhuish and Bean (1971), Okon et al (1972, 1973), Ercegovich et al (1973), Le Tourneau (1976, 1978), Igwegbe et al (1977), Fellman and Tourneau (1983) have reported that sclerotial formation of *Sclerotium rolfsii* in culture is inhibited by low concentrations of some unrelated chemicals some of which reduced mycelial growth.

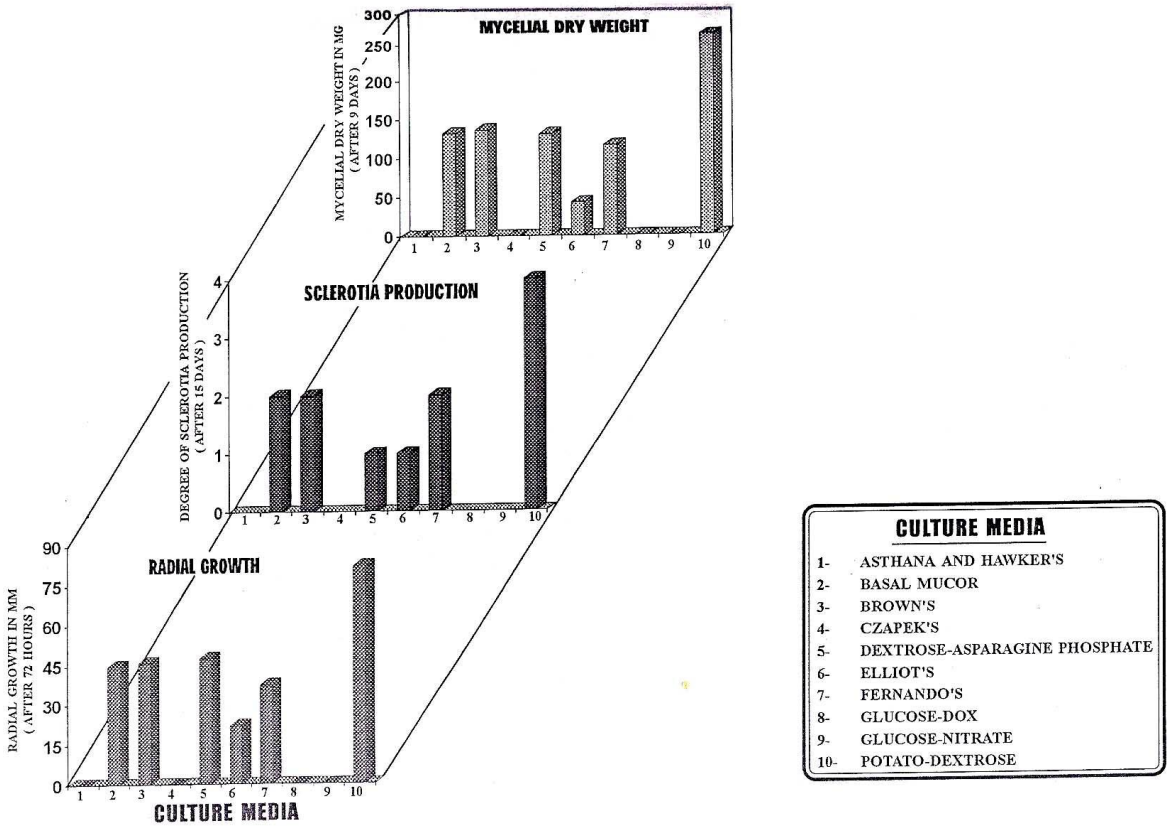

Figure 1: Effect of different media on the growth and sclerotia production of S*clerotium Rolfsii*

Effect of Temperature

The effect of different temperatures, viz, 15, 20, 25, 30, 35, 40 and 45^{0}C was tried to study their influence on the growth and sclerotia production. The data are presented in Table 3 and represented graphically in Fig 2 and photographically in plate 3.

In respect of radial growth at various temperatures, it was observed that at 25, 30 and 35^{0}C temperatures, the growth was recorded after 12 hours of inoculation and at low temperature i.e. at 15 and 20^{0}C, it was able to grow after 48 and 36 hours of inoculation respectively. From the results, it is evident that radial growth has always been correlated with mycelial dry weight.

From the results, it is clear that *Sclerotium rolfsii* grew poorly at 15^0C. Above 15^0C temperatures, the growth was gradually increased with increased in temperature upto 30^0C and then slightly declined at 35^0C. Above 35^0C the higher temperature i.e. 40 and 45^0C, the pathogen was unable to grow.

Table 3: Effect of different temperatures on the growth and sclerotia production of *Sclerotium Rolfsii*

Temperature (^{0}C)	Radial Growth in mm*						Radial Growth (mm/hr.)	Sclerotia production (After 15 days)	Mycelial dry weight in mg (After 9 days)
	Hours after inoculation								
	12	24	36	48	60	72			
15	0.0	0.0	0.0	3.0	5.5	7.5	0.03	-	120.65
20	0.0	0.0	2.5	9.5	19.0	31.2	0.16	1^+	168.26
25	4.5	10.4	20.2	32.2	44.3	57.3	0.59	3^+	230.75
30	7.0	22.0	37.7	56.2	73.0	82.0	1.00	4^+	267.30
35	5.5	11.5	24.5	37.2	50.2	65.2	0.68	4^+	242.61
40	0.0	0.0	0.0	0.0	0.0	0.0	0.00	-	0.00
45	0.0	0.0	0.0	0.0	0.0	0.0	0.00	-	0.00

* After deducting the inoculum disc of 8.0 mm diameter

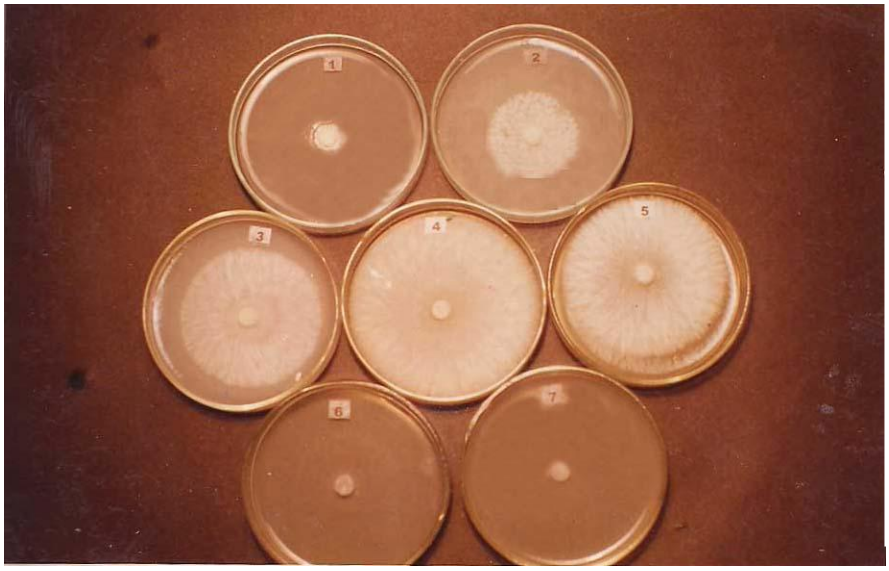

Figure 3: Showing radial growth of *Sclerotium Rolfsii* at different temperature (After 72 hours of inoculation)

1. 15^0C
2. 20^0C
3. 25^0C
4. 30^0C
5. 35^0C
6. 40^0C
7. 45^0C

Sclerotial production was also investigated at different temperatures on Potato dextrose agar medium. It was observed that slightly higher temperature was needed for the development of Sclerotia, as sclerotia

were started to produce when culture plates incubated at 20^0C. At higher temperature i.e. 30 to 35^0C, sclerotia produced abundently in culture, therefore proved to be the best suitable temperature for production of sclerotia.

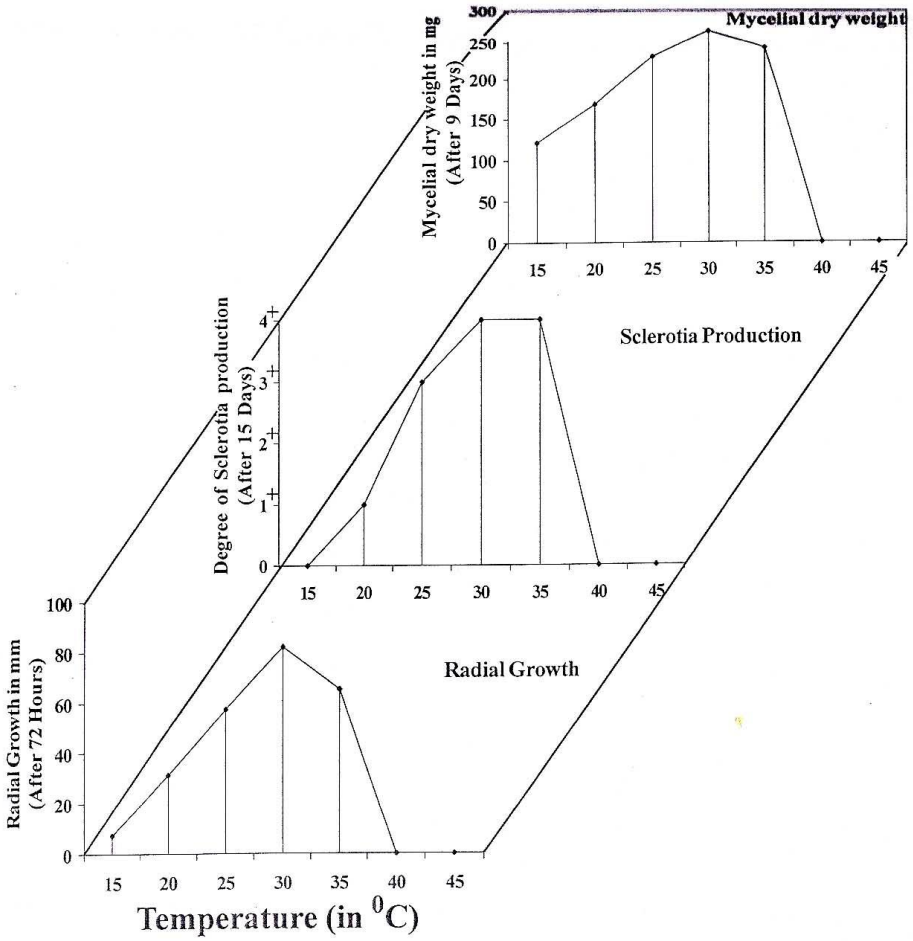

Figure 2: Effect of different temperatures on the growth and sclerotia production of *Sclerotium Rolfsii*

From these results it is concluded that the 30^0C was proved to be the best suitable temperature for the growth as well as the production of sclerotia. The growth was slightly declined at 35^0C but sclerotia produced excellently. 40 to 45^0C temperatures were found to be detrimental for growth and sclerotia production. Almost similar results have been recorded by Abeygunawardena and wood (1957), Mishra and Haque (1962), Mathur and Sorbhoy (1976), Sharma and Kaushal (1979) and Zoben (1980) with different isolates of *Sclerotium rolfsii*.

Effect of pH

In the present study, an attempt has been made to understand the influence of different pH (ranging from pH 3.0 to 9.0) on the growth and sclerotia production of *Sclerotium rolfsii* (Table 4, Fig. 3 and plate 4).

From the peruasal of data, it is apparent that radial growth of the pathogen was always correlated with mycelial dry weight. The *Sclerotium rolfsii* was able to grow over a wide range of pH, i.e., between pH 3.0 to 9.0. At low pH value i.e., 3.0, growth of pathogen was satisfactory. The radial growth as well as dry mycelial weight gradually increased with the increase in pH values up to 5.0. At pH 5.0, the maximum

growth of *Sclerotium rolfsii* was recorded. On further increase in pH value upto 9.0, the growth gradually decreased. The results of mycelial growth in present study, agree with the findings of Richharia (1984), who has reported that pH 5.0 was the optimum for mycelial growth of *Sclerotium rolfsii*. Wide range of pH with optimum near 6.0 for the growth of various isolates of *Sclerotium rolfsii* have been reported by Aycock (1966), Narasimhan (1969), Sharma and Kaushal (1979) and Punja (1984).

Table 4: Effect of different pH on the growth and sclerotia production of *Sclerotium Rolfsii*

pH	Radial Growth in mm* Hours after inoculation						Radial Growth (mm/hr.)	Sclerotia production (After 15 days)	Mycelial dry weight in mg (After 9 days)
	12	24	36	48	60	72			
3.0	0.0	2.5	7.5	15.0	24.5	34.1	0.24	-	130.38
4.0	5.5	11.0	21.2	31.3	42.4	54.2	0.59	4^+	235.84
5.0	7.0	22.0	37.7	56.2	73.0	82.0	1.00	4^+	267.30
6.0	6.2	16.0	27.2	39.3	51.6	64.3	0.74	4^+	242.12
7.0	5.1	10.6	21.0	31.0	41.2	51.4	0.57	4^+	220.45
8.0	3.5	10.0	19.1	28.2	38.1	48.2	0.51	3^+	185.60
9.0	2.0	8.0	16.5	23.5	31.0	38.5	0.41	2^+	155.50

* After deducting the inoculum disc of 8.0 mm diameter

Figure 3: Showing radial growth of *Sclerotium Rolfsii* at different pH (after 72 hours of inoculation).

1. pH 3.0
2. pH 4.0
3. pH 5.0
4. pH 6.0
5. pH 7.0
6. pH 8.0
7. pH 9.0

The sclerotia production of *Sclerotium rolfsii* was also affected by different pH values. From the results, it is clear that the pH range 4.0 to 7.0 was found to be the most fabourable for sclerotia production, as excellent degree of sclerotia production has been observed. Good degree of sclerotia formation was recorded at pH 8.0. Quite a fair degree of sclerotia production has been observed at pH 9.0. But higher acidic pH value i.e. pH 3.0 was recorded as detrimental and unfavourable for sclerotia development.
Different workers have reproted different optima for sclerotial formation by their isolate of Sclerotium rolfsii. Mathur and Sorbhoy (1976) have reported excellent sclerotial formation on double maxima, i.e., at pH 3.5 and pH 6.5 in case of sugar beet isolate of *Sclerotium rolfsii*. Sharma and Kaushal (1979) have

observed maximum sclerotial development between pH 5.2 to 5.8, in *Sclerotium rolfsii* isolated from sunflower. In the present study, pH4.0 TO 7.0 were found to be most favourable for the production of sclerotia.

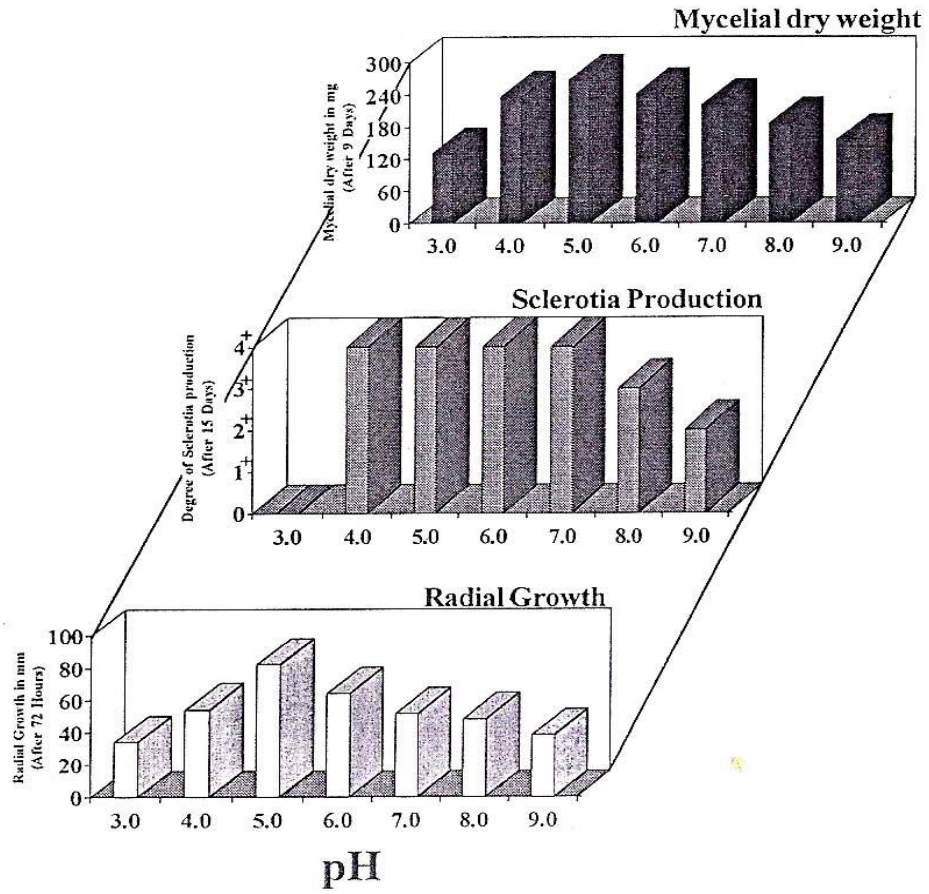

Figure 4: Effect of different pH on the growth and sclerotia production of *Sclerotium Rolfsii*

On the whole, it is concluded that *Sclerotium rolfsii* was able to grow and sporulate in the wide range of pH. The pH 5.0 was optimum for mycelial growth while the pH 4.0 to 7.0 were found to be most favourable for an excellent degree of sporulation. In higher acedic pH value i.e. pH 3.0, sclerotia did not produced.

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