

Cultural and physiological studies on *Sclerotium rolfsii* causing scleotium wilt of potato

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SUMMARY

Sclerotium wilt of potato caused by *Sclerotium rolfsii* Sacc. is one of the most important soil borne diseases which is attaining the major status in potato in Karnataka. Cultural characters were studied on fourteen different solid and liquid media. Among solid media tested against *Sclerotium rolfsii*, the maximum radial growth was observed on oat meal agar (90 mm), potato dextrose agar (90 mm) and Sabouraud's agar (90 mm) followed by carrot dextrose agar (88 mm). These four were at par with one another and significantly superior over the rest of all other media tested. Among liquid media tested the maximum mycelial weight was observed on oat meal extract broth (190.9 mg), potato dextrose broth (190.4 mg), host leaf extract (188.1 mg). These were at par with one another and significantly superior over the rest of all other liquid media tested. The maximum growth of the fungus was observed at a temperature of 30°C (538.60 mg) which was significantly superior to all other temperature levels tested. followed by 25°C (480.16 mg), 35°C (380.40 mg) and 20°C (349.20 mg). The maximum dry mycelial weight of the fungus was noticed at a pH level of 5.0 (547.00 mg) followed by 4.0 (493.33 mg) and 6.0 (355.66 mg). The exposure of the fungus to alternative cycles of 12 hrs light and 12 hrs darkness for ten days resulted in the maximum dry matter (387.62 mg) which was significantly superior over other two treatment tested. The dry mycelial weight of fungus exposed to continuous light resulted in moderate growth (329.72 mg) and continuous darkness resulted in minimum growth (134.07 mg). The exposure of the fungus to alternate cycles of 12 hrs light and 12 hrs darkness for ten days resulted in maximum radial growth of *Sclerotium rolfsii* i.e., 89.42 mm, which was significantly superior over other two treatments tested. The radial growth of fungus exposed to continuous light resulted in colony diameter of 86.05 mm.

Key Words : *Sclerotium rolfsii*, Light, Temperature, pH

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Potato (*Solanum tuberosum* L.) is one of the important commercial vegetable crops in India. It is the world's fourth important food crop owing to its great yield potential and high nutritive value and accounts for nearly half of the world's annual output of all root and tuber crops (Thortan and Sieczka, 1980).

Commercially potato is propagated through tubers. Many

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disease causing agents viz., viruses, fungus, bacteria, nematode, viroids and phytoplasmas are reported on potato. Among the fungal diseases, wilt caused by *Sclerotium rolfsii* Sacc. has attained the economic importance. In recent years, this disease is increasing and causing huge losses in potato (Paul Khurana, 1998). To findout the strategy for management of the disease it is necessary to evaluate fungicides and bioagents etc. against the pathogen *in vitro*. Therefore, the present study was undertaken to findout which media, temperature, pH and light sources support maximum mycelial growth of the fungus.

MATERIALS AND METHODS

Growth characters on solid media :

The growth characters of *Sclerotium rolfsii* were studied on fourteen solid media viz.,

- Basal agar media
- Carrot agar
- Corn meal agar
- Czapek's Dox agar
- Elliot's agar
- Host leaf extract agar
- Malt extract agar
- Oat meal extract agar
- Potato dextrose agar
- Richards's agar
- Rose bengal agar
- Sabouroud's agar
- Tochinai's agar
- Yeast extract agar

To carryout the study, 20 ml of each of the sterilized medium was poured in 90 mm Petriplates. Such Petriplates were inoculated with 5 mm disc cut from periphery of actively growing culture and incubated at 27±1°C. Each treatment was replicated thrice. Observations were taken when the fungus covered complete Petriplate in any one of the media. The data on radial growth were analyzed statistically.

Growth phase :

Each of the 150 ml flasks was inoculated aseptically with five mm culture disc. The flasks were incubated at room temperature (27±1°C), observations with respect to colony characters and sclerotial formation were taken everyday.

The mycelial mat was filtered through Whatman No. 42 filter paper discs dried to a constant weight at 60°C prior to filtration. The mycelial mat on the filter paper was washed thoroughly with distilled water to remove any salts likely to be associated with the mycelium and dried to a constant weight in an electrical oven at 60°C, cooled in a dessicator and weighed immediately on an analytical electrical balance. Harvesting and assessing the weight of the growth of the fungus was started from fourth day of incubation and was discontinued after 14th day of incubation since the weight of the fungal growth fall down after attaining the maximum growth on tenth day.

Growth characters on liquid media :

The liquid media used were same as that of solid media except agar was not added to the liquid media.

All the liquid media were sterilized and each treatment was replicated thrice. The weight of dry mycelium was recorded by above said procedure and the data were statistically analyzed. The best synthetic medium was found out and used for further studies as basal medium.

Temperature requirement :

Richards's liquid medium was used in this experiment. The different temperatures tried for the growth of the fungi were 10, 15, 20, 25, 30, 35, 40°C. for each temperature level three replications were maintained. The dry mycelial weight was recorded and results were analyzed statistically.

Hydrogen ion concentration :

The Richards's liquid medium was used in this experiment. The pH of the liquid media was adjusted by using 0.1 N alkali (NaOH) or 0.1 N acid (HCl). The pH of the medium used ranged

from 3 to 9. Each treatment was replicated thrice. The 250 ml flasks each containing 30 ml medium of respective hydrogenion concentration were sterilized, inoculated, incubated and mycelial weight was recorded. The data were analyzed statistically.

Light requirement :

Potato dextrose broth and agar were used in this experiment. Conical flasks of 150 ml capacity and each containing 30 ml of liquid medium were inoculated and exposed to different lengths of light hours, viz., altenrtae cycles of twelve hours light and 12 hours darkness, continuous light and continuous darkness in an environmental chamber. Each treatment was replicated seven times and incubated for 7 days. Dry mycelial weight was obtained as described earlier and results were analyzed statistically.

To carryout the study on solid media, 20 ml of potato dextrose agar was poured in 90 mm Petriplates. Such Petriplates were inoculated and incubated at different light intensities. Each treatment was replicated seven times and incubated for ten days. Colony diameter was recorded and results were analyzed statistically.

RESULTS AND DISCUSSION

The findings of the present study well as relevant discussions have been presented under following heads:

Growth characters on solid media :

The results (Table 1) of the cultural studies on solid media indicated that, the radial growth of *S. rolfsii* was maximum on oat meal agar (90 mm), potato dextrose agar (90 mm) and Sabouraud's agar (90 mm) followed by carrot dextrose agar (88 mm). These four were at par with one another and

Table 1: Mean colony diameter of *Sclerotium rolfsii* on different solid media

Sr. No.	Media	Mean colony diameter (mm)
1.	Basal agar	74.00
2.	Carrot dextrose agar	88.00
3.	Corn meal agar	76.50
4.	Czapek's Dox agar	79.00
5.	Elliot's agar	22.00
6.	Host leaf extract agar	80.00
7.	Malt extract agar	78.00
8.	Oat meal agar	90.00
9.	Potato dextrose agar	90.00
10.	Richards's agar	80.50
11.	Rose Bengal agar	40.10
12.	Sabouraud's agar	90.00
13.	Tochinai's agar	66.00
14.	Yeast extract agar	72.50
	S.E.±	1.86
	C.D. (P=0.01)	7.27

significantly superior over the rest of all other media tested. The results are in confirmation with that of Sengupta and Das (1970), Sulladmath *et al.* (1977) and Lingaraju (1977).

Growth phase :

The results (Table 2) indicated that dry mycelial weight of the *Sclerotium rolfii* was minimum on 4th day after inoculation and on subsequent harvest, it was significantly increased and finally reached maximum on 10th day. Later the dry mycelial weight started decreasing. Maximum dry mycelial weight of 188.4 mg was observed on tenth day of incubation and was significantly superior to all other treatments tested. This period was used as maximum growth period for further studies. Bagyaraj and Sirsi (1965) while working with an isolate of *S. rolfii* Sacc. observed maximum growth of that fungus with in eight to ten days of incubation.

Table 2 : Mean dry mycelial weight of *Sclerotium rolfii* Sacc. on potato dextrose broth

Day	Dry mycelial weight (mg)
4	69.00
5	100.00
6	107.20
7	134.20
8	158.20
9	173.90
10	188.40
11	188.00
12	177.10
13	158.70
14	120.90
S.E.±	0.70
C.D. (P=0.01)	2.79

Growth characters on liquid media :

The results (Table 3) indicated that, maximum dry mycelial weight of fungus was obtained in oat meal extract broth (190.9 mg), potato dextrose broth (190.4 mg), host leaf extract (188.1 mg). These were at par with one another and significantly superior over the rest of all other liquid media tested. The results are in conformation with Sulladmath *et al.* (1977), Tokahashi (1927) and Weber (1931) who also noticed the response of *S. rolfii* to different media.

Among the synthetic media tested, the maximum mycelial weight of the fungus was obtained in Richards's broth (174.8 mg) which was selected as best liquid medium for further studies.

Table 3 : Mean dry mycelial weight of *S. rolfii* in different liquid media

Sr. No.	Media	Mean dry mycelial weight (mg)
1.	Basal broth	44.8
2.	Carrot broth	184.3
3.	Corn meal broth	122.4
4.	Czapek's Dox broth	167.7
5.	Elliot's broth	47.3
6.	Host leaf extract broth	188.1
7.	Malt extract broth	107.9
8.	Oat meal broth	190.9
9.	Potato dextrose broth	190.4
10.	Richards's broth	174.8
11.	Rose Bengal broth	60.6
12.	Sabouraud's broth	149.2
13.	Tochinai's broth	87.7
14.	Yeast extract broth	142.3
	S.E.±	1.40
	C.D. (P=0.01)	5.49

Effect of temperature :

The maximum growth (Table 4) of the fungus was observed at a temperature of 30°C (538.60 mg) which was significantly superior to all other temperature levels tested. This was followed by 25°C (480.16 mg), 35°C (380.40 mg) and 20°C (349.20 mg). These results are in confirmation with the results of Sulladmath *et al.* (1977), Hari *et al.* (1988), Prasad *et al.* (1986) and Tripathi and Khare (2006).

Table 4 : Mean dry mycelial weight of *Sclerotium rolfii* at different temperatures

Temperature (°C)	Dry mycelial weight (mg)
10	101.66
15	212.96
20	349.20
25	480.16
30	538.60
35	380.40
40	107.70
S.E.±	1.27
C.D. at (P=0.1)	5.08

Effect of hydrogen ion concentration :

The effect of different pH values (Table 5) on the growth of the fungus was significant. The maximum dry mycelia weight of the fungus was noticed at a pH value of 5.0 (547.00 mg) followed by 4.0 (493.33 mg) and 6.0 (355.66 mg). The decrease

in the dry mycelial weight of the fungus was seen after a pH value of 6.0 (355.66 mg). The least dry weight was recorded at a pH of 9.0 (138.00 mg). Similar results were obtained by Tripathi and Khare (2006). But, Lingaraju (1977) found that optimum pH for growth of *S. rolfisii* ranged from 2.8 to 5.8; from pH 5.8 onwards the growth decreased suddenly and at pH 7.6 little rise in growth was observed. Prasad *et al.* (1986) and Dey *et al.* (1992) also observed maximum dry mycelial growth of the fungus at pH ranged from 4.0 to 7.0 in *S. rolfisii* isolated from tomato fruit.

Table 5 : Mean dry mycelial weight of *Sclerotium rolfisii* at different pH values

Sr. No.	pH	Mean dry mycelial weight (mg)
1.	3	181.33
2.	4	493.33
3.	5	547.00
4.	6	355.66
5.	7	235.66
6.	8	153.66
7.	9	138.00
	S.E.±	3.34
	C.D. (P=0.01)	14.10

Effect of light :

On dry mycelial weight :

The exposure of the fungus to alternative cycles of 12 hrs light and 12 hrs darkness for ten days resulted in the maximum dry matter (387.62 mg) which was significantly superior over other two treatment tested (Table 6). The dry mycelial weight of fungus exposed to continuous light resulted in moderate growth (329.72 mg) and continuous darkness resulted in minimum growth (134.07 mg).

Table 6 : Effect of light on growth of *Sclerotium rolfisii*

Treatments	Mean dry mycelial weight (mg)	Mean colony diameter (mm)
Continuous light	329.72	86.05
Continuous dark	128.57	71.38
Alternate cycle of 12 hour light and 12 hour darkness	387.62	89.42
S.E.±	1.17	0.21
C.D. (P=0.01)	4.68	1.84

On radial growth :

The exposure of the fungus to alternate cycles of 12 hrs light and 12 hrs darkness for ten days resulted in maximum radial growth of *Sclerotium rolfisii* i.e., 89.42 mm, which was significantly superior over other two treatments tested. The

radial growth of fungus exposed to continuous light resulted in colony diameter of 86.05 mm. The least colony diameter of 71.38 mm was recorded in treatments where the plates were kept continuously in darkness. Light has a profound effect on growth of fungi. Similar observations were made by Punja (1985).

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