

## Physiological studies of *Sclerotinia sclerotiorum* causing stem rot of fennel (*Foeniculum vulgare* Mill.)

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### ABSTRACT

Glucose sodium chloride agar medium supported maximum growth and sclerotia formation of the fungus. In liquid medium study, Glucose sodium chloride was also produced highest number of sclerotia with maximum dry weight, while Richard's medium supported maximum dry mycelial weight. The fungus grew best at pH 4.0, while sclerotia formation was found best at pH 6.0. The maximum fungal mycelial growth was observed at 25°C temperature, while higher number of sclerotia and sclerotial dry weight was recorded at 15°C temperature.

**Key words :** *Sclerotinia sclerotiorum*, fennel, temperature, pH and media.

### INTRODUCTION

Fennel (*Sclerotinia sclerotiorum*) is one of the most important seed spices in India. Gujarat is one of the major states cultivating fennel crop and contributing about 86.00 per cent of the total country production. It is attacked by many fungal and bacterial diseases. Stem rot of fennel caused by *Sclerotinia sclerotiorum* (lib.) de Bary is one of the most important disease and cause considerable damage to the fennel crop in some parts of Gujarat State. Not much work have been done on this disease except report of this disease in fennel crop. Hence, this disease present investigation were carried out on physiological aspects i.e. effect of different media, pH levels, and temperature on growth and sporulation of *S. sclerotiorum*.

### MATERIALS AND METHODS

#### Effect of different media

Effect of nine different solid and liquid media on growth and sclerotia formation of *S. sclerotiorum* were employed. For solid media, agar at the rate of two per cent was added in each of the medium. After sterilization of media, before cool it at 45 °C temperature, 20 ml of each media was poured separately in sterilized Petri plates aseptically. In liquid media study, 40 ml of the medium was poured in each conical flask (250 ml). Four replications per treatment were maintained. After solidifying the media, 4 mm bit of 8 days old culture of pathogen were cut with the help of sterilized cork borer and placed in the center of each plate or each flask and were incubated at 25 + 2°C temperature in an incubator. In the study of solid media, after five days of incubation, observation on radial growth and no of sclerotia of fungus were recorded. In liquid media study, after seven days of inoculation, dry

weight of mycelium and sclerotia were recorded.

#### Effect of pH

The effect of pH on growth and sclerotia formation of *S. sclerotiorum* was studied by growing fungus at different pH levels viz., 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 in liquid Richard's medium. The 100 ml PDA was filled in 250 ml flasks. The pH levels were adjusted by using 0.1 N HCl or 0.1 N NaOH solution with the help of pH meter. After adjusting pH, the media were sterilized in an autoclave. Inoculated flasks were incubated at 25± 2°C temperature for 7 and 14 days. Observations were recorded as described in media study.

#### Effect of temperature

To study the effect of different temperature on growth and sclerotia formation of *S. sclerotiorum*, the experiment was conducted by growing the fungus at seven levels of temperatures viz., 5, 10, 15, 20, 25, 30 and 35 °C in Richard's medium. Inoculated flasks as well as Petri dishes were incubated at different levels of temperatures in B.O.D. incubators for a period of 7 and 14 days. Observations were recorded as described in media study.

### RESULTS AND DISCUSSION

#### Effect of different media

##### (A) Solid media

The results revealed that there was a considerable variability in growth, sclerotial formation and dry weight of sclerotia of the pathogen on different solid media tested (Table 1). After three days of incubation at 25 ± 2°C temperature the growth of the pathogen on Glucose sodium chloride agar medium was maximum (86.33 mm) and significantly superior over all other media tested however

**Table 1.** Average colony diameter, number of sclerotia and dry weight of sclerotia of *S. sclerotiorum* on different solid media

No.	Media	Colony diameter (mm)*		Number of sclerotia*		Dry weight of sclerotia (mg)*	
		3 days	5 days	7 days	14 days	7 days	14 days
1.	Potato dextrose agar	68.00	86.33	6.06 (36.33)**	6.46 (41.33)**	180.33	229.33
2.	Fennel stem decoction agar	82.67	89.67	5.11 (25.67)	6.12 (37.00)	141.67	198.00
3.	Fennel leaf decoction agar	78.00	87.67	3.89 (14.67)	3.93 (15.00)	168.00	223.00
4.	Czapek's agar	20.00	34.33	4.02 (15.67)	3.85 (24.33)	58.00	178.33
5.	Richard's agar	74.33	86.67	4.84 (23.00)	5.81 (33.33)	247.33	395.67
6.	Glucose sodium agar	28.33	42.00	3.07 (9.00)	3.43 (11.33)	28.67	135.33
7.	Glucose sodium chloride agar	86.33	90.00	6.72 (44.67)	6.89 (47.00)	560.67	692.00
8.	Asthana and Hawker's agar	10.67	20.00	1.46 (1.67)	1.77 (2.67)	7.00	15.33
9.	Corn meal agar	15.67	23.67	2.48 (5.67)	2.67 (6.66)	9.00	18.00
	S. Em. ±	1.38	1.72	0.07	0.06	0.45	5.08
	C.D. at 5 %	4.12	5.11	0.20	0.17	1.36	15.10
	C.V.%	4.66	4.79	2.92	2.30	0.51	3.80

\* Average of three replications;

\*\* Figures in parentheses are retransformed value.

it was at par with Fennel stem decoction agar (82.67 mm) and followed by Fennel leaf decoction agar (78.00 mm) media, Richard's agar (74.33 mm) and Potato dextrose agar (68.00 mm). Significantly least growth (10.67 mm) was observed on Asthana and Hawker's agar medium followed by Corn meal agar (15.67 mm) and Czapek's agar (20.00 mm) media. But after five days of incubation, maximum growth of the pathogen was observed on Glucose sodium chloride agar (90 mm), Fennel stem decoction agar (89.67 mm), Fennel leaf decoction agar

(87.67 mm), Richard's agar (86.67 mm) and Potato dextrose agar media (86.33 mm) and there was no significant difference amongst them. Significantly maximum sclerotia were produced on Glucose sodium chloride agar medium after seven days (44.67) and after fourteen days (47.00) of incubation at  $25 \pm 2^\circ\text{C}$  temperature. This was followed by Potato dextrose agar, Fennel stem decoction agar and Richard's agar which produced 41.33, 37.00 and 33.33 sclerotia, respectively after fourteen days of incubation. The highest sclerotial dry weight was

**Table 2.** Average dry mycelial weight, number of sclerotia and dry weight of sclerotia of *S. sclerotiorum* in different liquid media

No.	Media	Dry mycelial weight (mg)*		Number of sclerotia*		Dry weight of sclerotia (mg)*	
		7 days	14 days	7 days	14 days	7 days	14 days
1.	Potato dextrose	289.67	135.00	4.29 (18.00)**	4.91 (23.67)**	365.33	690.33
2.	Fennel stem decoction	370.33	276.00	3.76 (13.67)	4.05 (16.00)	204.67	346.33
3.	Fennel leaf decoction	259.33	232.33	2.85 (7.67)	2.91 (8.00)	190.33	220.00
4.	Czapek's	220.33	241.00	2.19 (4.33)	3.12 (9.33)	115.67	174.67
5.	Richard's	432.00	669.00	3.07 (9.00)	4.33 (18.33)	360.00	674.00
6.	Glucose sodium nitrate	331.33	288.00	2.30 (5.00)	2.33 (5.00)	159.00	172.33
7.	Glucose sodium chloride	345.00	264.00	5.86 (34.00)	6.49 (41.67)	683.00	867.00
8.	Asthana and Hawker's	80.67	57.00	1.77 (2.67)	2.11 (4.00)	16.00	31.00
9.	Corn meal	139.00	69.00	2.07 (3.67)	2.54 (6.00)	35.67	57.00
	S. Em. ±	5.79	4.82	0.16	0.11	3.83	6.487
	C.D. at 5 %	17.21	14.32	0.47	0.33	11.38	19.27
	C.V. %	3.66	3.37	8.85	5.40	2.70	3.12

\* Average of three replications;

\*\* Figures in parentheses are retransformed value.

observed on Glucose sodium chloride agar after seven days (560.67 mg) and after fourteen days (692.00 mg) of incubation at  $25 \pm 2^\circ\text{C}$  temperature and was found significantly superior over all other media tested. Next best media were Richard's agar, Potato dextrose agar and Fennel leaf decoction agar media which were yielded 395.67 mg, 229.39 mg and 223.00 mg dry weight of sclerotia, respectively after fourteen days of incubation.

(B) Liquid media

In this study, maximum dry mycelial weight (432.00 mg) was recorded in Richard's medium after seven days of incubation at  $25 \pm 2^\circ\text{C}$  temperature and was found significantly superior over all other media tested (Table 2). This was followed by Fennel stem decoction medium (370.33 mg), Glucose sodium chloride medium (345.00 mg) and Glucose sodium nitrate medium (331.33 mg). The least dry mycelium weight was recorded in Asthana and Hawker's medium (80.67 mg) followed by Corn meal

**Table 3.** Effect of different H-ion concentrations on growth and sclerotia formation of *S. sclerotiorum* in liquid Richard's medium

No.	pH levels	Dry mycelial weight (mg)*		Number of sclerotia*		Dry weight of sclerotia (mg)*	
		7 days	14 days	7 days	14 days	7 days	14 days
1.	2.0	49.33	109.33	0.71 (0.00)**	0.71 (00.00)**	000.00	000.00
2.	3.0	251.00	485.00	2.19 (4.33)	3.08 (9.00)	87.67	428.67
3.	4.0	288.33	644.33	2.37 (5.13)	3.51 (11.83)	101.00	528.67
4.	5.0	294.67	470.33	2.94 (8.76)	4.08 (16.16)	185.50	668.83
5.	6.0	214.00	265.00	2.62 (12.66)	4.76 (22.16)	164.67	580.33
6.	7.0	171.00	216.33	1.67 (2.33)	2.18 (4.33)	39.00	204.33
7.	8.0	134.67	166.67	0.71 (0.00)	0.71 (00.00)	000.00	000.00
8.	9.0	81.33	108.33	0.71 (00.00)	0.71 (00.00)	000.00	000.00
	S. Em. ±	3.85	5.84	0.04	0.06	0.34	0.34
	C.D. at 5 %	11.54	17.53	0.13	0.20	1.04	1.04
	C.V.%	3.59	3.29	4.15	4.74	0.84	0.20

\* Average of three replications;

\*\* Figures in parentheses are retransformed value.

medium (139.00 mg).

After fourteen days of incubation, maximum dry mycelial weight (669.00 mg) was recorded in Richard's medium and was significantly superior over all other media tested and followed by Glucose sodium nitrate medium (288.00 mg), Fennel stem decoction medium (276.00 mg) and Glucose sodium chloride (264.00 mg). Significantly

maximum number of sclerotia were formed on Glucose sodium chloride medium after seven days (34.00) and after fourteen days (41.67) of incubation at 25 ± 2°C temperature. This was followed by Potato dextrose, Richard's and Fennel stem decoction which produced 23.67, 18.33 and 16.00 sclerotia, respectively after fourteen days of incubation. The highest sclerotial dry

**Table 4:** Effect of different temperatures on growth and sclerotia formation of *S. sclerotiorum* on solid Richard's medium

No.	Temperature (°C)	Colony diameter (mm)*		Number of sclerotia*		Dry weight of sclerotia (mg)*	
		3 days	5 days	7 days	14 days	7 days	14 days
1.	5	13.67	37.66	0.71 (00.00)**	0.71 (00.00)**	000.00	000.00
2.	10	17.33	62.63	1.77 (02.67)	1.85 (03.00)	024.33	55.00
3.	15	67.00	88.00	5.24 (26.00)	6.01 (35.67)	236.00	429.00
4.	20	72.00	89.17	5.01 (24.67)	5.87 (34.00)	215.00	365.00
5.	25	75.00	90.00	4.74 (22.00)	5.61 (31.00)	190.00	283.33
6.	30	00.00	00.00	0.71 (00.00)	0.71 (00.00)	000.00	000.00
7.	35	00.00	00.00	0.71 (00.00)	0.71 (00.00)	000.00	000.00
	S. Em. ±	0.41	0.76	0.05	0.07	1.76	2.28
	C.D. at 5 %	1.25	2.31	0.16	0.21	5.35	6.92
	C.V.%	2.06	2.52	3.49	3.99	3.21	2.44

\* Average of three replications;

\*\* Figures in parentheses are retransformed value.

weight was observed on Glucose sodium chloride medium after seven days (683.00 mg) and after fourteen days (867.00 mg) of incubation at 25 ± 2°C temperature and was found significantly superior over all other media tested. Next best media were Potato dextrose and Richard's agar which yielded 690.33 mg and 674.00 mg dry weight of sclerotia, respectively after fourteen days of incubation. Thus, Glucose sodium chloride supported maximum growth and sclerotia formation of the fungus. The liquid

medium Glucose sodium chloride also produced highest number of sclerotia with maximum dry weight, while liquid Richard's medium supported maximum dry mycelial weight. Potato dextrose medium also supported good fungal growth and sclerotial formation. Kirsanov (4) reported that solid and semi-liquid media containing the hydrolysate with 0.5 per cent sodium chloride and 3 per cent glucose gave good growth of *S. sclerotiorum*. Jani (2) also reported that the best growth and sclerotia

**Table 5.** Effect of different temperatures on growth and sclerotia formation of *S. sclerotiorum* in liquid Richard's medium

No.	Temperature (°C)	Dry mycelial weight (mg)*		Number of sclerotia*		Dry weight of sclerotia (mg)*	
		7 days	14 days	7 days	14 days	7 days	14 days
1.	5	70.66	158.00	0.71 (0.00)**	1.35 (1.33)	000.00	12.67
2.	10	152.00	322.67	1.22 (01.00)	1.67 (2.33)	005.00	25.00
3.	15	547.67	635.67	3.67 (13.00)	3.89 (14.67)	192.00	331.33
4.	20	585.33	640.67	3.23 (10.00)	3.63 (12.67)	146.00	272.67
5.	25	684.00	740.33	2.78 (07.33)	3.07 (9.00)	140.00	232.67
6.	30	62.67	80.00	0.71 (0.00)	0.71 (0.00)	000.00	000.00
7.	35	52.67	69.67	0.71 (0.00)	0.71 (0.00)	000.00	000.00
	S. Em. ±	4.10	5.88	0.10	0.07	1.36	2.07
	C.D. at 5 %	12.44	16.34	0.32	0.23	4.13	6.29
	C.V.%	2.31	2.47	10.01	6.27	3.42	2.88

\* Average of three replications;

\*\* Figures in parentheses are retransformed value.

formation of the fungus was obtained on Glucose sodium chloride agar and Richard's liquid medium, supported maximum mycelial growth, while Glucose sodium chloride medium produced highest number of sclerotia with maximum dry weight. Thus, the present findings are in agreement with the findings of above research workers.

#### Effect of pH

The fungus grew on a wide range of pH from 2.0 to 9.0 (Table 3). Significantly maximum dry mycelial weight was recorded at pH 5.0 (294.67 mg) and it was at par with

pH 4.0 (288.33 mg) followed by pH 3.0 (251.00 mg) after seven days of incubation. Least fungal growth was recorded at pH 2.0 (49.33 mg) followed by pH 9.0 (81.33 mg). Then after fourteen days of incubation, significantly maximum dry mycelial weight was recorded at pH 4.0 (644.33 mg) followed by pH 3.0 (485.00 mg) and pH 5.0 (470.33 mg). Least fungal growth was recorded at pH 9.0 (108.33 mg) followed by pH 2.0 (109.33 mg).

Significantly maximum number of sclerotia were formed at pH 6.0 (12.66) after seven day and fourteen

days (22.16) of incubation at  $25 \pm 2^\circ\text{C}$  temperature. This was followed by pH 5.0 (8.16) after seven days and fourteen days (16.16) of incubation. The highest sclerotial dry weight was recorded at pH 5.0 (185.50 mg) after seven days and after fourteen days (686.83 mg) of incubation. This was followed by pH 6.0 after seven days (164.67 mg) and after fourteen days (580.33 mg) of incubation. No sclerotial formation was recorded at pH 2.0, 8.0 and 9.0.

The fungus *S. sclerotiorum* studied by various workers differed in their pH requirement for growth and sclerotia formation. Jani (2) reported that *S. sclerotiorum*, the causal agent of stem blight of mustard grew best between 3.0 to 5.0 pH and pH 5.0 to 6.0 was optimum for higher number of sclerotia and maximum dry weight of the sclerotia. Kumar *et al.* (5) reported that pH range of 4.0 to 6.0 was found optimum for growth and formation of sclerotia of *S. sclerotiorum* from broccoli. Marukawa *et al.* (1975) obtained highest number of sclerotia at pH 4.0 to 6.0 of the substrate.

In the present study, the fungi differ in their pH requirements for growth at 4.00 and sclerotial formation was recorded at pH 6.0, while higher dry weight of sclerotia was recorded at pH 5.0. Sclerotial formation did not occur at pH 2.0, 8.0 and 9.0.

#### Effect of temperature

It is evident from the data in Table 4 that the significantly maximum growth (75.00 mm) was recorded at  $25^\circ\text{C}$  temperature followed by  $20^\circ\text{C}$  (72.00 mm) and  $15^\circ\text{C}$  (67.00 mm) after three days of incubation. Poor growth of the fungus was recorded at  $5^\circ\text{C}$  temperature (13.67 mm) and no growth at  $30^\circ\text{C}$  and  $35^\circ\text{C}$  temperature. Then after five days of incubation, maximum growth (90.00 mm) was recorded at  $25^\circ\text{C}$  temperature which was statistically at par with  $20^\circ\text{C}$  temperature (89.17 mm) and  $15^\circ\text{C}$  temperature (88.00 mm). Poor growth of the fungus was recorded at  $5^\circ\text{C}$  temperature (37.66 mm) and no growth at  $30^\circ\text{C}$  and  $35^\circ\text{C}$  temperature.

Significantly maximum sclerotia were recorded at  $15^\circ\text{C}$  temperature after seven days (26.00) and after fourteen days (35.67) of incubation. This was followed by  $20^\circ\text{C}$  temperature (34.00) and  $25^\circ\text{C}$  temperature (31.00) after fourteen days of incubation. The highest sclerotial dry weight was recorded at  $15^\circ\text{C}$  temperature after seven days (236.00 mg) and after fourteen days (429.00 mg) of incubation. This was followed by  $20^\circ\text{C}$  temperature (365.00 mg) and  $25^\circ\text{C}$  temperature (283.33 mg) after fourteen days of incubation. Fungus failed to proliferate at  $30^\circ\text{C}$  and  $35^\circ\text{C}$  temperature and failed to form sclerotia at  $5^\circ\text{C}$ ,  $30^\circ\text{C}$  and  $35^\circ\text{C}$  temperatures.

In liquid Richard's media, significantly maximum growth was obtained at  $25^\circ\text{C}$  temperature after seven days (684.00 mg) and after fourteen days (740.33 mg) of

incubation. This was followed by  $20^\circ\text{C}$  temperature (640.67 mg) and  $15^\circ\text{C}$  temperature (635.67 mg) after fourteen days of incubation. Significantly poor growth was recorded at  $35^\circ\text{C}$  (69.97 mg) which was at par with  $30^\circ\text{C}$  (80.00) after fourteen days of incubation (Table 5).

Significantly maximum numbers of sclerotia were formed at  $15^\circ\text{C}$  temperature after seven days (13.00) and after fourteen days (14.67) of incubation. This was followed by  $20^\circ\text{C}$  (12.67) and  $25^\circ\text{C}$  (9.00) after fourteen days of incubation. No sclerotial formation took place at  $5^\circ\text{C}$ ,  $30^\circ\text{C}$  and  $35^\circ\text{C}$  temperature after seven days of incubation, but few sclerotia were formed at  $5^\circ\text{C}$  (1.33) temperature after fourteen days of incubation.

The highest sclerotial dry weight was recorded at  $15^\circ\text{C}$  temperature after seven days (192.00 mg) and after fourteen days (331.33 mg) of incubation. This was followed by  $20^\circ\text{C}$  temperature (272.67 mg) and  $25^\circ\text{C}$  temperature (232.67 mg) after fourteen days of incubation. The present investigation revealed that the optimum temperature requirement of *S. sclerotiorum* for mycelial growth is certainly higher ( $25^\circ\text{C}$ ) than sclerotial formation ( $15^\circ\text{C}$ ).

Kheswala (3) reported  $20^\circ\text{C}$  to  $25^\circ\text{C}$  as optimum temperature for the growth of *S. sclerotiorum*. Bedi (1) reported highest number of sclerotia of *S. sclerotiorum* at  $15^\circ\text{C}$  temperature. Van Den Borg and Lentz (9) observed that the fungus died in a few days at  $35^\circ\text{C}$ . Naito and Sugimoto (6) observed a luxuriant growth of *S. sclerotiorum* at  $25^\circ\text{C}$  and slight at  $30^\circ\text{C}$ . Jani (2) reported that  $25^\circ\text{C}$  as optimum temperature for growth and  $15^\circ\text{C}$  for sclerotia formation of *S. sclerotiorum*. The growth and sclerotial formation of *S. sclerotiorum* was found at optimum temperature range of  $20^\circ\text{C}$  to  $25^\circ\text{C}$ , whereas maximum number and dry weight of sclerotia was at  $15^\circ\text{C}$  to  $20^\circ\text{C}$  (Sharma, 7 and Sharma and Kapoor, 8). Kumar *et al.* (5) reported that  $20^\circ\text{C}$  as optimum temperature for growth and  $20^\circ\text{C}$  to  $25^\circ\text{C}$  for sclerotia formation of *S. sclerotiorum*. Such variations in temperature requirement for mycelial growth and sclerotial formation should be attributed to the type of strains handled by the researchers.

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Received : Dec. 2011; Revised : Feb. 2012;  
Accepted : May 2012.