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Effect of different media, temperature, pH and illumination on the growth of *Fusarium oxysporum* f. sp. *zingiberi* causing wet rot of ginger

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Abstract
Laboratory studies were conducted to study the effect of different culture, media, pH and temperature levels on mycelia growth of *Fusarium oxysporum* f. sp. *zingiberi*. The fungus grew the best on Potato dextrose agar followed by Richards's agar among ten different culture media were tested. Growth of *F. oxysporum* was maximum at 25 °C (90.00 mm) after seven days of inoculation, which was reduced drastically below 15 °C and above 40 °C. The most suitable pH level for growth of fungus was 7 followed by 6.5 with 86.17 mm and 84.83 mm mycelia growth respectively. The growth of *F. oxysporum* f. sp *zingiberi* is maximum under continuous light compared with other alternate light and dark condition.

Keywords: *F. oxysporum* f. sp. *zingiberi*, various medium, pH, temperature, illumination

Introduction
Ginger (Zingiber officinale Rose) is one of the most important spices crops in India. It is grown throughout the country. In northern part of the country, the parmers cultivate it as a cash crop. Ginger has special significance for tropical countries, where it is produced and consumed in large quantities. It has medicinal value too. At present ginger is also being used for chewing purpose. In India, ginger is cultivated with an area of 1,68,000 ha, with the production of 10,76,000 MT and productivity of 6.4 MT/ha.

Materials and Methods
Isolates of *Fusarium oxysporum* f. sp. *zingiberi* were recovered from diseased ginger rhizomes from Bidar region during survey. Small pieces of discolored rotted rhizomes of diseased plants were placed on potato dextrose agar (PDA) and incubated at 25 ± 5 °C in the dark for four days. Culture was identified as *Fusarium oxysporum* f. sp. *zingiberi* by referring to the 'Illustrated genera of Imperfect fungi" (Barnett and Hunter, 1972) [2]. A single microconidial culture was prepared from each isolate. Studies of the following physiological aspects of *Fusarium oxysporum* f. sp. *zingiberi* were conducted in laboratory.

Effect of culture media
Following ten culture media were used to find out the most suitable one for the mycelial growth and sporulation. Each culture medium was prepared in 1 liter of water and autoclaved at 121.6 ºC at 15 psi for 20 min. These were cooled to 45 ºC and then poured in 90 mm Petri dishes for solidification.

1. Potato dextrose agar (PDA) medium (Peeled and sliced potato 200 g, Dextrose 20 g, Agar-agar 20 g).
2. Potato carrot agar (Agar-Agar 20.00 g, Dextrose 20.00 g, Carrot 100.00 g, Potato 100.00 g)
3. Host extract agar (Ginger rhizome 200.00 g, Dextrose 20.00 g, Agar-agar 20.00 g)
4. Corn meal agar (Maize 200 g, Dextrose 20 g, Agar – agar 20 g)
5. Oat meal agar (Oat meal powder 40.0 g, Agar-agar 20.0 g)
6. Richards's agar (RA) medium (Potassium nitrate 10 g, Potassium monobasic phosphate 5 g, Magnesium sulphate 2.5 g, Ferric chloride 0.02 g, Sucrose 50 g, Agar-agar 20 g).
7. Czapeks dox agar (CDA) medium (Sodium nitrate 2 g, Di potassium hydrogen phosphate 1g, Magnesium sulphate 0.5 g, Potassium chloride 0.5 g, Ferrous sulphate 0.01 g, Sucrose 30 g, Agar-agar 20 g).
8. Sabouraud's agar (Dextrose 40.00 g, Peptone 10.00 g, Agar- agar 20.00 g)

9. V8 juice agar (V8 juice 200 g, $CaCo_3$ 2 g, Agar agar 20 g)
10. Asthana and Hawker's medium (D-Glucose 5 g, Potassium nitrate 3.50 g, Potassium dihydrogen Phosphate 1.75 g, Magnesium sulphate 0.75 g, Agar-agar 20 g).

Effect of temperature

The temperature study was conducted by using suitable agar medium. For this purpose Petri plates (90 mm) containing 20 ml of the media was inoculated aseptically with uniform mycelial discs (9 mm diameter) taken from the margins of an actively growing culture of isolate and incubated in BOD at different temperatures *viz*., 15, 20, 25, 30, 35, 40 and 45 °C. Radial growth of the pathogen was recorded at eight days. Each treatment was replicated three times.

Effect of pH

The pH study was conducted using suitable agar medium. For this purpose Petri plates (90 mm) containing 20 ml of the media was inoculated aseptically with uniform mycelial discs (9 mm diameter) taken from the margins of an actively growing culture and incubated at different pH *viz*., 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8 respectively, using hydrochloric acid (0.1 N) or sodium hydroxide (0.1 N). The pH was measured using electrical pH meter and set before sterilization in autoclave. Radial growth and sporulation of the pathogen was recorded at eight days. Each treatment was replicated three times.

Effect of illumination

The growth was tested on the PDA in selected light conditions of continuous light, continuous dark, 6 hr alternate light and dark, 12 hr alternate light and dark, 24 hr alternate light and dark, 36 hr alternate light and dark and 48 hr alternate light and dark conditions. The light conditions were adjusted artificial light. From fifteen days old culture five mm mycelial disc of *F. oxysporum* f. sp. *zingiberi* was inoculated into plates containing 20 ml of PDA at different light conditions. Three replications were maintained. The plates after incubation at 27 ± 1 °C for 12 days, the mycelial growth and sporulation were recorded.

Results and Discussion

In case (Table 1) of *F. oxysporum*, maximum radial growth was observed in PDA (89.19 mm) followed by Richards agar (85.16 mm), Czapek's agar (84.46 mm). Minimum radial growth was observed in Corn meal agar (7.16 mm). Conidial production was maximum with the one having higher radial growth *i. e.,* on PDA. Varied mycelial characteristics were observed on the different media tested. On Potato dextrose agar mycelia was white to slight brownish coloured with fluffy growth. Where as in V8 juice agar medium uniform, fluffy dense mass of mycelium was observed and mycelium was cottony white in colour with smooth margin. Other colony characteristics were presented in Plate 1.

These results are in confirmation with findings of Rekha (2013) [9]. Who reported that maximum radial growth of *F. oxysporum* was observed in Potato dextrose agar, Czapek's, Richards's and Glucose asparagine agar and were also favoroured in producing conidia. Jamaria (1972) also reported maximum growth and sporulation of *F. oxysporum* f. sp. *vanillae* on Potato dextrose agar, Richard's agar and Czapek's dox agar. Khare *et al.* (1975) [6] reported maximum growth of *Fusarium oxysporum* f. sp. *lentis* on PDA followed by lentil extract and Richard's agar. Anjaneya Reddy (2002) [1], observed maximum growth of *F. udum* on Richard's agar and potato dextrose agar**.**

The pathogen, *F. oxysporum* f. sp. *zingiberi* grew well at temperature of 25 °C. The fungus grew at the temperature range of 15 °C – 45 °C. It was observed that at 25 °C and 30 °C, the fungus attained the maximum growth 90.00 and 89.67 mm respectively. With respect to sporulation, higher sporulation was noticed at temperature 25 °C and 30 °C in both the pathogen. While moderate sporulation observed at temperature of 20 °C and 35 °C. At temperature 15 °C, 40 °C and 45 °C less sporulation was recorded. Finally, the study revealed that 25 °C is most favorable temperature for the growth of the fungus followed by 30 °C. Reduced growth was observed at temperatures 15 °C, 35 °C and 40 °C whereas, there was moderate growth of the fungus at temperatures of 20 °C and least mycelial growth recorded at temperature 45 °C. The results are depicted in Table 2, and Plate 2.

The effect of temperature of *F. oxysporum* f. sp. *ciceris* was studied by Landa *et al.* (2001) [8]. They found the disease development was greater at 25 °C compared with 20 and 30 °C. Scott *et al.* (2010) [10] studied effect of temperature on *Fusarium* wilt of lettuce (*Lactuca sativa*), caused by *F. oxysporum* f. sp. *lactucae*, were observed to increase from 10 °C up to

Table 1: Effect of different solid media on mycelial growth and sporulation of *F. oxysporum* f. sp. *zingeberi*

Sl. No.	Media	Mycelial growth (mm)*	Mycelial character	Sporulation
1	V8 juice agar	80.35	White fluffy growth	+++
2	Czapek's agar	84.46	Fluffy white cottony growth	++
3	Potato dextrose agar	89.19	Sparse white slight brownish cottony growth	+++
4	Corn meal agar	7.16	Transparent white cottony growth	_
5	Host extract agar	33.07	white cottony radial growth	+
6	Oat meal agar	54.98	Sparse white cottony growth	+
7	Asthana and Hawks agar	81.39	White fluffy growth	++
8	Sabouraud's agar	50.23	White fluffy growth	+
9	Potato carrot agar	55.12	Pinkish white colony growth	+
10	Richards's agar	85.16	White cottony growth with irregular margin	+++
Mean		62.11		
S. Em± = 0.19			CD at 1% = 0.80	

*Mean of three replications

Sporulation: +++ - High ++ - Medium + - Low - - No

Table 2: Effect of temperature regimes (ºC) on radial growth and sporulation of *F. oxysporum* f. sp. *Zingeberi*

Sl. No.	Temperature level (ºC)	Mycelial growth (mm)*	Sporulation
1	15	31.41	+
2	20	72.73	++
3	25	90.00	+++
4	30	89.67	+++
5	35	43.41	++
6	40	35.00	+
7	45	20.03	+
Mean		54.60	
S. Em± = 0.40		CD at 1% = 1.69	

*Mean of three replications
Sporulation: +++ - High ++ - Medium + - Low - - No

Plate 1: Effect of different solid media on mycelial growth and sporulation of *F. oxysporum* f. sp. *zingeberi*

Plate 2: Effect of temperature regimes (ºC) on radial growth and sporulation of *F. oxysporum* f. sp. *zingeberi*

an apparent maximum near 25 °C. The aim of this work was to study the effect of temperatures ensure the elimination of *F. oxysporum* f. sp. *ciceri.* Results are in confirmation with Imran Khan *et al.* (2011) [4] showed the *F. oxysporum* f. sp. *ciceri* grew highest at 30 °C.

Growth and sporulation of the *F. oxysporum* f. sp. *zingiberi* was obtained at all the pH levels. In case of *F. oxysporum* f. sp. *zingiberi* mycelial growth was highest at pH 7.0 (86.17 mm) after 7 days of inoculation, which is on par with pH 6.5 (84.83 mm) and pH 7.5 (83.00 mm). At pH 8.0 mycelial growth recorded was 73.50 mm. While at pH 4.0 growth of mycelium recorded was 22.80 mm. Moderate mycelial growth was recorded at pH 5.5 (65.50 mm) and at pH 5.0 (51.17 mm) least growth was recorded. With respect to sporulation, higher sporulation recorded at pH 6.5, 7.0 and 7.5 while moderate sporulation showed at pH 5.5 and 5.0. At pH 4.0 no sporulation was recorded. Growth of the test fungus was decreased by increasing or decreasing the pH level from the 6.5 level. The foremost acidic and alkaline pH is not suitable for the growth and sporulation of pathogen. The results are depicted in Table 3 and Plate 3.

The results of the present study are in agreement with those achieved by Imran khan *et al.* (2011) [4] who also reported optimum pH for growth of *Fusarium oxysporum* f. sp. *ciceri* ranged from 6.5 to 7.0. Khilare and Ahmed, (2012) [7] reported most suitable pH level for growth of *Fusarium oxysporum* f. sp. *ciceri* was 6.0 and 6.5. Gangadhara *et al.*

(2010) [3] studied effect of pH levels on growth of *F. oxysporum* f. sp. *vanillae* isolates.

In case of *F. oxysporum* f. sp. *zingiberi* mycelial growth observation was taken at seven days after inoculation. The maximum mycelia growth seen in complete light (89.85 mm) followed by 48 h alternate light and dark (84.33 mm) and least mycelia growth seen in 6 h alternate light and dark (68.40 mm). The sporulation in case of *F. oxysporum* f. sp. *zingiberi* was maximum at complete light, 24 h alternate light and dark and lowest spoulation is seen in 6 h alternate light and dark and complete dark. The results are depicted in Table 4 and Plate 4.

The results revealed that maximum fungal growth and sporulation were observed under continuous light conditions. This might be due to more responsiveness of fungus under light conditions. Kausar *et al.* (2009) [5] found that continuous light was more suitable for maximum growth of *Fusarium solani* with colony diameter of 76.67 mm when exposed for seven days.

Table 3: Effect of different pH levels on mycelial growth and sporulation of *F. oxysporium* f. sp. *Zingeberi*

Sl. No.	pH levels	Mycelial growth(mm)*	Sporulation
1	4	22.80	_
2	4.5	42.83	+
3	5	51.17	++
4	5.5	65.50	++
5	6	75.50	++
6	6.5	84.83	+++
7	7	86.17	+++
8	7.5	83.00	+++
9	8	73.50	++
Mean		65.03	
S. Em± = 1.14		C.D. at 1% = 4.67	

*Mean of three replications

Sporulation: +++ - High ++ - Medium + - Low - - No

Table 4: Effect of illumination on mycelial growth and sporulation of *F. oxysporium* f. sp. *zingeberi*

Sl. No.	Treatments	Mycelial groth (mm)*	Sporulation
1	Complete light	89.85	+++
2	Complete dark	78.50	+
3	6 hrs alternate light and dark	68.40	+
4	12 hrs alternate light and dark	72.17	++
5	24 hrs alternate light and dark	78.40	+++
6	36 hrs alternate light and dark	81.53	++
7	48 hrs alternate light and dark	84.33	++
Mean		79.02	
S.Em± = 0.41		CD at 1% = 1.74	

*Mean of three replications

Sporulation: +++ - High ++ - Medium +- Low - - No

Plate 3: Effect of different pH levels on mycelial growth and sporulation of *F. oxysporium* f. sp. *zingeberi*

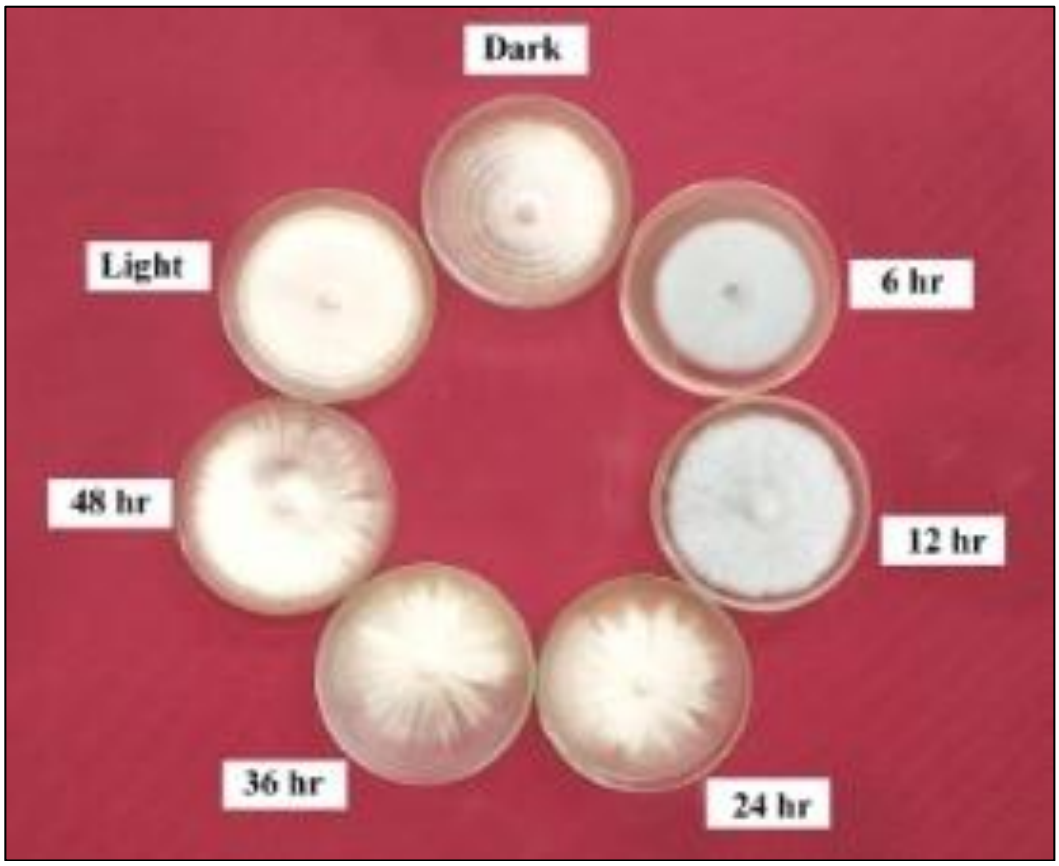

Plate 4: Effect of illumination on mycelial growth and sporulation of *F. oxysporium* f. sp. *Zingeberi*

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