



## Physiological studies of fungicide resistant and sensitive *Fusarium oxysporum f. sp. Spinaciae* U.N. Bhale

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*In vitro* studies were conducted on the effect of temperature, pH and light levels on mycelial growth and spore germination of *Fusarium oxysporum f. sp. spinaciae*. Sensitivity of 12 isolates were tested against Benomyl (50%WP) by food poisoning technique. Some of these isolates were resistant (NMU Fo 1) to fungicide whereas others were sensitive (Fo1). Radial growth and spore germination of fungicide resistant and sensitive isolates were checked by using Czapek Dox Agar (CZA) medium. The results revealed that radial growth and spore germination was maximum temp at 25 °C, followed by 15 °C. Maximum radial growth & spore germination was found at 6.5, followed by 7.5 pH. Continuous light and white light were found ideal for maximum radial growth and spore germination of *F. oxysporum*. It was seen that the radial growth, spore germination of resistant isolate was higher than that of the sensitive one.

**Keywords:** *Fusarium oxysporum f. sp. spinaciae*, physical factors, radial growth, spore germination.

### INTRODUCTION

Spinach (*Spinacea oleracea* L.) is one of the important vegetable crops. It is attacked by *Fusarium oxysporum f. spinaciae* (Sherb) Synder and Hansen, causing wilt of spinach is of the paramount importance in India. It is the member of family Chenopodiaceae and is related to Swiss chard, sugar beet and table beet. Although many diseases have been reported as externally and internally seed had borne (1, 2, 3, 4 & 5), which causes discoloration, spoilage of seeds, germination effects, rotting & wilting of seedlings, stem gall, blight, leaf spot, powdery mildew, downy mildew, rust and anthracnose. The disease is reported to be severe (6). Crop loss due to this disease is substantial as it leads to capital losses 20 to 60%. The disease is of great importance as the area under replanting and new clearing is ever increasing. Fungi in general can utilize a variety of physical factors for their growth and reproduction. Quantification of critical environmental factors responsible for initiation & subsequent spread of the disease. It is possible that the resistant pathogen may alter its physiological and biochemical characteristics. An attempt was made to study the optimum condition required for growth, and spore germination of sensitive and resistant *F. oxysporum* under laboratory conditions.

### MATERIALS AND METHODS

Wilt of spinach (*Spinacea oleracea* L.) caused by *Fusarium oxysporum f. sp. spinaciae*. Infected material was collected from various part of Marathwad region of Maharashtra. Twelve isolates were screened against Benomyl (50% WP) by food poisoning technique (7). Sensitive (Fo 1) and resistant (NMU Fo 1) isolates were used for environmental factors viz. tem, pH and light by using Czapek Dox Agar (CZA) medium. Radial growth of the fungus was measured on 2, 4, 6, 8, 10, and 12<sup>th</sup> day after inoculation and spore germination was also checked on 12<sup>th</sup> day of incubation. The 8 mm discs of 7 days old culture of sensitive and resistant isolates of *F. oxysporum* was inoculated in each experiment. Fresh culture of *F. oxysporum* was dispensed uniformly in sterilized distilled water in culture tube (1000-2050 spore / ml) to obtained 70-50 spores / microscopic field (400 x ) one ml of this spore suspension was mixed with one ml spore germination stimulant (glucose 0.1 %) (8).

### PHYSIOLOGICAL STUDIES

**Effect of temperature:** To test the optimum range of temp in the sterilized petridishes containing CZA culture medium. Sensitive (Fo 1) and resistant (NMU Fo 1) isolates of *F. oxysporum* were inoculated and incubated at different temperature viz., 0, 5, 10, 15, 25, 35 & 40°C for 12 days. Three replications were maintained for each

treatment to test for spore germination, the effect of temperature, cavity slides containing spore suspension were kept at different temp from 0 to 40 °C for 12 d for spore germination.

**Effect of pH:** Czapek Dox Agar was used as a basal medium to study the effect of pH on the growth of *F. oxysporum*. The pH of sterilized CZA medium was adjusted with 1 N HCL and 1 N NaOH at 4.5, 5.5, 6.5, 7.5, 8.5 and 9.5 before sterilization of medium. For pH, one ml of spore suspension was prepared by adding 1N HCL and 1N NaOH for each, and pH was adjusted as per said above, Two drops of this suspension were placed in a cavity slide and incubated at 28 ±1 °C for 12 d. .

**Effect of light:** For light, sterilized petridishes containing CZA medium wrapped with red, yellow, green blue and white cellophane sheets. The petridishes were kept at 28 ±1 °C for 12 days and observations on colony diameter were recorded. The effect of light colors on spore germination was studied in the growth chamber. Cavity slides containing spore suspension were kept in plastic petir plates lined with moistened blotter papers. The plates were exposed to different light colors viz., red, yellow, green, blue, white, continuous light and alternate light and darkness (12/12h). For this purpose petriplates containing cavity slides were wrapped with colored cellophane sheets and kept for 12 h in light and then 12 h in darkness and checked after 12 d.

#### Statistical analyses

Statistical analyses of the experiments were performed using the Handbook of Biological Statistics (9, 10).

## RESULTS AND DISCUSSION

**Effect of temperature:** Among the different temperature ranges, radial growth of *F. oxysporum f.spinaciae* was maximum at 25 °C followed by 15 °C. Poor growth was recorded at 0 & 5 °C. Higher temp was not favored for mycelial growth of test pathogen. Spores were germinated only at 15-25 °C and maximum was found at 25 °C (Table 1).

**Table No.1: Effect of temperature on mycelial growth and spore germination of *F. oxysporum*.**

Temp (°C)	Isolates	Radial growth (mm)	Spore germination (%)
0	S	21.66 (23.60)	00
	R	22.66 (24.75)	00
5	S	35.66 (40.23)	00
	R	37.00 (41.46)	00
15	S	44.66 (42.70)	10.00
	R	46.66 (54.02)	12.00
25	S	87.00 (99.21)	80.00
	R	90.00 (99.98)	87.00
35	S	00	00
	R	00	00
40	S	00	00
	R	00	00
CD (P=0.05)	S	34.32	
	R	34.48	

S = Sensitive, R = Resistant

Figures in parentheses are arcsine transformed value of radial growth.

**Effect of pH:** It is evident from the results that, *F. oxysporum* was found maximum radial growth at pH 6.5, followed by pH 7.5 (Table 2).

**Table No.2: Effect of pH on mycelial growth and spore germination of *F. oxysporum***

pH	Isolates	Radial growth (mm)	Spore germination (%)
4.5	S	18.66 (20.21)	05.00
	R	19.33 (20.21)	07.00
5.5	S	20.66 (22.46)	14.00
	R	23.00 (24.75)	17.00
6.5	S	84.00(97.44)	70.00
	R	89.00 (99.87)	74.00
7.5	S	48.66 (56.56)	30.00
	R	50.66 (59.12)	33.00
8.5	S	17.00 (17.95)	07.00
	R	19.00 (20.19)	08.00
9.5	S	14.00 (14.64)	05.00
	R	16.33 (16.86)	05.00
CD (P=0.05)	S	28.98	
	R	30.21	

S = Sensitive, R = Resistant

Figures in parentheses are arcsine transformed value of radial growth.

Least growth was found at lower and higher pH level. Spore germination also maximum at pH 6.5 than others.

**Effect of light:** Continuous and white light were enabled radial growth and spore germination of *F. oxysporum*. Continuous and white light showed a non significant difference. All the other light conditions reduced the mycelial and spore germination (Table 3).

**Table No.3: Effect of light on mycelial growth and spore germination of *F. oxysporum*.**

Light	Isolates	Radial growth (mm)	Spore germination (%)
Red	S	50.66 (59.12)	10.00
	R	51.66 (60.41)	11.00
Yellow	S	44.00 (50.20)	08.00
	R	45.00 (51.04)	10.00
Green	S	47.00 (54.01)	14.00
	R	48.00 (55.28)	16.00
Blue	S	42.66 (48.94)	15.00
	R	45.66 (52.74)	17.00
White	S	74.33 (87.85)	42.00
	R	80.66 (95.02)	44.00
Continuous	S	79.00 (93.15)	66.00
	R	88.00 (99.60)	69.00
CD (P=0.05)	S	16.89	
	R	20.21	

S = Sensitive, R = Resistant

Figures in parentheses are arcsine transformed value of radial growth

The results of the present studies are in agreement with those achieved the results (11). Similar findings were reported regarding temperature requirements to this fungus (12). Soil temperature relationship indicated that suitable temperature for development of chickpea wilt is 25-30°C (13). *Fusarium* fungus can tolerate a wide range of pH 5.0–6.5 (14). Growth of *F. oxysporum* was maximum at 30°C after seven days of inoculation, which was reduced drastically below 15°C and above 35°C and suitable pH level for growth of fungus was 7.0 and 6.0 (15). In general, the fungi grow well between pH 4.0-8.8, but pH of 6.4-7.6 has been found to be the best (16). Temp and pH are the two crucial components that influence the *in vitro* growth and various biological activities of microorganisms e.g. fungicide sensitivity (17). Similar results were reported that pH, temp and light parameters also influence the *in vivo* pathogenicity of *Ascochyta rabiei* (18). Maximum temp (20 °C), diffused light and 75% relative humidity were found ideal for maximum spore germination & germ tube length of uredospores of *Uromyces acori* (19). It was reported that darkness favors maximum uredospore germination of legume rust pathogen *Uromyces viciae-fabae* (20). Severity of the disease due to *Thielaviopsis paradoxa* was highest at 35 °C, while highest severity of *Fusarium semitectum* was recorded in the fruits at 30 °C (21).

## CONCLUSION

From the present study it was concluded that, tem 20 to 28°C, pH 6 to 7 and continuous and white light were enabled for radial growth and spore germination of *F. oxysporum*. The radial growth and spore germination of resistant isolate was higher than that of the sensitive one. Environmental factors were influenced by very significantly ( $P > 0.05$ ) as a part of its nutrition on the growth metabolism of the pathogen.

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