



EFFECT OF DIFFERENT MEDIA, PH AND TEMPERATURE ON THE GROWTH OF *FUSARIUM OXYSPORUM* F.SP. *CICERI* CAUSING CHICKPEA WILT

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ABSTRACT

Laboratory studies were conducted to study the effect of different culture, media, pH and temperature levels on mycelial growth of *Fusarium oxysporum* f.sp. *ciceri*. The fungus grew the best on Czapek dox agar and PDA media among six culture media were tested. The most suitable pH level for growth of fungus was 6.0 and 6.5 with 24.7 conidia/μl. Growth of *F. oxysporum* was maximum at 30°C (24.7 conidia/μl.) after seven days of inoculation, which was reduced drastically below 15°C and above 35°C.

KEYWORDS: *Fusarium oxysporum* f.sp. *ciceri*, various medium, pH, temperature, growth

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the world's third most important pulse crop and remained virtually stagnant over recent decades because of its susceptibility to wilt caused by *Fusarium oxysporum* f. sp. *ciceri*. Chickpea wilt is a major constrain to chickpea production globally. India accounts for approximately 75 percent of the world chickpea production (Singh, *et al.*, 2006). *F. oxysporum* f.sp. *ciceri* is a soil borne root pathogen colonizing xylem vessels, blocking them and causing wilting (Bateman *et al.*, 1996). The disease is important in dry and warm season. Although actual yield loss is estimated to be 10 to 12 percent globally (Nene and Reddy, 1987). Generally chickpea is cultivated as a rain fed crop in Maharashtra State and yield losses amounted to 10 to 15 percent (Khilare, *et al.* 2009). Present work depicts the role of different pH, temperature and media to understand

ecological survival of pathogen which will be helpful in management strategy and laboratory evaluation.

MATERIALS AND METHODS

Isolates of *F. oxysporum* f.sp. *ciceri* were recovered from diseased chickpea plants from Marathwada region during 2004 to 2007 (Table 1). Small pieces of discolored vascular tissue from lower part of the stems of diseased plants were placed on potato dextrose agar (PDA) and incubated at 25 ± 5°C in the dark for four days. Isolates were identified as *F. oxysporum* f.sp. *ciceri* by morphological criteria (Leslie and Summerell, 2006). A single microconidial culture was prepared from each isolate. Altogether 24 isolates were purified and tested for their virulence against susceptible variety JG-62 by using percent disease index (PDI) in field (Nene *et al.*, 1981). Studies of the following physiological aspects of *F. oxysporum* f. sp. *ciceri* isolates were conducted in laboratory.

TABLE 1. Survey of Chickpea wilt in Marathwada (2004 – 2007)

No. of villages surveyed	Area in acre	Percent wilt	Category against <i>F.oxysporum</i> f.sp. <i>ciceri</i>
48	123.7	9.64	Resistant
		16.42	Moderately resistant
		26.05	Susceptible

Effect of culture media

Following six 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 120°C at 15 psi for 20 min. These were cooled to 45°C and then poured in 8 cm Petri dishes for solidification.

1. Ashby's agar medium (Mannitol 20g, Di potassium phosphate 0.2g, Magnesium sulphate 0.2g, Sodium chloride 0.2g, Potassium sulphate 0.1g, Calcium

carbonate 5g, Agar-agar 15g, final pH (at 25°C) 7.4±0.2).

2. Asthana & Hawker's medium (D-Glucose 5g, Potassium nitrate 3.50g, Potassium dihydrogen phosphate 1.75g, Magnesium sulphate 0.75g, Agar-agar 20g).
3. Czapeks Dox agar (CDA) medium (Sodium nitrate 2g, Di potassium hydrogen phosphate 1g, Magnesium sulphate 0.5g, Potassium chloride 0.5g, Ferrous sulphate 0.01g, Sucrose 30g, Agar-agar 20g).

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4. Richards's agar (RA) medium (Potassium nitrate 10g, Potassium monobasic phosphate 5g, Magnesium sulphate 2.5g, Ferric chloride 0.02g, Sucrose 50g, Agar-agar 20g).
5. Potato Dextrose agar (PDA) medium (Peeled and sliced potato 200g, Dextrose 20g, Agar-agar 20g)
6. Martins Rose Bengal medium (Glucose 10g, Peptone 5g, Di potassium phosphate 0.5g, Potassium dihydrogen phosphate, Magnesium sulphate 0.5g, Rose Bengal 0.3g, Agar-agar 20g)

Effect of temperature and pH

Various temperatures were arranged in BOD incubator. The *F. oxysporum* f. sp *ciceri* was inoculated on Czapek Dox agar plates. The Petri plates were incubated for a week and radial growth was recorded every day. The pH

was adjusted in CZA medium by adding 1 N HCL and 1N NaOH. The *F. oxysporum* f. sp *ciceri* was inoculated at the center of the agar plates. The plates were incubated for a week in order to see the growth. Sporulation was calculated with the help of hemocytometer.

RESULTS

Survey of chickpea wilt

Chickpea is cultivated in Marathwada region as *rabi* crop. Table 1 depicts the survey of chickpea wilt in Marathwada. Altogether 48 villages were selected from 2004-05, 2005-06, 2006-07 for the survey of chickpea wilt. The fields showed 26.05 percent susceptible, 10.42 percent moderate and 9.64 percent minimum wilt.

TABLE 2. Effect of different pH on radial growth and sporulation of *F. oxysporum* f.sp. *ciceri*

pH	*Growth (mm)			Sporulation**
	5 DAI	7 DAI	9 DAI	Conidia/μl
4.0	07.00	08.00	09.00	03.00
4.5	25.00	35.00	45.00	11.60
5.0	40.00	63.00	72.00	19.00
5.5	59.00	71.00	76.00	20.30
6.0	66.00	75.00	89.66	24.70
6.5	54.00	68.00	79.00	21.60
7.0	48.00	57.00	68.00	18.36
7.5	36.00	51.00	63.00	17.10
8.0	32.00	45.00	57.00	15.40
P= 0.05	08.85	16.91	22.39	06.17
0.01	13.40	25.61	33.90	09.34

*Average of three replications,

**Average sporulation per microscopic field

DAI- Days after inoculation

Effect of pH

It was depicted in Table 2. The effect of pH is observed highest at 6 with sporulation 24.70 conidia/μl. However it was found that the range from 4.5 to 8.0 is suitable for the growth of *F. oxysporum* f. sp. *ciceri*. The foremost acidic and alkaline pH is not suitable for the growth of pathogen.

Effect of temperature

Growth of *F. oxysporum* f. sp. *ciceri* isolates was studied from 10 to 40°C. The result are presented in Table 3. It was seen that there was quite a large variation in the growth of these isolate at different temperature after 9 days. Temperatures from 25 to 35°C were most favorable for the growth of these isolates. The highest growth of pathogen was recorded at 30°C with higher sporulation 27.90 conidia/μl.

TABLE 3. Effect of different temperature on radial growth and sporulation of *F. oxysporum* f.sp. *ciceri*

Temp. °C	*Growth in (mm)			Sporulation**
	5 DAI	7 DAI	9 DAI	Conidia/μl
10	07.00	15.66	19.66	08.10
15	15.66	36.33	54.33	13.70
20	20.23	49.00	68.66	17.20
25	27.66	50.33	77.33	24.30
30	36.66	71.66	89.66	27.90
35	21.66	58.33	73.66	21.80
40	09.33	23.00	28.33	11.50
P= 0.05	13.30	15.32	17.23	04.62
P= 0.01	19.32	22.28	25.06	06.72

*Average of three replications, **Average sporulation per microscopic field, DAI- Days after inoculation

Effect on different media

A total of six media were used for studying the growth of twenty four isolates. The results are given in Table 4. On an average it was noted that Ashby's agar (Max. growth 82 mm with mean 66.3 mm), Asthana & Hawker's (Max. growth 88 mm with mean 77.9 mm), Richard agar medium (Max. growth 88 mm with mean 72.5 mm) and Martins Rose Bengal agar medium (Max. growth 83 mm with mean 69.7 mm) showed maximum growth only in one isolate namely, Nagpur, Parbhani, Ahmednagar and

Nanded respectively. Czapek's Dox agar medium and Potato Dextrose agar medium was more favorable for the growth of *F. oxysporum* f. sp. *ciceri* than other medium. The isolates from Ahmednagar, Amrawati, Beed, Nashik, Parbhani and Osmanabad showed higher growth. The highest growth was observed in Czapek's Dox agar medium (90 mm). Martins Rose Bengal agar was less favorable for the growth of many isolates (mean growth 69.7mm).

TABLE 4. Effect of solid media on growth of *FOC* from different districts of Maharashtra State

FOC Isolate	Location	Radial growth of colonies on different media (mm)*					
		Ashby's agar	Asthana & Hawker's	CZA	Richard agar	PDA	Martins Rose Bengal
1	Ahmednagar	64	85	90	88	81	72
2	Akola	68	70	84	65	65	72
3	Amrawati	63	82	90	71	78	69
4	Aurangabad	62	83	87	75	91	61
5	Beed	70	78	90	70	75	66
6	Buldhana	71	77	79	72	77	79
7	Dhule	67	74	87	73	77	75
8	Hingoli	61	73	72	68	59	66
9	Jalgaon	70	77	85	65	73	86
10	Jalna	40	74	71	71	55	56
11	Kolhapur	70	57	89	78	72	50
12	Latur	72	73	85	71	54	57
13	Nagpur	82	83	88	74	76	69
14	Nanded	67	75	88	72	75	83
15	Nashik	73	87	90	72	90	71
16	Osmanabad	76	79	88	81	90	81
17	Parbhani	63	88	90	77	90	61
18	Pune	75	85	83	81	77	71
19	Sangli	60	86	79	79	70	73
20	Satara	66	79	88	64	83	81
21	Solapur	64	72	81	71	72	78
22	Wardha	69	83	83	55	87	71
23	Washim	71	83	88	71	79	71
24	Yavatmal	73	68	81	72	55	55
	Mean	67.3	77.9	84.8	72.3	75	69.7

*Average of three replications

DISCUSSION**Effect of different media**

These results were in confirmation with Ingole (1995) who reported that PDA and Richard's agar supported best mycelial growth of *F. udum*. Jamaría (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) reported maximum growth of *Fusarium oxysporum* f. sp. *lentis* on PDA followed by lentil extract and Richard's agar. Anjaneya Reddy (2002) observed maximum growth of *F. udum* on Richard's agar and potato dextrose agar. Gangadhara, *et al* (2010) studied effect of temperature on growth of *F. oxysporum* f. sp. *vanillae* isolates. The fungus showed best growth on Richard's agar and potato dextrose agar media. Maximum growth was at 25°C after seven days of inoculation, which was reduced drastically below

15°C and showed zero growth at 40°C. The most suitable pH level for growth of fungus was 5.0 and 6.0. Recently Imran Khan *et al.*, (2011) studied effect of media on *F. oxysporum* f.sp. *ciceri* and found that PDA is best for the growth of different isolates. The present study indicated that Czapek's Dox agar and potato dextrose agar were best medium for growth of *F. oxysporum* f.sp. *ciceri*.

Effect of different pH:

Effect of pH are in confirmation with the findings of Moore (1924) who reported that two strains of *F. coeruleum* could tolerate a pH range of 3.0 to 11.0. The studies conducted by Jamaría (1972) on *F. oxysporum* f. sp. *nivium* indicated that, as the pH decreases or increases from the optimum, the rate of amount of growth gradually decreases. Gangadhara, *et al* (2010) studied effect of pH levels on growth of *F. oxysporum* f. sp. *vanillae* isolates. The fungus showed best growth pH at 5.0, Least growth of

all the isolates was recorded at 9.0 pH. Imran Khan *et al.*, (2011) showed optimum pH for growth of *F. oxysporum* f.sp. *ciceri* ranged from 6.5 to 7.0.

Effect of different temperature

Studies conducted by Chi and Hansen (1964) indicated that *F. solani* isolates grew well at higher temperature of 28°C. the fungus grew at the temperature range of 10–35°C. However, growth of the fungus was drastically reduced below 15°C and started to decline above 30°C and become zero at 40°C, as these temperatures did not favour for growth of the fungus. It was observed that at 25°C and 30°C, the fungus attained the maximum growth 76.8 and 85.4 mm while at 25°C, it was 59.3 mm after seven days of inoculation. Soil temperature relationship indicated that suitable temperature for development of chickpea wilt is 25-30°C. Gupta *et al.* (1986) reported similar findings regarding temperature requirements to this fungus. These studies are in confirmation with Anjaneya Reddy (2002) who reported that growth of 40 isolates of *F. udum* differed in their temperature requirement which varied from 20°C to 35°C. The effects of temperature of *F. oxysporum* f. sp. *ciceris* was studied by Landa *et al.* (2001). They found the disease development was greater at 25°C compared with 20 and 30°C. Scott, *et al.* (2010) studied effect of temperature on *Fusarium* wilt of lettuce (*Lactuca sativa*), caused by *F. oxysporum* f. sp. *lactucaae*, were observed to increase from 10°C up to 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) showed the *F. oxysporum* f.sp. *ciceri* grew highest at 30°C.

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