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Morphological and physiological variation of *Fusarium oxysporum* f. sp. *ciceri* isolates causing wilt disease in chickpea

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***Abstract*—** *Nine isolates of Fusarium oxysporum f. sp. ciceri infecting chickpea were collected from major chickpea growing areas of Bangladesh and their cultural, morphological, physiological and pathogenic characteristics were described. The isolates varied significantly in their cultural, morphological and physiological traits, i.e. colony color, shape, margin and texture; mycelial radial growth and spore production. Laboratory studies were conducted to study the effect of different culture media, pH and temperature levels on mycelial growth and sporulation of Fusarium oxysporum f. sp. ciceri. Mycelial radial growth and sporulation of F. oxysporum was maximum for all the isolates at 25°C after seven days of inoculation, which was reduced drastically below 15°C and above 35°C. No growth and sporulation was observed at 5 °C temperature for all the isolates. The most suitable pH level for growth and sporulation of the fungus was at pH 6.0. The fungus grew well on oat meal agar medium among seven culture media tested. No sporulation was observed on WA medium. The highest number of macro spores (3.27 x 10^5 ml^{-1}) and micro spores (4.06 x 10^5 ml^{-1}) were produced on PDA. Among the nine tested isolates, only one isolate (FOC-1) found to be highly virulent (HV) type on reaction on chickpea variety BARI Chola –1.*

Keywords*— *Fusarium oxysporum, variation, morphology, physiology, pathogenicity.

I. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important grain legume crop in the world and first in the Mediterranean basin and South Asia (Saxena, 1990). Chickpea is a cool season food legume crop grown on 10 million ha in 45 countries in the world and producing 93,13,043 tones of grain in the world (FAO, 2008). Chickpea is considered as one of the most important legume crops in Bangladesh. Despite of the large area under chickpea cultivation in the world, the total production and productivity are quite low in most of the chickpea growing areas (Pande *et al*., 2006). The climate and agro-ecological conditions of South Asian countries including Bangladesh favors the rapid growth and development of various plant pathogens (Ahmed, 1996). So, vulnerability of chickpea plant to a number of fungal pathogens from seedling stage to maturity is the primitive cause of low yield. Although a number of biotic and abiotic factors contribute for low chickpea production but endemic occurrence of wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri* is of significant importance. Chickpea is reported to be affected by more than 52 pathogens (Nene *et al*., 1984). Among these, wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is a wide spread soil borne diseases, and is reported from many parts of India with intensity ranging from 10 to 100 percent (Singh *et al*., 1986). *Fusarium* wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* is an important disease in many chickpea growing areas. This fungus is able to survive in the soil for long period of time by forming resting spores, thick walled reproductive structures. The wilt pathogen is soil-borne and survives through chlamydospores in seed and dead plant debris in soil (Haware *et al*., 1978). Since, the fungus can survive in the soil for several years; it is not possible to control the disease through normal crop rotations. Although a number of chickpea lines have been reported as resistant to wilt from different countries of the world (Nene *et al*., 1981), but their success has been highly localized due to location-specific races of the pathogen (Singh and Reddy, 1991). It is important to know which isolate to use in the screening process, how the resistance is expressed and inherited. In view of the above facts, the present research work was aimed to carry out comprehensive investigation on the cultural, morphological, physiological and pathogenic variation of *Fusarium oxysporum* f. sp. *ciceri*.

II. MATERIALS AND METHODS

Isolation and identification of the pathogen

Wilt infected plant samples were collected from nine locations covering four chickpea growing districts of Bangladesh. The pathogens that causes wilt disease in chickpea were isolated using tissue culture techniques. The infected chickpea roots were washed and placed into petriplates containing PDA media and incubated at 25 °C under near ultraviolet (NUV) light following ISTA rules (ISTA, 1996). Seven days after incubation, the fungal culture were studied under stereoscopic (Model: Olympus, SZ 61, Japan) and compound microscope (Model: Olympus, CX 21 FSI, Tokyo, Japan) for identification of the desired pathogens. Then the pathogen purified by single spore culture technique, preserved in PDA slants at 4 °C for further study.

Morphological variability

Nine isolates of *Fusarium oxysporum* f. sp. *ciceri* isolates were observed on PDA medium after 7 days of inoculation on the basis of colony color, shape, texture, margin, conidial color, size, shape and color of conidiophores.

Effect of culture media on radial mycelial growth of Fusarium oxysporum f. sp. ciceri

Seven culture media viz. potato dextrose agar (PDA) medium (Slice potato–200 g, dextrose–20 g, agar–20 g and distilled water– 1000 ml), Czapek'sdox agar (CDA) medium (Sucrose – 30 g, sodium nitrate – 2 g, di-potassium phosphate – 1 g, magnesium sulphate – 0.5 g, potassium chloride – 0.5 g, ferrous sulphate – 0.01 g, agar – 15 g and distilled water – 1000 ml), malt extract dextrose agar (MDA) medium (Malt extract – 20 g, peptone – 2 g, dextrose – 20 g, agar – 20 g, and distilled water – 1000 ml), corn meal agar (CMA) medium (Corn meal infusion form–50 g, agar–15 g and distilled water–1000 ml), oat meal agar (OMA) medium (Oat meal – 60 g, agar – 12.5 g and distilled water – 1000 ml), V_8 juice agar (V_8JA) medium [V_8 juice (100 ml) – 8.3 g, L-asparagine – 10 g, yeast extract – 2 g, calcium carbonate –2 g, glucose –2 g, agar –20 g and distilled water – 1000 ml)] and water agar (WA) medium (Agar – 20 g and distilled water – 1000 ml) were used to find out the most suitable one for the mycelia growth of the fungus.

Effect of temperature on radial mycelial growth and sporulation of Fusarium oxysporum *f. sp.* ciceri

The fungus was inoculated in PDA media using seven different levels of temperature viz., 5, 10, 15, 20, 25, 30 and to determine the temperature effect on radial colony growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri*.

Effect of pH levels radial mycelial growth of Fusarium oxysporum *f. sp.* ceceri

The isolates were inoculated in PDA medium having six pH levels viz., 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 in 9 cm diameter glass petriplates and incubated at 25±0.5 °C with alternating 12 hours of light and 12 hours of dark period in an incubator. The different level of pH were maintained by adding 0.1 N NaOH or 0.1N HCl.

For all the tests, 16 ml of medium were poured into each Petri plates using media dispenser having three replications. The medium autoclaved at 121 °C for 30 minutes at 15 PSI and then allowed for solidification in laminar airflow cabinet. Five mm diameter of mycelial disc were cut from the periphery of 7 days old culture of *Fusarium oxysporum* f. sp. *ciceri* with the help of a flame sterilized cork borer and then transferred into the centre of the petriplates containing solidified PDA medium. Then the plates were placed in an incubator maintaining required temperature level for temperature study. Data were noted on mycelial radial growth of *Fusarium oxysporum* f. sp. *ciceri* after two day of incubation till covering the entire petriplates of any isolates. The number of spores of *Fusarium oxysporum* f. sp. *ciceri* on different temperature and pH levels were counted using haemacytometer after 7 days of incubation. One (1) ml distilled water was poured in each test-tube and 5mm block of *Fusarium oxysporum* f. sp. *ciceri* isolates were put into the test-tube. Then the test-tubes were shaken by vortex shaker. After shaking, spores were counted using haemacytometer. The spore counting process was repeated 10 times of each replication.

Pathogenic variability

Plastic pots (8×10 cm) were used to grow chickpea plants in the pot house of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur. The pots were filled with 500 gm sterilized soil with well decomposed organic matter. In order to get a huge amount of inocula of *Fusarium oxysporum* f. sp. *ciceri*, isolates were sub-cultured on PDA medium and incubated for 10 days. One pertriplate (90 mm) full of inocula (mycelial mat and spores) were scraped by a plastic scrapper, wrapped with alluminium foil and preserved in the room temperature. Previously prepared inocula were incorporated into the sterilized soil. Five seeds of BARI Chola-1 were sown in each pot having three replications. Prior to sowing seeds were surface sterilized with Clorox (0.1% available chlorine) for 50 seconds and were rinsed by sterile distilled water for three times. The pots were kept in the net house of the Plant Pathology Division, BARI. Wilt incidence were recorded at 30, 45 and 60

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DAI but aggressiveness of the tested isolates were measured considering wilt incidence only at 60 DAI (days after inoculation). Koch's postulates were proved and pathogenic nature of each isolate was established.

III. RESULTS AND DISCUSSION

Morphological variability

Fusarium oxysporum f. sp. *ciceri* exhibited variations in colony characteristics such as color, shape, margin and texture. Colony colors were purplish white, whitish orange, creamy white, cottony white. Colony shapes were irregular, regular, regular with sector, regular without sector. Colony margins were irregular, entire and wavy. Colony textures were fluffy, flat/velvet (Table 1). In past studies various type of pigmentations (yellow, brown, crimson) in culture has been recorded (Saxena and Singh, 1987). Chauhan (1962) found variation among 22 isolates with respect to their mycelium type, colony colour, toxin production and pathogenicity. *Fusarium* wilt isolates were highly variable in their colony growth pattern, size of colony and pigmentations. The current findings were well supported by Dubey *et al*., (2010). In this experiment it was observed that the length of micro conidia varied from 5.00-14.00 µm. The breadth of micro conidia was 1.00-4.00 µm. Micro conidia was 0-2 septed. The length and breadth of macro conidia ranged from 9.00-26.00 µm and 1.00-5.00 µm respectively. The number of septation of macro conidia ranged from 1-5. *F. oxysporum* f. sp. *ciceri* showed variations in the size of micro and macro-conidia of 9 isolates with three replications was also studied. The largest size of the micro-conidia was obtained from the isolate Foc-14 (3.7× 4.5, 3.1 × 5.0 µm) and the smallest size was from isolates FOC-21 (3.0 × 3.7 µm). Whereas, the biggest size 7.5× 20.10 µm of the macro-conidia was obtained from the isolates Foc-25 and the smallest size of 3.5× 22.5 µm conidia were obtained from isolates Foc-11(Table 1).

Effect of temperature on mycelial radial growth and sporulation

As evident from Fig. 1, the fungus grew at the temperature range of 10–35°C. Maximum growth was found between 25°C and 30 °C for all the 9 isolates after 7 days of incubation. At 25°C maximum colony diameter (78.00 mm) was obtained in isolate FOC-2 followed by FOC-9 (76.67 mm). The lowest colony growth (9.66 mm) was noted at 35 °C in FOC-2. The present findings agreed with the findings of Farooq *et al*., (2005). They reported that the growth of the *F. oxysporum* f. sp. *ciceri* was drastically reduced below 15 °C and started to decline above 35 °C, as these temperatures did not favor for growth of the fungus. It was observed that at 25°C and 30°C, the fungus attained the maximum growth of 76.8 and 85.4 mm while at 15°C, it was 59.3 mm after seven days of inoculation. No growth was observed at 5 °C. The highest (6.78 x 10^5 ml^{-1}) sporulation of micro conidia was observed in FOC-3 at 25 °C followed by FOC-6 (6.00 x 10^5 ml^{-1}); FOC-1 (5.13 x 10^5 ml^{-1}) and FOC-7 (3.70 x10^5 ml^{-1}) after seven days of incubation period. The minimum (3.30 x 10^3 ml^{-1}) sporulation was observed in FOC-4 at 15 °C. Spore production was not observed in isolates at 5 °C in FOC-1 and FOC- 2 at10°C, in FOC-9 at 15°C and in FOC-4, FOC-5, FOC-7 at 35 °C (Table 2).The maximum (3.43 x 10^6 ml^{-1}) sporulation of macro conidia was observed in FOC-1 followed by FOC-6 (6.66 x 10^5 ml^{-1}) and FOC-9 (5.58 x 10^5 ml^{-1}) at 25 °C after seven days of incubation period. The minimum (1.66 x 10^3 ml^{-1}) sporulation was observed in FOC-5 and FOC-8 at 15 °C and FOC-2 (1.66 x 10^3 ml^{-1}) at 35 °C. All the nine isolates failed to produce any spore at 5 °C temperature (Table 3). Abundant sporulation of this fungus was found after seven days of incubation at 27±2 °C on potato dextrose agar medium (Barhate, 2006). This observation supports the result obtained from this study. Khilare and Rafi Ahmed (2012) stated the highest growth of pathogen was recorded at 30 °C with higher sporulation 27.90 conidia μl^{-1} and after seven days of incubation, which was reduced drastically below 15 °C and above 35 °C. Chauhan (1963) and Desai et al., (1994) found that 25 °C is the optimum temperature for growth of Fusarium wilt. Similarly, Sharma et al., (2005) verify that a temperature around 25 °C is optimum for disease development. While, Mina and Dubey (2010) observed maximum colony diameter (85 mm) at 28 °C. From this experiment, it appeared that 25 °C temperature is suitable for mycelial radial growth and spore production of *Fusarium oxysporum* f. sp. *ciceri*.

Table.1: Morphological variability in the isolates of Fusarium oxysporum f. sp. ciceri

Isolates	Cultural characters				Dimension and septation					
					Micro conidia			Macro conidia		
	Color	Shape	Margin	Texture	Length (µm)	Breadth (µm)	Septation	Length (µm)	Breadth (µm)	Septation
FOC 1	Purplish white	Irregular	Irregular	Fluffy	6-14	2-4	0-1	12-25	1.5-5	3-5

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FOC 2	Whitish orange	Regular	Entire	Fluffy	6-14	1.5-3	0-2	11-25	2-4	2-5
FOC 3	Creamy white	Regular	Entire	Flat/Velvet	5-10	1-3	0-1	9-15	2-3	1-4
FOC 4	Creamy white	Regular without sector	Wavy	Flat/Velvet	5-9	1.5-3	0	10-18	2-3	1-4
FOC 5	Cottony white	Regular without sector	Wavy, entire	Flat/Velvet	6-8	1.5-3	0-1	11-25	2-4	1-4
FOC 6	Creamy white	Regular	Wavy	Flat/Velvet	6-11	1.5-3	0-1	12-25	1-4	2-5
FOC 7	Whitish orange	Irregular	Irregular	Fluffy	5-12	1-3	0-1	15-26	2-5	2-5
FOC 8	Cottony white	Regular with sector	Wavy, entire	Fluffy	7-11	1-3	0-1	12-25	2-4	2-5
FOC 9	Cottony white	Irregular	Irregular	Fluffy	5-10	1-3	0-1	11-16	2-3	1-3

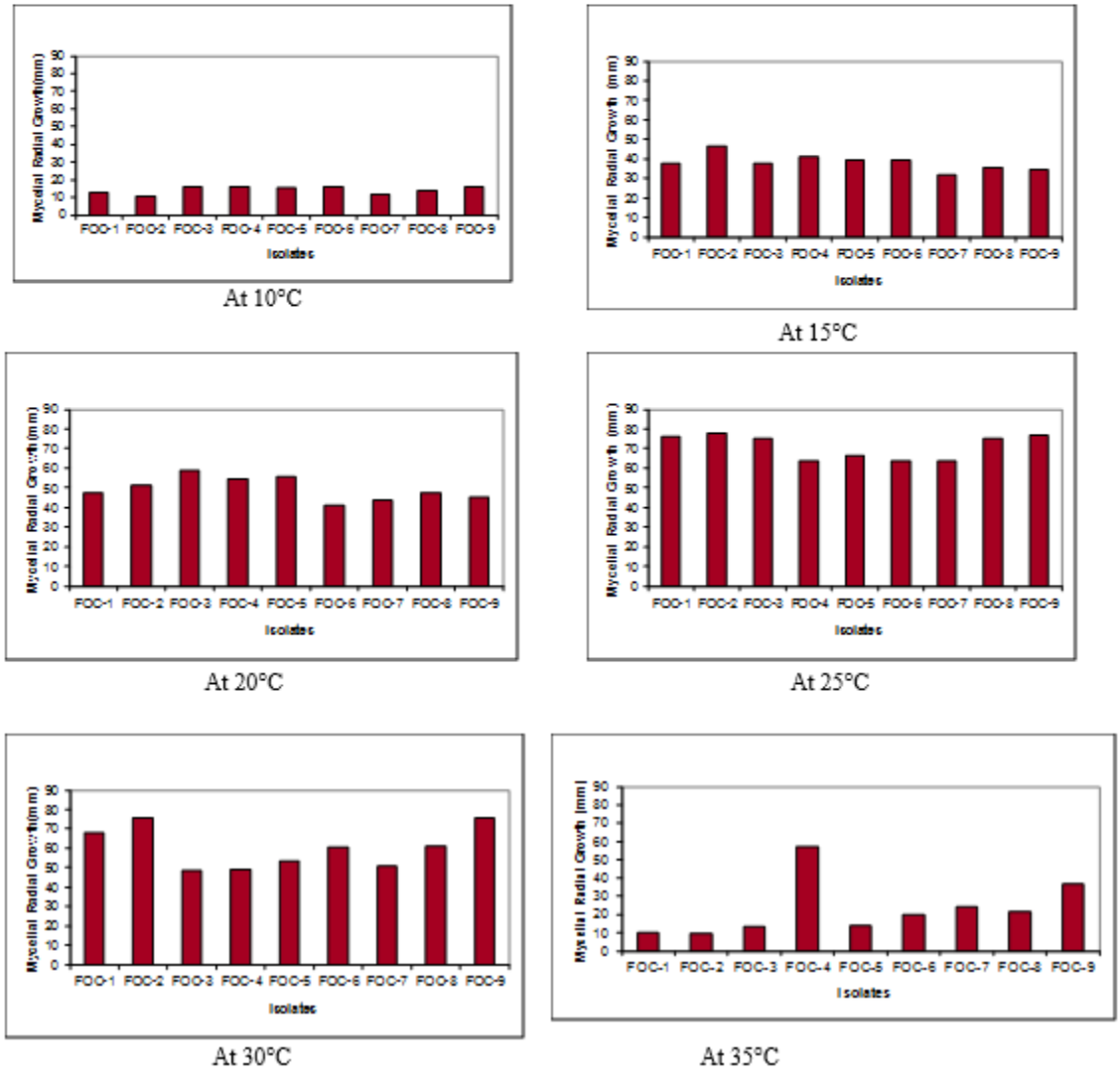

Fig.1: Radial mycelial growth of Fusarium oxysporum f. sp. ciceri at different temperature (°C) levels.

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Table.2: Effect of temperature on production of micro conidia of nine Fusarium oxysporum f. sp. ciceri isolates

Isolates	Production of micro conidia (ml^{-1}) at different temperature levels (°C)						
	5	10	15	20	25	30	35
FOC-1	*	*	3.33 $x10^4$	6.38 $x10^4$	5.13 $x\ 10^5$	1.12 $x\ 10^5$	3.38 $x\ 10^4$
FOC-2	*	*	2.61 $x10^4$	7.33 $x10^4$	1.66 $x\ 10^5$	9.38 $x\ 10^4$	5.61 $x\ 10^4$
FOC-3	*	**	6.27 $x10^3$	8.25 $x\ 10^3$	6.78 $x\ 10^5$	3.86 $x\ 10^4$	2.71$x10^4$
FOC-4	*	**	3.30 $x\ 10^3$	1.00 $x10^4$	1.25 $x\ 10^5$	1.83 $x10^4$	*
FOC-5	*	**	4.00 $x10^4$	1.36 $x\ 10^5$	3.63 $x\ 10^5$	2.98 $x\ 10^5$	*
FOC-6	*	**	3.66 $x10^4$	1.01 $x\ 10^5$	6.00 $x\ 10^5$	3.60 $x\ 10^5$	1.83 $x10^5$
FOC-7	*	**	5.33 $x\ 10^4$	2.85 $x\ 10^5$	3.70 $x10^5$	1.38 $x10^5$	*
FOC-8	*	**	4.16 $x10^4$	7.66 $x\ 10^4$	2.61 $x\ 10^5$	8.50 $x10^4$	3.83 $x\ 10^4$
FOC-9	*	**	*	1.16 $x\ 10^4$	9.66$x10^4$	5.16 $x\ 10^4$	8.33 $x\ 10^3$

* No sporulation
** Very few sporulation

Table.3: Effect of temperature on production of macro conidia of nine Fusarium oxysporum f. sp. ciceri isolates

Isolates	Production of macro conidia (ml^{-1}) at different temperature levels (°C)						
	5	10	15	20	25	30	35
FOC-1	*	**	2.11×10^4	5.22×10^4	3.43 $x\ 10^6$	1.64 $x\ 10^5$	1.33 $x\ 10^4$
FOC-2	*	**	2.44 $x\ 10^4$	7.33 $x\ 10^4$	1.56 $x\ 10^5$	1.03 $x\ 10^5$	1.66 $x\ 10^3$
FOC-3	*	*	6.44 $x\ 10^3$	1.06 $x\ 10^4$	4.33 $x\ 10^4$	1.97 $x\ 10^4$	*
FOC-4	*	**	2.50 $x\ 10^4$	8.66 $x\ 10^4$	1.33 $x\ 10^5$	2.66 $x\ 10^4$	1.66 $x\ 10^4$
FOC-5	*	*	1.66 $x\ 10^3$	3.33 $x\ 10^3$	2.00 $x\ 10^4$	1.00 $x\ 10^4$	*
FOC-6	*	*	*	6.66 $x\ 10^3$	6.66 $x\ 10^5$	1.66 $x\ 10^3$	*
FOC-7	*	**	*	1.00 $x\ 10^4$	2.70 $x\ 10^5$	3.33 $x\ 10^3$	3.33 $x\ 10^3$
FOC-8	*	**	1.66 $x\ 10^3$	3.33 $x\ 10^3$	3.83 $x\ 10^4$	*	*
FOC-9	*	*	5.33 $x\ 10^4$	1.73 $x\ 10^5$	5.58 $x\ 10^5$	2.61 $x\ 10^5$	6.33 $x\ 10^4$

* No sporulation
** Very few sporulation

Effect of pH on mycelial radial growth and sporulation

The results of this experiment indicated that luxuriant radial growth exhibited in all of the isolates at pH 6.0 and pH 6.5 (Fig. 2). The highest colony diameter was noted for the isolate FOC-2 at pH 6.0 (87.83 mm) followed by FOC-1 (86.17mm) at pH 6.0 and FOC-8 (84.50 mm) at pH 6.0. The lowest mycelial radial growth was recorded in isolate FOC-1 (24.83mm) at pH 4.5. Farooq *et al*., (2005) reported that *F. oxysporum* f. sp. *ciceri* can grow well at pH 7 where the radial growth was 80 mm after seven days of inoculation. They also observed that the growth of the fungus decreased by increasing or decreasing the pH level from the neutral level. Imran Khan *et al*., (2011) showed optimum pH for growth of *F. oxysporum* f. sp. *ciceri* ranged from pH 6.5 to 7.0. *F. oxysporum* f. sp. *ciceri* has ability to tolerate pH 5.0–6.5, at a wide range (Shaikh, 1974). Maximum (3.03 x 10^5 ml^{-1}) micro conidia was produced by FOC-7 at pH 6.0 followed by FOC-5 (1.86 x 10^5 ml^{-1}) and minimum sporulation was observed on FOC-3 (8.87x 10^3 ml^{-1}) at pH 4.5 after seven days of incubation period (Table 4). Maximum (7.06 x 10^5 ml^{-1}) macro conidia were produced by FOC-9 at pH 6.0 and minimum sporulation was observed on FOC-6 (1.66 x 10^3 ml^{-1}) at pH 4.5. No macro conidia was produced by FOC-9 at 4.5; FOC-7 and FOC-8 at pH 6.5 and FOC-4, FOC-5 and FOC-8 at pH 7.0 (Table 5). Khilare and Rafi Ahmed (2012) reported that the highest sporulation of *F. oxysporum* f. sp. *ciceri* was 24.70 conidia μl^{-1} at pH 6.0. T. Swati and P. Rajan (2014) found that the Maximum sporulation of the macro conidia and micro conidia was observed at pH 6.5 (5.06 and 122.4 spore/100 mL of medium respectively) and minimum sporulation occurred in pH 2.0 (0.47 and 2.42 spore/100 mL of medium respectively). Chaudhary (1971) and Prasad *et al*. (1992) reported 6.0 pH level as the best for the growth and sporulation of *Fusarium moniliforme v subglutinanse*.

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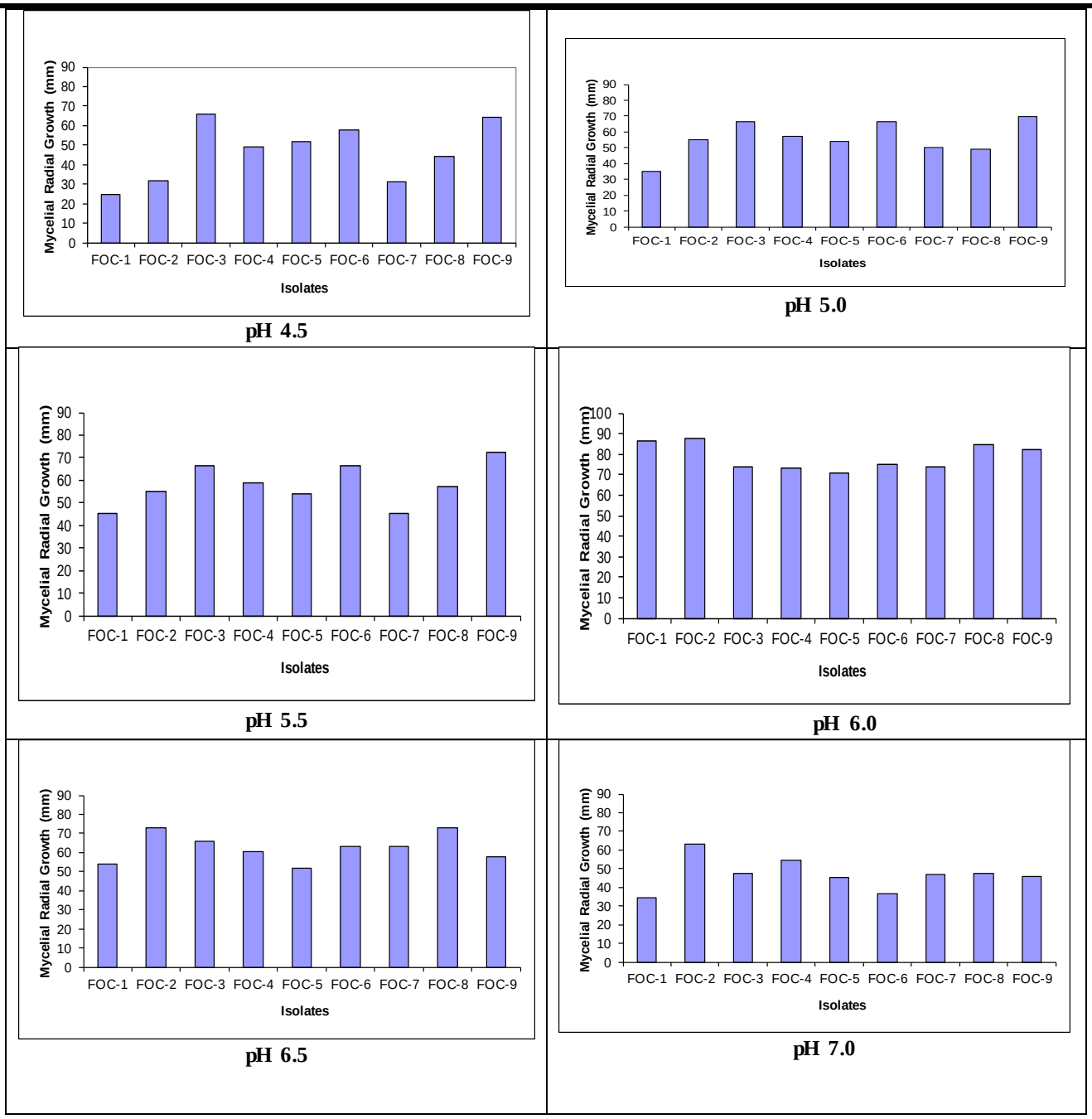

Fig.2: Effect of pH on mycelial radial growth of Fusarium oxysporum f. sp. ciceri isolates.

Table.4: ***Effect of*** *pH* ***on production*** *of micro conidia of Fusarium oxysporum f. sp. ciceri isolates*

Isolates	Production of micro conidia (ml^{-1}) at different pH levels					
	4.5	5.0	5.5	6.0	6.5	7.0
FOC-1	5.61x 10^4	5.77x 10^4	6.72 x 10^4	8.00 x 10^4	6.72 x 10^4	5.27 x 10^4
FOC-2	3.94x 10^4	4.72x 10^4	7.50x10^4	7.50 x 10^4	5.88 x 10^4	5.77 x 10^4
FOC-3	8.87x 10^3	1.06 x 10^4	1.33x 10^4	8.03 x 10^4	1.61 x 10^4	1.31 x 10^4
FOC-4	4.50x 10^4	5.00x 10^4	6.00 x 10^4	1.51 x 10^5	9.50 x 10^4	5.00 x 10^4
FOC-5	3.66x 10^4	3.83x10^4	5.83 x 10^4	1.86 x 10^5	6.16 x 10^4	6.00 x 10^4
FOC-6	2.50x 10^4	4.00 x 10^4	7.16 x 10^4	1.56 x 10^5	8.83 x 10^4	7.33x 10^4
FOC-7	6.16x 10^4	7.33 x 10^4	1.03 x 10^5	3.03 x 10^5	1.61 x 10^5	1.58x 10^5

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FOC-8	1.16x 10^4	3.50x 10^4	3.66 x 10^4	7.33 x 10^4	6.66 x 10^4	6.83 x 10^4
FOC-9	1.16x 10^4	2.33 x 10^4	3.00 x 10^4	1.01 x 10^5	8.83 x 10^4	6.50x 10^4

Table.5: Effect of pH on production of macro conidia of 9 Fusarium oxysporum f. sp. ciceri isolates

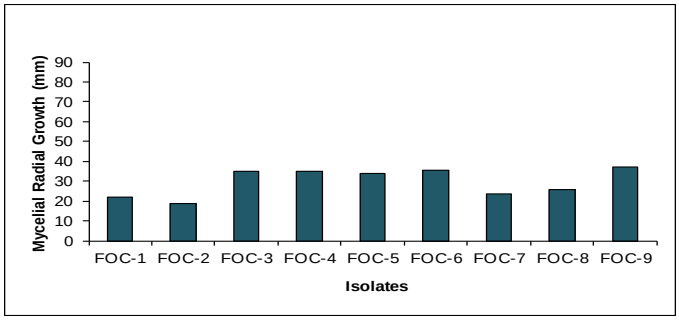
Isolates	Production of macro conidia (ml^{-1}) at different pH levels					
	4.5	**5.0**	**5.5**	**6.0**	**6.5**	**7.0**
FOC-1	2.50x10^4	5.72 x 10^4	1.72 x 10^5	3.90 x 10^5	1.38 x 10^4	1.00 x 10^4
FOC-2	2.00 x 10^4	5.05 x 10^4	8.61 x 10^4	4.76 x 10^5	8.33 x 10^4	1.33 x 10^4
FOC-3	1.00 x 10^4	1.35 x 10^4	5.47 x 10^4	1.50 x 10^5	2.30 x 10^4	1.16 x 10^4
FOC-4	5.00 x 10^3	6.66 x 10^3	3.25 x 10^5	3.80 x 10^5	6.66 x 10^3	*
FOC-5	3.50 x 10^4	9.33 x 10^4	6.66 x 10^3	2.30 x 10^5	1.66 x 10^3	*
FOC-6	1.66 x 10^3	1.66 x 10^3	5.00x 10^3	7.00 x 10^4	1.00 x 10^4	8.33 x 10^3
FOC-7	3.33 x 10^3	8.33 x 10^3	9.83 x 10^4	1.65 x 10^5	*	2.00 x 10^4
FOC-8	1.66 x 10^3	3.33 x 10^3	5.66 x 10^4	8.66 x 10^4	*	*
FOC-9	*	1.42 x 10^3	5.21 x 10^5	7.06 x 10^5	6.00 x 10^4	3.33x 10^3

* No sporulation

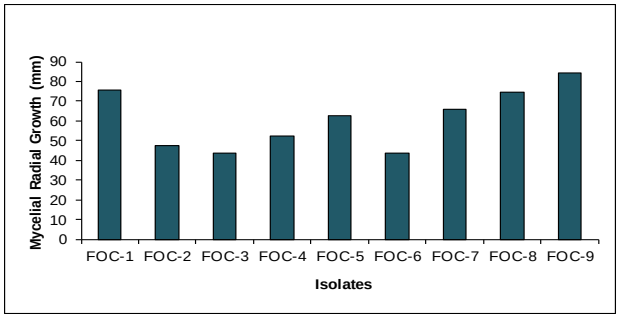
Effect of culture media on mycelial radial growth and sporulation

The results of the experiment revealed that the most effective medium supporting the growth of the fungus was oat meal agar medium (OMA) followed by Czapek's dox agar (CDA) medium which gave 90.00 mm and 84.50 mm mycelium colony growth of *F. oxysporum* f. sp. *ciceri* after an incubation of seven days respectively (Fig. 3). The results of the present study are in agreement with those achieved by Farooq *et al*., (2005). He mentioned that Minimum fungal growth was observed on PDA and the Czapeck's dox agar and CSMA media were the best for the radial growth of *F. oxysporum* as this fungus gave maximum growth of 85 and 80 mm, respectively. Maximum (4.06 x 10^5 ml^{-1}) micro conidia was produced by FOC-5 at PDA medium and minimum (2.41 x 10^3 ml^{-1}) sporulation was observed on FOC-3 at CDA medium. No micro conidia were produced by FOC-4 at V_8 JA and all isolates of WA medium (Table 6).

The highest sporulation of macro conidia was observed on FOC-1 (3.27 x 10^5 ml^{-1}) at PDA medium and the lowest sporulation was observed on FOC-3 (1.08 x 10^3 ml^{-1}) at V_8 JA medium. No sporulation was observed on FOC-8 at CDA; FOC-5, FOC-6, FOC-8 at MDA; FOC-6 at OMA and all isolates of WA medium (Table 7). 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. Khilare and Rafi Ahmed (2012) found that the fungus grew the best on Czapek's dox agar and PDA media among six culture media were tested. Jamaria (1972) 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.



PDA



CDA

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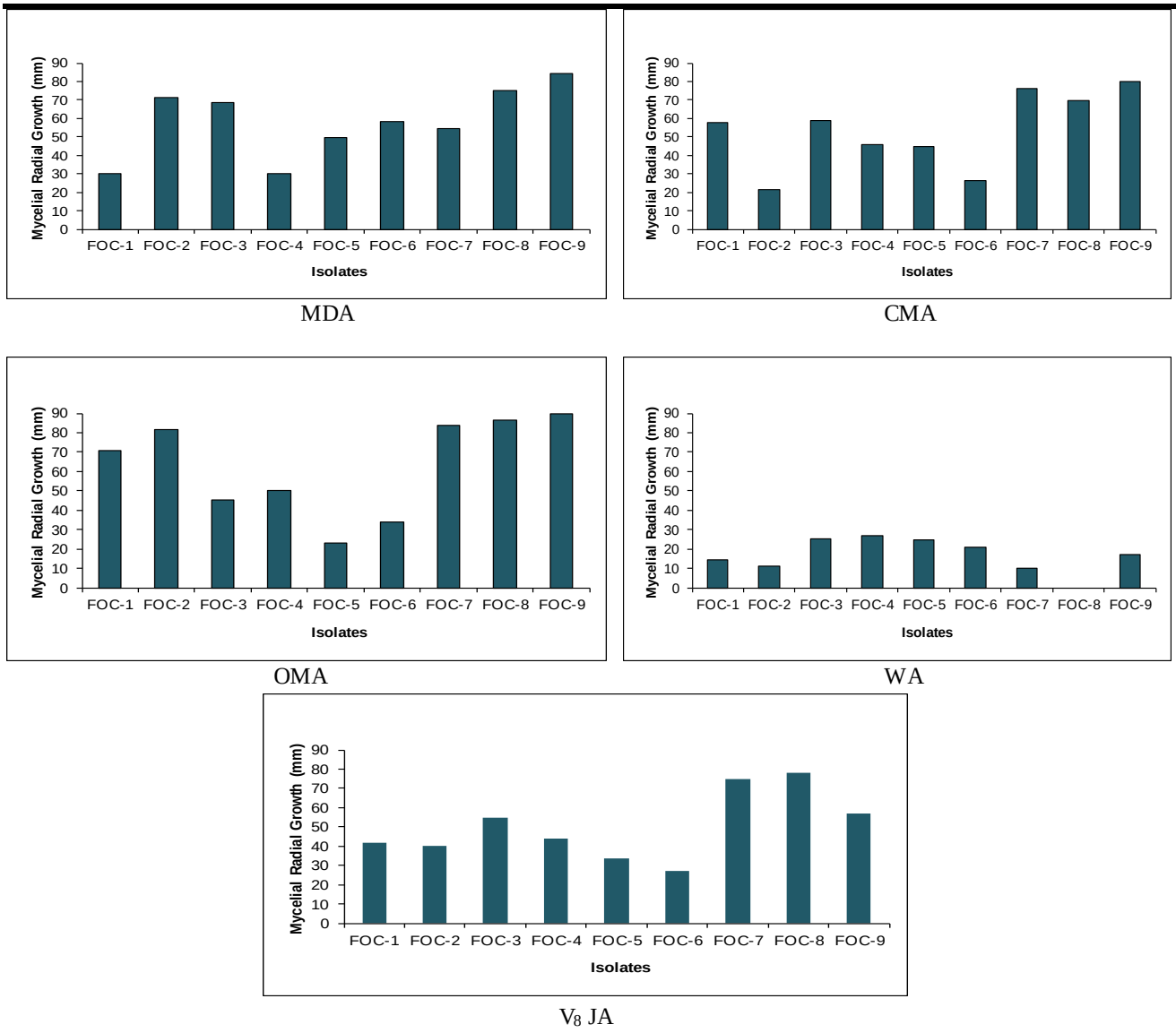

Fig.3: Effect of culture media on mycelial radial growth and sporulation of nine Fusarium oxysporum f. sp. ciceri isolates.

Table.6: Effect of culture media on production of micro conidia of nine Fusarium oxysporum f. sp. ciceri isolates

Isolates	Production of micro conidia (ml^{-1}) on different media						
	PDA	CDA	MDA	CMA	OMA	WA	V_8 JA
FOC-1	1.82x 10^5	1.33x 10^4	1.27x 10^4	1.55x 10^4	2.16x 10^4	*	7.22x 10^3
FOC-2	1.13x 10^5	1.33x 10^4	1.88x 10^4	1.00x 10^4	2.05x 10^4	*	6.11x 10^3
FOC-3	3.28x 10^4	2.41x 10^3	5.25x 10^3	4.32x 10^3	1.11x 10^4	*	4.32x 10^3
FOC-4	3.83x 10^4	2.83x 10^4	3.33x 10^3	8.33x 10^3	1.33x 10^4	*	*
FOC-5	4.06x 10^5	1.16x 10^4	8.33x 10^3	1.00x 10^4	2.16x 10^4	*	1.16x 10^4
FOC-6	4.66x 10^4	1.00x 10^4	6.66x 10^3	2.00x 10^4	1.33x 10^4	*	8.33x 10^3
FOC-7	5.50x 10^4	2.00x 10^4	1.83x 10^4	1.16x 10^4	1.00x 10^4	*	5.00x 10^3
FOC-8	7.50x 10^4	6.66x 10^3	2.33x 10^4	3.66x 10^4	2.00x 10^4	*	2.66x 10^4
FOC-9	5.83x 10^4	5.16x 10^4	8.33x 10^3	5.00x 10^3	2.33x 10^4	*	3.33x 10^3

* No sporulation

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Table.7: Effect of culture media on production of macro conidia of nine Fusarium oxysporum f. sp. ciceri isolates

Isolates	Production of macro conidia (ml $^{-1}$) on different media						
	PDA	CDA	MDA	CMA	OMA	WA	V_8 JA
FOC-1	$3.27x10^5$	$4.38x10^4$	$2.44x10^4$	$5.55x\ 10^3$	$3.94x\ 10^4$	*	$1.11\ x\ 10^3$
FOC-2	$9.07x10^4$	$4.50x\ 10^4$	$3.50x\ 10^4$	$3.33x\ 10^3$	$3.77x\ 10^4$	*	$6.66\ x\ 10^3$
FOC-3	$1.75x10^5$	$1.02x\ 10^4$	$4.46x\ 10^3$	$1.49x\ 10^3$	$4.96x\ 10^3$	*	$1.08\ x\ 10^3$
FOC-4	$1.58\ x\ 10^5$	$1.50x\ 10^5$	$1.16x\ 10^4$	$1.66x\ 10^3$	$2.66x\ 10^4$	*	$5.00\ x\ 10^3$
FOC-5	$9.00\ x\ 10^4$	$5.00x\ 10^3$	*	*	$8.33x\ 10^3$	*	$1.66\ x\ 10^3$
FOC-6	$5.00\ x\ 10^4$	$5.00x\ 10^4$	*	$8.33x\ 10^3$	*	*	$3.33\ x\ 10^3$
FOC-7	$3.33x10^3$	$5.00x\ 10^4$	$5.33x\ 10^4$	$1.66x\ 10^3$	$2.00x\ 10^4$	*	$3.33\ x\ 10^3$
FOC-8	$8.33\ x\ 10^3$	*	*	$6.66x\ 10^3$	$1.00x\ 10^4$	*	$6.66\ x\ 10^3$
FOC-9	$1.06\ x\ 10^5$	$1.55x\ 10^5$	$6.16x\ 10^4$	$1.66x\ 10^3$	$7.50x\ 10^4$	*	$3.33\ x\ 10^3$

* No sporulation

Pathogenic variability

In the present study it was observed that *Fusarium* wilt infected seedlings collapse and lies flat on the ground surface retaining their dull green color. Adult plants showed typical wilt symptoms of drooping of petioles, rachis and leaflets. The roots of the wilted plants did not show any external rotting but when split open vertically, dark brown discoloration of internal xylem was observed. According to these observations it was confirmed that *F. oxysporum* f. sp. *ciceri* is pathogenic to chickpea, which has also been supported by the findings of Nene (1980), who after making detailed symptomatolgical studies observed diagnostic symptoms of wilt at seedling stage (3-5 weeks after sowing). The present study indicates that wilt incidence at 30 DAI and 60 DAI varied from 0% to 13.33%, at 45 DAI it was 6.67% to 53.33% whereas at 60 DAI it ranged from 13.33% to 86.67% (Table 8). The most virulent isolates were Foc-1 (86.67% wilt incidence), Foc-7 (73.33%) and Foc-8 (73.33%) while, the least virulent isolate was Foc-6 (13.33% wilt incidence). The remaining isolates showed intermediate response of variation in virulence. Ahmad (2010) noted that the pathogenic variability of 27 isolates against differential chickpea cultivars, the most virulence isolates was observed Foc-2 (AZRI, Bahawalpur), whereas, the least virulence was Foc-4 (Chakwal). Shehabu *et al.*, (2008) studied 24 isolates for wilt resistance on 10 chickpea lines and eight improved varieties and found F13, F20 and F22 most virulent isolate. Haware *et al.*, (1992) also found pathogenic diversity among chickpea wilt isolates.

Table.8: Wilt incidence and aggressiveness of nine Fusarium oxysporum f. sp. ciceri isolates on BARI Chola-1 at 30, 45 and 60 DAI

Isolates	Wilt incidence (%) at different days after inoculation (DAI)			Aggressiveness
	30 DAI	45 DAI	60 DAI	
FOC-1	13.33	53.33	86.67	HV
FOC-2	0.00	13.33	40.00	MV
FOC-3	6.67	13.33	20.00	LV
FOC-4	0.00	13.33	20.00	LV
FOC-5	13.33	40.00	46.67	MV
FOC-6	0.00	6.67	13.33	LV
FOC-7	6.67	53.33	73.33	V
FOC-8	6.67	53.33	73.33	V
FOC-9	0.00	13.33	20.00	LV
Control	0.00	0.00	0.00	-

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