MORPHOLOGICAL AND PHYSIOLOGICAL VARIATION OF *FUSARIUM OXYSPORUM* F. SP. *CICERI* ISOLATES CAUSING WILT DISEASE IN CHICKPEA

By

NIBEDITA NATH
Registration No. 05-01821

DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
SHER-E-BANGLA NAGAR
DHAKA-1207, BANGLADESH

JUNE, 2011

MORPHOLOGICAL AND PHYSIOLOGICAL VARIATION OF *FUSARIUM OXYSPORUM* F. SP. *CICERI* ISOLATES CAUSING WILT DISEASE IN CHICKPEA

NIBEDITA NATH

DEPARTMENT OF PLANT PATHOLOGY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

SHER-E-BANGLA NAGAR

DHAKA-1207, BANGLADESH

JUNE, 2011

Dedicated to

My Beloved Parents

ACKNOWLEDGEMENTS

The author sincerely expresses her highest sense of gratitude to "**Almighty God**" to let her successfully duly complete her research work and submit the thesis paper for the degree of Master of Science (MS) in Plant Pathology from Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

The author is very grateful to express her sincere appreciation, deepest sense of gratitude and immense indebtedness to her scholastic supervisor Dr. Ashraf Uddin Ahmed, Principal Scientific Officer, Bangladesh Agriculture Research Institute, Joydebpur, Gazipur and to her reverend teacher and co-supervisor Dr. F. M. Aminuzzaman, Associate Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their systematic planning, kind and painstaking guidance, encouragement, valuable suggestions and constructive criticisms in completing the research work and compilation of this thesis.

The author expresses her heart-felt thanks to Prof. Dr. Md. Rafiqul Islum, Prof. Mrs. Nasim Akhter, Assoc. Prof. Nazneen sultana, Assoc. Prof. Dr. M. Salahuddin M. Chowdhury, Asstt. Prof. Khadija akhter, Asstt. Prof. Abu Noman Faruq Ahmmed, Asstt. Prof. Md. Tohidul Islam, Lecturer Shukti Rani Chowdhury, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their valuable teaching, kind support and inspirations through the courses of study and research work.

The author convey heartfelt thanks to her husband Sujan Debnath; brother Suman; Tapan Kumer Dey, Chief Scientific Officer, Plant Pathology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur; Mst. Arifunnahar, Scientific Officer, Plant Pathology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur;

friends- Rila, mouri, Ruma, Sontosh, Dhreti and many more for their painstaking cooperation for smooth running of the research work.

The author also extended thanks to Mr. Kalam, laboratory attendant; Mrs. Mala, Mr. Jahangir and other laboratory associates, Plant Pathology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur for their assistance to carryout the whole research work.

The Author

MORPHOLOGICAL AND PHYSIOLOGICAL VARIATION OF *FUSARIUM OXYSPORUM* F. SP. *CICERI* ISOLATES CAUSING WILT DISEASE IN CHICKPEA

By

NIBEDITA NATH
Registration No. 05-01821

ABSTRACT

A total of nine isolates of *Fusarium oxysporum* f. sp. *ciceri* infecting chickpea were collected from major chickpea growing areas of Bangladesh and were characterized in terms of cultural, morphological, physiological characteristics and pathogenicity. 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 observed on WA medium. The highest number of macro spores (3.27×10^5 ml^{-1}) and micro spores (4.06×10^5 ml^{-1}) were produced 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.

TABLE OF CONTENTS

List of Tables

List of Plates

List of Figures

LIST OF SOME ABBREVIATED FORMS AND THEIR ELLABORATIONS

Abbreviated Form	Elaboration
%	Percentage
@	At the Rate
µg	Microgram
µl	Microlitre
µm	Micromililitre
°C	Degree Celsius
AV	Avirulent
BARI	Bangladesh Agricultural Research Institute
$CaCO_3$	Calcium Carbonate
CDA	Czapek's dox agar
Cm	Centimeter
CMA	Corn Meal Agar
DAI	Days After Inoculation
DAS	Days After Sowing

DMRT	Duncan’s Multiple Range Test
Et al.	And Others
F. oxysporum	*Fusarium oxysporum*
f. sp.	*formae speciales*
FAO	Food and Agricultural Organization
G	Gram (s)
Ha	Hectare (s)
HCl	Hydrochloric Acid
$HgCl_2$	Mercury Chloride
HV	Highly Virulent
Hrs	Hours
ISTA	International Seed Testing Association
Kv	Kilovolt
LSD	Least Significant Difference
LV	Low Virulent
mA	Miliampere
MDA	Malt extract Dextrose Agar
ml^{-1}	Per Mililitre
Mm	Milimiter
MV	Moderately Virulent
NaOH	Sodium Hydroxide
OMA	Oat Meal Agar
PDA	Potato Dextrose Agar
PSI	Pound Per Square Inch
SEI	Secondary Electron Image
SEM	Scanning Electron Microscope
V	Virulent
V_8 JA	V_8 Juice Agar
Viz.	Videlicet
WA	Water Agar

CHAPTER 1
INTRODUCTION

CHAPTER 1
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. This crop is valued for it's nutritive seeds with high protein (23%), carbohydrates (64%), fat (5%), crude fiber (6%), ash (3%) and various minerals like P, Ca, Mg, Fe, Zn (FAO, 2008).

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 wilt is worldwide in occurrence and has been reported from many countries of the world (Nene *et al*., 1984).

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. This research will determine the morphological and physiological variation in *Fusarium oxysporum* f. sp. *ciceri* isolates from Bangladesh.

In view of the above facts, the present research work was aimed to carry out comprehensive investigation on the variability of the isolates of *Fusarium oxysporum* f. sp. *ciceri* with the following objectives.

Objectives

1. To determine the cultural, morphological and physiological variation of *Fusarium oxysporum* f. sp. *ciceri*.

2. To determine the pathogenic variation of *Fusarium oxysporum* f. sp. *ciceri*.

CHAPTER 2
LITERATURE REVIEW

CHAPTER 2
LITERATURE REVIEW

The wilt disease of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* is known to occur in almost all chickpea growing areas. A detailed study was undertaken on the disease and pathogen and the review pertaining to *Fusarium* sp. are given below.

2.1. Symptomatology of Fusarium wilt disease

Pande *et al.* (2007) stated that Fusarium wilt of chickpea is seed-borne and seeds harvested from wilted plants when mixed with healthy seeds can carry the wilt fungus to new areas and can establish the disease in the soil to economic threshold levels within three seasons.

Haware (1993) reported that pathogen gets entry into xylem vessel and invades whole vascular system, inducing symptoms of yellowing and wilting. In the absence of host plant the pathogen can survive up to six years.

Saxena and Singh (1987) found that internal discoloration of pith and xylem can be seen if stem and root of the wilted plants split vertically.

Westerlund *et al.* (1974); Prasad and Padwick *et al.,* (1939) found that the disease occurs at seedling and flowering stage of plant growth. The symptoms which can be observed are drooping of petioles and rachis, yellowing and drying of leaves from base to upward, browning of vascular bundles, improper branching, withering of plants and finally death of plants.

Erwin (1957) characterized chickpea wilt by yellowing of leaves and necrosis of the xylem. Leaves of the wilted plants turned grayish green, then became dull yellow and wilted. The xylem and pith become darkened and discolored.

2.2. Variability of *F. oxysporum* f .sp. *ciceri*

2.2.1. Morphological and cultural variability

Ahmad, M.A. (2010) showed that the size of micro and macro-conidia of 27 isolates were 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 were obtained from isolates Foc-11 respectively. The rest of isolates had intermediate size.

Dubey *et al*. (2010) described that Fusarium wilt isolates were highly variable in their colony growth pattern, size of colony and pigmentations. The size of micro conidia varied from 5.1-12.8 2.5- 5.0 µm, whereas macro conidia ranged from 16.5-37.9 4.0-5.9 µm with 1-5 septations.

Kulkarni, S.P. (2006) stated that micro conidia were abundant, hyaline, continuous, or 1 septate, ovoid and ovate and measured 3.2 – 5.4 x 1.1 – 2.4 µm (Average 4.3 x 1.75 µm). Macro conidia were scarce, often lacking and variable, 3 – septate measuring 19 – 21.0 x 3.1 – 4.2 µm (average 20.0 x 3.65µm). Chlamydospores were hyaline, usually vacuolated, spherical and 1 – celled,

produced either singly or in chain and 7 – 10 µm in diameter (average 8.5µm diameter).

Kistler (1997) and Tantaoui *et al.* (1996) described that the Fusarium wilt fungus is an asexual species. Isolates are more similar genetically within a *forma specialis* and are assumed to have a monophyletic origin.

Patel (1991) reported considerable variation among the 13 isolates of *F. solani*, but 3 isolates of *F. moniliforme* Shed and *F. oxysporum.* f .sp. *ciceri* appeared similar.

Gupta *et al.* (1987) studied that in disease plants in which leaves turn yellow and straw colored. Macro conidia 3 to 5 septate, thin walled, pointed both ends, fused and measuring 3.5 to 4.5 x 25 to 65 µm. Macro conidia are fewer than micro conidia, borne on branched in old culture. These are rough or smooth walled, intercalary or terminal and may be formed singly, in chains or pairs.

Saxena and Singh (1987) reported that *F. oxysporum* is septate, profusely branched growing on potato sucrose/dextrose agar at 25 °C initially white turning light buff or deep brown later, fluffy or submerged. The growth becomes felted or wrinkled in old cultures. Various types of pigmentation (yellow, brown, crimson) can be observed in culture. On solid medium, micro-conidia are borne on simple and short conidiophores which arise laterally on the hypha. Macro-conidia are borne on the conidiophores, which are thin walled, 3 to 5 septate, with both ends pointed and measuring 3.5-4.5 x 25–65 µm. In old cultures chlamydospores are formed, which are rough or smooth walled, intercalary or terminal and may be formed singly, in chains or pairs.

Gupta *et al*. (1986) reported that morphological studies of the six isolates of *F. oxysporum* f. sp. *ciceri* revealed the variation in size of micro and macro conidia, growth pattern, sporulation and pigmentation of medium which varied from normal white to pale cream dark brown, crimson and middle buff.

Murumkor and Chavan (1985) reported growth of fungus at 25 °C on chickpea meal agar appears cottony white, wrinkled in old culture. The fungus hypha is septate and profusely branched. On simple, short conidiophores micro conidia are produced. Micro conidia and macro conidia appear generally spare on solid medium. These are straight to curved or oval to cylindrical in shape measuring 2.5-3.5 x 5-11 μm.

Chauhan (1962) found that variation among 22 isolates in respect of their mycelium type, colony color, toxin production and pathogenicity. In *F. oxysporum*, there is a considerable variation in cultural characteristics. Isolates also vary in their physiology and virulence.

Venkataraman (1955) showed that culture of Fusarium wilt of muskmelon produced fluffy mycelium with sparse number of conidia differing with 'wild type' strain having abundant sporulation.

Mc. Culloch (1944) observed that in cultures, the pathogen *F. oxysporum* f .sp. *gladioli* produced white to peach pale salmon or purple mycelium. Micro conidia were abundant, hyaline, ovoid to ovate. Macro conidia were scarce, often lacking and variable, 3- septate. Chlamydospores were hyaline, usually vacuolate and spherical.

Wardlaw (1931) described the cultural characters shown by five strains of *Fusarium cubense*, when grown under uniform conditions on standard media. Important differences were observed in vegetative growth, color production, formation of chlamydospores, sclerotia and sporulation and stated that two strains were not identical.

Massey (1926) studied the cultural and morphological characters of *F. oxysporum* f. sp. *gladioli* and found more number of micro conidia and scanty macro conidia during isolation.

2.3. Physiological Variability

2.3.1. Effect of temperature on *Fusarium oxysporum* f. sp. *ciceri*

Khilare, V.C. and Rafi Ahmed (2012) reported that the growth of *F. oxysporum* was maximum at 30 °C and reduced drastically below 15 °C and above 35 °C.

Ahmad, M.A. (2010) reported that the growth of Fusarium wilt fungus took place at all temperature levels but the most suitable temperature supporting the maximum mycelial growth of the pathogen was found between 25 °C–30 °C as the diameter at this temperature was 76.8 to 85.4 mm, respectively.

Mina and Dubey (2010) studied on the environmental factors in relation to wilt disease development in chickpea and observed that the maximum colony diameter (85 mm) at 28 °C (in-vitro).

Gangadhara *et al.* (2010) studied that the effect of temperature on growth of *F. oxysporum* f. sp. *vanillae* isolates. 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.

Scott *et al.* (2010) studied that the 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 an apparent maximum near 25 °C.

Navas-Cortes *et al.* (2007) stated that most suitable soil temperature between 22-26 °C for infection and disease development.

Landa *et al.* (2006) reported that chickpea cultivar Ayala in a greenhouse experiments in Israel was found moderately resistant to Fusarium wilt at a day/night temperature regime of 24/21 °C but it was found highly susceptible to this fungus at 27/25 °C.

Farooq *et al*. (2005) reported that growth of *F. oxysporum* was maximum at 30 °C and reduced below 15 °C and above 35 °C. No growth was observed at 5 °C.

Sharma *et al.* (2005) verify that a temperature around 25 °C is optimum for the wilt disease development in chickpea.

Landa *et al.* (2001) found the disease development was greater at 25 °C compared with 20 and 30 °C.

Desai *et al*. (1994) reported that maximum growth of all the four races of *F. oxysporum* f. sp. *ciceri* was recorded at 25 °C.

Sugha *et al.* (1994a) reported that soil temperature in the range of 24.8 to 28.5 °C and moisture within the water holding capacity of soil were most conducive to chickpea wilt. Temperature and soil moisture levels above and below this range delayed the incidence and slowed down the progress of wilt.

Sowmya (1993) noticed that maximum growth of four isolates of *F. oxysporum* f .sp. *cubense* at 35 °C.

Bhatti and Kraft (1992) found wilt symptoms were more severe at high temperature (25 and 30 °C) than those at lower temperature (10, 15 and 20 °C).

Gupta *et al.* (1986) reported that at 25 °C and 30 °C, the fungus attained the maximum growth 76.8 and 85.4 mm while at 35 °C, it was 59.3 mm after seven days of inoculation. No growth was observed at 5 °C.

Dhingra *et al*. (1974) reported all the 8 strains of *F. oxysporum* f .sp *lentis* showed maximum growth at 25 °C. The strain 6 yielded the maximum mycelium weight at 15 °C and it decreased as the temperature increased.

Sinha and Dahiya (1973) found that 25 °C is the optimum temperature for growth of Fusarium wilt.

Chauhan (1965) stated that soil temperature relationship indicated that suitable temperature for development of chickpea wilt is 25-30 °C.

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 favor for growth of the fungus.

Chauhan (1963) and Sinha and Dahiya (1973) studied that 25 °C is the optimum temperature for the growth of the *F. oxysporum* fungus.

2.3.2. Effect of pH on *Fusarium oxysporum* f. sp. *ciceri*

Khilare and Rafi Ahmed (2012) stated that the most suitable pH level for growth of fungus was 6.0 and 6.5.

Imran Khan *et al.* (2011) showed that the optimum pH for growth of *F. oxysporum* f. sp. *ciceri* ranged from 6.5 to 7.0.

Ahmad, M.A. (2010) showed that effect of pH levels against Fusarium wilt showed significant difference at all pH levels. Observations revealed that fungus growth was maximum (88.33 mm) at pH 7 and pH 6 (83 mm). The fungus growth was decreased by increasing or decreasing pH levels from neutral level.

Farooq *et al*. (2005) conducted an experiment on the effect of pH levels on mycelial growth of *F. oxysporum* f. sp. *ciceri* and observed that the most suitable pH level for growth of fungus was 7.0 and 6.0.

Desai *et al.* (1994) reported that all the four races of *F. oxysporum* f. sp. *ciceri* recorded maximum growth at pH 6.0.

Sugha *et al.* (1994b) studied that maximum wilting occurs at soil pH 5.2 with a slight decline towards neutrality.

Hayes (1978) noted that the growth of the fungus was obtained at all the pH levels tested but it was maximum at pH 7 where it was 80 mm after seven days of

inoculation. pH 6 (74 mm) and pH 8 (65 mm) were also favorable. Growth of the fungus decreased by increasing or decreasing the pH level from the neutral level.

Shaikh (1974) experimented on *F. oxysporum* f .sp. *ciceri* the causal agent of wilt of chickpea and observed that this fungus can tolerate a wide range of pH 5.0–6.5.

Sinha (1973) reported that in pot experiment, soil pH of 3.4 - 9.2 reduced the incidence of wilt significantly without adverse affects on yield; shoot dry weight, seeds per pod, number of pods and crop yield.

Jamaria (1972) reported that *F. oxysporum* f .sp. *niveum* could grow well on wide range of pH varying from 3.2 to 8.3 and the optimum was between pH 5.5 to 6.5.

Gangadhara *et al.* (2010) showed that the most suitable pH level for growth of *F. oxysporum* f. sp. *vanillae* isolates were 5.0 and 6.0. The fungus showed best growth pH at 5.0 and least growth of all the isolates was recorded at 9.0 pH.

2.3.3. Effect of culture media on *Fusarium oxysporum* f. sp. *ciceri*

Khilare, V.C. and Rafi Ahmed (2012) conducted an experiment to study the effect of different culture media on mycelial growth of *Fusarium oxysporum* f. sp. *ciceri*. The fungus grew the best on Czapek's dox agar and PDA media among six culture media were tested.

Imran Khan *et al.* (2011) studied that the effect of media on *F. oxysporum* f. sp. *ciceri* and found that PDA is best for the growth of different isolates.

Gangadhara *et al.* (2010) showed that the best growth of *F. oxysporum* f. sp. *vanillae* isolates on Richard's agar and potato dextrose agar media.

Farooq *et al*. (2005) stated that the fungus *F. oxysporum* f. sp. *ciceri* grew the best on Czapek's dox agar and chickpea seed-meal agar media among eight culture media that were tested.

Dikkar and Deshmukh (2003) found best mycelial growth of fungus on Richard agar medium followed by PDA and Czapeck's agar medium. Among liquid media he found best growth on Richard's medium.

Paulkar *et al.* (2002) studied the effect of temperature, carbon and nitrogen sources and found best growth of *F. oxysporum* f. sp. *ciceris* on mannitol, dextrose, sucrose and potassium nitrate nutrients, between 25 to 30 °C.

Ingole (1995) reported that PDA and Richard's agar supported best mycelial growth of *F. udum*.

Desai *et al*. (1994) recorded that maltose and succinic acid supported good growth of race three of *F. oxysporum* f .sp. *ciceri.* He also claimed maltose for maximum growth and sporulation.

Desai *et al.* (1994) recorded that race 1 of *F. oxysporum.* f. sp. *ciceri* (Padwick) grew better on potassium nitrate and L – aspartic acid when compared to other races.

Sowmya (1993) studied four isolates of banana panama wilt pathogen on different nutrient media and observed maximum growth and sporulation of the pathogen on potato sucrose agar and Richards's agar, respectively.

Patel (1991) demonstrated that among carbon sources, maltose and mannitol were best utilised by *F. solani* where as mannitol and dextrose were most preferred source for *F. moniliforme* and *F. oxysporum*. f. sp. *ciceri* respectively.

Saheb *et al*. (1987) stated that *F. oxysporum.* f. sp. *lupini* grew faster on potato dextrose agar than on Czapek's medium and spore production was also greatest on potato dextrose agar.

Haware *et al*. (1986) had modified cape do agar medium by adding PCNB, streptomycin and malachite green. This medium is highly effective for the growth of *F. oxysporum*.

Mahendrapal and Grewal (1975) showed that ammonium salts in general supported growth of *F. oxysporum* f .sp. *ciceri*. Its growth was meager on calcium nitrate, whereas moderate to good growth of the fungus was recorded on monosodium dicarboxylic acids and amides.

Khare *et al.* (1975) reported that maximum growth of *Fusarium oxysporum* f. sp. *lentis* on PDA followed by lentil extract and Richard's agar.

Joffee *et al*. (1974) studied the development of *Fusarium poae* isolates on potato dextrose agar and maltose agar media and all of them showed best growth at 24 – 25 °C.

Shaikh (1974) stated that different synthetic and non-synthetic media have profound influence on cultural and morphological characters of the pathogen.

Rawal and Parmar (1973) studied on *F. oxysporum*. f. sp. *ciceri* and reported that poor growth and sporulation occurs in lactose.

Jamaria (1972) reported that Potato Dextrose Agar, Richards's Agar and Czapek's Agar provided maximum growth and sporulation of *F. oxysporum*. f. sp. *niveum.* Jamaria (1972) also reported that maximum growth and sporulation of *F. oxysporum* f. sp. *vanillae* on potato dextrose agar, Richard's agar and Czapek's dox agar.

2.4. Pathogenicity of *Fusarium oxysporum* f. sp. *ciceri*

Ahmad, M.A. (2010) showed that the pathogenic variability of 27 isolates against 10 genotypes, 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 genotypes and eight varieties and found F13, F20 and F22 most virulent isolate. Isolates with Race 3 were observed in all of the sick plots.

Sharma *et al.* (2005) describe that optimum inoculum load of micro or macro conidia for disease development must be 10^4–10^5.

Jimenez-Gasco *et al.* (2004) described that plant pathogens often exhibit variation in virulence, the disease causing ability on plant host with specific resistance, which is evident from the diversity of races observed within pathogenic species of monophyletic lineage in Fusarium wilt.

Navas-Cortes *et al.* (1998) and Navas- Cortes *et al.* (2000) stated that the disease control was dependent on a number of factors including virulence, concentration of the pathogen and the susceptibility of cultivar used.

Patel *et al.* (1985) screened 34 resistant germplasm lines from ICRISAT for wilting at 40 and 80 days after sowing in infested soil and reported 6 lines free from wilt, while, remaining entries showed 20-100 percent wilt (all at 80 days after sowing).

Halila *et al.* (1984) conducted experiment to find out resistant sources against chickpea wilt in Kabuli genotypes and observed significant difference between entries on the basis of wilt score 1 (resistant) to 9 (killed).

CHAPTER 3
MATERIALS AND METHODS

CHAPTER 3
MATERIALS AND METHODS

The experiment was conducted to record the cultural, morphological, physiological and pathogenic variability of nine *Fusarium oxysporum* f. sp. *ciceri* isolates collected from major chickpea growing areas of Bangladesh.

3.1. Experimental site

The experiment was laid during January to June 2010 at the Plant Pathology Laboratory and pot house of Bangladesh Agricultural Research Institute, Joydebpur, Gazipur.

3.2. Collection of isolates

Wilted plants of Chickpea showing typical symptoms were collected from nine (9) locations of four Upazillas of 4 districts of Bangladesh (Table-1). The *Fusarium oxysporum* f. sp. *ciceri* isolates were collected during the month of November 2009 from the infected chickpea field. Then the samples were taken to the Plant Pathology Laboratory, BARI, kept in brown paper packet and store at room temperature until isolation of the fungus. Pure culture of all the nine (9) *Fusarium oxysporum* f. sp. *ciceri* isolates were maintained for cultural, morphological, physiological and pathogenic variability studies.

Table 1. List of nine *Fusarium oxysporum* f. sp. *ciceri* isolates with their locations

Isolates	Locations	
	District	**Upazilla**
FOC-1	Pabna	Ishurdi
FOC-2	Jessore	Jessore Sadar
FOC-3	Munsigonj	Sirajdikhan
FOC-4	Munsigonj	Sirajdikhan
FOC-5	Jessore	Jessore Sadar
FOC-6	Munsigonj	Sirajdikhan
FOC-7	Gazipur	Gazipur Sadar
FOC-8	Gazipur	Gazipur Sadar
FOC-9	Munsigonj	Sirajdikhan

3.3. Isolation and Identification of the pathogens

The pathogens that causes wilt disease in chickpea were isolated using tissue culture techniques. The surface of working clean bench was sterilized with ethanol (70%). Then the infected chickpea roots were taken into the clean bench and cut into small pieces (0.5-1.0 cm). The cut pieces were sterilized in $HgCl_2$ solution (1:1000) for one and half minutes and then taken out with the help of sterile forceps and placed on sterile distilled water in order to wash the samples in three times. After washing, these cut pieces were placed on sterilized blotter paper in petridishs and also 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.

3.4. Purification and preservation of *Fusarium oxysporum* f. sp. *ciceri* isolates

After identification of *Fusarium oxysporum* f. sp. *ciceri* was purified and preserved for further study. Purification of *Fusarium oxysporum* f. sp. *ciceri* was done following standard single spore isolation technique. The stock culture of the isolates was preserved on Potato Dextrose Agar (PDA) in test tubes slant at 4 ± 0.5 °C in a refrigerator for further use.

3.5. Preparation of culture medium and culture plates

Extra pure dehydrated potato dextrose agar (PDA) manufactured by Hi-media was used for this experiments. The dehydrated potato dextrose agar was hydrated in distilled water @ 40g litre^{-1} and cooked for 5 minutes in a microwave oven (Model: 3D Power, Rangs). The pH of the medium was adjusted with 0.1N HCl or NaOH solution using a pH metre (Hariba pH metre, Model D-12). After adjustment of the required levels of pH (6.0) the medium was poured into a series of conical flasks (250 ml) and autoclaved (Model: HL 36-E, Tokyo, Hirayama manufacturing corporations, Japan) at 121 °C under 15 PSI for 30 minutes.

3.6. Cultural and morphological variations of *Fusarium oxysporum* f. sp. *ciceri*

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

3.7. Size of micro and macro conidia

Micro and macro conidia size was measured with ocular micrometer (Iqbal *et al*., 2005). The data for mycelial growth were analyzed statistically to check the difference.

3.8. Scanning electron microscope (SEM), stereo and compound microscope study of *Fusarium oxysporum* f. sp. *ciceri* isolates

A double sided adhesive carbon cement tape was fixed on an aluminium SEM stub. A loop full of *Fusarium oxysporum* f. sp. *ciceri* pure culture (sporulating plate, 10 days old culture) was taken out with the help of a tungsten loop and gently placed on to the adhesive carbon cement tape. Then aluminium SEM stubs were placed in a platinum coater (Model: JEOL JFC-1600, auto fine coater) and provided 10 mA current flow and 5±0.5 Pa pressure for 10 seconds to make the test samples conductive. After coating the samples the stub was placed into the SEM (Model: JEOL JSM-6490 LA, Analytical Scanning Electron Microscope) for obtaining the image. For getting a clear SEM image working distance, spot size and accelerating voltage was maintained 40, 12 and 10 Kv respectively in high vacuum condition. The pathogen of *Fusarium oxysporum* f. sp. *ciceri* was also studied under stereo (Model: Olympus, SZ 61, Japan) and compound (Model: Olumpus, CX 21 FSI. Tokyo, Japan) microscope.

3.9. Effect of different temperature levels on mycelial radial growth and sporulation of nine *Fusarium oxysporum* f. sp. *ciceri* isolates

The fungus was incubated in PDA media using seven different levels of temperature viz., 5, 10, 15, 20, 25, 30 and 35 °C were studied for its impact on radial colony growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri*. Sixteen (16) ml of PDA medium were poured into the Petri plates using media dispenser (Model: Rudolf, GMBH & Co.) having three replications for each temperature and autoclaved at 121 °C for 30 minutes at 15 PSI and then taken out and shifted into the laminar airflow cabinet for solidification. Five (5) 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 (Model: DB-3153, Delux Automatic B.O.D. Incubator, ®Yorco) maintaining required temperature level. 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 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* (7 days old) 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.

3.10. Effect of pH levels on mycelial radial growth and sporulation of nine *Fusarium oxysporum* f. sp. *ciceri* isolates

The isolates were inoculated into PDA medium having pH levels viz., 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 in 90 mm 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 (Model: DB-3153, Delux Automatic B.O.D.Incubator, ®Yorco). The different level of pH were maintained using 0.1 N NaOH or 0.1N HCl. The design of experiment was the same as mentioned under temperature studies. After two day of incubation, mycelial radial growth data recording were started and continued till covering the whole petriplates of any isolates of *Fusarium oxysporum* f. sp. *ciceri*. The number of spores were counted as same procedure followed in temperature studies.

3.11. Effect of different nutrient media on mycelial radial growth and sporulation of nine *Fusarium oxysporum* f. sp. *ciceri* isolates

Seven different culture media such as potato dextrose agar (PDA), Czapek's dox agar (CDA), malt extract dextrose agar (MDA), corn meal agar (CMA), oat meal agar (OMA), V_8 juice agar (V_8JA) and water agar (WA) media were used in this experiment and composition of this media are described in Table 2. The experimental design used for this study was completely randomized block design (CRD) having 3 replications. Data were recorded on mycelial radial growth of *Fusarium oxysporum* f. sp. *ciceri* after two day of incubation till covering the entire petriplates of any isolates. The spore production of *Fusarium oxysporum* f. sp. *ciceri* on different culture media were counted following the same procedure as stated earlier.

Table 2. Composition of nutrient media used in the experiment

Culture Media	Composition
PDA	Slice potato–200 g, dextrose–20 g, agar–20 g and distilled water– 1000 ml
CDA	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
MDA	Malt extract – 20 g, peptone – 2 g, dextrose – 20 g, agar – 20 g, and distilled water – 1000 ml
CMA	Corn meal infusion form–50 g, agar–15 g and distilled water– 1000 ml
OMA	Oat meal – 60 g, agar – 12.5 g and distilled water – 1000 ml
V_8JA	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
WA	Agar – 20 g and distilled water – 1000 ml

3.12. Pathogenicity test of *Fusarium oxysporum* f. sp. *ciceri* isolates on chickpea

3.12.1. Collection of chickpea seeds

For testing the virulence levels of *Fusarium oxysporum* f. sp. *ciceri* isolates seeds of BARI Chola-1 was collected from Pulse Research Centre, Bangladesh Agricultural Research Institute (BARI).

3.12.2. Preparation of inoculum

Nine isolates of *Fusarium oxysporum* f. sp. *ciceri* (FOC 1-9) were obtained from wilt infected chickpea plants from different chickpea growing regions of Bangladesh. Isolations were made from the wilted plants on potato dextrose agar (PDA) medium.

In order to get a huge amount of inocula of *Fusarium oxysporum* f. sp. *ciceri* isolates. Each isolate was sub-cultured on PDA medium and incubated at least 10 days of incubation, inocula (mycelial mat and spores) were scraped by a plastic scrapper, wrapped with alluminium foil and preserved in the room temperature.

3.12.3. Inoculation and seed sowing

Pathogenic variability of nine *F. oxysporum* f. sp. *ciceri* isolates was tested against BARI Chola-1. Plastic pots (15×20 cm) were used to grow chickpea plants in the pot house of Plant Pathology Division, BARI, Gazipur. The pots were filled with sterilized soil with well decomposed organic matter. Then 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 five seeds of BARI Chola-1 were surface sterilized with Clorox (0.1% available chlorine) and were sown in pots filled with inoculated soil. These pots were kept in the net house of the Plant Pathology Division of BARI.

3.12.4. Conformation of disease

To confirm the symptoms shown on plants that were caused by test isolates, infected plant parts were collected and causal organisms were re-isolated, following the standard isolation procedure (Nene *et al*., 1984).

3.12.5. Assessment of wilt incidence

Data on the number of wilted seedlings in each pot for nine isolates with control were recorded at 30, 45 and 60 days after sowing (DAS). The wilt incidence of each isolate was calculated by the following formula:

$$\text{Wilt incidence} = \frac{\text{No. of plants wilted}}{\text{Total number of plants}} \times 100$$

The level of virulence of each tested isolates was determined by using a 1-5 rating scale described by A. U. Ahmed, PSO, BARI (personal contact).

Where,

1= Highly virulent (HV), 76-100% plants wilted

2= Virulent (V), 51-75% plants wilted

3= Moderately virulent (MV), 26-50% plants wilted

4= Low virulent (LV), 1-25% plants wilted and

5= Avirulent (AV), no plants wilted.

Wilt incidence were recorded at 30, 45 and 60 DAI but aggressiveness of the tested isolates were measured considering wilt incidence only at 60 DAI.

CHAPTER 4
RESULTS

CHAPTER 4
RESULTS

4.1. Study on symptomology of Fusarium Wilt Disease

The symptoms of chickpea wilt was studied and characterized by yellowing and drying of leaves from base to upward, drooping of petioles and rachis, browning of vascular bundles, improper branching, withering of plants and finally death of plants (Plate-1 A-D). Leaves of the wilted plants turned grayish green, then became dull yellow and wilted. Pathogen gets entry into xylem vessel and invades whole vascular system inducing symptoms of yellowing and wilting. The xylem and pith become darkened and discolored. Moreover, internal discoloration of pith and xylem can be seen if stem and root of the wilted plants split vertically (Plate-1 E). The disease occurs at seedling and flowering stage of plant growth.

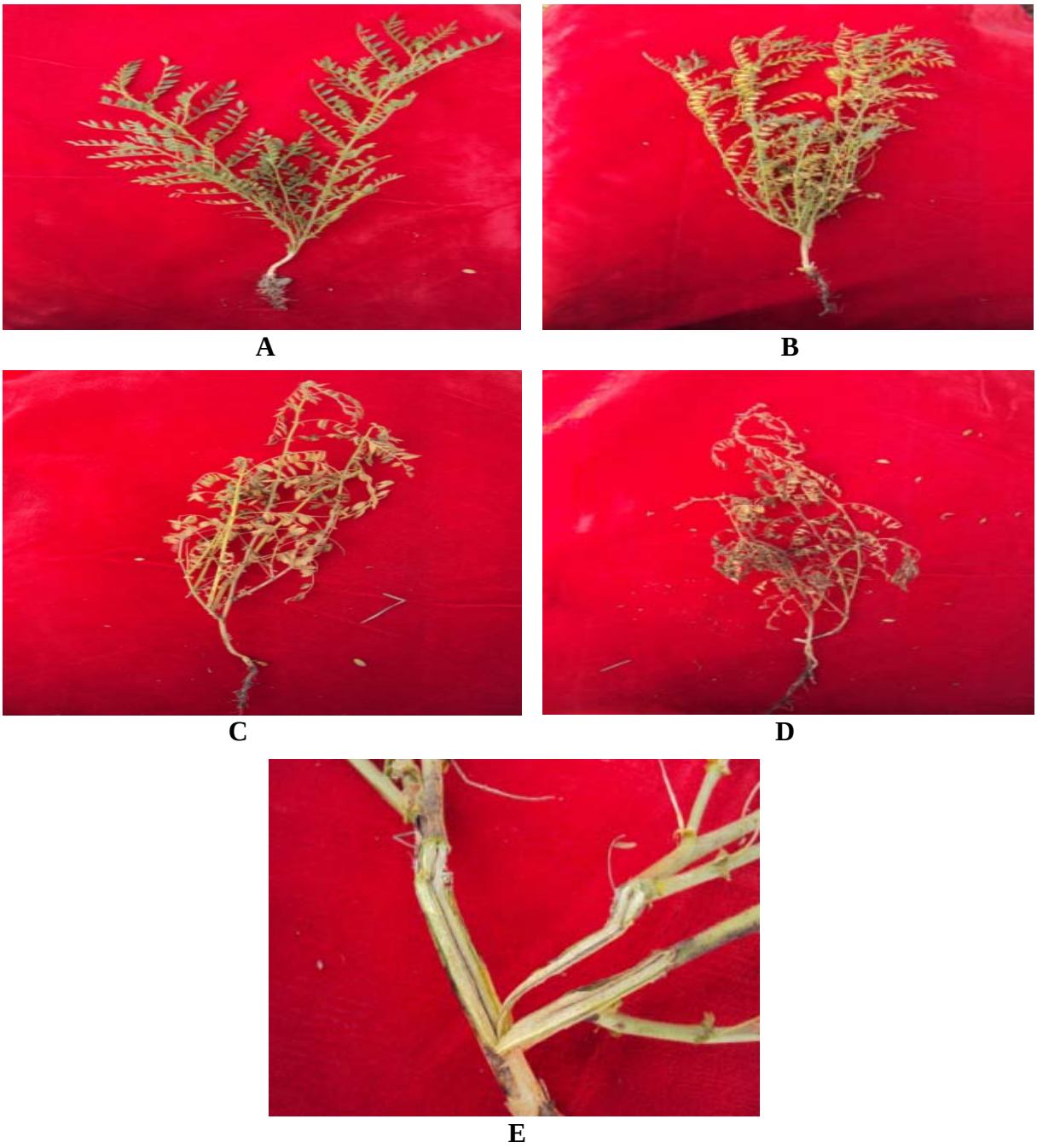


Plate 1. Wilt disease symptom in chickpea: A. Healthy chickpea plant, B. Symptoms expression at primary stage, C. Browning of leaves, D. Dead plant, E. Wilted plants split vertically

4.2. Cultural characteristics of *Fusarium oxysporum* f. sp. *ciceri* isolates

All the isolates of *Fusarium oxysporum* f. sp. *ciceri* exhibited variation in colony characteristics such as color, shape, margin and texture (Plate 2).

Colony color

Colony color was observed purplish white in isolate FOC-1, whitish orange in FOC-2 and FOC-7, creamy white in FOC-3, FOC-4, FOC-6 and cottony white in FOC-5, FOC-8 and FOC-9 (Table 3).

Colony shape

Distinct differences were found in colony shape. Irregular colonies were found in FOC-1, FOC-7 and FOC-9. Regular colonies were observed in FOC-2, FOC-3, FOC-6. Regular with sector was observed in FOC-8 and regular without sector in FOC-4 and FOC-5 (Table 3).

Colony margin:

Colony margin was observed irregular in FOC-1, FOC-7 and FOC-9. Entire colony margin was found in FOC-2 and FOC-3. Both wavy and entire colony margin were found in FOC-5, FOC-8. Only wavy margin was observed in isolates FOC-4 and FOC-6 (Table 3).

Colony texture:

Fluffy colony texture was found in FOC-1, FOC-2, FOC-7, FOC-8 and FOC-9. While flat/velvet colony texture was observed in FOC-3, FOC-4, FOC-5 and FOC-6 (Table 3).

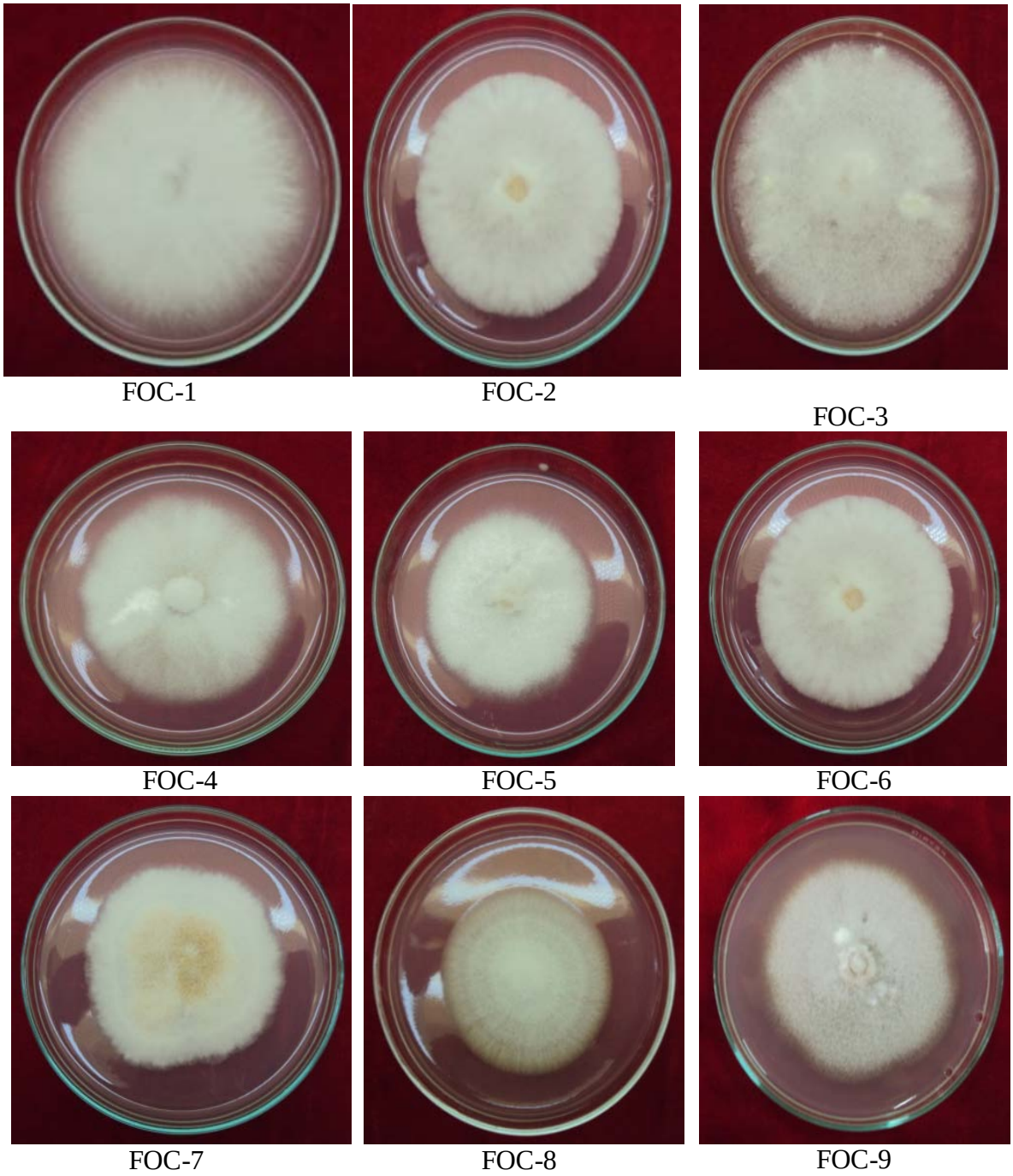


Plate 2. Cultural characteristics of nine *Fusarium oxysporum* f. sp. *ciceri* isolates on potato dextrose agar (PDA) medium

Table 3. Colony characteristics of nine *Fusarium oxysporum* f. sp. *ciceri* isolates grown on PDA medium

Isolates	Colony characteristics			
	Color	**Shape**	**Margin**	**Texture**
FOC 1	Purplish white	Irregular	Irregular	Fluffy
FOC 2	Whitish orange	Regular	Entire	Fluffy
FOC 3	Creamy white	Regular	Entire	Flat/Velvet
FOC 4	Creamy white	Regular without sector	Wavy	Flat/Velvet
FOC 5	Cottony white	Regular without sector	Wavy, entire	Flat/Velvet
FOC 6	Creamy white	Regular	Wavy	Flat/Velvet
FOC 7	Whitish orange	Irregular	Irregular	Fluffy
FOC 8	Cottony white	Regular with sector	Wavy, entire	Fluffy
FOC 9	Cottony white	Irregular	Irregular	Fluffy

4.3. Stereo, compound and scanning electron microscope (SEM) study of *Fusarium oxysporum* f. sp. *ciceri*

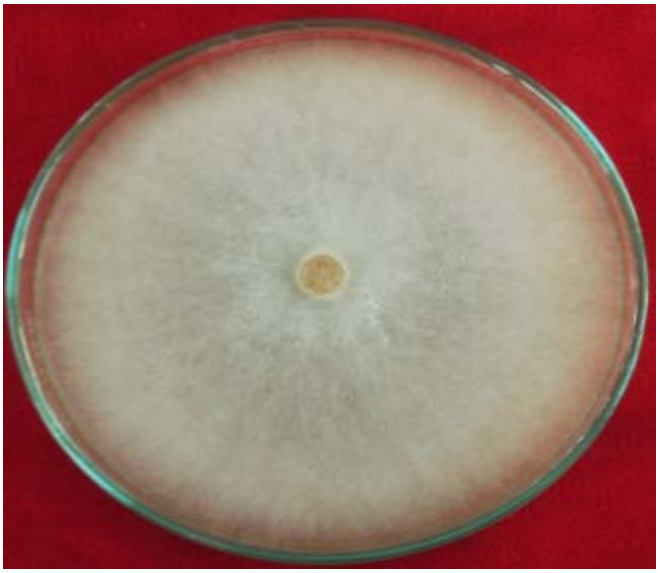
Characteristics features were investigated by studying *Fusarium oxysporum* f. sp. *ciceri* under stereo, compound and SEM.

The fungus hyphae were septate and profusely branched. The fungus produced micro conidia, macro conidia and chlamydospores (Plate 3 A-D).

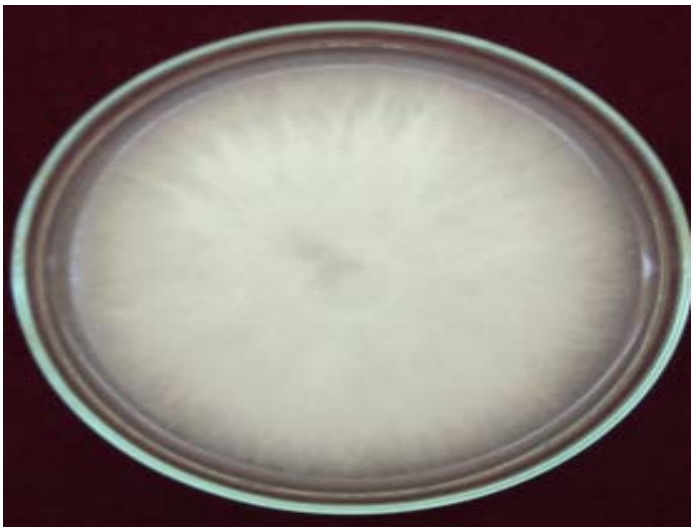
Chlamydospores are formed in old cultures, which are smooth or rough walled, usually vacuolated and spherical, terminal or intercalary and may form singly, in pairs or in chain (Plate 3 D).

Micro conidia were abundant, hyaline, continuous or 1-2 septate, borne on simple short conidiophores, arising laterally on the hyphae, oval to cylindrical, straight to slightly curved (Plate 4 A).

Macro conidia were lesser in number than micro conidia, 3-5 septate, borne on branched conidiophores, thin-walled (Plate 4 A-B).

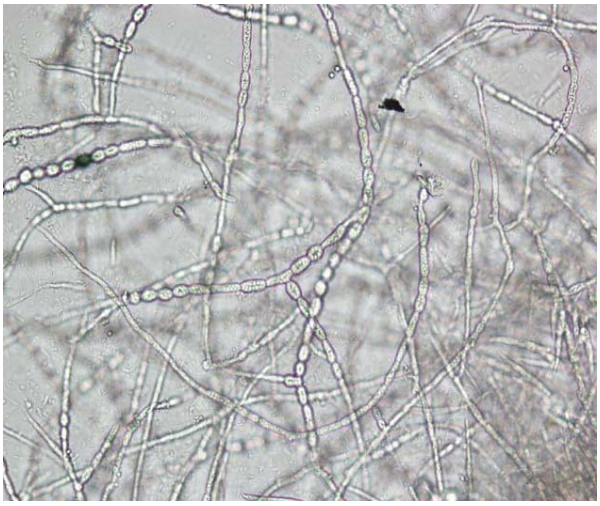


A



B

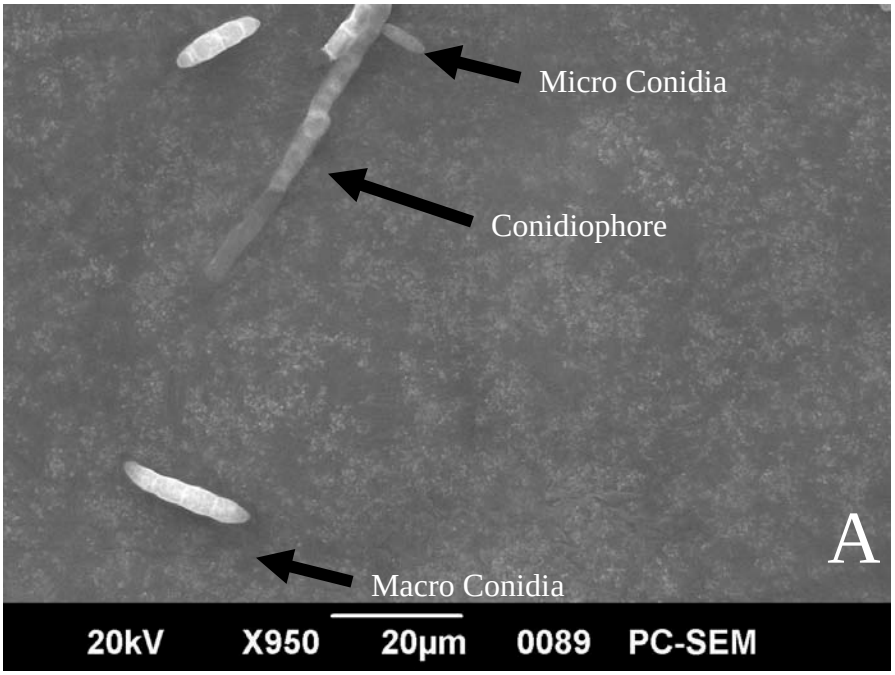
C



D

Plate 3. Microscopic and visual observation of *F. oxysporum* f. sp. *ciceri*
A & B - Colony growth of *F. oxysporum* f. sp. *ciceri*
C - Conidia of *F. oxysporum* f. sp. *ciceri*

D – Chlamydospores of *F. oxysporum* f. sp. *ciceri*



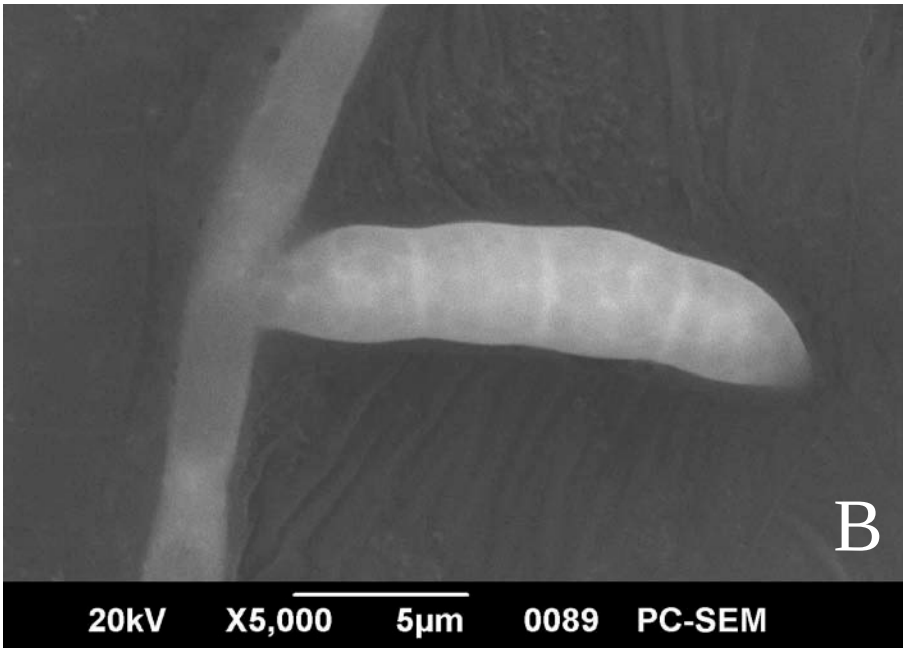


Plate 4. Secondary electron image of *Fusarium oxysporum* f. sp. *ciceri*

A. Micro conidia, macro conidia and conidiophore
B. Conidiophore bearing conidia

4.3.1. Conidial dimensions of nine isolates of *Fusarium oxysporum* f. sp. *ciceri*

Remarkable variations were observed in length, breadth and septation of both micro and macro conidia of nine *Fusarium oxysporum* f. sp. *ciceri* isolates (Table 4 and 5).

The length of micro conidia varied from 5.00-14.00 µm (average 6.25- 11.00 µm). Highest (11.00 µm) average length of micro conidia was observed in FOC-1 and lowest (6.25 µm) average length was observed in FOC-4. The breadth of micro conidia was 1.00-4.00 µm (average 1.95-2.80 µm). Micro conidia was 0-2 septed.

The length of macro conidia ranged from 9.00-26.00 µm (average 12.00- 20.45 µm). Highest (20.45 µm) average length of macro conidia was observed in FOC-7 and lowest (12.00 µm) average length was observed in FOC-3. The breadth of macro conidia was 1.00-5.00 µm (average 2.45-3.60 µm) and Macro conidia was 1-5 septed.

Table 4. Dimension and septation of micro conidia of nine *Fusarium oxysporum* f. sp. *ciceri* isolates

Isolate No.	Micro conidia					
	Length (µm)		Breadth (µm)		Septation	
	Average	Range	Average	Range	Average	Range
FOC 1	11.00	6-14	2.80	2-4	0.15	0-1
FOC 2	9.90	6-14	1.95	1.5-3	0.85	0-2
FOC 3	7.30	5-10	2.28	1-3	0.15	0-1
FOC 4	6.25	5-9	2.18	1.5-3	0.00	0
FOC 5	6.90	6-8	2.28	1.5-3	0.15	0-1
FOC 6	7.75	6-11	2.10	1.5-3	0.10	0-1
FOC 7	7.40	5-12	1.95	1-3	0.25	0-1
FOC 8	8.25	7-11	2.00	1-3	0.25	0-1
FOC 9	7.55	5-10	1.98	1-3	0.30	0-1

Table 5. Dimension and septation of macro conidia of nine *Fusarium oxysporum* f. sp. *ciceri* isolates

Isolate No.	Macro conidia					
	Length (µm)		Breadth (µm)		Septation	
	Average	Range	Average	Range	Average	Range
FOC 1	17.55	12-25	3.03	1.5-5	3.50	3-5
FOC 2	17.30	11-25	3.03	2-4	3.15	2-5
FOC 3	12.00	9-15	2.45	2-3	1.50	1-4
FOC 4	14.15	10-18	2.58	2-3	2.15	1-4
FOC 5	16.35	11-25	2.70	2-4	2.70	1-4
FOC 6	18.55	12-25	3.00	1-4	3.40	2-5
FOC 7	20.45	15-26	3.60	2-5	3.65	2-5
FOC 8	18.90	12-25	3.30	2-4	3.50	2-5
FOC 9	13.15	11-16	2.58	2-3	1.90	1-3

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

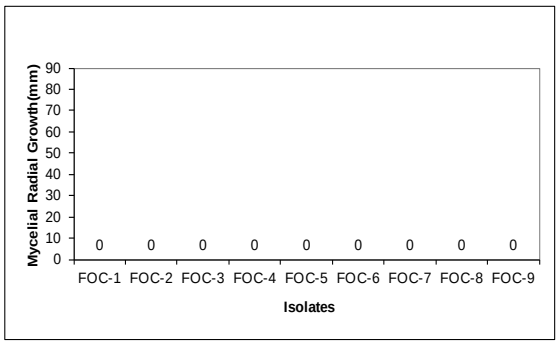
The effect of temperature on the growth of 9 *F. oxysporum* f .sp. *ciceri* isolates was studied and results are presented in Fig. 1 and Plate 5. As evident from the study the fungus grew at the temperature range of 10–35 °C. It was seen that there was quite a large variation in the growth of these isolates at different temperature after 7 days of incubation. Maximum growth was found between 25 and 30 °C for all the 9 isolates after 7 days of incubation. The radial growth increased with the increase of temperature up to 25 °C and decreased thereafter. However, other temperature below 25 °C and above 30 °C retarded the colony growth. Highest (78.00 mm) radial growth was obtained in isolate FOC-2 followed by FOC-9 (76.67 mm) at 25 °C. The lowest radial growth (9.66 mm) was noted at 35 °C in case of FOC-2.

Production of micro and macro conidia of *F. oxysporum* f. sp. *ciceri* varied greatly with different temperature levels. Production of micro and macro conidia of *F. oxysporum* f. sp. *ciceri* at different temperature level are represented in Tables 6 and 7. Micro spore production was increased with the increase of temperature up to 25 °C and declined thereafter in all the isolates. The maximum (6.78×10^5 ml^{-1}) sporulation was observed in FOC-3 at 25 °C followed by FOC-6 (6.00×10^5 ml^{-1}); FOC-1 (5.13×10^5 ml^{-1}) and FOC-7 (3.70×10^5 ml^{-1}) after seven days of incubation period. The minimum (3.30×10^3 ml^{-1}) sporulation was observed in FOC-4 at 15 °C. Very few sporulation occurs in FOC-3, FOC-4, FOC-5, FOC-6, FOC-7, FOC-8, FOC-9 at 10 °C. Spore production was not observed in isolates at

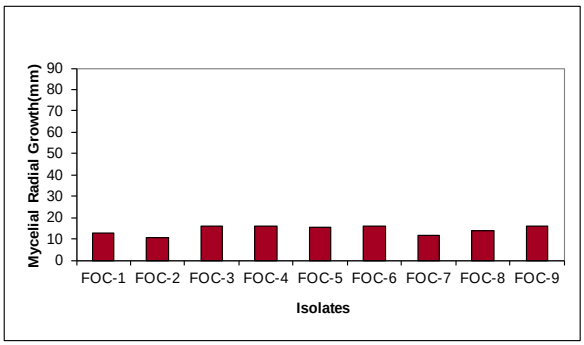
5 °C in FOC-1 and FOC- 2 at10 °C, in FOC-9 at 15 °C and in FOC-4, FOC-5, FOC-7 at 35 °C.

Macro spore production was increased with the increase of temperature up to 25 °C and declined thereafter in all the isolates. The maximum (3.43 x 10^6 ml^{-1}) sporulation 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. FOC-1, FOC-2, FOC-4, FOC-7 and FOC-8 produced very few spore at 10 °C. All the nine isolates failed to produce any spore at 5 °C temperature, FOC-6 and FOC-7 at 15 °C; FOC-8 at 30 °C; FOC-3, FOC-5, FOC-6 and FOC-8 at 35 °C temperature.

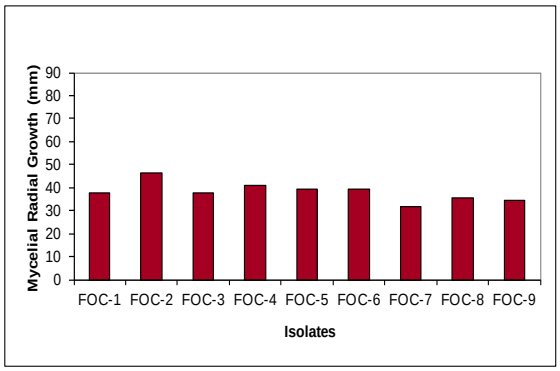
From this experiment, it appeared that 25 °C temperature is suitable for mycelial radial growth and spore production of *Fusarium oxysporum* f. sp. *ciceri.*



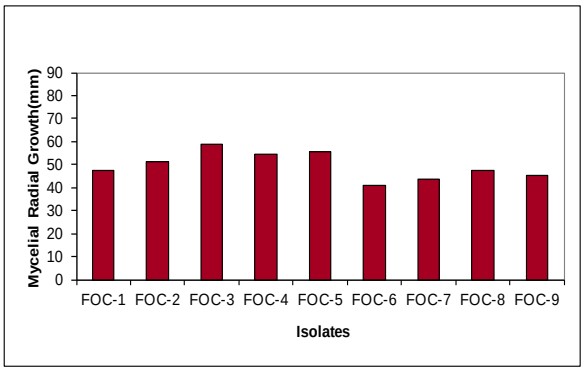
At 5°C



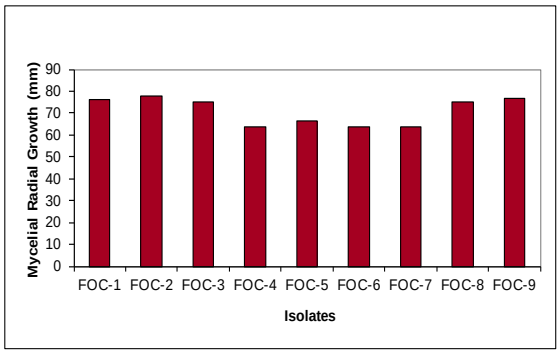
At 10°C



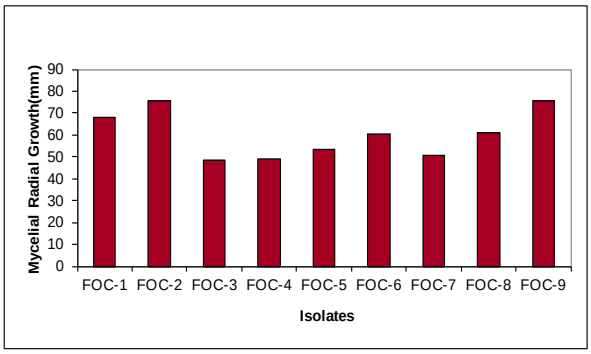
At 15°C



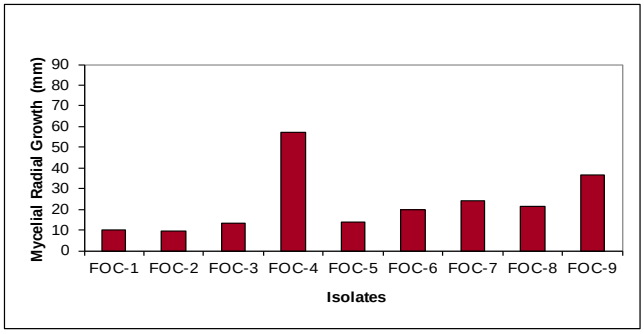
At 20°C



At 25°C

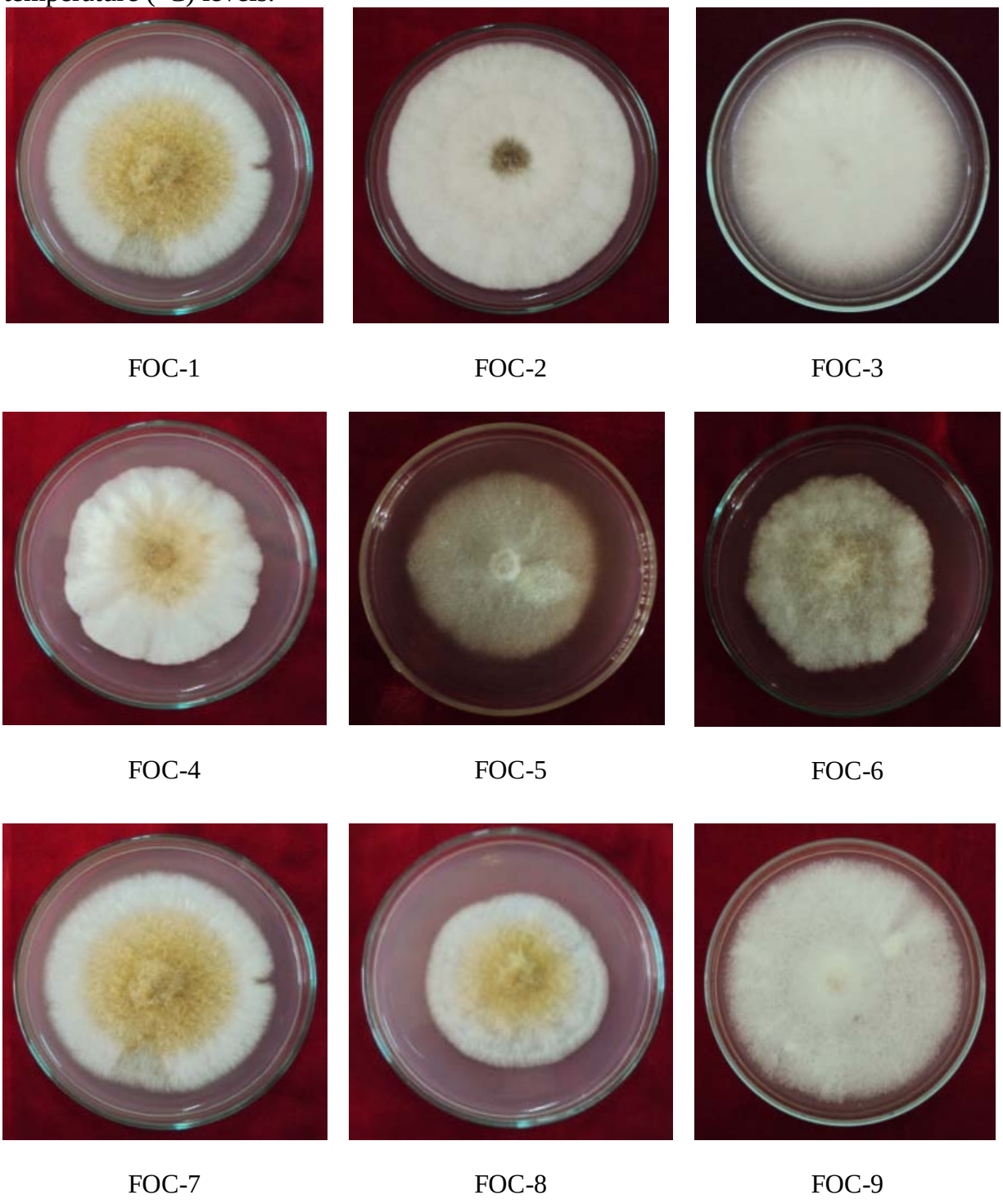


At 30°C



At 35°C

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



FOC-1 FOC-2 FOC-3

FOC-4 FOC-5 FOC-6

FOC-7 FOC-8 FOC-9

Plate 5. Radial mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* at 25 °C temperature (°C) level.

Table 6. 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	*	*	3.33×10^4	6.38×10^4	5.13×10^5	1.12×10^5	3.38×10^4
FOC-2	*	*	2.61×10^4	7.33×10^4	1.66×10^5	9.38×10^4	5.61×10^4
FOC-3	*	**	6.27×10^3	8.25×10^3	6.78×10^5	3.86×10^4	2.71×10^4
FOC-4	*	**	3.30×10^3	1.00×10^4	1.25×10^5	1.83×10^4	*
FOC-5	*	**	4.00×10^4	1.36×10^5	3.63×10^5	2.98×10^5	*
FOC-6	*	**	3.66×10^4	1.01×10^5	6.00×10^5	3.60×10^5	1.83×10^5
FOC-7	*	**	5.33×10^4	2.85×10^5	3.70×10^5	1.38×10^5	*
FOC-8	*	**	4.16×10^4	7.66×10^4	2.61×10^5	8.50×10^4	3.83×10^4
FOC-9	*	**	*	1.16×10^4	9.66×10^4	5.16×10^4	8.33×10^3

* No sporulation
** Very few sporulation

Table 7. Effect of temperature on production of macro conidia of 9 *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	3.43×10^6	1.64×10^5	1.33×10^4
FOC-2	*	**	2.44×10^4	7.33×10^4	1.56×10^5	1.03×10^5	1.66×10^3
FOC-3	*	*	6.44×10^3	1.06×10^4	4.33×10^4	1.97×10^4	*
FOC-4	*	**	2.50×10^4	8.66×10^4	1.33×10^5	2.66×10^4	1.66×10^4
FOC-5	*	*	1.66×10^3	3.33×10^3	2.00×10^4	1.00×10^4	*
FOC-6	*	*	*	6.66×10^3	6.66×10^5	1.66×10^3	*
FOC-7	*	**	*	1.00×10^4	2.70×10^5	3.33×10^3	3.33×10^3
FOC-8	*	**	1.66×10^3	3.33×10^3	3.83×10^4	*	*
FOC-9	*	*	5.33×10^4	1.73×10^5	5.58×10^5	2.61×10^5	6.33×10^4

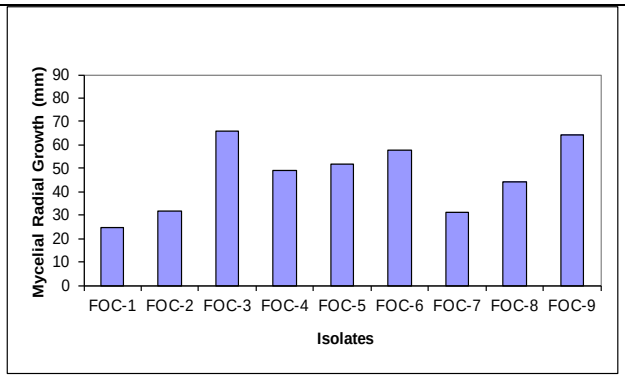
* No sporulation
** Very few sporulation

4.5. Effect of pH on mycelial radial growth and sporulation of nine *Fusarium oxysporum* f. sp. *ciceri* isolates

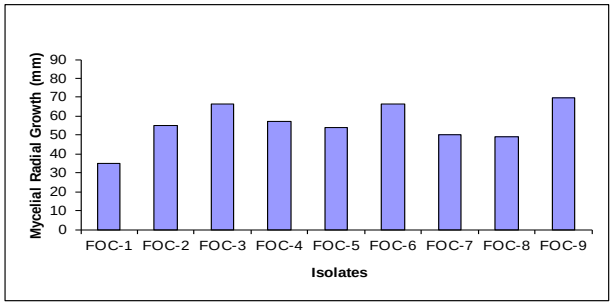
The fungus grew well on PDA medium with a wide range of pH 4.5 to pH 7.0 in this study (Fig. 2 and Plate 6). Among the pH levels, luxuriant radial growth exhibited in all of the isolates at pH 6.0 and pH-6.5. The highest colony diameter was noted from the isolate FOC-2 at pH 6.0 (87.83 mm) followed by FOC-1 at pH 6.0 (86.17mm) and FOC-8 (84.50 mm). The lowest mycelial radial growth was recorded in isolate FOC-1 at pH 4.5 (24.83mm) and the second lowest was in FOC-7 at pH-4.5 (31.00 mm).

The micro conidia production of *F. oxysporum* f. sp. *ciceri* isolates varied with different pH levels (Table 8). 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}) after seven days of incubation period. The minimum sporulation was observed on FOC-3 (8.87x 10^3 ml^{-1}) at pH 4.5.

The macro conidia production of *F. oxysporum* f. sp. *ciceri* isolates also varied with different pH levels. The effect of six different pH levels on macro conidia production of *F. oxysporum* f. sp. *ciceri* isolates on PDA are presented in Table 9. Maximum (7.06 x 10^5 ml^{-1}) macro conidia was produced by FOC-9 at pH 6.0 after seven days of incubation period. The 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.



pH 4.5



pH 5.0

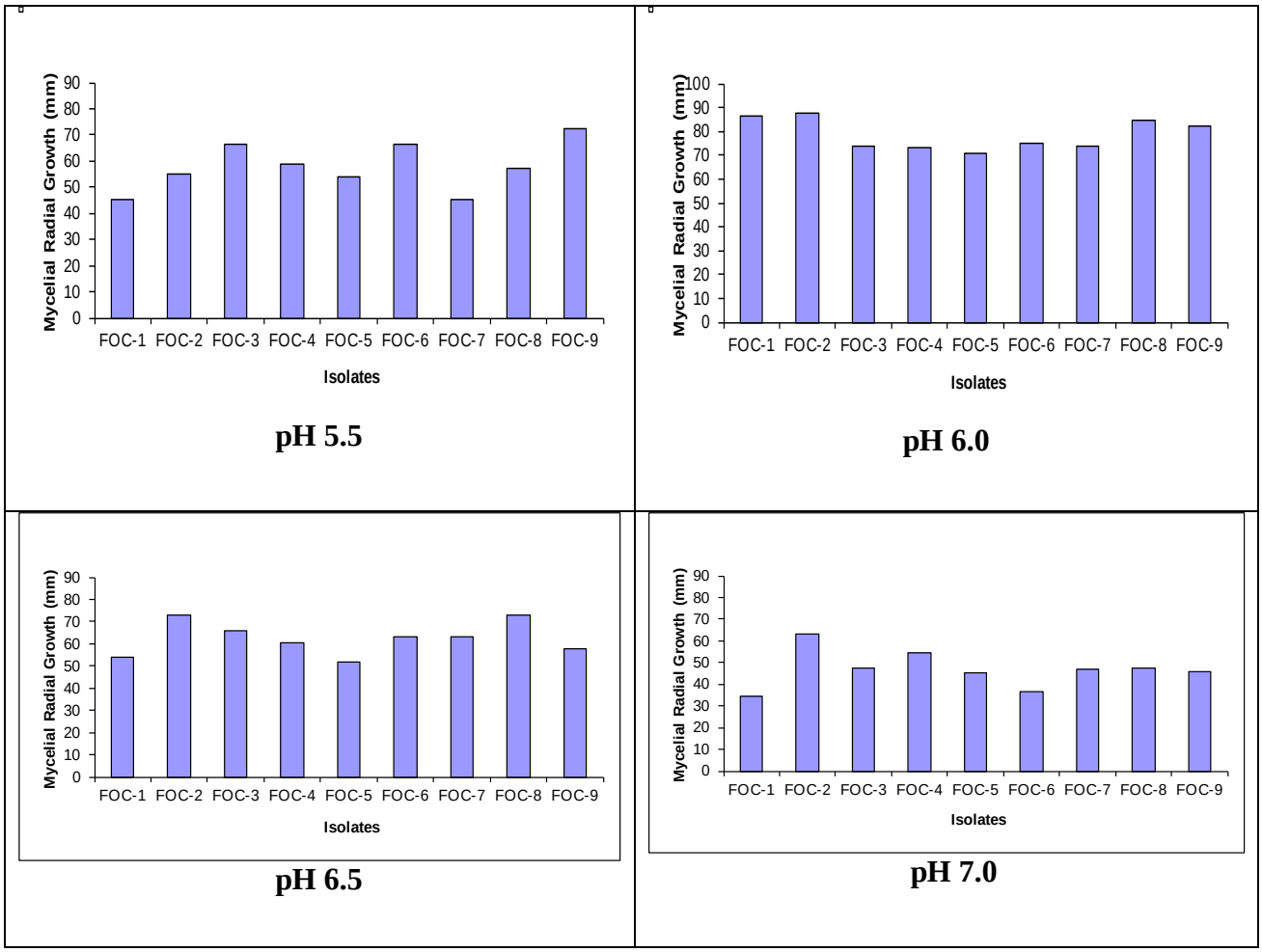


Figure 2. Effect of pH on mycelial radial growth of *Fusarium oxysporum* f. sp. *ciceri* isolates.

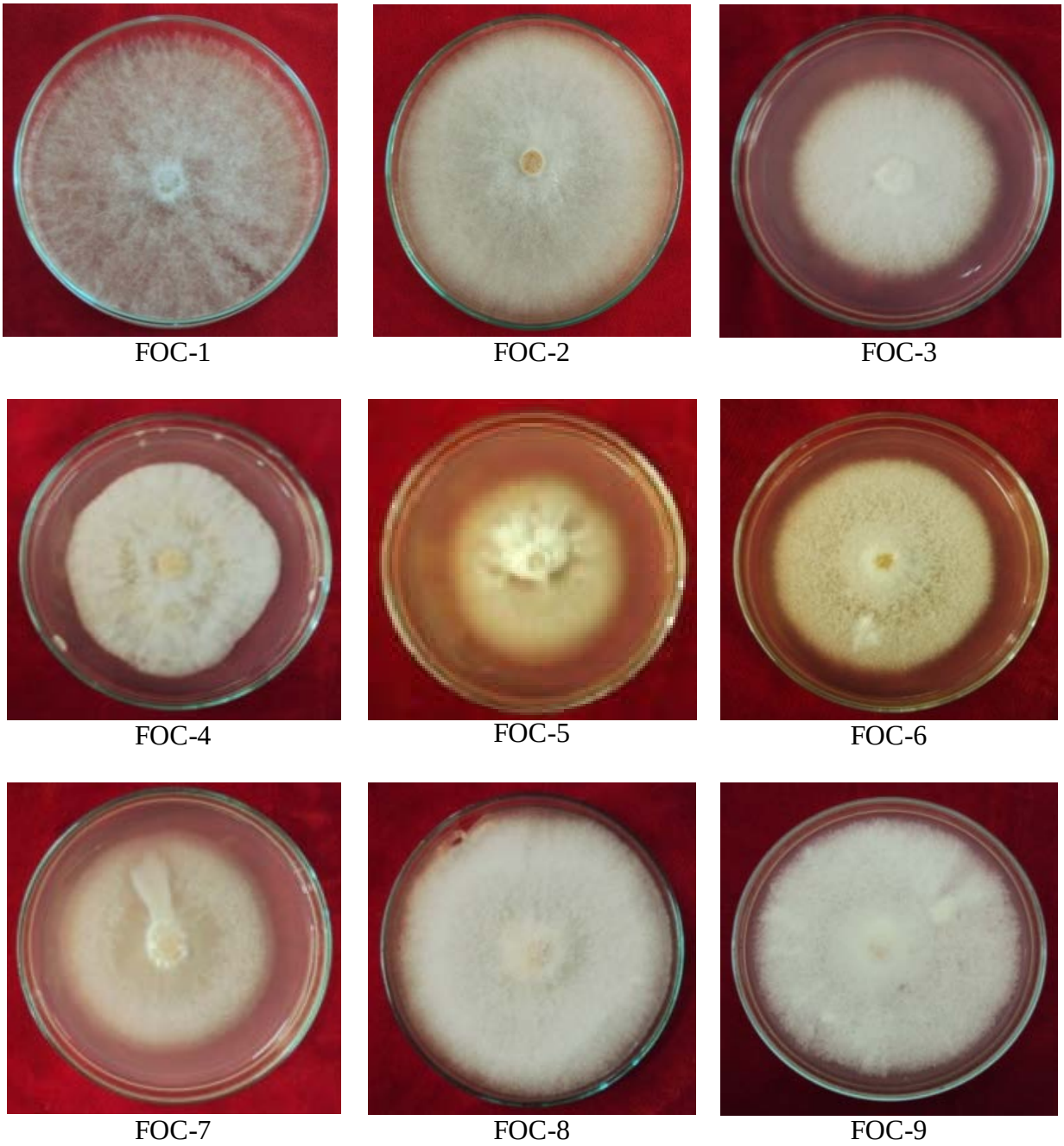


Plate 6. Radial mycelial growth of 9 *Fusarium oxysporum* f. sp. *ciceri* isolates at pH 6.0.

Table 8. 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	5.61×10^4	5.77×10^4	6.72×10^4	8.00×10^4	6.72×10^4	5.27×10^4
FOC-2	3.94×10^4	4.72×10^4	7.50×10^4	7.50×10^4	5.88×10^4	5.77×10^4
FOC-3	8.87×10^3	1.06×10^4	1.33×10^4	8.03×10^4	1.61×10^4	1.31×10^4
FOC-4	4.50×10^4	5.00×10^4	6.00×10^4	1.51×10^5	9.50×10^4	5.00×10^4
FOC-5	3.66×10^4	3.83×10^4	5.83×10^4	1.86×10^5	6.16×10^4	6.00×10^4
FOC-6	2.50×10^4	4.00×10^4	7.16×10^4	1.56×10^5	8.83×10^4	7.33×10^4
FOC-7	6.16×10^4	7.33×10^4	1.03×10^5	3.03×10^5	1.61×10^5	1.58×10^5
FOC-8	1.16×10^4	3.50×10^4	3.66×10^4	7.33×10^4	6.66×10^4	6.83×10^4
FOC-9	1.16×10^4	2.33×10^4	3.00×10^4	1.01×10^5	8.83×10^4	6.50×10^4

Table 9. 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	$2.50x10^4$	5.72×10^4	1.72×10^5	3.90×10^5	1.38×10^4	1.00×10^4
FOC-2	2.00×10^4	5.05×10^4	8.61×10^4	4.76×10^5	8.33×10^4	1.33×10^4
FOC-3	1.00×10^4	1.35×10^4	5.47×10^4	1.50×10^5	2.30×10^4	1.16×10^4
FOC-4	5.00×10^3	6.66×10^3	3.25×10^5	3.80×10^5	6.66×10^3	*
FOC-5	3.50×10^4	9.33×10^4	6.66×10^3	2.30×10^5	1.66×10^3	*
FOC-6	1.66×10^3	1.66×10^3	$5.00x\ 10^3$	7.00×10^4	1.00×10^4	8.33×10^3
FOC-7	3.33×10^3	8.33×10^3	9.83×10^4	1.65×10^5	*	2.00×10^4
FOC-8	1.66×10^3	3.33×10^3	5.66×10^4	8.66×10^4	*	*
FOC-9	*	1.42×10^3	5.21×10^5	7.06×10^5	6.00×10^4	$3.33x\ 10^3$

* No sporulation

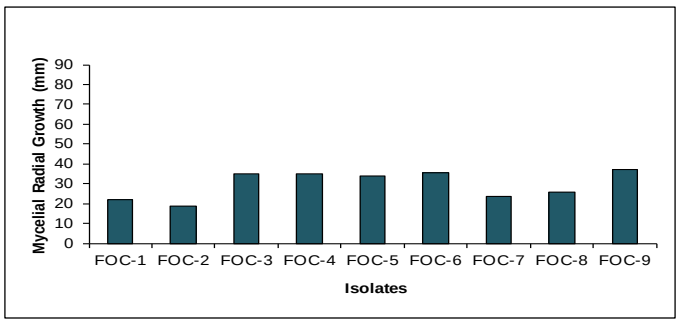
4.6. Effect of culture media on mycelial radial growth and sporulation of nine *Fusarium oxysporum* f. sp. *ciceri* isolates

Seven culture media were tested to find out the most suitable one for the mycelial growth of the fungus. Mycelial colony growth of *F. oxysporum* f. sp. *ciceri* varied greatly with different culture media (Fig. 3 and Plate 7). The most effective medium for 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.

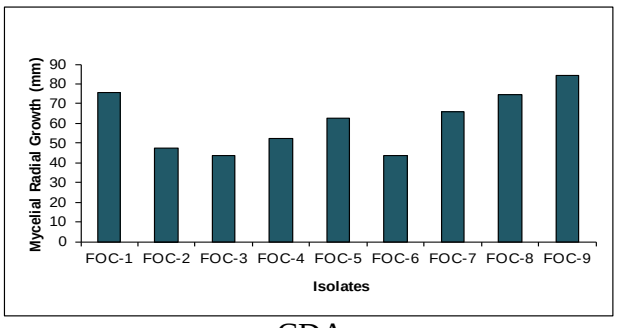
It was followed by 84.33 mm in malt extract dextrose agar (MDA), 80.00 mm in corn meal agar (CMA) and 78.33mm in V_8 juice agar (V_8 JA) medium. The highest radial growth was observed in FOC-9 at OMA (90.00mm) medium and the lowest mycelial growth was noted in FOC-8 at WA (0.00 mm) medium.

Results of effect of seven culture media on the production of micro conidia of *F. oxysporum* f. sp. *ciceri* isolates on PDA are presented in Table 10. Maximum (4.06×10^5 ml^{-1}) micro conidia was produced by FOC-5 at PDA after seven days of incubation. The minimum (2.41×10^3 ml^{-1}) sporulation was observed on FOC-3 at CDA medium. No micro conidia was produced by FOC-4 at V_8 JA and all isolates of WA medium.

Maximum (3.27×10^5 ml^{-1}) macro conidia was produced by FOC-1 on PDA after seven days of incubation. However, minimum (1.08×10^3 ml^{-1}) sporulation of macro conidia was observed on FOC-3 at V_8 JA medium. No macro conidia was produced by FOC-8 on CDA; FOC-5, FOC-6, FOC-8 on MDA; FOC-6 on OMA and all isolates on WA medium (Table 11).



PDA



CDA

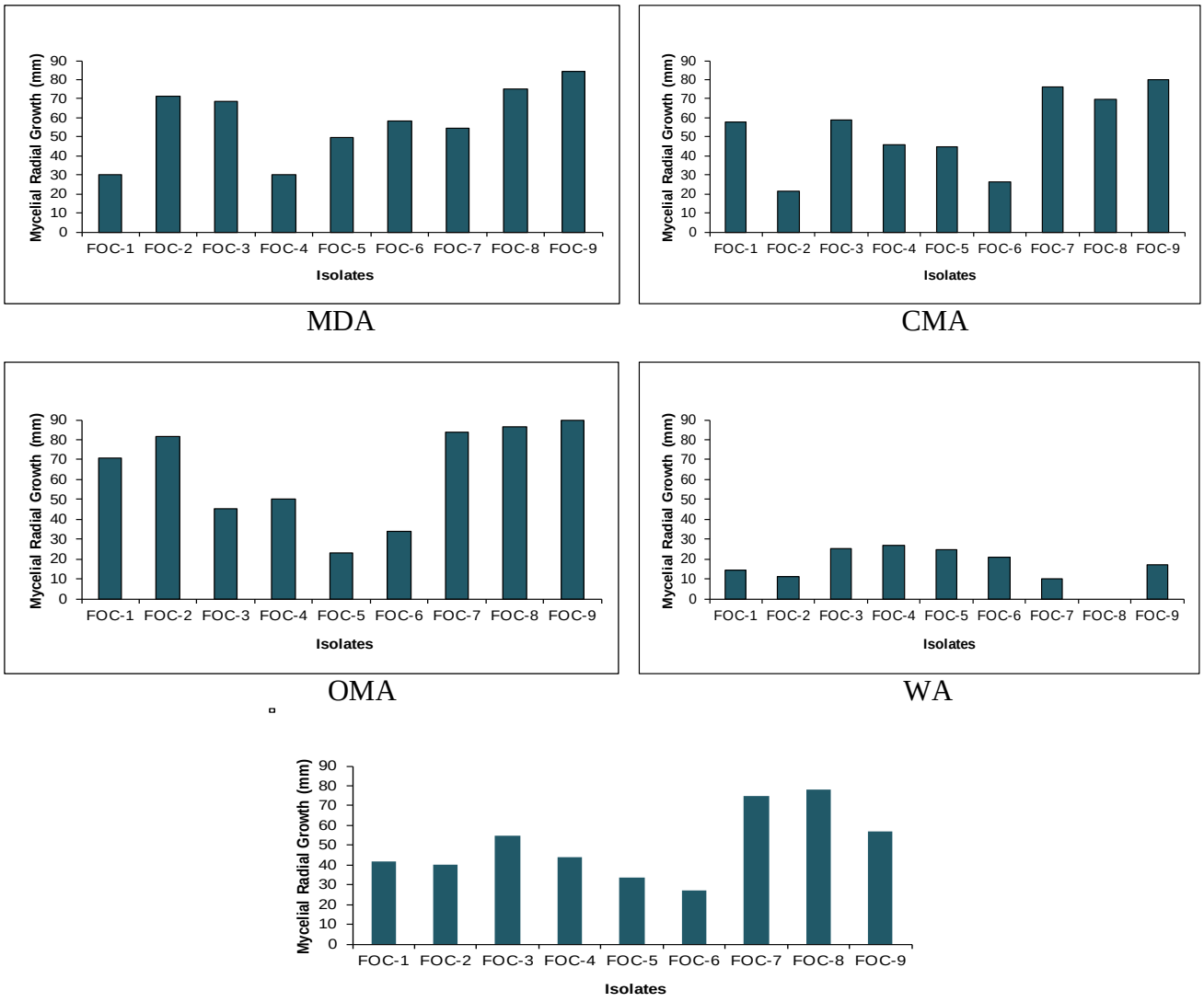


Figure 3. Effect of culture media on mycelial radial growth and sporulation of 9 *Fusarium oxysporum* f. sp. *ciceri* isolates.

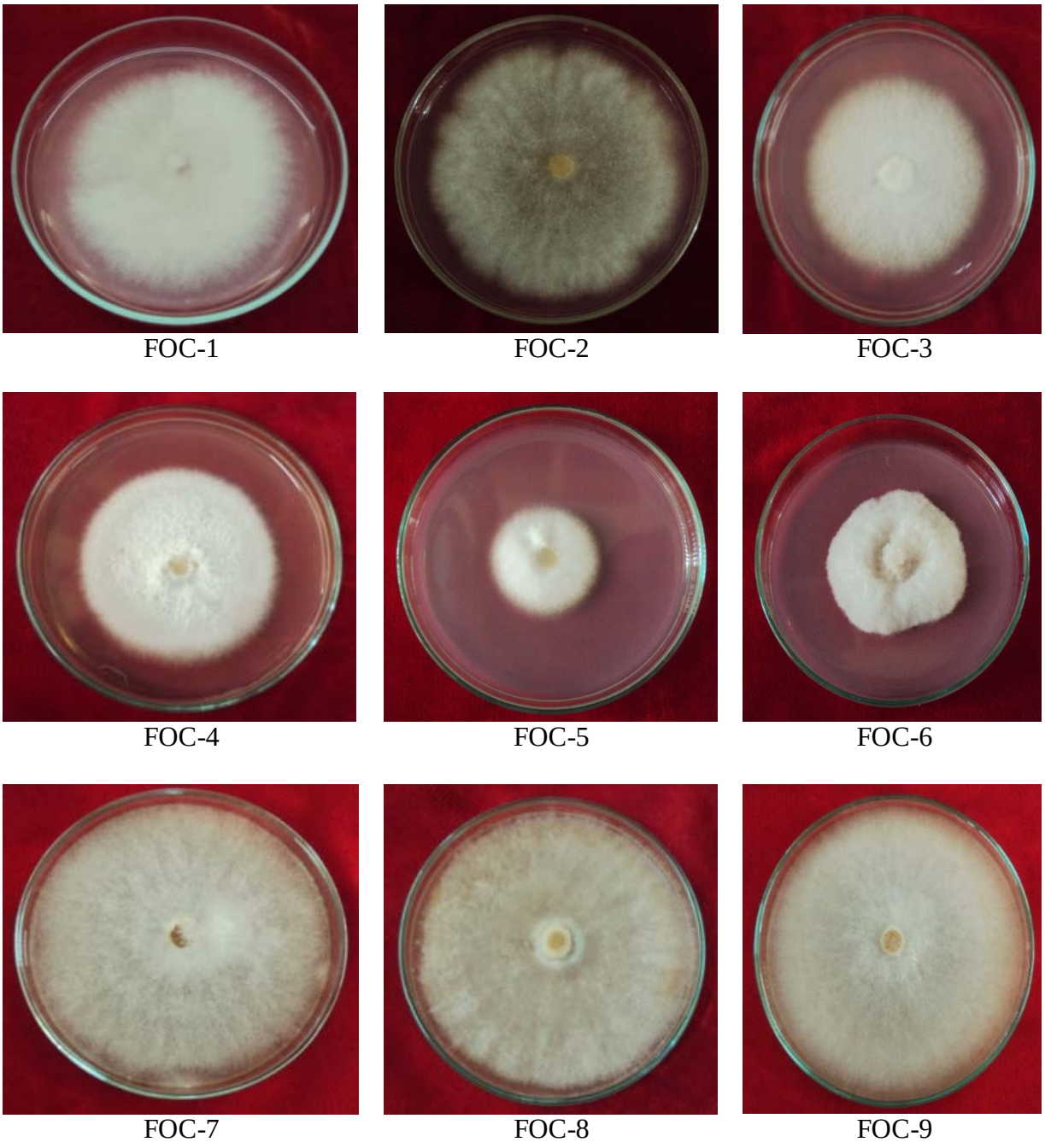


Plate 7. Radial mycelial growth of 9 *Fusarium oxysporum* f. sp. *ciceri* isolates at Oat Meal Agar (OMA) media.

Table 10. Effect of culture media on production of micro conidia of *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

Table 11. Effect of culture media on production of 9 macro conidia of *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	8.33×10^3	*	*	6.66×10^3	1.00×10^4	*	6.66×10^3
FOC-9	1.06×10^5	1.55×10^5	6.16×10^4	1.66×10^3	7.50×10^4	*	3.33×10^3

* No sporulation

4.7. Pathogenic Variation

There was significant variation among isolates in their disease reaction. The wilt disease incidence caused by nine isolates of *Fusarium oxysporum* f. sp. *ciceri* at 30, 45 and 60 DAI. Wilt incidence at 30 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%. The highest (86.67%) disease incidence was recorded in chickpea by FOC-1 at 60 DAI followed by 73.33% by FOC-7 and FOC-8. The least wilt incidence (13.33%) was noted by FOC-6 at 60 DAI. No wilt disease was observed in control (uninoculated) pot.

Variation in disease rating of each *F. oxysporum* f. sp. *ciceri* isolate tested exhibited a large variability. Only 1 isolate (FOC-1) was found to be the highly virulent (HV) with 86.67% wilt incidence after 60 DAI, while, 2 isolates (FOC-2 and FOC-5) showed moderately virulent (MV), 4 isolates (FOC-3, FOC-4, FOC-6 and FOC-9) showed low virulent (LV) and 2 isolates (FOC-7 and FOC-8) showed virulent (V) type of aggressiveness in their pathogenicity. None of the nine tested isolates was found to be avirulent (AV) in this study (Table 12).

Table 12. 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	-

CHAPTER 5
DISCUSSION

CHAPTER 5
DISCUSSION

In the present research work, all nine isolates (FOC-1to FOC-9) showed variations in respect of cultural, Morphological, physiological and pathologic characteristics.

5.1. Cultural variability in *F. oxysporum* f. sp. *ciceri*

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. 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).

5.2. Morphological variability in *F. oxysporum* f. sp. *ciceri*

In this experiment it was observed that the length of micro conidia varied from 5.00-14.00 µm (average 6.25- 11.00 µm). The breadth of micro conidia was 1.00-4.00 µm (average 1.95-2.80 µm). Micro conidia was 0-2 septed. The length of macro conidia ranged from 9.00-26.00 µm (average 12.00- 20.45 µm). The breadth of macro conidia was 1.00-5.00 µm (average 2.45-3.60 µm) and Macro

conidia was 1-5 septed. *F. oxysporum* f. sp. *ciceri* showed variations in the size of micro and macro-conidia of 27 isolates 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× 20.10µm of the macro -conidia was obtained from the isolates Foc-25 and the smallest size of 3.5× 22.5µm were obtain ed from isolates Foc-11 respectively. The rest of isolates had intermediate size. (Ahmad, 2010). While, Saxena and Singh (1987) described that on solid medium, micro conidia were oval to cylindrical, straight or curved and measure 2.5 -3.5 × 5–11 µm and macro conidia were thin walled, septate with 3–5 septa, pointed at both ends and measures 3.5 – 4.5 × 25 – 65 µm. Gupta *et al.,* (1987) reported similar observations the conidiophores thin walled, 3 – 5 septate, fused, pointed both ends and measures 3.5 – 4.5 × 25 – 65 µm. Macro conidia are fewer than micro conidia. Isolates also showed differences in spore size of micro- and macro conidia. (Edel *et al.,* 2000; Jimenez *et al.,* 1993; Haware and Nene, 1992).

5.3. Physiological variability in *F. oxysporum* f. sp. *ciceri*

5.3.1. Temperature

The fungus grew at the temperature range of 10–35 °C. Maximum growth was found between 25 and 30 °C as the fungus diameter at this temperature was 78.00 and 75.67 mm 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 case of FOC-2. The

present findings agreed with Farooq *et al.*, (2005). He reported that the growth of the fungus 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 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. Gupta *et al.,* (1986) reported similar findings regarding temperature requirements to this fungus. Soil temperature relationship indicated that suitable temperature for development of chickpea wilt is 25-30 °C (Chauhan, 1965). The growth of Fusarium wilt fungus took place at all temperature levels but the most suitable temperature supporting the maximum mycelial growth of the pathogen was found between 25 °C–30 °C as the diameter at this temperature was 76.8 to 85.4 mm, respectively. Similar studies have also been demonstrated by various workers (Navas-Cortes *et al*., 2007; Landa *et al*., 2006; Farooq *et al.*, 2005; Bhatti and Kraft, 1992; Chi and Hansen, 1964 and Agrios, 2005). Whereas, Chauhan (1963), Sinha and Dahiya (1973) 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. Imran Khan *et al.,* (2011) showed the *F. oxysporum* f. sp. *ciceri* grew highest at 30 °C. The effects of temperature of *F. oxysporum* f. sp. *ciceri* was studied by Landa *et al.,* (2001).They found the disease development was greater at 25 °C compared with 20 and 30 °C. Anjaneya Reddy (2002) reported that growth of 40 isolates of *F. udum* differed in their temperature requirement which varied

from 20 °C to 35 °C. Scott *et al.,* (2010) 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 an apparent maximum near 25 °C. The maximum sporulation was observed on FOC-1 (3.43 x 10^6 ml^{-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. However, minimum sporulation was observed on FOC-5 and FOC-8 (1.66 x 10^3 ml^{-1}) at 15 °C and FOC-2 (1.66 x 10^3 ml^{-1}) at 35°C. Very few sporulation occurs on FOC-1, FOC-2, FOC-4, FOC-7, FOC-8 at 10 °C. All the nine isolates of *F. oxysporum* f. sp. *ciceri* failed to produce any spore at 5 °C temperature. Abundant sporulation of this fungus were 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}.

5.3.2. pH

The results of this experiment indicated that luxuriant radial growth exhibited in all of the isolates at pH 6.0 and pH 6.5. The highest colony diameter was noted for the isolate FOC-2 at pH 6.0 (87.83 mm) followed by FOC-1 at pH 6.0 (86.17mm) and FOC-8 at pH 6.0 (84.50 mm). The lowest mycelial radial growth was recorded in isolate FOC-1 at pH 4.5 (24.83mm). 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). This study is well supported by Imran Khan *et al.,* (2011), Moore (1924) and Shaikh (1974). Sinha (1973) reported that soil pH of 3.4–9.2 significantly reduced the incidence of Fusarium wilt without adverse effect on yield. Whereas, Sugha *et al.,* (1994b) described that maximum wilting occurs at pH. 5.2 with a slight decline towards neutrality. Studies conducted by Jamaria (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. The maximum sporulation of macro conidia was observed on FOC-9 (5.21×10^5 ml^{-1}) at pH 5.5 and the minimum sporulation was observed on FOC-9 (1.42×10^3 ml^{-1}) at pH 5.0. No sporulation was observed on FOC-7 and FOC-8 at pH 6.5. This findings are in aggrement with Khilare and Rafi Ahmed (2012) who reported that highest at pH 6.0 with sporulation 24.70 conidia μl^{-1}.

5.3.3. Culture media

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 90.00mm and 84.50 mm mycelium colony growth of *F. oxysporum* f. sp. *ciceri* after an incubation of seven days respectively. The results of the present study are in agreement with those achieved by Farooq *et al.*, (2005). He mentioned that Minimum fungul 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, after seven days of inoculation followed by corn meal agar and malt extract agar media which showed growth of 70 and 65 mm, respectively. Different synthetic and non synthetic cultural media have profound influence on cultural and morphological characteristics of fungus (Shaikh, 1974). Haware *et al.*, (1986) had modified cape do agar medium by adding PCNB, streptomycin and malachite green. This medium is highly effective for the growth of *F. oxysporum*. 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 *et al.,* (2012) indicated that Czapek's dox agar and potato dextrose agar were best medium for growth of *F. oxysporum* f. sp. *ciceri.* Dikkar and Deshmukh (2003) found best *F. oxysporum* f. sp. *ciceri* growth on Richard's agar medium followed by PDA and Czapek's Dox agar medium. 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) reported maximum

growth of *Fusarium oxysporum* f. sp. *lentis* on PDA followed by lentil extract and Richard's Agar. The highest sporulation of macro conidia was observed on FOC-1 (3.27×10^5 ml^{-1}) at PDA medium after seven days of incubation and the lowest sporulation was observed on FOC-3 (1.08×10^3 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. Desai *et al.,* (1994) claimed maltose for maximum growth and sporulation, however poor growth and sporulation on lactose was reported by Rawal and Parmar (1973).

5.4. Pathogenic variability of *F. oxysporum* f. sp. *ciceri*

In these experiments 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%. The most virulent isolates found were Foc-1 (86.67%), Foc-7 (73.33%) and Foc-8 (73.33%) while, the least virulent isolate was Foc-6 (13.33%). 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). Haware *et al.*, (1992) also found pathogenic diversity among chickpea wilt isolates. Similarly, 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. Race 3, isolates were found in all of the wilt sick plots and varieties, among these var. Arerti and DZ-10-4 were found resistant and var. DZ-10-11 and Maryie susceptible against all the isolates. Navas-Cortes *et al*., (2007) describe difference in virulence of Fusarium wilt by yellowing or wilting symptom.

CHAPTER 6
SUMMARY AND CONCLUSION

CHAPTER 6
SUMMARY AND CONCLUSION

Chickpea is considered as one of the most important legume crops in Bangladesh. Fusarium wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* is most devastating disease in many chickpea growing areas. In Bangladesh, there has been sharp decline in chickpea area and production due to serious outbreaks of Fusarium wilt. Nine *Fusarium oxysporum* f. sp. *ciceri* isolates collected from major chickpea growing areas of Bangladesh. The present investigation include different studies viz. symptomology of Fusarium wilt disease and variability of cultural, morphological, physiological and pathogenic characteristics of all the nine isolates. Colony of *Fusarium oxysporum* f. sp. *ciceri* isolates were purplish white, whitish orange, creamy white, cottony white colored colony were irregular, regular, regular with sector, regular without sector and irregular, entire and wavy margins; fluffy, flat/velvet texture was observed. Fungus produced three kinds of spores viz., micro conidia, macro conidia and chlamydospores. The length of micro conidia varied from 5.00-14.00 µm (average 6.25- 11.00 µm). The breadth of micro conidia was 1.00-4.00 µm (average 1.95-2.80 µm). Micro conidia was 0-2 septed. The length of macro conidia ranged from 9.00-26.00 µm (average 12.00-20.45 µm). The breadth of macro conidia was 1.00-5.00 µm (average 2.45-3.60 µm) and macro conidia was 1-5 septed.

The fungus grew well in a wide range of temperature, pH and nutrient media. The maximum colony diameter was found at 25 °C, pH 6.0 and OMA media for most of the isolates. No growth was observed at 5 °C temperature. The highest number of macro spores were noted at 25 °C (3.43×10^6 ml^{-1}), at pH 6.0 (7.06×10^5 ml^{-1}) and on PDA (3.27×10^5 ml^{-1}) media. The highest number of micro spores were observed at 25 °C (6.78×10^5 ml^{-1}), at pH 6.0 (3.03×10^5 ml^{-1}) and on PDA (4.06×10^5 ml^{-1}) media. No sporulation observed at 5 °C and on WA media for all the isolates.

The isolates varied markedly in their test of aggressiveness. All the isolates (FOC-1 to FOC-9) were able to infect the chickpea variety (BARI Chola-1). The maximum aggressiveness was found in case of isolate FOC-1. Isolate FOC-1 was found to be the highly virulent while isolates FOC-3, FOC-4, FOC-6 and FOC-9 showed low virulent type of reaction.

At the end of the above results it can be concluded that-

- Variability exist in *Fusarium oxysporum* f. sp. *ciceri* prevailing in the chickpea growing areas of Bangladesh.
- Optimum temperature for the growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri* is 25 °C.
- Optimum pH for the growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri* is 6.0.

- Oat meal agar media is most suitable for the growth and PDA media is most suitable for sporulation of this fungus.
- All the isolates were not virulent equally to the tested chickpea cultivar (BARI Chola-1).
- The most virulent isolate observed on FOC-1 which was collected from Ishurdi Sadar, Pabna.
- Molecular characterization should be done to confirm the variability exist in *Fusarium oxysporum* f. sp. *ciceri* isolates.

CHAPTER 7
LITERATURE CITED

CHAPTER 7

LITERATURE CITED

Agrios, G. N. (2005). Environmental effects on the development of the infectious disease (*in*) *Plant* Pathology, 5th edn, Elesvier Acad. Press. Burlington, Mass, USA. 251–62.

Ahmed, A. U. (1996). Variation of twenty seven isolates of *Bipolaris sorokiniana* from wheat. MS Thesis, Bangabandhu Sheikh Mujibur Rahman Agricultural University (Former IPSA), Salna, Gazipur, Bangladesh.

Ahmad, M. A. (2010). Variability in *Fusarium oxysporum* f. sp. *ciceris* for Chickpea Wilt Resistance in Pakistan. Ph. D thesis. Quaid-i-Azam University, Islamabad, Pakistan.

Anjaneya Reddy, B. (2002) Variability of *Fusarium udum* and evaluation of pigeonpea (*Cajanus cajan* (L). Mills) genotypes. *M.Sc (Agri) Thesis*, Univ. Agril. Sci. Bangalore. pp 115.

Barhate, B. G., Dake, G. N., Game, B. C. and Padule, D. N. (2006). Variability for Virulence in *F. oxysporum* f. sp. *ciceri* Causing Wilt of Chickpea. *Legume Res*. 29 (4): 308 – 310.

Bhatti, M. A. and Kraft, J. M. (1992). Effect of inoculum density and temperature on root rot and wilt of chickpea. *Pl. Dis*. 76: 960-963.

Chauhan, S. K. (1962). Physiologic variation in *F. orthoceras* App. Wr. Var. *ciceris* causing wilt of gram. *Proc. Natn. Acad. Sci. Sec. B*. 22:78-84.

Chauhan, S. K. (1963). Influence of different soils temperatures on the incidence of *Fusarium* wilt of gram (*C. arietinum* L.) Proc. *Indian. Acad. Sci*. 8, 33:552-554.

Chauhan, S. K. (1965). The interaction of certain soil conditions in relation to the occurrence of *Fusarium* wilt of gram. *Indian J. Agric. Sci*. 35: 52.

Chi, C. and Hassan, E. W. (1964). Relation of temperature, pH and nutrition to growth and sporulation of *Fusarium* spp. from red clover. *Phytopathology* 54(9): 1053-58.

Desai, S., Nene, N. L. and Ramachandra Reddy, A. G. (1994). Races of *Fusarium oxysporum* causing wilt in chickpea. *Indian Journal of Mycology and Plant Pathology* 24: 120 – 127.

Dhingra, O. D., Agrawal, S. C., Khare, M. N. and Kushwaha, L. S. (1974). Temperature requirements of eight strains of *Fusarium oxysporum* f .sp. *lentis* causing wilt of lentil. *Indian Phytopathology* 27 : 408 – 410.

Dikkar, M. and Deshmukh, V. V. (2003). Nutrional requirement of *Fusarium* wilt of chickpea. *Crop Res*. 25(1): 197-199.

Dubey S. C., Singh, S. R; Singh, B. (2010). Morphological and pathogenic variability of Indian isolates of *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. Archives of *Phytopathology and Plant Protection* 43(2): 174 – 190.

Edel, V, Steinberg, C., Gautheron, N., Recorbet, G. and Alabouvette, C. (2000). Genetic diversity of *F. oxysporum* populations isolated from different soils in France. FEMS. *Microbiol. Ecol*. 36: 61-71.

Erwin, D. C. (1957). *Fusarium* and *Verticillium* wilt diseases of *Ciceris arietinum L Phytopathology* 47: 10.

FAO (2008). Food and Agriculture Organization Yearbook Production. Rome, Italy: 46: 105-115.

Farooq, S., Iqbal, S.M. and Rauf, A. (2005). Physiological Studies *of Fusarium oxysporum* f. sp. *ciceri. Int. J. Agri. Biol.* 7(2): 275-277.

Gangadhara, N. B., Nagaraja, R., Basavaraja M. K. And Krishna N. R. (2010) Variability studies of *Fusarium oxysporum* f. sp. *vanillae* isolates. *Int. J. of Science and Nature* 1(1):12-16.

Gupta, O., Kahre, M. N., and Kotashane, S. R., (1986), Variability among six isolates of *Fusarium oxysporum* f .sp. *ciceri* causing vascular wilt of chickpea. *Indian Phytopathology* 39: 279 – 281.

Gupta, O. M., Kosthane, S. R. and Khare, M. N. (1987). Survey of *Fusarium* wilt of chickpea in Mahya Pradesh, India. 27:21-23.

Halila, H. M., Girdley, H. E. and Houdiald, P. (1984). Sources of resistance to *Fusarium* wilt in kabuli chickpea. *Int. Chickpea Newslet*. 10:13–14.

Haware, M. P; Nene, Y. L. and R. Rajeshware. (1978). Eradication of *Fusarium oxysporum* f. sp. *ciceri* transmitted in chickpea seed. *Phytopathology* 68: 1364–7.

Haware, M. P., Nene, Y. L. and Mathur, S. B. (1986). Seed–borne diseases of chickpea. *Techn. Bullet. No.1, ICRISAT,* India. pp 32.

Haware, M. P., Nene, Y. L., Pundir, R. P. S. and Narayana, J. (1992). Screening of world chickpea germplasm for resistance to *Fusarium* wilt. *Field Crops Res*. 30: 147– 154.

Haware, M. P. (1993). *Fusarium* diseases of crops of India. *Indian Phytopathology* 46:101-109.

Hayes, W. A. (1978). Nutrition, substrates and principles of disease control. *In*: *The Biology and Cultivation of Edible Mushrooms.* Academic press. New York. pp 202–220.

Ingole, M. N. (1995) Estimation of losses, variability among isolates and management of pigeon pea wilt caused by *Fusarium udum* Butler. M. Sc. (Ag.) *Thesis*, Dr. PDKV, Akola.146.

Imran Khan, H. S., Saifulla M., Mahesh, S. B. and Pallavi, M. S. (2011). Effect of different media and environmental conditions on the growth of *Fusarium oxysporum* f. sp. *ciceri* causing *Fusarium* wilt of chickpea. pp 67-78.

Iqbal, S. M., Rauf C. A., Bakhsh, A. and Iqbal, U. (2005). Variability in *F. oxysporum* f. sp. *ciceris*. *Mycopath*. 3(1-2):47-51.

Jamaria, S. L. (1972). Nutritional requirement of *Fusarium oxysporum* f. sp. *niveum*. *Indian Phytopathology* 25: 29 – 32.

Jimenez-Diaz, R. M., Hervas, A. A. and Trapero-Casas, J. C. (1993). Pathogenic variability and host resistance in *F. oxysporum* f. sp. *ciceris, C. arietinum* pathosystem. Hodowla Rosin. Aklimatyzacja Nasiennictwo. 37: 87-94.

Jimenez-Gasco, M. M., Navas-Cortes, J. A. and Jimenez-Díaz, R. M. (2004). The *F. oxysporum* f. sp. *ciceriss / C. arietinum* pathosystem: a case study of the evolution of plant-pathogenic fungi into races an pathotypes. *Int. Microbio*. 7:95–104.

Joffee, A. Z., Palti, J. and Arbel–Sherman, R. (1974). *Fusarium oxysporum* Schlecht in Isreal. *Phytoparasitica* 2: 91 – 107.

Khare, M. N., Agarwal, S. C., Dhyra O. D. and Kushwaha, L. S. (1975). Variability in the growth of light strains of *Fusarium oxysporum* f. sp. *lentis* as different solid media. *Indian phytopathology* 28: 126-128.

Khilare, V. C. and Rafi Ahmed (2012). Effect of different media, pH and temperature on the growth of *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. *Int. J. of Advance Biological Research* 2(1): 99-102.

Kistler, H. C. (1997). Genetic diversity in the plant pathogenic fungus *F. oxysporum. Phytopathology* 87: 474 -479.

Kulkarni, S. P. (2006). Studies on *Fusarium oxysporum* Schlecht Fr f.sp. *gladioli* (Massey) Snyd. & Hans. Causing wilt of gladiolus. *Thesis* submitted to Department of Plant Pathology, College of Agriculture, dharwad, University of Agricultural Sciences. 22-24.

Landa, B. B., Navas-Cortés, J. A., Hervás, A., and Jimenez-Díaz, R. M. (2001). Influence of temperature and inoculum density of *Fusarium oxysporum* f. sp. *ciceris* on suppression of *Fusarium* wilt of chickpea by rhizosphere bacteria. *Phytopathology* 91:807-816.

Landa, B. B. Navas-Cortes, J. A., Jimenez-Gasco, M. M., Katan, J. and Jimenez-Díaz, R. M. (2006). Temperature Response of Chickpea Cultivars to Races of *F. oxysporum* f. sp. *ciceriss*, Causal Agent of *Fusarium* Wilt. Pl. Dis. 90(3) 365-374.

Mahendrapal and Grewal, J. S. (1975). Utilisation of different nitrogen sourcer by *Fusarium oxysporum* f. sp. *ciceri* causing wilt in chickpea. *Indian Phytopathology* 28: 419 – 421.

Massey, L. M. (1926), *Fusariu*m rot of gladiolus corms. *Phytopatholog*y 16: 509 – 523.

Mc Culloch, L. (1944). A vascular disease of gladiolus caused by *Fusarium. Phytopathology* 34: 263 – 287.

Mina, U. and Dubey, S. C. (2010). Effect of environmental variables on development of *Fusarium* wilt in chickpea (*Cicer arietinum*) cultivars. *Indian J. Agric. Sci*. 80(3):231-234.

Moore, E. S. (1924). The physiology of *Fusarium coeruleum. Annals of Botany.* 149: 137-161.

Murumkor, C. V. and Chavan, I. D. (1985). Physiological changes in chickpea level infected by *Fusarium* wilt. *Biovegyanam* 11(1):118-120.

Navas-Cortes, J. A., Hau, B., and Jimenez-Díaz, R. M. (1998). Effect of sowing date, host cultivar, and race of *F. oxysporum* f. sp. *ciceriss* on development of *Fusarium* wilt of chickpea. *Phytopathology* 88: 1338-1346.

Navas-Cortes, J. A., Landa, B. B., Mendez-Rodriguez, M. A. and Jimenez-Diaz, R. M. (2007). Quantitative modeling of the effects of temperature and inoculum density of *F. oxyxporum* f. sp. *ciceriss* races 0 and 5 in chickpea cultivars. *Phytopathology* 97: 564-573.

Nene, Y. L. and Haware, M. P. (1980). Screening chickpea for resistance to wilt. *Pl. Dis.* 64: 379–380.

Nene, Y. L., Haware, M. P. and Reddy, M. V. (1981). Chickpea diseases: resistance screening techniques. Information Bulletin No. 10, Int. Crop Res. Inst. for the Semi Arid Tropics, Patancheru. 1–10.

Nene, Y. L., Shiela, V. K. and Sharma, S. B. (1984). World list of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan*) pathogens. ICRISAT. Pulse Pathology Programme report, 32: 23 pp.

Pande, S., Galloway, Gaur, P.M., Siddique, K. H. M., Tripathi, H. S., Taylor, P., MacLeod, M. W. J., Basadrai, A. K., Bakr, M. A., Joshi, S., Kishore, K. G., Isenegger, D.A., Narayana, J. and Sharma, M. (2006). Botrytis gray mold of chickpea: a review of biology, epidemiology and disease management. *Australian J. Agril. Res*. 57: 1137-1150.

Pande, S., Rao, J. N. and Sharma, M. (2007). Establishment of the Chickpea Wilt Pathogen *F. oxysporum* f. sp. *ciceriss* in the Soil through Seed Transmission. *Plant Pathol. J.* 23(1): 3-6.

Patel, S. T. (1991). Studies on some aspects of wilt of chickpea. *Ph.D. Thesis*, University of Agricultural Sciences, Dharwad. 15 –25.

Patel, H., Patel, B. K., Thakar, N. A. and Patel, C. C. (1985). Reactions of few chickpea lines to *Fusarium* wilt. *Int. Chickpea Newslet.* 13:16-17.

Paulkar, P. K. Raut, B. T. and Kale, K. B. (2002). Variability in chickpea wilt isolates. *Karnatka J. Agric. Sci.* 15: 389-390.

Prasad, N. and Padwick, G. W. (1939). The genus *Fusarium* 11. A species of *Fusarium* as a cause of wilt of gram (*C. arietinum* L.). *Indian Agri. Sci*. 9: 731.

Rawal, G. S. and Parmar, (1973). Utilization of some carbon and nitrogen compounds by *Fusarium aroseum*. *Indian J. Mycol. Pl. Pathol*. 3: 97-99.

Saheb, A. F., Fahim, M. M., Osman, H. R. and Abdel–Kader, M. H. (1987). *In vitro* studies on *Fusarium oxysporum* f .sp. *lupini* Munifiya. *J. of Agril. Res.* **7**: 41 – 45.

Saxena, M. C. (1990). Problems and potential of chickpea production in the nineties. p. 13–27. In H. A. van Rheenen and M. C. Saxena (ed.) Chickpea in the nineties: Proceedings of the Second Interna- tional Workshop on Chickpea Improvement. 4–8 Dec. (1989). ICRI- SAT, Patancheru, India. Chickpea diseases and their con RAPD markers. pp 116.

Saxena, M. C. and Singh, K. B. (1987). The chickea published by *C.A.B. Int.* ICARDA. pp 250-252.

Scott, J. C., Gordon**,** T. R.**,** Shaw**,** D. V. and Koike**,** S. T. (2010). Effect of temperature on severity of Fusarium wilt of lettuce caused by *Fusarium oxysporum* f. sp. *lactucae*. *Plant Disease* 94 (1): 13-17.

Sharma, K. D., Chen, W. and Muehlbauer, F. J. (2005). Genetics of chickpea resistance to five races of *Fusarium* wilt and a concise set of race differentials for *F. oxysporum* f. sp. *ciceriss. Pl. Dis*. 89:385-390.

Shehabu, M., Ahmed, S. and Sakhuja, P. K. (2008). Pathogenic variability in Ethiopian isolates of *F. oxysporum* f. sp. *ciceriss* and reaction of chickpea improved varieties to the isolates. *Int. J. Pest Management*. 54(2):143–149.

Shaikh, M. H. (1974). Studies on wilt of gram (*Cicer arietinum* L.) caused by *F. oxysporum* f. sp. *ciceris* in Marathwada region. M.Sc (Agri.) *Thesis*, Marathwada Krishi vidysapeeth, Parbhani, India. pp 78-89.

Singh, F., Singh, N. and Gupta, P. K. (1986). *Fusarium* wilt of chickpea - Current status of the methods of study and measures to reduce crop losses. In: P. K. Gupta and J. Bahel (eds.) Genetics and Crop Improvement. Rastogi and Co., Meerut, PP. 133-148.

Singh, K. B. and M. V. Reddy, (1991). Advances in disease resistance breeding in chickpea. Advances in *Agron.*45: 191–222

Sinha, S. (1973). Some factors of the soil in relation to *Fusarium* wilt of Bengal gram. Symposium on wilt problem and breeding for wilt resistance in Bengal gram. September, 1973 at Indian Agri. Res. Inst., New Delhi, India. 9-11(Abst).

Sinha, S. and Dahiya, B. S. (1973). Breeding for wilt resistance in chickpea Symposium on wilt problem and breeding for wilt resistance in Bengal gram. Indian Agri. Inst. New Delhi, India.13-14.

Sowmya, G. S. (1993). Studies on Panama disease of banana caused by *Fusarium oxysporum* f .sp. *cubense*. M.Sc. (Agri) *Thesis* submitted to University of Agricultural Sciences, Bangalore. pp 11 – 27.

Sugha, S. K., Kapoor, R. J. and Singh, M. B. (1994a). Factors influencing *Fusarium* wilt of chickpea (*C. arietinum* L) *Indian Jour. Mycol. and Pl. Pathol*. 24(2): 97-102.

Sugha, S. K., Kapoor, S. K. and Singh, M. B. (1994b). Soil characteristics and their relation to *Fusarium* wilt of chickpea (*C. arietinum* L). *Trop. Sci*., 24(3): 282-288.

Tantaoui, A., Ouinten, M., Geiger, J. P. and Fernandez, D. (1996). Characterization of a single clonal lineage of *F. oxysporum* f. sp. *albedinis* causing bayoud disease of date palm in Morocco. *Phytopathology*. 86: 787–92.

Venkatraman, C. S. (1955). Variation in the cultural characteristics of *Fusarium lini*. *Phytopathology* 45: 240.

Wardlaw, C. M. (1931). *Fusarium cubense*. *Tropical Agriculture*. 3: 54 – 60.

Westerlund, F. V., Campbell, R. N. and Kimble, K. A. (1974). Fungal root rot and wilt of chickpea in California. *Phytopathology* 64: 432–436.