

**INVESTIGATIONS ON WILT OF
SAFFLOWER (*Carthamus tinctorius* L.)
CAUSED BY *Fusarium oxysporum* Schle.
f.sp. *carthami* Klisiewicz and Houston.**

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By

Miss. WAGHMARE SUNITA JAIKRISHAN



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DISSERTATION

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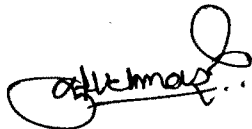
*Dedicated to My
Beloved Parents
and
Father-In-Law*

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*I hereby declare that the entire work embodied in
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PLACE : PARBHANI

DATE : 15/06/2007.


(Waghmare S. J.)

Dr. V.V. Datar

M.Sc. (Agri.), Ph.D.,
Associate Dean & Principal,
College of Agriculture, Latur,
Marathwada Agricultural University,
Parbhani- 431 402 (M.S.) India.

CERTIFICATE I

This is to certify that **Miss. WAGHMARE SUNITA JAIKRISHAN** has satisfactorily prosecuted her course and research for a period of not less than four semesters and that the dissertation entitled "**INVESTIGATIONS ON WILT OF SAFFLOWER (*Carthamus tinctorius* L.) CAUSED BY *Fusarium oxysporum* Schle f.sp. *carthami* Klisiewicz and Houston.**" submitted by her is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination.

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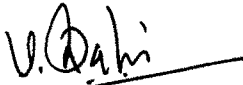
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Research Guide


CERTIFICATE -II

This is to certify that the dissertation entitled "INVESTIGATIONS ON WILT OF SAFFLOWER (*Carthamus tinctorius* L.) CAUSED BY *Fusarium oxysporum* Schle.f.sp. *carthami* Klisiewicz and Houston" submitted by Miss. WAGHMARE SUNITA JAIKRISHAN to the Marathwada Agricultural University, Parbhani in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY (Agriculture) in the subject of PLANT PATHOLOGY has been approved by the student's advisory committee after oral examination in collaboration with the external examiner.



External Examiner



(V. V. Datar)
Research Guide

Members of Advisory Committee :


(G.D. Deshpande)


(F.S. Patil)


(U.V. Kale)


Associate Dean (P.G.),
College of Agriculture,
M.A.U., Parbhani

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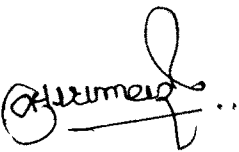
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(**S. J. Waghmare**)

LIST OF ABBREVIATIONS

%	-	Per cent
$\mu\text{g ml}^{-1}$	-	Micro gram per milliliter
μg	-	Micro gram
$\mu\text{l ml}^{-1}$	-	Micro liter per milliliter
μl	-	Micro liter
/	-	Per
@	-	at the rate of
CD	-	Critical difference
Cv	-	Cultivar
DAI	-	Days After Incubation
<i>et al.</i>	-	And associates
<i>etc.</i>	-	etcetera
Fig.	-	Figure (s)
FSM	-	Fusarium Selective Medium
g	-	Gram
ha	-	Hectare(s)
<i>i.e.</i>	-	That is
ISSR	-	Inter Simple Sequence Repeats
kg	-	Kilogram
lbs	-	Pounds
M.S.	-	Maharashtra State
mm	-	Millimeter
N.S.	-	Non signifiant
ng	-	Nanogram
No.	-	Number
$^{\circ}\text{C}$	-	Degree Celsius
OPA	-	Operon Primer Kit – A
OPC	-	Operon Primer Kit – C
PCR	-	Polymerase Chain Reaction
kg/cm^2	-	Kilogram per centimeter square
RAPD	-	Random Amplified Polymorphic DNA
RBD	-	Randomized block design
rpm	-	revolution per minuet
S.E.	-	Standard error
SEm	-	Standard Error of mean
sp.	-	Species
<i>Viz.</i>	-	Namely

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INTRODUCTION



CHAPTER-I

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is one of the most important rabi oilseed crops cultivated from centuries in India for its valuable oil and also orange colour dye. Safflower, apart from its superior adaptability to scanty moisture conditions produces oil, rich in polyunsaturated fatty acids which play an important role in reducing the blood cholesterol level.

India plays a vital role in international and national economy and ranks first in safflower hecterage and second in productivity (Weiss, 1971). India is the largest producer of safflower in the world accounting for 66 per cent of area and 50 per cent of total production. In India, the crop is grown on an area of 425.30 thousand ha with the production of 201.20 thousand tonnes, with an average productivity of 473 kg/ha (Anonymous, 2002). Recent statistics showed an area of 4.03 lakh ha in India with the production of 2 lakh tonnes with an average productivity of only 465 kg/ha (Anonymous, 2003). Maharashtra, Andhra Pradesh and Karnataka are the major safflower growing states of India. In Andhra Pradesh, the crop is grown on an area of 16.20 thousand ha with a productivity of 406 kg/ha (Anonymous, 2002). Maharashtra has an unique position of 72 per cent of area of safflower in India. Therefore, the role of Maharashtra in oil economy of safflower in India is decisive one (Anonymous, 2003).

In Marathwada region, area under this crop was 1.95 lakh ha with the production of 0.982 lakh tonnes (Anonymous, 1998).

Safflower is known to suffer from many fungal, bacterial, viral and phytoplasma diseases at different stages of crop growth. The crop is mainly affected by *Fusarium* wilt (*Fusarium oxysporum* f.sp. *carthami* (Klisiewicz and Houston)), *Alternaria* leaf spot (*Alternaria carthami* Choudhary), Rust (*Puccinia carthami* (Hutz.) Corda), Bud blight (*Phytophthora drechsleri* Tucker), *Ramularia* leaf spot (*Ramularia carthami* Zaprometov), *Cercospora* leaf spot (*Cercospora carthami* Subram. and Ramkr). Powdery mildew (*Erysiphe cichoracearum* f.sp. *carthami* Milovtsova), root rot (*Rhizoctonia bataticola* (Taub) Butler), Bacterial spot (*Pseudomonas syringae* Van Hall) and Mosaic (Cucumber mosaic virus Nitzany). Among all these, wilt caused by *Fusarium oxysporum* f.sp. *carthami* is an important soil borne disease recorded for the first time in India by Singh *et al.* (1975). It is spreading widely and causing yield losses up to 80 per cent threatening the crop cultivation (Kalpana Sastry and Chattopadhyay (1997) and reduction in kernel weight, 100 seed weight, oil quality and yield losses to an extent of 72-80 per cent (Kalpana Sastry and Ramachandran, 1994) and extinction of safflower in the traditional zones of Indian Peninsula (Kalpana Sastry, 1997). The disease is problematic in safflower growing areas, where it is cultivated without crop rotation. The disease is found to be endemic in India particularly in Maharashtra and Uttar Pradesh with an incidence ranging from 5-40 per cent (Singh *et al.*, 1975 and Deokar *et al.*, 1998).

Even though, resistant varieties have been evolved against wilt, yet all varieties are not performing well in all the areas. The variety which is resistant in one locality is susceptible in other localities due to variability in pathogen, but information on this aspect is meager. Species of *Fusarium* are widely distributed both in temperate and tropical climatic regions of the world and are mainly soil inhabitant fungi. Many species cause globally economically significant diseases on most of the world's important crop plant affecting heavy qualitative and quantitative losses (Booth,1971). *Fusarium* wilt appeared to be the result of recent introduction rather than an independent local origin of the pathotype. A sexual propagation is the dominant influence on population structure in *F. oxysporum* and the absence of sexual reproduction is not likely to prevent this pathogen from continuing to infact cause significant damage on susceptible crop hosts. Due to soil borne nature of the pathogen, chemical control is not feasible. From time to time, variability has been induced into the pathogen, because of the genetic diversity in host and the environment and thus all along the evolution process, a natural balance was maintained. Biological control is one of the viable preposition for management of soil borne disease like *Fusarium* wilt. In host parasite interaction, maintenance of genetic variability is crucial for the pathogen for better colonization of the host.

Studies on molecular variability in *F. oxysporum* are numerous and vary depending upon the objectives and desired applications. Molecular markers have been used to characterize genetic diversity of different isolates of *Fusarium oxysporum* f.sp. and races within form specialis.

The increasing demands made on agriculture, due to the ever increasing population, make it imperative to search for effective and ecologically sound crop protection measures. Breeding for disease resistance is one among the few cheapest, effective and powerful tools in the disease control. Unfortunately, the acquired variability at the pathogen's end to counter the stress due to resistant genes had been posing a serious problem to both pathologists and breeders.

Variation is well known among the isolates of the same forma-specialis in *Fusarium* (Booth, 1971), but information with reference to variability of *Fusarium oxysporum* f.sp. *carthami* is lacking.

Keeping in view the importance of this disease in safflower with special reference to variability and management, the present study was taken up with the following objectives.

1. Survey of farmers fields to record incidence of safflower wilt and to collect diseased samples.
2. Isolation and purification of the pathogen from wilted safflower roots from safflower growing areas of Marathwada region.
3. To prove Koch's postulates of isolates of the pathogen.
4. To detect the seed borne nature of *Fusarium oxysporum* f.sp. *carthami*.
5. To investigate the morphological, cultural and pathogenic variations among the isolates.
6. To investigate the DNA sequence of *Fusarium oxysporum* f.sp. *carthami* isolates.
7. Screening for host resistance against the isolates of *Fusarium oxysporum* f.sp. *carthami*.

8. To study efficacy of *T. harzianum*, *T. viride*, *T. hamatum*, *T. koningi* and *T. lignoum* against the isolates of *Fusarium oxysporum* f.sp. *carthami*
9. To investigate *in vitro* evaluation of different fungicides against *Fusarium oxysporum* f.sp. *carthami* isolates.

REVIEW OF LITERATURE



CHAPTER-II

REVIEW OF LITERATURE

The available literature pertaining to the present investigation is reviewed under the following heads.

- 2.1. Distribution of the disease
- 2.2. Economic importance of the disease
- 2.3. Symptomatology
- 2.4. Pathogen
- 2.5. Host
- 2.6. Etiology of the pathogen
- 2.7. Isolation of the pathogen and establishment of pathogenicity
- 2.8. Seed borne nature of *Fusarium oxysporum* f.sp. *carthami*
- 2.9. Morphological and cultural characteristics
- 2.10. Pathogenic variability
- 2.11. Genetic variability
- 2.12. Screening for host resistance
- 2.13. Biological management through *Trichoderma* spp.
- 2.14. *In vitro* evaluation of fungicides

2.1. Distribution of the Disease

Safflower wilt caused by *Fusarium* spp. was reported for the first time from Sacramento valley of California, USA by Klisiewicz

and Houston (1962). Since then the incidence has been reported from California (Zimmer *et al.*, 1963) and Yolo (Stout, 1963).

Singh *et al.* (1975) reported wilt disease of safflower for the first time from Varanasi in India.

Chakrabarti and Basuchaudhary (1978) reported the wilt disease from three distinct agricultural tracts that included fields of the Banaras Hindu University farm (tract A), the irrigated upland plain (tract B) and the Ganges river bank (tract C) in Varanasi area of Uttar Pradesh.

Zayed *et al.* (1980) reported safflower wilt disease from Egypt.

The disease was also reported from Marathwada region of Maharashtra with an incidence of 25 per cent by Nirmal *et al.* (1989).

Pedgaonkar *et al.* (1990) observed safflower wilt disease from Jalna, Parbhani and Latur districts of Maharashtra state with an incidence of 5.71 to 18.88 per cent. Incidence of safflower wilt disease up to 40 per cent was also recorded in Solapur, Osmanabad, Latur, Parbhani and Buldhana districts of Maharashtra (Deokar *et al.*, 1998).

The disease has been reported to cause severe losses in Ranga Reddy district of Andhra Pradesh when the crop was grown in vertisols (Kalpana Sastry and Chattopadhyay, 1997; and Nageshwar Rao, 2000).

2.2. Economic importance

Nirmal *et al.* (1989) reported 25 per cent wilt incidence in Marathwada region and disease spreads in epidemic proportions in Parbhani and Latur districts of Maharashtra.

Safflower wilt incited by *Fusarium oxysporum* f.sp. *carthami* Klisiewicz and Houston is an economically important disease in safflower growing areas of India causing yield loss of 7.2 to 100 per cent (Kalpana Sastry and Ramchandran, 1994) and in other countries like USA (Klisiewicz and Houston, 1962) and Egypt (Zayed *et al.*, 1980). Over the last ten years, the disease has been spreading widely threatening the extinction of safflower in the traditional zones of Indian Penninsula (Kalapna Sastry, 1997).

In India, this disease causes huge losses, often to the tune of 40-86 per cent, threatening the crop cultivation (Kalpana Sastry and Chattopadhyay, 1997). The disease is endemic in India, particularly in Maharashtra and Uttar Pradesh with an incidence ranging from 5-40 per cent (Singh, 1975 and Deokar *et al.*, 1998).

2.3. Symptomatology

Thomas (1959) was the first worker who reported consistent infection of safflower in acidic soils having pH range of 4.3 to 5 and noted the necrosis of root tips and areas of epidermis and cortex in affected area.

Klisiewicz and Houston (1962) recorded for the first time safflower wilt in California and noted that the symptoms are unilateral yellowing which begins in lower leaves and progresses upwards on one side of the plant with subsequent browning and wilting.

A streak is also seen on infected stem (Nirmal *et al.*, 1989).

Pedgaonkar *et al.* (1990) reported the symptoms of *Fusarium oxysporum* f.sp. *carthami* associated with unilateral yellowing of the foliage followed by premature wilting of the plants. Further they reported that yellowing begins on the lower leaves and progresses upwards on one side of the plant. The affected leaves turn brown and die and as the disease progress the entire plant becomes wilted. A brown discolouration of the vascular bundles appeared on one side of the root and stem of plants with unilateral top symptoms. The pathogen also produces rotting of roots and rootlets.

Nageshwara Rao (2003) described the wilt symptoms in safflower Cv. Nira at seedling and adult plant stage. Affected plants showed the formation of streaks on the stem followed by bronzing and yellowing of leaves, partial or complete wilting of the plants.

2.4. Pathogen

The causal organism of wilt of safflower was earlier described as *Fusarium oxysporum* Schelcht. Klisiewicz and Houston (1962) revised it as *F. oxysporum* f.sp. *carthami* due to its host specificity of infecting only safflower.

The *Fusarium oxysporum* produced both macro and micro conidia as well as chlamydospores. Klisiewicz and Houston (1962) noted the average length of 25-40 μm and breadth of 3 to 4 μm for macroconidia. They noted length of 4-6 μm and breadth of 2 μm for oval microconidia. However, they also noted globose microconidia. The macroconidia were typically sickle shaped. They further added that round to oval chlamydospores were also produced by *Fusarium oxysporum* f.sp. *carthami* (Klisiewicz and Houston, 1962; Chakrabarti and Basuchaudhary, 1980; Gore, 1998 and Somwanshi, 2000).

Klisiewicz and Houston (1962) proved the pathogenicity of *Fusarium oxysporum* f.sp. *carthami* with inoculum load of 10^5 spore/ml of liquid culture which was containing 50 per cent mature macroconidia. Millar and Cooper (1967) could prove the pathogenicity of *Fusarium* by root dipping for 5 min in liquid inoculum containing 50,000 conidia/ml.

2.5. Host

While testing introductions against *Fusarium* wilt Knowles *et al.* (1968) recorded that, there were 841 introductions from India which had incidence from 0 to 100 per cent with mean incidence of 53 per cent.

Klisiewicz and Urie (1982) observed low seed oil percentage as the characteristics of the selections due to high ratio of pericarp (hull) tissue to oil bearing (embryo) tissue. They further commented that these normal hull selections could be crossed readily

to reduced hull, high oil seed types. The germplasm group was comprised of spiny and spineless types.

2.6. Etiology of the pathogen

The taxonomic studies of *Fusarium* began with the description of the genus by Link (1809). In the beginning, there was confusion and disorder in *Fusarium* with forms named on the basis of superficial observations. The necessity for a precise and reliable system of classification became apparent when it was realized that *Fusaria* caused serious diseases of many plants, German Mycologist Wollenweber (1913) took up this challenge and started a detailed study of *Fusaria* there by classifying them in some system.

Morphological character of the genus *Fusarium* defined by Wollenweber (1913) still continue to be important in any new consideration of *Fusarium* taxonomy and nomenclature. The genus *Fusarium* was reduced to 143 species, 55 varieties and few forms grouped in to 16 sections based on shape of the macroconidia and microconidia, formation of sclerotia, chlamydospores, sporodochia, colour of the colony and mycelium (Wollenweber and Reinking, 1935). The number of species within the genus *Fusarium* was reduced to nine by Snyder and Hansen (1940) who started work with section *Elegans* and reduced all members of section *Elegans* into one species i.e. *Fusarium oxysporum* based on spore shape and selective pathogenicity to respective hosts.

Bilai (1954) reported that, host specificity should form the basis of forma-specialis and he classified the genus, *Fusarium* into 9 sections and 29 varieties. Subsequently Snyder and Hansen (1954) classified the genus into 9 sections on the sole basis of morphology and created 40 forma-specialis based on pathogenesis.

Klisiewicz and Houston (1963) reported a form of *Fusarium oxysporum* and classified the safflower wilt incitant *Fusarium* as forma-specialis *carthami*. The isolate from safflower was pathogenic only on species of *carthamus* and named it as *Fusarium oxysporum* Schelcht f.sp. *carthami* on the basis of selective pathogenicity and in accordance with the species concept in *Fusarium* presented by Snyder and Hansen (1940).

Singh *et al.* (1975) described *Fusarium* wilt in safflower to be caused by *Fusarium oxysporum* f.sp. *carthami*.

2.7. Isolation of the pathogen and establishment of pathogenicity

Klisiewicz and Houston (1962) isolated *Fusarium oxysporum* from safflower wilt affected plants and its pathogenicity was demonstrated by soil inoculation method. Further they reported that isolate from safflower was pathogenic only to species of *Carthamus*.

Klisiewicz and Thomas (1970a) isolated *Fusarium oxysporum* f.sp. *carthami* from diseased safflower plants and collected 12 isolates from different areas of Sacramento valley (USA) and isolated on PDA medium. Pathogenicity was established on three susceptible

safflower cultivars viz., Pacific, Gilla and US 10 with soil inoculation method by addition of inoculum @ 1 to 3 g/100 g of sterilized soil.

Singh *et al.* (1975) isolated *Fusarium oxysporum* f.sp. *carthami* from wilt affected safflower plants and cultured on PDA medium.

Pedgaonkar *et al.* (1990) isolated *Fusarium oxysporum* f.sp. *carthami* from wilt affected safflower plants and cultured on PDA for further confirmation of the disease.

2.8. Seed borne nature of Pathogen

Klisiewicz (1963) was the first pioneer pathologist, who demonstrated seed borne nature of *Fusarium oxysporum* f.sp. *carthami* through seeds of safflower (*Carthamus tinctorius* L.). Klisiewicz (1963) further noted that 42 per cent of the seeds harvested from infected seed heads carried the fungus. He observed mycelium and spores on the surface of some seeds. He also employed histopathological techniques and showed the presence of fungus hyphae in infected seed tissues. He could detect 8 per cent internal seed transmission.

The pathogen has been stated to be externally seed borne (Vaidehi *et al.*, 1985).

In addition to *Fusarium oxysporum* f.sp. *carthami* other species of *Fusarium* viz. *Fusarium moniliformae* and *Fusarium semitectum* have also been reported on the surface of safflower seed (Awadhiya, 1992).

2.8.1. Detection of seed borne nature of *Fusarium oxysporum* f.sp. *carthami*

Various methods of detection of seed mycoflora have been exhaustively enlisted by Neergaard (1977, 1988) and Agrawal and Sinclair (1987, 1997). They noted examination of dry seed indicating seed discolouration, morphological abnormalities, mixed fungal fruiting structures, bright field microscopy and observing seed vigour index. Near Ultraviolet Light (NUV) may give indication of the quality of seed. They further added that softening or soaking of seeds, study of seed washes may give additional information on the spectrum of seed borne fungi involved. Various incubation methods were also tried by international scientific community which included incubation by Blotter Method and Agar Plate Method, Seedling symptom by Rolled paper towel, Blotter, Soil, Fluorescence, Histo-pathological, embryo count test and NaOH seed soak method.

Limonard (1968) studied the detection methods of *Fusarium* spp. Deep Freeze Blotter (DFB) method developed by him was an alternative to 2, 4-D blotter method for detection of *Fusarium*.

Millar *et al.* (1951) commented that oxgall-PDA was better than PDA alone for detecting *Fusarium* sp. Nash and Snyder (1962) noted that Peptone-Quintozene Agar (PQA) used for detection of soil borne *Fusaria* can also be used for detection of seed carried *Fusarium* spp. PQA was also suitable for enumeration of *Fusarium* spp. from wheat seeds. Agrawal and Singh (1974) reported selective medium for

isolation of *F. moniliforme* from maize, pearl millet, sorghum and soybean seeds.

2.9. Morphological and cultural characteristics

Singh *et al.* (1975) reported morphology and cultural characteristics of pure culture of *Fusarium oxysporum* f.sp. *carthami* on PDA as delicate pink mycelial mass or white usually with a purple tinge. Hyphae of safflower *Fusarium* are septate, branched and produces sparse to abundant mycelium. The pathogen produces three types of spores viz., microconidia, macroconidia and chlamydospores. Microconidia are oval to elliptical, one celled, slightly curved borne on simple phialides arising laterally on the hyphae or on short sparsely branched conidophores. They measure 5 to 16 x 2.2 to 3.5 μ in size. Macroconidia are hyaline, 3-5 septate constricted at septa borne in sporodochia straight or curved, often pointed at the tip with round base measuring 10 to 36 x 3 to 6 μ mostly 28 x 4 to 5 μ . Chlamydospores are one celled, smooth, faintly coloured and 5 to 13 x 10 μ in size found terminal or intercalary usually solitary but occasionally formed in chains.

Nelson *et al.* (1983) reported terminal or intercalary chlamydospore in the genus *Fusarium*.

Fusarium oxysporum f.sp. *carthami* Klisiewicz and Houston, the incitant of safflower wilt is characterized by delicate white or peach coloured mycelium usually with a purplish tinge-sparse to abundant, floccose becoming felted, sometimes wrinkled in old cultures. Microconidia borne on simple phialides arising laterally on hyphae or on

short sparsely branched conidiophores, abundant oval ellipsoid, cylindrical, straight to curved, measures 5 – 12 x 2 to 3.5 μ . Macroconidia are sparse borne on more elaborately branched conidiophores, 3-5 septate, fusoid subovoid and pointed at both ends. Chlamydospores are smooth walled, generally abundant and are borne terminal and intercalary, usually solitary but occasionally formed in pairs (Anonymous, 2002).

Variation in cultural characteristics is a common phenomenon in the genus *Fusarium* (Oswald, 1949). Booth (1971) reported cultural variation among isolates of the same forma-specialis in *Fusarium*. The more common variations are reduction in aerial mycelium and growth rate, increase in microconidia and macroconidia production and colony pigmentation.

The isolates of *Fusarium oxysporum* f.sp. *udum* with luxuriant mycelial growth were found to be weakly to moderately pathogenic whereas the isolate with scanty mycelium was highly pathogenic. No correlation could be found between intensity of sporulation and pathogenicity of the isolates (Shit and Sen Gupta, 1978).

Burgess *et al.* (1989) studied variability and stability of morphological characters of *Fusarium oxysporum* and reported that the colony morphology and pigmentation vary extremely and thus these characters can not be considered as basis for species identification.

Desai *et al.* (1994) reported that growth variability was found useful in distinguishing four races of *Fusarium oxysporum* f.sp. *ciceri* (Padwick) Snyder and Hansen the incitant of chickpea wilt and found that dry mycelial weights of the four races differed significantly at different intervals.

Bai and Shaner (1996) reported that the isolates from different geographic areas exhibited variation in yield of conidia, growth rate of mycelium and pigmentation in growth media.

Fusarium oxysporum could clearly be distinguished from all the other species of *Fusarium*, mainly with the presence of 3 septate macroconidia (Gottwald *et al.*, 2001).

Desai *et al.* (2003) reported variability among 15 isolates of *Fusarium oxysporum* f.sp. *ricini* Nanda and Prasad in respect of cultural and morphological characters. They reported that six isolates produced moderate to profuse fluffy white mycelium while nine isolates produced thin flat to slight fluffy pinkish mycelium on PDA, colony diameter (62.67 to 73.67 mm), dry mycelial weights (106.00 to 214.67 mg) number of spores (2.18 to 23.82 million/ml) size of microconidia and macroconidia ranged from 5.25 – 14.00 x 3.50 – 7.00 μ and 17.50 – 70.00 x 3.50 – 5.25 μ , respectively. They also reported that highly virulent isolate produced abundant sporulation whereas moderately virulent isolates produced poor sporulation.

Dubey *et al.* (2006) studied morphological and cultural variability of 25 isolates of *Fusarium oxysporum* f.sp. *ciceris*.

Significant variability in respect of colony characters, mycelial growth, size of conidia and chlamydospores was observed among 25 isolates and these were considered for differentiating the isolates.

2.10. Pathogenic variability

Armstrong and Armstrong (1968) classified pathogen specificity based on the species level into forma-specialis (f.sp) and in some cases into physiological races based on the reaction of differential genotypes of the host plant.

Knowles *et al.* (1968) examined several seed samples of safflower for *Fusarium oxysporum* f.sp. *carthami* under laboratory conditions and reported that genetic stock numbers 4 (-2) -4 (-6) were resistant to wilt pathogen. Up to 1970, three races with *Fusarium oxysporum* f.sp. *carthami* were recognized by Klisiewicz and Thomas (1970^{a&b}). Klisiewicz (1975) recognized fourth race in 1975. These races were recognized on the basis of differential reaction on and cultivars namely Gila, Nebraska 6, US Biggs and UC-41. Race-1 has selective infectivity to Gila while it can not infect other host differentials like Nebraska 6, US Biggs and UC-41. Race-2 has selective infectivity to Gila and Nebraska 6 while it can not infect US-41. Race-3 has selective infectivity to Gila, Nebraska 6 and US Biggs while it can not infect UC-41. Race-4 can however, infect all the host differentials viz. Gila, Nebraska 6, US Biggs and UC-41.

Klisiewicz and Thomas (1970a) distinguished two pathogenic races of *Fusarium oxysporum* f.sp. *carthami* among 13

isolates which were highly virulent on three susceptible safflower varieties viz., pacific, Gila and US10.

Klisiewicz and Thomas (1970b) reported a third pathogenic race of *Fusarium oxysporum* f.sp. *carthami* by its virulence on A14154, a safflower cultivar resistant to two races of the pathogen differentiated by their virulence on cultivar Nebraska 6.

Klisiewicz and Thomas (1970b) screened various breeding material, for reaction of *Fusarium oxysporum* f.sp. *carthami* causing wilt and observed that there were two different pathogenic races on the basis of reaction on the breeding line. They reported that Nebraska 4051 was resistant to both the pathogenic races. They further reported the presence of third pathogenic race which was more virulent in nature and stated that cultivars A 49 and 2151 were good sources of resistance to all the races.

Klisiewicz (1975) identified a 4th race of *Fusarium oxysporum* f.sp. *carthami* with UC-41 a selection from P-I-30447 and reported that safflower plant introductions and cultivars that are highly resistant to races 1, 2 and 3 are either susceptible or only partially resistant to race 4.

Variability of population of the *Fusarium oxysporum* f.sp. *carthami* was reported by Chakrabarti (1979).

Kalpana Sastry and Chattopadhyay (2003) tested geographical isolates of *Fusarium oxysporum* f.sp. *carthami* (FOC) collected from different safflower growing regions and studied for their

pathogenic variability on six host differentials viz., Tara, Manjira, A-1, II-13-2A, VB-75-4 and 86-93-36A and the results revealed that Tara, Manjira and A-1 were susceptible while 83-93-36A was resistant to all the geographical isolates and differentiation on the isolates was made possible through the reaction of II-13-2A and isolates were grouped into four pathotypes and confirmed the existence of variability in the FOC population in different parts of the safflower growing areas.

Prameela *et al.* (2005) studied pathogenicity. Among all isolates, one isolate FOC-1 (DOR) was found to induce a highly susceptible reaction in all safflower cultivars. Two other isolates, FOC-5 and FOC-11 induced moderately susceptible reactions. Wilt incidence among 11 isolates ranged from 20 to 67 per cent in moderately resistant reactions in CVS, HUS-305 and A-1 and from 73 to 100 per cent in susceptible *Nira* and *Manjira*.

2.11 Genetic variability

Studies on molecular variability in *Fusarium* spp. are numerous (Guadet *et al.*, 1989 and O'Donnell, 1996) and vary depending upon the objectives and desired applications.

RAPD analysis was found to be useful in distinguishing non pathogenic *Fusarium oxysporum* isolates from *Fusarium oxysporum* f.sp. *dianthi*. (Manulis *et al.* 1994 and Kalc Wright *et al.* 1996), *Fusarium oxysporum* f.sp. *vasinfectum* (Assigbetse *et al.*, 1994). *Fusarium oxysporum* f.sp. *ciceris* (Kelly *et al.*, 1994), *Fusarium oxysporum* f.sp. *albendinis* (Fernandez and Tantaoui, 1994).

RAPD markers were found for these pathogenic races of *Fusarium oxysporum* f.sp. *vasinfectum* (Assigbetse *et al.*, 1994) and two different pathotypes of *Fusarium oxysporum* f.sp. *ciceris* (Kelly *et al.*, 1994).

Bentley *et al.* (1995) have analysed genetic variation within a world wide collection of isolates of *Fusarium oxysporum* f.sp. *cubense* which represented races 1, 2, 4 and 11 different vegetative compatibility groups using RAPD-PCR. Comparison of banding patterns both visually and by phenotypic analysis the isolates were sub divided into two major groups. The RAPD analysis helped in differentiating isolates within a vegetative compatibility group also.

Molecular markers have been used to characterize genetic diversity of different isolates of *Fusarium oxysporum* (Edel *et al.*, 1995; Kistler, 1997 and Alves Santos *et al.*, 1999), forma-specialis (Gherbawy, 1999; Alves Santos *et al.*, 2002) and races (Kelley *et al.*, 1994; Jimenez Gasco *et al.*, 2001; Jimenez Gasco and Jimenez – Diaz, 2003).

Dubey *et al.* (2006) studied genetic variability within 24 isolates representing seven races of *Fusarium oxysporum* f.sp. *ciceris* by RAPD technique.

Prasad *et al.* (2004) studied molecular variability among 45 isolates of *Fusarium* species belonging to 8 different sections studied by using universal rice primers (URPs) designed from the rice microsatellites. Amplification products obtained from the two URPs 2R

and 6R ranged between 0.3 kb to 3.5 kb and total number of loci amplified were 104 and 207, respectively.

2.12 Screening for host resistance

Immediately after the report of first epidemic of *Fusarium* wilt in California in 1962, Klisiewicz and Houston (1962) noted that all commercial safflower varieties viz. pacific 1, Gila, US-10, Pacific 2, pacific 7 and Nebraska 10 were susceptible to the disease.

Thomas and Hill (1977) studied the effect of the plant age on reaction of eight safflower cultivars to *Fusarium* wilt. He compared the inoculation of plants by planting seed in infected soil and plants grown for 21 days in non infected soil and then inoculated by root dip procedure. He observed four types of reactions of susceptibility and resistance on the basis of cultivar reactions. He finally concluded that inoculation of plant at both the ages is needed to evaluate cultivar resistance accurately.

Klisiewicz and Urie (1982) tested germplasm of safflower against *Fusarium oxysporum* f.sp. *carthami* in field and in green house test by artificial inoculation of the pathogen. Some of the genotypes were identified as resistant to pathogen.

Klisiewicz and Urie (1982) registered *Fusarium* resistant germplasm. These were the selections from safflower introduction in USA. These germplasm lines expressed resistance to all four races existing in USA. These germplasm selections were released jointly in 1981 by AR-SEA USDA and California Agric. Exp. Stn.

Nirmal (1985) screened fifty two safflower varieties for resistance against *Fusarium* wilt in pot culture and reported that NS-1016, HOO-22, MVI-28, BLY-211 and BLY-1080, were resistant to disease.

Kulkarni (1987) screened 12 safflower varieties for varietal reaction against *Fusarium oxysporum* f.sp. *carthami* and found that genotype BLY-1080 showed resistant reaction, while cultivars N-248, JLSF-1, HOP-116 were moderately resistant.

Mehetre (1988) screened 34 genotypes by water culture technique against *Fusarium oxysporum* f.sp. *carthami* and found that one genotype was moderately resistant, one genotype was tolerant and rest of the genotypes were susceptible and highly susceptible to the pathogen.

Pedgaonkar and Mayee (1989) screened 34 safflower genotypes *in vitro* by water culture technique. Lines viz. JLSF-88 and N-248 showed 20 to 40 per cent wilting respectively as against 100 per cent wilting in all the other genotypes. In field test none of the accessions was found free from wilt. However, 14 cultivars were moderately resistant and 13 were tolerant to wilt disease.

Nagale (1989) screened 34 safflower genotypes against *Fusarium* wilt by water culture technique and reported that none of the genotypes had shown resistant type of reaction. However, JLSF-88, 708, CTV-9, N-887 and BLY-652 were observed tolerant (40 per cent

wilt mortality). Cultivars Annigiri, Tara and JLSF-94 were susceptible and remaining 20 were highly susceptible.

Phad and Jagtap (1990) screened safflower germplasm lines against wilt disease and reported that the lines SSF-16, JSLF-47, P-57-2, NS -10-60 had showed high susceptibility where as NS-133 (Check) showed 40 per cent wilting.

Kalpana Sastry and Ramchandran (1992) in India evaluated safflower lines. They classified five entries as immune/tolerant exhibiting low/no disease at one or more crop growth stages. Three genotypes were recorded under moderately resistant category; moderately susceptible category was comprised of 25 genotypes and susceptible category was represented by eleven genotypes.

Mogle and Nirmal (1992) screened eighty two safflower germplasm lines against wilt disease under natural field conditions. Following maximum wilting percentage was considered for categorizing varieties. Immune (0 per cent incidence), resistant (0-2 per cent incidence), moderately resistant (2-5 per cent incidence and susceptible (more than 5 per cent incidence) ten lines viz. SSF-16, JSF-9, BS-506, B-20, ISA-300, SSF-48, NS-10-60, NS-99-A, JLSF-47 and PST-2 recorded less than 2 per cent mortality for three consecutive seasons while other germplasm lines showed high susceptibility. NS-133 (Check) showed mortality to the tune of 15 per cent.

Dhokne (1993) screened 170 safflower genotypes against *F. oxysporum* f.sp. *carthami* by water culture technique and found that five genotypes viz., GMU-840, 850, 861, 875 and CTV-218 were free from wilt reaction while GMV-841, 909, 946 and 1582 were moderately resistant. Genotype GMU-1071 was observed to be tolerant. Rest of the genotypes recorded susceptible and highly susceptible reaction.

Kalpana Sastry and Chattopadhyay (2003) screened 51 promising safflower germplasm lines in *Fusarium* wilt infested plots which resulted in identification of highly wilt resistant selection viz., 86-93-36A, 237550, VI-92-4-2 and II-13-2A, with some moderate resistance in HUS-305.

2.13. Biological management through *Trichoderma* spp.

Investigation on interactions between pathogens and potential bioagents has involved the use of laboratory and field experiments. The possible mechanisms of antagonism under *in vitro* condition include production of diffusible antibiotics (Dennis and Webster, 1971a) and mycoparasitism (Dennis and Webster, 1971b).

The concept of biological control in recent years developed into a viable and promising approach for disease control used individually or in combination with other systems (Cook and Baker, 1983 and Papavizas and Lewis, 1988). The use of chemicals has an adverse effect on beneficial organism in soil and creating residual problems. Under the circumstances antagonists can be effectively used to minimize the inoculum potential of the pathogen.

Potentiality of *Trichoderma* as biocontrol agent was reported by Weindling (1932). *T. viride* was utilized in the control of wilt of flax (Fedorinchik and Vader Plank, 1954 and Fedorinchik, 1961) and lentil (Mehrotra and Cladius, 1972).

Hanso and Karoles (1980) reported that *T. viride* was found inhibitory to *Fusarium avenaceum*, *Fusarium oxysporum* and *Fusarium moniliforme* under *in vitro* conditions.

Mahalinga (1982) reported that *T. viride* inhibited the growth of *Fusarium* spp. and also overgrew on its colony.

Rod (1984) reported antagonistic activity of *T. harzianum* against *Fusarium oxysporum* in onions. *T. harzianum* was utilized as a successful antagonist against *Fusarium oxysporum* f.sp. *radicis lycopersici* in tomato causing crown and root rot of tomato (Sivan and Chet, 1986 and Harman and Fletcher, 1991).

In vitro experiments can be useful in elucidating the mechanism of antagonism of biocontrol agents (Duchesne *et al.*, 1989).

Mukhopadhyay *et al.* (1989) studied the biocontrol potentiality of *T. harzianum* against chickpea wilt *in vitro* as well as under field conditions. They recorded an excellent control of disease due to this bioagent.

Chattopadhyay and Sastry (1990) reported that safflower wilt incidence was reduced by 82 per cent by integrating treatment of

safflower seed with Carbendazim (0.1%), *T. viride* and soil application of KCl at 20 g/ha.

Clavet *et al.* (1990) observed inhibition in the growth of *Fusarium oxysporum* f.sp. *ciceri* by *T. harzianum* isolate.

Dhendi *et al.* (1990) reported inhibition of *F. oxysporum* f.sp. *ciceri* in the laboratory by the metabolites from *Trichoderma* spp.

Katragudda and Murugesan (1996) reported the mechanism of antagonism between the mycoparasite *T. harzianum* and cotton wilt pathogen *Fusarium oxysporum* f.sp. *vasinfectum* and concluded that upon contact the mycoparasite produces lytic enzyme which degrade the cell walls of pathogen.

Chattopadhyay and Kalpana Sastry (1997) studied the effect of naturally occurring antagonists and some botanicals on reduction of wilt disease in safflower and microscopic studies revealed a mechanism of mycoparasitism and it was the first successful attempt for control of wilt by seed dressing with *T. viride*. They also reported crude extracts of botanicals to be more effective than their commercial formulations against safflower wilt pathogen.

Charati *et al.* (1998) studied the effect treatment of *Trichoderma* spp. on cotton and reported that treatment with *T. harzianum* gave maximum germination percentage and lowest wilt incidence.

Biswas and Das (1999) tested the efficacy of *Trichoderma* spp. as a bio-control agent against the wilt pathogen *Fusarium udum* of pigeonpea, using 5 species of *Trichoderma* in *in vitro* in dual culture. The conclusion was that *T. harzianum* was most effective antagonist by *T. longicornis* while *T. koningii* and *T. viride* were ineffective.

Karunanithi and Mohammed Usman (1999) reported that nine isolates of *Trichoderma* spp. were screened *in vitro* for efficacy in suppressing the growth of *Fusarium oxysporum* f.sp. *sesami* the incitant of sesamum wilt. *T. viride* was found most effective and also reported that the isolates *T. viride* (C₁), *T. harzianum* (C₂) and *T. viride* (M₂) were effective in reducing radial growth by producing volatile and non volatile compounds.

Behere (2002) reported that seed treatment with bioagents significantly reduced the safflower wilt incidence. *T. viride* -5 recorded highest seed germination and also increased the shoot and root length of safflower.

Gowdar and Kulkarni (2003) reported that *Fusarium oxysporum* f.sp. *udum* inciting pigeon pea wilt was significantly controlled by *T. harzianum*.

Prasad (2003) reported that *T. viride* increased seed germination of safflower, increased vigour index and reduced wilt incidence as compared to control.

Raju *et al.* (2003) studied the effect of biocontrol agents against *Fusarium oxysporum* f.sp. *carthami* causing wilt of safflower and

reported that seed treatment with Thiram + *T. harzianum* + *T. viride* completely inhibited the disease which is on par with seed treatment with *T. viride*, *T. harzianum* and Carbendazim.

Sharma *et al.* (2003) studied antagonistic activity of eight species of *Trichoderma* against *Fusarium oxysporum* f.sp. *lini* (Bolley) Synd. and Hans. Maximum inhibition of *Fusarium oxysporum* f.sp. *lini* (Bolley) Synd. and Hans was recorded by *T. viride* (P) followed by *T. harzianum* (P), *T. viride* (K) and *T. harziannum* (K) in a descending order.

Shalini and Dohroo (2005) studied efficacy of *Trichoderma harzianum* and *T. viride* against *Fusarium oxysporum* f.sp. *pisi* (Synd. and Hans) by dual culture method found *Trichoderma harzianum* and *T. viride* exhibited the maximum antagonistic activity against *Fusarium oxysporum* f.sp. *pisi* (Synd. and Hans).

2.14. *In vitro* evaluation of fungicide

Valaskova (1983) tested five *Fusarium* species in *in vitro* trials. MBC (Carbendazim) was most effective followed by benomyl and TB-Z (thiobendazole). Tolerance was induced in *Fusarium oxysporum* most rapidly with Bavistin and slowly with Benlate.

Sinha (1974) studied the efficacy of benlate (Benomyl) against *Fusarium oxysporum* f.sp. *udum* causing wilt of pigeonpea and found that seed treatment with Benlate was ineffective.

Chakrabarti *et al.* (1975) tested the efficacy of different fungicides in controlling safflower wilt disease caused by *Fusarium*

oxysporum f.sp. *carthami*. They reported that seed dressing with Benomyl as well as furrow application of benomyl was highly effective.

Zayed *et al.* (1980) tested the systemic fungicide, Baycor which proved to be effective in controlling safflower wilt.

Desai (1986) concluded that Bavistin was highly toxic to *Fusarium udum*. Next best was Thiram followed by Topsin-M, Agrozim, Benlate and Foltaf.

Mehetre (1988) could control seed borne infection of *Fusarium oxysporum* f.sp. *carthami* in safflower with Thiram 75 WP.

Mathur and Tyagi (1988) noted that Benlate (Benomyl) was translocated from roots to the aerial parts of the seedlings but maximum accumulation was in the roots followed by shoots and leaves.

Kapoor and Kumar (1991) demonstrated by "Poisoned food technique" that Carbendazim and benomyl were most toxic to *Fusarium oxysporum*.

Hyas *et al.* (1992) evaluated some fungicides against *Fusarium oxysporum* f.sp. *carthami* and found that, Benomyl, Topsin-M (thiophnate methyl), Tilt (Propiconazole) and Rhizolax (Rhizolax, tolcofos- methyl) were most effective. In soil drench tests, benomyl was most effective followed by Rhizolex and Thiophanate methyl.

MATERIALS AND METHODS



CHAPTER-III

MATERIALS AND METHODS

The experiments described in this chapter were conducted during *rabi* 2005-06 and 2006-07 in the Department of Plant Pathology, College of Agriculture, Marathwada Agricultural University, Parbhani in collaboration with Plant Pathology Section, Directorate of Oilseeds Research (DOR), Rajendranagar, Hyderabad, Andhra Pradesh (India).

The general laboratory techniques followed for the present study were, those described by Nene and Thapliyal (1993), Dhingra and Sinclair (1993) and Aneja (2001) for preparation of media, sterilization, isolation and maintenance of fungal cultures with slight modifications wherever, necessary.

The materials and methods used in the present investigations were broadly described under the following heads :

- 3.1 Glassware
- 3.2 Sterilization of media and glassware
- 3.3 Collection of diseased samples
- 3.4 Isolation and identification of the pathogen isolates
- 3.5 Detection of seed borne nature of *Fusarium oxysporum* f.sp. *carthami*
- 3.6 Morphological characteristics
- 3.7 Cultural characteristics
- 3.8 Pathogenic variability
- 3.9 Genetic variability
- 3.10 Screening for disease resistance



3.11 Management through *Trichoderma* spp.

3.12 *In vitro* evaluation of fungicides

3.13 Statistical analysis

3.1 Glassware

The glassware used in the present study comprised of Petri plates, Ehrlenmeyer conical flasks, test tubes, beakers and measuring cylinders of Corning and Borosil make.

3.1.1. Seeds

The seeds of safflower for laboratory and glass house studies were obtained from All India Coordinated Safflower Project Dept. of Botany, Marathwada Agricultural University (MAU), Parbhani.

3.2 Sterilization of media and glassware

Liquid materials such as media and distilled water were sterilized in an autoclave at 1.1 kg/cm² for 20 minutes. Glasswares were sterilized in hot air oven at 180°C for two hours after wrapping in aluminum foil.

For surface sterilization 0.1 per cent mercuric chloride (HgCl₂) was used for plant material such as root and rectified spirit, for other material like inoculation needles, forceps, inoculation chamber and hands.

Soil was sterilized using 5 per cent formaldehyde solution by thoroughly mixing the chemical with soil and covering the soil heap with polythene sheet for seven days. The cover was removed and allowed for five days for evaporation of chemical fumes.

3.3 Collection of diseased samples

Wilt affected safflower plant samples were collected during the survey from safflower growing areas of Marathwada region during *rabi* 2005-2006 and 2006-2007. The number of places of survey are given in Table 1.

These diseased samples were preserved at 4°C in refrigerator and used for further studies.

The disease incidence in these locations was calculated by using the following formula.

$$\text{Per cent Disease Incidence} = \frac{\text{Number of plants infected with wilt pathogen}}{\text{Total number of plants observed}} \times 100$$

Table 1. Details of locations of collection of infected samples of safflower for isolation of *Fusarium oxysporum f.sp. carthami* from different areas of Marathwada region.

Sample No.	Name of the Farmer	District	Taluka/Village
1.	Pandurang Kashinath Shinde	Beed	Majalgaon/ Lahul
2.	Atmaram Sarjerao Solanke	Beed	Majalgaon/ Hingani
3.	Gajanan Bhagwan Deshmukh	Beed	Ambejogai/ Mamdapur
4.	Bhagwan Digambar Thorat	Beed	Ambejogai/ Kumbhephal
5.	Suryakant Babasaheb Kale	Beed	Kej / Dhanegaon
6.	Bhanudas Gajanan Shingare	Beed	Kej / Aavasgaon
7.	Bhaurao Narayan Patange	Hingoli	Kalamnuri / Digras
8.	Gulab Gangadhar Buthar	Hingoli	Kalamnuri/ Sawangi

9.	Nurshan Akbar Shan	Hingoli	Sengaon/ Kendra
10.	Sanjay Shrawan Rathod	Hingoli	Sengaon / Kolsa
11.	Subhash Muralidhar Konde	Hingoli	Aundha/ Shahapur
12.	Sikandar Taterao Khillare	Hingoli	Aundha / Kalamb
13.	Hiraman Bhikaji Bhalerao	Parbhani	Selu/ Walur
14.	Janardhan Bhaurao Magar	Parbhani	Selu/ Sonna
15.	Sidharth Babasaheb Magar	Parbhani	Manvat / Savangi
16.	Shankar Ramkishan Avchar	Parbhani	Manvat/ Devalgaon
17.	Govind Ramrao Shinde	Parbhani	Pathri / Wadi
18.	Prakash Ghadge	Parbhani	Pathari/ Pathargavhan
19.	Shashikant Bhimrao Pawar	Osmanabad	Kalamb/ Lomatejawala
20.	Dnyaneshwar Aabasaheb Ritapure	Osmanabad	Kalamb / Mangrul
21.	Bibhishan Narayanrao Hange	Osmanabad	Paranda/ Sonari
22.	Sadashiv Balasaheb Patil	Osmanabad	Paranda / Handagali
23.	Ramdas Nivattirao Shinde	Osmanabad	Bhoom / Wangi
24.	Ashok Bhaurao Jadhav	Osmanabad	Bhoom / Samsapur
25.	Gajanan Marotrao Kadam	Nanded	Nanded / Khadki
26.	Pravin Narayan Pohale	Nanded	Nanded / Maralak
27.	Ramnath Laxmanrao Shinde	Nanded	Kandhar/ Manaspuri
28.	Trimbak Shaukarrao Kadam	Nanded	Kandhar / Bachoti
29.	Ramesh Vitthal Pawde	Nanded	Ardhapur / Pimpalgaon
30.	Tukaram Sambhaji Kadam	Nanded	Ardhapur / Delub
31.	Kartik Shivappa Biradar	Latur	Nilanga / Aansarwada
32.	Ganesh Ramrao Jadhav	Latur	Nilanga/ Makni
33.	Manik Bhimrao Mehetre	Latur	Ausa / Wanwada
34.	Dnyanoba Keshav Patil	Latur	Ausa / Nagarsoga
35.	Shivaji Mahadev Bhosale	Latur	Udgir / Handargali

36.	Kundlik Baliram Gore	Latur	Udgir / Kharwadi
37.	Raukhmaji Bhimrao Barkule	Jalna	Partur / Khandvi
38.	Santaji Sham Shinde	Jalna	Partur / Satona
39.	Sonaji Rambhau More	Jalna	Mantha /Aakni
40.	Samadhan Arun Gondage	Jalna	Mantha/ Gevrai
41.	Ramdas Bhagwan Thokre	Jalna	Ghansawangi/ Hiwara
42.	Samadhan Santaji Sonwane	Jalna	Ghansawangi / Ekclaira
43.	Bhima Uttamrao Singh	Aurangabad	Aurangabad / Karmad
44.	Balasaheb Shamrao Pawar	Aurangabad	Aurangabad / Satola
45.	Arjun Sarjerao Ghavate	Aurangabad	Paithan /Anandpur
46.	Uttam Trimbakrao Ukirde	Aurangabad	Paithan / Katpur
47.	Ashok Ramkisan Narake	Aurangabad	Vaijapur/ Jabargaon
48.	Jaganath Aatmaram Wakade	Aurangabad	Vaijapur/ Rotegaon

3.4 Isolation and identification of the pathogen isolates

3.4.1. Media used

Potato Dextrose Agar (PDA) medium was used for isolation and maintenance of pure cultures whereas, Potato Dextrose Broth (PDB) was used for multiplication of pathogen. *Fusarium* Selective Medium (FSM) was used for detection of seed borne (external or internal) nature of *Fusarium oxysporum* f.sp. *carthami*. The seed borne nature of *Fusarium oxysporum* f.sp. *carthami* was detected by Blotter paper method suggested by Agrawal and Sinclair (1987) and by Agar plate method Nash and Snyder (1962).

Fusarium selective Medium (FSM)

Peptone

15.0 g

KH ₂ PO ₄	1.0 g
MgSO ₄ , 7H ₂ O	0.5 g
Agar Agar	20.0 g
Distilled water	950 ml.

(Sterilize at 15 psi for 20 min)

Antibiotics and inhibitor

PCNB	0.5 g
(Brassicol 75 wp)	
Oxgall	0.5 g
Streptomycin sulphate	0.1 g
Chlorotetracyclin hydrochloride	0.5 g

Above antimicrobial agents (antibiotics and inhibitors) were added to 50 ml of sterilized water which was then mixed in 950 ml of medium.

PDA

Potato (peeled, washed & sliced)	200 g
Dextrose (Qualigens Fine Chemicals, Mumbai)	20 g
Agar agar (Qualigens Fine Chemicals, Mumbai)	20 g
Distilled water	1 lit.

PDA medium was prepared by boiling 200 g of peeled, washed and sliced potato in 500 ml of distilled water for about 30 minutes. In other conical flask containing 500 ml of distilled water, 20 g agar was melted. Both of these were filtered through muslin cloth into a beaker and 20 g of dextrose was added to the filtrate. The final volume

of the medium was made up to one liter by adding distilled water. The pH of the medium was adjusted to 6.5 before sterilization by adding 1 N HCl or 1 N NaOH as the case may be. The medium was sterilized in an autoclave at 15 pound per square inch (psi) (121°C) for 20 minutes.

PDB

Potato (peeled, washed & sliced)	200 g
Dextrose (Qualigens Fine Chemicals, Mumbai)	20 g
Distilled water	1 lit.

PDB was prepared similarly as PDA but without the addition of agar; sterilized in an autoclave at 15 pounds per square inch (psi) (121°C) for 20 minutes.

3.4.2. Isolation

Isolations were made from wilt affected safflower plant parts i.e. roots and crown region which were collected from eight districts of Marathwada region selected for isolation of pathogen.

The infected plant parts were washed thoroughly under running tap water and transferred to blotting paper. They were cut into 0.20 cm thick pieces and surface sterilized with 0.1 per cent mercuric chloride solution for one minute followed by three washings with sterile distilled water and were plated on potato dextrose agar medium under aseptic conditions and incubated at $25 \pm 1^\circ\text{C}$.

3.4.3. Purification of the pathogen isolates

All the isolates of the pathogen were purified by hyphal tip method (Dohroo and Sharma, 1992).

Sufficient care was taken to maintain the purity of the isolates throughout the study.

3.4.4. Identification and maintenance of the pathogen isolates

The isolates of the pathogen were identified based on colony characters and spore measurement by using monograph (Booth, 1971). Measurements of conidia of the isolates were taken with the help of ocular micrometer after calibration of the microscope. microphotographs of the *Fusarium oxysporum* f.sp. *carthami* isolates were taken to describe spore morphology. All the isolates were maintained on PDA medium at 4°C and subcultured every three months.

3.4.5. Pathogenicity test

Pathogenicity of different isolates of *Fusarium oxysporum* f.sp. *carthami* was proved by soil inoculation method and water culture technique on variety Nira (Nene *et al.*, 1981)..

3.4.5.1. Soil inoculation method

Nineteen isolates were multiplied on PDB (Potato Dextrose Broth) for 15 days in 250 ml ehrlenmeyer conical flask containing 100 ml PDB/flask. For each isolates three pots were used with sterile soil keeping one pot as control for each isolate. In pots sterile soil and PDB was added. To allow maximum multiplication of inoculum the pots were adequately watered with sterile water daily. On 4th days five healthy seeds of variety Nira were sown per pot. Plants emerged out of soil within five days and few plants were found wilted after few days of emergence. Plants wilted from each isolate were collected separately. They were subjected to isolation by 0.1 per cent HgCl₂ surface

sterilization and three changes of sterile water and finally 19 isolates were recovered from artificially inoculated plants and confirming the pathogenicity of *Fusarium oxysporum* f.sp. *carthami* of the 19 isolates.

The recovered isolates were maintained on PDA slants.

3.4.5.2. Water culture technique

Water culture technique suggested by Nene *et al.* (1981) was used which is as follows.

Potato dextrose broth (PDB) was prepared in conical flask. Mycelial discs of 5 mm diameter were cut from the margin of the seven day old culture of each isolate of the pathogen and transferred to the conical flask containing the sterilized medium under aseptic conditions. The flasks were incubated at 25 + 1°C in an incubator for 10 days. The culture filtrate 50 ml was added to 50 ml sterile water and only sterile water tubes without culture filtrate were maintained as control.

The seedlings of safflower cultivar Nira grown in sterile culture using sterile water were transferred after 15 days of emergence to culture filtrate tubes and the observations on the number of seedlings wilted were recorded after 5 days of transplanting. Per cent disease incidence was calculated by using the following formulae.

$$\text{Per cent disease incidence} = \frac{\text{Number of wilted plants}}{\text{Total number of plants}} \times 100$$

3.5 Detection of seed borne nature of *Fusarium oxysporum* f.sp. *carthami*

Blotter paper method and Agar plate method were employed for detection of seed borne *Fusarium*.

3.5.1. Blotter paper method

This method is widely used. Seeds (400) were placed on well water soaked blotter paper and incubated at $20 \pm 2^{\circ}\text{C}$ in Petri dish usually for 7 days in alternating cycles of 12 h light and 12 hr darkness. The individual seed was examined under stereo-microscope and fungi were identified based on their characters.

3.5.2. Effect of seed treatment on the incidence of seed mycoflora of safflower on CV. Nira by Agar plate method.

To study the effect of seed treatment with fungicides, chemical and hot water on seed borne mycoflora of safflower, the seeds of safflower cultivar Nira were treated with fungicides Carbendazim (0.3 %), Thiram (0.3 %), Carbendazim (0.15 %) + Thiram (0.15 %), Captan (0.3 %), surface sterilized with 0.1 % (HgCl_2) and treated with hot water at 52°C (before treatment the seeds were soaked in water for over night). Unsterilized / untreated seeds served as control. After seed treatments and sterilization, the seeds were placed in Petri plates containing *Fusarium* Selective Medium (FSM). Observation on colony development of *Fusarium oxysporum* f.sp. *carthami*, *Rhizopus* spp. and *Aspergillus* spp. were noted.

3.6 Morphological characteristics

To study morphology of macroconidia, microconidia and chlamydospore fifteen day old culture of each isolate grown on PDA medium was stained with 0.1 per cent lactophenol cotton blue and observed under compound microscope. Observation on length, breadth and septa of microscopic structures were recorded.

3.6.1. Preparation of lactophenol

Phenol (crystal)	:	20 g
Lactic acid	:	20 ml
Glycerol	:	40 ml
Distilled water	:	20 ml

Cotton blue crystals (0.05 g) were added in 50 ml lactophenol and mixed by vortex mixer to dissolve it. The stain was then filtered through Whatman No. 1 filter paper to make it dust free and transferred to a stoppered bottle.

3.6.2. Preparation of slides

A small amount of pure culture was taken using a sterile needle and transferred to a clean glass slide. The culture was taken from four position of the culture plate two at the right angle to each other, one from very close to inoculation point and another from the mid point of the radius. Total three culture plates of each isolate were used for the morphological studies after incubation at $25 \pm 1^{\circ}\text{C}$. The culture was stained with 0.1 per cent lactophenol cotton blue and observed for the macroconidia, microconidia and chlamydo spores using a compound microscope.

3.6.2.1. Microconidia

Microconidia of all the isolates were measured with the help of an ocular micrometer after calibrating the microscope. For each isolate length, breadth and septation of 75 conidia were measured.

3.6.2.2. Macroconidia

Length, breadth and septation of 75 macroconidia were measured for 19 isolates of *F. o. f.sp. carthami*.

3.6.2.3. Chlamydo spores

Presence of chlamydo spores was observed in culture of 19 *F. o. f.sp. carthami* isolates and were recorded.

3.7 Cultural characteristics

For the cultural studies all the isolates of *F. o. f.sp. carthami* were grown on PDA and PDB medium.

Observations on colony growth, colony colour, diameter of the colony, mycelial dry weight and sectoring in the culture of each isolate were recorded. Whereas, characteristic like sporulation was visually observed and recorded.

3.7.1. Colony diameter, colony growth and colour

Twenty ml of lukewarm PDA medium was poured in sterilized petri plates and allowed to solidify. Mycelial discs of 5 mm diameter were cut from the margin of seven days old culture of *Fusarium oxysporum f.sp. carthami* for each isolate and placed in the centre of Petri plate under aseptic conditions. The plates were incubated for 10 days in an incubator at $25 \pm 1^{\circ}\text{C}$ and the diameter of the colony was measured and recorded. Three replications of each isolate were maintained for the study.

Colony growth of each isolate was recorded as felty, fluffy while, the colour of the colony was recorded as whitish or light whitish or pink whitish.

3.7.2. Sectoring in the culture

Presence or absence of distinct sectors in pure culture of each isolate grown on PDA medium was observed and recorded.

3.7.3. Mycelial dry weight and sporulation

To study the mycelial dry weight of each of the isolate, PDB was poured in 250 ml conical flask, plugged and sterilized. Mycelial discs of 5 mm diameter were cut from the margin of the seven day old culture of each isolate of the pathogen and transferred to the conical flask containing the sterilized medium under aseptic conditions. The flasks were incubated at $25 \pm 1^{\circ}\text{C}$ in an incubator for 10 days. Mycelial mat was removed aseptically washed thoroughly with distilled water and dried in an oven at 60°C for 12 hr. Dried mycelial weight of each isolate was taken and recorded using digital electronic balance.

Five mm discs of the fungal culture grown on PDA medium were cut by a sterilized cork borer from the center to the margin at three different equidistant places along the radius. The three discs were taken in a test tube containing 15 ml sterile distilled water and crushed by a glass rod. The spore suspension was shaken by a vortex mixer to dislodge the conidia. The spore suspension (0.02 ml) was placed with a graduated 0.1 ml pipette in one chamber of the Haemocytometer. The number of spores in a small square at the centre were counted and calculated for the total number of spore ($n \times 10^4$; where n = number of spore per small square) in 1 ml of the spore suspension. Three replications of each isolate were observed.

The extent of spore production was assayed with respect to each isolate by counting the number of spores present in one milliliter of

spore suspension. The sporulation of each isolate was designated as excellent, good, moderate and poor based on the relative number of spores counted.

3.8 Pathogenic variability

Pathogenic variability of different *Fusarium oxysporum* f.sp. *carthami* isolates was studied in water culture technique.

3.8.1. Host differentials

Five safflower cultivars were selected as host differentials to study the pathogenic variations in test isolates.

- | | | | |
|----|---------|---|----------------------|
| 1. | A-1 | } | Moderately resistant |
| 2. | PBNS-40 | | |
| 3. | Bhima | | |
| 4. | Nira | } | Susceptible |
| 5. | Manjira | | |

Seeds were surface sterilized with 0.1 per cent mercuric chloride then washed with sterile distilled water and dried at room temperature before sowing. Seeds were sown equidistantly in each pot and watered regularly. Seedling were raised and used for transplanting.

3.8.2. Water culture technique

Water culture technique suggested by Nene *et al.* (1981) was used which is as follows.

Potato dextrose broth (PDB) was prepared in conical flask. Mycelial discs of 5 mm diameter were cut from the margin of the seven day old culture of each isolate of the pathogen and transferred to the conical flask containing the sterilized medium under aseptic condition. The flasks were incubated at 25 +1°C in an incubator for 10 days. The

culture filtrate 2.5 ml was added to 97.5 ml sterile water and only sterile water tubes without culture filtrate were maintained as control.

The seedlings of safflower cultivars viz. A-1, PBNS-40, Bhima, Nira and Manjira, grown in sterile culture using sterile water were transferred after 15 days of emergence to culture filtrate tubes and the observations on the number of seedlings wilted were recorded after 5 days of transplanting. Per cent disease incidence was calculated by using the following formulae.

$$\text{Per cent disease incidence} = \frac{\text{Number of wilted plants}}{\text{Total number of plants}} \times 100$$

3.8.3. Disease reaction

Virulence of the pathogen isolates and disease reaction against five safflower differentials (A-1, PBNS-40, Bhima, Nira and Manjira) was recorded following the scale proposed by Mayee and Datar (1986).

Per cent disease incidence	Disease reaction / response
0	Highly resistant / immune
1 or less	Resistant
1-10	Moderately resistant
11-20	Tolerant
20-50	Susceptible
51 or more	Highly Susceptible

3.9 Genetic variability

Random amplified polymorphic DNA (RAPD) analysis was used to detect the variations among the isolates of *Fusarium oxysporum*

f.sp. *carthami*. Standard protocols were used for the isolation of DNA and RAPD analysis (Williams *et al.*, 1990).

3.9.1. Fungal isolates and media

The information on isolates is given in Table 2. *Fusarium oxysporum* f.sp. *carthami* isolates were isolated on potato dextrose agar (PDA) from infected safflower samples collected from various locations of safflower growing areas (As mentioned in Table 2)

3.9.2. Total genomic DNA extraction

DNA was extracted using the CTAB (Hexa-decyl tri-methyl ammonium bromide, (Sigma Chemical Co., St. Louis, USA) method of Lee and Taylor (1990) with slight modifications.

For DNA extraction, fungal tissue was harvested from the isolates grown in potato dextrose broth medium incubated for 3-5 days in orbital shaker (Kuhner, Switzerland make) at 200 rpm and 26°C. After incubation the fungal mat was filtered through sterile cheese cloth and was grinded to a fine powder in the presence of liquid nitrogen prior to DNA extraction.

Approximately two g of grinded mycelium was taken into a 30 ml Oakridge centrifuge tube in which 10 ml of CTAB extraction buffer (200 mM Tris-HCl pH 8.0, 1M NaCl, 50 mM EDTA pH 8.0 and 2 % CTAB) was added. The sample was incubated in water bath at 65°C for 60 min. Preparations were homogenized by vortexing prior to the addition of 10 ml of chloroform + ISO-amyl alcohol (24:1, v/v) and separated by centrifugation at 15000 x g for 10 min in centrifuge (Sorval RC 5 B plus, USA). ISO-amyl alcohol acts as a stabilizer and protects DNA from degradation, whereas, chloroform facilitated the separation of

the contaminating proteins that sediment together with polysaccharides. The upper aqueous phase was transferred into a clean 30 ml oakridge centrifuge tube using a wide bore 10 ml pipette tip and DNA was precipitated with 6 ml cold iso-propanol. The precipitate was collected by centrifugation at 13000 x g for 5 min. The resulting pellet was washed with 70 per cent cold ethanol, dried and re-suspended in 500 μ l TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0).

3.9.3. Assessment of DNA quality and quantity

The extracted DNA was qualified by running 2 μ l of each DNA sample on 0.7 per cent agarose gel with lambda uncut DNA at 30 and 60 ng to get a final concentration of 15 – 20 ng/ μ l.

To assess the quality, the DNA was diluted with TE buffer and loaded on 1 per cent agarose gels in 0.5 x TAE buffer (pH 8.0) in a gel electrophoresis apparatus (Biorad, USA) and electrophoresed for 30 min under constant voltage of 70 v. The ethidium bromide (final concentration of 0.3 μ g/ml) was added to the molten agarose at the time of gel casting. Upon the completion of electrophoresis, the gel was visualized on a UV transilluminator, and gel images were taken using a gel documentation system (multi-image light cabinet, Alpha Innotech Corporation, USA). Subsequently, the gel images were printed directly or saved on diskette as TIF files for later analysis.

Table 2 Sources of isolates of *Fusarium oxysporum* f.sp. *carthami* used for molecular analysis

Sr. No.	Isolate	Place	Collection year	No. /code
1.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Dhanegon	2006	FOC-5
2.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Kolsa	2006	FOC-10
3.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Kalamb	2006	FOC-12
4.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Sonna	2006	FOC-14
5.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Devalgaon	2006	FOC-16
6.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Pathargavhan	2006	FOC-18
7.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Sonari	2006	FOC-21
8.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Samasapur	2006	FOC-24
9.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Manaspuri	2006	FOC-27
10.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Pimpalgaon	2006	FOC-29
11.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Delub	2006	FOC-30
12.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Makni	2006	FOC-32
13.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Handargali	2006	FOC-35
14.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Khardwadi	2006	FOC-36
15.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Aakni	2006	FOC-39
16.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Hiwara	2006	FOC-41
17.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Eklaira	2006	FOC-42
18.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Satola	2006	FOC-44
19.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Jabargam	2006	FOC-47
20.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	DOR	2005	---
21.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	DOR	2005	---
22.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Solapur	2002	---
23.	<i>F. oxysporum</i> f.sp. <i>carthami</i>	Solapur	2002	---

24.	<i>F. oxysporum</i>	CBS, Netherlands	1938	ITCC 179
25.	<i>F. moniliformae</i>	---	1970	ITCC 1526
26.	<i>F. graminearum</i>	Wellington T.N.	1985	ITCC 3437
27.	<i>F. solani</i>	Raichur	2001	ITCC 5219
28.	<i>F. equiseti</i>	New Delhi	1973	ITCC 1806
29.	<i>F. moniliformae</i> f. sp. <i>subglutinans</i>	Hyderabad	1990	ITCC 4019
30.	<i>F. chlamydosporum</i>	Kanpur	2000	ITCC 4969

3.9.4. Random primers and PCR conditions

Twenty 10 – mer primers of OPA and OPC series of Operon Technologies Inc., Alameda, CA, USA were used in the present study.

The RAPD-PCR amplifications were carried out in a total volume of 25 µl containing 15-25 ng of genomic DNA (Williams *et al.* 1990). The reaction buffer consisted of 2.5 µl of 10 x PCR buffer [(500 mM KCl, 15 mM MgCl₂, 100 mM Tris – HCl (pH 8.0)], 0.2 µM RAPD primers (0.2 mM of each dNTP mix (Fermentas Inc., MD., USA)) and 0.2 µl of taq polymerase (Banglore Genei, Bangalore, India). The PCR amplifications were performed using thermal cycler (biometra) programmed for initial denaturation at 94°C for 5 min, 45 cycles at 94°C for 1 min, and 35°C for 1 min, 72°C for 2 min and a final cycle at 72°C for 7 min. All amplified DNA products were resolved by electrophoresis

on agarose gel (1.4 %) in TAE (0.5 x) buffer, stained with ethidium bromide and photographs were taken by using Gel Documentation System.

3.9.5. Microsatellite primers and PCR conditions

Primer CCCGCATCC (CA)₉ Oligonucleotide repeat based on a previous study (Prasad *et al.*, 2004) was got synthesized from Bioserve Biotechnologies, Hyderabad (Table 3).

PCR was performed in a T-Gradient Thermal Cycler (Biometra, Germany). Each reaction contained 10 to 30 ng of genomic DNA, 4 U_M primer, 200 μM and dNTPs, 2.5 units of Taq DNA polymerase (Bangalore Genei, India), 50 mM KCl, 10 mM Tris (pH 8.3), 2 mM MgCl₂ in a final volume of 25 μl. PCR reactions were performed for 35 cycles consisting of initial denaturation of template DNA, 94°C for 1 min, primer annealing at 10°C below the T_m of each oligonucleotide primer (Table 3) for 1 min, 72°C for 2 min and one final cycle of 72°C for 7 min. The PCR products were then analyzed by agarose (1.4 %) gel electrophoresis for 3 h at 80 v in 0.5 x TAE and visualized by ethidium bromide staining.

Table 3. Microsatellite (SSR repeats) primers used for amplification of genomic DNA of *Fusarium* isolates.

Sr. No.	Primer sequence (SSR repeat) 5'-3'	Melting temperature (T _m °C)	Annealing Temp. Gradient surveyed (°C)	Best Annealing Temp (°C)	Total number of bands scored
1.	CCCGCATCCC ACACACACAC ACACACA	64	45-55	53	187

3.9.6 Data analysis

The relatedness of 30 isolates was estimated by means of scorable bands amplified from random and microsatellite primers. Each band was considered as a character which had two possible states of presence (coded as 1) and absence (coded as 0). Only major bands were counted.

3.10 Screening for disease resistance

To find out the sources of resistance in safflower genotypes and breeding material against *Fusarium oxysporum* f.sp. *carthami* screening of safflower genotypes was done in laboratory by water culture technique (Nene *et al.*, 1981).

One hundred fifty safflower genotypes were selected for screening. The details of the technique were described earlier in pathogenic variability.

Observations were recorded for seedling mortality (Wilting up to five days. Per cent disease incidence and disease reaction was rerecorded as described earlier in pathogenic variability.

3.11. *In vitro* evaluation of fungal bioagents against two isolates of *Fusarium oxysporum* f.sp. *carthami*

3.11.1. Management through *Trichoderma* spp.

The potential antagonistic activity of five *Trichoderma* spp. collected from Department of Plant Pathology, MAU, Parbhani culture

collections was tested against nineteen isolates of *Fusarium oxysporum* f.sp. *carthami*.

The antagonistic potential of *Trichoderma* spp was assessed against *Fusarium oxysporum* f.sp. *carthami* by dual culture technique on PDA medium as per procedure described by Stack *et al.* (1986). For this, 20 ml of sterilized and cooled medium (PDA) was poured in each; allowed to solidify. A 5 mm disc of *Fusarium oxysporum* f.sp. *carthami*, was placed at one end of the medium with the help of sterilized needle. Just opposite to it, 5 mm disc of the respective *Trichoderma* spp. was placed. For this, a week old culture of *Fusarium oxysporum* f.sp. *carthami* isolates and respective *Trichoderma* spp. was used. Controls i.e. without inoculation of the *Trichoderma* spp. fungus were also maintained.

Observations regarding per cent inhibition and colony radius of *Fusarium oxysporum* f.sp. *carthami* isolates of *Trichoderma* spp. were recorded after 3, 6 and 9 days after inoculation by incubating at $25\pm 1^{\circ}\text{C}$ temperature. The per cent inhibition of the pathogen will be calculated by using following formula (Vincent, 1972).

$$\text{PI} = \frac{\text{C} - \text{T}}{\text{C}} \times 100$$

PI = Percent inhibition

C = Radius of *Fusarium* colony in control

T = Radius of *Fusarium* colony in treatment

3.12 *In vitro* evaluation of fungicides

The fungicides used in the present study along with particulars of trade name, ingredient of the chemical in the formulation and source of supply are presented in (Table 4).

For evaluation of different fungicides poisoned food technique (Nene and Thapliyal, 1993) was adopted to determine the sensitivity of the 19 isolates of *Fusarium oxysporum* f.sp. *carthami*.

3.12.1. Poisoned Food Technique

The required quantity of fungicide was added to the PDA medium at luke warm stage to get 100 µg/ml concentration on active ingredient basis. The stock solution was prepared on whole chemical basis. From this stock, serial dilutions were made by adding required quantity of PDA so as to get 1000, 500, 100, 50, 25, 10 and 1 µg/ml concentrations. Three replications were maintained in respect of each isolate and each concentration.

Five mm discs of the test fungal isolates of *Fusarium oxysporum* f.sp. *carthami* were cut with sterile cork borer and transferred aseptically to the center of poisoned medium. Similarly controls were maintained by placing five mm discs of all the test isolates in the centre of the non-poisoned PDA medium.

All the Petri plates were incubated at 25 + 1°C in BOD incubator for 10 days. The diameter of fungal colony was measured in each of the treatment.

Table 4. Particulars of fungicides employed in the investigation

Sr. No.	Common name	Trade name	Chemical name	Active ingredient	Source of supply
1.	Carbendazim	Bavistin	Methyl-benzimidazole 2 yl carbamate	50 % WP	BASF India Ltd. Mumbai
2.	Copper oxychloride	Blitox	Copper oxychloride	50 % WP	Rallis India Ltd. Mumbai
3.	Captan	Captaf	N –(Trichloro methylthio) cyclohexene-1,2-dicarboximide	50 % WP	Rallis India Ltd. Mumbai
4.	Thiram	Thiram	Tetramethyl thiuram disulphide	75 % WP	Rallis India Ltd. Mumbai
5.	Propiconazole	Tilt	Propiconazole	25 EC	Syngenta India Ltd. Mumbai

3.13 Statistical analysis

The data obtained in different experiments was statistically analyzed following completely randomized block design as per procedures suggested by Panse and Sukhatme (1985). The data pertaining to percentage was angularly transformed whenever necessary.

Cluster analysis with the unweighed pair group method with an arithmetic average (UPGMA) algorithm was performed using NTSYS-PC (ver. 2.0) (Rohlf, 1993) to produce a dendrogram. To determine robustness of the dendrogram, we bootstrapped the data with 2000 replications in the computer programme WINBOOT (Yap and Nelson, 1996).

RESULTS





Fig.1. Map showing locations surveyed for collection of wilt infected samples of safflower from different areas of Marathwada region

CHAPTER-IV

RESULTS

The present study was undertaken to obtain isolates of *Fusarium oxysporum* f.sp. *carthami* from various locations of Marathwada region and study their seed borne nature, morphological, cultural and pathogenic variations, DNA sequence, screening for disease resistance, biological management and chemical management in laboratory. Study was conducted during *rabi* season in the year 2005-2006 and 2006-2007. Results of these experiments are compiled and statistically analysed, wherever necessary and the inferences drawn are presented on following pages.

4.1. Survey for wilt incidence and collection of diseased samples

A random survey was undertaken during *rabi* 2005-06 and 2006-07 of safflower wilt in farmer's fields of Marathwada region (Table 1 and Fig. 1). The incidence of wilt in different cultivars on farmer's field is given in Table 5. The results indicated that farmers had grown Local (62.5 %), Sharda (20.82 %), Bhima (10.41 %) and PBNS-12 (4.16 %) cultivars during 2005-2006 and Local (70.83 %), Bhima (18.75 %) and Sharda (8.33 %) during 2006-2007.

The incidence of wilt disease varied from 1.66 to 11.66 per cent in eight districts of Marathwada region during *rabi* 2005-2006. In 2005-06, the maximum (11.66 %) incidence of the disease was observed on safflower cultivar Local at Kolsa village of Taluqua Sengaon in Hingoli district while the least incidence (1.66 %) was observed on cultivar Sharda at Nagarsoga village of Ausa Taluqua in Latur district.

Table 5. Incidence of safflower wilt in farmers' fields in Marathwada during *rabi* 2005-06 and 2006-07.

Sample No.	Designation of isolate from samples collected in 2005-06	Previous crop (2005-06 and 2006-07)	Variety		Disease incidence (%)		Result of isolation
			2005	2006	2005	2006	
1	---	Soybean	PBNS-12	Local	6	5	-
2	---	Soybean	Bhima	Bhima	3.33	5	-
3	---	Soybean	Bhima	Bhima	5	6	-
4	---	Soybean	Local	Local	6.66	6	-
5	FOC-5	Tur, mung	Bhima	Local	3.33	3.33	+
6	---	Soybean	Local	Local	4	5	-
7	---	Cotton soybean	Local	Local	10	12	-
8	---	Soybean	Local	Local	8.33	10	-
9	---	Soybean	Local	Local	12	10	-
10	FOC-10	Soybean	Local	Local	11.66	12	+
11	---	Cotton soybean	Local	Local	6	5	-
12	FOC-12	Soybean	Sharda	Bhima	3.33	3.33	+
13	---	Soybean	Local	Local	5	6	-
14	FOC-14	Soybean cotton	Local	Local	6	5	+
15	---	Cotton	Local	Local	3.33	3.33	-
16	FOC-16	Soybean	Local	Local	8.33	10	+
17	---	Soybean	Local	Local	10	10	-
18	FOC-18	Cotton	Local	Local	5	6	+
19	---	Soybean	PBNS-12	Local	5	4	-
20	---	Mung, soybean	Bhima	Bhima	3.33	5	-
21	FOC-21	Soybean	Local	Local	8.33	10	+
22	---	Cotton	Local	Local	3.33	4	-
23	---	Mung, udid	Local	Local	4	5	-

Sample No.	Designation of isolate	Previous crop	Variety		Disease incidence (%)		Result of isolation
			2005	2006	2005	2006	
24	FOC-24	Cotton	Local	Local	10	8.33	+
25	---	Cotton	Sharda	Bhima	10	6.66	-
26	---	Cotton	Sharda	Bhima	6.66	6	-
27	FOC-27	Soybean	Local	Local	10	12	+
28	---	Cotton	Local	Local	6	4	-
29	FOC-29	Cotton	Sharda	Bhima	4	4	+
30	FOC-30	Cotton	Local	Local	10	6	+
31	---	Soybean	Sharda	Sharda	5	3.33	-
32	FOC-32	Cotton	Sharda	Sharda	6	6	+
33	---	Soybean	Local	Local	3.33	5	-
34	---	Soybean	Sharda	Sharda	1.66	3.33	-
35	FOC-35	Cotton	Local	Local	10	12	+
36	FOC-36	Soybean	Local	Local	8.33	6	+
37	---	Soybean	Bhima	Local	5	3.33	-
38	---	Soybean	Local	Local	5	6	-
39	FOC-39	Mung, udid	Local	Local	8.33	6	+
40	---	Cotton	Local	Local	6	5	-
41	FOC-41	Cotton	Local	Local	3.33	5	+
42	FOC-42	Soybean	Local	Local	6	6	+
43	---	Cotton	Sharda	Bhima	5	6	-
44	FOC-44	Cotton	Sharda	Bhima	6.66	6	+
45	---	Soybean	Local	Local	6	5	-
46	---	Cotton	Sharda	Sharda	4	6	-
47	FOC-47	Cotton	Local	Local	5	3.33	+
48	---	Cotton	Local	Local	4	4	-

+ - Presence of *Fusarium oxysporum* f.sp. *carthami*

- - Absence of *Fusarium oxysporum* f.sp. *carthami*

The incidence of wilt disease varied from 3.33 per cent to 12 per cent in eight districts of Marathwada region during *rabi* 2006-07. The maximum (12 %) incidence was recorded in Digras village of Taluqa Kalamnuri in Hingoli district followed by Kolsa village of Taluqa Sengaon in Hingoli district, Manaspuri village of Taluqa Kandhar in Nanded district and Handarguli Village of Taluqa Udgir in Latur district. The least (3.33 %) incidence was recorded from different districts (Table 5). Most of the farmers have grown the same variety of safflower during both years of survey. In most of the fields visited, the use of same variety has shown increase in wilt incidence. Most of the *Fusarium oxysporum* f.sp. *carthami* isolates obtained were from local safflower cultivar followed by Sharda and Bhima.

Diseased samples exhibited typical symptoms of safflower wilt like unilateral yellowing of plant, yellowing and complete wilting and brown discoloration of stem (Plate 1).

4.2. Isolation and identification of the *Fusarium oxysporum* f.sp. *carthami* isolates

Fusarium oxysporum f.sp. *carthami* could be easily isolated by tissue isolation method (root infected) (Plate 2 A, B) from diseased samples of safflower wilt. Nineteen isolates of *Fusarium oxysporum* f.sp. *carthami* were obtained and designated as FOC (Plate 2 C).

Growth of fungus was observed 3-4 days after incubation at $25 \pm 1^{\circ}\text{C}$ in all the FOC isolates. The maximum colony growth was obtained ten days after planting. Thereafter, the growth of culture ceased. The culture was raised fealty or fluffy in its growth. While, the



A. Partial wilting

B. Complete wilting



C. Yellowing and wilting

D. Brown discolouration of stem



Plate 1. Typical symptoms of safflower wilt

colony colour was dull white, Pinkish white and pink depending upon the isolates. On the basis of macroconidia, micro conidia their size, shape and septation, the isolates were identified as *Fusarium oxysporum* f.sp. *carthami* and the morphological characteristics feature mentioned in monograph by Booth (1971). The isolates were purified and mass multiplied for further studies.

4.3. Pathogenicity test

The pathogenicity test was proved by soil inoculation method and water culture technique on safflower variety Nira.

4.3.1. Soil inoculation method

Nineteen isolates were multiplied on PDB (Potato Dextrose Broth) for 15 days in 250 ml Ehrlenmeyer conical flask containing 100 ml PDB with sterile soil keeping one pot as control for each isolate. In pot sterile soil and PDB was added. To allow maximum multiplication of inoculum the pots were adequately watered with sterile water daily. On 4th day 4 healthy seeds of Nira variety were sown per pot. Another pot without addition of inoculum was kept as control.

The observations on germination and per cent wilting were recorded on 3rd and 5th day of sowing respectively (Plate 3A). The results of pathogenicity test are given in Table 6. The results indicated that all the isolates were pathogenic to safflower. FOC-14 was less virulent than other isolates.

Table 6. Pathogenicity of *Fusarium oxysporum* f.sp. *carthami* isolates in soil inoculation test.

S. No.	Isolate No.	No. of Seeds sown	No. of seeds germinated	Per cent germination	No. of seedling wilted	Per cent Wilting
1	FOC-5	4	2	50	2	100
2	FOC-10	4	2	50	2	100
3	FOC-12	4	2	50	2	100
4	FOC-14	4	3	75	2	66
5	FOC-16	4	2	50	2	100
6	FOC-18	4	2	50	2	100
7	FOC-21	4	2	50	2	100
8	FOC-24	4	2	50	2	100
9	FOC-27	4	2	50	2	100
10	FOC-29	4	2	50	2	100
11	FOC-30	4	2	50	2	100
12	FOC-32	4	2	50	2	100
13	FOC-35	4	2	50	2	100
14	FOC-36	4	2	50	2	100
15	FOC-39	4	2	50	2	100
16	FOC-41	4	2	50	2	100
17	FOC-42	4	2	50	2	100
18	FOC-44	4	2	50	2	100
19	FOC-47	4	2	50	2	100
20	Control	4	4	100	0	0

4.3.2. Pathogenicity by water culture technique

Water culture technique suggested by Nene *et al.* (1981) was used to confirm pathogenicity of FOC isolates. Cultural filtrate of the *Fusarium oxysporum* f.sp. *carthami* was obtained by growing *F.o.* f.sp. *carthami* for 15 days on PDB fifty per cent sterile water and fifty per cent cultural filtrate taken. Fifteen days old seedling of the variety Nira raised in pots were removed from the pots; roots were washed and dipped in tube containing 50 per cent diluted cultural filtrate. Observations on number of seedling wilted are given in Table 7 and Plate 3B.

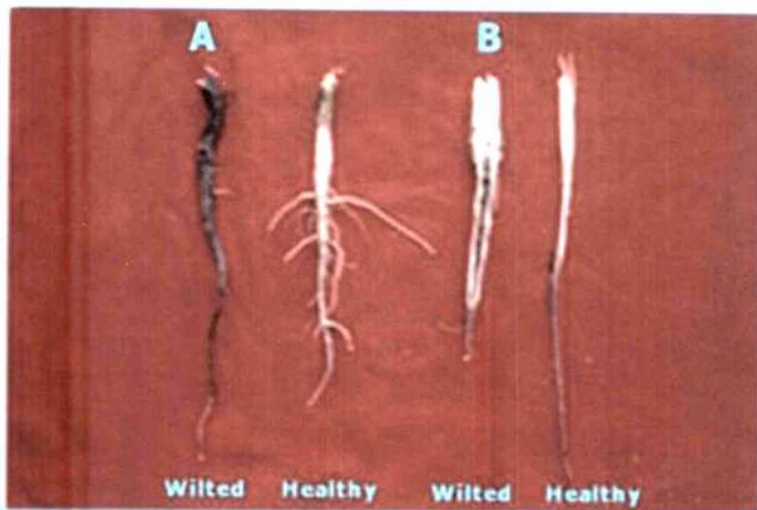
The results indicated that all *F.o.* f.sp. *carthami* (FOC) isolates were pathogenic resulting in 100 per cent wilting in 24 hr.

4.3.3. Reisolation

From artificially inoculated and wilted plants reisolations were undertaken. These reisolated cultures were morphologically studied and compared with original isolates and used for inoculation in pathogenicity tests. The comparison revealed that original isolates and reisolated cultures were identical thus proving Koch's postulate. The reisolated cultures were thereafter maintained on PDA slants.

4.3.4. Maintenance of *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates

The culture of each isolate was maintained on PDA medium by frequent sub-culturing and storing at 4°C in a refrigerator. The cultures were revived easily on PDA medium whenever required.



- A. Naturally wilt affected root
 B. Longitudinal section of root of wilted safflower plant showing brown discoloration



- C. *Fusarium oxysporum* f.sp. *carthami* isolates of Marathwada region

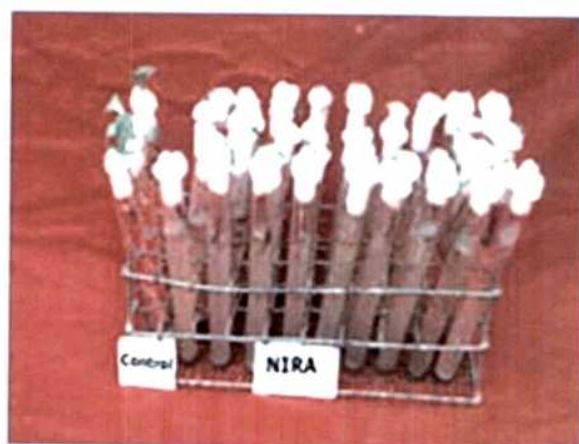


- D. Intravascular growth of *Fusarium oxysporum* f.sp. *carthami* in vascular bundles of safflower

Plate 2.

Table 7. Pathogenicity of *Fusarium oxysporum* f.sp. *carthami* isolates in water culture technique.

Sr. No.	Isolate No.	No. of Seeding	No. of seedling wilted
1	FOC-5	3	3
2	FOC-10	3	3
3	FOC-12	3	3
4	FOC-14	3	3
5	FOC-16	3	3
6	FOC-18	3	3
7	FOC-21	3	3
8	FOC-24	3	3
9	FOC-27	3	3
10	FOC-29	3	3
11	FOC-30	3	3
12	FOC-32	3	3
13	FOC-35	3	3
14	FOC-36	3	3
15	FOC-39	3	3
16	FOC-41	3	3
17	FOC-42	3	3
18	FOC-44	3	3
19	FOC-47	3	3
20	Control	19	0



A. Soil inoculation method B. Water culture method

Plate 3. Pathogenicity test on safflower variety Nira



Plate 4A. Seed mycoflora on safflower seeds by Blotter paper method

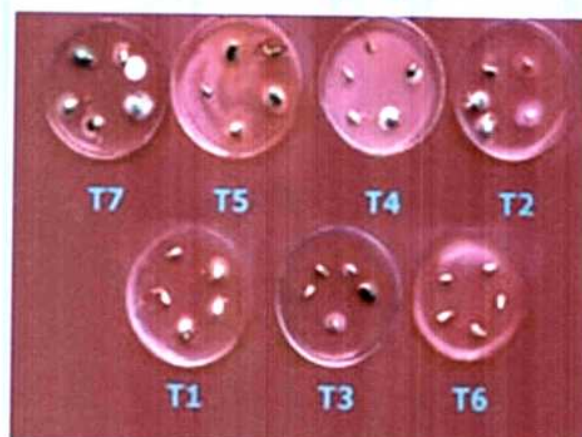


Plate 4B. Seed mycoflora on safflower cv. Nira seeds by Agar plate method with seed treatments

4.4. Detection of seed borne nature of *Fusarium oxysporum* f.sp. *carthami*

Detection of seed borne nature of *Fusarium oxysporum* f.sp. *carthami* was studied by using Blotter paper and Agar plate method.

4.4.1. Blotter paper method

For detection of seed borne nature of the *Fusarium oxysporum* f.sp. *carthami*, 400 seeds of five varieties of safflower were used for Blotter paper method. Four hundred seeds of each variety were placed in Petri dishes on Blotter paper. Observations on incidence of *Fusarium oxysporum* f.sp. *carthami*, *Rhizopus* spp and *Aspergillus* spp. were noted and the results were presented in Table 8. From Table 8 (Plate 4A), it can be revealed that significantly highest incidence of *Fusarium oxysporum* f.sp. *carthami* was observed in safflower variety Manjira and was at par with PBNS-12. Lowest incidence of *Fusarium oxysporum* f.sp. *carthami* was observed in the variety HUS-305 and it was at par with PBNS-40.

As regards incidence of *Rhizopus* spp. was observed, lowest incidence was observed in variety Manjira followed by PBNS-12 while HUS-305 was observed to have highest incidence.

Highest incidence of *Aspergillus* spp. was observed in variety HUS-305 and it was followed by PBNS-40 and Bhima. Lowest incidence of *Aspergillus* spp was observed in variety Manjira and it was at par with PBNS-12.

Table 8. Incidence of *Fusarium oxysporum f.sp. carthami*, *Rhizopus* sp. and *Aspergillus* sp. detected by Blotter paper method

Sr. No.	Variety	Per cent seed mycoflora		
		<i>Fusarium oxysporum f.sp. carthami</i>	<i>Rhizopus</i>	<i>Aspergillus</i>
1	Bhima	70 (56.99)	10 (18.34)	20 (26.52)
2.	PBNS-12	80 (63.54)	8.75 (17.20)	11.25 (19.58)
3.	Majira	85 (68.08)	5 (12.87)	10 (18.40)
4.	HUS-305	47.5 (43.55)	22.5 (28.29)	30 (33.17)
5.	PBNS-40	60 (50.78)	17.5 (24.71)	22.5 (28.29)
	S.E. \pm	3.17	0.80	1.05
	CD at 1 %	14.46	3.65	4.79
	CV (%)	9.72	6.88	7.26

Figures in parenthesis are angular transformed values

4.4.2. Effect of seed treatment on the incidence of seed mycoflora of safflower on Cv. Nira by Agar plate method.

Effect of seed treatment on seed mycoflora of safflower by Agar plate method on *Fusarium* selective medium (FSM) was studied. The seeds of safflower cultivar Nira were treated with fungicides Carbendazim (0.3 per cent), Thiram (0.3 per cent), Carbendazim (0.15 per cent) + Thiram (0.15 per cent), Captan (0.3 per cent), surface sterilization with 0.1 per cent mercuric chloride and seeds treated with hot water at 52°C. After seed treatment the seeds of Nira cultivar were placed in petri plates containing FSM. Observation on colony development of *Fusarium oxysporum* f.sp. *carthami*, *Rhizopus* spp. and *Aspergillus* spp. were noted.

The results from Table 9 and (Plate 4B) indicated that the incidence of *Fusarium oxysporum* f.sp. *carthami* was maximum in unsterilized seeds. It was followed by seeds sterilized with mercuric chloride and Captan. Lowest incidence of *Fusarium oxysporum* f.sp. *carthami* was observed in seeds sterilized with hot water treatment at 52°C. In case of *Rhizopus* spp. lowest incidence was observed in seed treated with Carbendazim followed by unsterilized seeds, while maximum incidence was observed in seed sterilized with mercuric chloride. In case of *Aspergillus* spp. lowest incidence was observed in seeds treated with carbendazim + Thiram followed by seeds treated in hot water at 52°C and Captan. Maximum incidence was observed in unsterilized seeds.

Table 9. Effect of seed treatment on seed mycoflora of safflower by Agar plate method

Sr. No.	Treatments	Per cent seed mycoflora		
		<i>Fusarium oxysporum</i> f.sp. <i>carthami</i>	<i>Rhizopus</i> spp.	<i>Aspergillus</i> spp.
1.	Carbendazim	53.33 (46.90)	6.00 (14.14)	20.66 (27.03)
2.	Thiram	60.66 (51.15)	11.33 (19.65)	27.66 (31.71)
3.	Carbendazim + Thiram	40.66 (39.61)	20.66 (27.03)	10.66 (19.04)
4.	Captan	70.00 (56.79)	18.00 (28.07)	12.00 (20.22)
5.	Mercuric chloride	76.33 (60.89)	28.33 (32.15)	38.66 (38.44)
6.	Hot water treatment at 52°C	10.33 (18.71)	21.33 (27.50)	11.66 (19.94)
7.	Unsterilized seeds	88.00 (69.76)	10.33 (18.71)	40.66 (39.61)
	S.E. ±	0.73	0.65	0.70
	CD at 1 %	3.12	2.78	3.00
	CV (%)	2.60	4.86	4.33

Figures in parenthesis are angular transformed values

4.5. Morphological variability of *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates

Investigations on morphological characteristics of 19 isolates of FOC grown on PDA medium were made by recording the length, breadth and septation of macroconidia, microconidia and presence or absence of chlamydospore. The isolates differed distinctly in respect of morphological characteristics.

4.5.1. Macroconidia

The perusal of the data presented in Table 10 indicated that there was significant difference in size of macroconidia of 19 isolates of *Fusarium oxysporum* f.sp. *carthami*. The mean length of macroconidia of the different isolates ranged from 19.33 to 28.60 μm and mean breadth ranged from 2.52 to 4.18 μm . Minimum length of macroconidia was observed in FOC-16. Isolate FOC-32 was recorded statistically significantly superior over rest of the isolates (Plate 5A).

The breadth of macroconidica of the isolate FOC-36 was least (2.52 μm). It was at par with FOC-5,12,14, 16, 21, 29, 35, 42 and 47, while it was maximum (4.18 μm) in isolate FOC-32. It was at par with FOC- 10, 18, 24, 27, 30, 39, 41 and 44. However, isolate FOC-32 was recorded significantly superior in respective of breadth over isolate FOC-12,16,36 and 42. Likewise FOC- was significant over isolate FOC-12, 36 and 42.

The macroconidia of all the isolates were falcate in shape (Table 11 and Plate 5B). The number of septa in macroconidia ranged from 3 to 4. FOC-10, 14 and 35 had 4 septa. Rest of the isolates had 3 septa. There was no distinct variation among the isolates with respect to

Table 10. Length (μm), breadth (μm) in macroconidia of 19 *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates

Isolate No.	Length (μm)*		Breadth (μm)*	
	Range	Mean	Range	Mean
FOC-5	22.0 -23.4	23.10 ^a	2.2 – 4.4	3.49 ^a
FOC-10	22.0 – 29.3	25.80 ^a	3.6 – 4.4	4.04 ^b
FOC-12	20.5 – 22.0	21.10 ^a	2.5 - 3.3	2.83 ^a
FOC-14	22.0 – 23.4	22.80 ^a	2.2 – 4.4	3.31 ^a
FOC-16	18.3 – 19.8	19.33 ^a	2.2 – 3.3	2.94 ^a
FOC-18	20.5 – 24.2	22.40 ^a	3.3 – 4.4	3.98 ^b
FOC-21	22.7 – 26.4	24.90 ^a	2.2 – 4.4	3.57 ^a
FOC-24	22.0 – 28.6	25.10 ^a	3.6 – 4.4	4.03 ^b
FOC-27	20.5 – 24.2	22.60 ^a	2.2 – 4.4	3.81 ^b
FOC-29	19.8 – 26.4	23.30 ^a	2.2 – 4.4	3.54 ^a
FOC-30	22.7 – 26.4	24.60 ^a	3.6 – 4.4	4.03 ^b
FOC-32	26.4 – 30.8	28.60 ^b	3.6 – 4.4	4.18 ^b
FOC-35	22.0 – 26.4	24.70 ^a	2.9 – 4.4	3.66 ^a
FOC-36	18.3 – 21.2	19.70 ^a	2.2 – 3.6	2.52 ^a
FOC-39	22.7 – 25.6	24.20 ^a	3.3 – 4.4	3.91 ^b
FOC-41	19.8 – 24.2	21.90 ^a	3.3 – 4.4	3.88 ^b
FOC-42	22.0 – 26.4	24.20 ^a	2.2 – 3.3	2.74 ^a
FOC-44	19.8 – 24.2	22.70 ^a	3.3 – 4.4	3.95 ^b
FOC-47	19.8 – 22.0	21.00 ^a	2.2 – 4.4	3.52 ^a
S.E. \pm		1.58		0.29
CD at 1 %		6.20		1.13
CV (%)		1.18		1.41

* - Mean of 75 macro conidia

Mean with same letter are statistically at par.

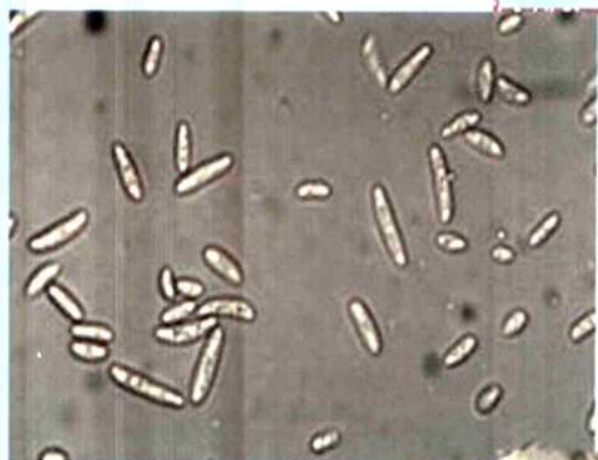
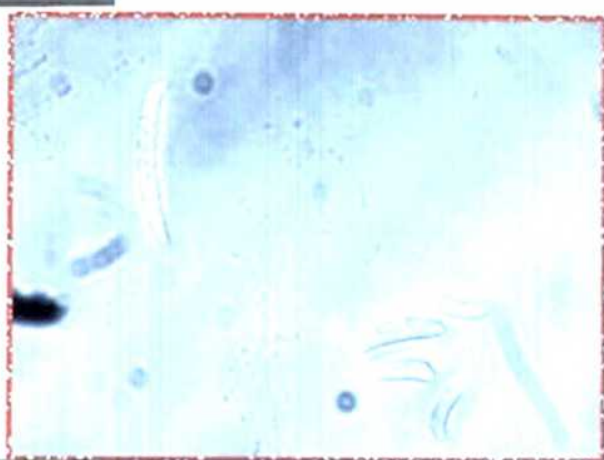
Table 11. Shape and septation of macroconidia in 19 *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates

Isolate No.	Shape of macroconidia	No. of septa per macroconidia
FOC-5	Falcate	3
FOC-10	Falcate	4
FOC-12	Falcate	3
FOC-14	Falcate	4
FOC-16	Falcate	3
FOC-18	Falcate	3
FOC-21	Falcate	3
FOC-24	Falcate	3
FOC-27	Falcate	3
FOC-29	Falcate	3
FOC-30	Falcate	3
FOC-32	Falcate	3
FOC-35	Falcate	4
FOC-36	Falcate	3
FOC-39	Falcate	3
FOC-41	Falcate	3
FOC-42	Falcate	3
FOC-44	Falcate	3
FOC-47	Falcate	3



5A. Microphotograph of isolates 32 showing maximum length macrocondia (450x)

5B. Microphotograph showing sickle (Falcate) shape macrocondia (450x)



5C. Microphotograph of isolates FOC 21 showing maximum length of microcondia (450x)

5D. Microphotograph of isolates FOC 35 showing minimum length of microcondia (450x)



Plate 5.

colour of the macroconidia, as the colour of macroconidia of all the isolates was hyaline.

4.5.2. Microconidia

Significant variations in the length of the microconidia among 19 isolates of FOC were recorded. The mean length of microconidia of different isolates varied from 4.51 to 9.79 μm and breadth from 1.65 to 3.79 μm (Table 12). Maximum length of microconidia (9.79 μm) was observed in the isolate FOC-21 (Plate 5C). It was at par with the isolates FOC-18 and 41. Minimum length (4.51 μm) was recorded in the isolates FOC 35 (Plate 5D) which was statistically at par with isolates FOC-5, 14, 27, 35, 42 and 44.

The mean maximum breadth of microconidia (3.82 μm) was recorded in the isolate FOC-21. It was at par with the isolate FOC-10 and 41. Mean minimum length (1.65 μm) was observed in the isolate FOC-35 which was statistically at par with FOC-5, 12, 14, 16, 24, 36, 39, 42 and 47.

The microconidia were found to be oval in shape and having 1 to 2 septa. Two septa were found only in isolate FOC-14 and 39 (Table 13). There was no distinct variation among the isolates with respect to colour of the microconidia, as the colour of microconidia of the isolates was hyaline.

4.5.3. Chlamyospore

All the isolates of *Fusarium oxysporum* f.sp. *carthami* produced chlamyospore except one isolate FOC-41. In isolate FOC-35, terminal chlamyospore was observed (Plate 6B) whereas

Table 12. Length (μm) and breadth (μm) in microconidia of 19 *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates

Isolate No.	Length (μm)*		Breadth (μm)*	
	Range	Mean	Range	Mean
FOC-5	4.4 – 5.5	4.71 ^a	1.4 – 2.2	1.89 ^a
FOC-10	5.8 – 7.3	6.83 ^b	3.3 – 4.4	3.79 ^c
FOC-12	5.1 – 8.8	6.74 ^b	1.4 – 2.9	1.90 ^a
FOC-14	4.4 – 7.3	5.60 ^a	1.1 – 2.2	1.76 ^a
FOC-16	5.8 – 8.8	7.15 ^b	1.4 – 2.9	2.06 ^a
FOC-18	6.6 – 11.0	8.88 ^c	2.2 – 4.4	3.43 ^c
FOC-21	6.6 – 11.7	9.79 ^c	3.3 – 4.4	3.82 ^c
FOC-24	2.9 – 8.8	6.68 ^b	1.1 – 2.2	1.76 ^a
FOC-27	4.7 – 6.6	5.80 ^a	2.2 – 2.9	2.75 ^b
FOC-29	4.4 – 8.8	6.68 ^b	2.2 – 4.0	3.06 ^b
FOC-30	4.4 – 8.8	7.59 ^b	1.4 – 3.3	2.56 ^b
FOC-32	5.1 – 8.0	6.71 ^b	2.2 – 3.3	2.68 ^b
FOC-35	2.2 – 7.3	4.51 ^a	1.4 – 2.2	1.65 ^a
FOC-36	4.4 – 8.8	6.71 ^b	1.1 – 3.6	2.28 ^a
FOC-39	4.4 – 7.3	6.14 ^b	1.1 – 3.3	2.18 ^a
FOC-41	6.6 – 11.0	8.80 ^c	2.2 – 4.4	3.43 ^c
FOC-42	4.4 – 6.6	5.74 ^a	1.4 – 2.2	1.86 ^a
FOC-44	2.2 – 8.8	4.92 ^a	2.5 – 3.6	2.87 ^b
FOC-47	2.2 – 7.3	6.10 ^b	1.4 – 2.2	1.89 ^a
S.E. \pm		0.38		0.17
CD at 1 %		1.49		0.66
CV (%)		9.94		1.96

* - Mean of 75 micro conidia

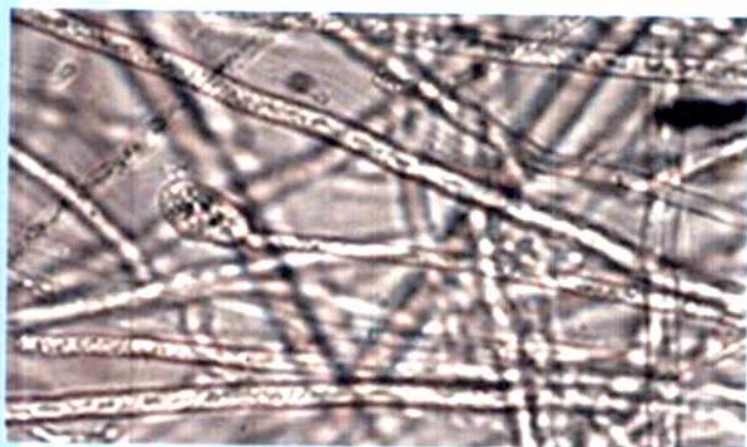
Mean with same letter are statistically at par

Table 13. Morphological characteristics of micro conidia of 19 isolates of *Fusarium oxysporum* f.sp. *carthami*

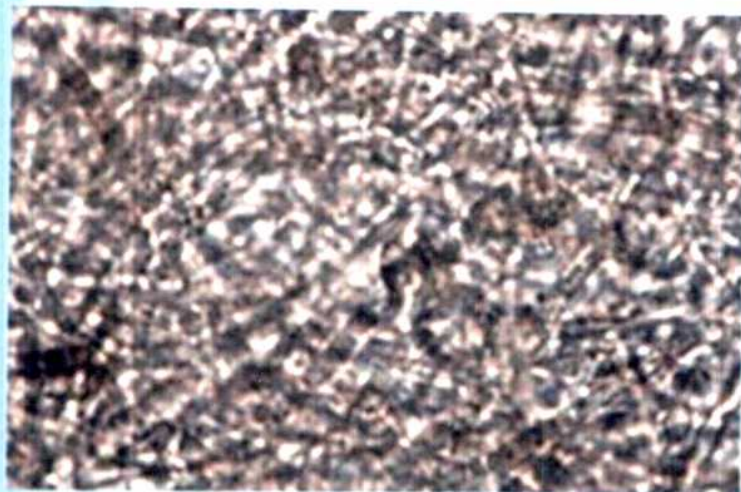
Isolate No.	Shape of microconidia	No. of septa per microconidia
FOC-5	Oval	1
FOC-10	Oval	1
FOC-12	Oval	1
FOC-14	Oval	2
FOC-16	Oval	1
FOC-18	Oval	1
FOC-21	Oval	1
FOC-24	Oval	1
FOC-27	Oval	1
FOC-29	Oval	1
FOC-30	Oval	1
FOC-32	Oval	1
FOC-35	Oval	1
FOC-36	Oval	1
FOC-39	Oval	2
FOC-41	Oval	1
FOC-42	Oval	1
FOC-44	Oval	1
FOC-47	Oval	1



6A. Microphotograph showing polymorphism in *Fusarium oxysporum* f.sp. *carthami* (450x)



6B. Microphotograph of isolate FOC-35 showing Terminal chlamydospore (450x)



6C. Microphotograph showing Intercalary Chlamydospore (450x)

intercalary chlamydospore (Plate 6C) were observed in rest of the isolates.

4.6. Cultural variability *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates

4.6.1. Colony characteristics on PDA and PDB medium

Studies on cultural characteristics of 19 isolates of FOC of the pathogen grown on PDA medium were made by recording colony diameter, colour of the colony, growth of colony or topography, shape of colony, sectoring, sporulation and mycelial dry weight.

4.6.1.1. Colony diameter topography and colour

Significant variation was observed with respect to colony diameter of the isolates 10 days after incubation on PDA medium. It is evident from the data presented (Table 14 and Fig. 2) that diameter of colony of the isolates ranged between 42.66 to 85.00 mm. The isolate FOC-35 showed maximum growth (85.00 mm) followed by isolates FOC-29 (82.66 mm) and FOC-44 (78.00 mm). The isolate FOC-5,0,12,14,16,18,21,24,27,29,30,32,35,36,41,42,44 and 47 were recorded significant over only one isolate FOC-39.

Variation was observed with respect to colony growth or topography and colour of colony among the *Fusarium oxysporum* f.sp. *carthami* isolates studied (Table 14) and (Plate 7A). Most of the isolates produced Felty growth on PDA medium while, the isolates FOC-21, 32, 39 and 47 produced Fluffy raised growth. Colours of the cultures were dull whitish to whitish and pinkish white to pink. Isolates FOC-36, 41 and 42 were pinkish white in colour while isolate FOC-35 was pink in colour (Plate 6).

Table 14. Colony characteristics of 19 *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates on PDA medium at 25 ± 1°C.

Sr. No.	Isolate No.	Colony diameters (mm)*	Colony growth/ Topography	Colony colour
1	FOC-5	64.00 ^b	Felty	Dull white
2	FOC-10	70.33 ^c	Felty	White
3	FOC-12	60.66 ^b	Felty	Dull white
4	FOC-14	68.00 ^c	Felty	Dull white
5	FOC-16	52.66 ^a	Felty	Dull white
6	FOC-18	73.00	Felty	White
7	FOC-21	51.66 ^a	Fluffy, raised	Dull white
8	FOC-24	67.00 ^c	Felty	Dull white
9	FOC-27	61.66 ^b	Felty	Dull white
10	FOC-29	82.66 ^d	Felty	Dull white
11	FOC-30	66.00 ^c	Felty	White
12	FOC-32	59.00 ^b	Fluffy, raised	Dull white
13	FOC-35	85.00 ^d	Felty	Pink
14	FOC-36	54.33 ^a	Felty	Pinkish white
15	FOC-39	42.66	Fluffy, raised	White
16	FOC-41	66.66 ^c	Felty	Pinkish white
17	FOC-42	58.66 ^b	Felty	Pinkish white
18	FOC-44	78.00	Felty	Dull white
19	FOC-47	67.66 ^c	Fluffy, raised	Dull white
	S.E.±	0.65		
	CD at 1 %	2.55		
	C.V. (%)	1.76		

* - Means of three replications

Means with same letter are statistically at par

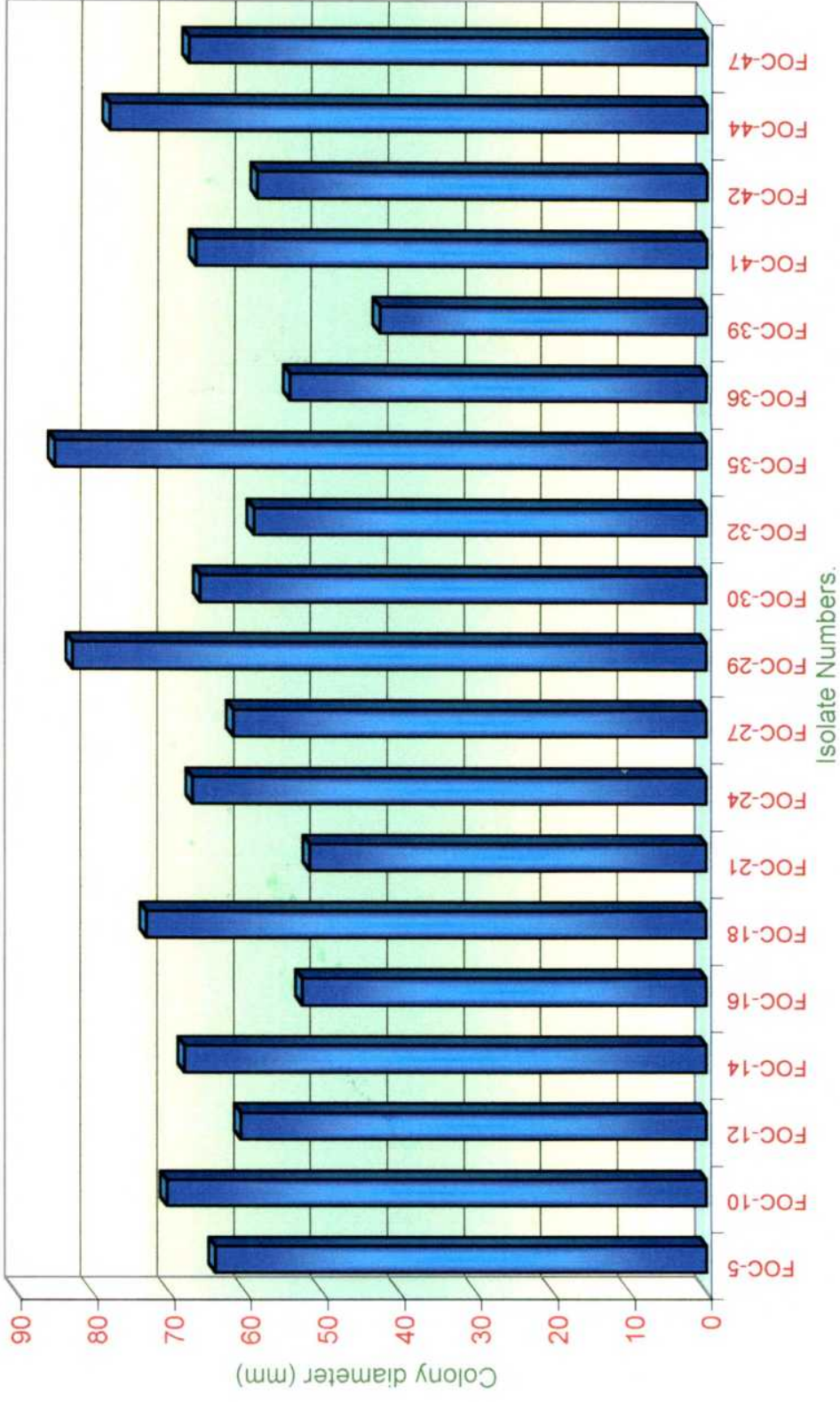


Fig.2. Colony diameter of 19 *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates on PDA medium after 10 days incubation at $25 \pm 1^{\circ}\text{C}$.



Plate 7A. Cultural characteristics of FOC 19 isolates of *Fusarium oxysporum* f.sp. *carthami*

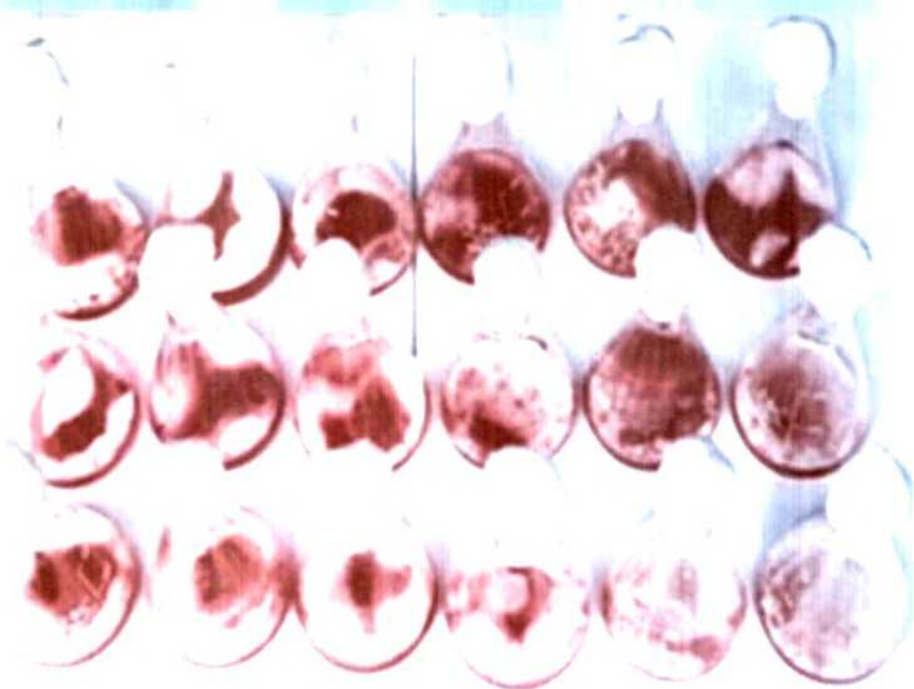


Plate 7B. Mycelial growth of *Fusarium oxysporum* f.sp. *carthami* isolates potato dextrose broth (PDB)

4.6.1.2. Shape and Sectoring

The shape of colony among the 19 isolates of FOC was found varying. Based on the shape of colony the isolates could be divided into two groups (Table 15) and (Plate 7A).

Group I - FOC-5, 10, 12, 16, 18, 21, 24, 27,
29, 30 32, 36, 44, 47 (circular shape)

Group II - FOC-14, 35, 39, 41 and 42 (Irregular shape)

As regards sectoring in the cultures, of the 19 isolates of FOC studied, from Latur district (FOC-35, 36) and from Jalna district (FOC-41, 42) had showed sectoring in the culture within 7-8 days after incubation at $25 \pm 1^{\circ}\text{C}$ on PDA medium (Table 15 and Plate 7A).

4.6.1.3 Mycelial dry weight and sporulation

A large variation was observed in the mycelial dry weight among the isolates, with minimum dry weight of the mycelium recorded with the isolate FOC-42 (166.67 mg). It was at par with isolates FOC^{5,} 14, 18²¹, 24²⁹, 32³⁵, 36, 39, 41, ~~42~~⁴⁴ and 47. Maximum dry mycelial weight was observed in the isolate FOC-27 (583.33 mg). It was at par with FOC-10¹² and 16²⁰ isolates from Hingoli district (FOC-10 and 12), Pabhani district (FOC-16) and Nanded district (FOC-27 and 30) put forth maximum mycelial dry weight than the isolates from other districts (Table 16, Fig.3 and Plate 7B).

It is evident from the data presented (Table 16) that excellent macrocondial sporulation ($> 70 \times 10^4$ macroconidia ml^{-1}) was observed in cultures of FOC-12, after 15 days of incubation on PDA medium. Good sporulation ($40-70 \times 10^4$ macrocondia ml^{-1}) was observed in isolates FOC-14, 16, 21, 35 and 30. Whereas, moderate

Table 15. Cultural characteristics of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* on PDA medium at 25 ± 1°C.

Sr. No.	Isolate No.	Shape of colony	Sectoring
1	FOC-5	Circular	Absent
2	FOC-10	Circular	Absent
3	FOC-12	Circular	Absent
4	FOC-14	Irregular	Absent
5	FOC-16	Circular	Absent
6	FOC-18	Circular	Absent
7	FOC-21	Circular	Absent
8	FOC-24	Circular	Absent
9	FOC-27	Circular	Absent
10	FOC-29	Circular	Absent
11	FOC-30	Circular	Absent
12	FOC-32	Circular	Absent
13	FOC-35	Irregular	Present
14	FOC-36	Circular	Present
15	FOC-39	Irregular	Absent
16	FOC-41	Irregular	Present
17	FOC-42	Irregular	Present
18	FOC-44	Circular	Absent
19	FOC-47	Circular	Absent

Table 16. Mycelial dry weight (mg) and sporulation in 19 isolates of *Fusarium oxysporum* f.sp. *carthami* in potato dextrose broth.

Sr. No.	Isolate No.	Mycelial dry weight (mg)*	Sporulation	
			Macroconidia	Microconidia
1	FOC-5	423.33 ^a	++	++
2	FOC-10	573.33 ^b	++	++
3	FOC-12	523.33 ^b	++++	++++
4	FOC-14	343.33 ^a	+++	++++
5	FOC-16	563.33 ^b	+++	+++
6	FOC-18	336.67 ^a	++	++++
7	FOC-21	400.00 ^a	+++	+++
8	FOC-24	183.33 ^a	++	+++
9	FOC-27	583.33 ^b	++	+++
10	FOC-29	353.33 ^a	++	++
11	FOC-30	496.67 ^b	++	+++
12	FOC-32	210.00 ^a	+	++
13	FOC-35	353.33 ^a	+++	+++
14	FOC-36	196.67 ^a	++	++++
15	FOC-39	340.00 ^a	+++	++++
16	FOC-41	305.33 ^a	++	++
17	FOC-42	166.67 ^a	+	+
18	FOC-44	410.00 ^a	++	+++
19	FOC-47	190.00 ^a	+	+
	S.E. \pm	66.00		
	CD at 1 %	259.1		
	CV (%)	3.12		

* - Mean of three replications

Mean with same letter are statistically at par

Macroconidia

- ++++ -Excellent ($>100 \times 10^4 \text{ ml}^{-1}$)
- +++ -Good ($40-70 \times 10^4 \text{ ml}^{-1}$)
- ++ -Moderate ($20-40 \times 10^4 \text{ ml}^{-1}$)
- + -Poor ($< 30 \times 10^4 \text{ ml}^{-1}$)

Microconidia

- ++++ -Excellent ($>70 \times 10^4 \text{ ml}^{-1}$)
- +++ -Good ($80-100 \times 10^4 \text{ ml}^{-1}$)
- ++ -Moderate ($35-80 \times 10^4 \text{ ml}^{-1}$)
- + -Poor ($< 20 \times 10^4 \text{ ml}^{-1}$)

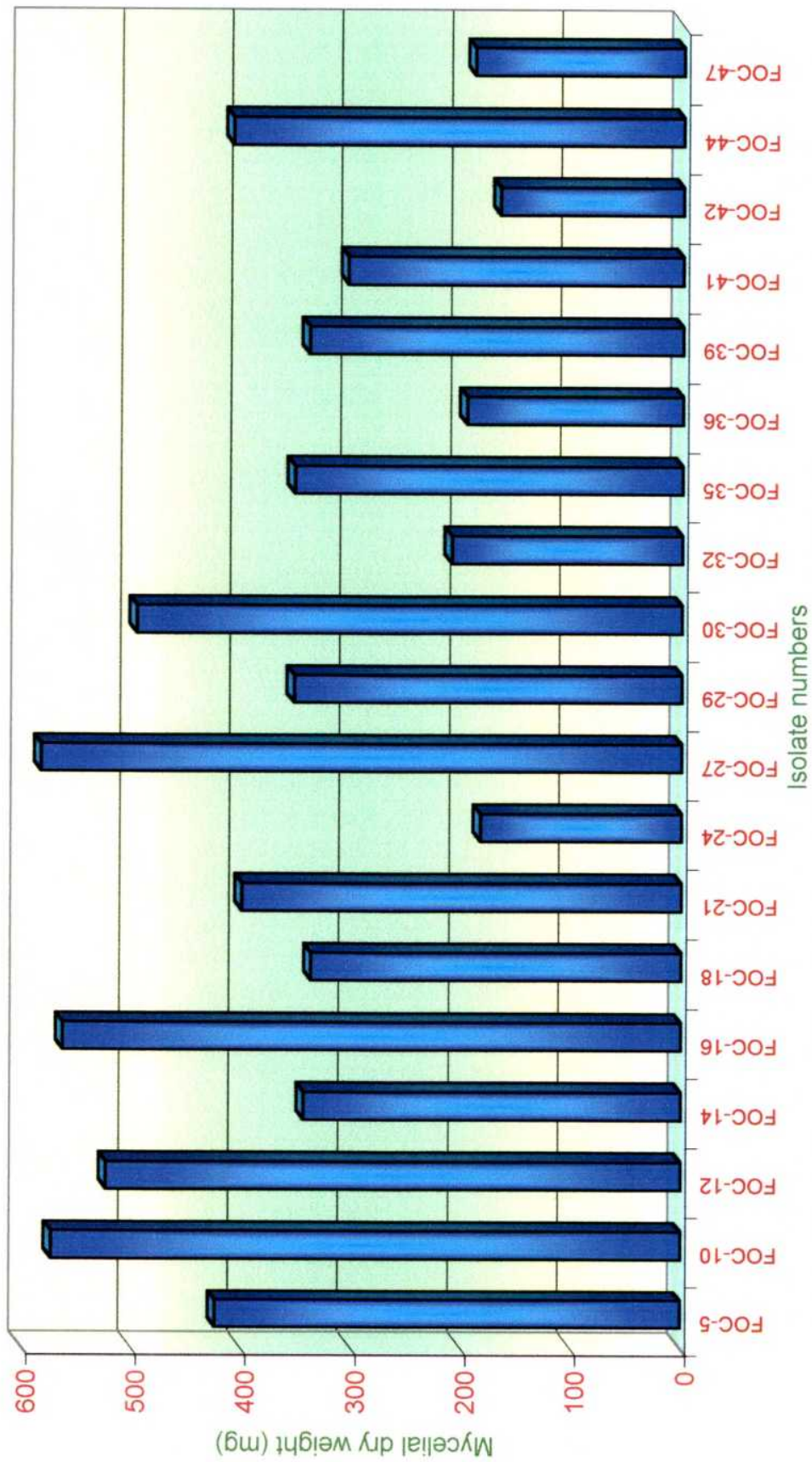


Fig.3. Mycelial dry weight (mg) and sporulation in 19 isolates of *Fusarium oxysporum* f.sp. *carthami* in potato dextrose broth after 10 days incubation at $25 \pm 1^\circ\text{C}$.

sporulation ($20-40 \times 10^4$ macroconidia ml^{-1}) was observed in isolates FOC-5, 10, 18, 24, 27, 29, 30, 36, 41 and 44 and poor ($< 20 \times 10^4$ macroconidia ml^{-1}) in the FOC-32, 42 and 47.

Excellent microconidial sporulation ($>100 \times 10^4$ microconidia ml^{-1}) was observed in culture of FOC-12, 14, 18, 36 and 39 after 15 days of incubation on PDA medium. Good sporulation ($80-100 \times 10^4$ microconidia ml^{-1}) was observed in isolates FOC-16, 21, 24, 27, 30, 35 and 44. Whereas, moderate sporulation $6.35 - 80 \times 10^4$ microconidia ml^{-1}) was observed in isolates FOC-42 and 47. Sporulation was observed as minute droplets scattered all over the colony.

4.7. Pathogenic variability

Pathogenic variability of the 19 isolates of *Fusarium oxysporum* f.sp. *carthami* was tested using three moderately resistant safflower cultivars (A-1, PBNS-40 and Bhima) and two susceptible cultivars (Nira and Manjira) (Plate 8A).

4.7.1. Virulence of *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates on safflower cultivar A-1

Significant differences in virulence among the 19 isolates of FOC were observed on safflower cultivar A-1 (Table 17 and Fig.4). In all the isolates no wilting of the safflower plant was observed up to 2 days but wilting was observed after 5 days. The highest wilting percentage (90 %) was observed in the isolates FOC-12, 24 and 36. That is highly susceptible reaction to these isolates. It indicated that isolate FOC-12, 24 and 36 were highly virulent while FOC-18 and 30 were less virulent.

Table 17. Virulence of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* (FOC) on safflower Cv. A-1.

Sr. No.	Isolate No.	No. of plants (out of 10) wilted after 5 days)	Wilting (%)	Disease score
1	FOC-5	7	70 (56.99)	9
2	FOC-10	7	70 (52.76)	9
3	FOC-12	9	90 (71.94)	9
4	FOC-14	4	40 (39.20)	7
5	FOC-16	5	50 (44.99)	7
6	FOC-18	1	10 (18.41)	3
7	FOC-21	7	70 (56.85)	9
8	FOC-24	9	90 (72.14)	9
9	FOC-27	4	40 (39.17)	7
10	FOC-29	2	20 (26.54)	5
11	FOC-30	1	10 (18.41)	3
12	FOC-32	7	70 (56.99)	9
13	FOC-35	2	20 (26.48)	5
14	FOC-36	9	90 (71.94)	9
15	FOC-39	7	70 (56.99)	9
16	FOC-41	4	40 (39.20)	7
17	FOC-42	5	50 (44.99)	7
18	FOC-44	4	50 (39.20)	7
19	FOC-47	5	50 (44.99)	7
	S.E. +	---	2.37	---
	CD at 1 %	---	9.30	---
	CV (%)	---	8.88	---

- Based on ten seedlings for each isolate
- The wilting percentage in sterilized water (control) was nil.
- Figures in parenthesis are angular transformed and statistics was applied to them

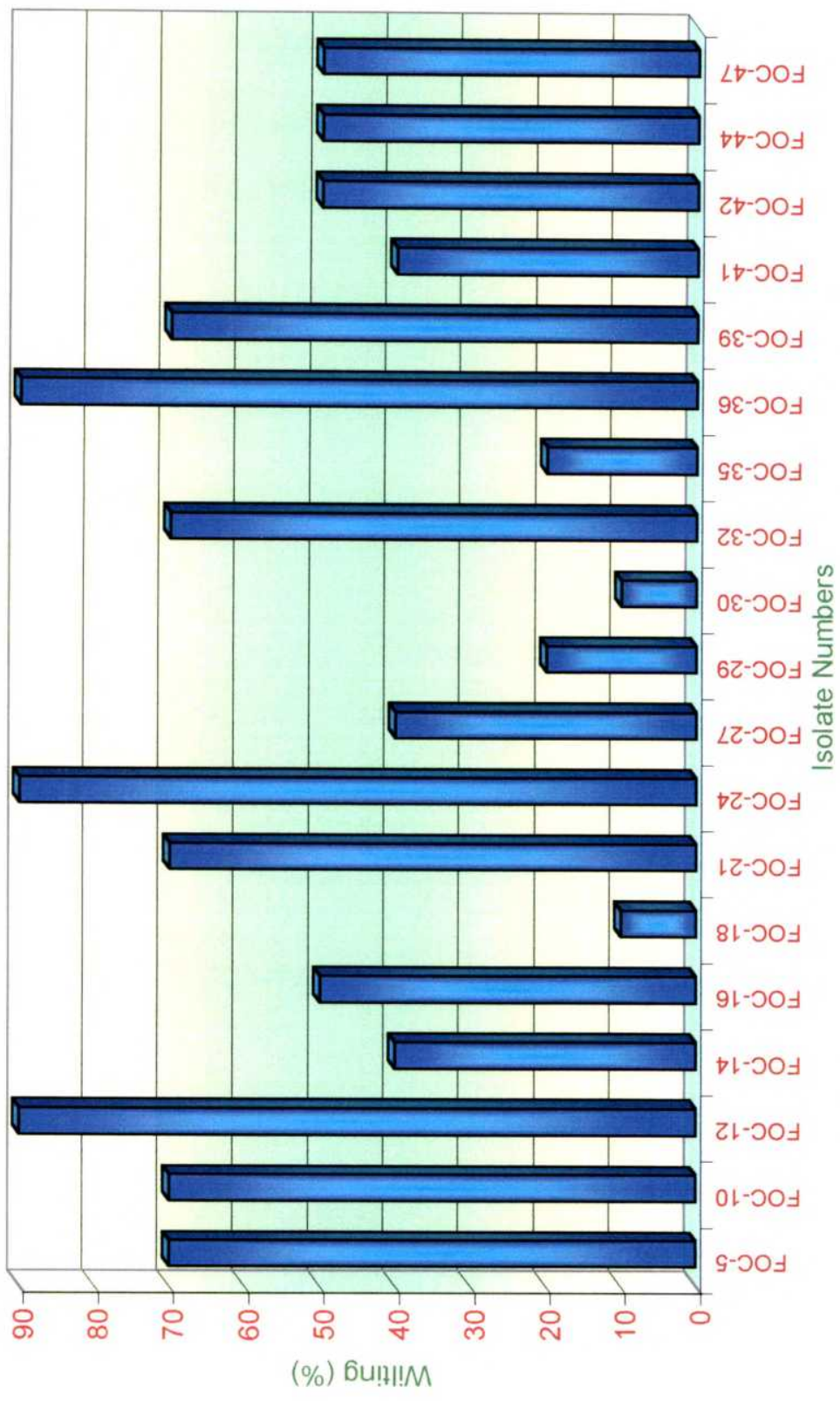


Fig.4. Virulence of the 19 isolates of *Fusarium oxysporum* f.sp. *carthami* on safflower Cv. A-1.

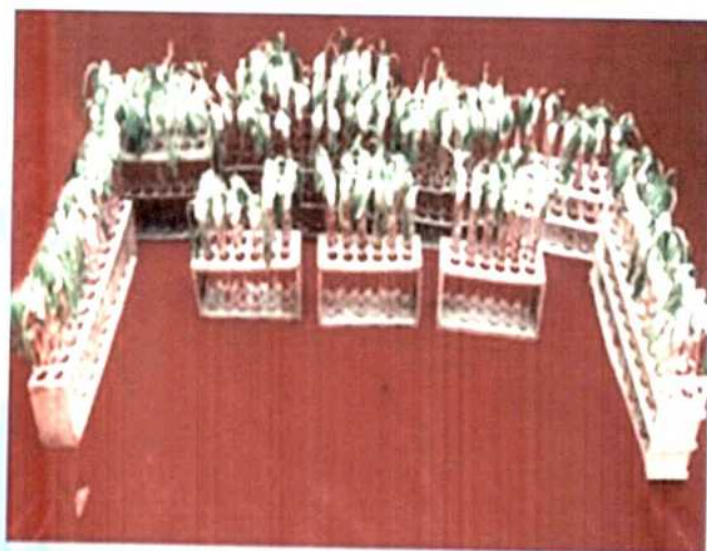


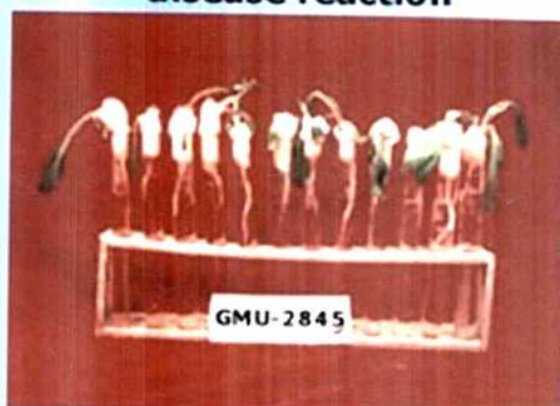
Plate 8A. Pathogenic variability of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* by water culture technique on



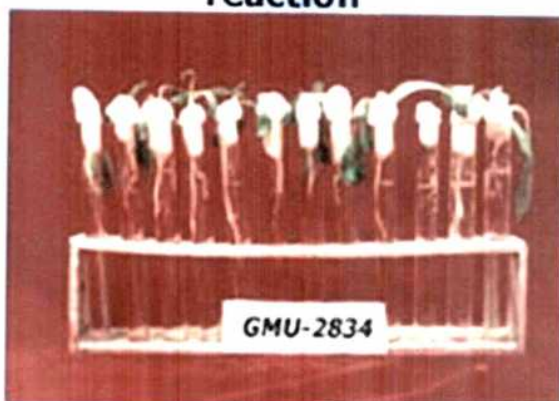
a. GMU 2873 showing moderately resistant disease reaction



b. GMU 2881 showing tolerant disease reaction



c. GMU 2845 showing highly susceptible disease reaction



d. GMU 2834 showing susceptible disease reaction

Plate 8B. Screening of germplasm lines by water culture technique at 2.5 % concentration

Wilting percentage (50 to 70 %) was recorded in FOC-5, 10, 16, 21, 32, 39, 42 and 47 disease reaction showed was highly susceptible.

Isolate FOC-14, 16, 27, 41,42, 44 and 47 showed 40-50 per cent, wilt. Isolate FOC-29 and FOC-35 (wilting 20 %) showed tolerant to wilt of safflower. FOC-18 and 30 isolates were recorded only 10 per cent wilting that is moderately resistant reaction against these two isolates.

4.7.2. Virulence of *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates on safflower cultivar PBNS-40

In respect of the isolates of *Fusarium oxysporum* f.sp. *carthami*, wilting percentage was recorded after 5th day (Table 18 and Fig.5).

Among different isolates maximum wilting percentage (90 %) was recorded in the isolates viz. FOC-5, 14, 21, 41 and 47 indicating that they were most virulent. Isolates viz. FOC-10, 12, 16, 27, 39 and 42 caused 70 per cent wilting. Per cent wilting of 50 per cent and above showed highly susceptible reaction.

The wilting percentage 21-50 per cent was found in the isolates FOC-24, 29, 32, 36 and 44. Whereas 20 per cent wilting was observed in the isolates FOC-18, 30 and 35 which showed tolerant reaction against wilt.

Table 18. Virulence of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* (FOC) on safflower Cv. PBNS-40

Sr. No.	Isolate No.	No. of plants (out of 10) wilted after 5 days)	Wilting (%)	Disease score
1	FOC-5	9	90 (70.68)	9
2	FOC-10	7	70 (56.99)	9
3	FOC-12	7	70 (56.99)	9
4	FOC-14	9	90 (71.94)	9
5	FOC-16	7	70 (56.83)	9
6	FOC-18	2	20 (26.54)	5
7	FOC-21	9	90 (71.94)	9
8	FOC-24	4	40 (39.20)	7
9	FOC-27	7	70 (56.82)	9
10	FOC-29	4	40 (39.22)	7
11	FOC-30	2	20 (26.52)	5
12	FOC-32	4	40 (39.21)	7
13	FOC-35	2	20 (26.54)	5
14	FOC-36	5	50 (44.99)	7
15	FOC-39	7	70 (56.85)	9
16	FOC-41	9	90 (71.94)	9
17	FOC-42	7	70 (56.83)	9
18	FOC-44	5	50 (44.99)	7
19	FOC-47	9	90 (72.40)	9
	S.E. +	---	2.33	---
	CD at 1 %	---	9.14	---
	CV (%)	---	7.78	---

- Based on ten seedlings for each isolate
- The wilting percentage in sterilized water (control) was nil.
- Figures in parenthesis are angular transformed and statistics was applied to them

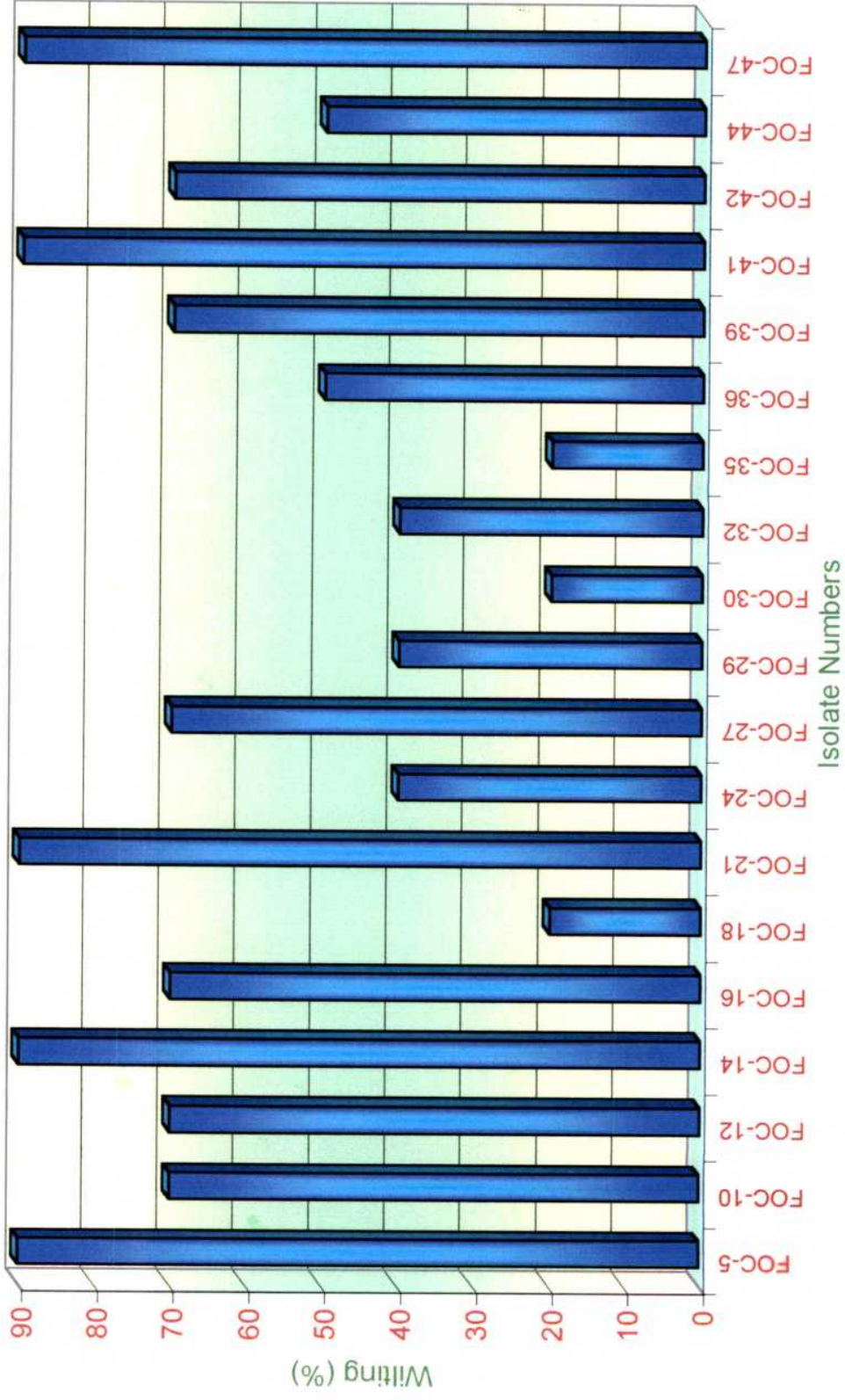


Fig.5. Virulence of the 19 isolates of *Fusarium oxysporum* f.sp. *carthami* on safflower Cv. PBNS-40

4.7.3. Virulence of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* (FOC) on safflower Cv. Bhima

Significant differences in virulence among the 19 isolates of *Fusarium oxysporum* f.sp. *carthami* were observed (Table 19 and Fig.6).

Minimum wilting per cent (10 %) was observed in the isolate FOC-30, which showed moderately resistant disease reaction. Maximum wilting per cent (90 %) was observed in the isolates FOC-16, 24, 27, 39, 41, 42 and 47 indicating that these isolates are most virulent. Isolates FOC-12, 14, 21, 32, 36 and 44 were exhibited 70 per cent wilting.

Up to 20 per cent wilting was observed in the isolates FOC-10, 18, 29 and 35 which showed tolerant reaction.

4.7.4. Virulence of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* (FOC) on safflower cultivar Nira

Of the nineteen FOC isolates tested for their virulence on safflower cv. Nira the isolates FOC-10, 14, 21, 24, 32, 36, 42 and 47 caused 50 per cent wilting after 5 days while the remaining isolates FOC-16, 29, 30 and 44 caused 90 per cent wilting after 5 days and were considered most virulent isolates (Table 20 and Fig.7).

Based on disease reacting of safflower Cv. Nira showed susceptible to highly susceptible reaction.

Table 19. Virulence of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* (FOC) on safflower Cv. Bhima

Sr. No.	Isolate No.	No. of plants (out of 10) wilted after 5 days)	Wilting (%)	Disease score
1	FOC-5	4	40 (39.14)	7
2	FOC-10	2	20 (26.44)	5
3	FOC-12	7	70 (56.99)	9
4	FOC-14	7	70 (56.83)	9
5	FOC-16	9	90 (71.94)	9
6	FOC-18	2	20 (26.54)	5
7	FOC-21	7	70 (56.83)	9
8	FOC-24	9	90 (71.94)	9
9	FOC-27	9	90 (71.94)	9
10	FOC-29	2	20 (26.52)	5
11	FOC-30	1	10 (18.43)	3
12	FOC-32	7	70 (56.99)	9
13	FOC-35	2	20 (26.55)	5
14	FOC-36	7	70 (56.99)	9
15	FOC-39	9	90 (71.94)	9
16	FOC-41	9	90 (71.94)	7
17	FOC-42	9	90 (71.94)	9
18	FOC-44	7	70 (56.99)	9
19	FOC-47	9	90 (71.94)	9
	S.E. ±	---	2.72	---
	CD at 1 %	---	10.68	---
	CV (%)	---	9.00	---

- Based on ten seedlings for each isolate
- The wilting percentage in sterilized water (control) was nil.
- Figures in parenthesis are angular transformed and statistics was applied to them

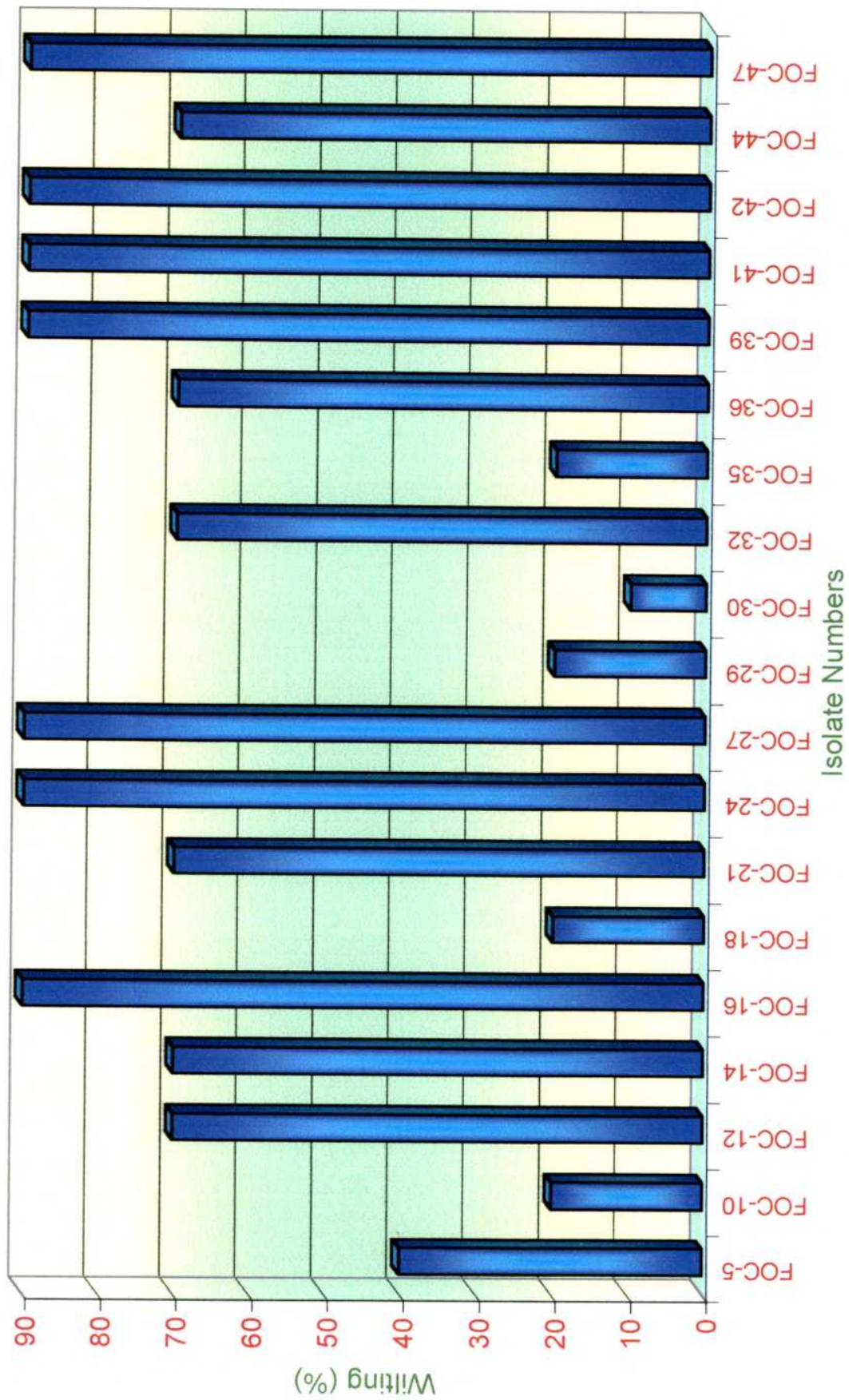


Fig.6. Virulence of the 19 isolates of *Fusarium oxysporum* f.sp. *carthami* on safflower Cv. Bhima

Table 20. Virulence of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* (FOC) on safflower Cv. Nira

Sr. No.	Isolate No.	No. of plants (out of 10) wilted after 5 days)	Wilting (%)	Disease score
1	FOC-5	7	70 (56.79)	9
2	FOC-10	5	50 (44.99)	9
3	FOC-12	9	90 (71.94)	9
4	FOC-14	5	50 (44.99)	7
5	FOC-16	9	90 (72.14)	9
6	FOC-18	7	70 (56.91)	9
7	FOC-21	5	50 (44.99)	7
8	FOC-24	5	50 (44.99)	7
9	FOC-27	7	70 (56.80)	9
10	FOC-29	9	90 (71.79)	9
11	FOC-30	9	90 (71.94)	9
12	FOC-32	5	50 (44.99)	7
13	FOC-35	7	70 (56.81)	9
14	FOC-36	5	50 (44.99)	7
15	FOC-39	7	70 (56.80)	9
16	FOC-41	7	70 (50.77)	9
17	FOC-42	5	50 (49.99)	7
18	FOC-44	9	90 (71.94)	9
19	FOC-47	5	50 (44.99)	7
	S.E. ±	---	2.01	---
	CD at 1 %	---	7.89	---
	CV (%)	---	6.27	---

- Based on ten seedlings for each isolate
- The wilting percentage in sterilized water (control) was nil.
- Figures in parenthesis are angular transformed and statistics was applied to them

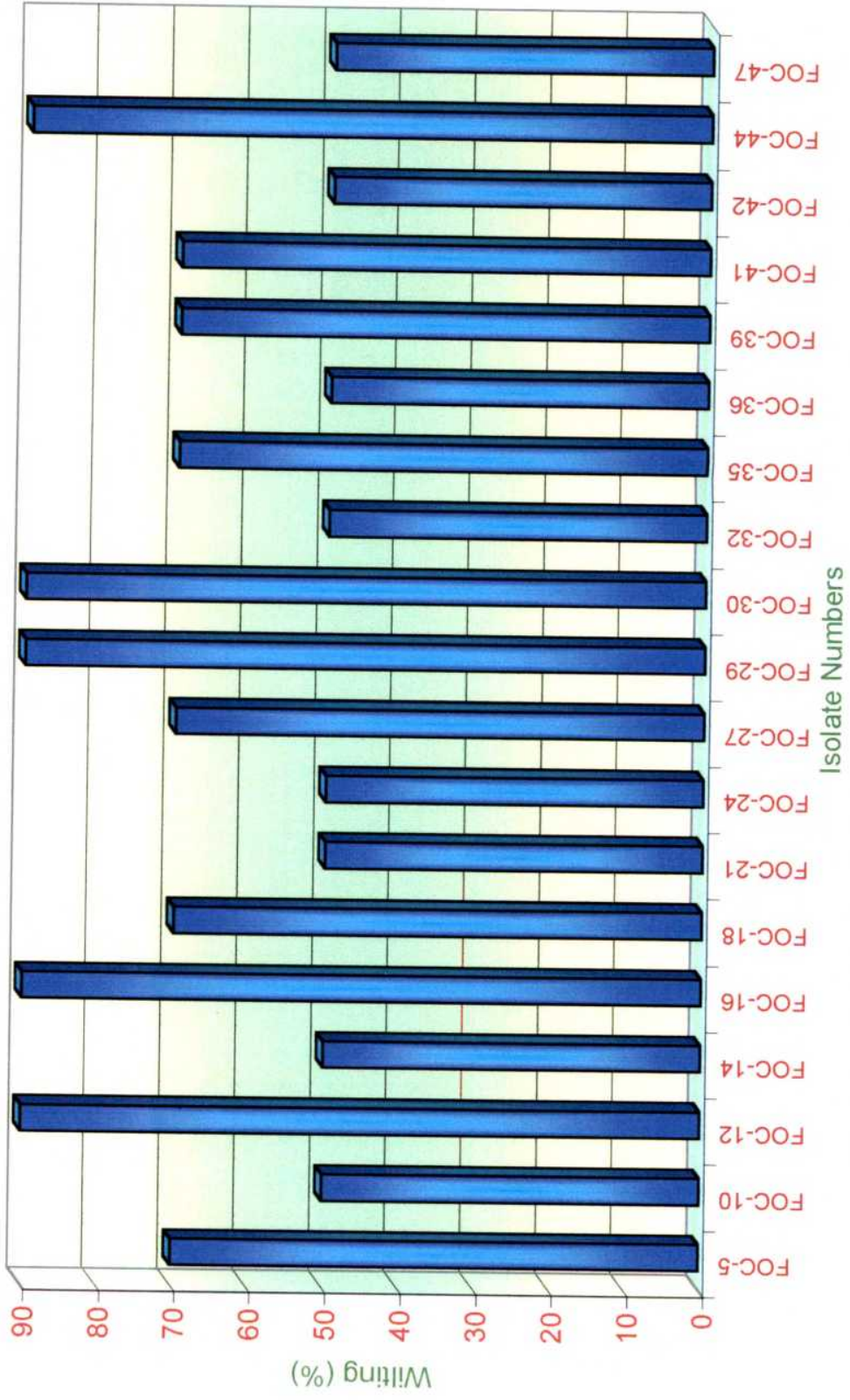


Fig.7. Virulence of the 19 isolates of *Fusarium oxysporum* f.sp. *carthami* on safflower Cv. Nira

4.7.5. Virulence of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* (FOC) on safflower cultivar Manjira

Significant differences in virulence among the 19 isolates of the pathogen were observed on safflower Cv. Manjira (Table 21 and Fig.8). FOC-12, 14, 21, 39, 42 and 47 showed 90 per cent wilting and were considered most virulent isolates. Isolate FOC-30 was showed 50 per cent wilting per cent, disease reaction showed susceptible reaction. And remaining isolates were showed 70 per cent wilting per cent.

Based on disease reaction of safflower Cv. Manjira, all isolates showed highly susceptible reaction except one isolate was susceptible reaction.

4.7.6. Mean virulence of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* over five safflower cultivars

Mean per cent wilt induced by 19 isolates of FOC in five cultivars of safflower is presented in Table 22. A perusal of Table 22 indicated that FOC 12 and 39 were most virulent isolates causing 82 and 78 per cent wilt respectively over five cultivars. They were followed by FOC 16 and 21. Lowest wilt per cent was observed in FOC 30 followed by FOC 18 and 35 which less virulent.

4.7.7. Reaction of five safflower cultivars to test isolates of *Fusarium oxysporum* f.sp. *carthami*

Results in Table 23 revealed that all the cultivars were highly susceptible to isolate FOC 12, 39 and 41 indicating that these three isolates were most virulent. A-1, PBNS-40 and Bhima shown moderately resistant reaction. Variety PBNS-40 was shown tolerant reaction against isolates FOC-18, 30 and 35. Variety Bhima exhibited

Table 21. Virulence of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* (FOC) on safflower Cv. Manjira

Sr. No.	Isolate No.	No. of plants (out of 10) wilted after 5 days)	Wilting (%)	Disease score
1	FOC-5	7	70 (56.99)	9
2	FOC-10	7	70 (56.83)	9
3	FOC-12	9	90 (71.94)	9
4	FOC-14	9	90 (71.94)	9
5	FOC-16	7	70 (56.83)	9
6	FOC-18	7	70 (56.99)	9
7	FOC-21	9	90 (71.94)	9
8	FOC-24	7	70 (56.99)	9
9	FOC-27	7	70 (56.99)	9
10	FOC-29	7	70 (56.99)	9
11	FOC-30	5	50 (44.99)	7
12	FOC-32	9	90 (71.94)	9
13	FOC-35	7	70 (56.83)	9
14	FOC-36	7	70 (56.83)	9
15	FOC-39	9	90 (71.94)	9
16	FOC-41	7	70 (56.99)	9
17	FOC-42	9	90 (71.94)	9
18	FOC-44	7	70 (56.99)	9
19	FOC-47	9	90 (71.94)	9
	S.E. +	---	3.03	---
	CD at 1 %	---	11.89	---
	CV (%)	---	8.49	---

- Based on ten seedlings for each isolate
- The wilting percentage in sterilized water (control) was nil.
- Figures in parenthesis are angular transformed and statistics was applied to them

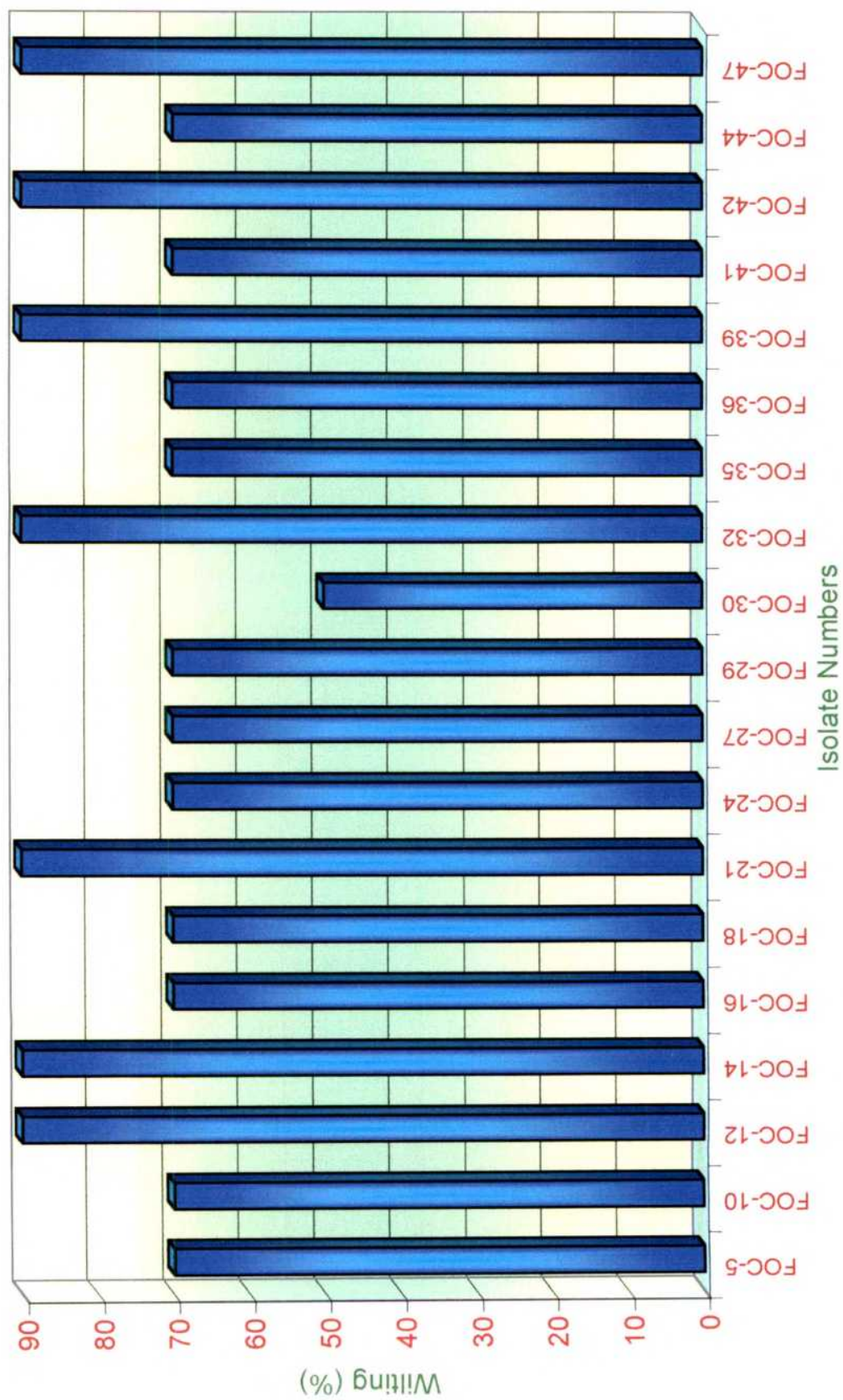


Fig.8. Virulence of the 19 isolates of *Fusarium oxysporum* f.sp. *carthami* on safflower Cv. Manjira

Table 22. Per cent wilt induced by *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates in five cultivars of safflower

Isolate No.	Wilting (%) after 5 days in safflower cultivars					Mean
	A-1	PBNS-40	Bhima	Nira	Manjira	
FOC-5	70	90	40	70	70	68
FOC-10	70	70	20	50	70	56
FOC-12	90	70	70	90	90	82
FOC-14	40	90	70	50	90	68
FOC-16	50	70	90	90	70	74
FOC-18	10	20	20	10	70	38
FOC-21	70	90	70	50	90	74
FOC-24	90	40	90	50	70	68
FOC-27	40	70	90	70	70	68
FOC-29	20	40	20	90	70	48
FOC-30	10	20	10	90	50	36
FOC-32	70	40	70	50	90	64
FOC-35	20	20	20	70	70	40
FOC-36	90	50	70	50	70	66
FOC-39	70	70	90	70	90	78
FOC-41	40	90	90	70	70	72
FOC-42	50	70	70	50	90	66
FOC-44	50	50	90	90	70	70
FOC-47	50	90	70	50	90	70

Table 23. Disease reaction of five safflower cultivars to test isolates of *Fusarium oxysporum* f.sp. *carthami*

Isolate No.	Safflower cultivars				
	A-1	PBNS-40	Bhima	Nira	Manjira
FOC-5	HS	HS	S	HS	HS
FOC-10	HS	HS	T	S	HS
FOC-12	HS	HS	HS	HS	HS
FOC-14	S	HS	HS	S	HS
FOC-16	S	HS	HS	HS	HS
FOC-18	MR	T	T	HS	HS
FOC-21	HS	HS	HS	S	HS
FOC-24	HS	S	HS	S	HS
FOC-27	S	HS	HS	HS	HS
FOC-29	T	S	T	HS	HS
FOC-30	MR	T	MR	HS	S
FOC-32	HS	S	HS	S	HS
FOC-35	T	T	T	HS	HS
FOC-36	HS	S	HS	S	HS
FOC-39	HS	HS	HS	HS	HS
FOC-41	HS	HS	HS	HS	HS
FOC-42	S	HS	HS	S	HS
FOC-44	S	S	HS	HS	HS
FOC-47	S	HS	HS	S	HS

MR – Moderately resistant – 1 – 10 %
T – Tolerant – 11 – 20 %
S – Susceptible – 20 – 50 %
HS – Highly susceptible – 51 or more %

tolerant reaction to FOC 10, while other varieties are highly susceptible to susceptible against FOC 10. Similarly variety A-1 and Bhima were tolerant to isolate FOC 29 while other varieties were either highly susceptible or susceptible. Thus, A-1, PBNS-40 and Bhima can be used as differential for differentiating pathotypes for *Fusarium oxysporum* f.sp. *carthami*.

On the basis of pathogenic variability on five cultivars A-1, Bhima, PBNS-40, Nira and Manjira, the isolates can be grouped in to four races. Race-I includes FOC 18, 30 and 35 which showed susceptible to highly susceptible reaction on Nira and Majira. Race-II which included FOC 29 which showed tolerant reaction to A-1 and Bhima and susceptible to highly susceptible to PBNS-40, Nira and Manjira. Race-III which include FOC 10 which showed tolerant to safflower Cv. Bhima and susceptible to highly susceptible to A-1, PBNS-40, Nira and Manjira. Race-IV which include FOC-5, 12, 14, 16, 21, 24, 27, 32, 36, 39, 41, 42, 44 and 47 showed susceptible to highly susceptible reaction to all the cultivars that is A-1, Bhima, PBNS-40, Nira and Manjira.

4.8. Genetic variability

Significant polymorphism was observed between the isolates from different agroecological situations of Marathwada region.

4.8.1. Random amplified polymorphic DNA (RAPD) analysis

RAPD analysis of 23 isolates of *Fusarium oxysporum* f.sp. *carthami* and 7 isolates of *Fusarium* spp. was done with two random primers that is OPA-18 and OPC-11 selected for amplification. OPA-18 and OPC-11 were selected after screening of 20 primers as they have

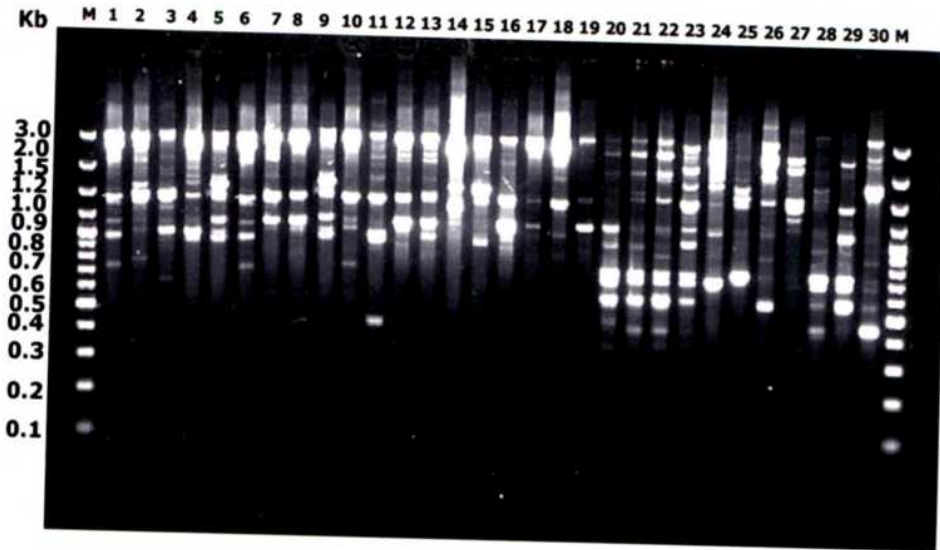


Fig.9. OPA-18 RAPD marker profiles of isolates of *Fusarium oxysporum* f.sp. *carthami* M, molecular weight markers of a 1 kb DNA ladder

1-23-*Fusarium oxysporum* f.sp. *carthami* (1-19 isolates from Parbhani; 20 & 21 isolates-DOR, Hyderabad; 22 & 23 isoaltes from Solapur); 24 *F. oxysporum*, 25- *F. moniliforme*, 26- *F. graminearum*; 27 - *F. solani*; 28 - *F. equiseti*, 29 - *F. moniliforme* f.sp. *subglutinans*; 30-*F. chlamyosporum*

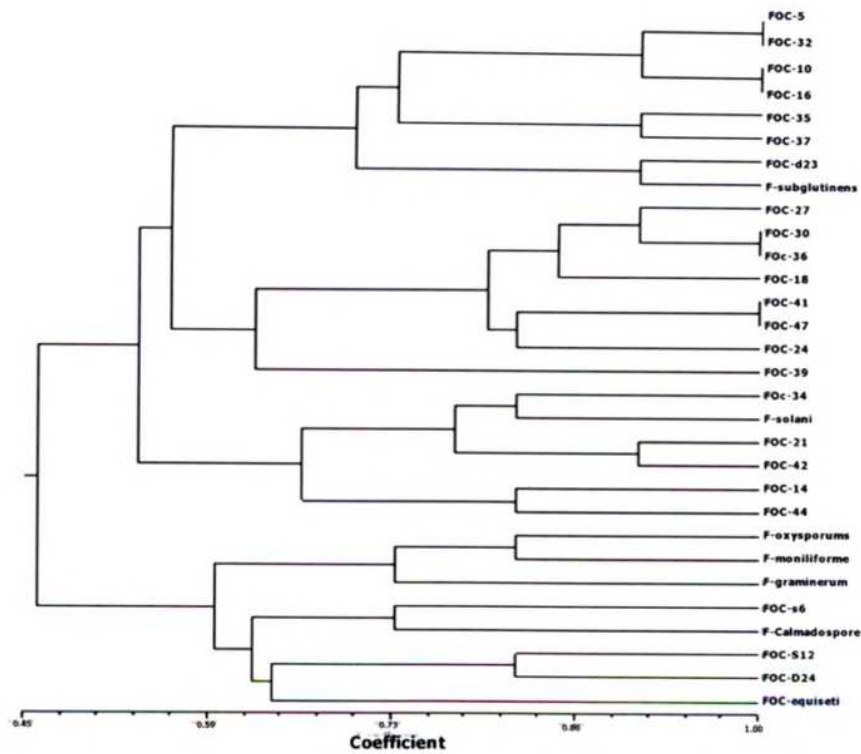


Fig.10. Dendrogram of OPA-18 RAPD marker of 23 isolates of *Fusarium oxysporum* f.sp. *carthami* constructed using NT-SYS. 5 groups were resolved by UPGMA analysis. Numbers of the termini of branches are the isolate numbers

shown good polymorphic bands. The illustration of the significant differences in RAPD profiles of 23 samples of various *Fusarium oxysporum* f.sp. *carthami* isolates along with some known species other than *carthami* like *F. oxysporum*, *F. moniliformae* f.sp. *subglutinans*, *F. chlamydosporum* with primer OPC 18 is given in Fig 9. The primer, OPA-18 produced polymorphic chain reaction (PCR) products of molecular weight ranging from 0.4 kb to 3.0 kb in different isolates with diverse fingerprinting patterns. The amplification products of the above 2 primer ranged between 0.3 kb and 3.0 kb. the total numbers of loci amplified by 2 primers were 263.

A dendrogram for the 23 *Fusarium oxysporum* f.sp. *carthami* isolates along with *F. oxysporum*, *F. moniliformae*, *F. graminearum*, *F. solani*, *F. equiseti*, *F. moniliformae* f.sp. *subglutinans*, *F. chlamydosporum* was prepared by using the similarity coefficients of markers generated by 2 primers (Fig.10 and 12).

As per cluster analysis, 30 isolates of *Fusarium* formed 4 main group (Fig. 10). Based on this analysis of *Fusarium oxysporum* f.sp. *carthami*, seven isolates of *Fusarium oxysporum* f.sp. *carthami* with 70 to 100 per cent genetic similarity with a bootstrap value of 100 formed one group. The second group comprised of 8 isolates of *Fusarium oxysporum* f.sp. *carthami* with 60-100 per cent genetic similarity among them. The group I, group II and group III contained most of FOC isolates, the group IV has more diverse isolates comprising *F. chlamydosporum*, *F. equiseti*, *F. moniliformae* and *F. graminearum*. The isolates 12 and 24 were diverse and have around 75 per cent genetic similarity with group IV. Isolates No. 6 shared 73 per

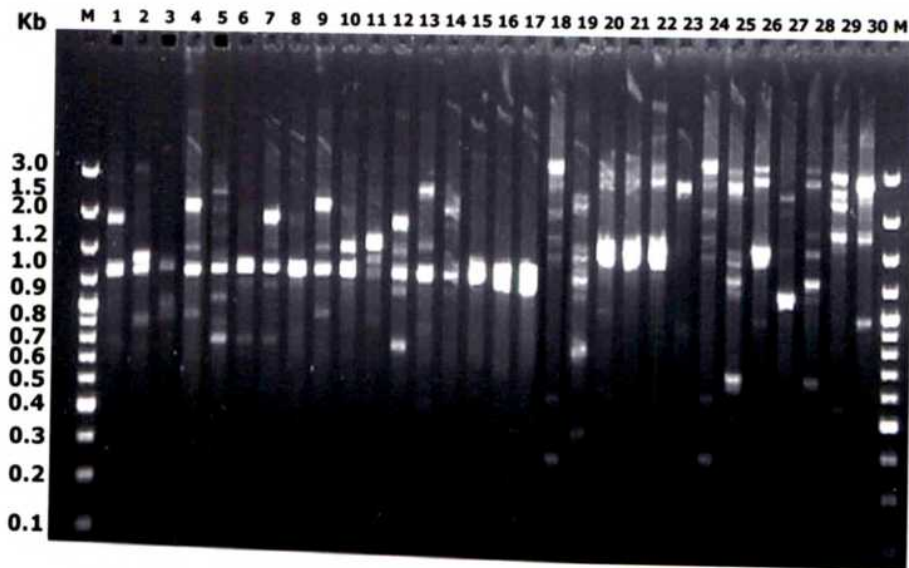


Fig.11. OPC-11 RAPD marker profiles of isolates of *Fusarium oxysporum* f.sp. *carthami* M, molecular weight markers of a 1 kb DNA ladder

1-23-*Fusarium oxysporum* f.sp. *carthami* (1-19 isolates from Parbhani; 20 & 21 isolates-DOR, Hyderabad; 22 & 23 isoaltes from Solapur); 24 *F. oxysporum*, 25- *F. moniliforme*, 26- *F. graminearum*; 27 - *F. solani*; 28 - *F. equiseti*, 29 - *F. moniliforme* f.sp. *subglutinans*; 30-*F. chlamydosporum*

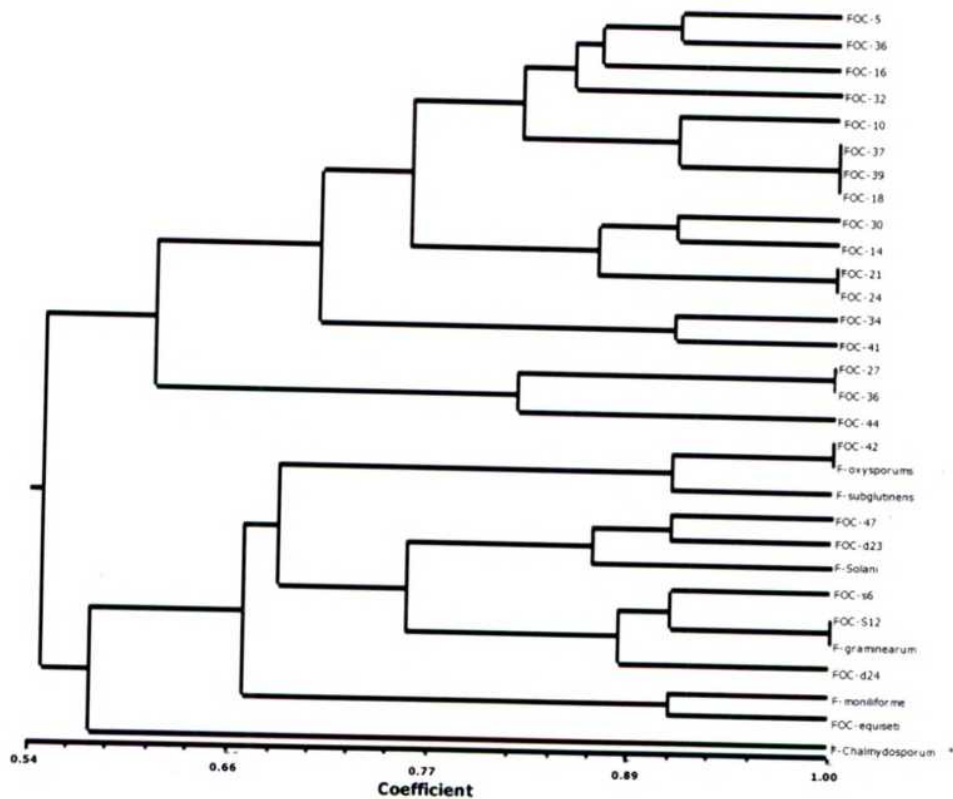


Fig.12. Dendrogram of OPC-11 RAPD marker of 23 isolates of *Fusarium oxysporum* f.sp. *carthami* constructed using NT-SYS. 5 groups were resolved by UPGMA analysis. Numbers of the termini of branches are the isolate numbers

cent similarly with *F. chlamydosporum*. The dendrogram Fig.12 obtained grouped all isolates in a single large cluster consisting of 2 groups with a similarity values ranging between 50 – 100 per cent. The group I contained most of the FOC isolates, the group II have other species of *Fusarium*. The group I comprised of 17 isolates with 70 to 100 per cent genetic similarity amongst them.

4.8.2. Microsatellite analysis

In preliminary experiment inter simple sequence repeats (ISSR) were used for the study, as they have produced scorable and reproducible bands. The amplification products of these primer ranged between 0.3 kb and 3.0 kb. The total number of loci amplified by ISSR were 187 respectively Fig.13.

The dendrogram for the *Fusarium* isolates was prepared by using the similarity coefficients of DNA markers generated by ISSR (Fig. 14). As per cluster analysis, isolates (23 *Fusarium oxysporum* f.sp. *carthami* isolates from safflower and 7 standards of *F. oxysporum*, *F. moniliformae* f.sp. *subglutinans*, *F. moniliformae*, *F. solani*, *F. graminearum*, *F. chlamydosporum* and *F. equisetii*) formed 2 main groups. The group I has 3 sub groups (Fig. 14). The group Ia comprised of 15 isolates sharing more than 75 % genetic similarity. Group Ib has 4 isolates with more than 75 per cent similarity. Group Ic has 2 isolates with more than 85 per cent similarity among them. These three sub groups have shown distinct banding pattern separating one group from another. The main group I has more than 60 per cent similarity among its isolates. The group II has more diverse isolates comprising of isolates 6 and 12 from safflower and some of the

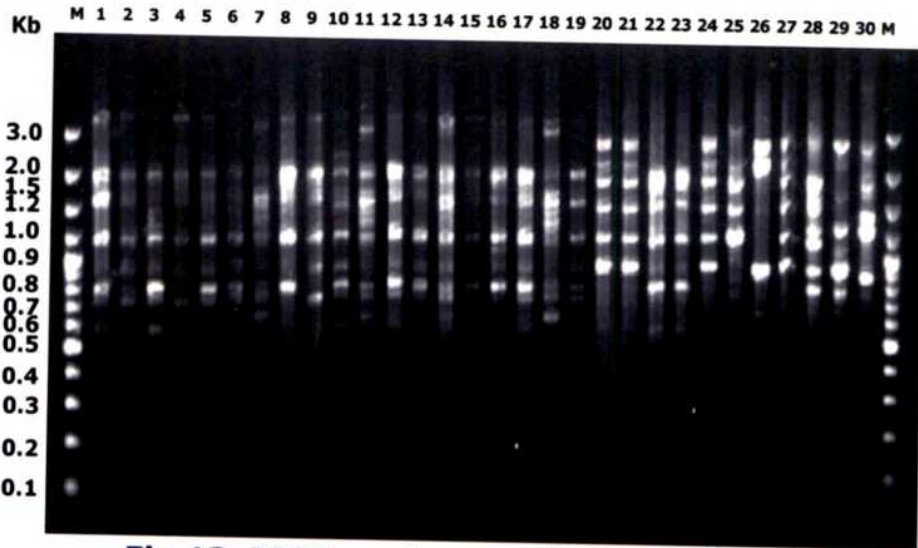


Fig.13. ISSR marker profiles of isolates of *Fusarium oxysporum* f.sp. *carthami* M, molecular weight markers of a 1 kb DNA ladder

1-23-*Fusarium oxysporum* f.sp *carthami* (1-19 isolates from Parbhani; 20 & 21 isolates-DOR, Hyderabad; 22 & 23 isoaltes from Solapur); 24 *F. oxysporum*, 25- *F moniliforme*, 26- *F. graminearum*; 27 - *F. solani*; 28 - *F. equiseti*, 29 - *F. moniliforme* f.sp. *subglutinans*; 30-*F. chlamyosporum*

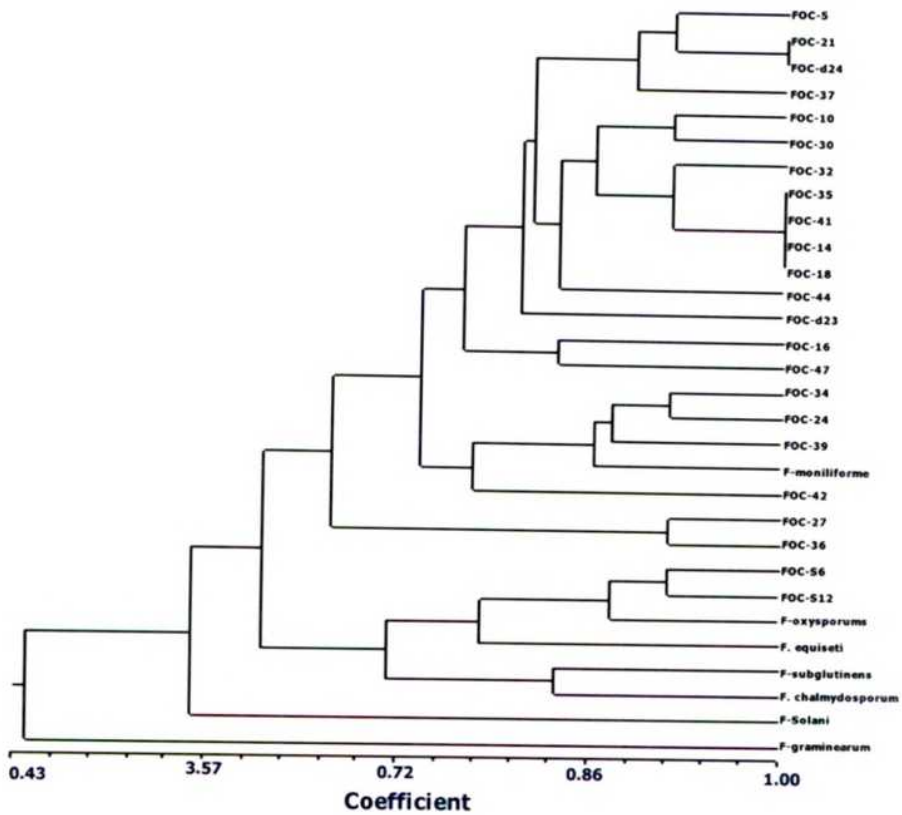


Fig.14. Dendrogram of ISSR marker of 23 isolates of *Fusarium oxysporum* f.sp. *carthami* constructed using NT-SYS. 5 groups were resolved by UPGMA analysis. Numbers of the termini of branches are the isolate numbers

standard isolates. The second group comprised of 2 FOC isolates with 90-100 per cent similarity among them.

Microsatellite analysis has clearly showed one main group among *Fusarium oxysporum* f.sp. *carthami* isolates from safflower.

4.9. Screening for disease resistance

Results (Table 24 and plate 8B) indicated that safflower genotypes have variable reaction to *Fusarium oxysporum* f.sp. *carthami*. In general, variation in wilting percentage at the end of five days in water culture screening ranged from 10 to 100 per cent between genotypes. Safflower genotypes continued exhibiting wilt symptoms over an observation period of five days. Within first three days of observation, majority of the genotypes exhibited wilting symptoms.

Out of 150 safflower genotypes three genotypes viz. GMU-2794, 2828, 2850 were free from wilt at the end of five days. Genotypes GMU 2765, 2873, 2886, 2901, 2906, 2921 and 2924 were observed moderately resistant and genotypes GMU 2866, 2867, 2880 and 2881 were tolerant, 39 genotypes were susceptible and 97 genotypes were highly susceptible at the end of five days.

It is seen from results depicted in Table 25 that three genotypes had shown no wilting up to 5 days. Observations indicating 0 per cent wilt was noticed in three genotypes. Moderately resistant reaction (per cent wilt incidence 1-10%) was noticed in seven genotypes. Tolerant reaction (Per cent wilt incidence 11 to 20 %) was noticed in four genotypes. Thirty nine genotypes were susceptible, while 97 genotypes showed highly susceptible reaction (wilt incidence 51 % or more).

Table 24. Screening of safflower genotypes against FOC-12 (*Fusarium oxysporum* f.sp. *carthami*) wilt by water culture technique

Sr. No.	Genotypes	No. of plants (out of 10) wilted after 5 days	Wilting per cent	Disease score
1	GMU - 2752	7	70 (56.78)	9
2	GMU - 2753	10	100 (89.99)	9
3	GMU - 2755	8	80 (63.42)	9
4	GMU - 2756	10	100 (89.99)	9
5	GMU - 2757	3	30 (33.20)	7
6	GMU - 2759	10	100 (89.99)	9
7	GMU - 2761	3	30 (33.20)	7
8	GMU - 2763	7	70 (56.78)	9
9	GMU - 2765	1	10 (18.43)	9
10	GMU - 2766	3	30 (33.20)	7
11	GMU - 2767	7	70 (56.78)	9
12	GMU - 2768	7	70 (56.78)	9
13	GMU - 2769	9	90 (71.55)	9
14	GMU - 2770	9	90 (71.55)	9
15	GMU - 2771	7	70 (56.78)	9
16	GMU - 2772	9	90 (71.55)	9
17	GMU - 2773	10	100 (89.99)	9
18	GMU - 2774	10	100 (89.99)	9

Sr. No.	Genotypes	No. of plants (out of 10) wilted after 5 days	Wilting per cent	Disease score
19	GMU - 2776	7	70 (56.78)	9
20	GMU - 2777	10	100 (89.99)	9
21	GMU - 2778	3	30 (33.20)	7
22	GMU - 2779	7	70 (56.78)	9
23	GMU - 2780	7	70 (56.78)	9
24	GMU - 2781	10	100 (89.99)	9
25	GMU - 2782	3	30 (33.20)	7
26	GMU - 2783	9	90 (71.55)	9
27	GMU - 2784	7	70 (56.78)	9
28	GMU - 2785	4	40 (39.22)	7
29	GMU - 2786	10	100 (89.99)	9
30	GMU - 2787	4	40 (39.22)	7
31	GMU - 2788	9	90 (71.55)	9
32	GMU - 2789	3	30 (33.20)	7
33	GMU - 2790	10	100 (89.99)	9
34	GMU - 2791	7	70 (56.78)	9
35	GMU - 2793	9	90 (71.55)	9
36	GMU - 2794	0	0 (0.00)	0
37	GMU - 2795	9	90 (71.55)	9
38	GMU - 2796	3	30 (33.20)	7

Sr. No.	Genotypes	No. of plants (out of 10) wilted after 5 days	Wilting per cent	Disease score
39	GMU - 2798	7	70 (56.78)	9
40	GMU - 2799	9	90 (71.55)	9
41	GMU - 2800	4	40 (39.22)	7
42	GMU - 2801	10	100 (89.99)	9
43	GMU - 2802	7	70 (56.78)	9
44	GMU - 2803	3	30 (33.20)	7
45	GMU - 2804	7	70 (56.78)	9
46	GMU - 2806	9	90 (71.55)	9
47	GMU - 2807	7	70 (56.78)	9
48	GMU - 2808	10	100 (89.99)	9
49	GMU - 2809	9	90 (71.55)	9
50	GMU - 2811	3	30 (33.20)	7
51	GMU - 2812	10	100 (89.99)	9
52	GMU - 2813	7	70 (56.78)	9
53	GMU - 2814	9	90 (71.55)	9
54	GMU - 2815	3	30 (33.20)	7
55	GMU - 2817	10	100 (89.99)	9
56	GMU - 2818	4	40 (39.22)	7
57	GMU - 2819	7	70 (56.78)	9
58	GMU - 2820	9	90 (71.55)	9

Sr. No.	Genotypes	No. of plants (out of 10) wilted after 5 days	Wilting per cent	Disease score
59	GMU - 2821	3	30 (33.20)	7
60	GMU - 2822	9	90 (71.55)	9
61	GMU - 2823	10	100 (89.99)	9
62	GMU - 2824	7	70 (56.78)	9
63	GMU - 2826	4	40 (39.22)	7
64	GMU - 2827	9	90 (71.55)	9
65	GMU - 2828	0	0 (0.00)	0
66	GMU - 2829	9	90 (71.55)	9
67	GMU - 2830	4	40 (39.22)	7
68	GMU - 2831	10	100 (89.99)	9
69	GMU - 2832	9	90 (71.55)	9
70	GMU - 2833	7	70 (56.78)	9
71	GMU - 2834	4	40 (39.22)	7
72	GMU - 2836	9	90 (71.55)	9
73	GMU - 2838	3	30 (33.20)	7
74	GMU - 2840	10	100 (89.99)	9
75	GMU - 2841	9	90 (71.55)	9
76	GMU - 2843	3	30 (33.20)	7
77	GMU - 2845	10	100 (89.99)	9
78	GMU - 2846	3	30 (33.20)	7

Sr. No.	Genotypes	No. of plants (out of 10) wilted after 5 days	Wilting per cent	Disease score
79	GMU - 2847	9	90 (71.55)	9
80	GMU - 2848	4	40 (39.22)	7
81	GMU - 2849	10	100 (89.99)	9
82	GMU - 2850	0	0 (0.00)	0
83	GMU - 2851	9	90 (71.55)	9
84	GMU - 2852	7	70 (56.78)	9
85	GMU - 2853	3	30 (33.20)	7
86	GMU - 2854	9	90 (71.55)	9
87	GMU - 2855	9	90 (71.55)	9
88	GMU - 2856	3	30 (33.20)	7
89	GMU - 2857	7	70 (56.78)	9
90	GMU - 2858	9	90 (71.55)	9
91	GMU - 2859	3	30 (33.20)	7
92	GMU - 2860	10	100 (89.99)	9
93	GMU - 2862	3	30 (33.20)	7
94	GMU - 2863	9	90 (71.55)	9
95	GMU - 2864	7	70 (56.78)	9
96	GMU - 2865	7	70 (56.78)	9
97	GMU - 2866	2	20 (26.56)	5
98	GMU - 2867	2	20 (26.56)	5

Sr. No.	Genotypes	No. of plants (out of 10) wilted after 5 days	Wilting per cent	Disease score
99	GMU - 2869	9	90 (71.55)	9
100	GMU - 2870	3	30 (33.20)	9
101	GMU - 2871	10	100 (89.99)	9
102	GMU - 2872	9	90 (71.55)	9
103	GMU - 2873	1	10 (18.43)	9
104	GMU - 2874	7	70 (56.78)	9
105	GMU - 2875	4	40 (39.22)	7
106	GMU - 2876	10	100 (89.99)	9
107	GMU - 2877	7	70 (56.78)	9
108	GMU - 2878	4	40 (39.22)	7
109	GMU - 2879	9	90 (71.55)	9
110	GMU - 2880	2	20 (26.56)	5
111	GMU - 2881	2	20 (26.56)	5
112	GMU - 2882	10	100 (89.99)	9
113	GMU - 2883	9	90 (71.55)	9
114	GMU - 2884	7	70 (56.78)	9
115	GMU - 2886	1	10 (18.43)	9
116	GMU - 2887	9	90 (71.55)	9
117	GMU - 2888	7	70 (56.78)	9
118	GMU - 2889	3	30 (33.20)	7

Sr. No.	Genotypes	No. of plants (out of 10) wilted after 5 days	Wilting per cent	Disease score
119	GMU - 2890	10	100 (89.99)	9
120	GMU - 2891	3	30 (33.20)	7
121	GMU - 2892	7	70 (56.78)	9
122	GMU - 2893	3	30 (33.20)	7
123	GMU - 2894	10	100 (89.99)	9
124	GMU - 2895	9	90 (71.55)	9
125	GMU - 2896	3	30 (33.20)	7
126	GMU - 2897	7	70 (56.78)	9
127	GMU - 2898	3	30 (33.20)	7
128	GMU - 2899	4	40 (39.22)	7
129	GMU - 2900	9	90 (71.55)	9
130	GMU - 2901	1	10 (18.43)	9
131	GMU - 2902	10	100 (89.99)	9
132	GMU - 2903	3	30 (33.20)	7
133	GMU - 2904	7	70 (56.78)	9
134	GMU - 2905	10	100 (89.99)	9
135	GMU - 2906	1	10 (18.43)	9
136	GMU - 2907	9	90 (71.55)	9
137	GMU - 2908	4	40 (39.22)	7
138	GMU - 2909	7	70 (56.78)	9

Sr. No.	Genotypes	No. of plants (out of 10) wilted after 5 days	Wilting per cent	Disease score
139	GMU - 2910	9	90 (71.55)	9
140	GMU - 2911	3	30 (33.20)	7
141	GMU - 2912	7	70 (56.78)	9
142	GMU - 2913	9	90 (71.55)	9
143	GMU - 2914	3	30 (33.20)	7
144	GMU - 2917	10	100 (89.99)	9
145	GMU - 2918	3	30 (33.20)	7
146	GMU - 2919	9	90 (71.55)	9
147	GMU - 2920	7	70 (56.78)	9
148	GMU - 2921	1	10 (18.43)	9
149	GMU - 2923	10	100 (89.99)	9
150	GMU - 2924	1	10 (18.43)	9
S.E. ±			0.93	
CD at 1 %			3.42	
CV %			63.30	

* Based on ten seedlings of each genotypes.

* The wilting percentage in sterilized water (control) was nil

Figures in parenthesis are angular transformed value and statistics was applied to them

Table 25. Categorization of safflower genotypes screened against safflower wilt by water culture technique

Reaction to wilt	Per cent wilt incidence	No. of genotypes
Highly resistant	0	3
Resistant	1 % or less	Nil
Moderately resistant	1 – 10 %	7
Tolerant	11-20 %	4
Susceptible	21 – 50 %	39
Highly susceptible	51 % or more	97

Present findings clearly indicated that water culture technique is most useful, suitable and economic for initial evaluation of screening of large number of genotypes and breeding material in laboratory to avoid laborious and time consuming job in the field.

4.10 Biological management through *Trichoderma* spp

4.10.1. Efficacy of *Trichoderma harzianum* against *Fusarium oxysporum* f.sp. *carthami* in vitro.

The effect of biocontrol agent *Trichoderma harzianum* was tested against the 19 isolates of FOC by dual culture technique and per cent inhibition after 3, 6 and 9 days of incubation is presented in Table 26, 27 and 28.

Table 26. Mean colony radius (mm) of FOC isolates and *T. harzianum* (3 DAI) with per cent inhibition of FOC isolates

Isolate No.	Growth of <i>F. oxysporum</i> f.sp. <i>carthami</i> *	Growth of <i>T. harzianum</i> *	Radius (mm) of only pathogen	per cent inhibition
FOC-5	12.66	21.00	9.16	-38.15
FOC-10	13.66	19.66	9.08	-50.44
FOC-12	6.33	32.33	10.00	36.7
FOC-14	6.66	21.66	9.16	27.34
FOC-16	11.33	23.66	10.91	-3.80
FOC-18	8.30	21.66	9.25	10.27
FOC-21	10.00	22.33	9.16	-9.17
FOC-24	7.33	23.66	10.91	32.81
FOC-27	5.33	22.66	11.91	55.24
FOC-29	8.66	18.66	11.16	22.40
FOC-30	11.00	22.00	12.00	8.33
FOC-32	11.33	19.33	10.5	-7.90
FOC-35	14.00	13.00	12.5	-12.00
FOC-36	7.33	28.66	7.66	4.30
FOC-39	5.33	21.66	12.75	58.19
FOC-41	5.33	21.33	12.25	56.48
FOC-42	5.00	22.66	10.91	54.17
FOC-44	5.66	22.33	13.5	58.07
FOC-47	5.33	23.33	12.66	57.89
S.E. \pm	0.54	0.83	---	---
CD at 1 %	2.12	3.25	---	---

* - Mean of three replications
DAI – Days after incubation

Table 27. Mean colony radius (mm) of FOC isolates and *T. harzianum* (6 DAI) with per cent inhibition of FOC isolates

Isolate No.	Growth of <i>F. oxysporum</i> f.sp. <i>carthami</i> *	Growth of <i>T. harzianum</i> *	Radius (mm) of only pathogen	per cent inhibition
FOC-5	16.33	33.00	18.75	12.90
FOC-10	19.66	29.00	10.83	-81.53
FOC-12	9.66	40.00	21.16	54.34
FOC-14	7.66	40.66	20.5	62.63
FOC-16	12.00	42.33	16.0	25.00
FOC-18	10.66	43.00	12.33	13.54
FOC-21	11.66	44.66	12.25	4.81
FOC-24	9.66	54.33	16.5	41.45
FOC-27	9.00	46.33	19.58	54.03
FOC-29	12.33	49.00	17.33	28.85
FOC-30	12.33	43.66	15.75	21.71
FOC-32	13.66	31.33	12.00	-13.83
FOC-35	26.00	18.66	20.58	-26.33
FOC-36	8.66	31.33	12.08	28.31
FOC-39	7.00	53.00	14.75	52.54
FOC-41	9.66	44.00	19.16	49.58
FOC-42	5.00	46.33	16.33	69.38
FOC-44	7.00	46.66	17.25	59.42
FOC-47	9.33	41.00	16.33	42.86
S.E. \pm	0.74	0.77	---	---
CD at 1 %	2.90	3.02	---	---

* - Mean of three replications
DAI – Days after incubation

Table 28. Mean colony radius (mm) of FOC isolates and *T. harzianum* (9 DAI) with per cent inhibition of FOC isolates

Isolate No.	Growth of <i>F. oxysporum</i> f.sp. <i>carthami</i> *	Growth of <i>T. harzianum</i> *	Radius (mm) of only pathogen	per cent inhibition
FOC-5	22.00	43.33	34.25	35.76
FOC-10	23.66	33.66	16.50	-43.39
FOC-12	12.00	58.33	30.00	60.00
FOC-14	10.33	62.33	31.00	66.67
FOC-16	14.00	62.00	31.25	55.20
FOC-18	12.66	63.00	15.91	20.42
FOC-21	16.00	61.33	16.41	2.49
FOC-24	11.33	64.00	34.83	67.47
FOC-27	13.00	68.00	35.08	62.94
FOC-29	17.66	63.66	38.41	54.02
FOC-30	21.00	63.66	34.16	38.52
FOC-32	23.00	48.33	29.08	20.90
FOC-35	30.00	23.66	36.66	18.16
FOC-36	10.33	44.00	20.55	49.73
FOC-39	10.00	60.66	40.08	75.04
FOC-41	12.66	59.00	38.41	67.03
FOC-42	6.00	69.66	26.25	77.14
FOC-44	9.66	72.00	37.08	73.94
FOC-47	14.00	56.66	31.25	55.20
S.E. \pm	0.76	0.73	---	---
CD at 1 %	2.98	2.86	---	---

* - Mean of three replications
DAI – Days after incubation



Plate 9A. Testing of Bioagent (*T. harzianum*) against 19 isolates of *Fusarium oxysporum* f.sp. *carthami*



Plate 9B. Testing of Bioagent (*T. viridae*) against 19 isolates of *Fusarium oxysporum* f.sp. *carthami*

Perusal of results from Table 26 indicated that growth of FOC isolates was significantly influenced by *T. harzianum* under study at all the observation periods. At 3 days of incubation the results indicated that the per cent inhibition in growth of *Fusarium oxysporum* f.sp. *carthami* due to *Trichoderma harzianum* ranged from -3.80 to 58.19 per cent. Maximum per cent inhibition being in the isolate FOC 39 and minimum in FOC 16.

At 6 DAI, the growth of all the isolates except FOC 10, 32 and 35 exhibited inhibition in growth ranging from 4.81 per cent in FOC 21 to 69.38 per cent in FOC 42 (Table 27).

At 9 DAI, *Trichoderma harzainum* inhibited the growth of all isolates except one. The per cent reduction ranged from 2.49 per cent in FOC 21 to 77.14 per cent in FOC 42 (Table 28) and (Plate 9A).

4.10.2. Efficacy of *Trichoderma viride* against *Fusarium oxysporum* f.sp. *carthami* in vitro

The effect of biocontrol agent *T. viride* was tested against the 19 isolates of FOC by dual culture technique and per cent inhibition after 3, 6 and 9 days of incubation is presented in Table 29, 30 and 31.

Perusal of results indicated that growth of *Fusarium oxysporum* f.sp. *carthami* was significantly inhibited by *T. viridae* under study at all the observation periods. Per cent inhibition in growth of FOC isolates due to *T. viridae* varied from 0.72 per cent in isolate FOC 41 to 57.36 per cent in FOC 44. The biocontrol agent could not inhibit the growth of FOC 16, 18, 36 and 42 at 3 DAI (Table 29).

Table 29. Mean colony radius (mm) of FOC isolates and *T. viride* (3 DAI) with per cent inhibition of FOC isolates

Isolate No.	Growth of <i>F. oxysporum</i> f.sp. <i>carthami</i> *	Growth of <i>T. viride</i> *	Radius (mm) of only pathogen	Per cent inhibition
FOC-5	6.66	23.66	9.75	31.69
FOC-10	8.33	20.00	10.50	20.66
FOC-12	7.66	32.33	10.58	27.59
FOC-14	10.66	35.00	11.08	3.79
FOC-16	13.33	35.33	11.33	-17.65
FOC-18	11.33	20.33	9.25	-22.48
FOC-21	5.33	32.33	8.25	35.39
FOC-24	5.66	28.66	11.25	49.68
FOC-27	7.00	27.66	10.41	32.75
FOC-29	9.66	22.33	10.91	11.45
FOC-30	5.66	33.66	11.58	51.12
FOC-32	5.66	24.00	8.41	32.69
FOC-35	10.33	28.00	12.41	16.76
FOC-36	11.00	16.33	10.25	-7.31
FOC-39	7.00	20.00	11.75	40.42
FOC-41	11.00	8.00	11.08	0.72
FOC-42	13.00	8.33	9.16	-41.92
FOC-44	5.33	15.00	12.5	57.36
FOC-47	6.33	18.33	12.25	48.32
S.E. \pm	1.02	1.31	---	---
CD at 1 %	4.00	5.14	---	---

* - Mean of three replications
DAI – Days after incubation

Table 30. Mean colony radius (mm) of FOC isolates and *T. viride* (6 DAI) with per cent inhibition of FOC isolates

Isolate No.	Growth of <i>F. oxysporum</i> f.sp. <i>carthami</i> *	Growth of <i>T. viride</i> *	Radius (mm) of only pathogen	Per cent inhibition
FOC-5	12.33	42.66	19.33	36.21
FOC-10	10.66	32.33	15.08	29.31
FOC-12	8.66	52.33	18.75	53.81
FOC-14	14.66	56.00	19.66	25.45
FOC-16	16.67	48.00	18.00	7.38
FOC-18	15.00	33.33	11.50	-30.43
FOC-21	9.00	45.00	10.50	14.28
FOC-24	8.66	60.33	15.91	45.60
FOC-27	9.66	51.33	19.91	51.50
FOC-29	13.66	30.00	17.00	19.64
FOC-30	8.00	47.66	17.25	53.62
FOC-32	9.00	33.33	14.00	35.71
FOC-35	15.66	35.00	22.08	29.07
FOC-36	17.66	25.00	12.91	-36.79
FOC-39	9.66	33.33	17.25	44.00
FOC-41	17.66	13.33	16.00	-10.37
FOC-42	19.66	13.00	15.08	-30.37
FOC-44	9.00	32.00	20.25	55.55
FOC-47	10.66	33.33	20.16	47.14
S.E. \pm	1.24	1.32	---	---
CD at 1 %	4.86	5.18	---	---

* - Mean of three replications
DAI – Days after incubation

Table 31. Mean colony radius (mm) of FOC isolates and *T. viride* (9 DAI) with per cent inhibition of FOC isolates

Isolate No.	Growth of <i>F. oxysporum</i> f.sp. <i>carthami</i> *	Growth of <i>T. viride</i> *	Radius (mm) of only pathogen	per cent inhibition
FOC-5	17.66	62.33	36.08	51.05
FOC-10	16.66	51.66	19.16	13.07
FOC-12	12.33	71.66	31.16	60.44
FOC-14	22.66	73.33	34.91	35.10
FOC-16	20.00	70.00	31.75	37.00
FOC-18	23.33	52.00	20.41	-14.28
FOC-21	13.00	72.00	16.25	20.00
FOC-24	10.66	73.00	35.08	69.61
FOC-27	14.00	65.00	37.08	62.24
FOC-29	22.00	42.00	36.50	39.72
FOC-30	11.66	69.33	32.50	64.12
FOC-32	12.33	63.66	28.41	56.61
FOC-35	24.33	61.33	35.83	32.09
FOC-36	31.00	41.66	20.55	-50.82
FOC-39	12.33	62.00	37.00	66.67
FOC-41	30.00	23.33	35.83	16.27
FOC-42	33.33	16.00	30.41	9.58
FOC-44	12.33	63.00	37.91	67.48
FOC-47	13.00	63.66	36.25	64.13
S.E. \pm	1.41	1.07	---	---
CD at 1 %	5.53	4.20	---	---

* - Mean of three replications
DAI – Days after incubation

At 6 DAI all the FOC isolates except FOC 18, 36, 41 and 42 exhibited reduction in mycelial growth ranging from 7.38 per cent in FOC 16 to 55.55 per cent in FOC 44 (Table 30).

At 9 DAI, mycelial growth of all FOC isolates except FOC 18 and 36, was reduced from 9.58 per cent in FOC 42 to 69.61 per cent in FOC 24 (Table 31) and (Plate 9B).

4.10.3. Efficacy of *Trichoderma hamatum* against *Fusarium oxysporum* f.sp. *carthami* in vitro

The effect of biocontrol agent *T. hamatum* was tested against the 19 isolates of FOC by dual culture technique and per cent inhibition after 3, 6 and 9 days of incubation is presented in Table 32, 33 and 34.

Perusal of results indicated that growth of *Trichoderma hamatum* significantly reduced the growth of *Fusarium oxysporum* f.sp. *carthami* under study at all the observation periods. At 3 DAI, *Trichoderma hamatum* reduced the mycelial growth of all the FOC isolates except FOC 10, 21 and 32. Maximum inhibition per cent reduction was observed in FOC 30 followed by FOC 47 and FOC 36. While the lowest inhibition per cent was observed in FOC 5 (Table 32).

At 6 DAI, the mycelial growth of all the FOC isolates except FOC 10 and FOC 21, was reduced in the range of 11.76 per cent in isolate FOC 24 to 55.40 per cent the isolate in FOC 29 (Table 33).

At 9 DAI, the mycelial growth of all the isolates except FOC 10 and 21, was reduced 30.34 per cent in the isolate FOC 32 to 72.14 per cent in the isolate FOC 29 (Table 34) and (Plate 9C).

Table 32. Mean colony radius (mm) of FOC isolates and *T. hamatum* (3 DAI) with per cent inhibition of FOC isolates

Isolate No.	Growth of <i>F. oxysporum</i> f.sp. <i>carthami</i> *	Growth of <i>T. hamatum</i> *	Radius (mm) of only pathogen	per cent inhibition
FOC-5	8.66	20.66	10.25	15.51
FOC-10	9.00	23.66	7.75	-16.12
FOC-12	8.00	21.33	10.91	26.71
FOC-14	8.00	19.00	10.41	23.19
FOC-16	7.00	22.66	12.33	43.22
FOC-18	5.66	16.33	7.75	26.96
FOC-21	9.33	13.66	7.75	-20.38
FOC-24	7.66	18.00	12.25	37.46
FOC-27	6.00	22.66	10.75	44.18
FOC-29	5.33	11.33	9.08	41.29
FOC-30	6.66	13.00	13.75	51.56
FOC-32	8.00	21.33	6.16	-29.78
FOC-35	6.66	23.66	12.25	45.63
FOC-36	5.66	27.00	10.75	47.34
FOC-39	6.66	19.00	10.25	35.02
FOC-41	6.66	24.00	11.08	39.89
FOC-42	6.00	21.33	10.50	42.85
FOC-44	9.33	22.00	13.16	29.14
FOC-47	5.66	22.66	10.83	47.73
S.E. \pm	0.57	0.78	---	---
CD at 1 %	2.23	3.06	---	---

* - Mean of three replications

DAI – Days after incubation

Table 33. Mean colony radius (mm) of FOC isolates and *T. hamatum* (6 DAI) with per cent inhibition of FOC isolates

Isolate No.	Growth of <i>F. oxysporum</i> f.sp. <i>carthami</i> *	Growth of <i>T. hamatum</i> *	Radius (mm) of only pathogen	per cent inhibition
FOC-5	12.00	32.66	20.25	40.74
FOC-10	14.66	34.66	10.66	-37.47
FOC-12	11.66	36.00	19.83	41.20
FOC-14	11.33	40.66	20.25	44.04
FOC-16	9.33	42.66	17.66	47.19
FOC-18	8.00	24.00	11.50	30.43
FOC-21	14.00	21.33	-10.83	-29.27
FOC-24	15.00	34.00	17.00	11.76
FOC-27	12.33	38.66	20.91	41.05
FOC-29	8.66	17.33	19.41	55.40
FOC-30	11.33	22.66	17.16	34.00
FOC-32	10.66	30.66	12.25	12.97
FOC-35	11.33	42.33	21.25	46.68
FOC-36	9.66	43.66	12.91	25.21
FOC-39	10.00	28.66	16.25	38.46
FOC-41	9.33	42.66	17.58	46.92
FOC-42	8.33	44.00	17.66	52.85
FOC-44	12.66	33.00	16.50	23.27
FOC-47	9.33	36.00	18.00	48.16
S.E. \pm	0.48	0.70	---	---
CD at 1 %	1.88	2.74	---	---

* - Mean of three replications

DAI – Days after incubation

Table 34. Mean colony radius (mm) of FOC isolates and *T. hamatum* (9 DAI) with per cent inhibition of FOC isolates

Isolate No.	Growth of <i>F. oxysporum</i> f.sp. <i>carthami</i> *	Growth of <i>T. hamatum</i> *	Radius (mm) of only pathogen	Per cent inhibition
FOC-5	18.66	44.00	35.58	47.55
FOC-10	23.33	48.00	17.00	-37.23
FOC-12	17.00	53.33	30.25	43.80
FOC-14	18.66	49.00	32.00	41.68
FOC-16	14.66	62.66	32.58	55.00
FOC-18	11.33	33.66	17.75	36.16
FOC-21	23.66	40.66	-15.33	-54.33
FOC-24	21.33	52.00	34.66	38.47
FOC-27	16.66	60.66	35.83	53.50
FOC-29	11.33	26.00	40.66	72.14
FOC-30	20.66	43.33	34.25	39.67
FOC-32	19.33	48.66	27.75	30.34
FOC-35	17.00	62.66	37.25	54.36
FOC-36	14.00	61.66	21.08	33.58
FOC-39	15.00	48.66	39.50	62.02
FOC-41	12.00	72.66	37.00	67.56
FOC-42	12.00	70.00	27.16	55.83
FOC-44	24.66	41.33	37.16	33.65
FOC-47	12.66	56.66	32.41	60.95
S.E. \pm	0.72	0.65	---	---
CD at 1 %	2.82	2.55	---	---

* - Mean of three replications
DAI – Days after incubation

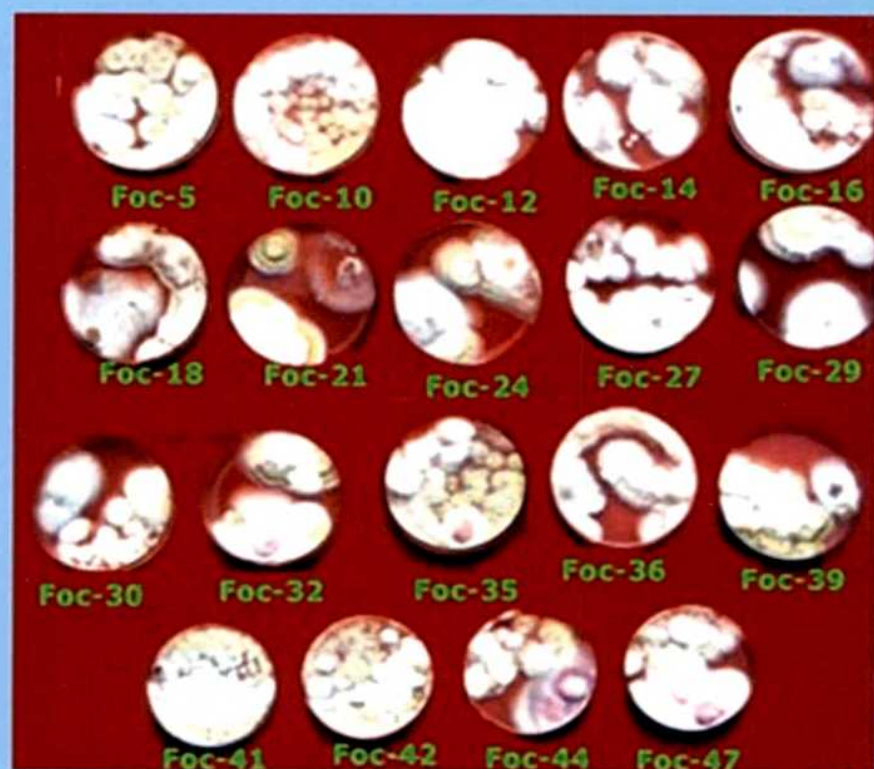


Plate 9C. Testing of Bioagent (*T. hamatum*) against 19 isolates of *Fusarium oxysporum* f.sp. *carthami*

4.10.4. Efficacy of *Trichoderma koningii* against *Fusarium oxysporum* f.sp. *carthami* in vitro

The effect of biocontrol agent *Trichoderma koningii* was tested against the 19 isolates of FOC by dual culture technique and per cent inhibition after 3, 6 and 9 days of incubation is presented in Table 35, 36 and 37.

As regards *T. koningii* at 3 DAI, the growth of all the FOC isolates except FOC-18, 36, 39, 42, 44 and 47 was reduced. The reduction ranged from 3.90 per cent (FOC-32) to 48.54 per cent isolate FOC 30 (Table 35).

At 6 DAI, *Trichoderma koningii* could reduce the mycelial growth of FOC-16, 18, 27, 29, 30, 35, 36 and 41 only. The minimum reduction of 1.03 per cent was observed in the isolate FOC 16 while the maximum reduction 43.45 per cent was observed in the isolate FOC 30 (Table 36).

At 9 DAI, *Trichoderma koningii* could reduce the mycelial growth of FOC-5, 14, 16, 29, 30, 35, 36 and 41. The minimum reduction was observed to be 5.60 per cent in the isolate FOC 5 and maximum reduction was observed in the isolate FOC 41 that is 34.24 per cent (Table 37).

Table 35. Mean colony radius (mm) of FOC isolates and *T. koningii* (3 DAI) with per cent inhibition of FOC isolates

Isolate No.	Growth of <i>F. oxysporum</i> f.sp. <i>carthami</i> *	Growth of <i>T. koningii</i> *	Radius (mm) of only pathogen	Per cent inhibition
FOC-5	9.33	21.66	10.5	11.14
FOC-10	6.00	22.33	11.5	47.82
FOC-12	8.00	23.66	10.16	21.30
FOC-14	8.00	18.66	10.5	23.80
FOC-16	7.33	22.00	10.0	26.70
FOC-18	9.00	28.66	8.08	-11.38
FOC-21	7.33	21.66	9.25	20.75
FOC-24	11.00	22.66	12.33	10.78
FOC-27	8.66	22.33	11.16	22.44
FOC-29	9.66	23.66	11.66	17.19
FOC-30	5.66	20.00	11.00	48.54
FOC-32	10.33	32.33	10.75	3.90
FOC-35	11.00	35.00	11.66	5.70
FOC-36	10.33	36.33	9.41	-9.72
FOC-39	13.00	17.66	12.00	-8.33
FOC-41	9.00	23.66	11.16	19.39
FOC-42	14.00	30.00	9.83	-42.42
FOC-44	16.66	25.66	15.33	-8.67
FOC-47	14.00	10.00	11.33	-23.56
S.E. \pm	0.74	1.02	---	---
CD at 1 %	2.90	4.00	---	---

* - Mean of three replications
DAI – Days after incubation

Table 36. Mean colony radius (mm) of FOC isolates and *T. koningii* (6 DAI) with per cent inhibition of FOC isolates

Isolate No.	Growth of <i>F. oxysporum</i> f.sp. <i>carthami</i> *	Growth of <i>T. koningii</i> *	Radius (mm) of only pathogen	Per cent inhibition
FOC-5	22.00	31.33	20.00	-10.00
FOC-10	15.00	28.66	12.91	-16.15
FOC-12	22.00	27.00	14.25	-54.38
FOC-14	23.66	32.66	17.75	-33.29
FOC-16	16.33	23.00	16.50	1.03
FOC-18	11.33	18.00	11.50	1.47
FOC-21	13.66	20.00	10.91	-25.16
FOC-24	19.00	22.66	15.00	-26.66
FOC-27	12.33	16.00	16.50	25.27
FOC-29	13.33	22.00	20.41	34.71
FOC-30	9.33	23.66	16.50	43.45
FOC-32	19.66	33.66	16.50	-19.15
FOC-35	12.66	32.33	15.66	19.18
FOC-36	16.00	31.33	16.41	2.52
FOC-39	21.33	25.33	14.83	-43.83
FOC-41	13.00	16.33	16.50	21.21
FOC-42	22.66	26.00	16.25	-39.44
FOC-44	21.33	31.33	18.66	-14.28
FOC-47	24.33	36.00	17.91	-35.81
S.E. \pm	0.84	0.81	---	---
CD at 1 %	3.29	3.18	---	---

* - Mean of three replications
DAI – Days after incubation

Table 37. Mean colony radius (mm) of FOC isolates and *T. koningii* (9 DAI) with per cent inhibition of FOC isolates

Isolate No.	Growth of <i>F. oxysporum</i> f.sp. <i>carthami</i> *	Growth of <i>T. koningii</i> *	Radius (mm) of only pathogen	Per cent inhibition
FOC-5	30.66	45.33	32.50	5.66
FOC-10	22.00	36.00	19.50	-12.82
FOC-12	25.00	41.66	16.33	-53.09
FOC-14	31.33	43.66	31.83	1.57
FOC-16	22.00	36.33	25.57	13.98
FOC-18	19.33	27.00	17.25	-12.05
FOC-21	20.66	36.33	20.41	-1.20
FOC-24	26.33	41.33	26.33	0.00
FOC-27	23.33	30.66	21.58	-8.10
FOC-29	22.00	36.00	32.08	31.42
FOC-30	18.00	33.00	25.91	30.54
FOC-32	23.66	40.66	22.75	-4.00
FOC-35	16.33	45.66	21.50	24.05
FOC-36	21.33	50.66	24.91	14.39
FOC-39	30.66	41.33	27.00	-13.55
FOC-41	20.66	26.00	31.41	34.24
FOC-42	29.33	38.00	22.75	-28.92
FOC-44	27.00	43.33	21.25	-27.05
FOC-47	32.33	50.00	22.33	-44.78
S.E. \pm	0.85	0.82	---	---
CD at 1 %	3.33	3.22	---	---

* - Mean of three replications
DAI – Days after incubation

4.10.5 Efficacy of *Trichoderma lignorum* against *Fusarium oxysporum* f.sp. *carthami* in vitro.

The effect of bio control agent *Trichoderma lignorum* was tested against the 19 isolates of FOC by dual culture technique and per cent inhibition after 3, 6 and 9 days of incubation is presented in Table 38, 39, 40.

In case of *Trichoderma lignorum* at 3 DAI the mycelial growth of the isolates FOC 12, 14, 21, 32, 35 and 47 was reduced. The minimum reduction was observed in the isolate FOC-35 (1.43 per cent) while maximum reduction of 31.00 per cent was observed in the isolate FOC 47 (Table 38).

At 6 DAI, the mycelial growth of FOC-21, 32 and 47 isolates was reduced while in other isolates of FOC the growth was enhanced. The minimum growth inhibition of 0.48 per cent was observed in the isolate FOC 21 while maximum reduction of 38.48 per cent was observed in the isolate FOC 47 (Table 39).

At 9 DAI, *Trichoderma lignorum* could reduced the mycelial growth of the isolates FOC 12, 21, 42 and 47 only. In rest of the isolates the growth was enhanced. The minimum reduction in mycelial growth was 8.95 per cent in the isolate FOC-21 to 33.54 per cent in the isolates FOC-47 (Table 40).

Table 38. Mean colony radius (mm) of FOC isolates and *T. lignorum* (3 DAI) with per cent inhibition of FOC isolates

Isolate No.	Growth of <i>F. oxysporum</i> f.sp. <i>carthami</i> *	Growth of <i>T. lignorum</i> *	Radius (mm) of only pathogen	per cent inhibition
FOC-5	10.66	20.66	10.00	-6.60
FOC-10	20.66	22.66	14.75	-40.06
FOC-12	14.00	21.33	15.41	9.18
FOC-14	9.00	16.66	9.41	4.41
FOC-16	9.00	24.66	7.91	-13.71
FOC-18	21.33	21.33	11.16	-91.12
FOC-21	10.66	16.33	11.16	4.48
FOC-24	15.33	20.66	11.50	-33.30
FOC-27	9.33	16.33	8.00	-16.62
FOC-29	14.33	21.33	10.00	-43.30
FOC-30	16.00	20.66	12.16	-31.53
FOC-32	8.33	22.00	8.50	2.00
FOC-35	11.00	18.66	11.16	1.43
FOC-36	11.33	16.00	9.33	-22.22
FOC-39	19.00	23.00	12.16	-56.25
FOC-41	12.66	17.33	11.33	-11.73
FOC-42	17.33	20.66	12.16	-42.72
FOC-44	13.66	22.66	12.66	-7.85
FOC-47	9.66	31.33	14.00	31.00
S.E. \pm	0.65	0.80	---	---
CD at 1 %	2.55	3.14	---	---

* - Mean of three replications
DAI – Days after incubation

Table 39. Mean colony radius (mm) of FOC isolates and *T. lignorum* (6 DAI) with per cent inhibition of FOC isolates

Isolate No.	Growth of <i>F. oxysporum</i> f.sp. <i>carthami</i> *	Growth of <i>T. lignorum</i> *	Radius (mm) of only pathogen	Per cent inhibition
FOC-5	17.00	33.66	15.50	-9.67
FOC-10	24.66	31.66	15.41	-60.02
FOC-12	20.66	26.33	20.25	-2.02
FOC-14	19.66	22.66	11.33	-73.52
FOC-16	15.66	32.66	11.50	-36.17
FOC-18	30.00	25.00	16.50	-81.81
FOC-21	16.33	22.00	16.41	0.48
FOC-24	22.33	28.00	15.63	-42.86
FOC-27	16.00	22.66	11.33	-41.21
FOC-29	21.33	30.00	16.50	-29.27
FOC-30	19.66	27.33	16.16	-21.65
FOC-32	13.00	31.33	14.41	9.78
FOC-35	18.66	24.00	16.00	-16.62
FOC-36	20.66	20.00	11.50	-79.65
FOC-39	25.00	36.33	15.83	-57.92
FOC-41	20.66	22.66	16.00	-29.12
FOC-42	18.66	27.33	17.83	-4.65
FOC-44	23.66	30.66	20.00	-18.30
FOC-47	12.66	42.00	20.58	38.48
S.E. \pm	0.81	0.90	---	---
CD at 1 %	3.18	3.53	---	---

* - Mean of three replications
DAI – Days after incubation

Table 40. Mean colony radius (mm) of FOC isolates and *T. lignorum* (9 DAI) with per cent inhibition of FOC isolates

Isolate No.	Growth of <i>F. oxysporum</i> f.sp. <i>carthami</i> *	Growth of <i>T. lignorum</i> *	Radius (mm) of only pathogen	per cent inhibition
FOC-5	26.00	44.00	21.58	-20.48
FOC-10	31.33	40.66	22.66	-38.23
FOC-12	22.66	33.33	31.16	27.29
FOC-14	22.66	30.00	16.58	-36.67
FOC-16	20.66	40.66	20.16	-2.45
FOC-18	36.66	31.00	21.16	-73.22
FOC-21	20.33	30.00	22.33	8.95
FOC-24	29.33	36.33	21.50	-36.41
FOC-27	18.66	29.33	17.75	-5.12
FOC-29	30.66	37.33	20.25	-51.40
FOC-30	27.33	34.00	21.25	-28.61
FOC-32	20.66	41.33	17.75	-16.39
FOC-35	27.33	34.66	23.83	-14.68
FOC-36	26.33	30.00	14.66	-79.57
FOC-39	31.33	42.00	21.66	-44.62
FOC-41	25.33	36.66	22.41	-13.02
FOC-42	21.33	33.00	24.00	11.12
FOC-44	30.66	41.33	21.91	-39.91
FOC-47	18.66	61.33	28.08	33.54
S.E. \pm	0.78	0.87	---	---
CD at 1 %	3.06	3.41	---	---

* - Mean of three replications
DAI – Days after incubation

4.11. Evaluation of fungicides *in vitro*

Five fungitoxicants namely Carbendazim, Copper oxychloride, Captan, Thiram and Propiconazole were evaluated at 1, 10, 25, 50, 100, 500 and 1000 $\mu\text{g} / \text{ml}$ concentrations against 19 isolates of FOC and per cent inhibition in mycelial growth is presented in following pages. From the results it was observed that growth inhibition in all the isolates increased with corresponding increase in concentration of the fungicides.

4.11.1 Evaluation of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* (FOC) to Carbendazim

Perusal of results presented in Table 41 and Fig.15 indicated that at lower concentration ($1 \mu\text{g}/\text{ml}^{-1}$) of Carbendazim, the isolate from Parbhani district FOC-18 showed maximum inhibition of mycelial growth (100 %) followed by the isolate from Beed district FOC-5 (88.88 %). On the other hand the isolates FOC-14, 29, 39 and 42 observed minimum growth inhibition than other isolates of FOC.

The isolates FOC 18 showed highest growth inhibition (100 %) at all concentrations of Carbendazim. The lowest growth inhibition was observed FOC 39 (67.03 %) at $10 \mu\text{g}/\text{ml}^{-1}$ of Carbendazim concentration. At $25 \mu\text{g} / \text{ml}^{-1}$ concentration isolate FOC 18 was completely inhibited followed by isolates FOC 21 with 90.74 per cent, FOC 24 with 90.07 per cent, FOC 30 with 92.96 per cent and FOC 36 with 91.85 per cent inhibition. All above isolates had significantly more per cent inhibition than rest of the isolates.

The isolates FOC 18 showed maximum (100 per cent) growth inhibition at $50 \mu\text{g} / \text{ml}^{-1}$ concentration. The same trend was

Table 41. Per cent growth inhibition of FOC isolates with different concentrations of carbendazim

Isolate No.	*1 µg	*10µg	*25 µg	*50 µg	*100 µg	*500 µg	*1000 µg	Mean
FOC-5	88.88 (70.56)	89.62 (71.20)	89.62 (71.20)	90.74 (72.29)	91.11 (72.66)	93.33 (75.28)	93.33 (72.28)	90.95
FOC-10	79.25 (62.96)	82.59 (65.35)	84.81 (67.08)	88.14 (69.85)	91.48 (73.03)	92.96 (74.70)	94.81 (76.98)	87.72
FOC-12	77.03 (61.35)	80.74 (63.98)	86.66 (68.57)	89.25 (70.86)	90.74 (72.28)	94.07 (76.02)	100.00 (89.98)	88.35
FOC-14	60.74 (51.19)	72.59 (58.43)	75.92 (60.60)	79.05 (62.75)	82.96 (62.63)	88.14 (70.02)	93.33 (75.13)	78.96
FOC-16	83.70 (66.23)	86.66 (68.57)	89.25 (70.86)	89.99 (71.67)	92.96 (74.70)	93.70 (75.73)	94.07 (76.02)	90.04
FOC-18	100.00 (89.98)	100.00 (89.98)	100.00 (89.98)	100.00 (89.98)	100.00 (89.98)	100.00 (89.98)	100.00 (89.98)	100.00
FOC-21	87.40 (69.23)	88.88 (70.52)	90.74 (72.29)	94.07 (76.50)	94.07 (75.92)	94.44 (76.38)	100.00 (89.98)	92.80
FOC-24	88.14 (69.85)	90.00 (71.61)	90.07 (71.63)	92.22 (73.88)	93.33 (75.05)	93.70 (75.57)	94.07 (75.92)	91.74
FOC-27	73.33 (58.92)	76.29 (60.85)	80.00 (63.43)	82.22 (65.07)	90.74 (72.33)	93.70 (75.57)	94.07 (75.92)	84.33
FOC-29	65.18 (53.83)	70.36 (57.01)	74.81 (59.91)	79.25 (62.89)	82.59 (65.35)	94.80 (76.97)	91.48 (73.03)	79.78
FOC-30	87.40 (69.23)	90.74 (72.29)	92.96 (74.70)	93.33 (75.28)	93.70 (75.73)	94.44 (76.38)	100.00 (89.98)	93.22
FOC-32	78.14 (62.12)	81.11 (64.23)	82.96 (65.63)	86.66 (68.57)	88.14 (69.94)	90.37 (71.92)	93.33 (75.13)	85.81
FOC-35	77.03 (61.36)	80.74 (63.96)	86.66 (68.65)	89.23 (70.84)	90.74 (72.33)	94.07 (76.02)	95.55 (77.86)	87.72
FOC-36	87.03 (68.89)	89.25 (70.86)	91.85 (73.42)	92.59 (74.28)	93.70 (75.48)	9.07 (76.02)	94.44 (77.04)	91.84
FOC-39	64.44 (53.40)	67.03 (54.95)	69.25 (56.31)	72.59 (58.43)	76.29 (60.86)	82.58 (65.34)	89.99 (71.56)	74.59
FOC-41	76.29 (60.85)	79.25 (62.89)	82.96 (65.63)	86.29 (68.26)	89.22 (70.87)	90.74 (72.29)	92.22 (73.88)	85.28
FOC-42	63.70 (52.95)	67.40 (55.17)	71.48 (57.71)	77.03 (61.35)	81.48 (64.50)	85.92 (67.98)	90.74 (72.29)	76.82
FOC-44	85.55 (67.65)	88.88 (70.52)	90.74 (72.29)	91.85 (73.42)	94.07 (76.50)	95.55 (78.02)	100.00 (89.98)	92.37
FOC-47	77.03 (61.35)	79.62 (63.16)	82.96 (65.63)	85.18 (67.38)	90.36 (71.91)	89.62 (71.20)	91.85 (73.42)	85.23
Mean	79.96	82.19	84.93	87.35	89.87	92.43	94.90	87.23
S.E. ±	0.93	0.59	0.80	1.13	1.18	1.35	1.21	
CD at 1 %	3.44	2.18	2.96	4.19	4.37	5.00	4.48	

* - Mean of three replications

- Figures in parenthesis are angular transformed values.

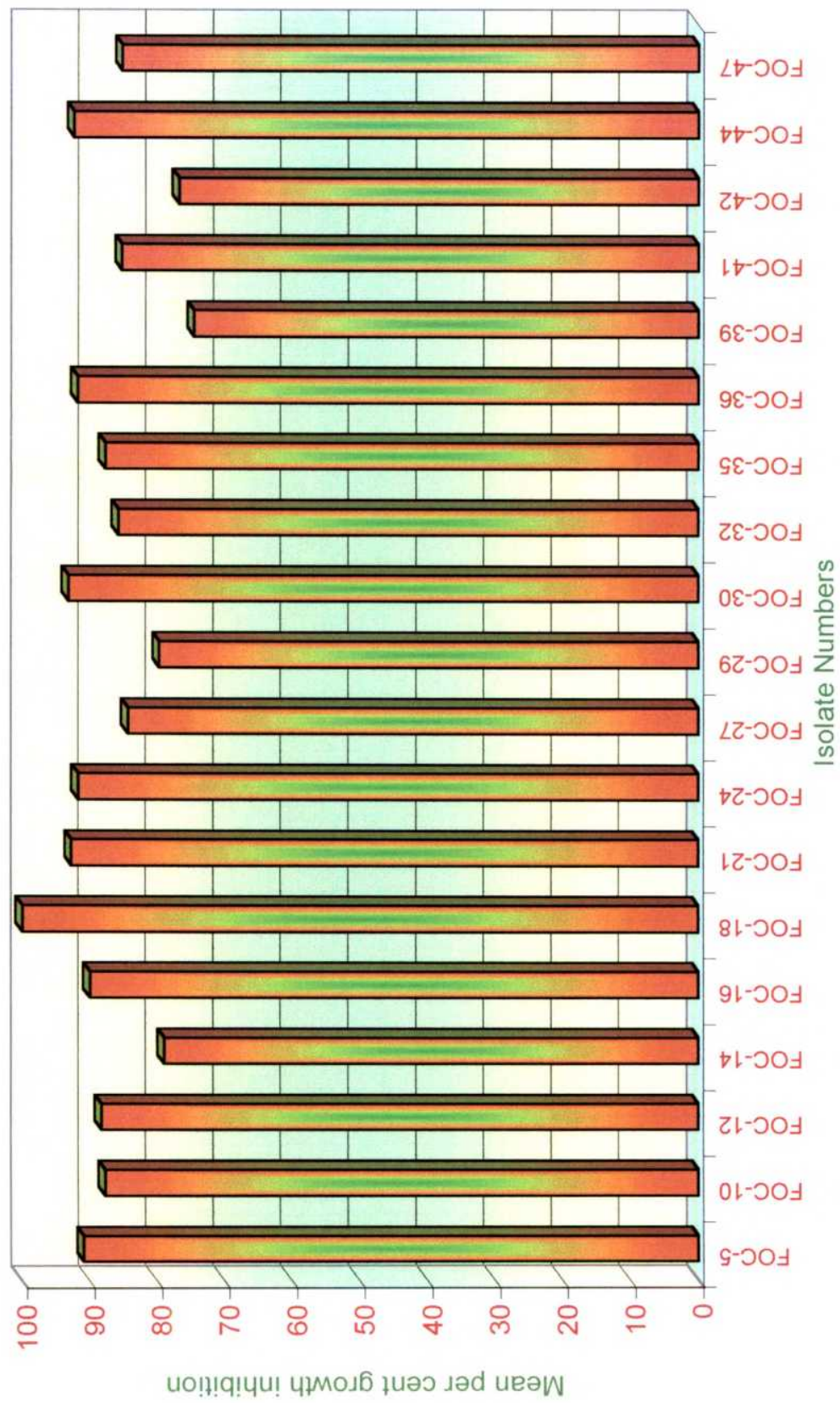


Fig.15. Mean per cent growth inhibition of *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates with carbendazim

observed even at 100, 500 and 1000 $\mu\text{g}/\text{ml}^{-1}$ of Carbendazim concentrations also.

At 1000 $\mu\text{g}/\text{ml}^{-1}$ of Carbendazim, highest growth inhibition (100 %) was observed in the isolates FOC 12, 18, 21, 30 and 44.

Based on mean per cent growth inhibition the isolate from Parbhani district FOC 18 (Plate 10A) was highly sensitive to Carbendazim followed by the isolates FOC 5, 16, 21, 30, 36 and 44.

Mean per cent growth inhibition at different concentrations in all the isolates varied from 79.96 to 94.90 per cent. The mean growth inhibition increased with increase in concentration of Carbendazim.

4.11.2 Evaluation of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* (FOC) to Copper oxychloride

Perusal of results from Table 42 and Fig.16 indicated that at lower concentration (1 $\mu\text{g}/\text{ml}^{-1}$) of Copper oxychloride the isolate from Osmanabad district FOC 24 exhibited maximum growth inhibition (18.88 %) and minimum growth inhibition (1.85 %) was observed in the isolate FOC 18 from Parbhani district.

At 10 $\mu\text{g}/\text{ml}^{-1}$ of Copper oxychloride, maximum inhibition was observed in the isolate FOC 24 followed by other isolates FOC 32, 41 and 44.

FOC 24 isolate showed maximum growth inhibition at 1 $\mu\text{g}/\text{ml}^{-1}$ to 500 $\mu\text{g}/\text{ml}$ than other isolates. At 500 $\mu\text{g}/\text{ml}$ concentration, maximum growth inhibition was noticed in the isolates FOC 5 (52.22 %) and FOC 10 (42.96 %) and minimum growth inhibition was noticed in the isolates FOC 42 (11.85 %).

Table 42. Per cent growth inhibition of FOC isolates with different concentrations of copper oxychloride

Isolate No.	*1 µg	*10µg	*25 µg	*50 µg	*100 µg	*500 µg	*1000 µg	Mean
FOC-5	8.14 (16.55)	8.88 (17.32)	11.11 (19.45)	14.81 (22.62)	17.40 (24.65)	52.22 (46.26)	68.14 (55.63)	25.81
FOC-10	7.03 (15.37)	7.40 (15.77)	7.77 (16.17)	10.74 (19.12)	12.96 (21.09)	42.96 (40.94)	54.07 (47.33)	20.41
FOC-12	4.81 (12.63)	7.03 (15.37)	9.25 (17.70)	8.14 (16.57)	10.71 (19.10)	18.88 (25.74)	45.55 (42.44)	14.91
FOC-14	2.96 (9.89)	4.81 (12.66)	6.29 (14.52)	8.14 (16.57)	8.88 (17.32)	22.96 (28.61)	52.22 (46.26)	15.18
FOC-16	2.59 (9.24)	6.29 (14.52)	8.51 (16.95)	10.33 (18.74)	12.96 (21.09)	21.48 (27.60)	42.96 (40.94)	15.01
FOC-18	1.85 (7.81)	4.44 (12.15)	6.29 (14.52)	7.40 (15.77)	10.74 (19.12)	35.18 (36.37)	52.96 (46.69)	16.98
FOC-21	9.62 (18.06)	10.00 (18.41)	12.96 (21.08)	12.96 (21.09)	14.81 (22.62)	21.85 (27.86)	48.51 (44.14)	18.67
FOC-24	18.88 (25.74)	20.37 (26.82)	21.11 (27.34)	22.96 (28.61)	24.81 (29.85)	27.40 (31.55)	30.74 (33.66)	23.75
FOC-27	3.33 (10.50)	5.92 (14.05)	7.03 (15.37)	10.37 (18.77)	10.37 (18.77)	13.70 (21.71)	20.74 (27.08)	10.20
FOC-29	5.18 (13.15)	7.03 (15.37)	9.25 (17.70)	12.59 (20.77)	15.18 (22.92)	20.74 (27.08)	38.51 (38.34)	15.49
FOC-30	6.29 (14.52)	7.40 (15.77)	10.37 (18.77)	11.48 (19.79)	12.96 (21.08)	16.29 (23.79)	29.99 (33.19)	13.54
FOC-32	8.14 (16.55)	11.48 (19.79)	17.40 (24.64)	19.25 (26.01)	19.62 (26.28)	24.07 (29.32)	37.77 (37.91)	19.67
FOC-35	6.29 (14.52)	9.62 (18.06)	9.99 (18.41)	12.22 (20.44)	15.92 (23.48)	29.62 (22.95)	52.96 (46.69)	19.52
FOC-36	3.70 (11.05)	9.25 (17.70)	9.62 (18.06)	12.96 (21.08)	14.81 (22.66)	17.03 (24.36)	41.85 (40.30)	15.60
FOC-39	8.14 (16.57)	8.80 (17.24)	12.96 (21.08)	15.92 (23.50)	17.77 (24.90)	20.37 (26.82)	28.51 (32.26)	16.07
FOC-41	11.85 (20.12)	15.18 (22.92)	17.40 (24.64)	18.14 (25.20)	19.62 (26.29)	21.85 (27.86)	25.18 (30.10)	18.46
FOC-42	7.03 (15.37)	8.51 (16.95)	8.51 (16.95)	9.62 (18.06)	10.37 (18.78)	11.85 (20.12)	21.48 (27.60)	11.05
FOC-44	9.62 (18.06)	12.59 (20.77)	14.44 (22.32)	15.92 (23.50)	17.40 (24.62)	20.74 (27.08)	32.96 (35.02)	17.66
FOC-47	4.81 (12.66)	8.51 (16.95)	10.36 (18.76)	12.96 (21.08)	14.07 (22.01)	24.07 (29.36)	37.77 (37.91)	16.08
Mean	6.85	9.13	11.08	12.99	14.80	24.38	40.15	17.05
S.E. ±	0.34	0.36	0.39	0.44	0.64	0.65	0.61	---
CD at 1 %	1.26	1.33	1.44	1.63	2.37	2.41	2.26	---

* - Mean of three replications

- Figures in parenthesis are angular transformed values.

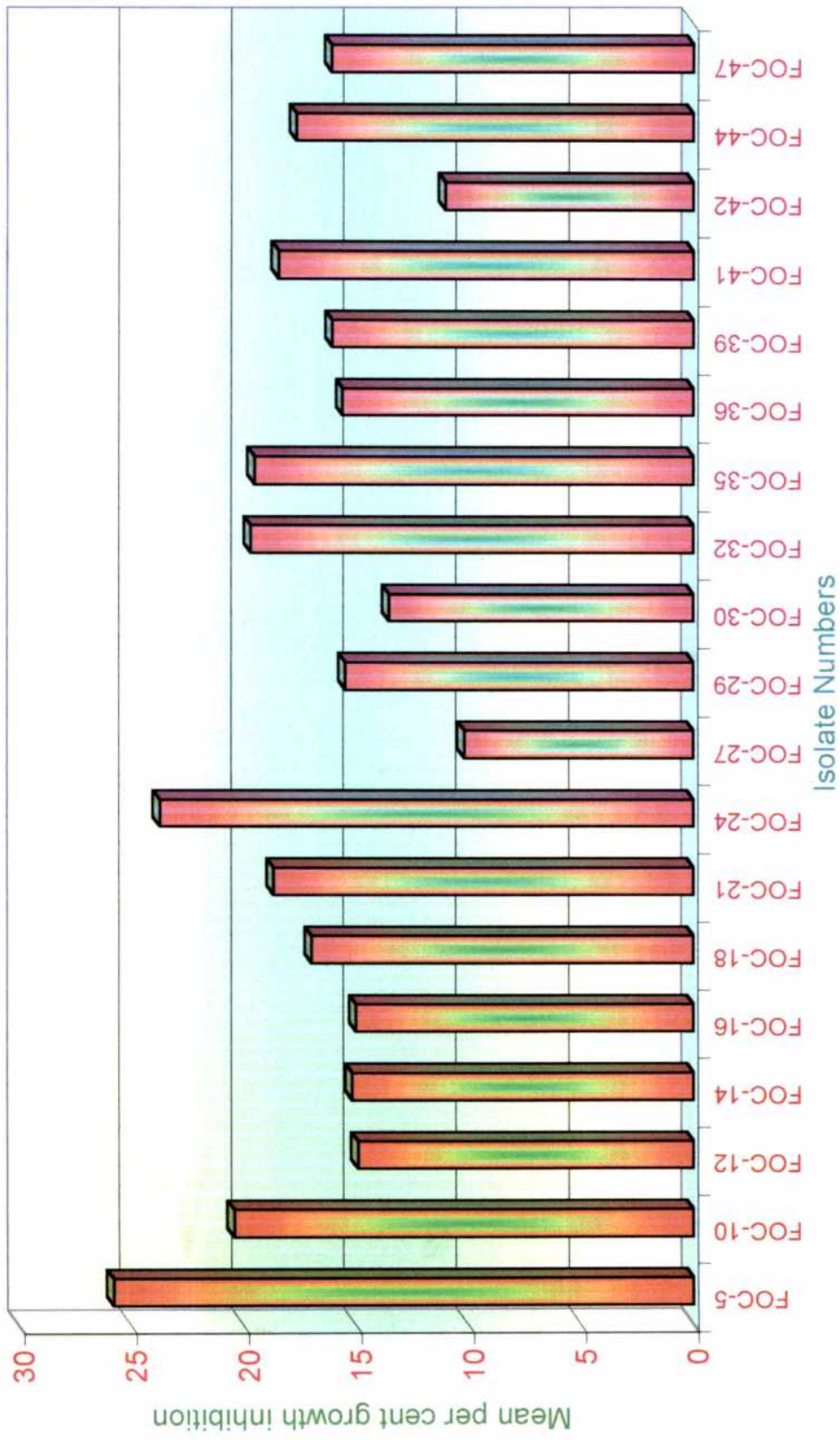


Fig.16. Mean per cent growth inhibition of *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates with Copper oxychloride

At 1000 $\mu\text{g/ml}^{-1}$ concentration, maximum growth inhibition was noticed in the isolate FOC 5 (68.14 %) followed by FOC 10 (54.07 %), FOC 14 (52.22 %), FOC 18 (52.96 %) and FOC 35 (52.96 %). The least growth inhibition was noticed in the isolate FOC 27 (13.70 %).

The mean per cent growth inhibition among the isolates varied from 10.20 to 25.81 per cent. Least inhibition was noticed in the isolate FOC 27 (Plate 10B) and highest inhibition was noticed in the isolate FOC 5.

Mean per cent growth inhibition among the isolates at different concentrations of Copper oxychloride varied from 6.85 to 40.15 per cent. The growth increased with corresponding increase in concentrations of Copper oxychloride.

4.11.3. Evaluation of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* (FOC) to Captan

Perusal of results from Table 43 and Fig. 17 indicated that maximum per cent growth inhibition was recorded in the isolate FOC 39 (78.51 %) followed by isolate FOC 44 (72.22 %) and minimum per cent inhibition was recorded in the isolate FOC 10 (2.96 %) at 1 $\mu\text{g /ml}^{-1}$ of Captan. At 10 $\mu\text{g/ml}^{-1}$ concentration maximum growth inhibition was observed in the isolate FOC 32 (87.03 %) followed by isolate FOC 39 (81.48 %) and minimum growth inhibition was observed in the isolate FOC 18 (5.92%). The isolate FOC 32 (91.85 %) was highly sensitive at 25 $\mu\text{g/ml}^{-1}$. At 25 $\mu\text{g/ml}^{-1}$, minimum or least growth inhibition was observed in the isolate FOC 42 (9.62 %) which was highly insensitive.

Table 43. Per cent growth inhibition of FOC isolates with different concentrations of Captan

Isolate No.	*1 µg	*10µg	*25 µg	*50 µg	*100 µg	*500 µg	*1000 µg	Mean
FOC-5	4.44 (12.15)	20.74 (27.08)	31.85 (34.35)	34.07 (35.68)	40.36 (39.43)	51.48 (45.84)	60.36 (50.97)	34.75
FOC-10	2.96 (9.89)	10.36 (18.76)	26.66 (31.08)	37.40 (37.69)	41.48 (40.08)	57.03 (49.03)	63.33 (52.73)	34.17
FOC-12	12.22 (20.44)	14.81 (22.62)	22.96 (28.61)	41.10 (39.86)	44.44 (41.80)	48.88 (44.35)	54.07 (47.33)	34.07
FOC-14	14.81 (22.60)	16.29 (23.79)	21.48 (27.60)	29.99 (33.19)	33.33 (35.24)	36.29 (37.03)	43.33 (41.15)	27.93
FOC-16	4.44 (12.15)	9.62 (18.06)	15.18 (22.92)	21.48 (27.60)	22.22 (28.10)	24.81 (30.28)	28.14 (32.03)	17.98
FOC-18	4.44 (12.15)	5.92 (14.07)	21.48 (27.60)	32.59 (34.80)	42.96 (40.94)	44.44 (41.79)	51.48 (45.84)	29.04
FOC-21	8.14 (16.57)	19.62 (26.28)	28.14 (32.03)	33.33 (35.24)	36.29 (37.03)	42.96 (40.94)	45.92 (42.65)	30.63
FOC-24	31.11 (33.89)	31.85 (34.35)	33.33 (35.24)	35.92 (36.81)	39.25 (38.78)	44.44 (41.79)	44.44 (41.80)	37.19
FOC-27	4.81 (12.63)	18.14 (25.20)	28.51 (32.26)	33.33 (35.24)	44.81 (42.39)	51.48 (45.84)	55.18 (47.96)	33.75
FOC-29	19.25 (26.02)	21.85 (27.86)	22.96 (28.61)	24.44 (29.61)	25.18 (30.11)	27.77 (31.78)	31.85 (34.35)	24.75
FOC-30	5.18 (13.15)	8.85 (17.29)	10.36 (18.76)	13.32 (21.39)	16.29 (23.79)	20.00 (26.55)	22.20 (28.09)	13.74
FOC-32	35.92 (36.81)	87.03 (68.89)	91.85 (72.72)	92.59 (74.28)	93.62 (75.47)	94.44 (76.38)	100 (89.98)	85.06
FOC-35	12.96 (21.08)	15.55 (23.21)	20.00 (26.55)	60.74 (51.19)	72.59 (58.43)	75.72 (60.55)	79.25 (63.25)	48.11
FOC-36	9.62 (18.06)	12.59 (20.77)	15.92 (23.50)	17.77 (24.92)	19.25 (26.01)	22.22 (28.10)	23.33 (28.84)	17.24
FOC-39	78.51 (62.37)	81.48 (64.50)	83.70 (66.23)	86.29 (68.26)	88.51 (70.22)	90.37 (72.32)	92.22 (73.88)	85.86
FOC-41	14.07 (22.01)	14.81 (22.62)	17.03 (24.36)	20.74 (27.07)	22.96 (28.61)	27.40 (31.55)	31.11 (33.89)	21.16
FOC-42	5.55 (13.61)	6.66 (14.94)	9.62 (18.06)	11.11 (19.45)	14.81 (22.62)	16.29 (23.79)	21.10 (27.32)	12.16
FOC-44	72.22 (58.19)	74.81 (59.91)	77.03 (61.35)	79.62 (63.19)	82.59 (65.35)	84.44 (66.87)	87.40 (69.36)	79.73
FOC-47	4.81 (12.63)	7.40 (15.77)	11.11 (19.45)	14.07 (22.01)	15.55 (23.19)	18.51 (25.47)	22.59 (28.36)	13.43
Mean	18.18	25.17	31.00	37.88	41.92	46.26	53.46	36.26
S.E. ±	0.46	0.54	0.57	0.80	0.76	1.14	1.19	---
CD at 1 %	1.70	2.00	2.11	2.96	2.81	4.22	4.41	---

* - Mean of three replications

- Figures in parenthesis are angular transformed values.

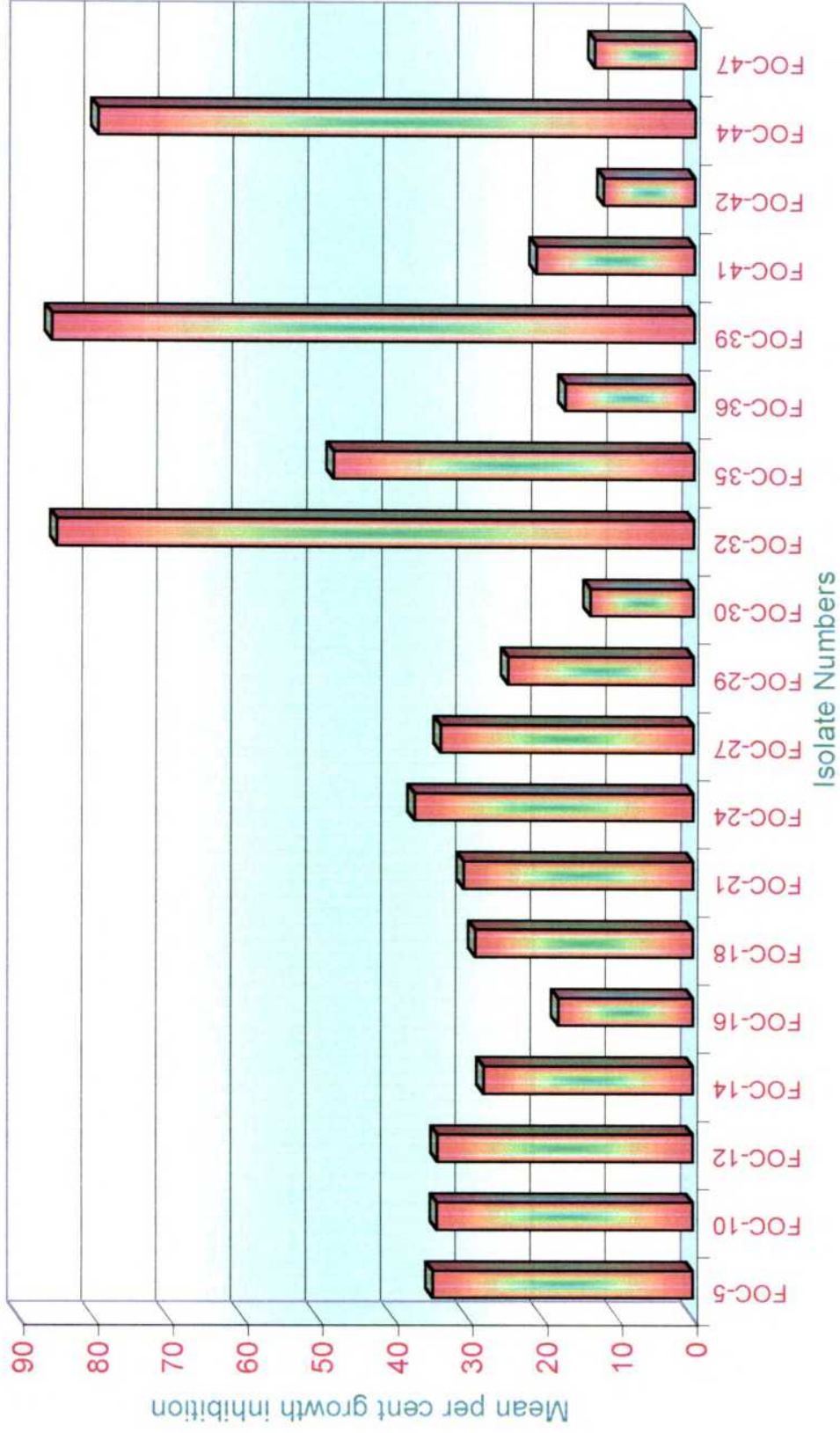


Fig.17. Mean per cent growth inhibition of *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates with Captan

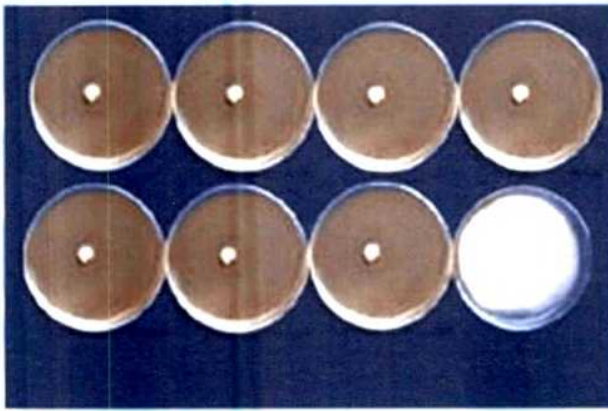


Plate 10 A. Effect of different concentrations of Carbendazim against mycelial growth of isolate FOC-18

Plate 10 B. Effect of different concentrations of Copper oxychloride against mycelial growth of isolate FOC-27

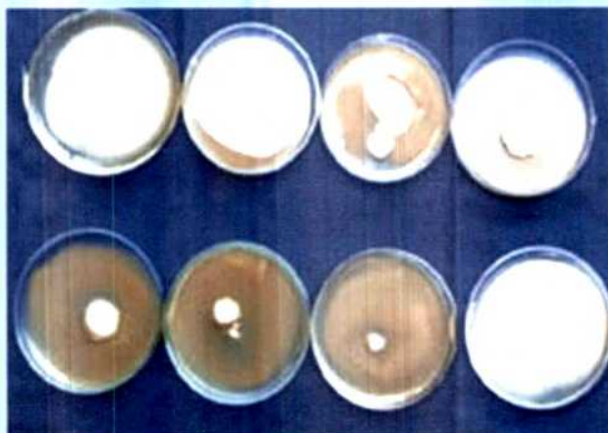
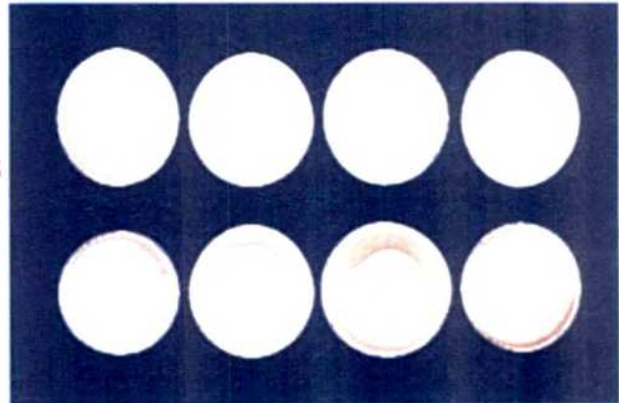


Plate 10 C. Effect of different concentrations of Capatan against mycelial growth of isolate FOC-32

Plate 10 D. Effect of different concentrations of Thiram against mycelial growth of isolate FOC-05

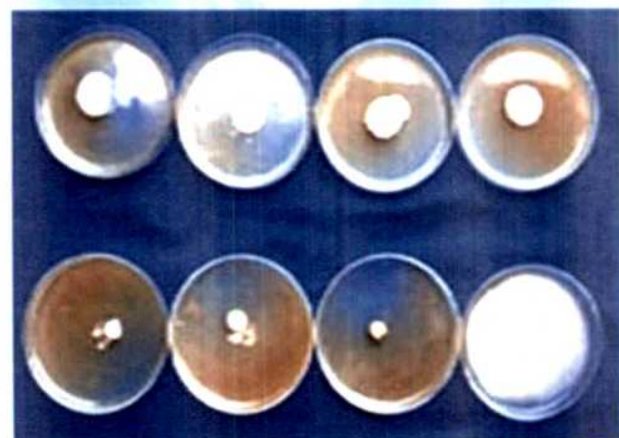


Plate 10.

At 50 $\mu\text{g/ml}^{-1}$ the isolate FOC 32 showed maximum inhibition of mycelial growth (92.59 %) and isolate FOC 42 showed minimum (11.11 %). Maximum inhibition was observed in FOC 32 at 100, 500 and 1000 $\mu\text{g/ml}^{-1}$ concentration while minimum in FOC 42 at above concentrations. This indicates that FOC 42 is resistant to Captan while FOC 32 and 39 is sensitive.

The mean growth inhibition among the isolate was maximum in the isolate was maximum in the isolate FOC 39 (85.86 %) and minimum in the isolate FOC 42 (12.16 %).

The mean growth inhibition among the isolates at different concentration varied from 18.18 to 53.46 per cent. The mean growth inhibition increased with increase in concentration of Captan.

4.11.4 Evaluation of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* (FOC) to Thiram

Perusal of results from Table 44 and Fig. 18 indicated that at lower concentration (1 $\mu\text{g/ml}$), the isolate FOC 5 (62.00 %) showed maximum growth inhibition, while the isolates FOC 44 and 47 showed no mycelial growth inhibition. At 10 $\mu\text{g/ml}^{-1}$ concentration FOC 5 showed maximum growth inhibition (63.00 %) and FOC 47 showed minimum growth (0.0%).

At 25 $\mu\text{g/ml}^{-1}$ of Thiram, maximum growth inhibition was observed in the isolate FOC 5 (64.33 %) followed by isolate FOC 14 (63.66 %) and minimum growth inhibition was observed in the isolate FOC 47 (9.00 %). At 50 and 100 $\mu\text{g/ml}^{-1}$ concentration of Thiram isolate

Table 44. Per cent growth inhibition of FOC isolates with different concentrations of Thiram

Isolate No.	*1 µg	*10µg	*25 µg	*50 µg	*100 µg	*500 µg	*1000 µg	Mean
FOC-5	62.00 (51.94)	63.00 (52.53)	64.33 (53.34)	69.33 (56.36)	72.66 (58.47)	73.66 (59.13)	85.66 (67.94)	70.09
FOC-10	46.67 (43.07)	48.00 (43.84)	58.66 (49.98)	63.33 (52.73)	68.33 (55.86)	76.00 (60.67)	82.00 (64.90)	63.28
FOC-12	30.66 (33.61)	42.66 (40.77)	60.66 (51.15)	62.66 (52.33)	71.66 (57.83)	77.00 (61.51)	78.66 (62.51)	60.57
FOC-14	57.66 (49.43)	62.33 (52.13)	63.66 (52.93)	64.66 (53.53)	70.33 (56.99)	72.66 (58.47)	81.33 (64.39)	67.52
FOC-16	41.33 (40.01)	42.33 (40.58)	52.33 (46.33)	57.33 (49.21)	68.33 (55.76)	72.66 (58.47)	73.33 (58.92)	58.23
FOC-18	28.66 (32.34)	40.66 (39.61)	52.33 (46.33)	53.00 (46.71)	60.66 (55.15)	68.33 (55.76)	72.66 (58.47)	53.76
FOC-21	39.66 (39.01)	48.66 (44.22)	50.66 (45.37)	58.66 (49.98)	63.00 (52.53)	69.33 (56.39)	75.33 (60.22)	57.90
FOC-24	38.33 (38.23)	38.33 (40.13)	51.33 (45.75)	62.66 (52.33)	71.33 (57.61)	71.33 (57.62)	71.66 (57.83)	57.85
FOC-27	26.66 (31.08)	49.66 (44.79)	50.66 (45.37)	57.66 (49.40)	60.66 (51.15)	67.33 (55.13)	71.33 (57.62)	54.85
FOC-29	31.33 (34.03)	36.00 (36.85)	40.66 (39.60)	40.66 (39.61)	43.66 (41.34)	62.00 (51.94)	67.33 (55.13)	45.95
FOC-30	24.66 (29.75)	30.66 (33.61)	43.00 (40.96)	45.33 (42.31)	58.66 (49.98)	68.66 (55.96)	69.00 (56.18)	48.57
FOC-32	34.33 (35.86)	38.66 (38.40)	41.33 (40.00)	43.33 (41.15)	52.33 (46.33)	52.33 (46.33)	63.00 (52.53)	45.61
FOC-35	25.66 (30.42)	26.66 (31.07)	28.66 (32.35)	30.66 (33.61)	48.66 (44.22)	51.33 (45.75)	59.66 (50.57)	38.76
FOC-36	18.66 (25.58)	21.33 (27.49)	28.66 (32.34)	31.33 (34.03)	49.00 (44.43)	56.66 (48.84)	65.66 (54.13)	38.76
FOC-39	13.66 (21.67)	16.00 (23.56)	23.66 (29.07)	32.66 (34.84)	41.33 (40.00)	43.66 (41.34)	49.33 (44.61)	31.47
FOC-41	11.66 (19.95)	17.00 (24.34)	17.33 (24.57)	20.00 (26.54)	39.00 (38.63)	46.00 (42.70)	53.66 (47.09)	29.23
FOC-42	14.00 (21.83)	16.33 (23.82)	19.33 (26.06)	21.33 (27.49)	29.33 (32.76)	31.33 (34.03)	41.33 (40.00)	24.71
FOC-44	0.00 (0.00)	6.66 (14.92)	10.00 (18.41)	11.00 (19.35)	12.66 (20.83)	12.66 (20.80)	31.33 (34.03)	12.04
FOC-47	0.00 (0.00)	0.00 (0.00)	9.00 (17.45)	22.66 (28.41)	33.00 (35.04)	30.66 (33.60)	42.66 (40.77)	19.71
Mean	28.71	33.94	40.32	44.64	53.39	58.08	64.99	46.29
S.E. ±	1.04	1.05	0.81	0.75	1.04	1.13	1.04	
CD at 1 %	3.85	3.89	3.00	2.78	3.85	4.19	3.85	

* - Mean of three replications

- Figures in parenthesis are angular transformed values.

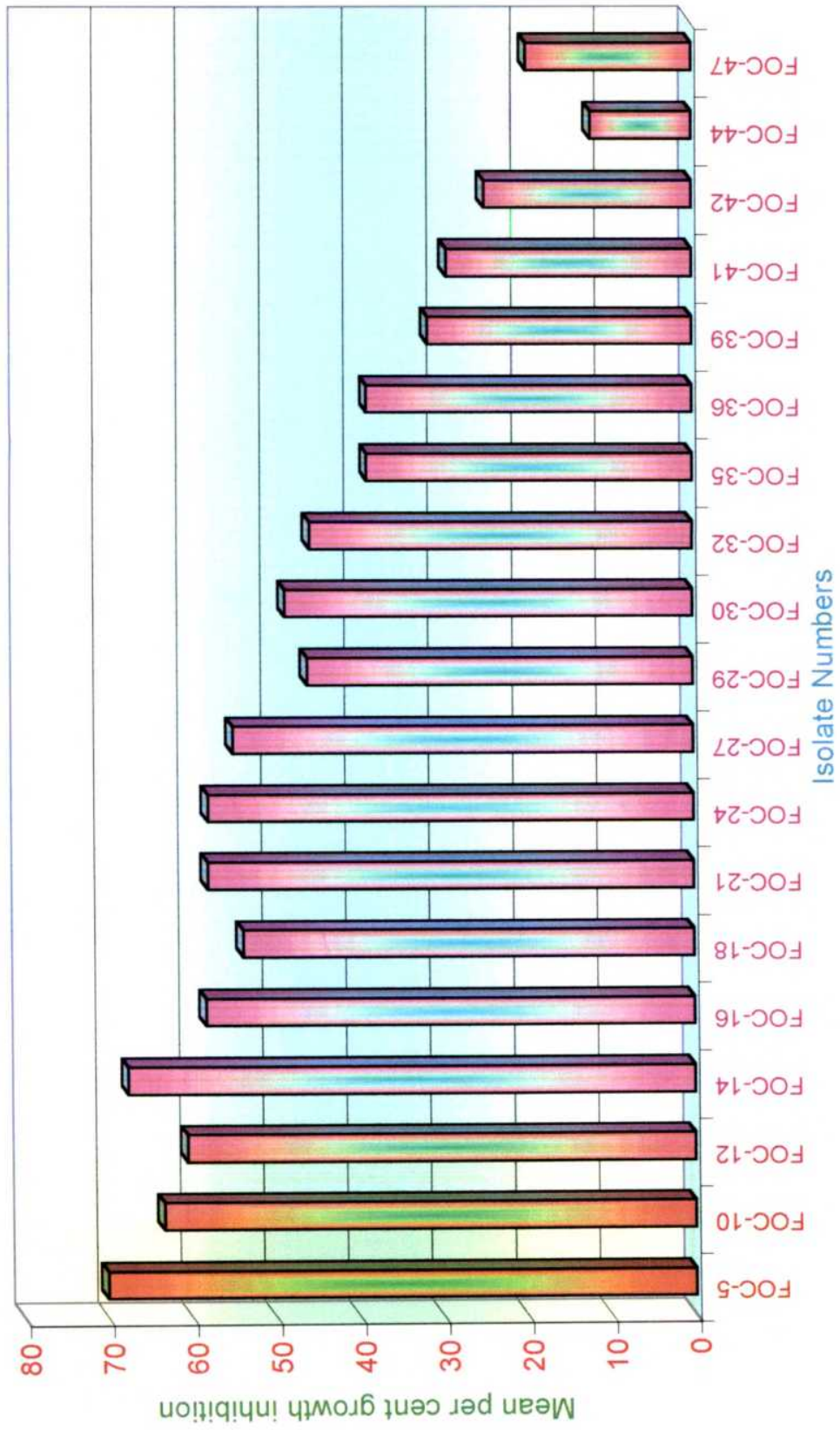


Fig.18. Mean per cent growth inhibition of *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates with Thiram

FOC 5 showed maximum inhibition and FOC 47 showed minimum inhibition.

At 500 $\mu\text{g/ml}^{-1}$ of Thiram, high growth inhibition was observed in the isolate FOC 12 (77.00 %) followed by isolates FOC 10 (76.00%), FOC-5 (73.66 %). Least growth inhibition was observed in the isolate FOC 44 (12.66%). At 1000 $\mu\text{g/ml}$ of Thiram, maximum growth inhibition was observed in the isolate FOC 5 (85.66 %) followed by isolate FOC 10 (82.00 %), FOC 14 (81.33 %) and FOC 12 (78.66 %) having more growth inhibition than other isolates.

Based on mean per cent growth inhibition, the isolate from Beed district (FOC 5) was highly sensitive to Thiram while the isolates from Aurangabad district (FOC 44 and 47) were least sensitive to the fungicide.

Mean per cent growth inhibition among the isolates at different concentrations varied from 28.71 to 64.99 per cent. The mean growth inhibition increased with corresponding increase in concentrations.

4.11.5 Evaluation of 19 isolates of *Fusarium oxysporum* f.sp. *carthami* (FOC) to Propiconazole

Perusal of results from Table 45 Fig. 19 indicated that at lower concentration 1 $\mu\text{g/ml}^{-1}$ the isolate FOC 47 (65.92 %) showed maximum growth inhibition followed by isolate FOC 42 (52.59 %). Minimum growth inhibition was observed in the isolate FOC 18 (4.81 %).

At 10 $\mu\text{g/ml}^{-1}$, the isolate FOC 47 (68.14 %) again showed maximum growth inhibition followed by isolates FOC 30 (54.07 %), FOC

32 (54.07 %) and FOC 42 (54.81 %). And minimum growth inhibition was again observed in the isolate FOC 18 (9.36 %).

Isolate FOC 47 showed maximum growth inhibition and isolate FOC 18 minimum growth inhibition again at 25 and 50 $\mu\text{g/ml}^{-1}$ concentration of Propiconazole.

At 100 $\mu\text{g/ml}^{-1}$ of concentration of Propiconazole fungicide, maximum growth inhibition and highly sensitive reaction was observed in the isolate FOC 42 (79.25 %) followed by isolate FOC 47 (76.29 %). Minimum growth inhibition was observed in the isolate FOC 44 (27.03 %) followed by isolate FOC 18 (29.99 %).

Isolate FOC 42 (87.33 %) showed maximum growth inhibition followed by isolates FOC 29 (84.81 %), FOC 24 (82.22 %), FOC 47 (81.85 %) and FOC 36 (81.11 %). Minimum growth inhibition was observed in the isolate FOC 35 (40.36 %). At 1000 $\mu\text{g/ml}^{-1}$ concentration of Propiconazole, highest growth inhibition was observed in the isolates FOC 20, 36 and 42 (100 %) which were highly sensitive. The minimum growth inhibition was observed in the isolate FOC 35 (52.59 %).

Maximum growth inhibition was observed in the isolate FOC 47 (75.02 %) which was highly sensitive and FOC 18 which was least sensitive to the fungicide.

Mean per cent growth inhibition, of all the isolates at different concentration varied from 28.33 to 80.24 per cent. The mean growth inhibition was increased with increase in concentrations of Propiconazole.

Table 45. Per cent growth inhibition of FOC isolates with different concentrations of Propiconazole

Isolate No.	*1 µg	*10µg	*25 µg	*50 µg	*100 µg	*500 µg	*1000 µg	Mean
FOC-5	29.99 (33.03)	32.59 (34.80)	41.11 (39.87)	43.70 (41.37)	52.22 (46.26)	65.18 (53.83)	76.29 (60.86)	48.72
FOC-10	28.51 (32.24)	30.37 (33.42)	32.59 (34.80)	35.92 (36.81)	41.83 (40.29)	45.55 (42.44)	65.18 (53.85)	39.99
FOC-12	31.11 (33.89)	35.92 (36.81)	41.85 (40.30)	44.81 (41.62)	51.85 (46.05)	68.14 (55.63)	84.44 (66.87)	51.16
FOC-14	18.14 (25.18)	21.85 (27.86)	24.07 (29.36)	25.18 (30.10)	54.07 (47.33)	63.70 (52.95)	78.51 (62.41)	40.79
FOC-16	22.96 (28.61)	28.14 (32.02)	30.74 (33.66)	32.59 (34.80)	41.83 (40.29)	51.48 (45.84)	67.40 (55.18)	39.30
FOC-18	4.81 (12.63)	9.36 (17.81)	16.29 (23.79)	20.74 (27.08)	29.99 (33.19)	45.18 (42.22)	85.92 (67.98)	30.32
FOC-21	19.25 (26.00)	29.25 (32.72)	34.81 (36.14)	51.48 (45.84)	61.85 (51.84)	73.70 (59.16)	88.88 (70.56)	51.31
FOC-24	24.07 (29.32)	30.74 (33.66)	43.70 (41.37)	54.81 (47.76)	63.70 (52.95)	82.22 (65.07)	92.59 (74.28)	55.97
FOC-27	8.88 (17.25)	19.25 (26.01)	22.96 (28.61)	31.11 (33.89)	45.92 (42.65)	54.07 (47.33)	65.18 (53.83)	35.34
FOC-29	29.62 (32.93)	36.29 (37.02)	45.92 (42.64)	54.81 (47.76)	71.48 (57.71)	84.81 (67.17)	100.00 (89.98)	60.41
FOC-30	48.14 (43.92)	54.07 (47.33)	55.92 (48.39)	59.99 (50.76)	68.14 (55.64)	77.77 (61.87)	85.92 (67.96)	64.28
FOC-32	46.66 (43.07)	54.07 (47.33)	57.40 (49.25)	65.18 (53.83)	70.36 (57.01)	80.74 (64.00)	87.77 (69.53)	66.02
FOC-35	25.55 (30.34)	19.25 (25.97)	20.74 (27.08)	25.18 (30.10)	33.70 (35.46)	40.36 (39.43)	52.59 (46.47)	31.05
FOC-36	34.74 (36.08)	41.85 (40.30)	42.96 (40.94)	49.62 (44.77)	65.92 (54.29)	81.11 (64.23)	100.00 (89.98)	59.45
FOC-39	20.37 (26.78)	24.07 (29.36)	30.36 (33.42)	34.81 (36.15)	42.96 (40.94)	48.88 (44.35)	63.33 (52.73)	37.82
FOC-41	14.44 (22.32)	19.99 (26.55)	20.37 (26.82)	23.33 (28.84)	37.03 (37.47)	56.66 (48.82)	74.81 (59.89)	35.23
FOC-42	52.59 (46.47)	54.81 (47.75)	57.40 (49.45)	65.92 (54.28)	79.25 (62.91)	87.03 (68.92)	100.00 (89.98)	71.00
FOC-44	12.59 (20.74)	19.25 (26.00)	20.74 (27.08)	23.70 (29.11)	27.03 (31.31)	54.07 (47.33)	65.92 (54.28)	31.90
FOC-47	65.92 (54.28)	68.14 (55.64)	70.37 (57.02)	72.59 (58.43)	76.29 (60.88)	81.85 (64.78)	89.99 (71.60)	75.02
Mean	28.33	33.11	37.38	42.91	53.44	65.39	80.24	48.68
S.E. ±	1.19	0.83	0.74	0.84	0.79	0.89	0.97	--
CD at 1 %	4.41	3.07	2.74	3.11	2.93	3.30	3.59	--

* - Mean of three replications

- Figures in parenthesis are angular transformed values.

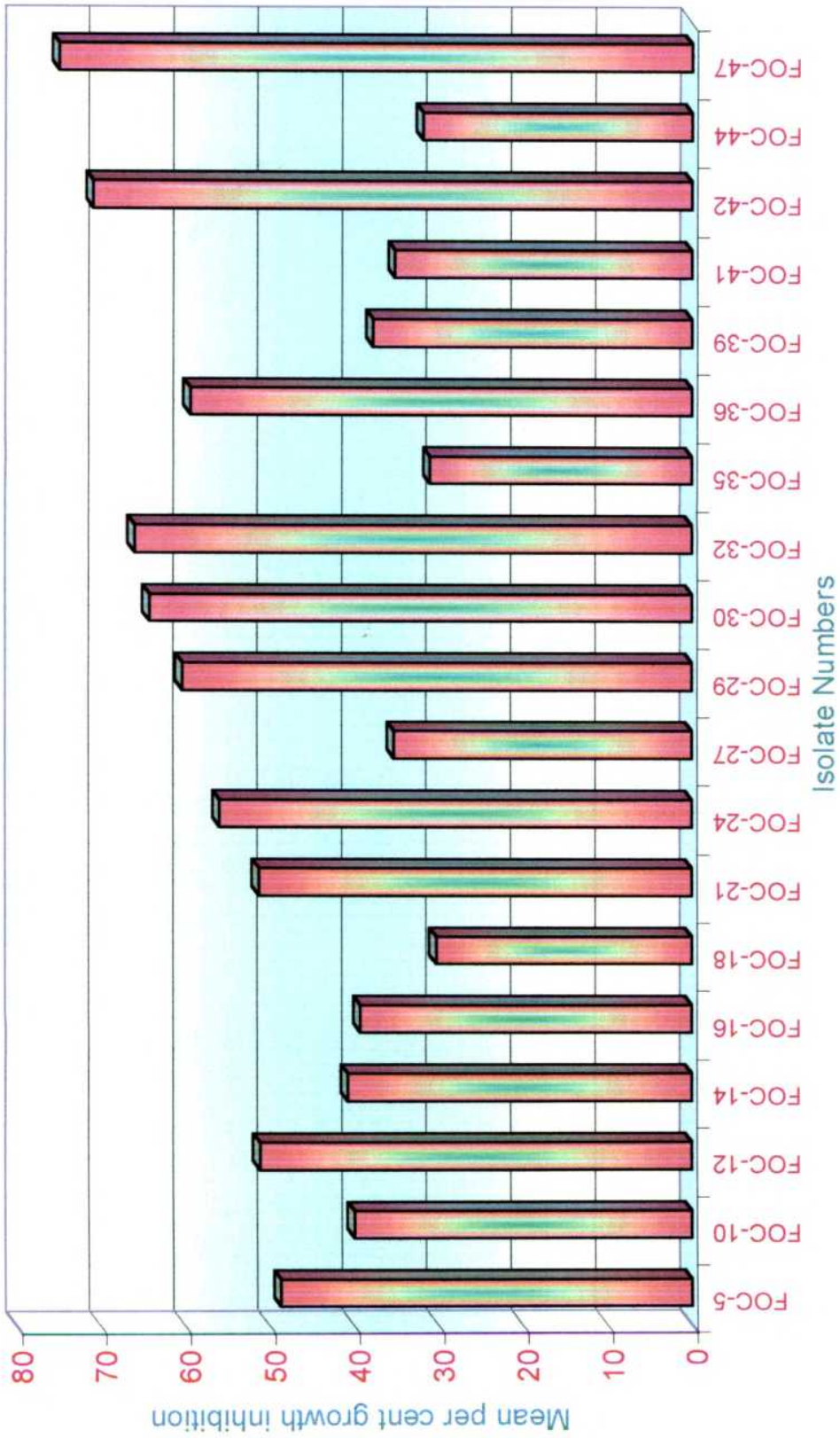


Fig.19. Mean per cent growth inhibition of *Fusarium oxysporum* f.sp. *carthami* (FOC) isolates with propiconazole

DISCUSSION



CHAPTER-V

DISCUSSION

The wilt of safflower (*Carthamus tinctorius* L.) caused by *Fusarium oxysporum* f.sp. *carthami* Klisiewicz and Houston is an important soil borne disease wherever the crop is grown. The disease is endemic in certain regions of India particularly in Maharashtra and Uttar Pradesh. It is spreading widely and causing yield losses up to 80 per cent (Kalpana Sastry and Chattopadhyay, 1997). Varietal resistance seems to be the most economical and effective means of control of the disease. Screening trials for evolving varieties resistant to wilt have often given inconsistent results. This could be due to the variability within the species, but information on this aspect is lacking. Hence, studies were undertaken with nineteen isolates of the *Fusarium oxysporum* f.sp. *carthami* obtained from safflower growing areas to determine their nature of seed borne, morphological, cultural and pathogenic variability, genetic variation, screening for host resistance, biological management through *Trichoderma* spp and *in vitro* evaluation of fungicides against pathogen were undertaken. The results of the experiments were critically examined and discussed here under.

5.1. Survey and collection of diseased samples

Random survey was taken during *rabi* 2005-06 and 2006-07 during safflower growing season to know the incidence of safflower wilt in farmer's field of Marathwada region.

The results indicated that, farmers grew Local, Sharada, Bhima and PBNS-12 varieties of safflower. The maximum incidence was recorded in Hingoli district in both year of survey. Most of the farmers have used the same variety and hence incidence has increased. The wilt disease of safflower has been reported by several workers like Singh *et al.* (1975); Chakrabarti and Basuchaudhary (1978) from Varanasi, Uttar Pradesh and in Maharashtra.

Zayed *et al.* (1980) reported the disease from Egypt. Nirmal *et al.* (1989) reported 29 per cent safflower wilt while Pedgaonkar *et al.* (1990) reported that wilt was present in Jalna, Parbhani and Latur district to extent of 5 to 18 per cent. Deokar *et al.* (1998) found up to 40 per cent wilt in safflower in Solapur, Osmanabad, Latur, Parbhani and Buldhana districts. The disease has also been reported from Ranga Reddy of Andhra Pradesh where the crop is grown in vertisols (Kalpana Sastry and Chattopadhyay, 1997 and Nageshwar Rao, 2000).

Disease sample exhibited typical symptoms of safflower wilt like unilateral yellowing of leaves, complete wilting and brown discolouration of stem. Pedgaonkar *et al.* (1990) reported unilateral yellowing of foliage followed by premature wilting of plant. They further observed that yellowing begins from the lower leaves and progresses upwards on one side of the plant. A brown discolouration of vascular bundles appeared on one side of the root and stem with unilateral top symptom. Nageshwar Rao (2003) reported bronzing and yellowing of leaves.

5.2 Isolation and identification

In the present investigation the fungus could be easily isolated by tissue isolation method and the pathogen was purified by Single hyphal tip method. Nineteen isolates of *Fusarium oxysporum* f.sp. *carthami* were obtained from the diseased samples of safflower wilt collected from survey. The isolates produced whitish to pink colour and produced macroconidia, microconidia and chlamydospore of typical *Fusarium oxysporum* f.sp. *carthami*. The identification was confirmed by compared with Monograph by Booth (1971).

The pathogenicity was proved by soil inoculation and water culture technique as described in materials and methods. The results indicated that all the 19 isolates were pathogenic to safflower variety Nira. Isolate FOC 14 was less virulent than other isolates. In water culture technique all the FOC isolates proved to be pathogenic in safflower variety Nira resulting in 100 per cent wilting in 24 hr.

Klisiewicz and Houston (1962) isolated *Fusarium oxysporum* f.sp. *carthami* from safflower wilt affected plant and its pathogenicity was demonstrated by soil inoculation method. Klisiewicz and Thomas (1970a) collected 12 isolates of *Fusarium oxysporum* f.sp. *carthami* from Sacramento valley (USA) and established pathogenicity on three safflower cultivars by soil inoculation method. Singh *et al.* and Pedgaonkar *et al.* (1975 and 1990, respectively) isolated *Fusarium oxysporum* f.sp. *carthami* on PDA from wilted safflower plants.

5.3. Etiology of pathogen

On the basis of the colony characters of the mycelium measurement of macroconidia, microconidia and chlamydospore the fungus was identified as *Fusarium oxysporum* f.sp. *carthami* (Snyder and Hanson, 1940, 1954 and Bilai, 1954).

Klisiewicz and Houston (1963) found that the isolates from safflower wilt was pathogenic to only *Carthamus* species and named it, as *Fusarium oxysporum* Schlect *carthami* on the basis of selective pathogenicity.

5.4. Seed borne nature of pathogen

5.4.1. By Blotter paper method

In the present investigation the pathogen present on the seed (externally seed borne) was studied in five varieties and it was found that in the cultivar Manjira seed borne pathogen was present to the extent of 85 per cent of seeds. It was followed by PBNS-12, Bhima and PBNS-40. Klisiewicz (1963) demonstrated for the first time seed borne nature of *Fusarium oxysporum* f.sp. *carthami* through seeds of safflower. He found 42 per cent of the seeds to be infected by this fungus externally. He also further reported that 8 per cent of the seed showed internal seed transmission. Vaidehi *et al.* (1985) also found the pathogen to be externally seed borne. Awadhiya (1992) also reported the pathogen to be externally seed borne.

5.4.2. Effect of seed treatments on seed mycoflora of safflower by Agar Plate method

To study the effect of seed treatments on seed mycoflora of safflower cultivar Nira by Agar plate method on *Fusarium* Selective Medium (FSM), four fungicides and seeds surface sterilized with chemical and hot water, treatments were studied for enumeration of *Fusarium oxysporum* f.sp. *carthami*. Unsterilized seeds served as control. The results indicated that there was minimum incidence of *Fusarium oxysporum* f.sp. *carthami* in hot water treatment. In case of fungicides the maximum incidence of *Fusarium oxysporum* f.sp. *carthami* was observed in Captan, it was followed by Thiram, Carbendazim and Carbendazim + Thiram. Highest incidence was recorded in unsterilized seeds followed by mercuric chloride.

Results indicated that Carbendazim + Thiram, Carbendazim, Thiram and Captan were more effective against *Fusarium oxysporum* f.sp. *carthami* while seeds treated with hot water are most effective against *Fusarium oxysporum* f.sp. *carthami*.

Nash and Snyder (1962) used peptone quitozene Agar media (PQA) for detection of seed borne nature of *Fusarium*.

5.5. Morphological variability

Morphological variability of 19 isolates of FOC pathogen was studied by measuring the length, breadth and septation of microconidia and microconida and presence or absence of chlamydospore.

5.5.1 Macroconidia

The results indicated that maximum length of macroconidia was observed in FOC-32 (28.60 μm) while minimum length was observed in FOC-16 (19.33 μm). While the maximum breadth of macroconidia was observed in FOC-32 (4.18 μm) while minimum was in FOC-36 (2.52 μm).

As regards septation of macroconidia, maximum septation (4 septa) were observed in FOC-10, 14 and 35. Rest of the isolates had three septa.

5.5.2 Microconidia

As regards microconidia, the maximum length was observed i.e. 9.79 μm in FOC-21 while minimum (4.51 μm) was observed in the isolate FOC-35. The maximum breadth 3.79 μm in isolate FOC-10 while minimum 1.65 μm in the isolate FOC-35.

As regards septation of microconidia, 2 septa were found in the isolate FOC-14 and 39. Rest of the isolates had only one septa.

5.5.3 Chlamydospores

All the isolates of *Fusarium oxysporum* f.sp. *carthami* produced chlamydospores except FOC-41. The chlamydospore was terminal only in isolate FOC-35 and in other it was intercalary.

Singh *et al.* (1975) reported that microconidia have the size of 5 – 16 in length x 2.3 – 3.5 μ in breadth. Macroconidia are 3 to 5 septate measuring 10 – 36 x 3 – 6 μ . Chlamydospores are terminal or intercalary. The present investigations are in general agreement of

Singh *et al.* (1975). Nelson *et al.* (1983) reported terminal or intercalary chlamydospore in the genus *Fusarium*.

5.6. Cultural variability

The colony growth of *Fusarium oxysporum* f.sp. *carthami* isolates differed with respect to colony diameter, colony growth / topography, colour, shape, sectoring, mycelial dry weight and sporulation.

In the present investigation, colony diameter ranged from 42 to 85 mm. Maximum growth was observed in FOC 35 followed by FOC 29 and 44. Most of the isolates produced Felty growth while isolate FOC-21, 32, 39 and 47 produced Fluffy raised growth. FOC-36, 41 and 42 had pinkish white in colour while isolate FOC-35 was dark pink.

FOC-14, 35, 39, 41 and 42 were irregular in shape while rest of the isolates was circular in shape. Sectoring was presented in FOC-35, 36, 41 and 42.

The mycelium dry weight ranged from 166.67 mg in FOC-42 to 583.33 mg in FOC-27. The isolates from Parbhani, Hingoli and Nanded district put fourth higher mycelial dry weight than other district. Excellent macroconidial spourlation was observed in FOC-12 followed by 14, 16 and 21. Maximum microconidial sporulation was observed in FOC-12 followed by FOC-14, 18, 36 and 39. Variation in cultural characteristics is a common phenomenon in genus *Fusarium*, Oswald (1949). Shit and Sen Gupta (1978) reported that isolates of *Fusarium udum* with luxuriant mycelial growth were weakly pathogenic whereas isolates with scanty mycelium with highly pathogenic similarly. There

were no correlation between intensity of sporulation and pathogenicity. Burgess *et al.* (1989) reported that morphology and pigmentation of colony varied extensively and can not be considered as basis of identification of species. Desai *et al.* (1994) reported dry mycelial weights of four races of *Fusarium oxysporum* f.sp. *ciceri* differed significantly. Bai and Shaner (1996) reported that isolates from different geographical areas varied in growth rate of mycelium and pigmentation. Desai *et al.* (2003) found that the isolates differed in morphological and cultural characteristics and highly virulent isolates produced abundant sporulation.

5.7 Pathogenic variability

Pathogenic variability within 19 isolates of *Fusarium oxysporum* f. sp. *carthami* was investigated by studying the pathogenicity on five cultivars of safflower.

Mean per cent wilt induced by 19 FOC isolates indicated that FOC 12 and 39 were most virulent isolates causing 82 to 78 per cent wilt respectively over five cultivars. They were followed by FOC 16 and 21. Lowest wilt per cent was observed in FOC 30, 18 and 35.

On the basis of reaction of five cultivars to 19 isolates they were grouped into four pathotypes I, II, III and IV. Race I, virulent on two cultivars i.e. Nira and Manjira. Race II virulent on three cultivars i.e. PBNS-40, Nira and Manjira. Race III, virulent on four cultivars i.e. A-1, PBNS-40, Nira and Manjira. Race IV virulent on all cultivars i.e. A-1, Bhima, PBNS-40, Nira and Manjira.

Klisiewicz and Thomas (1970a) distinguished two pathogenic races of *Fusarium oxysporum* f.sp. *carthami* among 30

isolates which were highly susceptible on three safflower variety. Klisiewicz and Thomas (1970b) they reported third pathogen race of *Fusarium oxysporum* f.sp. *carthami* by its virulence on a safflower cultivar which was resistant to earlier pathogen race. Klisiewicz (1975) reported fourth race of *Fusarium oxysporum* f.sp. *carthami* on the basis of susceptibility of cultivars which are resistant to earlier three races. Kalpana Sastry and Chattopadhyay (2003) also reported four pathotypes and confirmed the variability on different parts of safflower growing areas. Prameela *et al.* (2005) also reported variability in isolates of *Fusarium oxysporum* f.sp. *carthami*.

5.8. Genetic variability

RAPD analysis of 23 isolates of *Fusarium oxysporum* f.sp. *carthami* along with other *Fusarium* spp revealed that, there is variability between the isolates. A dendrogram was prepared by using the similarity coefficient of markers generated by two primers. As per cluster analysis with OPA-18 primer, the *Fusarium* isolated formed four main groups. In one and two group, 7 FOC isolates with 70 to 100 per cent similarity and to 8 isolates with 60 to 100 per cent similarity were included. Group three comprised of remaining FOC isolates and group four comprising of other species of *Fusarium*.

Dendrogram of OPC 11 RAPD marker revealed two groups with similarity value ranging between 50 to 100 per cent. Group one contained most of the FOC isolates and groups two other spp. of *Fusarium*. Group one comprising 17 isolates with 70 to 100 per cent genetic similarity.

Bentley *et al.* (1994) used RAPD PCR for differential *Fusarium oxysporum* f.sp. *cubens* races and 7 vegetative compatibility groups (VCG) known to occur in Australia. RAPD analysis was found to be useful in distinguishing non pathogenic *Fusarium oxysporum* isolates from *Fusarium oxysporum* f.sp. *dianthi* (Manulis *et al.*, 1994; Kalc Wright *et al.*, 1996), *F. oxysporum* f.sp. *vasinfectum* (Assigbetse *et al.*, 1994), *F. oxysporum* sp. *ciceris* (Kelly *et al.*, 1994), *Fusarium oxysporum* f.sp. *albendinis* (Fernandez and Tantaoui, 1994). There is no earlier research work on RAPD analysis of *Fusarium oxysporum* f.sp. *carthami* and this is for the first time such work has been done. Dubey *et al.* (2006) studied genetic variability within 24 isolates representing 7 races of *Fusarium oxysporum* f.sp. *ciceris* by RAPD technique.

Microsatellite analysis using (ISSR) inter simple sequence repeats were carried out to investigate variability in FOC isolates. The cluster analysis revealed that all the FOC isolates formed one group, which has three sub group I_A comprised 15 isolates, sharing more than 75 per cent genetic similarity. Group I_B has four isolates with more than 75 per cent similarity, Group I_C has two isolates with more than 85 per cent similarity, groups two has two FOC isolates having 90 to 100 per cent similarity and other spp of *Fusarium*.

Prasad *et al.* (2004) studied molecular variability among 45 isolates of *Fusarium* spp. belonging to 8 different sections by using Universal Rice Primers (URPs) designed from rice microsatellite.

5.9 Screening for host resistance

One hundred fifty safflower genotypes were screened using water culture technique for their reaction to wilt caused by *Fusarium*

oxysporum f.sp. *carthami*. The results indicated that three genotypes GMU 2794, 2828 and 2850 were free from wilt while genotypes GMU 2765, 2873, 2886, 2901, 2906, 2921 and 2924 were observed to be moderately resistant to wilt of safflower. Nirmal (1985) screened 52 safflower genotypes and found that NS 1016, H00-22, MVI-28, BLY 211 and BLY 1080 were resistant to disease. Similar results were reported by Kulkarni (1987), Pedgaonkar and Mayee (1989) screened 34 safflower genotypes by water culture technique. JSLF 88 and N-248 showed 22 to 40 per cent wilt as against 100 per cent wilt in another genotype. Similar results reported by Nagale (1989). Mogale and Nirmal (1992) screened 82 safflower germplasm lines against wilt disease and found that ten lines SSF16, JSF-9, BS-506, B-20, IJA-300, SSF-48, NS-10-60, NSS-99-A, JLSF-47 and PST-2 recorded less than 2 % mortality. Dhokne (1993) screened 170 safflower genotypes against *Fusarium oxysporum* f.sp. *carthami* by water culture technique while five genotypes viz. GMU 849, 850, 861, 875 and CTV-218 were free from wilt. Kalpana Sastry and Chattopadyay(2003) screened 51 promising safflower germplasm line in *Fusarium* sick plot and found that selections viz. 86-93-36A, 237550, VI-92-4-2 and II-13-2A were resistant to wilt while HUS-305 was moderately resistant.

5.10 Biological management through *Trichoderma* spp.

The effect of *Trichoderma* spp on mycelial growth of *Fusarium oxysporum* f.sp. *carthami* was investigated by dual culture technique.

Results indicated that at 9 DAI in *T. harzianum* growth reduction was observed in FOC 2 (77.14 per cent) it was followed by FOC-39 and FOC-44. In case of *T. viridae* at 9 DAI, maximum reduction was observed in FOC-24 followed by FOC-44 and FOC-47. In case of *T. hamatum* at 9 DAI, maximum reduction was observed in FOC 29 followed by FOC 39 and 41. In case of *T. koningii* at 9 DAI, maximum reduction was observed in FOC 41 followed by FOC 29 and 30. In case of *T. lignorum* at 9 DAI, maximum reduction was observed in FOC 47 followed by FOC 12 and 42.

Overall it was found that *T. harzianum* gave the maximum control of most of the isolates followed by *T. hamatum*, *T. viridae*, *T. lignorum* and *T. koningii*, *Trichoderma harzianum* has broad efficacy over 18 isolates out of 19. *Trichoderma viridae* has also broad spectrum efficacy over 17 isolates out of 19, *Trichoderma hamatum* was effective against 17 isolates out of 19, *Trichoderma lingnorum* selectively control isolate FOC 12 and 47 and *Trichoderma koningii* has selective activity against the isolates FOC 16, 29, 30, 35, 36 and 41. Potentiality of *Trichoderma* spp as biocontrol agent was reported by Weindling (1932). Rod (1984) reported antagonistic activity of *Trichoderma harzianum* against *Fusarium oxysporum* in onion. Mukhopadhyay et al. (1989) recorded excellent control of chickpea wilt *in vitro* by *Trichoderma harzianum*. Mukhopadhyay and Kaur (1990)

reported production of antibiotics by *T. harzianum* inhibiting the growth of *Fusarium oxysporum*. Similar results were reported by Clavet (1990) against *Fusarium oxysporum* f.sp. *ciceri*. Katragudda and Murugesan (1996) reported that *T. harzianum* contacts the cotton wilt pathogen *Fusarium oxysporum* f.sp. *vasinfectum* and produces lytic enzyme which degrade the cell wall of pathogen. Charati *et al.* (1998) reported maximum germination and lowest wilt in cotton due to seed treatment of *T. harzianum*.

Gowder and Kulkarni (2003) reported control of *Fusarium oxysporum* f.sp. *udum* causing pigeonpea wilt by *T. harzianum*. Shalini *et al.* (2005) reported that *T. harzianum* and *T. viridae* exhibited maximum antagonistic activity against *Fusarium oxysporum* f.sp. *pisi* by dual culture technique. There are several reports of *T. viridae* controlling several pathogens (Chattopadhyay and Kalpana Sastry 1997) against wilt of safflower, Karunanithi and Mohammed Usman (1999) against *Fusarium oxysporum* f.p. *sesami*, Behere (2002) against safflower wilt and Prasad (2003) against safflower wilt.

5.11. *In vitro* evaluation of fungicides

Five fungitoxicants namely Carbendazim, Copper oxychloride, Captan, Thiram and Propiconazole were evaluated at 1, 10, 25, 50, 100, 500 and 1000 $\mu\text{g/ml}^{-1}$ concentration against 19 FOC isolates and per cent inhibition was calculated.

From the results it was observed that, with increase in the concentrations of the fungitoxicant the per cent inhibition also increased. The results indicated that, Carbendazim was best fungicide for inhibiting the growth of *Fusarium oxysporum* f.sp. *carthami*. It was

followed by Propiconazole, Thiram, Captan and Copper oxychloride. One $\mu\text{g/ml}^{-1}$ of Carbendazim was equal or better than 1000 $\mu\text{g/ml}$ of rest of fungicide in inhibiting the mycelial growth of *Fusarium oxysporum f.sp. carthami*. Desai (1986) reported that Bavistin was effective to *F. udum* followed by Thiram. Mehetre (1980) could control seed borne infection of *Fusarium oxysporum f.sp. carthami* in safflower with Thiram 75 wp. Kapoor and Kumar (1991) found Carbendazim to be most toxic to *Fusarium oxysporum*. Hyas *et al.* (1992) found that Tilt (Propiconazole) to be most effective in controlling *Fusarium oxysporum f.sp. carthami*.

Future lines /Gaps

1. Survey can be continue
2. Biological control at pot and field level.
3. Effect of soil conditions on wilt of safflower.
4. Mycoflora associated with rhizosphere safflower and its interaction with pathogen

SUMMARY



CHAPTER-VI

SUMMARY

Wilt of safflower caused by *Fusarium oxysporum* f.sp. *carthami* Klisiewicz and Houston is an important soil borne disease where ever the crop is grown.

Random survey conducted during rabi 2005-06 and 2006-07 indicated that, farmers of Marathwada region predominantly grow local, Sharda, Bhima and PBNS-12 varieties of safflower. Maximum wilt incidence was recorded in Hingoli district in both the years of survey. Most of the farmers have used the same variety which resulted in increase in incidence of wilt. The disease samples collected from survey were subjected to isolation, purification and pathogenicity. Nineteen pathogenic isolates were selected for further studies.

On the basis of colony characters, colour of mycelium, pigmentation, measurement of macroconidia, microconidia and chlamydospores, the fungus was identified as *Fusarium oxysporum* f.sp. *carthami*.

Seed pathological studies indicated that seeds of safflower variety Manjira had maximum incidence of seed borne infection followed by PBNS-12, Bhima and PBNS-40. Effect of seed treatment with fungicides, mercuric chloride and hot water, Carbendazim + Thiram, Carbendazim, Thiram and Captan were effective to reduce the growth of *Fusarium oxysporum* f.sp. *carthami* while, the treatment of seeds with hot water at 52°C was found to be most effective.

Observations on length, breadth and septation of macroconidia and microconidia of the pathogen revealed that, there was great variability among the isolates which can be utilized to distinguish morphological groups in the FOC isolates. It was found that, most of the isolates produced intercalary chlamydospores. In case of cultural variability, it was observed that, the isolates differed with respect to colony diameter, topography, colour, shape, sectoring, mycelial dry weight and sporulation. In pathogenic variability, isolates of FOC-12 and FOC-39 were most virulent, while FOC 18 and FOC 35 were least virulent. On the basis of reaction on five cultivars, 19 isolates of FOC are grouped in four pathotypes I, virulent on two cultivars i.e. Nira and Manjira, Race II virulent on three cultivars i.e. Nira, Manjira and PBNS-40, Race III virulent on four cultivars viz., Nira, Manjira, A-1 and PBNS-40 and Race IV virulent on all five cultivars.

RAPD analysis of 23 isolates of *Fusarium oxysporum* f.sp. *carthami* and other *Fusarium* spp. revealed that by using OPA-18 primer, *Fusarium* isolates could be grouped into four groups. One group included seven isolates with 70 to 100 per cent genetic similarity, group two included eight isolates with 60 to 100 per cent genetic similarity, group three includes remaining FOC isolates and group four other species of *Fusarium*.

By using OPC 11 RAPD primer, two groups were revealed. Group one containing most of the FOC isolates and group two containing other spp. of *Fusarium*. Microsatellite analysis using ISSR revealed, two main groups. Group one includes most FOC isolates. Group I_A, 15 FOC isolates with more than 75 per cent genetic similarity,

group I_B includes four isolates with more than 75 per cent genetic similarity and group I_C includes two isolates with more than 80 per cent genetic similarity. Group II consists of two FOC isolates having 90 to 100 per cent genetic similarity and remaining other *Fusarium* spp.

One hundred and fifty genotypes of safflower were screened using water culture technique and the results indicated that, three genotypes viz. GMU-2794, GMU-2828 and GMU-2850 were free from wilt, while genotypes viz. GMU 2765, GMU 2873, GMU 2886, GMU 2901, GMU 2906, GMU 2921 and GMU 2924 were moderately resistant to wilt of safflower.

Effect of *Trichoderma* spp. on the mycelial growth of pathogen were studied by dual culture technique and it was found that at 9 DAI, the maximum growth reduction with *Trichoderma harzianum* was 77.14 per cent in FOC-42. In case of *Trichoderma viride* at 9 DAI, maximum reduction was observed in FOC 24 followed by FOC-44 and FOC 47. In case of *Trichoderma hamatum* at 9 DAI, maximum reduction was observed in FOC 29 followed by FOC 39 and FOC 41. In *Trichoderma koningii* at 9 DAI, maximum reduction was observed in FOC-41 followed by FOC-29 and FOC-30. In case of *Trichoderma lignorum* at 9 DAI, maximum reduction was observed in FOC-47 followed by FOC 12 and FOC 42. However, it was found that, *T. harzianum* gave maximum control of most of the isolates followed by *T. hamatum*, *T. viride*, *T. lignorum* and *T. koningii*. *Trichoderma harzianum* has broad spectrum efficacy over 18 isolates out of 19, *T. viridae* and *T. hamatum* had broad spectrum efficacy over 17 isolates out of 19. *T.*

koningii is selective against six isolates viz., FOC-16, 29, 30, 35, 36 and 41, while *T. lignorum* selectively controlled FOC 7 and FOC 12 isolates.

Five fungitoxicants viz., Carbendazim, Copper oxychloride, Captan, Thiram, Propiconazole were evaluated at 1, 10, 25, 50, 100, 500 and 1000 µg/ml concentration against 19 isolates of FOC and per cent inhibition was calculated. It was observed that, with increase in concentrations of fungitoxicants the per cent inhibition also increased. The results indicated that, Carbendazim was best fungicide for inhibiting the growth of *Fusarium oxysporum* f.sp. *carthami*, followed by Propiconazole, Thiram, Captan and Copper oxychloride. Even 1 µg/ml of Carbendazim was equal or better than 1000 µg/ml of rest of the fungicides in inhibiting the growth of *Fusarium oxysporum* f.sp. *carthami*.

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APPENDIX-II : Weekly weather data for the year 2005 at Parbhani

MW	Dates	RF (mm)	R. days	Temp °C		Humidity %		EVP (mm)	BSS (Hrs.)	W.S. (kmph)
				Max.	Min.	AM	PM			
01	01-07 Jan.	0.0	0	30.1	14.0	75	38	4.0	8.6	2.7
02	08-14 Jan.	0.0	0	30.2	9.1	76	32	4.2	10.6	2.8
03	15-21 Jan	0.0	0	28.9	9.2	74	27	4.3	10.3	3.4
04	22-28 Jan	10.0	1	30.4	15.4	67	41	4.6	9.2	3.8
05	29-04 Feb.	11.0	1	26.2	12.8	78	53	3.9	7.6	4.6
06	05-11 Feb	0.0	0	33.3	14.7	75	26	5.4	10.9	3.5
07	12-18 Feb.	0.0	0	36.1	12.2	66	24	5.8	11.1	3.4
08	19-25 Feb.	0.0	0	33.1	13.9	64	29	6.6	10.8	4.0
09	26-04 Mar.	2.4	0	35.7	17.7	60	32	6.9	8.6	4.0
10	05-11 Mar.	53.8	2	34.6	17.0	70	44	6.5	9.2	4.8
11	12-18 Mar.	0.0	0	35.4	14.7	61	27	7.1	11.5	3.5
12	19-25 Mar.	0.0	0	38.1	17.0	51	26	8.8	11.1	4.2
13	26-01 Apr.	0.0	0	38.4	15.8	48	16	9.2	11.4	3.9
14	02-08 Apr.	0.0	0	38.7	20.9	49	21	9.2	10.5	5.1
15	09-15 Apr.	18.8	1	38.3	19.7	50	24	8.4	10.0	5.0
16	16-22 Apr.	0.0	0	39.8	21.4	55	22	8.9	10.2	4.1
17	23-29 Apr.	2.4	0	38.9	21.2	63	25	8.1	9.2	4.9
18	30-06 May	0.0	0	38.9	22.3	53	22	9.8	10.4	4.9
19	07-13 May	0.0	0	41.0	24.7	45	18	11.0	10.4	7.4
20	14-20 May	0.0	0	43.2	22.2	39	15	12.9	11.9	6.0
21	21-27 May	0.8	0	43.0	24.2	44	18	11.0	9.0	5.1
22	28-03 June	0.0	0	41.4	26.1	42	22	11.2	7.3	6.9
23	04-10 June	4.4	1	42.2	25.4	47	20	11.6	10.2	8.0
24	11-17 June	28.8	3	38.9	24.1	66	33	9.2	6.5	8.3
25	18-24 June	0.0	0	38.1	24.5	66	36	8.8	10.8	8.5
26	25-01 July	18.0	3	33.7	23.2	80	51	4.7	1.7	9.1
27	02-08 July	7.2	1	33.4	22.4	77	53	4.8	3.7	10.2
28	09-15 July	214.9	5	30.0	21.3	92	75	3.8	2.6	6.0
29	16-22 July	70.0	3	32.0	22.0	90	65	4.3	6.2	3.9
30	23-29 July	500.3	5	27.2	21.0	97	84	2.7	0.7	7.0
31	30-05 Aug	81.3	3	28.9	21.1	93	83	3.3	2.0	6.3
32	06-12 Aug	20.1	2	29.2	20.8	86	68	3.2	4.6	4.2
33	13-19 Aug	20.6	3	30.4	21.5	85	70	3.9	5.2	4.6
34	20-26 Aug	42.2	4	30.3	20.5	93	65	3.7	5.8	4.1
35	27-02 Sept	9.2	1	34.0	21.4	84	50	5.0	8.9	3.0
36	03-09 Sep	24.2	3	31.8	22.0	93	64	4.9	5.0	3.1
37	10-16 Sep	14.3	4	31.0	21.3	90	68	4.3	3.7	4.3
38	17-23 Sep	100.0	2	29.1	20.4	84	72	3.8	4.9	6.8
39	24-30 Sep	0.0	0	31.0	18.9	83	58	4.7	10.6	3.8
40	01-07 Oct.	0.0	0	33.6	18.3	80	40	4.3	9.6	2.4
41	08-14 Oct.	12.2	1	34.0	17.0	84	49	5.8	9.7	5.0
42	15-21 Oct.	141.4	3	28.8	19.5	93	70	3.5	5.4	5.1
43	22-28 Oct.	0.0	0	31.0	15.0	73	48	4.4	9.6	3.7
44	29-04 Nov.	0.0	0	31.2	13.2	80	51	4.3	8.5	4.0
45	05-11 Nov.	0.0	0	30.2	8.9	81	38	4.4	10.5	4.0
46	12-18 Nov.	0.0	0	30.8	7.7	76	38	4.7	10.7	2.6
47	19-25 Nov.	0.0	0	31.2	7.9	78	33	4.9	10.9	3.5
48	26-02 Dec.	0.0	0	30.9	10.8	77	38	5.0	10.5	3.1
49	03-09 Dec.	0.0	0	30.5	12.2	76	42	4.7	8.8	3.6
50	10-16 Dec.	0.0	0	28.7	6.3	82	46	4.2	10.6	2.7
51	17-23 Dec.	0.0	0	29.0	8.8	77	40	4.1	10.1	2.7
52	24-31 Dec.	0.0	0	27.5	7.3	79	35	3.9	9.0	2.8
Total		1408.3	52							

APPENDIX-I : Weekly weather data for the year 2006 at Parbhani

MW	Dates	RF (mm)	Temp °C		Humidity %		EVP (mm)	BSS (Hrs.)	W.S. (kmph)
			Max.	Min.	AM	PM			
01	01-07 Jan.	0.0	28.9	8.7	76	34	4.8	10.2	3.4
02	08-14 Jan.	0.0	29.9	9.2	72	32	5.0	10.6	2.6
03	15-21 Jan.	0.0	32.4	13.5	71	28	5.1	10.4	2.3
04	22-28 Jan.	0.0	28.3	7.4	76	23	4.8	11.0	4.5
05	29-04 Feb.	0.0	31.0	9.4	71	24	5.3	10.9	2.9
06	05-11 Feb.	0.0	31.8	9.4	67	21	5.2	10.9	2.7
07	12-18 Feb.	0.0	33.8	12.2	58	20	5.3	10.9	2.6
08	19-25 Feb.	0.0	36.7	15.0	53	18	6.6	10.9	2.9
09	26-04 Mar.	16.2	36.0	17.5	60	32	6.3	9.8	4.5
10	05-11 Mar.	20.7	31.9	16.9	88	44	5.6	9.0	5.6
11	12-18 Mar.	0.0	31.3	21.5	83	36	5.4	10.3	4.4
12	19-25 Mar.	0.0	36.1	16.3	55	17	7.1	11.2	4.3
13	26-01 Apr.	0.0	38.1	18.9	51	15	7.7	10.7	3.6
14	02-08 Apr.	0.0	39.7	18.5	46	15	9.0	11.5	4.1
15	09-15 Apr.	0.0	40.4	20.8	47	16	9.2	10.0	3.9
16	16-22 Apr.	0.0	37.9	19.3	49	19	9.5	10.8	5.3
17	23-29 Apr.	0.0	40.7	21.8	42	16	10.4	11.0	4.8
18	30-06 May	0.0	42.5	24.0	40	14	12.0	10.7	5.6
19	07-13 May	0.0	43.1	22.5	43	15	12.7	11.8	6.7
20	14-20 May	4.2	41.7	22.2	51	20	12.2	11.6	7.4
21	21-27 May	28.1	37.6	22.5	71	43	9.0	8.8	9.6
22	28-03 June	0.0	36.2	23.2	66	40	7.6	8.1	7.2
23	04-10 June	0.0	36.8	24.3	66	36	9.3	9.3	9.0
24	11-17 June	7.2	37.9	25.8	60	30	9.8	8.9	7.7
25	18-24 June	57.0	36.6	23.1	78	43	7.4	7.1	5.9
26	25-01 July	66.2	32.4	23.4	87	64	4.4	5.0	5.2
27	02-08 July	71.7	31.3	23.2	86	64	4.1	4.4	8.0
28	09-15 July	0.0	33.7	23.8	79	53	5.8	4.9	8.1
29	16-22 July	12.8	32.0	22.4	80	64	4.6	3.8	7.1
30	23-29 July	12.0	31.1	22.2	85	67	3.8	2.5	6.6
31	30-05 Aug	182.1	28.4	21.5	88	83	2.6	1.4	7.2
32	06-12 Aug	271.1	27.6	21.6	86	72	2.8	3.3	6.8
33	13-19 Aug	1.6	31.0	22.2	82	61	3.9	5.2	5.9
34	20-26 Aug	1.6	31.7	20.9	79	57	4.4	7.6	5.2
35	27-02 Sept	3.0	31.1	21.0	84	56	4.3	4.4	7.8
36	03-09 Sep	13.6	32.9	22.6	79	57	5.0	9.2	5.2
37	10-16 Sep	16.7	32.0	22.1	85	65	4.3	8.2	3.3
38	17-23 Sep	54.1	30.9	21.6	93	69	3.6	3.7	3.7
39	24-30 Sep	66.4	31.2	21.2	89	75	3.6	7.5	4.9
40	01-07 Oct.	31.4	30.2	21.4	82	65	3.6	4.6	3.4
41	08-14 Oct.	18.8	33.4	19.9	83	46	4.7	10.0	2.1
42	15-21 Oct.	0.0	34.1	17.9	76	40	5.2	10.6	2.5
43	22-28 Oct.	0.0	32.9	15.6	79	40	4.9	10.3	3.4
44	29-04 Nov.	38.0	29.7	18.6	86	58	4.7	6.2	4.8
45	05-11 Nov.	0.0	30.5	16.5	81	51	4.8	8.7	3.1
46	12-18 Nov.	0.0	31.4	12.8	81	43	4.9	10.8	3.9
47	19-25 Nov.	0.0	30.3	14.1	82	49	4.2	7.7	2.2
48	26-02 Dec.	0.0	31.9	15.5	79	43	4.6	9.6	1.7
49	03-09 Dec.	0.0	31.0	12.8	79	36	4.6	10.1	2.5
50	10-16 Dec.	0.0	30.0	10.7	80	32	4.7	10.4	2.5
51	17-23 Dec.	0.0	29.0	8.8	77	40	4.1	10.1	2.7
52	24-31 Dec.	0.0	27.5	7.5	79	35	3.9	9.0	2.8
	Total	994.6							

APPENDIX-I : Weekly weather data for the year 2007 at Parbhani

MW	Dates	RF (mm)	Temp °C		Humidity %		EVP (mm)	BSS (Hrs.)	W.S. (kmph)
			Max.	Min.	AM	PM			
01	01-07 Jan.	0.0	28.8	9.0	78	37	4.1	10.3	2.7
02	08-14 Jan.	0.0	29.0	10.7	74	36	4.1	10.3	2.9
03	15-21 Jan	0.0	30.9	10.9	75	28	4.2	10.4	2.7
04	22-28 Jan	0.0	31.7	12.6	72	29	4.4	10.6	3.1
05	29-04 Feb.	0.0	32.5	15.1	72	27	5.7	10.2	3.8
06	05-11 Feb	0.0	33.3	14.9	72	24	5.9	10.3	3.5
07	12-18 Feb.	0.0	30.7	12.8	73	30	6.0	10.5	4.7
08	19-25 Feb.	0.0	32.1	13.1	70	23	6.3	10.9	4.7
09	26-04 Mar.	0.0	33.6	15.0	66	22	6.6	11.0	4.3
10	05-11 Mar.	0.0	9.9	4.9	18	3	1.9	3	1.2