

**Studies on Chickpea Wilt (*Fusarium oxysporum* f. sp.
ciceri) in Kashmir**

Shifa Showket
(2017-A-1173-M)



Division of Plant Pathology
Faculty of Agriculture
Sher-e-Kashmir University of Agricultural Sciences and
Technology of Kashmir
2020

**Studies on Chickpea Wilt (*Fusarium oxysporum* f. sp.
ciceri) in Kashmir**

Shifa Showket
(2017-A-1173-M)



Thesis

Submitted to

The Faculty of Agriculture

Sher-e-Kashmir University of Agricultural Sciences &

Technology of Kashmir

In partial fulfilment of requirements for the award of the degree of

Master of Science in Agriculture
(Plant Pathology)

2020



They are the ones who have been with me through everything in my journey, that's why I must be devoted to them. "My lord! Bestow on them thy mercy even as they cherished me in my childhood "(17:24)

Sher-e-Kashmir
University of Agricultural Sciences and Technology of Kashmir
Division of Plant Pathology, Faculty of Agriculture, Wadura
193201

Certificate – I

This is to certify that the thesis entitled, “**Studies on Chickpea Wilt (*Fusarium oxysporum f. sp. ciceri*) in Kashmir**” submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Agriculture (Plant Pathology)**, to the **Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** is a record of bonafide research work carried out by **Ms. Shifa Showket (Regd. No. 2017-A-1173-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or information received during the course of investigation has duly been acknowledged.

Dr. Sabiya Bashir
Chairman
Advisory Committee

Endorsed
Head,
Division of Plant Pathology

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Agriculture, Division of Plant Pathology, Wadura
193201

Certificate – II

We, the members of the Advisory Committee of **Ms. Shifa Showket (Regd. No. 2017-A-1173-M)** a candidate for the degree of **Master of Science in Agriculture (Plant Pathology)** have gone through the manuscript of the thesis entitled, “**Studies on Chickpea Wilt (*Fusarium oxysporum* f. sp. *ciceri*) in Kashmir**” and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of the degree.

Advisory Committee

Chairman

Dr. Sabiya Bashir

Assistant Professor (Plant Pathology)
SKUAST-K, DARS-Srinagar

Members

Dr. Muhammad Najeeb Mughal

Assistant Professor (Plant Pathology)
FoA, SKUAST-K

Dr Reyaz-ul-Rouf Mir

Associate Professor
Division of Genetics and Plant Breeding
FoA, SKUAST-K

Dr. Showkat Maqbool,

Associate Professor
Division of AGB
FVSc and AH, SKUAST-K

Dean’s Nominee

Dr. Tariq Ahmad Shah

Professor (Plant Pathology)
FoH, SKUAST-K.

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Agriculture, Division of Plant Pathology, Wadura
193201

Certificate – III

This is to certify that the thesis entitled, “**Studies on Chickpea Wilt (*Fusarium oxysporum* f. sp. *ciceri*) in Kashmir**” submitted by **Ms. Shifa Showket (Regd. No. 2017-A-1173-M)** to the **Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** in partial fulfilment of the requirements for the award of the degree of **Master of Science in Agriculture (Plant Pathology)** was examined and approved by the Advisory Committee and External Examiner on

Chairman
Advisory Committee

External Examiner

Head,
Division of Plant Pathology

Dean,
Faculty of Agriculture, Wadura
SKUAST-Kashmir

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Plant Pathology, Faculty of Agriculture, Wadura
193201

Name of the student : Shifa Showket
Registration No. : 2017-A-1173-M
Major Subject : Plant Pathology
Minor Subject : Genetics and Plant Breeding
Major Advisor : Dr. Sabiya Bashir
Assistant Professor (Plant Pathology)
SKUAST-K, DARS-Srinagar
Title of the Thesis : **“Studies on Chickpea Wilt (*Fusarium oxysporum* f. sp. *ciceri*) in Kashmir”**

ABSTRACT

Fusarium wilt is the most serious disease of chickpea causing 20 to 25 per cent yield losses each year. The fungus associated with the disease was isolated and identified as *Fusarium oxysporum* f. sp. *ciceri*. Microscopic examination revealed mycelium as septate producing microconidia, macroconidia and chlamydospores. The pathogenicity of the isolated fungus was proved by Koch's postulates. In order to determine the best solid media for the maximum growth and sporulation, the pathogen was grown on five different solid media viz., potato dextrose agar (PDA), oatmeal agar (OMA), chickpea leaf extract agar (CLA), Czapek dox agar (CDA) and dal weed sucrose agar (DWS). In each media average radial mycelial growth (mm) and sporulation were recorded after 7 and 15 days of incubation. The maximum mycelial growth (80 mm) was recorded on OMA. The highest number of microconidia ($8.55 \times 10^6 \text{ ml}^{-1}$) and macroconidia ($7.56 \times 10^6 \text{ ml}^{-1}$) was formed on PDA and minimum sporulation was recorded on DWS. The pathogen had a white cottony growth on PDA, OMA and CDA, pure white on CLA and DWS. Mycelium was dense and fluffy on OMA, PDA, CDA and CLA, however on DWS a sparse and fluffy mycelium was observed. PDA, DWS and CDA had irregular mycelial growth pattern while as OMA and CLA

had regular mycelial growth pattern. On evaluation of the liquid media, the maximum dry mycelial growth was obtained from oat meal broth (723.33 mg) followed by potato dextrose broth (710.33 mg), chickpea leaf extract broth (700.23 mg) and Czapek dox broth (680.53 mg), while lowest dry mycelial growth was recorded in dal weed sucrose broth (650.33 mg). Out of seventy five genotypes evaluated against Fusarium wilt under natural epiphytotic conditions, only two genotypes viz., FLIP10-382C and FLIP11-122C were highly resistant and nine genotypes viz., FLIP11-24C, FLIP11-23C, FLIP11-150C, FLIP11-77C, FLIP11-90C, FLIP11-176C, FLIP10-355C, FLIP11-40C and FLIP11-45C were resistant. Among the three bio-agents evaluated *in vitro* against *Fusarium oxysporum* f. sp. *ciceri*, the highest per cent mycelial growth inhibition (82.40%) was observed with *Trichoderma harzianum* followed by *Bacillus subtilis* with mean mycelial growth inhibition of 70.53 per cent. *Pseudomonas fluorescens* proved least effective with mean mycelial growth inhibition of 66.30 per cent. All the nine tested fungicides were significantly superior over control in inhibiting the mycelial growth of the fungus *in vitro*. Among non-systemic fungicides tested, mancozeb 63%+ carbendazim 12% WP proved most effective and resulted in 94.02 per cent mycelial growth inhibition while as among systemic fungicides carbendazim 50WP proved most effective by providing 99.15 per cent mycelial growth inhibition over control. On the basis of their efficacies *in vitro* mancozeb 63%+ carbendazim 12% WP, carbendazim 50WP and *Trichoderma harzianum* were further evaluated during Rabi 2018-19 as seed treatments and drenching, under field conditions against Fusarium wilt of chickpea. All the seed treatments and soil drenches were superior over the control in reducing the disease incidence. Combination treatment comprising of seed treatment with *Trichoderma harzianum* (10^8 conidia ml⁻¹) and drenching with carbendazim 50WP (0.1%) was most efficacious and resulted in lowest disease incidence (4.43%), while seed treatment with *Trichoderma harzianum* (10^8 conidia ml⁻¹) alone was least efficacious and resulted in highest disease incidence of 21.97 per cent. It was, however superior than control where disease incidence was 46.59 per cent.

Keywords: Bio-agents, Chickpea, Fungicides, Fusarium wilt, Screening.

Signature of Student

Dated: _____

Signature of Major Advisor

Dated : _____

ACKNOWLEDGEMENT

In the name of Allah, the most Beneficent and the most Merciful. I offer my prime salutations to Allah (SWT) for He has always answered my prayers and provided me good health to complete this phase of my life.

Every effort is motivated by an ambition and all ambitions have an inspiration behind. I deem it to be my profound privilege to express my deep sense of gratitude and profound personal regards to esteemed Vice Chancellor Dr. Nazir Ahmad, an eminent scientist and a fatherly figure who genuinely perceives the interest and wellbeing of his students as the most supreme.

I am short of words to express my gratitude to Dr. Sabiya Bashir Assistant Professor SKUAST-Kashmir, and Chairman of my advisory committee whose impeccable guidance, critical analysis, constructive criticism and unparalleled execution of the essential requisites during the entire course of study are beyond reach of my formal words. I have been extremely lucky to have a guide who cared so much about my work, and who responded to my questions and queries so promptly.

I extend my deepest gratitude and veneration to the esteemed members of my advisory committee Dr. Muhammad Najeeb Mughal, Dr. Tariq Ahmad Shah, Dr. Reyaz ul Rouf Mir and Dr. Showkat Maqbool for their valuable guidance, constant encouragement and suggestions during the study and for helping in finalization of the manuscript.

I cannot express enough thanks to Dr. Shaheen Kauser Jan Professor and Head, Division of Plant Pathology, FoA, SKUAST- K, Dr. Tanveer Ahmad Wani, Dr. Farooq Ahmad Bhat, Dr. Khursheed Ahmad Bhat, Dr Baby Summuna, and Dr. Zahoor Ahmad Baba, for their valuable suggestions and whole hearted cooperation during the entire period of study.

I wish to show my gratitude to Dean Faculty of Agriculture; Dean Student Welfare; Director, Research Station; And Division of Plant Pathology; And Division of Agricultural Microbiology, for providing me necessary facilities for smooth conduct of research work,

My completion of this project could not have been accomplished without the moral encouragement, support and constant inspiration of my friends especially Butulla Rasheed, Shafiya Rashid, Masrat Siraj, Ayman Javeed, Razia Rehman, Zakir Amin, Reshi Irshad, Ifra Hussain, Nusrat Allae, Burhana Rasheed, Liyaqat Ayoub, Fazil Fayaz, Raheeba tu Nisa, Danishta Aziz, Nahida Anjum, Farzana Hameed, Sumira Rafiqee, Nakeeb un Nisa Haziq Shabir, Gulzar Ahmad, Gowhar Malik, Wani Altaf, Bushra Rasool, Aabida Abbas, Ruksana Majid, Irfan Javaid, Hina Altaf, Tabasum Manzoor, and other batch mates.

I am also indebted to thank my seniors Shugufta Parveen, Musharib Gull, Nargis Nazir, Uzma Rasheed, Tabasia Amin and Rouf Ahmad Rather, for their constant help and support during my studies.

I am highly thankful to ARIS and Library staff members of SKUAST-K for their constant encouragement, valuable suggestions and generous help during preparation of this manuscript.

I am also thankful to all the non-teaching staff members of Division of Plant Pathology, FoA, SKUAST-K for their cooperation with special reference to Fayaz Ahmad, Farooq Ahmad and Firdous Ahmad.

Nobody has been more important to me in the pursuit of this project than the members of my family. I would like to thank my parents (Mr. Showket Ahmad Wani and Ms. Shahzada Akhter), my brother (Nadeem Wani) and my grandfather (Gh. Nabi Wani) whose love and guidance are with me in whatever i pursue. They are the ultimate role models, i owe this pride place to my revered family members whose encouragement, positive reception and trust upon me has always inspired me to move further. Last but not least i wish to thank Peerzada Aakif Hussain, who provides an unending inspiration.

Shifa Showket

Place: Wadura, Sopore

Dated:

CONTENTS

Chapter No.	Particulars	Page No.
1.	INTRODUCTION	1-4
2.	REVIEW OF LITERATURE	5-15
2.1	Causal organism	5
2.2	Symptomology	6
2.3	Morphology	7
2.4	Physiological studies	8
2.5	Host Germplasm Evaluation	9
2.6	Disease Management	11
3.	MATERIALS AND METHODS	16-24
3.1	Isolation, Purification and Identification of the Pathogen	16
3.2	Pathogenicity Test	17
3.3	Morphology and Physiology of Pathogen on Different Solid Media	18
3.4	Dry Mycelial Weight of Pathogen on Different Liquid Media	18
3.5	Screening of the Available Germplasm against the pathogen	19
3.6	Evaluation of Bio-control Agents and Fungicides	20
3.7	Statistical Analysis	24
4.	EXPERIMENTAL FINDINGS	25-44
4.1	Isolation, Purification and Identification of the Pathogen	25
4.1.1	Isolation of the causal pathogen	25
4.1.2	Purification and maintenance of the pathogen	25
4.1.3	Identification of the pathogen	25

4.2	Pathogenicity test	27
4.3	Morphology and physiology of the pathogen on different solid media	27
4.3.1	Effect of different solid media on mycelial growth and sporulation of the pathogen	27
4.3.2	Effect of different solid media on morphological characters of the pathogen	28
4.4	Dry mycelial weight of the pathogen on different liquid media	29
4.5	Screening of the available germplasm against the pathogen	30
4.6	Evaluation of bio-agents and fungicides against the pathogen	39
4.6.1	<i>In vitro</i> evaluation of bio-agents and fungicides	39
4.6.2	<i>In vivo</i> management of the disease	43
5.	DISCUSSION	45-50
6.	SUMMARY AND CONCLUSION	51-54
	LITERATURE CITED	i- vii

LIST OF TABLES

Table No.	Particulars	Page No.
1.	Morphological characteristics of <i>Fusarium oxysporum</i> f. sp. <i>ciceri</i> chickpea wilt	26
2.	Effect of different solid media on radial growth and sporulation of <i>Fusarium oxysporum</i> f. sp. <i>ciceri</i>	28
3.	Effect of different solid media on morphology and colony characters of <i>Fusarium oxysporum</i> f. sp. <i>ciceri</i>	29
4.	Average dry mycelial weight of <i>Fusarium oxysporum</i> f. sp. <i>ciceri</i> on different liquid media	30
5.	Reaction of genotypes in CIFWN-18 to Fusarium wilt during rabi 2017-18	32-33
6.	Categorisation of chickpea genotypes in CIFWN-18 on the basis of their reaction to Fusarium wilt	34
7.	Reaction of genotypes in CIEN-W-18 to Fusarium wilt during rabi 2017-18	36-37
8.	Categorisation of chickpea genotypes in CIEN-W-18 on the basis of their reaction to Fusarium wilt	38
9.	<i>In vitro</i> efficacy of bio-agents on mycelial growth inhibition of <i>Fusarium oxysporum</i> f. sp. <i>ciceri</i>	39
10.	<i>In vitro</i> evaluation of non- systemic fungicides for mycelial growth inhibition of <i>Fusarium oxysporum</i> f. sp. <i>Cicero</i>	41
11.	<i>In vitro</i> evaluation of systemic fungicides for mycelial growth inhibition of <i>Fusarium oxysporum</i> f. sp. <i>ciceri</i>	42
12.	<i>In vivo</i> /field evaluation of the bio-agent and fungicides against the wilt disease	44

LIST OF FIGURES

Figure No.	Particulars	After Page No.
01	<i>In vitro</i> efficacy of bio-agents on mycelial growth inhibition of <i>Fusarium oxysporum</i> f. sp. <i>ciceri</i>	39
02	<i>In vitro</i> evaluation of non-systemic fungicides for mycelial growth inhibition of <i>Fusarium oxysporum</i> f. sp. <i>ciceri</i>	42
03	<i>In vitro</i> evaluation of systemic fungicides for mycelial growth inhibition of <i>Fusarium oxysporum</i> f. sp. <i>ciceri</i>	42

LIST OF PLATES

Plate No.	Particulars	After Page No.
1.	Isolation, purification and maintenance of <i>Fusarium oxysporum</i> f. sp. <i>ciceri</i>	26
2.	Colony characteristics of <i>Fusarium oxysporum</i> f.sp. <i>ciceri</i> (on PDA)	26
3.	Morphological characteristics of <i>Fusarium oxysporum</i> f.sp. <i>ciceri</i>	26
4.	Pathogenicity test	28
5.	Radial growth of <i>Fusarium oxysporum</i> f .sp. <i>ciceri</i> on different solid media	28
6.	Average dry mycelial weight of <i>Fusarium oxysporum</i> f. sp. <i>Cicero</i>	29
7.	Screening of the available germplasm against the pathogen	31
8.	<i>In vitro</i> efficacy of bio-agents on mycelial growth inhibition of <i>Fusarium oxysporum</i> f. sp. <i>ciceri</i>	39
9.	<i>In vitro</i> evaluation of non-systemic fungicides for mycelial growth inhibition of <i>Fusarium oxysporum</i> f. sp. <i>ciceri</i>	42
10.	<i>In vitro</i> evaluation of systemic fungicides for mycelial growth inhibition of <i>Fusarium oxysporum</i> f. sp. <i>ciceri</i>	42

Chapter-1

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important and widely grown pulse crop. It belongs to family *Fabaceae* and is the third most important pulse crop after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.). It originated in South-Eastern Turkey (Ladizinsky, 1975).

India is the largest producer, consumer and importer of the chickpea in world, contributing 71 per cent in area and 71.95 per cent in production. Chickpea contributes nearly 48 to 52 per cent of total pulse production in India. The total area under chickpea cultivation in India is about 10.56 million hectares with annual production of 11.23 million tonnes and an average productivity of 1.063 tonnes ha⁻¹ (Anonymous, 2018). The production of chickpea in Jammu and Kashmir during the year 2011 was 0.002 million tonnes with average productivity of 0.710 tonnes ha⁻¹ from an area of 0.005 million hectares (Andrabi *et al.*, 2011). Commercially, chickpea is grouped into *Desi* (small, coloured seed coat) and *Kabuli* (large, white or cream seed coat) types. *Desi* occupies 85 per cent area while 15 per cent of the area is occupied by *Kabuli* type. Six states *viz.*, Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Karnataka and Andhra Pradesh together contribute 90 per cent of the area and 91 per cent of the production in the country (Singh, 2010).

Chickpea is mainly sown in the months of September-November and is harvested from February-April. The crop duration is 90-120 days, depending on the variety. *Desi* varieties are of short duration while *Kabuli* varieties take relatively longer period to mature. In cooler areas like Northern India it requires longer period for maturity as compared to Southern parts where relatively warm weather conditions prevail. Chickpea is a hardy deep-rooted dry land crop and can grow to full maturity despite conditions that would prove fatal for most crops. The

yield is maximum when the legume crop is grown in sandy-loam soils possessing an appropriate drainage system. Chickpea crop is very sensitive to excess water availability as well as very cold weather conditions, which result in great reduction in its productivity. It has an indeterminate growth habit, which means that the growth cycle extends as long as moisture is available.

From the nutritional point of view, chickpea acquires importance as it provides protein rich food for humans as well as for livestock. Furthermore, chickpea seed coats and pod covers can also be used as fodder. It is consumed as a fresh immature green seed, whole seed, dal and flour. The chickpea seed is a good source of carbohydrates and proteins, which together constitute 80% of the total dry seed weight. On an average, chickpea seed contains 64% total carbohydrates, 23% protein, 47% starch, 6% crude fibre, 6% soluble sugar, 5% fat and 3% ash. The mineral component is high in phosphorus (340 mg per 100 g), calcium (190 mg per 100 g), magnesium (140 mg per 100g), iron (7 mg per 100 g) and zinc (3 mg per 100 g). Chickpea protein has highest digestibility when compared to other dry edible legumes. The lipid fraction is high in unsaturated fatty acids, primarily linoleic and oleic acids (Ibriki *et al.*, 2003). Thus, chickpea serves as a main source of dietary protein for more than 80% of the Indian population which is vegetarian in nature. Since, chickpea plays the pivotal role of supplying protein source in the vegetarian diet, it is also called as the 'poor man's meat'. Supplementation of cereals with high protein legume is potentially one of the best solutions to protein-calorie malnutrition, particularly in the developing countries. It plays significant role in improving soil fertility by fixing atmospheric nitrogen. It meets 80 per cent of its nitrogen (N) requirement from symbiotic nitrogen fixation and can fix up to 140 kg N per hectare from air (Gaur *et al.*, 2010).

The yield of chickpea dwindles year after year due to diseases, pests and poor management practices. Amongst them, diseases play pivotal role in reducing yield potential of chickpea. More than 50 diseases have been reported to affect chickpea with economic importance. These include wilt [*Fusarium oxysporum* f.

sp. *ciceri* (Padwick) Synd & Hans.], black root rot [*Fusarium solani* (Mart.) Sacc.], wet root rot (*Rhizoctonia solani* Khun.), dry root rot [*Rhizactonia bataticola* (Taub.) Butler], Aschochyta blight [*Aschochyta rabie* (Pass.) Lab.], Pythium root and seed rot (*Pythium ultimum* Trow.) and collar rot (*Sclerotium rolfsii* Sacc.) which are of considerable importance (Nene and Haware, 1981). Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri*) is one of the most destructive and wide spread diseases of chickpea (Nene *et al.*, 1984). It was first reported from India by Butler in 1918. Under favourable conditions it causes heavy losses (20-100%) depending upon the stage of infection (Animisha *et al.*, 2012). Singh *et al.*, (2007) reported 60 per cent yield loss, however, under favourable weather conditions the yield loss may be upto 100 per cent (Kumari and Khanna, 2014). Chickpea wilt is known to be soil borne (Jimenez-Fernandez *et al.*, 2011) and internally seed borne (Pande *et al.*, 2007). The pathogen colonizes the xylem vessels of the plant and blocks them completely to cause wilting (Cho and Muehlbauer, 2004). Affected plants show no external rotting of root and pith at seedling stage, however, when the roots are split vertically internal discoloration may be seen in wilted plants but at adult stage the affected plants show typical wilting i.e. drooping of leaflets, rachis and lamina. Wilted plants when uprooted and split, exhibit more pronounced internal brown to black discoloration of the xylem vessels (Haware *et al.*, 1978)

The pathogen grows on potato dextrose agar at 25 °C and the colony appears as delicate, white and cottony which later becomes felted and wrinkled (Nath *et al.*, 2017). Fungal hyphae are septate and profusely branched. The fungus produces three types of spores viz., microconidia, macroconidia and chlamydospores. Microconidia are borne on simple short conidiophores, arising laterally on hyphae and are oval to cylindrical, straight or curved, 0-1 septate and 5-11×2.5-3.5µm in size. Macroconidia are thin walled, 3-5 septate, fusoid, pointed at both ends, and measure 25-65×3.5-4.5µm. Chlamydospores are formed in 15 days old culture, are smooth or rough walled, terminal or intercalary and formed

either singly or in pairs. The fungus survives in soil in the form of chlamydospores and mycelia (Singh *et al.*, 2007).

Though reports on different aspects of the disease are available from India and abroad, very scanty information is available on this disease from Jammu and Kashmir. Keeping in view, the importance of disease and socio-economic status of the crop, the present studies were undertaken with the following objectives:

- To study the morphology and physiology of the pathogen.
- To screen the available germplasm/ lines against the wilt causing pathogen.
- Evaluation of fungicides and bio-control agents against the pathogen.

Chapter-2

REVIEW OF LITERATURE

Chickpea is prone to more than 50 pathogens. These include wilt (*Fusarium oxysporum* f. sp. *ciceri*), black root rot (*Fusarium solani*), wet root rot (*Rhizoctonia solani*), dry root rot (*Rhizactonia bataticola*), Aschochyta blight (*Aschochyta rabie*), damping-off (*Pythium* spp) and collar rot (*Sclerotium rolfsii*) which are of considerable importance (Nene and Haware, 1981). Out of these diseases, Fusarium wilt is the most important disease of chickpea and is wide spread in chickpea growing areas, where the crop growing season is dry and warm (Nene *et al.*, 1984). Yield losses due to Fusarium wilt of chickpea have been observed as high as 60 per cent (Singh *et al.*, 2007). However, under congenial weather conditions the extent of loss may be upto 100 per cent (Kumari and Khanna, 2014). Research work on characterisation, screening, chemical and biological control of the disease has been carried out world-wide. The review of relevant literature pertaining to the disease and its management is discussed here under following heads:

2.1. Causal organism

The pathogen is a common soil inhabitant with taxonomic nomenclature *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Matuo and Sato (Snyder and Hansen, 1940). This disease was first reported from India by Butler in 1918. Prasad and Padwick (1939) observed the characters *viz.*, colour of mycelium, discoloration of the vascular bundles and conducted pathogenicity test. They reisolated the same fungus from the artificially infected plants. Based on the conidial characters and intercalary chlamydospores, they identified the fungus as *Fusarium*. Padwick (1940) critically studied the characters like colour of stroma, plectenchyma type and size of conidia and identified the species as *Fusarium orthoceras* var. *ciceri*. Following the classification of Snyder and Hansen (1940), Chattopadhyaya and

Sengupta (1967) renamed *Fusarium orthoceras* var. *ciceri* as *Fusarium oxysporum* f. sp. *ciceri*. *Fusarium oxysporum* f. sp. *ciceri* belongs to the Kingdom: Mycota, Division: Eumycota, Sub-division: Deuteromycotina, Class: Hyphomycetes, Order: Tuberculariales, Family: Tuberculariaceae and Genus: *Fusarium*. This change has been accepted by Booth (1971).

2.2. Symptomology

Fusarium wilt incited by *Fusarium oxysporum* f. sp. *ciceri* is one of the most damaging diseases of chickpea. It causes seedlings collapse without discoloration but at adult stage the affected plants show typical wilting i.e. drooping of leaflets, rachis and lamina. When uprooted, the affected seedlings usually show uneven shrinking of the stem above and below the collar region.

Nene *et al.*, (1984) conducted detailed symptomatological studies and observed diagnostic symptoms when the disease occurred at seedling stage. They reported that seedlings collapsed and lie flat on the soil surface, although they retained normal green colour. Such diseased seedlings, when uprooted generally showed uneven shrinking of the stem above and collar region. They further reported that affected seedlings did not exhibit any external root rot, however when split open vertically from collar region downwards, black discoloration of xylem vessels was observed. The seedlings of highly susceptible cultivars were reported to die within 10 days after emergence without black discoloration of internal tissues; however internal browning from root tip to upwards were noticed. They observed that when adult plants were infected, they exhibited drooping of petioles, rachis and leaflets. Initially, drooping was observed in the upper part of the plants, but within a short time (1-2 days) disease was visible in the entire plant. The lower leaves dried but were not shed at maturity. External root rot was not observed but the central inner portion showed dark brown to black discoloration of xylem below and above the collar region. Jalali *et al.*, (1980) reported that only a few branches of the plant were affected which resulted in

partial wilting of chickpea plants. Singh *et al.*, (2007) observed that the pathogen was primarily confined to the xylem vessels in which the mycelium branch and produce microconidia. The microconidia were reported to detach and carried upward in the vascular system until movement stopped, at which point they germinate and the mycelium penetrate the wall of the adjacent vessels.

According to Sonkar *et al.*, (2018) wilt symptoms in adult plants are very common at flowering and pod filling stages. The affected plants show characteristic wilting *viz.*, dropping of the petioles, rachis and leaflets. The lower leaves become chlorotic but most of the other leaves droop while still green. Gradually, all the leaves turn yellow and then light brown or straw coloured. No external rotting, drying or root discoloration is seen when the wilted plants are uprooted, however when the stem is split vertically, internal discoloration can be observed.

2.3. Morphology

Saxena and Singh (1987) reported that hypha of *Fusarium oxysporum* was septate, profusely branched on potato sucrose/dextrose agar at 25 °C initially white turning light buff or deep brown later, fluffy or submerged. The growth becomes felted or wrinkled in old cultures. Various types of pigmentation (yellow, brown, crimson) can be observed in culture. Booth (1971) reported the shape of microconidia of *Fusarium oxysporum* f. sp. *ciceri* as oval-ellipsoid, cylindrical straight to curved measuring 5-12×2.2-3.5 µm, whereas the macroconidia were fusoid and pointed at both ends and 3-5 septate. The three septate macroconidia measured 27-46 µm and 5 septate macroconidia measured 35-60×3.5 µm while that of macroconidia were 6-7 septate, 50-60×3.5-5.0 µm. Couteaudier and Alabouvette (1990) reported that the macro conidia are straight to slightly curved, slender, thin walled usually with three or four septa, a foot-shaped basal cell and curved apical cell. Further, they observed that the micro conidia are ellipsoidal and either have no septum or a single one. Both are formed

from phialides in false heads by basipetal division. They are important in secondary infection. They reported that the chlamydo spores are globose with thick walls and are formed from hyphae or alternatively by the modification of hyphal cells. They are important as endurance organs in soils where they act as inoculum in primary infection.

2.4. Physiological studies

Fusarium has some specific nutrient requirements for growth and sporulation. Farooq *et al.*, (2005) conducted *in vitro* studies on the effect of culture media, carbon and nitrogen sources, temperature and pH levels on mycelial growth of *Fusarium oxysporum* f. sp. *ciceri*. They reported that the fungus grew best on Czapek dox agar and chickpea seed-meal agar media among eight culture media that were tried. Glucose was found to be the best source of carbon whereas peptone was the best source of nitrogen. In 2012, Khilare and Ahmad studied the effect of different culture media, pH and temperature levels on mycelial growth of *Fusarium oxysporum* f. sp. *ciceri*. They reported that the fungus grew best on Czapek dox agar and PDA media among six tested culture media. They further reported that the most suitable pH level for the growth of fungus was 6.0 and 6.5 with 24.7 conidia μl^{-1} . Growth of the fungus was reported to be maximum at 30 °C (24.7 conidia μl^{-1}) after seven days of inoculation, which was reduced drastically below 15 °C and above 35 °C. Similarly Mohamed *et al.*, (2016) reported that the fungus grew best on potato dextrose agar medium (86.7 mm) followed by potato sucrose agar (78.2 mm). Thaware *et al.*, (2016) tested different culture media for mycelial growth of *Fusarium oxysporum* f. sp. *ciceri*, among which potato dextrose agar proved most efficacious media with mean mycelial growth of 89.66 mm followed by Richard's agar medium with 85.66 mm mean mycelial growth. Different culture media, pH and temperature levels on mycelial growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri* was studied by Nath *et al.*, (2017). Mycelial radial growth and sporulation of *Fusarium oxysporum* was reported to be maximum on oatmeal agar medium among seven

culture media tested. No sporulation was observed on water agar medium. Further, they reported that the highest number of macroconidia ($3.27 \times 10^5 \text{ ml}^{-1}$) and microconidia ($4.06 \times 10^5 \text{ ml}^{-1}$) were produced on PDA. Maitlo *et al.*, (2017) revealed that colony growth of *Fusarium oxysporum* f. sp. *ciceri* varied significantly with different culture media, temperatures, nutritional sources and with different pH levels. Moreover, conidial size and number of macro and micro conidia were also reported to vary with different growth conditions. Richard's agar and potato dextrose agar were reported to be the best media for mycelial growth, which produced rapid colony growth, biggest conidia and highest sporulation as compared to the other culture media.

2.5. Host Germplasm Evaluation

Importance of resistant cultivars in disease management is an established fact recognized by researchers. Therefore, identification and use of resistant sources against pests and diseases is an integral component of genetic improvement programme (Iqbal *et al.*, 2010) and various sources of resistance have been identified by Haware *et al* (1992), Pawar *et al.*, (1993) , Dey *et al.*, (1993).

Bajwa *et al.*, (2000) evaluated 32 genotypes against chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri*. Only one line (97021) was found resistant, four lines (97019, 97020, 97022 and 97023) tolerant and 27 lines were reported to be susceptible to highly susceptible. Ayyub (2001) evaluated 101 chickpea lines for wilt resistance in greenhouse. He reported five resistant lines, six lines exhibited moderately susceptible response, while 13 lines were found susceptible and 74 were reported to be highly susceptible. Gurha *et al.*, (2002) screened 570 chickpea genotypes for resistance to chickpea wilt at Kanpur and found 22 cultivars with stable resistance against *Fusarium* wilt. Similarly, Suryawanshi *et al.*, (2003) evaluated 53 genotypes, out of which the cultivars Phule G-9222-2, 97121, 9525-8-3-9, 9421-1, 9409-4, 9426-2, 9329-1, 92307, 96005, 97125,

950103-5-11 and Vijay were reported to be resistant to Fusarium wilt. Dubey and Birendra (2008) screened 600 chickpea genotypes against *Fusarium oxysporum* f. sp. *ciceri* in field conditions. Two genotypes, H 01-36 and PCS-8 were found resistant (<10% wilt) while eight genotypes were found moderately resistant (>10-20% wilt). The remaining genotypes were reported susceptible to highly susceptible against wilt. Subhani *et al.*, (2008) screened 371 cultivars of chickpea against Fusarium wilt and reported thirty-seven resistant lines, 112 moderately resistant, 139 susceptible and 83 were reported to be highly susceptible. Ahmad *et al.*, (2010) evaluated 321 genotypes from different sources under controlled condition to identify genetic sources of resistance against *Fusarium oxysporum* f. sp. *ciceri* at seedling and reproductive stage. They reported 173 resistant, 54 tolerant and 94 susceptible genotypes at seedling stage, whereas at reproductive stage, 102 genotypes were reported resistant, 36 tolerant and 183 susceptible. Eighty two genotypes were reported to be resistant at both stages. Iqbal *et al.*, (2010) reported 14 genotypes *viz.*, 90395, C-235, C-44, CM2000, ILC 182, FLIP97-129C, FLIP97-172C, FLIP98-107C, FLIP98-227C, FLIP98-230C, FLIP98-231C, FLIP98-38C, FLIP98-54C, ILC7374, KC-89 as resistant, 65 as tolerant and 66 as susceptible among the total of 145 lines evaluated. In an another study three hundred and ninety lines were evaluated against Fusarium wilt by Kaur *et al.*, (2012), out of which 48 were found resistant. Saifulla and Nagamma (2012) screened 111 chickpea genotypes against Fusarium wilt during 2011-2012 at Bangalore. Only one genotype, JG 27, was found resistant, fourteen genotypes moderately resistant, twenty one moderately susceptible, forty three susceptible while fourteen were reported to be highly susceptible to wilt. Again in 2013, Saifulla and Nagamma screened eleven chickpea advance breeding materials against Fusarium wilt and reported that genotype WR 315 as resistant while two genotypes *viz.*, L 550 and K 850 were found moderately susceptible and nine genotypes as highly susceptible. Saifulla and Nagamma (2014) screened 64 genotypes and reported three genotypes *viz.*, WR 315, GNG 2047 (R) and BG 3012 as resistant, and five genotypes *viz.*, CSJK 54 (R), GNG 2104, Vihar (Phule

G 95311), AKG 2002- IK and JGK 16 moderately resistant, sixteen genotypes moderately susceptible, sixteen genotypes susceptible while twenty four genotypes were reported to be highly susceptible.

Thaware *et al.*, (2017) screened the chickpea cultivars against *Fusarium oxysporum* f. sp. *ciceri* under natural epiphytotic conditions and reported that during Rabi 2013-14 all the 50 chickpea genotypes exhibited different reactions against *Fusarium oxysporum* f. sp. *ciceri*. Six genotypes (Vishal, BCP-10, BCP-21, BCP-49, BCP-60 and BCP-61) were found highly resistant, thirty one resistant, eight moderately resistant, two moderately susceptible and three were reported to be highly susceptible (JG-62, BDNGK-807 and AKG-1207). In the Rabi 2014-2015, they again evaluated 48 genotypes which exhibited different reactions against Fusarium wilt of chickpea. Single genotype (PG- 8108) was found highly resistant, twenty one resistant, eight moderately resistant, ten moderately susceptible, five susceptible and three were reported to be highly susceptible.

The development of resistant cultivars is the most effective method to manage chickpea wilt and to contribute to stabilizing chickpea yields (Warda *et al.*, 2017). The review of the work done on this aspect suggested that evaluation of a much broader range of germplasm of chickpea under *in vitro* and *in vivo* conditions is required for wilt resistance.

2.6. Disease Management.

2.6.1 Bio-agents

Researchers world-over have tried different bio-agents to effectively manage the chickpea wilt.

Rani and Mane (2014) evaluated two species of fungal bio-agents *viz.*, *Trichoderma viride*, *Trichoderma harzianum* and two bacterial bio-agents (*Pseudomonas fluorescens* and *Bacillus subtilis*) against *Fusarium oxysporum* f.

sp. *ciceri* in *in vitro* conditions using dual culture technique. The highest per cent mycelial growth inhibition was reported in *Trichoderma harzianum* (76.66%) followed by *Bacillus subtilis* (63.14%). The lowest growth inhibition was reported in *Pseudomonas fluorescens* (53.52%). Nagamani *et al.*, (2015) reported the biocontrol efficacy of antagonistic organism in managing the chickpea major root rot pathogens viz., *Fusarium oxysporum* f. sp. *ciceri*, *R. bataticola* and *S. rolfsii* under *in vitro* conditions and reported 81.1% efficiency of *Trichoderma* isolate, KNN 4, against *R. bataticola*. In an experiment, conducted by Suther *et al.*, (2017), *Bacillus subtilis* isolate K18 (BS-K18) was found effective antagonist against wilt pathogen Foc under *in vitro* conditions. The seed treatment with BS-K18 was reported to effectively reduce the onset of disease symptoms in susceptible genotype JG-62 up to 7 days after transplanting in Foc sick soil. Apart from improving disease tolerance, the BS-K18 isolate was reported to exhibit PGPR effect on both resistance and susceptible genotype where shoot length, root length, number of roots per plant, fresh weight and root volume were significantly improved as compare to control chickpea genotypes. Thaware *et al.*, (2016 b) evaluated *in vitro* efficacy of six fungal and two bacterial antagonists against Foc and reported that the *Trichoderma viride* showed highest mycelial growth inhibition (75.55%), followed by *Trichoderma harzianum* (73.77%). Bashir *et al.*, (2017) evaluated three strains of *Trichoderma viride* designated as Tv1, Tv2 Tv3 and two strain of *Trichoderma harzianum* designated as Th1 and Th2 against wilt pathogen of chickpea. They reported that Tv2 was most efficacious and resulted in 72.00 per cent mean inhibition of mycelial growth followed by Tv1 (68.83 %). *Trichoderma harzianum* 2 (Th2) was reported to be least efficacious with 44.40 per cent mean inhibition of mycelial growth only.

Sonkar *et al.*, (2018) reported that *Trichoderma* spp. secrete some compounds such as volatile, non-volatile, water soluble and secondary metabolites from a unique gene, called 'Gene cluster'. *Trichoderma* spp. have played a crucial role in parasitism behaviour for *Fusarium oxysporum* by different

mode of actions. They have regulated mechanism *viz.*, direct mechanism (competition, mycoparasitism, antibiosis and induce resistance of host plant) and indirect mechanism (inactivation of the enzymes produced by pathogen).

2.6.2 Bio-agents and Fungicides.

Andrabi *et al.*, (2011) evaluated different measures to manage the wilt disease in chickpea through various cultural practices, use of bio-control agents and fungicides under *in vitro* and *in vivo* conditions and reported superiority of *Trichoderma viride* over *Trichoderma virens* in controlling the pathogens *in vitro*. They further reported that carbendazim at 100, 200, 500 ppm caused maximum (100 per cent) mycelial growth inhibition of the pathogens under *in vitro* conditions. Fungicides applied as seed treatment were reported to reduce disease incidence. They further reported that seed treatment with carbendazim increased seed germination (71.24%), though it was at par with carbendazim + mancozeb (62.21%) and mancozeb (61.46%). Seed coating with *T. viride* was reported to result in minimum disease incidence (9.24%). They further reported maximum yield (10.10 q ha⁻¹) with the application of carbendazim, followed by carbendazim + mancozeb (9.77 q ha⁻¹) and *T. viride* (8.10 q ha⁻¹). Mondhe *et al.*, (2013) conducted an experiment to study the effect of antagonists, fungicides and botanical extracts against *Fusarium oxysporum* f. sp. *ciceri*. They reported that carbendazim seed treatment (2g/ kg seed) resulted in minimum wilt incidence (26.38%) and maximum yield (13.47q/ha) followed by *T. viride* + Carbendanzim, *T. viride* + Thiram and *Trichoderma viride* alone.

Efficacy of fungicides and biological control agents were tested side by side both in *in vitro* and in glass house against chickpea wilt pathogen by Mahmood *et al.*, (2015). Carbendazim was reported to be best in checking the mycelial growth (83.7%) over control and similar results were observed in glass house assay. In dual culture test of bio control agents *Pseudomonas fluorescens* was found to cause more mycelial growth inhibition (70.94%) followed by

Trichoderma harzianum (63.95%) and *Bacillus subtilis* (57.68%). Biswas and Ali (2017) conducted an experiment for the management of Fusarium wilt of chickpea under the undulating red and lateritic belt of West Bengal. Of all the biocontrol agents evaluated against *Fusarium oxysporum* f. sp. *ciceri*, *Trichoderma harzianum* was reported to exhibit maximum mycelial growth inhibition (80%) followed by *Pseudomonas fluorescens* (75.55%) and *Trichoderma viride* (64.44%). They further reported that carbendazim exhibited maximum fungal growth inhibition (100%) at 0.1, 0.15 and 0.2 per cent concentrations followed by combination product (carbendazim 12% + mancozeb 63%), which resulted in 88.7 per cent mycelial growth inhibition at 0.1 per cent concentration and 100 per cent growth inhibition at 0.15 and 0.2 per cent concentrations.

2.6.3 Fungicides.

Subhani *et al.*, (2011) evaluated fungitoxic effects of six fungicides, namely, Benomyl, Derosal, Ridomil, Cabrio Top, Vitavax and Prevent at different concentrations through poisoned food technique. They reported that the most effective fungicides in inhibiting the growth of the fungus, in descending order of efficacies were Derosal, Benomyl and Vitavax with 100, 95.81, and 93.80 per cent reduction in mycelial growth at 50 ppm concentration, respectively. The results of their field evaluation indicated that Derosal and Benomyl were most effective in reducing number of wilted plants followed by Vitavax, while the Cabrio Top was reported to be least effective. They further reported that Derosal and Benomyl exhibited 100 per cent reduction in disease incidence, while Vitavax and Cabrio Top exhibited 96.33 and 88.37 per cent reduction in disease incidence, respectively. Patra and Biswas (2016) evaluated ten fungicides against the chickpea wilt pathogen under *in-vitro* conditions at three concentrations. They reported that carbendazim exhibited 100% inhibition of fungus at 1000 and 1500 ppm while as copper oxychloride was found least effective with 51.11, 65.56 and 76.67 per cent inhibition at 500, 1000 and 1500 ppm concentrations, respectively. Carbendazim was reported to be most

the effective fungicide under *in vivo* evaluation with minimum wilt incidence (9.66%) while Chlorothalonil was found to be least effective fungicide for the management of disease with 15.18 per cent disease incidence.

Moreover, five non- systemic fungicides *viz.*, captan 50WP, copper oxychloride 50WP, dodine 65WP, mancozeb 75WP, antracol 70WP and five systemic fungicides *viz.*, bitertanol 25 WP, carbendazim 50WP, difenconazole 25EC, hexaconazole 5EC, and myclobutanil 10WP were evaluated against chickpea wilt by Bashir *et al.*, (2017) who reported dodine and captan as the most effective in inhibiting mean mycelial growth by 66.13 per cent followed by mancozeb (51.42%). They reported copper oxychloride as least effective among the tested non systemic fungicides causing only 23.79 per cent mean inhibition of mycelial growth. Among the systemic fungicides, they reported carbendazim as most effective exhibiting mean mycelial growth inhibition of 92.17 per cent followed by difenconazole and hexaconazole with 76.52 and 71.18 per cent, mean mycelial growth inhibition, respectively. Among the systemic fungicides, they reported myclobutanil 10WP as the least effective with only 64.38 per cent mean mycelial growth inhibition.

Golakiya *et al.*, (2018) conducted an *in vitro* experiment to find out effective fungicides for the management of chickpea wilt. Different fungicides were tested against *Fusarium oxysporum* f. sp. *ciceri* *viz.*, systemic, non-systemic and fungicide combination. Among systemic fungicides, tebuconazole 25.9% EC was reported as most effective with mean mycelial growth inhibition of 89.19 per cent, while among non-systemic group of fungicides copper oxychloride 50% WP was reported most effective with mean mycelial growth inhibition of 61.40 per cent. In case of combination fungicides, carbendazim 12% + mancozeb 63% WP was reported to cause a maximum mean mycelial growth inhibition of 73.50 per cent.

Chapter-3

MATERIALS AND METHODS

The present investigations on “**Studies on Chickpea Wilt (*Fusarium oxysporum* f. sp. *ciceri*) in Kashmir**” were undertaken during the year 2017-19 in the laboratory and experimental fields of Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Wadura. The materials used and methods followed during the course of investigations are described as under:

3.1 Isolation, Purification and Identification of the Pathogen

3.1.1 Isolation of the pathogen.

Chickpea plants showing typical disease symptoms were collected from experimental field at FoA, Wadura, and used for the isolation of the pathogen. The isolation of fungal pathogen was done by tissue bit transfer technique (Sicard *et al.*, 1997). The symptomatic diseased roots were cut into small bits (2-3 mm) with a sharp sterilized blade so that each diseased bit contained a portion of healthy tissue along with it. The bits were subjected to surface sterilization with 2 per cent Sodium hypochlorite solution for one minute followed by thrice rinses with distilled sterilized water to remove the last trace of sodium hypochlorite solution. The bits were blotter dried and later transferred aseptically to potato dextrose agar (PDA) medium in sterilized Petri plates and incubated at 25 ± 1 °C for periodic observations for colony colour, texture and sporulation.

3.1.2 Purification of the pathogen

The pure culture of the fungus was obtained by single spore transfer method (Jhonston and Booth, 1983). For this purpose, a spore suspension was evenly spread on the surface of plain agar medium in Petri plates for germination. The plates were incubated at 25 ± 1 °C and regularly observed under compound

microscope for spore germination. The germinating spores along with agar disc were lifted with the help of a sterilized inoculation needle, transferred to PDA medium plates under aseptic conditions and incubated at 25 ± 1 °C. The pure culture so obtained was maintained for further study in different experiments by sub-culturing regularly after every 30 days. The stock cultures in PDA slants were stored at 4 °C in refrigerator.

3.1.3 Identification of the pathogen.

The pathogenic isolate was identified on the basis of morphological characters of somatic and reproductive structures and compared with the monograph on *Fusarium* spp by Booth (1971).

3.2 Pathogenicity Test

Pathogenicity test was carried by inoculating conidial suspension in pots after 10 days of sowing. In order to raise the plants to conduct pathogenicity test, plastic pots were filled with sterilized soil consisting of soil + sand + FYM in the ratio of 2:1:1 (w/w/w). The soil was sterilized by autoclaving at 1.05 kg cm^{-2} for three consecutive days and 3 kg of the sterilized soil was put in each pot (having diameter of 40 cm). Spore suspension was prepared by brushing freshly prepared culture containing sterilized distilled water. Spores were washed from the medium and suspended in sterile distilled water. Conidial suspensions were adjusted to approximately 2.5×10^6 conidia ml^{-1} using haemocytometer. Inoculation was done after 10 days of sowing by drenching the conidial suspension of test fungus (2.5×10^6 conidia ml^{-1}) in the pots. The plant pots were kept in greenhouse. These plants were maintained for six weeks and were examined for symptom development. The symptoms developed were microscopically examined for presence of spores. The pathogenicity was confirmed through Koch's postulates.

3.3 Morphology and Physiology of Pathogen on Different Solid Media.

Five different solid culture media *viz.*, potato dextrose agar, Czapek dox agar, oatmeal agar, dal weed sucrose agar and chickpea leaf extract agar were used to study the morphological characters (colony colour, texture and shape), effect on mycelial growth and sporulation of the pathogen. Each of the media to be evaluated was poured separately in 250 ml conical flasks, plugged with non-absorbent cotton and autoclaved at 15 lbs pressure per square inch for 15 minutes. Thirty millilitre of each test media was poured separately into sterilized Petri plates (80 mm) under aseptic conditions. Inoculations were made with uniform mycelial discs (5 mm diameter) from seven days old culture. Each treatment was replicated three times in Completely Randomized Design (CRD). The inoculated Petri plates were incubated at 25 ± 1 °C for 7 days. The radial mycelial growth in all the three replications from each medium was recorded and average of the three replications was taken as final observation. The sporulation was studied, by thoroughly homogenizing a 5 mm mycelial disc in 3 ml of sterilized distilled water. The spore suspension thus obtained was used for counting the number of spores with the help of haemocytometer.

3.4 Dry Mycelial Weight of the Pathogen on Different Liquid Media.

Mycelial discs (5 mm) taken from the seven days old culture of the pathogen were inoculated on potato dextrose broth, oatmeal broth, chickpea leaf extract broth, dal weed sucrose broth and Czapek dox broth. The inoculated flasks were incubated for 15 days at 25 ± 1 °C. After 15 days fungal mycelia mats were harvested, washed and filtered through previously weighed Whattman's filter paper no.1. Mycelial mats along with filter papers were dried at 60 °C for 24 hours. After drying the mycelial mats were weighed and again dried in oven until the constant weights were obtained.

Weight of dry mycelial mat was calculated by the following formula:

$$DW = (W_2 - W_1)$$

Where,

DW = Dry weight of mycelial mat

W_2 = Weight of test fungus along with filter paper

W_1 = Weight of filter paper

3.5 Screening of the Available Germplasm against the Pathogen.

Seventy-five genotypes collected from ICARDA were screened against the pathogen in wilt sick plots under natural epiphytotic conditions at FoA, Wadura in Rabi season during 2017-18.

The genotypes were sown in two different nurseries *viz.*, Chickpea International Fusarium Wilt Nursery 2018 (CIFWN-18) and Chickpea International Elite Nursery Winter 2018 (CIEN-W-18). In CIFWN-18, there were forty test genotypes and one susceptible check *viz.*, ILC482. Two lines of test genotypes were alternated with one check and were planted in two replications. In CIEN-W-18, there were thirty-one test genotypes and four susceptible checks ILC482, FLIP82-150C, FLIP88-85C and FLIP93-93C. All the test genotypes were planted in two replications. Susceptible checks were planted in separate plots. In both the nurseries length of each row was 4 metre and width was 0.45 metre.

The observations on wilt were recorded as percentage of disease incidence by applying 0 – 5 point disease rating scale.

Disease rating scale for calculating per cent mortality of *Fusarium oxysporum* f. sp. *ciceri*

Grade	Per cent Mortality	Disease Reactions
0	No disease	Highly resistant
1	1 to 10	Resistant
2	10.1 to 20	Moderately resistant
3	20.1 to 30	Moderately susceptible
4	30.1 to 50	Susceptible
5	50 and above	Highly susceptible

(Anonymous, 1999)

Per cent disease incidence was calculated as:

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

3.6 Evaluation of Bio-control Agents and Fungicides.

The bio-control agents and fungicides were first evaluated under *in vitro* conditions and those found most effective were further evaluated under *in vivo* conditions.

3.6.1 Procurement and maintenance of bio-control agents

One fungal bio-agent *viz. Trichoderma harzianum* and two bacterial bio-agents *Bacillus subtilis* and *Pseudomonas fluorescens* were procured from bio-fertilizer unit of FoA, Wadura, SKUAST Kashmir. Bio-agents were sub-cultured on PDA and allowed to grow at 25±1 °C for ten days and preserved in refrigerator for *in vitro* and *in vivo* studies.

3.6.2 *In vitro* evaluation of bio-agents

Bio-control agents viz., *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against *Fusarium oxysporum* f. sp. *ciceri* through dual culture method (Dhingra and Sinclair, 1995). Culture discs (5 mm) of fungal antagonist and the pathogen were taken from the margin of the actively growing cultures and transferred to PDA medium in 80 mm Petri plates on opposite side, approximately at 10 mm from the wall of the plate while as bacterial bio agents were streaked on the opposite side of the pathogen. A check having the pathogen only was maintained under similar conditions for comparison. The Petri plates were subsequently incubated at 25±1° C till mycelial inhibition was observed. Colony diameter of the fungus in the dual culture as well as in control was recorded after seven days of incubation. Per cent growth inhibition of the pathogen over control was calculated according to the formula given by Vincent (1947) as:

$$I = \frac{C-T}{C} \times 100$$

Where,

I = Per cent Inhibition;

C= Colony diameter in control

T= Colony diameter in treatment

3.6.3 *In vitro* Evaluation of Fungicides.

Nine fungicides (four non systemic and five systemic) were evaluated *in vitro* for their efficacy against the mycelial growth inhibition of *Fusarium oxysporum* f. sp. *ciceri* by poisoned food technique (Nene and Thapliyal, 1979). Each fungicide was evaluated at three different concentrations. The non-systemic fungicides were evaluated at 500, 1000 and 1500 ppm while systemic fungicides were evaluated at 50, 150, and 250 ppm.

List of fungicides used in the experiment.

Fungicide	Trade name	Chemical name
Mancozeb 75 WP	Dithane M-45	Manganese ethylene bis (dithiocarbamate) polymeric complex with zinc
Copper Oxychloride 50 WP	Blue copper	Copper chloride oxide, hydrate
Mancozeb 63%+ Carbendazim 12% WP	Saathi	2-(Methoxy carbonyl) – benzimidazole + Manganese zinc ethylene bisdithiocarbamate
Chlorothalonil 75 WP	Kaavach	2,4,5,6-Tetrachloro isophthalonitrile
Carbendazim 50 WP	Bavistin	Methyl 1H-benzimidazole-2-ylcarbamate
Difenoconazole 25 EC	Score	Cis-trans-3-chloro-4[4-methyl-2-(1H-1,2,4-triazole-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl-4-chlorophenylether
Kresoxim methyl 44.3% SC	Tata Ergon	Methyl(E)-methoxyimino[α-(o-tolyloxy-o tolyl)]acetate
Tebuconazole 50 WG+ Trifloxystrobin 25 WG	Nativo	(RS)-1-(4-Chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazole-1-ylmethyl)pentan-3-ol + Benzeneacetic acid, (E,E) alpha(methoxyimino) 2-[[[1-{3(trifluoromethyl) phenyl}ethylidene]amino]oxy]methyl-, methylester
Metiram 55%+ Pyraclostrobin 5% WG	Cabrio Top	Zinc ammoniate ethylene bis (dithiocarbamate)-poly[ethylene bis thiuramdisulphide] + Methyl{2-[1-(4-chlorophenyl)pyrazole-3-ylloxymethyl]phenyl}(methoxy)carbamate

The required concentrations of the fungicides were prepared by adding appropriate amount of fungicides to sterilized molten PDA medium in conical flasks. Thirty millilitre of such amended PDA was aseptically poured in sterilized Petri plates. A 5 mm diameter mycelial disc of the pathogen was aseptically placed in the centre of each Petri plate. Suitable checks were kept where the culture discs were grown under same conditions on PDA without fungicides. Each treatment was replicated thrice and incubated at 25±1 °C for seven days. The comparative efficacy of fungicides was calculated as per cent inhibition of mycelial growth of the test fungus in each treatment as compared to check by the following formula (Vincent, 1947)

$$I = \frac{C-T}{C} \times 100$$

Where,

I = Per cent Inhibition;

C= Colony diameter in control

T= Colony diameter in treatment

3.6.4 *In vivo* evaluation of bio-agent and fungicides.

The bio-control agent (*Trichoderma harzianum*) and the fungicides (mancozeb 63% WP+ carbendazim 12% WP among non-systemic fungicide and carbendazim 50 WP among systemic fungicide) that proved most effective in *in vitro* studies were further evaluated under field conditions for their efficacy in controlling the disease. Field trial was conducted during Rabi 2018-19 at Faculty of Agriculture, SKUAST-K, Wadura. Field was equally divided into beds with plot size of 2 × 1m with each plot having 50 plants. Three replications for each treatment were maintained in Randomized Complete Block Design. The chickpea seeds of susceptible variety (ILC482) were sown after treatment with different combination of bio-agents, non-systemic and systemic fungicide as seed treatment

as well as drenching. The drenching was done after seven days of crop emergence. The plots where only water was given as treatment served as check. Observations on disease incidence were recorded ten days after treatment.

The details of treatments imposed in trial are here as under:

Details of treatment combinations:

S. No	Treatment
T1	Seed treatment with <i>Trichoderma harzianum</i> @10 ⁸ cfu
T2	Seed treatment with mancozeb 63% WP+ carbendazim12%WP @ 2g kg ⁻¹ of seed.
T3	Seed treatment with carbendazim 50WP @1g kg ⁻¹ of seed
T4	Seed treatment with <i>Trichoderma harzianum</i> @10 ⁸ cfu and drenching with same @10 ¹⁶ cfu
T5	Seed treatment with <i>Trichoderma harzianum</i> @10 ⁸ cfu and drenching with mancozeb 63% WP+ carbendazim12%WP @ 0.5%
T6	Seed treatment with <i>Trichoderma harzianum</i> @10 ⁸ cfu and drenching with carbendazim 50WP @ 0.1%
T7	Drenching with <i>Trichoderma harzianum</i> @10 ¹⁶ cfu
T8	Drenching with mancozeb 63% WP+ carbendazim12%WP @ 0.5%
T9	Drenching with carbendazim @ 0.1%
T10	Control (no treatment)

3.7 Statistical Analysis

The data generated from laboratory and field experiments were subjected to statistical analysis as per standard procedure given by Gomez and Gomez (1984) using OPSTAT software.

Chapter- 4

EXPERIMENTAL FINDINGS

The results of present study entitled “**Studies on Chickpea Wilt (*Fusarium oxysporum* f. sp. *ciceri*) in Kashmir**” are presented under following headings.

4.1 Isolation, Purification and Identification of the Pathogen

4.1.1 Isolation of casual pathogen

Wilt infected chickpea plants collected from the field were brought to the laboratory for isolation and further studies (Plate 1). The fungus associated with disease was isolated on PDA from black- brown necrotic tissues of infected roots of chickpea under aseptic conditions as described under materials and methods (section 3.3). The isolated fungus on the culture was examined periodically.

4.1.2 Purification and maintenance of the pathogen

The pure culture of the fungus obtained by using single spore technique was sub-cultured at monthly intervals and stored in refrigerator at 4 °C for further studies.

4.1.3 Identification of the pathogen

The isolated fungus was identified on the basis of various cultural and morphological studies, *viz.*, colony characteristics, mycelia growth, colour, size and sporulation of the fungus under compound microscope and also on the basis of the descriptions given in various monographs and literature (Booth, 1971).

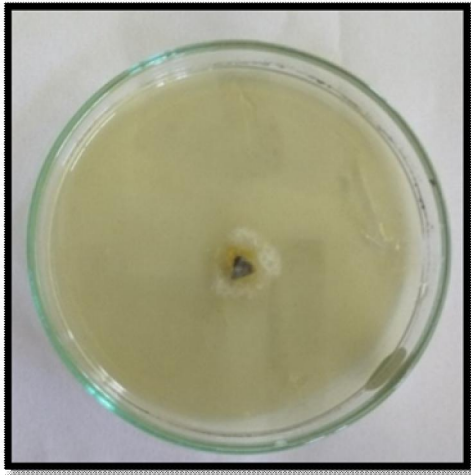
4.1.3.1 Morphological characteristics of the pathogen

The morphological characters of different structures *viz.*, mycelium, micro and macro conidia and chlamydo spores produced by the pathogen were studied using 5-10 day old culture (Table 1).

The pure culture of *Fusarium oxysporum* f. sp. *ciceri* was critically studied for macroscopic and microscopic morphological characters. The fungal colony was white, cottony with profuse, fluffy aerial mycelium up to 15 days of incubation. The colony gradually turned creamish in colour with time, became wrinkled and felted (Plate 2). Microscopic observations of the isolated fungus revealed that the mycelium was septate and hyaline with hyphal width of 5.2-7.6 μm . The pathogen produced three types of spores within the culture viz., microconidia, macroconidia and chlamydospores. Microconidia were hyaline, 0-1 septate and oval to ellipsoid in shape. The length \times breadth of the microconidia ranged from 7.10-10.48 \times 2.40-4.02 μm . Macroconidia too were hyaline with 4-5 septate and fusiform to curved in shape and the size ranged from 20.20-56.00 \times 2.93-6.20 μm . Chlamydospores were produced in 15-20 days old culture. They were globose, single celled, aseptate, produced terminally or intercalary with 7.98-11.77 \times 6.65-9.90 μm size (Plate 3).

Table1. Morphological characteristics of *Fusarium oxysporum* f. sp. *ciceri*

Morphological stage	Shape	Color	Size (μm)	Septation
Mycelium	-	Hyaline	Width 5.2-7.6	Septate
Microconidia	Oval to ellipsoid	Hyaline	7.10-10.48 \times 2.40 - 4.02	0-1
Macroconidia	Fusiform to curved	Hyaline	20.20- 56.00 \times 2.93-6.20	4-5
Chlamydospores	Globose	Hyaline	7.98-11.77 \times 6.65-9.90	Aseptate



(a)



(b)



(c)

Plate 1. (a) Isolation

(b) Purification

(c) Maintenance of *Fusarium oxysporum* f. sp. *ciceri*



(a)



(b)



(c)



(d)

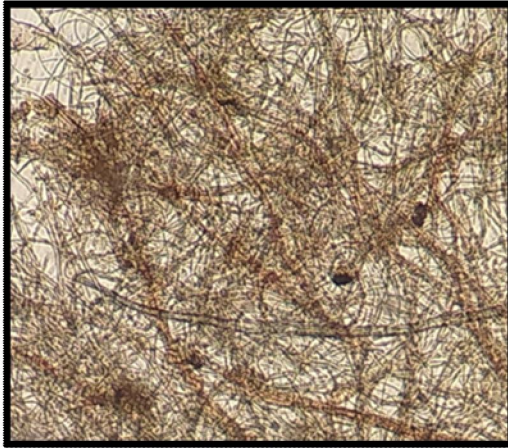
Plate-2: Colony characteristics of *Fusarium oxysporum* f.sp. *ciceri*

(a) 5 days old colony

(b) 15 days old colony

(c) 25 days old colony

(d) 30 days old colony



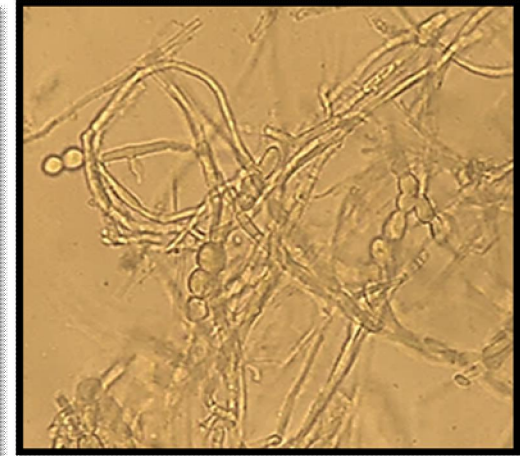
(a)



(b)



(c)



(d)

Plate 3. Morphological characteristics of *Fusarium oxysporum* f.sp. *ciceri*

- (a) Mycelium**
- (b) Microconidia**
- (c) Macroconidia**
- (d) Chlamydospores**

4.2 Pathogenicity Test

Chickpea plants inoculated with conidial suspension were regularly observed for development of symptoms after seven days of inoculation. Observations regarding the pathogenicity of the test fungus revealed initiation of typical symptoms of the disease after 15-20 days of inoculation (Plate 4). However, no symptoms were observed in control. The fungus on re-isolation from inoculated plants resembled initially isolated and inoculated pathogen and hence pathogenicity of the isolate was proved.

4.3 Morphology and Physiology of the Pathogen on Different Solid Media

The preference of *Fusarium oxysporum* f. sp. *ciceri* to various solid media were studied by allowing the test pathogen to grow separately on different media at 25 °C for 7 and 15 days and the data on radial mycelial growth, morphological characters and sporulation obtained was recorded.

4.3.1 Effect of different solid media on mycelial growth and sporulation of the pathogen

Persual of the data (Table 2, Plate 5) revealed that fungus can utilize number of media for its growth. The maximum mycelial growth was recorded on oatmeal agar (80.00 mm) followed by potato dextrose agar (77.63 mm). Minimum mycelial growth (60.40 mm) was observed on dal weed sucrose agar media. Radial mycelial growth on chickpea leaf extract agar and Czapek dox agar was 75.20 mm and 68.40 mm, respectively.

The data further revealed that the highest number of microconidia ($8.55 \times 10^6 \text{ ml}^{-1}$) and macroconidia ($7.56 \times 10^6 \text{ ml}^{-1}$) were formed on potato dextrose agar. dal weed sucrose agar media with microconidia and macroconidia of $4.46 \times 10^6 \text{ ml}^{-1}$ and $3.04 \times 10^6 \text{ ml}^{-1}$ respectively, proved least effective. On oatmeal agar, chickpea leaf extract agar and Czapek dox agar the number of microconidia was

$6.57 \times 10^6 \text{ ml}^{-1}$, $7.10 \times 10^6 \text{ ml}^{-1}$ and $7.44 \times 10^6 \text{ ml}^{-1}$ respectively, while as the number of macroconidia was $4.99 \times 10^6 \text{ ml}^{-1}$, $6.48 \times 10^6 \text{ ml}^{-1}$ and $6.68 \times 10^6 \text{ ml}^{-1}$ respectively.

Table 2. Effect of different solid media on radial growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri*.

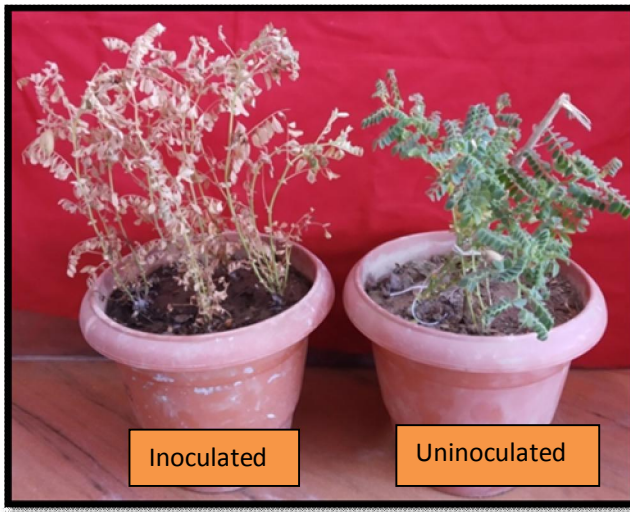
Cultural media	Radial mycelial growth (mm)*	No. of spores ($\times 10^6 \text{ ml}^{-1}$)**	
		Microconidia	Macroconidia
Oatmeal agar	80.00	6.57	4.99
Potato dextrose agar	77.63	8.55	7.56
Chickpea leaf extract agar	75.20	7.10	6.48
Czapek dox agar	68.40	7.44	6.68
Dal weed sucrose agar	60.40	4.46	3.04
CD ($p \leq 0.05$)	0.42	0.46	0.37

*after 7 days of incubation

**after 15 days of incubation

4.3.2 Effect of different solid media on morphological characters of the pathogen

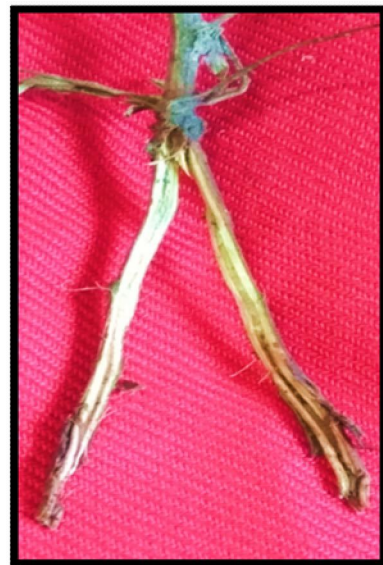
The Petri plates containing different solid media inoculated with test fungus were observed for colony colour, mycelial growth pattern and mycelium form. Observations were recorded after seven days. The results (Table 3) revealed that the fungus had a cottony white, dense and fluffy colony on oatmeal agar media, potato dextrose agar and Czapek dox agar. On dal weed sucrose agar media colony growth was sparse and fluffy. On oatmeal agar and chickpea leaf extract agar fungus a regular mycelial form while as an irregular mycelial form was observed in potato dextrose agar, Czapek dox agar and dal weed sucrose agar.



Healthy plant



Diseased plant



V.S of diseased stem

Plate 4. Pathogenicity test



(a)

(b)

(c)



(d)



(e)

Plate 5. Radial growth of *Fusarium oxysporum* f .sp. *ciceri* on different solid media

- (a) Oat meal agar
- (b) Potato dextrose agar
- (c) Chickpea leaf extract agar
- (d) Czapek dox agar
- (e) Dal weed sucrose agar

Table 3. Effect of different solid media on morphology and colony characters of *Fusarium oxysporum* f. sp. *ciceri*

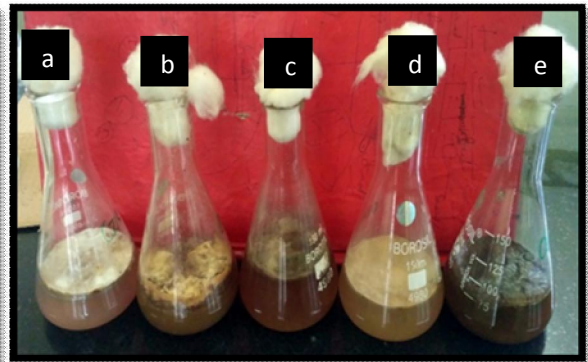
Media	Morphological characters		
	Colour	Mycelial growth pattern	Mycelium form
Oatmeal agar	Cottony white	Dense, compact and fluffy	Regular
Potato dextrose agar	Cottony white	Dense and fluffy	Irregular
Chickpea leaf extract agar	Pure white	Dense and fluffy	Regular with concentric rings
Czapek dox agar	Cottony white	Dense and fluffy	Irregular
Dal weed sucrose agar	Pure white	Sparse and fluffy	Irregular

4.4 Dry Mycelial Weight of the Pathogen on Different Liquid Media.

In order to ascertain the best liquid media for dry mycelial weight of the pathogen, it was allowed to grow separately on five liquid media *viz.*, potato dextrose broth, oatmeal broth, chickpea leaf extract broth, Czapek dox broth and dal weed sucrose broth for 15 days at 25 °C. After 15 days of incubation mycelial mats from each of the media were harvested, filtered and dried at 60 °C for 24 hours (Plate 6). The dry mycelial weight was then calculated by the procedure



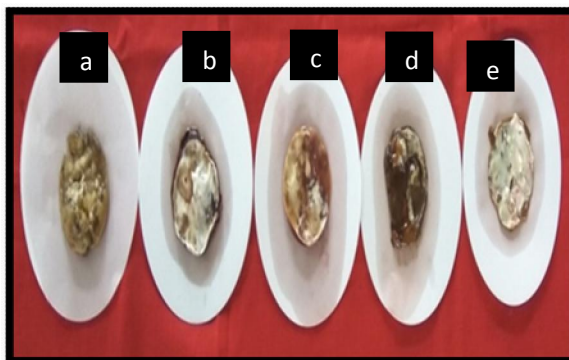
Before inoculation



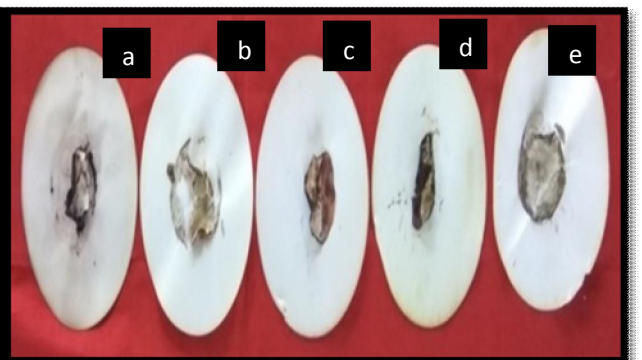
After incubation of 15 days



Filtration



Mycelial mats before drying



Mycelial mats after drying

Plate 6. Average dry mycelial weight of *Fusarium oxysporum* f. sp. *ciceri*

- a.
- b.
- c.
- d.
- e. Chickpea leaf extract broth

- Czapek dox broth
- Oat meal broth
- Dal weed sucrose broth
- Potato dextrose broth

mentioned under section 3. Persual of the data (Table 4) reveals that the oatmeal broth proved the most effective providing an average dry mycelial weight of 723.33 mg followed by potato dextrose broth providing an average dry mycelial weight of 710.33 mg whereas chickpea leaf extract broth and Czapek dox broth provided an average dry mycelial weight of 700.23 mg and 680.53 mg, respectively. Dal weed sucrose broth with an average dry mycelial weight of 650.33 mg proved to be the least efficacious.

Table 4. Average dry mycelial weight of *Fusarium oxysporum* f. sp. *ciceri* on different liquid media

Liquid media	Average dry mycelial weight (mg)*
Oatmeal broth	723.33
Potato dextrose broth	710.33
Chickpea leaf extract broth	700.23
Czapek dox broth	680.53
Dal weed sucrose broth	650.33
CD $p \leq 0.05$	0.47

*after 15 days of incubation.

4.5 Screening of the Available Germplasm against the Pathogen.

Seventy-five genotypes sown in two separate nurseries i.e. CIFWN-18 and CIEN-W-18 (Plate 7) were evaluated for their reaction to Fusarium wilt under natural epiphytotic conditions during Rabi 2017-18 and the observations on per cent incidence were recorded.

The data on the disease reaction at Wadura in CIFWN-18 nursery (Table 5) indicated that the disease incidence ranged from 0.00 to 64.50 per cent. The highest disease incidence of 64.50 per cent was recorded in genotypes ILC482,

while lowest disease incidence of 0.00 per cent was recorded in two genotypes namely FLIP10-382C and FLIP11-122C. The test genotypes were allotted to different reaction groups on the basis of per cent disease incidence in each genotype (Table 6). The data revealed that two genotypes *viz.*, FLIP10-382C and FLIP11-122C were highly resistant with 0.00 per cent disease incidence. Six genotypes *viz.*, FLIP11-23C, FLIP11-24C, FLIP11-77C, FLIP11-90C, FLIP11-150C and FLIP11-176C were resistant where disease incidence varied from 2.75 per cent in genotype FLIP11-24C to 9.00 per cent in genotype FLIP11-176C. Twelve genotypes *viz.*, FLIP10-354C, FLIP11-69C, FLIP11-52C, FLIP11-115C, FLIP11-123C, FLIP11-121C, FLIP11-83C, FLIP11-124C, FLIP11-142C, FLIP11-149C, FLIP11-159C and FLIP11-172C were moderately resistant with disease incidence ranging from 12.00 per cent in genotypes FLIP10-354C to 19.00 per cent in genotype FLIP11-159C. Thirteen genotypes *viz.*, FLIP10-350C, FLIP10-357C, FLIP10-376C, FLIP10-380C, FLIP11-48C, FLIP11-49C, FLIP11-68C, FLIP11-82C, FLIP11-143C, FLIP11-144C, FLIP11-152 and FLIP11-223C were moderately susceptible. The genotype ILC482 was highly susceptible having 64.50 per cent disease incidence.



Chickpea International Fusarium Wilt Nursery-18



**Plate 7. Screening of the available germplasm against the pathogen
Chickpea International Winter Elite Nursery -18**

Table 5. Reaction of genotypes in CIFWN-18 to Fusarium wilt during Rabi 2017-18

S. No	Genotype	Wilt incidence (%)	Disease reaction
01	FLIP10-382C	0	HR
02	FLIP11-122C	0	HR
03	FLIP11-24C	2.75	R
04	FLIP11-23C	3.00	R
05	FLIP11-150C	6.75	R
06	FLIP11-77C	8.00	R
07	FLIP11-90C	8.25	R
08	FLIP11-176C	9.00	R
09	FLIP10-354C	12.00	MR
10	FLIP11-69C	12.33	MR
11	FLIP11-123C	12.50	MR
12	FLIP11-124C	12.50	MR
13	FLIP11-121C	12.75	MR
14	FLIP11-172C	12.75	MR
15	FLIP11-83C	15.50	MR
16	FLIP11-142C	15.50	MR
17	FLIP11-52C	15.75	MR
18	FLIP11-149C	17.25	MR
19	FLIP11-115C	18.00	MR
20	FLIP11-159C	19.00	MR
21	FLIP10-357C	21.00	MS
22	FLIP10-380C	21.00	MS
23	FLIP11-152C	21.75	MS
24	FLIP11-223C	23.00	MS
25	FLIP11-144C	23.25	MS

26	FLIP10-376C	23.50	MS
27	FLIP11-48C	24.00	MS
28	FLIP10-350C	24.75	MS
29	FLIP11-116C	24.75	MS
30	FLIP11-49C	25.00	MS
31	FLIP11-68C	27.75	MS
32	FLIP11-82C	29.00	MS
33	FLIP11-143C	29.00	MS
34	FLIP11-35C	33.25	S
35	FLIP10-368C	34.25	S
36	FLIP10-358C	36.00	S
37	FLIP11-37C	38.00	S
38	FLIP11-204C	42.90	S
39	FLIP11-227C	44.00	S
40	FLIP11-186C	45.00	S
41	ILC482	64.50	HS

HR= Highly resistant, R= Resistant, MR= Moderately resistant, S= Susceptible,
HS= Highly susceptible.

Table 6. Categorization of chickpea genotypes in CIFWN-18 on the basis of their reaction to Fusarium wilt.

Category	Reaction	Average wilt incidence	Genotype
0	Highly resistant	0	FLIP10-382C and FLIP11-122C
1	Resistant	1-10	FLIP11-23C, FLIP11-24C, FLIP11-77C, FLIP11-90C, FLIP11-150C and FLIP11-176C
2	Moderately resistant	10-20	FLIP10-354C, FLIP11-69C, FLIP11-52C, FLIP11-115C, FLIP11-123C and FLIP11-121C, FLIP11-83C, FLIP11-124C, FLIP11-142C, FLIP11-149C, FLIP11-159C and FLIP11-172C
3	Moderately susceptible	20-30	FLIP10-350C, FLIP10-357C, FLIP10-376C FLIP10-380C, FLIP11-48C, FLIP11-49C, FLIP11-68C, FLIP11-82C, FLIP11-143C, FLIP11-144C, FLIP11-152C, FLIP11-116C and FLIP11-223C
4	Susceptible	30-50	FLIP10-358C, FLIP10-368C, FLIP11-37C, FLIP11-186C, FLIP11-204C and FLIP11-227C
5	Highly susceptible	50 & above	ILC482

Similarly in second nursery, CIEN-W-18, data (Table 7) indicated that disease incidence ranged from 4.75 to 55.75 per cent. The highest disease incidence of 55.75 per cent was recorded in genotypes FLIP11-08C and lowest disease incidence of 4.75 per cent was recorded in genotype FLIP10-355C. The

test genotypes were allotted to different reaction groups on the basis of per cent disease incidence in each genotype (Table 8). The data revealed that none of the genotype was found highly resistant. However, three genotypes *viz.*, FLIP10-355C, FLIP11-40C and FLIP11-45C were resistant with disease incidence ranging from 4.75 to 8.75 per cent and seven genotypes *viz.*, FLIP10-332C, FLIP10-343C, FLIP11-163C, FLIP11-175C, FLIP11-190C and FLIP11-231C were moderately resistant with disease incidence ranging from 12.50 to 18.00 per cent in genotype FLIP11-163C whereas fourteen genotypes *viz.*, FLIP11-09C, FLIP11-193C, FLIP11-22C, FLIP11-06C, FLIP11-134C, FLIP93-93C, FLIP10-318C, FLIP11-58C, FLIP11-11C, FLIP11-32C, FLIP10-333C, FLIP10-364C, FLIP11-05C and FLIP11-151C were moderately susceptible showing disease incidence of 21.00, 21.00, 21.25, 22.25, 22.50, 23.00, 23.50, 24.00, 24.75, 24.75, 25.00, 25.00, 27.75 and 28.00 per cent, respectively. Eight genotypes *viz.*, FLIP10-337C, FLIP10-345C, FLIP11-21C, FLIP11-102C, FLIP11-66C, FLIP88-85C, FLIP11-158C and FLIP11-150C were susceptible showing disease incidence of 35.00, 38.25, 40.50, 42.00, 43.50, 43.75, 45.00 and 49.00 per cent, respectively. Three genotypes namely FLIP10-338C, ILC482C and FLIP11-08C were found highly susceptible with disease incidence of 52.75, 53.00 and 55.75 per cent, respectively.

Table 7. Reaction of genotypes in CIEN-W-18 to Fusarium wilt during Rabi 2017-18.

S. No	Genotype	Per cent wilt incidence (%)	Disease reaction
01	FLIP10-355C	4.75	R
02	FLIP11-40C	7.75	R
03	FLIP11-45C	8.75	R
04	FLIP10-332C	15.75	MR
05	FLIP10-343C	13.00	MR
05	FLIP11-125C	12.50	MR
07	FLIP11-163C	18.00	MR
08	FLIP11-175C	17.75	MR
09	FLIP11-190C	11.00	MR
10	FLIP11-231C	16.25	MR
11	FLIP11-09C	21.00	MS
12	FLIP11-193C	21.00	MS
13	FLIP11-22C	21.25	MS
14	FLIP11-06C	22.25	MS
15	FLIP11-134C	22.50	MS
16	FLIP93-93C	23.00	MS
17	FLIP10-318C	23.50	MS
18	FLIP11-58C	24.00	MS
19	FLIP11-11C	24.75	MS
20	FLIP11-32C	24.75	MS
21	FLIP10-333C	25.00	MS
22	FLIP10-364C	25.00	MS
23	FLIP11-05C	27.75	MS
24	FLIP11-151C	28.00	MS
25	FLIP10-337C	35.00	S

26	FLIP10-354C	38.25	S
27	FLIP11-21C	40.50	S
28	FLIP11-102C	42.00	S
29	FLIP11-66C	43.50	S
30	FLIP11-158C	45.00	S
31	FLIP11-150C	49.00	S
32	FLIP88-85C	43.75	S
33	FLIP10-338C	52.75	HS
34	ILC482C	53.00	HS
35	FLIP11-08C	55.75	HS

HR= Highly resistant, R= Resistant, MR= Moderately resistant, S= Susceptible,
HS= Highly susceptible.

Table 8. Categorization of chickpea genotypes in CIEN-W-18 on the basis of their reaction to Fusarium wilt.

Category	Reaction	Average wilt incidence	Genotype
0	Highly resistant	0	-
1	Resistant	1-10	FLIP10-355C, FLIP11-40C and FLIP11-45C
2	Moderately resistant	10-20	FLIP10-332C, FLIP10-343C, FLIP11-125C, FLIP11-163C, FLIP11-175C, FLIP11-190C and FLIP11-231C
3	Moderately susceptible	20-30	FLIP10-318C, FLIP10-333C, FLIP10-364C, FLIP11-05C, FLIP11-06C, FLIP11-09C, FLIP11-11C, FLIP11-22C, FLIP11-32C, FLIP11-58C, FLIP11-134C, FLOP11-151C, FLIP11-193C and FLIP93-93C
4	Susceptible	30-50	FLIP10-337C, FLIP10-345C, FLIP11-21C, FLIP11-102C, FLIP11-66C, FLIP11-158C, FLIP82-150C and FLIP88-85C
5	Highly susceptible	50 & above	ILC482, FLIP11-08C and FLIP10-338C

4.6 Evaluation of Bio-agents and Fungicides against the pathogen.

4.6.1 *In vitro* evaluation of bio-agents and fungicides

4.6.1.1 Bio-agents.

One fungal bio-agent viz., *Trichoderma harzianum*, and two bacterial bio-agents *Pseudomonas fluorescens*, *Bacillus subtilis* (Plate 8) were screened *in vitro* against *Fusarium oxysporum* f. sp. *ciceri* for their antagonistic activity via dual culture technique on PDA as described under 3.7.2.

The data (Table 9, Fig 1.) revealed that after 07 days of incubation, radial mycelial growth in control was 80 mm. *Trichoderma harzianum* proved most effective exhibiting 82.40 per cent mycelial growth inhibition followed by *Bacillus subtilis* with 70.53 per cent mycelial growth inhibition. *Pseudomonas fluorescens* proved least efficacious with 66.30 per cent mycelial growth inhibition.

Table 9. *In vitro* efficacy of bio-agents on mycelial growth inhibition of *Fusarium oxysporum* f. sp. *ciceri*

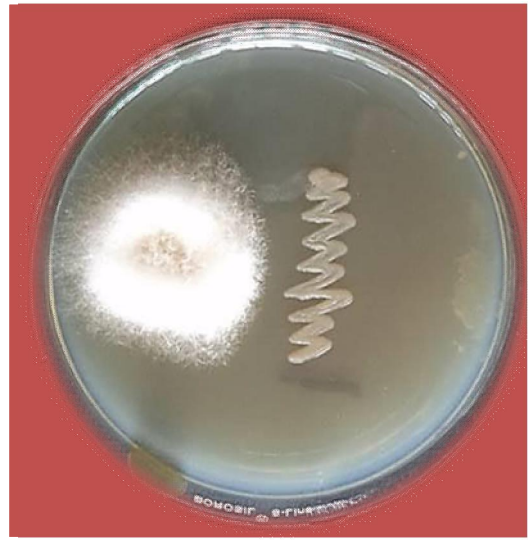
Bio-agent	Mean radial growth (mm)	Mycelial growth inhibition (%)
<i>Trichoderma harzianum</i>	14.08**	82.40** (65.32)*
<i>Bacillus subtilis</i>	23.57	70.53 (57.16)
<i>Pseudomonas fluorescens</i>	26.96	66.30 (54.60)
Control	80	-
CD (p≤0.05)		0.40

**average of five replications

*values in parenthesis are arcsine transformed.



Trichoderma harzianum



Bacillus subtilis



Pseudomonas fluorescens

Plate 8. *In vitro* efficacy of bio-agents on mycelial growth inhibition of *Fusarium oxysporum* f. sp. *ciceri*

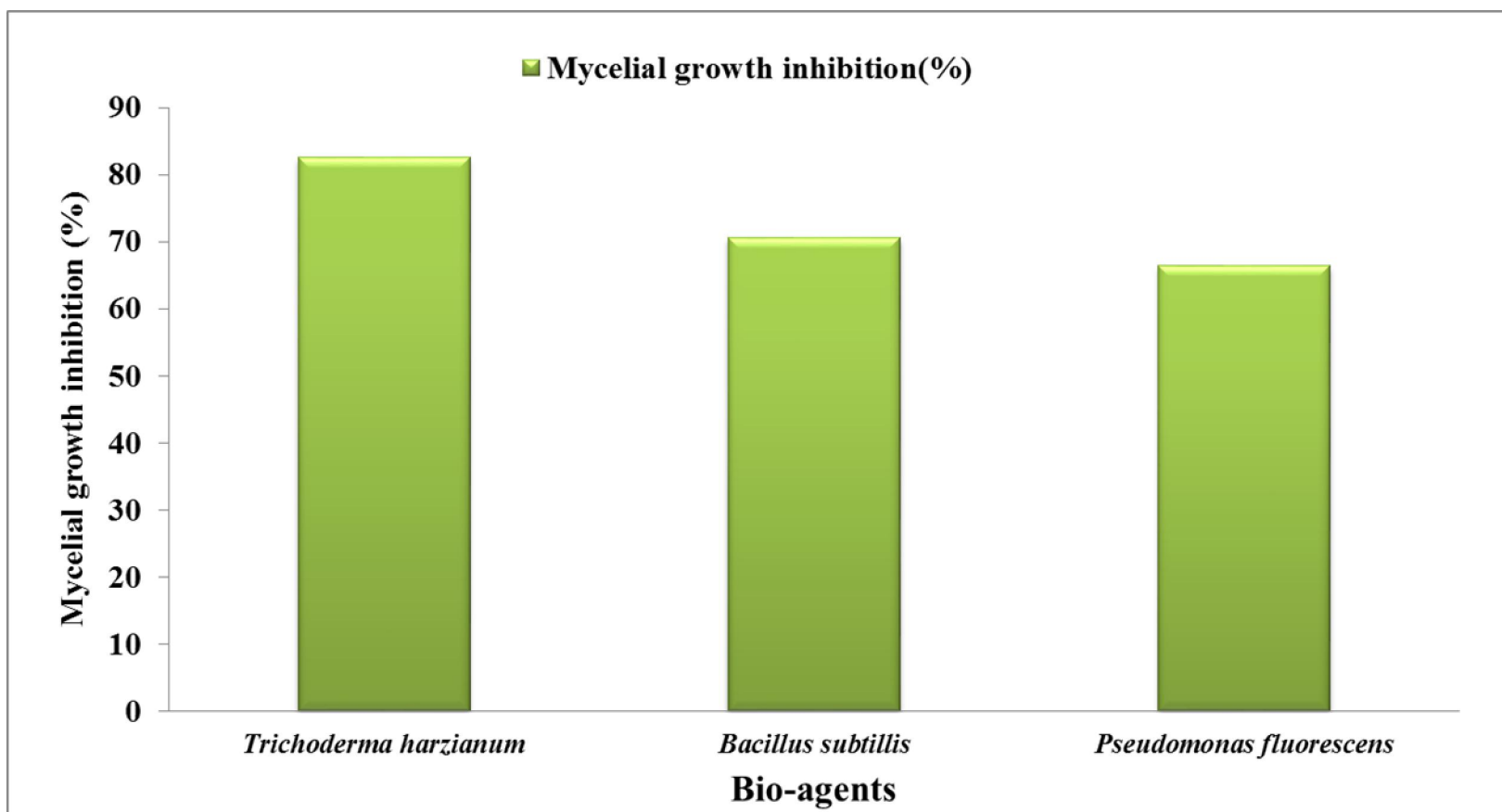


Fig 1. *In vitro* efficacy of bio-agents on mycelial growth inhibition of *Fusarium oxysporum* f. sp. *ciceri*

4.6.1.2 Fungicides

All the nine fungicides when tested *in vitro* through poisoned food technique were significantly superior over control in inhibiting the mycelial growth of the fungus.

4.6.1.3 *In vitro* evaluation of non-systemic fungicides

Perusal of the data (Table 10, Fig 2.) revealed that all the non-systemic fungicides differ significantly from each other (Plate 9). Among non-systemic fungicides tested, mancozeb 63%+ carbendazim12% WP proved to be most effective exhibiting mean mycelial growth inhibition of 94.02 per cent over control followed by mancozeb 75WP exhibiting 72.82 per cent mean mycelial growth inhibition. However, copper oxychloride 50WP proved to be least effective with only 67.46 per cent mean mycelial growth inhibition. There is also a significant interaction between fungicides and their concentrations. Mancozeb 63%+ carbendazim12% WP at each respective concentration was found to be statistically superior to all test fungicides. The data further reveals that mancozeb 63%+ carbendazim12% WP proved significantly most effective by providing 100 per cent mycelial growth inhibition at 1500 ppm concentration.

A minimum inhibition of 64.62 per cent was achieved at 500 ppm which increased gradually to 86.62 per cent at 1500 ppm concentration.

4.6.1.4 *In vitro* evaluation of systemic fungicides

Perusal of data (Table 11, Fig 3.) revealed that all the four systemic fungicides differ significantly from each other (Plate 9). Among the systemic fungicides tested, carbendazim 50 WP proved to be most effective by providing 99.15 per cent mycelial growth inhibition over control followed by difenconazole 25EC exhibiting 75.39 per cent growth inhibition, respectively. Trifloxystrobin 25% +tebuconazole 50% WP and metiram 55% + pyraclostrobin 5% WP inhibited mycelial growth by 58.05 and 48.15 per cent, respectively. However,

krexosim methyl 44.3% SC proved to be least effective with only 45.72 per cent inhibition of mycelial growth. On an overall basis the inhibitory effect of test fungicides increased with increase in concentration. Carbendazim 50 WP caused 100.00 per cent mycelial growth inhibition beyond 150 ppm. A minimum inhibition of 56.97 per cent was achieved at 50 ppm which increased gradually to 72.89 per cent at 250 ppm concentration.

Table 10. *In vitro* evaluation of non-systemic fungicides for mycelial growth inhibition of *Fusarium oxysporum* f. sp. *ciceri*

CD ($p \leq 0.05$) Concentration = 0.23 Fungi-toxicant = 0.18

S. No	Fungicide	Mycelial growth inhibition (%)*	Concentrations (ppm)		
			500	1000	1500
01	Mancozeb 63% + Carbendazim 12% WP	87.46**	94.62	100	94.02
		(69.27)	(76.62)	(90.02)	(78.64)
02	Mancozeb 75WP	57.27	74.62	86.57	72.82
		(49.20)	(59.77)	(68.56)	(59.18)
03	Chlorothalonil 50WP	58.46	69.10	80.76	69.44
		(49.86)	(56.24)	(64.01)	(56.70)
04	Copper oxychloride 50WP	55.30	67.95	79.13	67.46
		(48.06)	(55.55)	(62.84)	(55.48)
Mean		64.62	76.57	86.62	
		(56.58)	(64.10)	(72.57)	

(FxC) = 0.40

*Over control

**Average of three replication.

Table 11. *In vitro* evaluation of systemic fungicides for mycelial growth inhibition of *Fusarium oxysporum* f. sp. *ciceri*

S. No.	Fungicide	Mycelial growth inhibition (%)*			
		Concentrations (ppm)			Mean
		50	150	250	
01	Carbendazim 50WP	97.46	100.00	100.00	99.15
		(80.88)**	(90.00)	(90.00)	(86.96)
02	Difenconazole 25EC	70.55	73.93	81.68	75.39
		(57.14)	(59.30)	(64.70)	(60.38)
03	Trifloxstrobilin 25% + Tebuconazole 50% WP	44.95	59.37	69.84	58.05
		(42.10)	(50.40)	(49.73)	(47.73)
04	Metiram 55% + Pyraclostrobin 5% WG	39.22	50.00	55.22	48.15
		(41.12)	(47.23)	(45.34)	(43.44)
05	Kresoxim methyl 44.3% SC	32.70	46.73	57.74	45.72
		(34.88)	(43.13)	(49.46)	(39.49)
Mean		56.97	66.01	72.89	
		(49.75)	(57.71)	(61.59)	

CD ($p \leq 0.05$) Fungi-toxicant (F) = 0.14 Concentration(C) = 0.12
(F×C) = 0.24

*Over check

**Figures in parenthesis are arc sine transformed values.

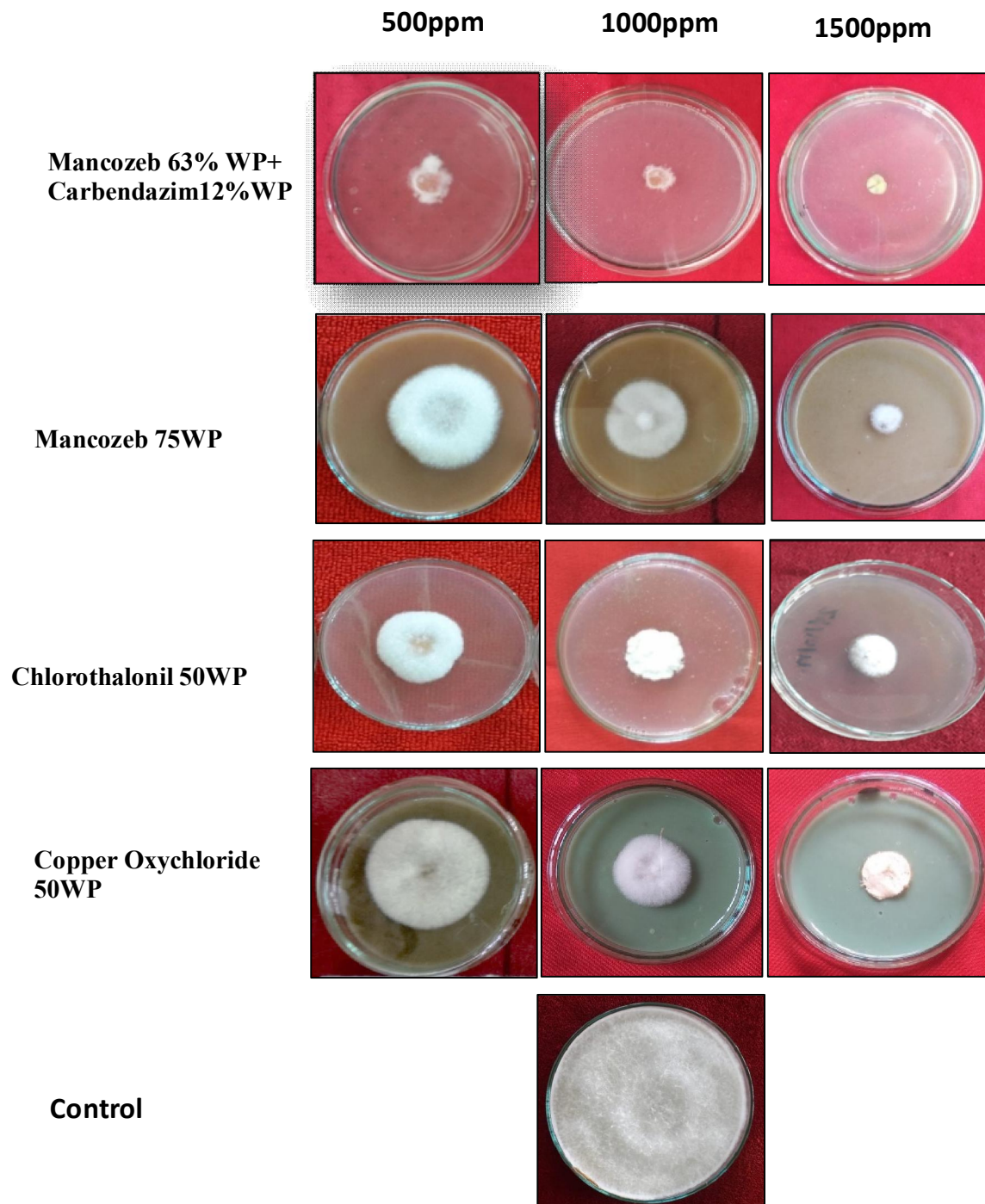
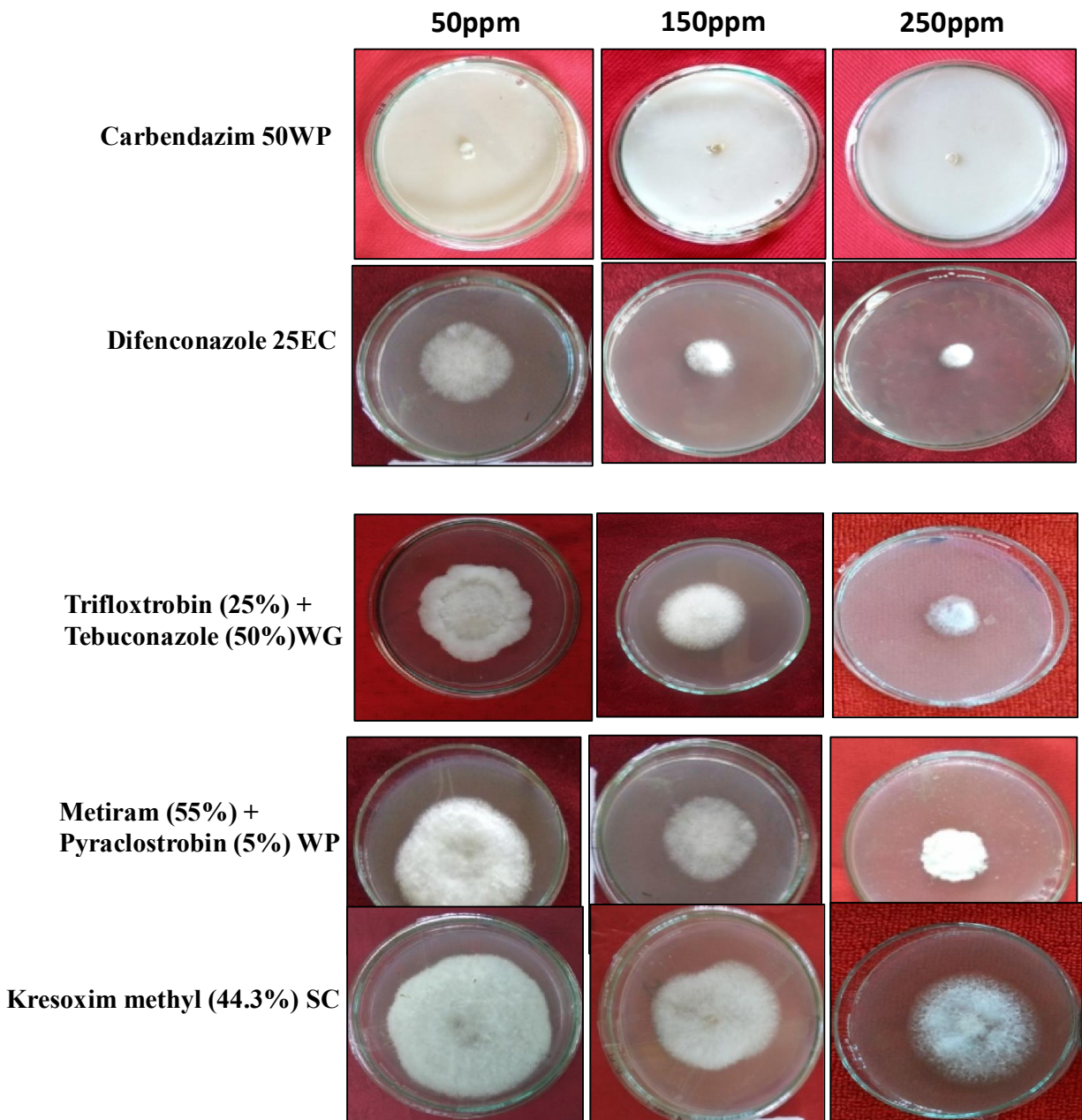


Plate 9. *In vitro* evaluation of non-systemic fungicides for mycelial growth inhibition of *Fusarium oxysporum* f. sp. *Ciceri*



Control



Plate 10. *In vitro* evaluation of systemic fungicides for mycelial growth inhibition of *Fusarium oxysporum* f. sp. *ciceri*

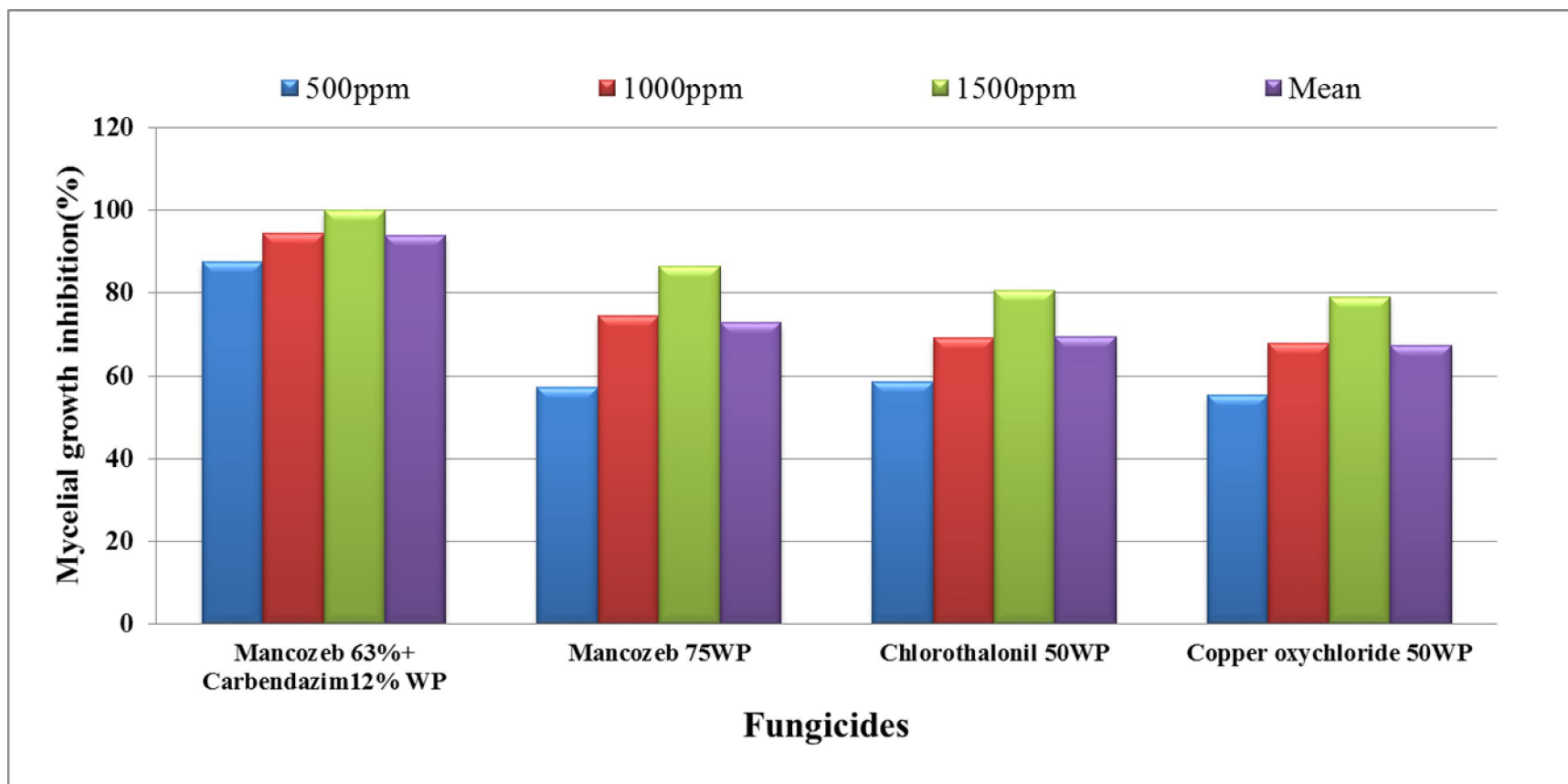


Fig 2. *In vitro* evaluation of non-systemic fungicides for mycelial growth inhibition of *Fusarium oxysporum* f. sp. *ciceri*

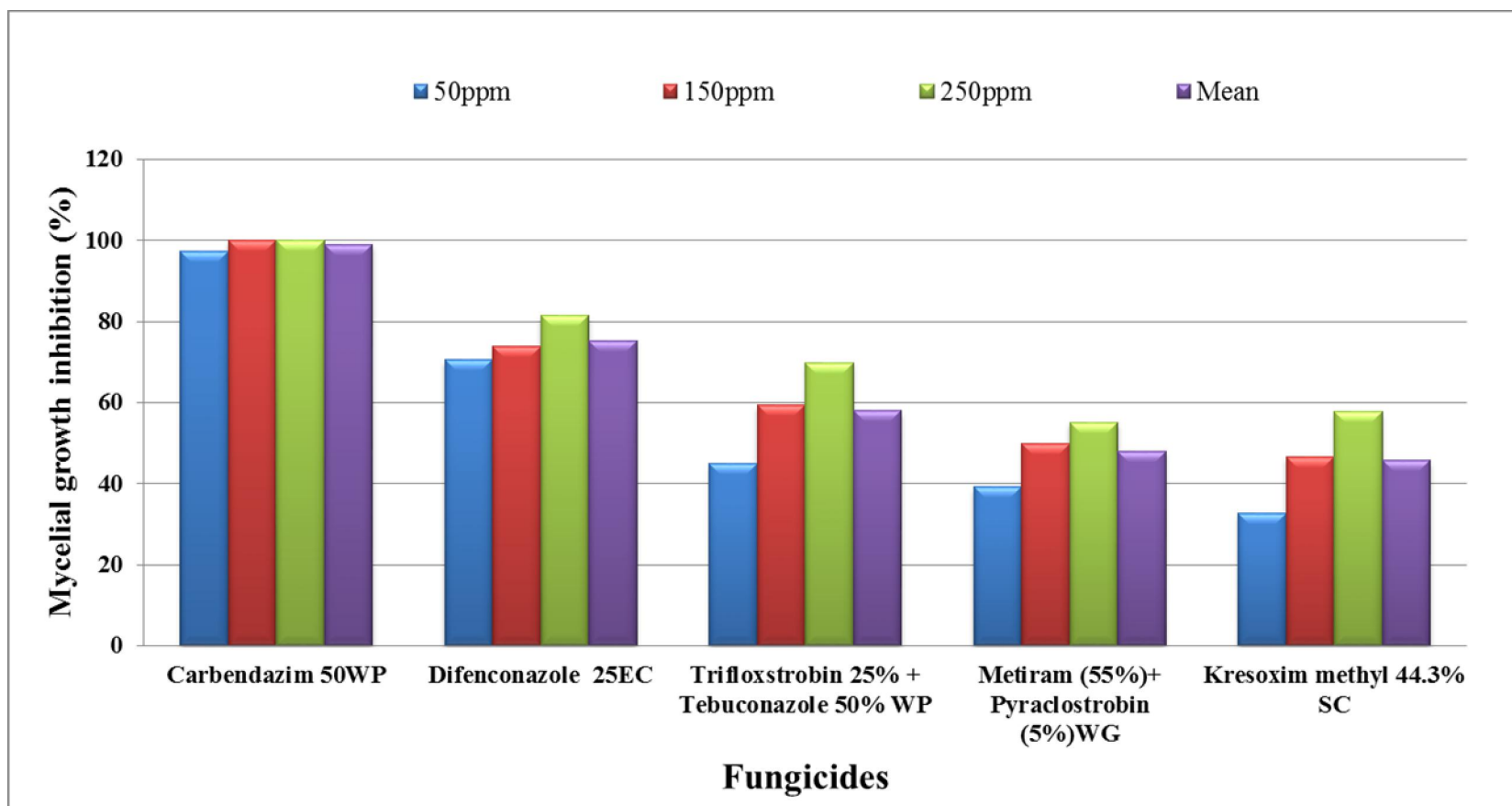


Fig 3. *In vitro* evaluation of systemic fungicides for mycelial growth inhibition of *Fusarium oxysporum* f. sp. *ciceri*

4.6.2 *In vivo* management of the disease

The experiments to evaluate the effect of promising bio-agents and fungicides, found effective under *in vitro* studies, were conducted in the experimental plots at Faculty of Agriculture, SKUAST-K, Wadura during Rabi 2018-19. The bio-agents and fungicides were used as seed treatment and drenching under field conditions against the Fusarium wilt of chickpea. The experiment was laid in Randomized Complete Block Design. Field was equally divided into raised beds with plot size of 2×1m and each plot having 50 plants and replicated thrice. The most effective non-systemic fungicide (mancozeb 63% + carbendazim 12% WP), most effective systemic fungicide (carbendazim 50WP) and most effective bio-agent (*Trichoderma harzianum*) were evaluated under field conditions to device disease management capsule.

The results on disease incidence are presented in Table 12. The data revealed that all the seed treatments and soil drenches were superior over the control in controlling the disease incidence. In the experiment the mean disease incidence ranged from 4.53 to 46.59 per cent. Lowest disease incidence of 4.53 per cent was recorded in treatment combination of seed treatment with *Trichoderma harzianum* @10⁸ cfu and drenching with carbendazim 50 WP @ 0.1 per cent. Highest disease incidence of 46.59 per cent was observed in check plots. Drenching with carbendazim 50 WP @ 0.5 per cent alone resulted in the disease incidence of 10.27 per cent followed by mancozeb 63% WP+ carbendazim12%WP @ 0.5 per cent and *Trichoderma harzianum* @10¹⁶ cfu with disease incidence of 11.74 per cent and 17.65 per cent, respectively. Further it was observed that amongst the seed treatments, least disease incidence of 13.67 per cent was observed when the seeds were treated with carbendazim 50 WP @1g kg⁻¹ of seed followed by mancozeb 63% WP + carbendazim 12% WP @ 2g kg⁻¹ seed and *Trichoderma harzianum* @10⁸ cfu with disease incidence of 16.26 and 21.97 per cent, respectively.

Table 12. *In vivo*/ field evaluation of the bio-agent and fungicide against the wilt disease

S. No	Treatment	Mean disease incidence (%)
T1	Seed treatment with <i>Trichoderma harzianum</i> @10 ⁸ cfu	21.97*(27.93)**
T2	Seed treatment with mancozeb 63% WP+ carbendazim 12% WP @ 2g kg ⁻¹ of seed	16.26(24.01)
T3	Seed treatment with carbendazim 50WP 1g/kg of seed	13.67(21.40)
T4	Seed treatment with <i>Trichoderma harzianum</i> @10 ⁸ cfu and drenching with same @10 ¹⁶ cfu	12.22(20.35)
T5	Seed treatment with <i>Trichoderma harzianum</i> @10 ⁸ cfu and drenching with mancozeb 63% WP+ carbendazim 12% WP @ 0.5%	8.59(16.03)
T6	Seed treatment with <i>Trichoderma harzianum</i> @10 ⁸ cfu and drenching with carbendazim 50WP @ 0.1%	4.53(12.21)
T7	Drenching with <i>Trichoderma harzianum</i> @10 ¹⁶ cfu	17.65(24.20)
T8	Drenching with mancozeb 63% WP+ Carbendazim 12% WP @ 0.5%	11.74(20.03)
T9	Drenching with carbendazim @ 0.1%	10.27(18.62)
T10	Control	46.59(42.65)
CD (p≤0.05)		0.34

*Average of three replications

**Figures in parenthesis are arc sine transformed value

Chapter-5

DISCUSSION

Chickpea (*Cicer arietinum* L.) is an important legume food crop cultivated in almost all over the world including temperate and sub-tropical regions. Chickpea is rich source of proteins, carbohydrates, starch, other minerals and essential amino acids. Chickpea plays the pivotal role of supplying protein source in the vegetarian diet and is also called as the 'poor man's meat' (Ibrikci *et al.*, 2003). It is affected by various diseases *viz.*, Fusarium wilt, Ascochyta blight, Phytophthora root rot, Botrytis grey mold, Damping off, Pythium rot and seed rot (Singh and Sumbali, 2007). Among them Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is the most important disease of chickpea and is wide spread in chickpea growing areas, where the crop growing season is dry and warm (Nene *et al.*, 1984). In India the chickpea wilt was first reported by Butler in 1918. Chickpea wilt is deemed as one of the severe, destructive and widespread fungal disease that cause heavy losses (20-100%) depending upon the stage of infection (Animisha *et al.*, 2012). Initially the disease causes yellowing of lower leaves followed by yellowing of upper leaves. On uprooting the affected seedlings no superficial signs of rotting was seen, however the seedlings had shrunken stems above and below soil level. At adult stage, affected plants showed characteristic wilting *viz.*, drooping of the petioles, rachis and leaflets. Gradually, all the leaves became yellow which later turned light brown or straw coloured. Brown to black discoloration of the internal tissue was seen when the uprooted roots were dissected longitudinally. The symptoms observed resembled with the Fusarium wilt as reported by the other workers (Jalali *et al.*, 1980; Nene *et al.*, 1984; Al-Taae *et al.*, 2013).

The causal organism *Fusarium oxysporum* f. sp. *ciceri* involved in chickpea wilt disease was isolated from the infected roots by standard tissue bit technique and subsequently maintained for further studies. The morphological

studies revealed that initially the colonies were cottony white, with profuse fluffy aerial mycelium. Colony gradually turned creamy in colour, became wrinkled and felted. Microscopic observations of the isolated fungus revealed that the mycelium was septate and hyaline with hyphal width of 5.2-7.6 μm . The pathogen produced three types of spores within the culture i.e. micro-conidia, macroconidia and chlamydo spores. Microconidia were hyaline, 0-1 septate and oval to ellipsoid in shape. The size of the microconidia ranged from 7.10-10.48 \times 2.40-4.02 μm . Macroconidia too were hyaline, 4-5 septate and fusiform to curved in shape ranged from 20.20-56.00 \times 2.93-6.20 μm in size. Chlamydo spores were produced after 15-20 days of incubation. They were globose, single celled, aseptate, produced terminally or intercalary with 7.98-11.77 \times 6.65-9.90 μm size. The above morphological characters of the pathogen resemble with the description given by Saxena and Singh (1987), Couteaudier and Alabouvette (1990) and Aminisha *et al.*, (2012). Conidial suspension of the test fungus was drenched in pots with chickpea in order to prove the pathogenicity. On the basis of morphology, colony characters, pathogenicity and comparison with authentic description, the pathogen was identified as *Fusarium oxysporum* f. sp. *ciceri*. Similar observations have also been made by Armstrong and Armstrong (1968) and Booth (1971).

Since, the pathogen exhibited variation in morphological and physiological attributes such as size/ septation, colony characteristics, sporulation, and requirement for optimal level of nutrients, microorganisms differ in their nutritional requirements as reported by Cochrane (1958). The pathogen produced maximum radial mycelial growth on oatmeal agar (80.00 mm) followed by potato dextrose agar (77.63 mm), while the least growth was recorded on dal weed sucrose agar medium (60.40 mm). The fungus, however, produced the highest number of microconidia ($8.55 \times 10^6 \text{ ml}^{-1}$) and macroconidia ($7.56 \times 10^6 \text{ ml}^{-1}$) on potato dextrose agar. The fungus produced the maximum dry mycelial (723.33 mg) on oatmeal broth followed by and potato dextrose broth (710.33 mg). Minimum dry mycelial weight was obtained from dal weed sucrose broth (650.33

mg). The results were in agreement with that of Nath *et al.*, (2017) who found the radial growth of the pathogen was maximum on oatmeal agar however the highest number of macro spores ($3.27 \times 10^5 \text{ ml}^{-1}$) and micro spores ($4.06 \times 10^5 \text{ ml}^{-1}$) were produced on PDA. Similar results were found by Thaware *et al.*, 2016, Mehmood *et al.*, 2016 and Maitlo *et al.*, (2017). It was observed that the fungus had a pure white colony colour on chickpea leaf extract agar and dal weed sucrose agar however on oatmeal agar, PDA and Czapek dox agar colony was cottony white in colour. On oatmeal agar, potato dextrose agar, chickpea leaf extract agar and Czapek dox agar the colony growth was dense and fluffy while as on dal weed sucrose agar colony was sparse and fluffy. The fungus had a regular mycelial form on oatmeal and chickpea leaf extract agar and irregular form on PDA, Czapek dox agar and dal weed sucrose agar.

In view of the fact that the Fusarium wilt of chickpea has been causing exorbitant losses over the world and also in Jammu and Kashmir, attempts to manage the disease have become inevitable. There are many ways of managing plant diseases but the cultivation of resistant varieties is by far the most reliable and the cheapest. Under natural epiphytotic conditions, the reaction of seventy-five chickpea genotypes, sown in two separate nurseries; CIFWN-18 and CIEN-W-18, was studied. The objective being to select the resistant ones which might serve as direct introduction or be used as parents for providing resistant genes for any hybridization programme.

In the present study, out of seventy-five genotypes only two genotypes *viz.*, FLIP10-382C and FLIP11-122C were highly resistant with no disease incidence. Nine genotypes *viz.*, FLIP11-23C, FLIP11-24C, FLIP11-77C, FLIP11-90C, FLIP11-150C, FLIP11-176C, FLIP10-355C, FLIP11-40C and FLIP11-45C were found resistant. Nineteen genotypes *viz.*, FLIP10-354C, FLIP11-69C, FLIP11-52C, FLIP11-115C, FLIP11-123C and FLIP11-121C, FLIP11-83C, FLIP11-124C, FLIP11-142C, FLIP11-149C, FLIP11-159C, FLIP11-172C, FLIP10-332C, FLIP10-343C, FLIP11-125C, FLIP11-163C, FLIP11-175C, FLIP11-190C and

FLIP11-231C. These results are in agreement with those of Dubey and Birendra (2008) who found only two genotypes as resistant and eight as moderately resistant out of six hundred genotypes. Iqbal *et al.*, (2010) evaluated one hundred and forty five genotypes and identified fourteen lines viz., 90395, C-235, C-44, CM-2000, ILC 182, FLIP 97-172C, FLIP 98-107C, FLIP 98-227C, FLIP 98 230C, FLIP98 231C, FLIP98-38C, FLIP98 54C, ILC 7374 and KC-89 as resistant to wilt disease. Thaware *et al.*, (2017) evaluated the fifty chickpea genotypes against *Fusarium oxysporum* f. sp. *ciceri* under natural epiphytotic conditions and found six genotypes as highly resistant, thirty-one as resistant and eight genotypes as moderately resistant. Sarwar *et al.*, (2012) found the similar results.

Chemical pesticides have its own limitations such as high cost, no availability, toxicity, development of resistant strains, environmental pollution and adverse effect on beneficial soil micro flora and fauna has compounded the problem in India and all other countries producing chickpea. Biological control is a potential alternative to chemical fungicides.

In the present study three bio-agents viz., *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* were evaluated *in vitro* against *Fusarium oxysporum* f. sp. *ciceri* inciting wilt of chickpea. The highest per cent growth inhibition (82.40%) was observed in *Trichoderma harzianum*. Minimum per cent growth inhibition (66.30%) was observed in *Pseudomonas fluorescens*. In case of *Bacillus subtilis* per cent growth inhibition of 70.53 per cent was observed. Similar results were found by Rani and Mane (2014) and Biswas and Ali (2017). The mechanism involved in antagonism behind *Trichoderma* might be biochemical and antibiosis effect rather than physical and chemical (Pandey *et al.*, 2005; Sonkar *et al.*, 2018). Organic amendments of soil enhance the activity of *Trichoderma*. Soil amendment of *Trichoderma* with various organic substrates have showed effectiveness against few soil borne diseases of various crops (Nikam *et al.*, 2007; Singh *et al.*, 2007).

Studies on *in vitro* evaluation of four non-systemic and five systemic fungicides at different concentrations against mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* were conducted to get a preliminary idea about fungicides to be used under natural field conditions against the disease. It was observed that all the nine fungicides when tested *in vitro* were significantly superior over control in inhibiting the mycelial growth of the fungus. Among non-systemic fungicides tested, mancozeb 63% WP + carbendazim 12% WP proved to be most effective as it provided 94.02 per cent mycelial growth inhibition followed by mancozeb 75 WP exhibiting 72.82 per cent mycelial growth inhibition. However, copper oxychloride 50 WP proved to be least effective with only 67.60 per cent mycelial growth inhibition mancozeb 63%+ carbendazim 12% WP at each respective concentration was found to be statistically superior to all test fungicides. The data further reveals that mancozeb 63%+ carbendazim 12% WP proved significantly most effective by providing cent per cent growth inhibition at 1500 ppm concentration. A minimum inhibition of 64.62 per cent was achieved at 500 ppm which increased gradually to 86.62 per cent at 1500 ppm concentration. Among the systemic fungicides tested, carbendazim 50 WP proved to be most effective by providing 99.15 per cent mycelial growth inhibition over control followed by difenconazole 25 EC exhibiting 75.39 per cent growth inhibition, respectively. Krexosim methyl 44.3% SC proved to be least effective with only 52.88 per cent inhibition of mycelial growth. On an overall basis the inhibitory effect of test fungicides increased with increase in concentration. A minimum inhibition of 56.97 per cent was achieved at 50 ppm which increased gradually to 72.89 per cent at 250 ppm concentration. A non-significant interaction existed between carbendazim concentrations with cent per cent inhibition beyond 150 ppm. The results are in agreement with that of Mondhe *et al.*, (2013) who reported a minimum wilt incidence of 26.38 per cent in case of seed treatment with carbendazim 50 WP @ 2g kg⁻¹. Similar inferences were drawn by Andrabi *et al.*, (2011). Patra and Biwas (2016) evaluated ten fungicides against the wilt pathogen and reported carbendazim as the best with

minimum wilt incidence of 100 per cent at 1000 and 1500 ppm under *in vitro* conditions and 9.66 per cent under *in vivo* conditions. Also, Biswas and Ali (2017) reported that carbendazim exhibited maximum fungal mycelial growth inhibition (100%) at 0.1, 0.15 and 0.2 per cent concentrations followed by combination product (carbendazim 12% + mancozeb 63%), which gave 88.7 per cent mycelial growth inhibition at 0.1 per cent concentration and 100 per cent mycelial growth inhibition at 0.15 and 0.2 per cent concentrations.

The bio-agent systemic and non systemic fungicides which proved best under *in vitro* studies were also evaluated in the field during 2019-19 as seed treatment and soil drenches. The disease incidence ranged from 4.53 to 46.59 per cent. Lowest disease incidence of 4.53 per cent was recorded in treatment combination of seed treatment with *Trichoderma harzianum* @ 10^8 cfu and drenching with carbendazim 50WP @ 0.1 per cent. Highest disease incidence of 46.59 per cent was observed in control plots. Amongst the soil drenches alone, drenching with carbendazim 50WP @ 0.5 per cent proved best with disease incidence of 10.27 per cent. Further it was observed that amongst the seed treatments, treatment with carbendazim 50 WP @ 0.1 per cent proved superior to the other seed treatments. Disease incidence in case of seed treatments was observed least in carbendazim 50WP @ 1 g kg^{-1} of seed (13.67%). The results were in agreement with that of Mahmood *et al.*, (2015) who found the seed treatment with *Trichoderma harzianum* followed by carbendazim drenching as most effective by increasing the disease reduction percentage up to 93.75 per cent. Seed treatment with carbendazim (2 g kg^{-1} seed) gave minimum wilt incidence of 26.38% (Mondhe *et al.*, 2013). Similar results were found by Biswas and Ali (2017).

Chapter- 6

SUMMARY AND CONCLUSION

Chickpea wilt, caused by *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Synd & Hans, one of the most important diseases of chickpea (*Cicer arietinum* L.) causes exorbitant yield losses under both temperate and sub-tropical conditions. Studies on morphological and physiological characteristics of pathogen, disease management through identification of resistant donors and management through non-systemic and systemic fungicides and bio-control agents were carried out in the experimental fields and laboratory of Faculty of Agriculture, Division of Plant Pathology, SKUAST-K, Wadura.

Pathogen causing chickpea wilt was isolated from the infected roots by standard tissue bit technique. The morphological studies revealed that initially the colony white, cottony with profuse, fluffy aerial mycelium. Colony gradually turned creamy in colour, became wrinkled and felted. Microscopic observations of the isolated fungus revealed that the mycelium was septate and hyaline with hyphal width of 5.2-7.6 μm . The pathogen produced three types of spores within the culture i.e. micro-conidia, macroconidia and chlamydo spores. Microconidia were hyaline, 0-1 septate and oval to ellipsoid in shape. The size of the microconidia ranged from 7.10-10.48 \times 2.40-4.02 μm . Macroconidia were hyaline, 4-5 septate and fusiform to curved in shape and the size ranged from 20.20-56.00 \times 2.93-6.20 μm . Chlamydo spores were produced 15-20 days after incubation. They were globose, single celled, aseptate, produced terminally or intercalary with 7.98-11.77 \times 6.65-9.90 μm size.

Conidial suspension of the test fungus was drenched in pots with chickpea in order to prove the pathogenicity. On the basis of the morphological characteristics, pathogenicity and comparison with authentic description, the pathogen was identified as *Fusarium oxysporum* f. sp. *ciceri*. Comparison between mycelial growth and sporulation of pathogen on different media revealed

that oatmeal agar showed the maximum mycelial growth (80.00 mm) whereas PDA was found to be effective media for sporulation of pathogen. Minimum mycelial growth of 60.40 mm was observed on dal weed sucrose agar media. Dry mycelial weight of the fungus was maximum on oatmeal broth (723.33 mg), whereas the dal weed sucrose broth proved least effective media showed minimum dry mycelial weight of 650.33 milligram.

Among seventy-five genotypes only two genotypes *viz.*, FLIP10-382C and FLIP11-122C were highly resistant with no disease incidence under natural epiphytotic conditions at FoA, Wadura. Nine genotypes *viz.*, FLIP11-23C, FLIP11-24C, FLIP11-77C, FLIP11-90C, FLIP110150C, FLIP11-176C, FLIP10-355C, FLIP11-40C and FLIP11-45C exhibited resistant reaction, whereas nineteen genotypes *viz.*, FLIP10-354C, FLIP11-69C, FLIP11-52C, FLIP11-115C, FLIP11-123C and FLIP11-121C, FLIP11-83C, FLIP11-124C, FLIP11-142C, FLIP11-149C, FLIP11-159C, FLIP11-172C, FLIP10-332C, FLIP10-343C, FLIP11-125C, FLIP11-163C, FLIP11-175C, FLIP11-190C and FLIP11-231C exhibited moderately resistant reaction. The genotypes ILC482, FLIP10-338C and FLIP11-08C gave highly susceptible reaction under natural epiphytotic conditions at FoA, Wadura.

Among the bio-agents evaluated *in vitro* the highest per cent growth inhibition of 82.40 per cent was observed in *Trichoderma harzianum* followed by *Bacillus subtilis* with 70.53 per cent growth inhibition. Minimum growth inhibition of 66.30 per cent was observed in *Pseudomonas fluorescens*.

Systemic and non systemic fungicides were evaluated at different concentrations for their *in vitro* efficacy in inhibiting mycelial growth of *Fusarium oxysporum* f. sp. *ciceri*. Among non-systemic fungicides, mancozeb 63% + carbendazim 12% WP proved highly effective in inhibiting the mycelial growth. The other fungicides in decreasing order of their efficacy were mancozeb 75WP > copper oxychloride 50WP > chlorothalonil 50WP. Among systemic

fungicides, carbendazim 50WP proved to be highly effective in inhibiting the mycelial growth. The other fungicides in decreasing order of their efficacy were difenconazole 25 WP > trifloxystrobin 25WG + tebuconazole 50 WG > metiram 55%+ pyraclostrobin 5% WG > krexosim methyl 44.3 SC.

The most effective treatment under field evaluation was seed treatment with *Trichoderma harzianum* (10^8 cfu) and drenching with carbendazim 50WP (0.1 %) which resulted in minimum disease incidence of 4.53 per cent. Highest disease incidence (46.59%) was observed in check plots.

In the light of the present investigations, the overall conclusion is that:

- Among the five tested solid media oatmeal agar proved best for the radial mycelial growth of *Fusarium oxysporum* f. sp. *ciceri*, however, sporulation of the pathogen was highest on PDA.
- Among the five tested liquid media, maximum dry mycelial weight was obtained on oatmeal broth.
- In CIFWN-18, two genotypes viz., FLIP10-382C and FLIP11-122C were highly resistant, six genotypes viz., FLIP11-24C, FLIP11-23C, FLIP11-150C, FLIP11-77C and FLIP11-90C were resistant and 12 genotypes namely FLIP10-354C, FLIP11-69C, FLIP11-123C, FLIP11-124C, FLIP11-121C, FLIP11-172C, FLIP11-83C, FLIP11-142C, FLIP11-52C, FLIP11-149C, FLIP11-115C and FLIP11-159C were moderately resistant.
- In CIEN-W-18, three genotypes namely FLIP10-355C, FLIP11-40C and FLIP11-45C were resistant and seven viz., FLIP10-332C, FLIP10-343C, FLIP11-125C, FLIP11-163C, FLIP11-175C, FLIP11-190C and FLIP11-231C were moderately resistant, none of the genotype was highly resistant.
- Under *in vitro* conditions, *Trichoderma harzianum* proved the best bio-agent while as mancozeb 63% WP + carbendazim 12%WP and

carbendazim 50WP proved most efficacious non-systemic and systemic fungicides, respectively.

- Under field conditions, the best disease management capsule was seed treatment with *Trichoderma harzianum* (10^8 cfu) and drenching with carbendazim 50WP (0.1%) after 7 days of emergence of seedlings.

LITERATURE CITED

- Ahmad, M. A., Iqbal, S. M., Ayub, N., Ahmad, Y. and Akram, A. 2010. Identification of resistant sources in chickpea against *Fusarium* wilt. *Pakistan Journal of Botany* **42**: 417-426.
- Al-Taae, A. K., Ali H. H. and Al-Jobory, S. A. E. 2013. Physiological Races of *Fusarium oxysporum* f. sp. *ciceri* in Iraq. *Journal of Life Sciences* **7**: 1070-1075.
- Andrabi, M., Vaid, A. and Kumar, R. V. 2011. Evaluation of different measures to control wilt causing pathogens in chickpea. *Journal of Plant Protection Research* **51**: 55-59.
- Animisha., Zacharia, S., Jaiswal, K. K. and Pandey, P. 2012. Integrated management of chickpea wilt incited by *Fusarium oxysporum* f. sp. *ciceri*. *International Journal of Agricultural Research* **7**: 284-290.
- Anonymous, 1999. Proceedings of technical programme for *Rabi* workshop organized by Indian Institute of Pulse Research Kanpur. pp.13.
- Anonymous, 2018. *Pulses Revolution- From Food to Nutritional Security*. Ministry of Agriculture and Farmers Welfare (DAC & FW), Govt. of India. pp. 3-4.
- Armstrong, G. M. and Armstrong, J. K. 1968. Formae specialis and races of *Fusarium oxysporum* causing a trachemycosis in the syndrome of disease. *Phytopathology* **68**: 1241-1246.
- Ayyub, M. A. 2001. *Evaluation of chickpea germplasm, fungicides, organic and inorganic materials for the management of wilt (F. oxysporum f. sp. ciceri)*. Ph.D thesis, University of Agriculture Faisalabad, Pakistan.

- Bajwa, K. M., Khan, I. A., Alam, S. S., Ahmed, I. and Gill, M. A. 2000. Screening against phytotoxins for resistance to *Fusarium* wilt in chickpea. *Pakistan Journal of Phytopathology* **12**: 62-65.
- Bashir, S., Mughal, M. N., Dar, S. A., Nissa, S., Hakeem, S., Wani, R. A., Baba, J. A. and Habib, M. 2017. *In vitro* efficacy of fungi-toxicants and bio-control agents against wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* (Foc). *International Journal of Current Microbiology and Applied Sciences* **6**: 1392-1399.
- Biswas, M. K. and Ali, S. K. J. 2017. Management of *Fusarium* wilt of chickpea (*Cicer arietinum* L.) under the undulating red and lateritic belt of West Bengal. *Journal of Mycopathological Research* **54**: 461-468.
- Booth, C. 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew England. pp. 37.
- Butler, E. J. 1918. *Fungi and Diseases of Plants*. Wallingford, UK, pp. 233-270.
- Chattopadhyaya, S. B. and Sengupta, P. K. 1967. Studies of wilt disease of pulses: Variation and taxonomy of *Fusarium* species associated with wilt disease of pulses. *Indian Journal of Mycological Research* **5**: 45-53.
- Cho, S. and Muehlbauer, F. J. 2004. Genetic effect of differentially regulated fungal response genes on resistance to necrotrophic fungal pathogens in chickpea (*Cicer arietinum* L.). *Physiological and Molecular Plant Pathology* **64**: 57-66.
- Cochrane, V. W. 1958. *Physiology of Fungi*. John Willey and Sons, New York, USA. pp. 524.

- Couteaudier, Y and Alabouvette, C. 1990. Survival and inoculums potential of conidia and chlamydospores of *Fusarium oxysporum* f. sp. *lini* in soil. *Canadian Journal of Microbiology* **36**: 551-556.
- Dey, T. K., Ayub, A., Ahmed, H. U. and Rahman, M. H. 1993. Screening of chickpea germplasm against Fusarium wilt. *Bangladesh Journal of Plant Pathology* **9**: 29-30.
- Dhingra, O. D and Sinclair J. B. 1995. *Basic Plant Pathology Methods*. CRS Press, Boca Raton, Florida USA. pp. 335.
- Dubey, S. C. and Birendra, S. 2008. Evaluation of chickpea genotypes against *Fusarium oxysporum* f. sp. *ciceri*. *Indian Phytopathology* **61**: 280-281.
- Farooq, S., Iqbal, S. M. and Rauf, C. A. 2005. Physiological Studies of *Fusarium oxysporum* f. sp. *ciceri*. *International Journal of Agriculture and Biology* **7**: 275-277.
- Gaur, P. M., Tripathi, S., Gowda, C. L. L., Ranga Rao, G. V., Sharma, H. C., Pande, S. and Sharma, M. 2010, *Chickpea Seed Production Manual*. International Crop Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India. pp. 28.
- Golakiya, B. B., Bhimani, M. D. and Akbari, L. F. 2018. Efficacy of different fungi-toxicants for the management of chickpea wilt (*Fusarium oxysporum* f. sp. *ciceri*). *International Journal of Chemical Studies* **6**: 199-205.
- Gomez, K. A. and Gomez, A. A. 1984. *Statistical Procedures for Agricultural Research*. John Wiley and Sons, New York, USA. pp. 316-356.

- Gurha, S. N., Singh, R. A., Naimuddin and Ghosh, A. C. 2002. Stable and broad based resistance against wilt in chickpea. *Annual Plant Protection Science* **10**: 18-90.
- Haware, M. P., Nene, Y. L. and Rajeshwari, R. 1978. Eradication of *Fusarium oxysporum* f. sp. *ciceri* transmitted in chickpea seed. *Phytopathology* **68**: 1364-1367.
- Haware, M. P., Nene, Y. L. and Rao, J. N. 1992. Screening of world chickpea germplasm for resistance to Fusarium wilt. *Field Crop Research* **30**: 147–154.
- Ibrikci, H., Knewton S. J. B. and Grusak, M. A. 2003. Chickpea leaves as a vegetable green for humans: evaluation of mineral composition. *Journal of the Science of Food and Agriculture* **83**: 945-950.
- Iqbal, S. M., Ghafoor, A., Bakhsh, A., Ahmad, I. and Sher, A. 2010. Identification of resistant sources for multiple disease resistance in chickpea. *Pakistan Journal of Phytopathology* **22**: 89-94.
- Jalali, B. L., Sangwan, M. S. and Khirbat, S. K. 1980. *Report on Rabi Pulse Pathology*. ICAR Project on Improvement of Pulses. pp. 29
- Jhonston, A. and Booth, C. 1983. *Plant Pathologist's Pocket Book*. Commonwealth Mycological Institute, Kew, England. pp. 439.
- Jiménez-Fernández, D., Montes-Borrego, M., Jiménez-Díaz, R. M., Navas-Cortés, J. A. and Landa, B. B. 2011. Plant and soil quantification of *Fusarium oxysporum* f. sp. *ciceri* and evaluation of Fusarium wilt resistance in chickpea with a newly developed quantitative polymerase chain reaction assay. *Phytopathology* **101**: 250-262.

- Kaur, L., Sirari, A., Singh, S., Singh, I., Kumar, D. and Sandhu, J. S. 2012. Status of resistance to *Ascochyta* blight and *Fusarium* wilt in large-seeded Kabuli. *Archives of Phytopathology and Plant Protection* **4**: 22-32.
- Khilare, V. C. and Ahmad, R. 2012. Effect of different media, pH and temperature on the growth of *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. *International Journal of Advanced Biological Research* **2**: 99-102.
- Kumari, S. and Khanna, V. 2014. Effect of antagonistic *Rhizobacteria* co-inoculated with *Mesorhizobium ciceri* on control of *Fusarium* wilt in chickpea (*Cicer arietinum* L.). *African Journal of Microbiological Research* **8**: 1255-1265.
- Ladizinsky, G., 1975. *The origin of chickpea (Cicer arietinum L.)*. Springer. pp. 211-217.
- Mahmood, Y., Khan, M. A., Javed, N. and Arif, M. J. 2015. Comparative efficacy of fungi-toxicants and biological control agents for the management of chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri*. *The Journal of Animal and Plant Sciences* **25**: 1063-1071.
- Maitlo, S., Rajput, A. Q., Syed, R. N., Khanzada, M. A., Rajput, N. A. and Lodhi, A. M. 2017. Influence of physiological factors on vegetative growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri*. *Pakistan Journal of Botany* **49**: 311-316.
- Mohamed, E. K., Haroun, N. E. and AbdAlla, M. A. E. 2016. Control of chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* with botanical extracts and fungi-toxicants. *International Journal of Current Microbiology and Applied Sciences* **5**: 360-370.

- Mondhe, M. K., Kamidi, D. R., Jadesha, G., Kshirsagar, D. N. and Thakur, K. D. 2013. Efficacy of botanicals, bio-agents and fungi-toxicants against *Fusarium oxysporum* f. sp. *ciceri*, in chickpea wilt sick plot. *Annals of Biological Research* **3**: 5390-5392.
- Nagamani, P., Biswas, M. K. and Bhagat S. 2015. Efficacy of native *Trichoderma* spp. for the management of root rot pathogens in chickpea (*Cicer arietinum*). *Progressive Research – An International Journal* **10**: 353-356.
- Nath, N., Ahmed, A. U. and Aminuzzaman, F. M. 2017. Morphological and physiological variation of *Fusarium oxysporum* f. sp. *ciceri* isolates causing wilt disease in chickpea. *International Journal of Environment Agriculture and Biotechnology* **2**: 202-211.
- Nene, Y. L. and Haware, M. P. 1981. Influence of wilt at different stages on the yield loss in chickpea. *Tropical Grain Legume Bulletin* **19**: 38-44.
- Nene, Y. L. and Thapliyal, P. N. 1979. Inhibition of plant pathogens by higher plant substances. *Journal of Scientific and Industrial Research* **26**: 289-299.
- Nene, Y. L., Sheilla, V. K. and Sharma, S. B. 1984. A World List of Chickpea and Pigeonpea Pathogens. *ICRISAT Pulse Pathology Progress Report* **8**: 9.
- Nikam, P. S., Jagtap, G. P. and Sontakke, P. L. 2007. Management of chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri*. *African Journal of Agricultural Research* **2**: 692-697.
- Padwick, G. W. 1940. A critical study of the fungus causing wilt of gram. *Indian Journal of Agricultural Sciences* **5**: 241-248

- Pande, S., Narayana, R. J. and Sharma, M. 2007. Establishment of the chickpea wilt pathogen *Fusarium oxysporum* f. sp. *ciceri* in the soil through seed transmission. *Plant Pathology Journal* **23**: 3-6.
- Pandey, K. K., Pandey, P.K. and Upadhyay, J. P. 2005. Mycoparasitism of *Trichoderma* spp. on *Fusarium* and *Rhizoctonia*. *Journal of Mycology and Plant Pathology* **35**:174-176.
- Patra, S. and Biswas, M. K. 2016. Efficacy of fungi-toxicants for the management of chickpea wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri*. *International Journal of Advanced Research* **4**: 1457-1461.
- Pawar, K. B., Bendre, N. J., Deshmukh, R. B. and Bhor, S. B. 1993. Field reactions of some chickpea lines to *Fusarium* wilt. *Journal of Maharashtra Agriculture University* **18**: 327-328.
- Prasad, N. and Padwick, G. W. 1939. The genus *Fusarium* II. A species of *Fusarium* as a cause of wilt of gram (*Cicer arietinum* L.). *Indian Journal of Agricultural Science* **9**: 371-380.
- Rani, R. R. and Mane, S. S. 2014. Efficacy of bio-agents against chickpea wilt pathogens. *International Journal of Applied Biology and Pharmaceutical Technology* **5**: 147-149.
- Saifulla, M. and Nagamma, G. 2012. Assessment of chickpea Desi genotypes for wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri*. *National symposium on heading towards horizons in Plant Pathology: Host Resistance, pathogen dynamics and Management* held at Sugarcane breeding Institute, Coimbatore, India. pp. 99.
- Saifulla, M. and Nagamma, G. 2013. Evaluation of chickpea genotypes for wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri*. *34th Annual*

conference and symposium, crop disease management: advances and challenges held at Navasari Agricultural University, Navasari, Gujarat, India. pp. 40.

- Saifulla, M. and Nagamma, G. 2014. Assessment of chickpea Kabuli genotypes for wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri*. *National conference on Productivity and sustainability: Role of Agriculturally important microorganism* held at Sugarcane breeding Institute, Coimbatore, India. pp.10.
- Sarwar, N., Akhtar, K. P., Shah, T. M. and Atta, B. M. 2012. Evaluation of chickpea advance genotypes against blight and wilt diseases under field conditions. *International Journal of Agriculture and Biology* **14**: 993–996.
- Saxena, M. C. and Singh, K. B. 1987. *The Chickpea*. CAB International, Oxon, UK. pp. 250-252.
- Sicard, D., Michalakis, Y., Dron, M. and Neema, C. 1997. Genetics diversity and pathogenic variation of *Colletotrichum lindemuthianum* in three centers of diversity of its host, *Phaseolus vulgaris*. *Phytopathology* **87**: 807-813.
- Singh, G., Chen, W., Rubiales, D., Moore, K., Sharma, Y. R. and Gan, Y. 2007. *24 Diseases and their Management*. Academic Press, New Dehli. pp. 497-519.
- Singh, N. P., 2010. *Chickpea Project Coordinator Report*, Patancheru, Hyderabad. pp. 45.
- Singh, Y. P. and Sumbali, G. 2007. Efficacy of leaf extract and essential oils of some plant species against *Penicillium expansum* rot of apples. *Annals of Plant Protection Sciences* **15**: 135-139.

- Snyder, W. C. and Hansen, H. N. 1940. The species concept in *Fusarium*. *American Journal of Botany* **27**: 64-67.
- Sonkar, P., Chandra., Singh R., and Kumar S. 2018. Study on management of *Fusarium oxysporum* through different mode of action of *Trichoderma* spp. *International Journal of Current Trends in Science and Technology* **8**: 20192-20200.
- Subhani, M. N., Chaudhry, M. A., Abdul, K. and Faqir M. 2008. Screening of promising varieties/lines of chickpea against Fusarium wilt. *Science International* **3**: 225-227.
- Subhani, M. N., Sahi, S. T., Hussain, S., Ali, A., Iqbal, J. and Hameed, K. 2011. Evaluation of various fungi-toxicants for the control of gram wilt caused by *Fusarium oxysporum* f. sp. *ciceris*. *African Journal of Agricultural Research* **6**: 4555-4559.
- Suryawanshi, A. V., Mandhare, V. K., Sanap, M. W. and Jamadagni, B. M. 2003. Reaction of chickpea entries to Fusarium wilt and gram pod borer. *Journal of Maharashtra Agricultural Universities* **28**: 213-214.
- Suthar, K. P., Patel, R. M., Singh, D. and Khunt, M. D. 2017. Efficacy of *Bacillus subtilis* isolate K18 against chickpea wilt *Fusarium oxysporum* f. sp. *ciceri*. *International Journal of Pure and Applied Biosciences* **5**: 838-843.
- Thaware, D. S., Gholve, V. M. and Ghante, P. H. 2017. Screening of chickpea varieties, cultivars and genotypes against *Fusarium oxysporum* f. sp. *ciceri*. *International Journal of Current Microbiology and Applied Sciences* **6**: 896-904.

- Thaware, D. S., Kohire, O. D. and Gholve, V. M. 2016 b. *In vitro* efficacy of fungal and bacterial antagonists against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. *International Journal of Agriculture Sciences* **12**: 215-218.
- Thaware, D. S., Kohire, O. D., Gholve, V. M., Wagh, S. S. and Chavan, A. A. 2016. Nutritional and physiological studies of *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Snyder and Hansen causing wilt of chickpea. *International Journal of Plant Sciences* **11**: 213-217.
- Vincent, J. H. 1947. Distortion of fungal hyphae in presence of certain inhibitors. *Nature* **15**: 850.
- Warda, J., Mariem, B., Amal, B., Mohamed, B. and Mohamed, K. 2017. Fusarium wilt affecting chickpea crop. *MDPI Journal Agriculture* **7**: 23.

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Plant Pathology, Faculty of Agriculture, Wadura
193201

CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner **Dr. Dinesh Singh, Principal Scientist, Division of Plant Pathology, IARI, New Delhi** during viva-voce examination held on 25th of February, 2020 have been incorporated in the manuscript entitled “**Studies on Chickpea Wilt (*Fusarium oxysporum* f. sp. *ciceri*) in Kashmir**” submitted by **Ms. Shifa Showket (Regd. No. 2017-A-1173-M)**.

(Dr. Sabiya Bashir)
Chairman
Advisory Committee



Sher-e-Kashmir
University of Agricultural Sciences and Technology of Kashmir
Division of Plant Pathology, Faculty of Agriculture, Wadura
193201

TO WHOM IT MAY CONCERN

This is to certify that the work and conduct of **Ms. Shifa Showket (Regd. No. 2017-A-1173-M)** a candidate for the degree of **Master of Science in Agriculture (Plant Pathology)**, under my supervision and guidance during the course of work remained **satisfactory**.

(Dr. Sabiya Bashir)

Chairman

Advisory Committee