STUDIES ON VARIABILITY AND MANAGEMENT OF FUSARIUM WILT OF CAPSICUM

Thesis

by

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(H-2021-47-M)

submitted to

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PLANT PATHOLOGY

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CERTIFICATE-I

This is to certify that the thesis titled, **"Studies on variability and management of Fusarium wilt of capsicum"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science (Agriculture) Plant Pathology** in the discipline of **Plant Protection** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP) - 173 230 is a bonafide research work carried out by **Mr. Yash Punia (H-2021-47-M)** son of Mr. Pawan Kumar under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

Place: Nauni, Solan
Date:

Dr. Arti Shukla
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CERTIFICATE-II

This is to certify that the thesis titled, **"Studies on variability and management of Fusarium wilt of capsicum"** submitted by **Mr. Yash Punia (H-2021-47-M)** son of Mr. Pawan Kumar to the Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP) - 173 230 India in partial fulfilment of the requirements for the degree of **Master of Science (Agriculture) Plant Pathology** in the discipline of **Plant Protection** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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Place: Nauni, Solan
Dated: / / 2023 **(Yash Punia)**

CONTENTS

LIST OF ABBREVIATIONS

@	:	At the rate of
%	:	Per cent
&	:	And
i.e.	:	That is
viz.	:	*Videlicet* (namely)
etc.	:	Et cetera
et al.	:	Co-workers
HP	:	Himachal Pradesh
ed.	:	Editors
var.	:	Variety
cv.	:	Cultivar
mg	:	Milligram
gm	:	Gram
kg	:	Kilogram
Q	:	Quintals
MT	:	Metric Ton
cm^2	:	Square centimetre
µm	:	Micro meter
Ha	:	Hectare (10,000 m^2)
L	:	Litre
ml	:	Millilitres
cm	:	Centimetre
µl	:	Micro litre
mm	:	Millimetres
Dia	:	Diameter
Cfu	:	Colony forming units
rpm	:	Revolutions per minute
+	:	Plus
PDB	:	Potato dextrose broth
bp	:	Base pairs
PDA	:	Potato Dextrose Agar
Psi	:	Per square inch

HRTS	:	Horticulture Research and Training Station
BOD	:	Biological Oxygen Demand
amsl	:	Above mean sea level
pH	:	*Puissance de Hydrogen* (Potential of hydrogen)
No.	:	Number (s)
ANOVA	:	Analysis of variance
DF	:	Degree of freedom
CD	:	Critical difference
CRD	:	Completely Randomized design
SS	:	Sum of Square
MSS	:	Mean Sum of Square
NCBI	:	National Centre for Biotechnology Information
^{0}C	:	Degree centigrade

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Chapter-1

INTRODUCTION

Capsicum or bell pepper (*Capsicum annuum* L.) is one of the most important vegetable crops of the Solanaceae family. It is observed to be originated in Mexico (Kraft et al. 2014) and in India, bell pepper was introduced by Britishers nearly in the 19th century in Shimla hills of Himachal Pradesh and therefore, commonly known as *Shimla Mirch*. Capsicum is a rich source of vitamins and minerals viz., vitamin A, vitamin C, vitamin E, calcium, phosphorus, iron, magnesium, sodium and selenium (Rubio et al. 2010). It contains high value of vitamin C which is five to six times more as compared to other vegetables which makes it ideal vegetable to prevent flu cold (Boselad and Votava, 2006).

According to FAO, worldwide total area under bell pepper cultivation is 19,90,926 hectares with a production of 3,80,27,164 MT. China is the leading producer of capsicum with annual production of 1,89,78,024 MT, followed by Mexico (FAO, 2019). Capsicum is a warm season crop which is planted from June to December in plains and February to June in hills. In India, it is grown over an area of 38,000 hectares with annual production of 5,63,000 MT (NHB, 2021-22). It is a high value cash crop grown as an off-season vegetable (April-October) in various agroclimatic zones of Himachal Pradesh which covers an area of 2,854 hectares with annual production of 56,011.7 metric tons (Directorate of Agriculture, 2021-22).

In Himachal Pradesh, due to monoculture of solanaceous crops, capsicum is severely affected by various soil borne diseases of which Fusarium wilt caused *Fusarium oxysporum* f.sp. *capsici* is the important one that poses serious threat to its cultivation and causes huge economical losses (Bose et al. 2002; Chadha, 2003; Gupta and Thind, 2006; Chauhan et al. 2021).

Fusarium oxysporum (Wongpia and Lomthaisong, 2010) is morphologically characterized by white colour colony culture which is converted into pink colour colony on PDA (Potato Dextrose Agar) media, that show septated, branched hypha under microscope and produce microconidia, macroconidia (sickle shape) and chlamydospores (Endriyas, 2019). Symptoms of the disease are expressed as yellowing of foliage, upward and inward rolling of leaves, browning or discoloration of the vascular tissue leading to wilting and death of plants (Gowda et al. 2020).

Although the disease can be managed through chemical methods, however, it is neither economical nor environment friendly (Paker et al. 1985). Over last few years, use of resistant varieties and biological methods are being used effectively for the management of soil borne diseases and these methods are safe and economical (EARO, 2004; Mamta et al. 2012). However, the pathogen being highly variable poses a major obstacle to resistance breeding programs (Buurlage et al. 2001). Hence in recent past, more emphasis is being given on use of biological control methods. Keeping this in mind, present investigation was proposed with the following objectives:

1. Survey of different capsicum growing areas of Solan district to record the incidence of the disease and to collect the isolates of *Fusarium oxysporum*
2. Cultural and morphological characterization of the isolates
3. Isolation of biocontrol agents from the rhizosphere of capsicum, their characterization and to test their efficiency against the pathogen under *in vitro* and pot house conditions

Chapter-2

REVIEW OF LITERATURE

Bell pepper is grown all over the world as a commercial crop and holds a huge economical value. The crop is susceptible to various disease caused by fungi, bacteria, and virus. However, major damage is caused by soil borne pathogens such as *Fusarium*, *Rhizoctonia*, *Verticillium* and *Pythium* etc of the various disease inflicting economical losses in bell pepper, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *capsici* is a major cause in mid hills of Himachal Pradesh. The relevant literature on Fusarium wilt has been discussed under the following headings.

GEOGRAPHICAL DISTRIBUTION

SYMPTOMATOLOGY

CAUSAL ORGANISM AND IDENTIFICATION

PATHOGENICITY

DISEASE MANGEMENT

GEOGRAPHICAL DISTRIBUTION

Fusarium wilt of capsicum was first reported in Mexico (Leonian, 1919). Mexico is one of the major producers of bell pepper plants in the world where it is cultivated over an area of approximately 1,76,517 ha of land.

In 1997, a study was conducted by Mushtaq and Hashmi in Mirpur Khas District of Sindh, Pakistan which showed that capsicum wilt is caused by *F oxysporum*. Which caused a huge loss to the farmers (Mushtaq and Hashmi, 1997).

In Southern Spain, bell pepper covers nearly 9,920 ha area under protected conditions with 35% incidence of Fusarium wilt (Perez-Hernandez et al. 2014). In 2014, Survey of various location of Mexico was done for collecting diseased plants samples from greenhouses based on symptoms (chlorosis, wilting and vascular necrosis). On the analysis of the samples, it was noticed that 88% of the samples were infected by *Fusarium oxysporum* (Velarde Felix et al. 2018).

Fusarium oxysporum, a pathogenic fungus, was found to infect greenhouse pepper plants (*Capsicum annuum*) in two commercial operations in Ontario. This resulted in plant death and significant yield losses. The *Fusarium oxysporum* fungus did not exhibit pathogenicity towards other greenhouse crops such as tomatoes, cucumbers, or eggplants. Furthermore, it did not cause harm to field crops like beans, chickpeas, or zucchini squash (Cerkauskas, 2017).

Fusarium oxysporum species cause main yield losses in the regions of Turkey, many hosts of the pathogen are present in the family Solanaceae (tomato, eggplant, and pepper). It causes losses in both greenhouse conditions as well as in field conditions. In 2015-2016, about 33% of the green houses and 55% of the field were infected by Fusarium wilt disease, which is caused by *Fusarium oxysporum* f.sp. *capsici* (Altinok et al. 2020).

In Brazil, nationwide surveys of the pathogens associated with vascular wilt of tomatoes was done which is caused by various races of *Fusarium oxysporum*. It was analyzed that about 60% of the yield losses were caused by the pathogen (Cabral et al. 2020).

In Ethiopia, hot pepper is one of the important cash crops for the farmers and for agriculture commodity that contribute to export earnings. The crop is infected by complex pathogens like powdery mildew, Phytophthora leaf blight, Fusarium wilt, Bacterial leaf spot, bacterial wilt, Bacterial soft rot, and pepper mottle virus. Among these, wilt caused by *Fusarium oxysporum* f.sp. *capsici* is one of the major diseases of the pepper crop. Due to Fusarium wilt, the economic yield losses up to 68 to 71% have been reported in Ethiopia (Gabrekiristos and Aryana, 2020).

In India, bell pepper was first introduced by the Britishers in 19th century in Shimla, so it is known as *Shimla Mirch*. Occurrence of Fusarium wilt in bell pepper and chilli has been reported from various parts of the country including Jammu and Kashmir, Karnataka, Himachal Pradesh and causes up to 25 per cent losses with wilt incidence up to 75 per cent (Najar et al. 2012; Sharma, 2018; Sharma, 2022).

During 2013, in Anantnag and Kulgam districts of Kashmir valley, a survey was conducted in vegetable growing areas. The fusarium wilt disease was first noticed in seedling stage and maximum infection of disease found during flowering/fruiting stage. Kulgam district showed maximum disease incidence of 6% and 40% was recorded during transplanting stage and flowering/fruiting stage, respectively. Whereas in the district

Anantnag, disease incidence was 4% and 24% during transplanting and flowering/fruiting stages, respectively (Baba et al. 2014).

In Andhra Pradesh, chili (*Capsicum annum* L.) is one of the important vegetable crops. During survey of major chili growing areas, it was noticed that Fusarium wilt is the major disease. With disease incidence ranging from 8 to 40%. The disease was observed to be high during November-December months (Bai et al. 2018).

In Himachal Pradesh, 25 per cent of losses were caused alone by Fusarium wilt of bell pepper, with disease incidence reaching up to 75 per cent (Sharma, 2022).

Fusarium wilt is a highly destructive disease that affects solanaceous crops worldwide. Solanaceous crops, which are commonly grown and highly productive vegetables globally, are extensively cultivated in India. Fusarium is recognized as the primary cause of Wilt, leading to significant losses of 50–80% annually (Parihar et al. 2022).

SYMPTOMATOLOGY

In older plants, vein clearing and leaf epinasty are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves and finally death of the entire plant. Browning of the vascular tissue is strong evidence of Fusarium wilt in pepper (Agrios, 1988; Endriyas, 2019).

Fusarium wilt is the most important disease caused by *F. oxysporum* in pepper plants. The wilt first appears as slight vein clearing on the outer portion of the younger leaves, followed by epinasty (downward drooping) of the older leaves. At the seedling stage, plants infected by *F. oxysporum* may wilt and die soon after the symptoms appear (Gowda et al. 2020).

CAUSAL ORGANISM AND IDENTIFICATION

Nelson (1994), showed that the apical cells of the macroconidia produced by *Fusarium oxysporum* were slightly curved and tapered, while the basal cells exhibited a slight foot shape in some instances. The mean length and width of 1-celled microconidia ranged from 7.07 to 9.0 µm and from 2.95 to 3.24 µm, respectively, while the 3-celled macroconidia measured between 17.36 and 23.99 µm in length and 3.65 to 4.18 µm in width.

The study conducted by Cha et al. (2007) identified and characterized *Fusarium oxysporum*, which was isolated from the decayed or infected stems and roots of *Capsicum* sp. The fungus displayed typical characteristics of *Fusarium oxysporum*, such as white aerial mycelia that eventually produced a dark violet pigment or pink pigment on PDA. The fungus also produced elliptical, aseptate microconidia and straight to slightly curved macroconidia with three septa, which were abundantly formed in sporodochia when cultured on potato dextrose agar media (PDA) at 25ºC for 10 days. Chlamydospores were formed singly on potato dextrose agar media after 5 days.

Microconidia were ellipsoidal to cylindrical in shape, and straight or curved, produced on short phialides, hyaline, and either had 0 or 1 septum. The macroconidia of F. *oxysporum* were fusiform, pointed at the ends, had pedicellate basal cells, and were hyaline with 2–4 septa. The fungus produced chlamydospores terminally, either singly or in chains, which were nearly spherical and hyaline (Teixeira, 2015).

The white or light mauve floccose mycelium of *F. oxysporum* produced oval or occasionally obovoid-shaped, 1 or 2-celled microconidia on short, subcylindrical monophialides that sometimes emerged laterally on the hyphae. The chlamydospores of Fusarium were smooth, globose, typically single, and had a light-yellow brown colour with thick walls (Cerkauskas, 2017). The fungus also formed straight to slightly curved macroconidia with thin walls, and sporodochia were present (Gabrekiristos et al. 2020).

When cultured in pure cultures, *Fusarium oxysporum* displayed white colonies initially, but later developed a peach-brown colour at the agar base and grew to 90 mm in 10 days of incubation at 25±1 °C. The mycelium of the fungus was smooth, cylindrical, septate, and branched, with a width ranging between 3.00- 4.80 µm (Hami et al. 2021; Sharma, 2022).

PATHOGENICITY

Abdel and Ismail (2010) studied the pathogenicity of ten different strains of *Fusarium* spp. causing root rot and wilt in pepper seedlings. Sterilized sandy loam soil was individually inoculated with each fungus which is grown on barley-sand medium (140 g barley grains, 60 g sand and 60 ml water) and placed in plastic pots. The pots were then placed at a temperature of 25±1°C for two weeks. Five pots were used for each fungus, while a control without any fungus was also included. Healthy pepper seedlings were planted in each pot at a

rate of five seedlings per pot. After 60 days of inoculation, the severity of root rot and wilt was assessed using the following scale: 0 indicated no root discoloration or leaf yellowing, 1 indicated 1-25% root discoloration or one yellowed leaf, 2 indicated 26-50% root discoloration or more than one yellowed leaf, 3 indicated 51-75% root discoloration or vascular discoloration plus one wilted leaf, 4 indicated 76 % root discoloration or more than one wilted leaf, and 5 indicated completely dead plants. The results of the pathogenicity tests revealed significant variations in the severity of root rot and wilt among the different isolates of *Fusarium*.

Evaluating the level of vascular discoloration offers a more comprehensive understanding of the isolates' pathogenicity. This assessment helps determine the extent of damage inflicted on the vascular system of the pepper plants. Vascular discoloration serves as a crucial indicator of the pathogen's ability to invade and colonize the plant's vascular tissues, leading to wilting and other associated disease symptoms (Ferniah, 2014).

Altinok et al. (2020) carried out pathogenicity of the 129 *F. oxysporum* isolates, 64 of them were found to be pathogenic to the pepper variety 'Atalante F1' and were identified as *F. oxysporum* f. sp. *capsici* (Foc). The seedlings that were not inoculated did not show any symptoms. The experiment revealed that the initial symptoms appeared 10 days after inoculation, characterized by mild yellowing of older leaves. The wilting symptoms continued to progress systematically until the 25th day, with some infected seedlings eventually collapsing. The symptoms observed in the inoculated plants resembled those observed in the field, including leaf yellowing, necrosis, leaf drop, and browning of the vascular bundles. It is worth noting that none of the 64 *F. oxysporum* isolates pathogenic to pepper induced any wilting symptoms in tomato and eggplant during cross-inoculation tests, confirming their specific designation as *F. oxysporum* f. sp. *capsici*. The disease progress of the *F. oxysporum* f. sp. *capsici* (Foc) isolates was evaluated using disease severity and the Area Under the Disease Progress Curve (AUDPC) as percentages of the maximum AUDPC values. A strong correlation was found between disease severity and incidence on the 25th day after inoculation (DAI). Virulence groups were identified based on percent disease severity. Classified as low-virulent (virulence group I; disease severity ranged from 28% to 42%), moderate-virulent (virulence group II; disease severity ranged from 43% to 52%), and highly virulent (virulence group III; disease severity ranged from 53% to 72%). The field observations of wilting symptoms were consistent with the virulence of the Foc isolates in the pathogenicity tests and wilting symptoms in the field

were generally at a moderate virulence level, and no severe wilting symptoms were observed.

The wilt incidence analysis for *Fusarium oxysporum* isolates showed that the majority of the isolates caused wilting in the pepper plants at 20 days after inoculation. Based on the level of wilt incidence, the isolates were classified into five pathogenic classes: non-pathogenic, less pathogenic, moderately pathogenic, pathogenic, and highly pathogenic. The numbers of isolates identified in each class were as follows: 1 non-pathogenic, 3 less pathogenic, 21 moderately pathogenic, 12 pathogenic, and 12 highly pathogenic isolates as reported by Gabrekiristos et al. (2020). According to this evaluation, Gabrekiristos et al. (2020) in their study found that all 48 isolates tested to be pathogenic to hot pepper plants, specifically to the Mareko Fana variety. These isolates caused the typical symptom of vascular discoloration on xylem vesicles. Among them, the isolates 3DGK, 4DGK, and 1DBG from Dugda, 3MDM, 4MDM, and 1MJD from Mareko, 3AA1, 1AA2, and 4AAT from Alaba, and 5MBG from Meskan were identified as the highly aggressive isolates.

In another study conducted by Parihar et al. (2022) *Fusarium oxysporum* develops its initial symptoms on the plant in the second week of inoculation. The initial symptoms showed light green to yellowish discoloration of leaves followed by their shriveling, drooping and finally death of whole plant at sixth week of inoculation.

DISEASE MANGEMENT

In vitro evaluation of antagonistic microorganisms against *Fusarium oxysporum*

The various methods by which bio-control agents combat plant pathogens include competing for resources and space, stimulating host plants to develop tolerance or resistance against the pathogen, as well as producing low molecular toxic compounds or enzymes through a process known as antibiosis and they also play role in enhance the growth of crop (Singh and Zaidi, 2002).

Antagonistic microorganisms, such as species of *Trichoderma*, *Bacillus* and *Pseudomonas* have been explored by many workers for biological control of plant pathogens.

The Fusarium genus comprises a wide range of species, and certain strains of *Fusarium oxysporum* are non-pathogenic and can even inhibit the growth of pathogenic

strains. These non-pathogenic strains have the potential to be employed as biological agents in controlling plant diseases (Fravel et al. 2003).

In terms of reducing the colony growth of *Fusarium oxysporum* in sweet pepper (*Capsicum annuum*), *T. viride* exhibited the most effective performance with a reduction rate of 62%. It was followed by *T. harzianum* at 36%, *T. aureoviride* at 24%, *T. koningii* at 18%, and *T. pseudokoningii* at 6%. These results highlight the varying levels of effectiveness among different *Trichoderma* species in suppressing the growth of *F. oxysporum* in sweet pepper plants (Sahi and Khalid, 2007).

In their study, Khan et al. (2018) showed that *Fusarium* is a complex genus of plant pathogens that cause significant agricultural losses. Controlling *Fusarium* infections with chemicals raises environmental and health concerns, leading to the exploration of biocontrol agents. In this study, four *Bacillus* strains were analyzed for their plant-growth promoting and biocontrol properties against *Fusarium*. Among them, *B. subtilis* 30VD-1 exhibited the most effective antagonism against *Fusarium* under laboratory conditions. The crude extract of its cell-free culture filtrate inhibited *Fusarium* growth by approximately 40%. Pea seeds inoculated with 30VD-1 showed reduced wilt severity and increased plant biomass in *Fusarium*-infested soil. Microscopic analysis revealed abnormalities in *Fusarium* hyphae when co-cultured with 30VD-1, indicating a multifaceted antagonistic mechanism involving chitinase, volatiles, and other antifungal compounds.

Evaluation of biocontrol agents against Fusarium wilt of Capsicum under pot culture conditions

The immersion of roots in a suspension of antagonistic species, such as *Trichoderma* spp., at a concentration of 10^6 colony-forming units per milliliter (cfu/ml), offers a dual advantage. It not only reduces the severity of disease but also promotes the growth of seedlings in crops like rice, tomato, brinjal, chilli, and capsicum. This approach provides a beneficial outcome in terms of disease management and enhanced seedling growth (Singh and Zaidi, 2002).

The utilization of antagonistic species in biological control is an environmentally friendly, ecologically sustainable, and economically viable method. It offers a favorable benefit: cost ratio and exhibits fewer negative impacts on the environment and lower toxicity compared to conventional disease control methods. In recent years, biological

control has gained significant recognition as a crucial approach in agricultural biotechnology for effectively managing numerous fungal plant pathogens (Abada and Eid, 2014).

Chowdhury et al. (2020) investigated the potential of *Bacillus* sp. LBF-01 as a biocontrol agent for preventing *Fusarium oxysporum* disease and promoting plant growth in *Capsicum annuum* plant. The strain demonstrated effective antifungal activity against *F. oxysporum*. In a pot experiment involving *C. annuum* plants, it was observed that disease incidence caused by *F. oxysporum* was reduced by 9.04 times compared to plants treated with 50% carbendazim. Furthermore, the strain positively influenced various plant growth parameters. The disease prevention and multiple growth-promoting characteristics of *Bacillus* sp. LBF-01 indicate its potential as a biocontrol agent for sustainable agriculture in *C. annuum* plants.

The efficiency of *Trichoderma* spp. having antagonistic capability against *Fusarium* spp. had been documented in cucumber also, under *in vitro* and pot culture conditions (Sharma, 2021).

During *in vitro* evaluation Venkataramanamma et al. (2022) found significant differences in the inhibition of *Fumarium oxysporum* growth among different isolates of *Bacillus*. The inhibition percentages varied from 2.3% to 74.36%. Among the isolates, *Bacillus*-7 exhibited the highest inhibition of pathogen growth, with a percentage of 74.36%. It was followed by *Bacillus*-5, *Bacillus*-6, and *Bacillus*-4, which showed inhibition percentages of 71.63%, 57.73%, and 52.89% respectively as compared to the control. There was no significant difference in the inhibition percentage between *Bacillus*-7 (*Bacillus cereus* strain, Accession number: KJ874357) and *Bacillus*-5 (*Bacillus cereus* strain, Accession number: KC248214). *Bacillus*-3 recorded an inhibition percentage of 51.85% and was not significantly different from *Bacillus*-4. The lowest percentage of inhibition was observed in the *Bacillus*-2 isolate (2.3%), followed by *Bacillus*-9 (6.2%), and these two isolates were not significantly different from each other. *Bacillus*-8 also reduced pathogen growth by 47.66%.

Chapter-3

MATERIALS AND METHODS

The present research on the topic entitled "**Studies on variability and management of Fusarium wilt of capsicum**" was conducted at experimental farm of the HRTS & KVK, Kandaghat and the Department of Plant Pathology, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, during 2021-22 and 2022-23. The methodologies used during studies are given under the following headings:

SURVEY

A survey of different capsicum growing regions of Solan district of Himachal Pradesh was conducted for recording the prevalence and incidence of capsicum wilt and to collect the isolates of the fungus based on visible symptoms under open and protected conditions.

The per cent disease incidence was calculated by the formula:

$$\text{Disease Incidence (\%)} = \frac{\text{Total number of plant infected}}{\text{Total number of plants observed}} \text{ X } 100$$

During survey, rhizosphere soil samples from healthy capsicum plants were also collected to isolate fungal and bacterial antagonists.

SYMPTOMATOLOGY

The above ground and below ground symptoms of the disease were studied on infected capsicum plants during the survey.

ISOLATION AND IDENTIFICATION OF PATHOGEN(S)

During surveys, diseased plants were collected in paper bags and brought to the laboratory for immediate isolation of the associated pathogen(s) and kept in the refrigerator for future use.

Isolation

The experiment involved isolation of associated fungus from wilted bell pepper plants on potato dextrose agar (PDA) medium. Small 2-3 mm bits were cut from the area where healthy and diseased tissues met in the collar and crown region. These bits were carefully

sterilized by dipping them in a 1% sodium hypochlorite solution for 30 seconds, followed by three washes with distilled water. Excess moisture was removed by placing the bits on sterilized filter paper. Under aseptic conditions, the sterilized bits were transferred to slants and Petri plates containing PDA medium and then incubated at a temperature of 25±2 ºC. The resulting cultures were further purified by using hyphal tip technique and maintained on PDA medium at a temperature of 4±2 ºC. The stock cultures were regularly sub-cultured every 20-25 days.

Identification

Cultural and morphological characterization

The pathogen isolated from the bell pepper plants were purified using the hyphal tip culture method. The morphological characteristics of the isolated fungi were examined on potato dextrose agar medium and under microscope. The cultural traits such as colony form, color, and margins were observed, while the shape, septation of microconidia and macroconidia, and the presence of chlamydospores were recorded under a Nikon microscope. The size of the spores was measured using NIS Elements software.

PATHOGENICITY

To confirm the pathogenicity of the fungi isolated from the root and collar region, pathogenicity tests were conducted under laboratory conditions using two different methods: soil inoculation with mass culture and spore suspension. Data in terms of incubation period (days) were recorded.

Inoculum Preparation

Preparation of Mass culture

The test pathogen isolated and propagated on a mixture of preboiled and sterilized Corn: Sand meal medium. Polypropylene bags, each containing 300 g of corn grains and sand in a 1:1 ratio, were autoclaved under a pressure of 1.05 kg/cm^2 for 1 hour. These bags were then inoculated with 5 mm discs of actively growing fungal cultures from 7-day-old cultures of each pathogen. The bags were incubated at a temperature of 25±2°C for 25 days to allow complete colonization of the grains by the fungus. Regular shaking of the bags was performed to ensure uniform colonization. Once the grains were fully colonized, the inoculum was air-dried in the shade. Subsequently, the colonized grains were separated into

individual kernels, placed in plastic bags, and stored at a temperature of 4°C until they were ready for use.

Preparation of spore suspension

To assess the pathogenicity of *Fusarium oxysporum*, a conidial suspension was prepared from one-week-old culture. Sterile water was added to each culture plate and the culture was gently scraped with a spatula to collect the spores. The spore suspension was gathered in a beaker and filtered through muslin cloth. The filtered solution was then resuspended in distilled water, and the spore concentration was adjusted to 1×10^7 conidia/ml using a haemocytometer.

Pathogenicity test

The pathogenicity of causal organisms *Fusarium oxysporum* was tested on bell pepper cv "Kandaghat Selection." The seedlings were procured from HRTS and KVK, Kandaghat.

Inoculation of soil with spore suspension

The *F. oxysporum* spore suspension (50ml) was poured into each polybag filled with sterilized soil. The polybags were then incubated for approximately seven days after inoculation to allow the inoculum to establish. Following this, two seedlings of bell pepper cv. Kandaghat Selection were transplanted into each polybag, and the polybags were placed in a glass culture to observe symptom development. Polybags without the spore suspension served as control. The pathogen(s) were reisolated from the infected plants, and their morphological characteristics were compared with the original cultures to fulfil the Koch's postulates, thereby confirming the pathogenicity of the isolated fungi.

Observation

i. Incubation period (days)

Inoculation of soil with mass culture

To conduct the pathogenicity test, the potting soil was sterilized using 5% formaldehyde solution. Separate polybags were filled with the sterilized soil and inoculated with the isolated fungus, specifically *F. oxysporum*, @ 5 g/kg of soil. To allow the inoculum

to establish, the polybags were incubated for 7 days before transplanting the seedlings. Regular watering was carried out to maintain optimal soil moisture levels. Two healthy seedlings of the cv. Kandaghat Selection of bell pepper were transplanted into each polybag. The development of wilt symptoms was observed and recorded at regular intervals. Subsequently, the pathogens were re-isolated from the infected plants, and their morphological characteristics were compared with the original cultures to fulfil the Koch's postulates, thereby confirming their pathogenic nature.

Observation

i. Incubation period (days)

DISEASE MANAGEMENT

Biological control

ISOLATION AND IDENTIFICATION OF BIOCONTROL AGENTS FROM RHIZOSPHERE

Biocontrol agents (Fungi and bacteria) were isolated from the capsicum rhizosphere using specific growth media by serial dilution technique (Kumar et al. 2015; Kannan et al. 2018). Based on the morphological and cultural characters, identification of the bioagents was done.

Isolation and identification of bacteria

10 grams of the rhizosphere soil was mixed with 90 ml of distilled water solution. The mixture was then placed on a shaker and agitated for one hour at 120 rpm. This process was done to mechanically separate the bacterial cells present in the soil. Subsequent dilutions of the suspension were prepared by manually shaking it for 10 seconds to resuspend the soil particles. Then transfer 1 ml an aliquot with a sterile pipette to 9 ml sterile distilled water in a test tube. This suspension was shaken manually for 10 seconds, and subsequent serial dilutions were prepared up to 10^{-9}. For isolation of bacteria spread 0.1 ml of soil suspension from 10^{-7} to 10^{-9} serial dilution on isolation selective agar medium (Bacillus media, Pseudomonas selective media). The number of bacteria colonies was estimated after 3-7 days of incubation at 28°C. Pick up the colonies to the same isolation medium on petri-dish. The isolates of bacteria were identified by using morphological characteristic as observation of cell shape by staining.

Isolation and identification procedure of fungi

10 grams of soil from the rhizosphere soil sample in 90 ml of distilled water than mix on shaker for one hour at 120 rpm. The soil extract was diluted from 10^{-1} to10^{-9}. Spread 0.1ml soil sample suspension from 10^{-3} to 10^{-5} serial dilution on to PDA medium with antibiotic. Then it was incubated at 28°c for 3 days. For pure isolation of fungus colonies were transferred on the same isolation media. The isolates of fungus were identified by using morphological characteristic and microscopic characteristics.

Molecular Characterisation

DNA Extraction

DNA extraction of the biocontrol agents was done by using Quick-DNA fungal/Bacterial miniprep kit designed by Zymo Research. For extraction of DNA standard procedure was followed as per protocol standardized by Zymo Research company, USA.

PCR amplification

Each PCR amplification reaction were performed in a 40µl mixture containing 5µl PCR buffer, 2µl deoxy nucleoside triphosphates (dNTPs), 1µl Taq Polymerase, 2µl each primer, 20 µl sterilised distilled water and 5µl genomic DNA. The primer pairs for amplifying full length ITS regions were ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). PCR consisted of initial denaturation at 94°C for 1 minute, followed by 35 cycles consisting of denaturing at 94°C for 30 seconds, annealing at 54°C for 30 seconds and extension at 72°C for 1 min, and a final extension for 7 minutes at 72°C. Amplified products were subjected to 2 per cent horizontal agarose gel electrophoresis in 1 per cent TAE buffer, pre-stained with ethidium bromide (5µl) and electrophoresis was carried out at 80V for 1.5-2 hours in TAE buffer. A ladder of 100 bp was used as a marker. The gel was observed in Gel Documentation System.

IN VITRO* EVALUATION OF BIOCONTROL AGENTS AGAINST *FUSARIUM OXYSPORUM

Evaluation of biocontrol agents

Effect of various fungal (*Trichoderma* spp.) and bacterial biocontrol agents (*Bacillus* spp. and *Pseudomonas* spp.) isolated from capsicum rhizosphere soil were checked under *in vitro* condition by using dual culture method and streak plate methods, respectively.

During the observations, the colony diameter of the test fungus as well as antagonist up to the zone of inhibition was measured and the pre cent growth inhibition of the test pathogen over the control was calculated as per the formula given by Vincent (1947):

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

Where,

I – Per cent inhibition in mycelial growth

C - Linear mycelial growth in control (mm)

T - linear mycelial growth in treatment (mm)

EVALUATION OF BIOCONTROL AGENTS AGAINST FUSARIUM WILT OF CAPSICUM UNDER POT CULTURE CONDITIONS:

Preparation of mass culture of fungal antagonist

The mass culture of fungal antagonists were prepared on corn seed: sand: sucrose (3:1:1 w/w/v) medium and autoclaved consecutively for two days. Mycelial discs (5 mm diameter) from the margins of actively growing 3-day old cultures were aseptically transferred into polypropylene bags containing autoclaved medium. After inoculation, polypropylene bags were incubated at 25±2° C for 15 days. After three days, the bags were shaken on a regular basis to ensure that the fungus grew uniformly.

Mass multiplication of bacterial bio control agents (*Bacillus* and *Pseudomonas*)

Bacterial biocontrol agents were mass multiplied in nutrient broth. Two loopful of 48 hour old bacterial culture were added to the nutrient broth medium and incubated for three days at 28 ± 2°C. The concentration of the medium was kept constant at 10^7 cfu/ml using the serial dilution method.

Evaluation of fungal and bacterial antagonists under pot culture condition

To evaluate efficacy of antagonists under pot culture conditions, sick pots were prepared as per method mentioned in pathogenicity trial. Then mass culture of *Trichoderma* spp. (50 g per Kg soil) and bacterial antagonists (50 ml per Kg soil) was added to the sick pots. Thereafter, seedlings of capsicum cv. Kandaghat Selection were transplanted in the sick

pots (2 seedling/pot). The experiment was conducted in completely randomized design. The treatments were replicated thrice having two seedlings per replication and pots were kept in the glasshouse for symptom development. Pots without inoculum and without biocontrol agents served as positive and negative controls, respectively. Data on disease incidence (%) was recorded three weeks after inoculation.

STATISTICAL ANALYSIS

Statistical analysis was performed on data collected from periodic surveys and *in vitro* experiments. The difference between treatments in various experiments were tested for significance at 5 % using the standard procedure described by Gomez and Gomez (1986). The ANOVA tables for the various experiments are presented in Appendix-II.

Chapter-4

RESULTS AND DISCUSSION

The results obtained during the current investigations are presented under the following headings:

SURVEY AND SURVEILLANCE

SYMPTOMATOLOGY

ISOLATION AND IDENTIFICATION

PATHOGENICITY

DISEASE MANAGEMENT

SURVEY AND SURVEILLANCE

To know the status of the wilt disease in the Solan district of Himachal Pradesh, surveys were conducted in different capsicum growing areas of the district periodically during cropping season of 2022-2023.

Scrutiny of the data (Table 1) voiced that disease was found to be widespread in all the surveyed regions (Plate 1 and Plate 2), showing varying levels of disease incidence which ranged from 6.22 to 38.88 percent. Maximum disease incidence under open filed conditions was recorded in Dadhog (30%) followed by Shatal (24.5%), Top ki ber (23.33%) and Dedgharat (21.5%) whereas, minimum disease incidence was recorded at Zadari (9.00%). Highest disease incidence in polyhouse condition was observed at Mansar (38.88%) followed by Padag (21%) while minimum disease incidence was observed at Diarag Bokhar (6.22%).

Relative analysis of data present in Table 1 revealed that mean of wilt incidence in polyhouse conditions (19.73%) was comparatively higher than in open field condition (18.31%) this could be ascribed to the prevalence of high temperature and moisture conditions under protected conditions which are congenial for the growth and survival of the pathogen. Further, high incidence at certain locations could be due to monoculture of susceptible cultivars leading to build up of soil borne inoculum.

Table 1: Incidence of Fusarium wilt of bell pepper at different locations of Solan district of Himachal Pradesh under open field and polyhouse conditions

Blocks	Location	Disease incidence (%) under open field conditions
Solan	Shatal	24.50
	Deothi	20.00
	Top ki ber	23.33
	Dadhog	30.00
	Dharon ki dhar	10.00
	Mathia	18.00
	Mansar	17.37
Kandaghat	Dedgharat	21.5.0
	Dhalli	9.44
	Zadari	9.00
	MEAN	**18.31**
Blocks	**Location**	**Disease incidence (%) under polyhouse conditions**
Solan	Grani	18.44
	Mansar	38.88
	Padag	21.00
	Dyarag bukhar	6.22
	Nauni	14.11
	MEAN	**19.73**

The occurrence of the disease has been reported from different capsicum growing regions of the world (Atiq et al. 2021). In India, Fusarium wilt incidence has been reported from Kashmir valley (Naik et al. 2008; Shaheen et al. 2021) and disease incidence ranging from 8.32 to 58.23 percent has been reported in Solan district of Himachal Pradesh (Sharma, 2022).

Table 2: Data on various crop parameters in relation to disease incidence

So no.	Name of farmer	location	Disease incidence (%)	Name of Variety	Time of plantation	Previous year/season crop	Type of cultivation	Years of growing	Types of fertilizers used in field
1	Rajesh	Shatal	24.50	Dollar	April	Cauliflower	Open field	3	Inorganic + FYM
2	Sanjay	Deothi	20.00	Dollar	April	Green pea	Open field	3	Inorganic + FYM
3	Hemant	Dadhog	30.00	Dollar	April	Cauliflower	Open field	4	Inorganic+ FYM
4	Sumit	Top ki ber	23.33	Dollar	April	Cauliflower/Cabbage	Open field	2	Inorganic+ FYM
5	Naresh	Mathia	18.00	Kandaghat Selection	April	Cauliflower	Open field	3	Organic
6	Dinesh	Mansar	17.37	Dollar	April	Cauliflower/ Green pea	Open field	3	Organic
7	Vijay	Dedgharat	21.50	Kandaghat selection	April	Cauliflower	Open field	3	Inorganic+ FYM
8	Ram Swaroop	Dhalli	09.44	Kandaghat selection	April	Green pea	Open field	2	Organic
9	Chandan	Zadari	09.00	Dollar	April	Cauliflower / Green pea	Open field	2	Organic + FYM
10	Puneet	Dharon ki dhar	10.00	Dollar	April	Cauliflower	Open field	2	Inorganic+ FYM
11	Kanti Swaroop	Grani	18.44	Paladin	Feb.	Capsicum	Polyhouse	2	Inorganic +FYM
12	Sham pal	Mansar	38.88	Orobelle /Bomby	Feb.	Capsicum	Polyhouse	4	Inorganic+ FYM
13	Aman dutt	Padag	21.00	Paladin/ Bachata	Feb.	Capsicum	Polyhouse	3	Inorganic+ FYM
14	Suresh	Nauni	14.11	Dollar	Feb.	Capsicum	Polyhouse	2	Inorganic+ FYM
15	Shalender	Dyarag bukhar	6.22	Paladin/ Bachata	Feb	Capsicum	Polyhouse	3	Natural formulations

Deothi

Top ki Ber

Shatal

Dadhog

Dharon ki Dhar

Dhalli

Dedghrat

Mansar

Plate 1: Incidence of Fusarium wilt of capsicum in district Solan under open field conditions

Grani

Padag

Mansar

Dyarag Bukhar

Nauni

Plate 2: Incidence of Fusarium wilt of capsicum in district Solan under protected conditions

Data presented in Table 2 revealed incidence of wilt at different location of solan districts of Himachal Pradesh w.r.t various crop parameters. The disease incidence varied from 9.00 to 30.00 per cent under open field conditions and under polyhouse conditions from 6.22 to 38.88 per cent. Perusal of the data revealed that higher disease incidence was recorded in the fields of farmers using inorganic fertilizers injudiciously and cultivating capsicum on same piece of land for 3 or more than three years whereas incidence was comparatively low in the fields of farmers using organic fertilizers or natural formulations. In general, high disease incidence was recorded in hybrids grown under polyhouse conditions as compared to open pollinated varieties under open field conditions.

SYMPTOMATOLOGY

During surveys, above and below ground symptoms were observed on disease plants as depicted in Plate 3 are described below:

The typical symptoms observed under field conditions are yellowing of the older leaves, epinasty of leaves, petioles, branches, wilting and death of the entire plant. Browning and discolouration of vascular bundle tissues was observed during vertically dissertation. Fruits on the infected plants were small, shrivelled and started rotting in later stages.

There are two types of symptoms (Primary and secondary) which are exhibited by wilt causing pathogens in the plant. Mostly *Fusarium* spp. show infection from roots and infect from inside to cortex. Wilting of plant is the result of xylem dysfunction of vascular wilt disease. Primary or typical symptoms of *Fusarium* spp. is browning of vascular bundle/discoloration of vascular tissue, yellowing of old leaf followed by upward or inward rolling leading to dropping of old leaves and subsequently wilting of plant (Gabrekiristos et al. 2020).

The characteristic systems of the disease as recorded during present investigations are in line with those described by other workers (Black and Rivelli 1990; Naik et al. 2008; Hami et al. 2021; Pandy et al. 2023).

Isolation and Identification

Isolation

The associated pathogen (s) was isolated from the vascular region of stem of infected plants collected from various locations by using standard procedure and pure cultures were

maintained on potato dextrose agar medium (PDA). The isolated fungus was purified by using hyphal tip method. To maintain the purity of the isolates, subculturing was done at 20-25 days intervals and for future use pure cultures were maintained in slants at 4+ 1°C for future use (Plate 4).

Cultural and morphological characters of pathogen

The associated pathogen isolates were isolated in pure form from the vascular tissues of the infected plants which showed wilt disease symptoms. The cultural characteristics of all the isolates were observed on PDA whereas the morphological characteristics were examined under a compound microscope. Details of cultural and morphological characters of isolates of the pathogen are as under Table 3.

Table 3: Cultural and Morphological characters of isolates of *Fusarium oxysporum*

Isolate	Colony characteristics			Microscopic observation								
	Growth after 3 days (cm)	Colony colour	Types of colony	Diameter of hyphae (µm)	Distance between two septa of hyphae (µm)	Types of Spores						
						Macroconidia				Microconidia		
						Length (µm)	Width (µm)	Septation	Macrospore count	Length (µm)	Width (µm)	Microspore count
1	5.15	White fluffy cottony	Raised	3.66	33.30	12.77	3.05	2	+	5.77	3.49	+
2	4.2	white cottony growth	Raised	3.52	32.94	8.78	4.28	2	+++	4.59	4.32	+++
3	4.75	Black, White cottony	Raised	2.88	18.16	12.59	4.84	2	++	6.93	5.24	++
4	4.75	Pink, White fluffy cottony	Raised	4.22	12.67	10.87	3.47	2	+++	5.79	2.86	+
5	5.4	White fluffy cottony	Raised	2.97	17.40	11.49	3.60	2-3	+++	4.66	3.61	+++
6	5.25	White fluffy cottony	Raised	4.60	39.41	11.49	4.74	2	++	5.28	3.61	++
7	4.25	White cottony	Raised	3.49	22.68	19.37	4.26	2	++	6.23	2.95	++
8	4.75	Pink, white fluffy cottony	Raised	4.83	31.32	13.31	4.15	2-3	+	7.02	2.52	+
9	4.9	Light pink, white cottony	Raised	2.94	53.96	10.45	4.00	2	+	3.00	2.69	+

*(+++ = high sporulation (>50 spores per microscopic field), ++ = medium sporulation (30-50 spores per microscopic field) and + = less sporulation (30 spores per microscopic field))

Perusal of the data (Table 3) reveal that growth of the colonies of isolates after 3 days of incubation varied between 4.2 cm to 5.4 cm. Colonies were raised and colour was white

Yellowing of leaves

Drooping of leaves

Completely wilted plants

Browning of the vascular tissue

Plate 3: Symptoms of Fusarium wilt of bell pepper

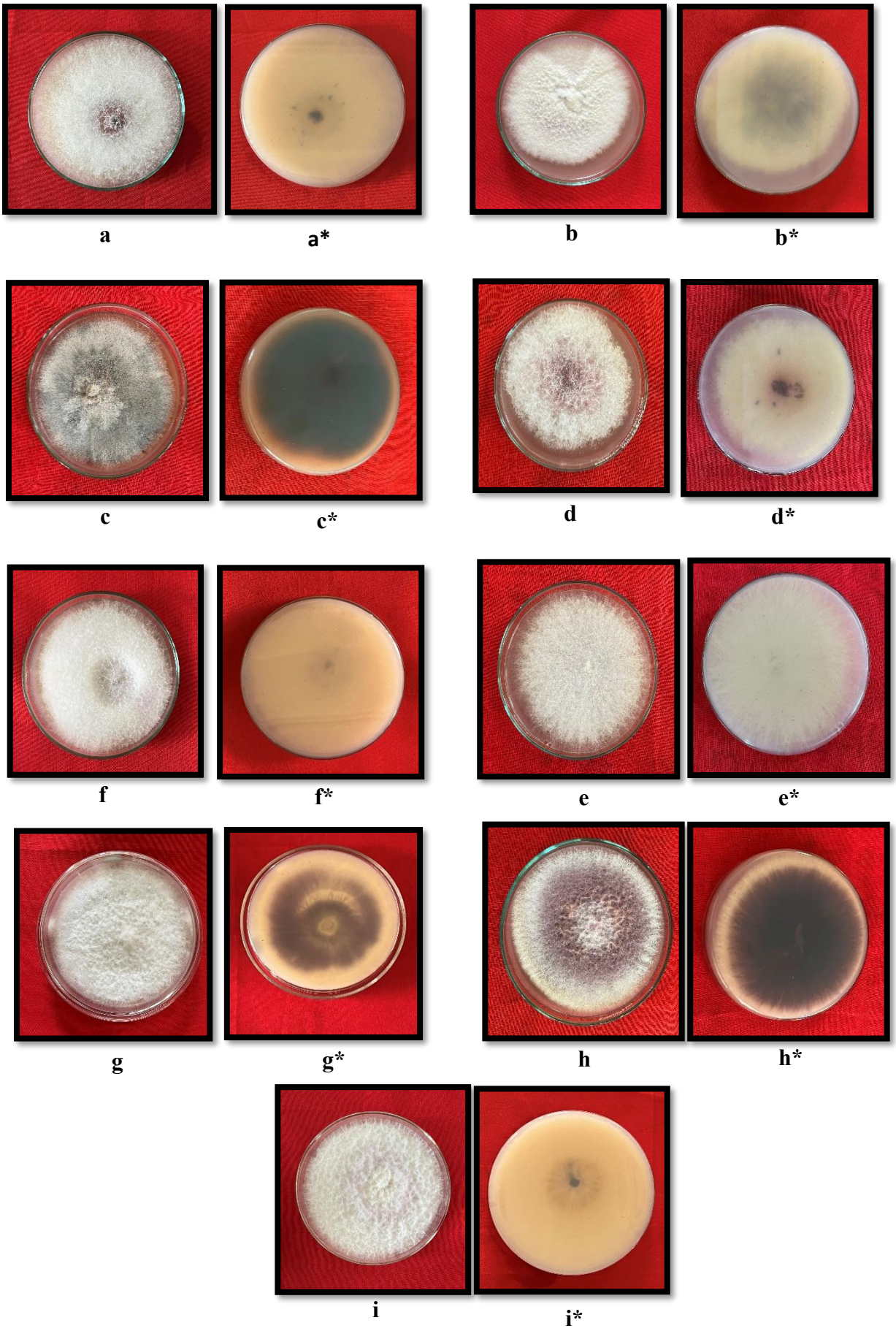


Plate 4: Dorsal and *ventral view of isolates of *Fusarium oxysporum*

fluffy cottony, pink, white fluffy cottony or blackish white cottony w.r.t different isolates. Under microscopic observations (Plate 5a and 5b) diameter of hyphae was observed to lie between 2.88 to 4.83 µm, distance between two septa of hyphae was 12.67 to 53.96 µm, length of macroconidia varied ranged between 8.78 to 19.37 µm, while width lied between 3.05 to 4.84 µm whereas, septation varied from 2 to 3. In case microconidia, length varied from 3.00 to 7.02 µm and width from 2.52 to 5.24 µm w.r.t different isolates. In some of the isolates, chlamydospores were observed in old culture which were terminal and inter-calary, globose to sub globose, thick walled and smooth.

The data obtained during present investigations are in consonance with Gowda et al. 2020; Sharma, 2022 who obtained cottony white colonies which later turned into pinkish white. The hyphae of the fungus were septate with average diameter of 3.67 µm. Microconidia size lied between 3.00 µm (length) x 2.69 µm (width) to 7.02 µm (length) x 2.52 µm (width). In case of macroconidia, size varied in between 8.78 µm (length) x 4.25 µm (width) to 19.37 µm (length) x 4.26 µm (width).

Based on these characteristic features and comparing them with the authentic descriptions and taxonomic keys (Booth et al. 1971; Anon, 2005), the fungus was identified as *Fusarium oxysporum* (Schlect.) Emend. Synd. and Hans. f. sp. *capsici* Riv.

Pathogenicity

The pathogenicity test of all *Fusarium oxysporum* isolates were performed on the bell pepper cv "Kandaghat Selection". The pathogenicity tests were carried out by using two methods viz. addition of mass culture of the pathogen and addition of spore suspension into pots which contained sterilized and healthy soil. Pathogenicity of the pathogen proved the Koch's Postulates (Plate 6). Data in terms of incubation period (days) was recorded and present in table 4.

Table 4: Pathogenicity test of the isolated fungi (*Fusarium oxysporum*) under pot culture condition

Method of inoculation	Incubation period (days)
Spore suspension	20
Mass culture	18

Perusal of the data reveal (Table 4) that inoculation by mass culture was found best with the incubation period of 18 days whereas, in case of soil inoculation by spore suspension method, incubation period of 20 days was recorded.

The findings of the present investigation are in consonance with other workers (Fadhal et al. 2019; Gabrekiristons et al. 2020; Sharma, 2022).

Table 5: Evaluation of pathogenicity of *Fusarium oxysporum* isolates collected from various capsicum growing areas in Solan district of Himachal Pradesh.

Isolates	location	Wilt incidence (%) 21 DAI
Isolate 1	Top ki ber	****
Isolate 2	Dharon ki dhar	***
Isolate 3	Dadhog	**
Isolate 4	Deothi	****
Isolate 5	Dedgharat	****
Isolate 6	Garhong	***
Isolate 7	Mansar	**
Isolate 8	Padag	**
Isolate 9	Shatal	****

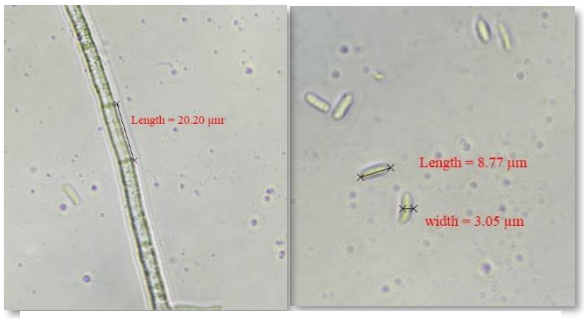
[DAI: Days After Inoculation, None pathogenic = None-patho , Less pathogenic (1-20 percent incidence) = * , Moderately pathogenic (21-50 percent incidence) = ** , Pathogenic (51-70 percent incidence) = ***, Highly pathogenic (71-100 percent incidence) = ****]

To ascertain the pathogenicity of all *Fusarium Oxysporum* isolates, soil inoculation by spore suspension was done, while soil inoculation by mass culture was done, only for those isolates which were found more aggressive in spore suspension method trial. These isolates were 1,4,5 and 9. (Table 5)

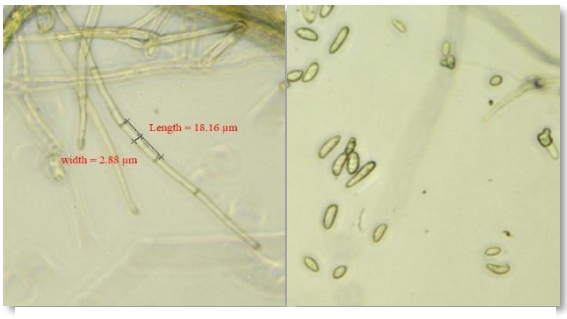
DISEASE MANAGEMENT

Biological control

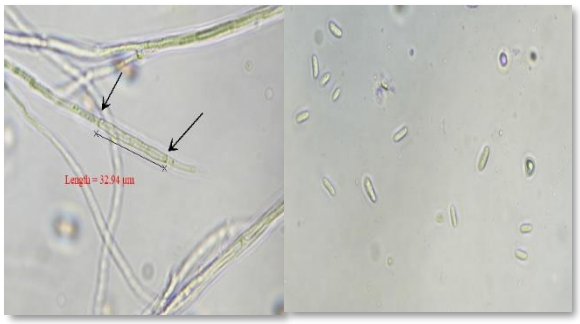
Various forms of biological control had been documented for their notable efficacy in combating plant pathogens across diverse crops, while also maintaining environmental safety (Lugtenberg et al. 2001). Numerous chemical fungicides, such as benzimidazoles and inhibitors of sterol biosynthesis, are currently employed to manage diseases in capsicum, both within India and internationally. The extensive application of chemical fungicides, along with fertilizers, pesticides and herbicides, had raised significant apprehensions due to its adverse impact on food production and the environment. Consequently, the exploration and creation of novel and promising biopesticides stand out as a practical pathway toward fostering sustainable agricultural and horticultural practices (Sang et al. 2007; Chowdhury et al. 2020).



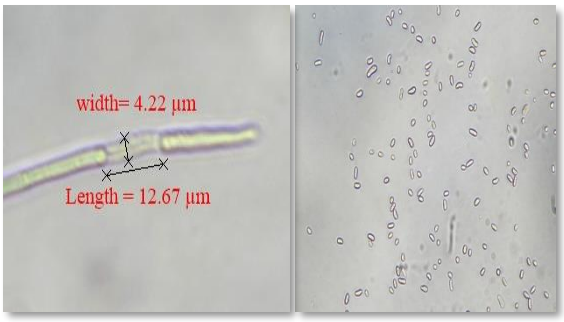
Isolate 1



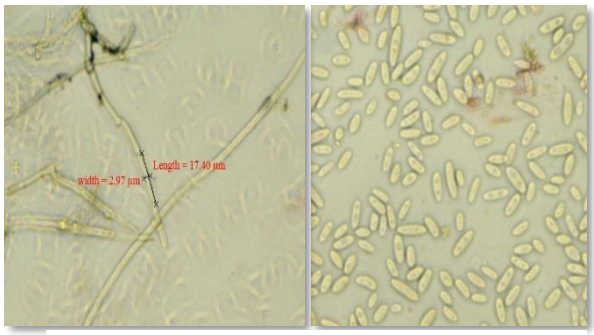
Isolate 2



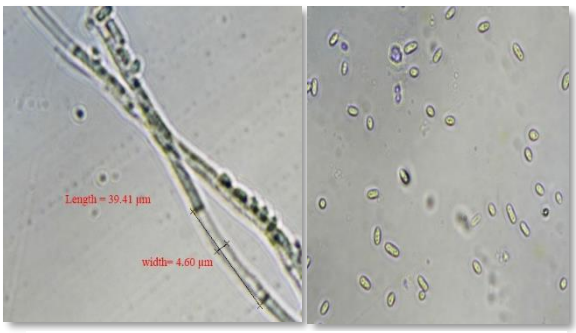
Isolate 3



Isolate 4

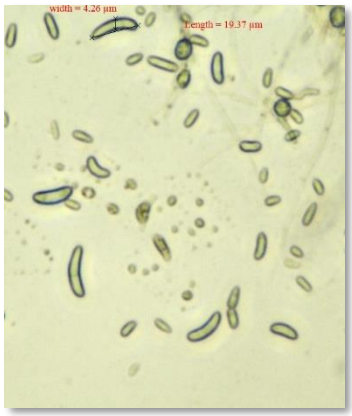


Isolate 5

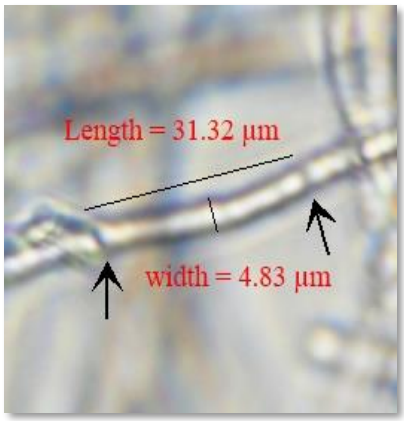


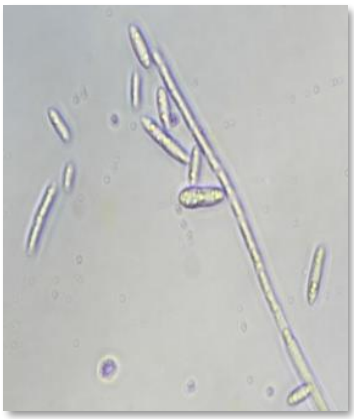
Isolate 6

Plate 5.a: Microscopic view of *F. oxysporum* isolates

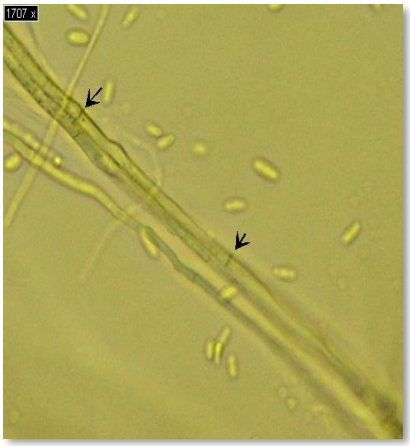


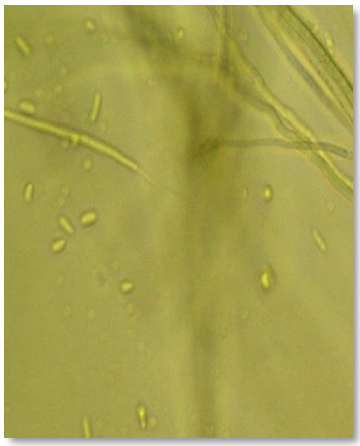
Isolate 7





Isolate 8





Isolate 9

Plate 5.b: Microscopic view of *F. oxysporum* isolates

Mass culture of *Fusarium oxysporum*

Pathogenicity test of *Fusarium* isolates

Healthy Plant

Infected Plant

Longitudinal section of plant showing healthy vascular region

Longitudinal section of plant showing infected vascular region

Plate 6: Pathogenicity test of *Fusarium oxysporum*

Isolation of biocontrol agents from rhizosphere

Biocontrol agents were isolated from the capsicum rhizosphere soil using specific growth media (appendix 1) by serial dilution technique.

In case of fungi, PDA media was used for isolation of *Trichoderma* from the soil. For isolation of *Trichoderma* from soil, flask containing water (90ml) and soil (10gm) was placed on an orbital shaker for 15 minutes at 240 rpm. Then, a series of dilutions (ranging from 10^{-5} to 10^{-7}) were prepared. Using 0.1 mL of suspensions from dilutions 10^{-5} to 10^{-7}, the samples were evenly spread onto potato dextrose agar (PDA) plates and placed in an incubator at a temperature of 26 ± 2°C for a duration of 3 to 7 days. Three *Trichoderma* spp. (Plate 7) were isolated from the soil at 10^{-7} dilution factor, which were identified on the basis of morphological and cultural characteristics as depicted in Table 6 such as the mycelial growth; lower and upper colony colour; colony texture; pigment secreted into the agar, conidia shape and size and the formation of distinct concentric rings (Plate 8).

Further DNA of two effective *Trichoderma* spp. (T1 and T2) against *Fusarium oxysporum* were isolated using Zymo research kit and PCR amplification of ITS region was carried out using both ITS primers. The amplified products were sent for purification and sequencing to Eurofins Genomics India Pvt. Ltd. After receiving the sequencing results, sequences were trimmed using Bio-edit software. BLASTn analysis of the sequences was carried out and the results indicated that *Trichoderma* sp.1 and *Trichoderma* sp.3 shared 97 -100 percent homology with the sequences reported from different regions in GenBank Database. The size of the DNA amplicons of *Trichoderma* sp.1 and *Trichoderma* sp.3 were approximately 650 bp. The sequences were submitted in the GenBank Database and accession numbers were obtained as mentioned in Table 6.1.

Table 6: Morphological and cultural characteristics of *Trichoderma* isolates

Bioagents	Culture observation			Microscopic observation		
	Growth after 4 days	Colour of colony	Types of colony	Diameter of mycelium (µm)	Size of conidia	
					Length (µm)	Width (µm)
Trichoderma sp. 1	Full plate growth (9 cm)	Light green	Raised	3.31	19.62	5.55
Trichoderma sp. 2	Full plate growth (9cm)	Dark green	Raised	3.97	13.73	3.02
Trichoderma sp. 3	Full plate growth (9cm)	White growth with green rings	Raised	4.78	15.37	3.92

Table 6.1: Accession numbers along with sequence IDs in NCBI GenBank of *Trichoderma* biocontrol agents

#Accession	Sequence ID	Biocontrol agent	Remarks
OR733695	Seq1	*Trichoderma brevicompactum* isolate Punia T1	Contain internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
OR733696	Seq2	*Trichoderma atroviride* isolate Punia T3	Contain small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.

In case of bacterial antagonist, isolation was done on specific media such as for *Bacillus*, it was Bacillus media and for *Pseudomonas*, it was Pseudomonas media by Serial dilution method was used followed by Aneja and Sharma (2010). Collection of rhizosphere soil of healthy plant was done from field of capsicum. For isolation of bacteria 10^{-7} to 10^{-9} serial dilution factor suspension was used. 0.1 ml of the suspension was used for spreading on the specific media and placed in an incubator at a temperature of 28 ± 2°C for 3 days. Seven isolates of bacteria (Plate 9) were isolated from the soil sample, which were identified on the basic of morphological and cultural characters as shown in Table 7.

Table 7: Morphological and cultural characters of bacterial isolates

Bacterial isolate	Culture observation		
	Colony morphology	Cell morphology	
		Shape	Gram staining
Bacillus sp. 1	White, circular, raised, entire	rod, chain	Gram +ve
Bacillus sp. 2	Light red, circular, convex, entire	rod, chain	Gram +ve
Bacillus sp. 3	Creamy, punctiform, flat, entire	rod, chain	Gram +ve
Bacillus sp. 4	Creamy white, punctiform, flat, entire	rod, chain	Gram +ve
Pseudomonas sp 1	Light orange, circular, raised, entire	rod, pair	Gram -ve
Pseudomonas sp 2	Slime white, circular, convex, entire	rod, single	Gram -ve
Pseudomonas sp 3	White, punctiform, raised, entire	rod, chain	Gram -ve

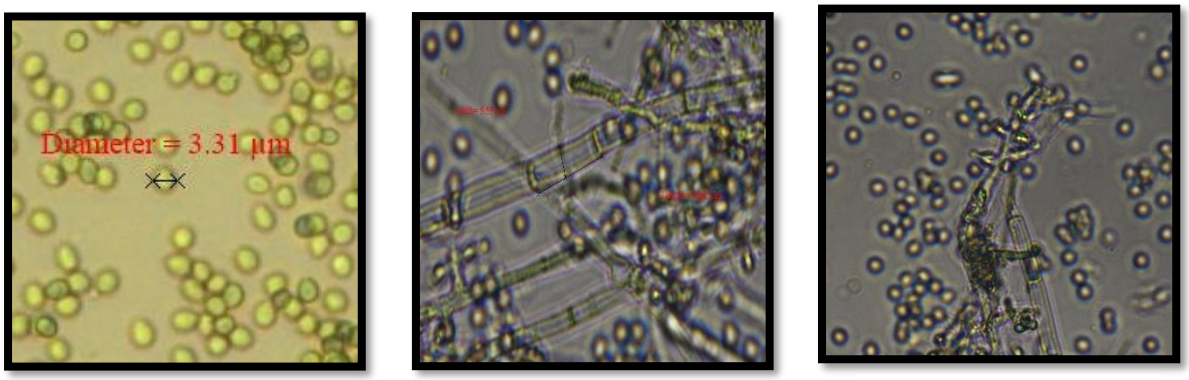
In Table 7, morphological and culture characteristics of seven bacterial isolates are given, out of which first four are Gram positive bacteria (*Bacillus* sp.1, *Bacillus* sp 2,

***Trichoderma* sp. 1**

***Trichoderma* sp. 2**

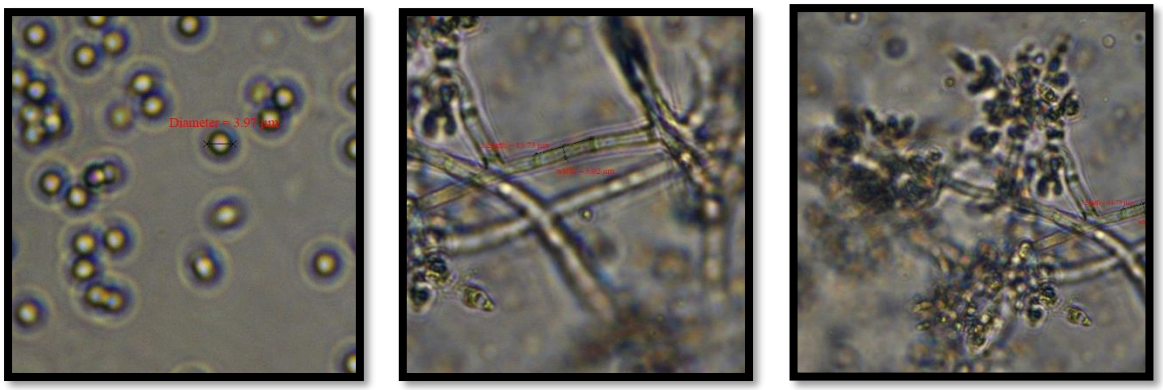
***Trichoderma* sp. 3**

Plate 7: Dorsal and Ventral view of *Trichoderma* isolates



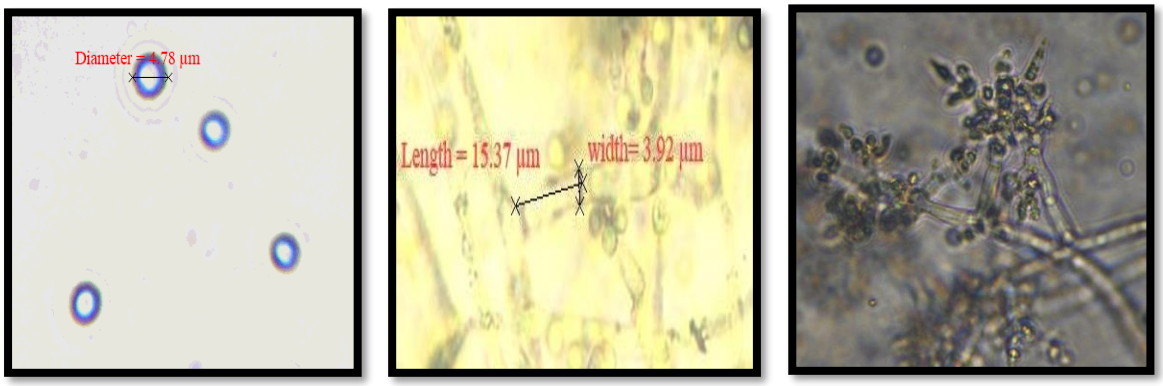
conidia Hyphae Conidiophore

Trichoderma sp. 1



conidia Hyphae Conidiophore

Trichoderma sp. 2



conidia Hyphae Conidiophore

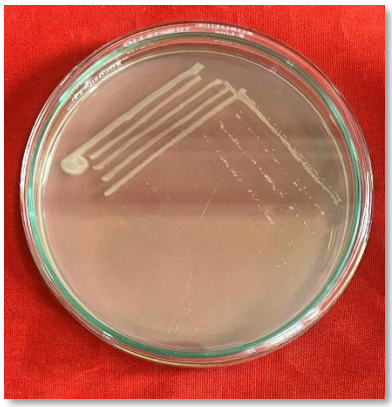
Trichoderma sp. 3

Plate 8: Microscopic view of *Trichoderma* sp. isolates

Bacillus sp. 1

Bacillus sp. 2

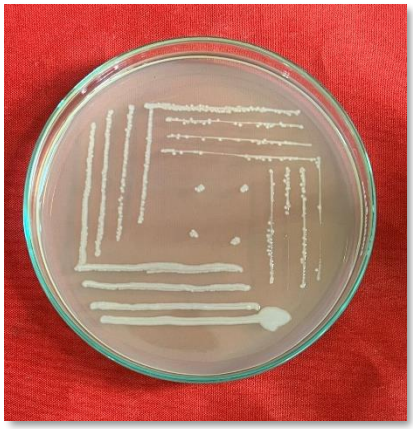
Bacillus sp. 3



Bacillus sp. 4

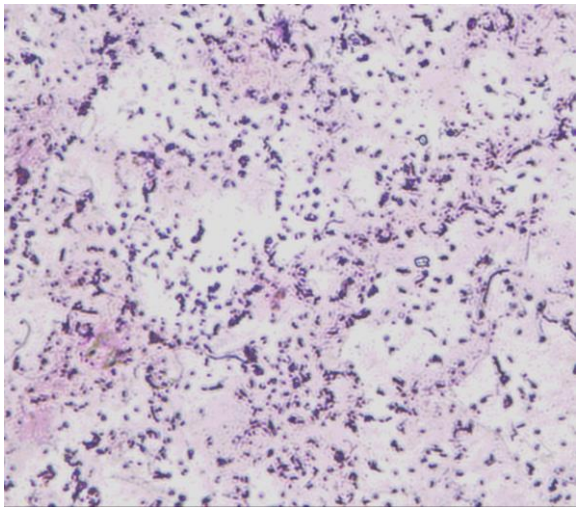
Pseudomonas sp. 1

Pseudomonas sp. 2

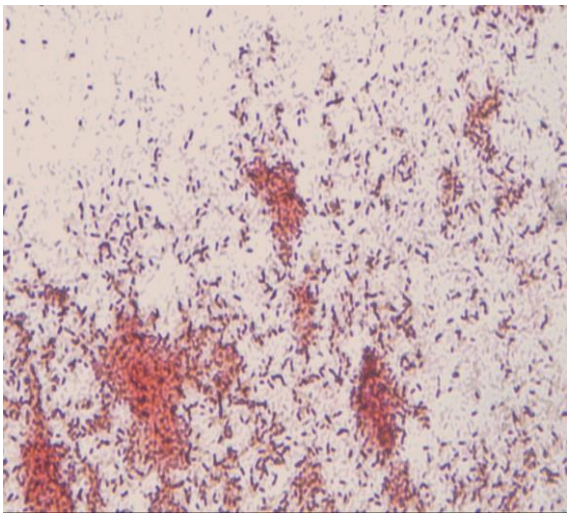


Pseudomonas sp. 3

Plate 9: Bacterial isolates



***Bacillus* sp.**



***Pseudomonas* sp.**

Plate 10: Microscopic view of bacterial isolates

Bacillus sp 3 and *Bacillus* sp 4) and three are Gram negative (*Pseudomonas* sp 1, *Pseudomonas* sp 2 and *Pseudomonas* sp 3) (Plate 10). All the bacterial isolates had varied colony morphology. The isolates were rod shaped. They were either single, in pair or in chains.

The colony and cell morphology of the isolates were in accordance with other workers (Swain and Ray, 2009; Trotel-Aziz et al. 2008; Passari et al. 2017; Zhao et al. 2018).

***In vitro* evaluation of biocontrol agents against *Fusarium oxysporum*:**

All the fungal (*Trichoderma* sp.) and bacterial isolates (*Bacillus* sp. and *Pseudomonas* sp.) were tested for their antagonist activity against the Fusarium isolates by adopting dual culture technique and streak plate method, respectively.

In case of fungal bio control agents, all three *Trichoderma* spp. isolates were tested against isolates of *Fusarium oxysporum* by adopting dual culture techniques. Data on mycelial growth of the pathogen were recorded and percentage of mycelial inhibition was calculated and presented in Table 8.

Perusal of the data (Table 8) revealed that all the *Trichoderma* spp. were able to inhibit the mycelial growth of *Fusarium oxysporum* isolates, however, there is difference in per cent inhibition provided by the different species w.r.t. isolates. As far as *Trichoderma* sp. 1 is concerned, it gave maximum inhibition for isolate 1 (65.18 %) followed by isolate 2 (63.88 %) however, these were statistically at par with each other while, minimum inhibition was recorded for isolate 8 (42.66%). In case of *Trichoderma* sp. 2, maximum inhibition (59.78%) was observed for isolate 3 followed by isolate 6 (52.34%) though these were statically at par while, minimum mycelium inhibition was recored (34.26%) for isolate 1. Minimum mycelial growth (23.66%) and maximum inhibition (73.31) was observered in isolate 4 for *Trichoderma* sp. 3 followed by isolate 2 i.e., 62.77 percent. However, minimum per cent inhibition was recorded for isolate 9 (53.70 %). (Plate 11,12 and 13).

Efficacy of *Trichoderma spp*. against *Fusarium oxysporum* could be ascribed to their higher competitive ability by different mechanisms viz. mycoparasitism, antibiosis and siderophore production (Sharma, 2019).

Table 8: *In vitro* evaluation of *Trichoderma* spp. against different isolates of *Fusarium oxysporum*

Fusarium Isolates	*Trichoderma* sp. 1		*Trichoderma* sp.2		*Trichoderma* sp. 3	
	Mycelial growth (mm)	Percent inhibition	Mycelial growth (mm)	Percent inhibition	Mycelial growth (mm)	Percent inhibition
Isolate 1	31.33	65.18	56.50	34.26	39.66	55.92
Isolate 2	32.50	63.88	47.50	44.11	33.50	62.77
Isolate 3	34.16	62.03	35.16	59.78	33.16	59.25
Isolate 4	36.66	59.25	43.83	48.42	23.66	73.71
Isolate 5	38.66	57.03	50.83	40.19	39.33	56.29
Isolate 6	39.50	56.11	40.50	52.34	35.00	61.11
Isolate 7	40.66	54.81	48.66	42.74	41.16	54.25
Isolate 8	42.66	52.59	48.50	42.93	33.83	62.40
Isolate 9	40.66	54.80	52.50	38.23	41.66	53.70
CONTROL	90.00	----	90.00	-----	90.00	-----
CD	4.48	4.98	6.94	8.17	5.63	6.70

The effectiveness of *Trichoderma* sp. against *Fusarium oxysporum* has been well documented in the literature (Sahi and Khalid, 2007; Tapwal et al. 2015; Raghu, 2016; Sharma, 2019; Anjum, 2020; Girma, 2022; Kim et al. 2023; Sharma et al. 2023).

In case of bacterial bio control agents, *Bacillus* spp. and *Pseudomonas* spp. were tested against isolates of *Fusarium oxysporum* by adopting streak plate method. Data on pathogen mycelial growth were recorded and percentage of mycelial inhibition was calculated as shown in Table 9.

Table 9: *In vitro* evaluation of bacterial bioagents against *Fusarium oxysporum* (Isolate 4)

Bacterial isolate (s)	Mycelial growth (mm)	Percent inhibition
Bacillus sp. 1	54.00	36.46 **(37.08)**
Bacillus sp. 2	54.50	35.87 **(36.48)**
Bacillus sp. 3	68.33	19.60 **(25.16)**
Bacillus sp. 4	74.66	12.15 **(20.29)**
Pseudomonas sp. 1	57.50	32.34 **(34.54)**
Pseudomonas sp. 2	66.66	21.56 **(27.22)**
Pseudomonas sp. 3	70.33	17.25 **(24.53)**
Control	85.00	----
CD	12.52	**(10.14)**

***Figures in the parentheses are arc sine transformed values**

Among the bacterial bioagents, *Bacillus* spp. were more effective in inhibiting the growth of *F. oxysporum* (isolate 4). Maximum inhibition (36.46) was recorded in *Bacillus* sp. 1 and minimum in *Bacillus* sp.4 whereas, *Pseudomonas* sp.1 having maximum per cent mycelial inhibition (32.34) (Plate 14a and 14b).

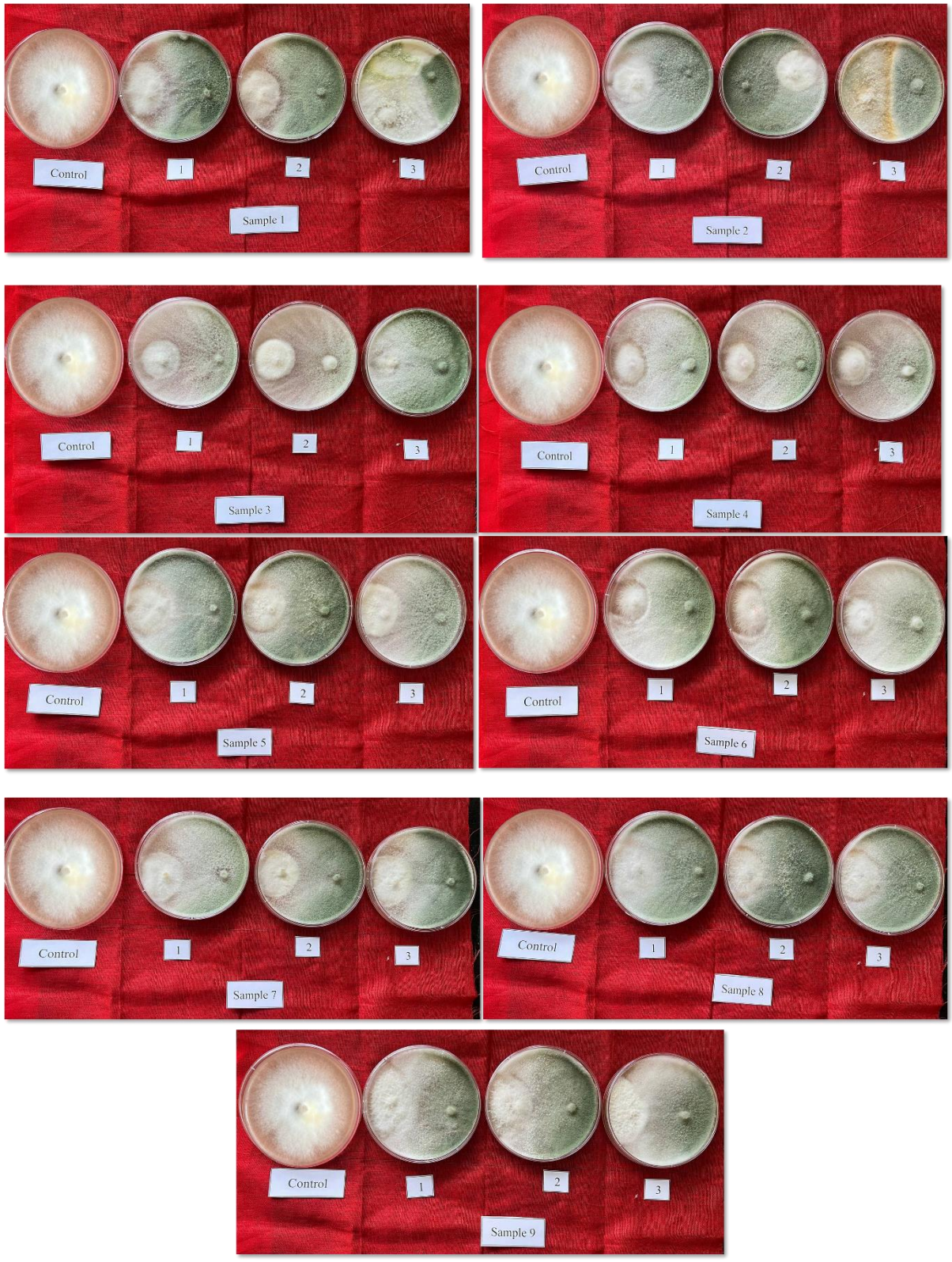


Plate 11: *In vitro* evaluation of *Trichoderma* sp. 1 against isolates of *Fusarium oxysporum*

Plate 12: *In vitro* evaluation of *Trichoderma* sp. 2 against isolates of *Fusarium oxysporum*

Plate 13: *In vitro* evaluation of *Trichoderma* sp. 3 against isolates of *Fusarium oxysporum*

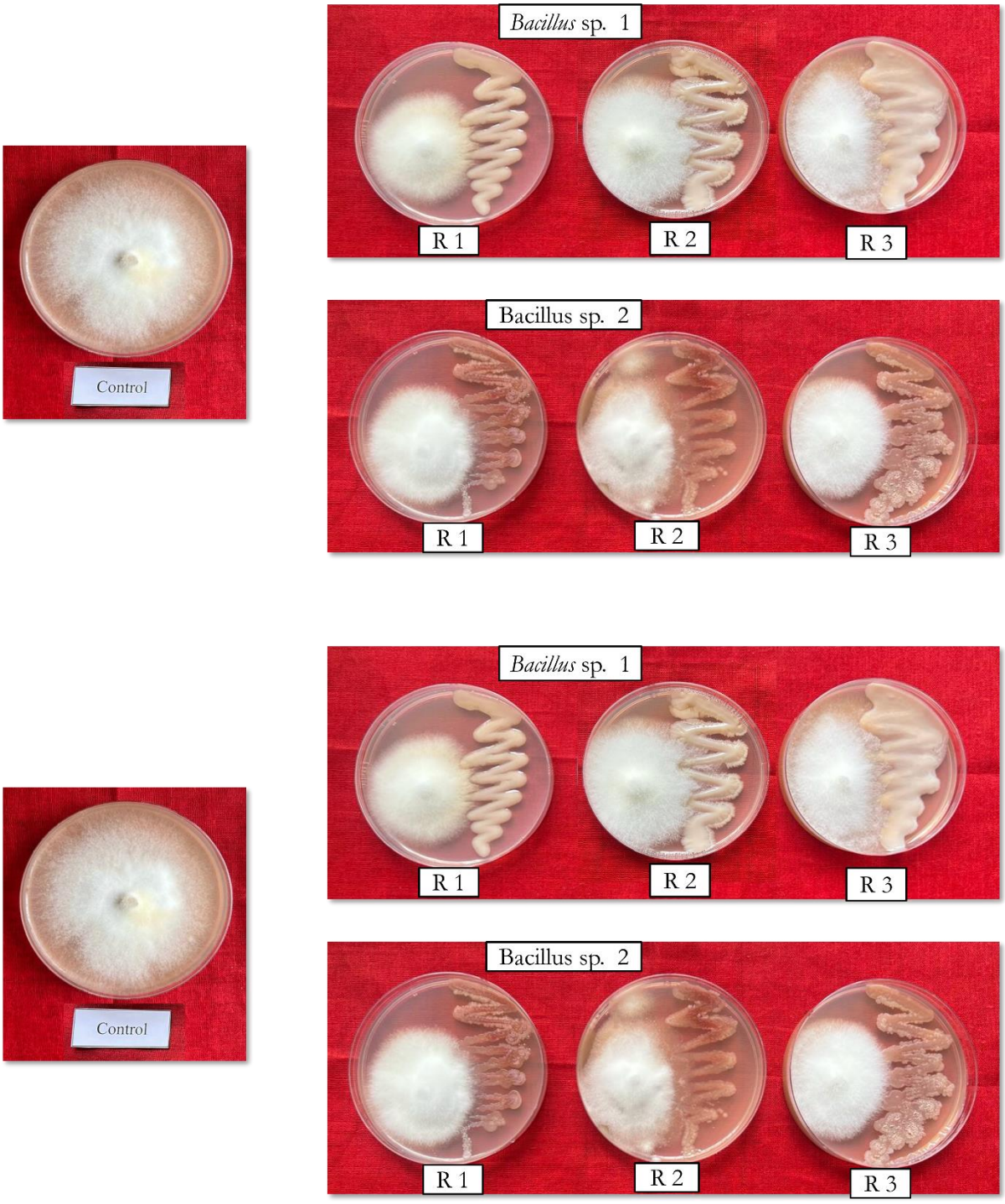


Plate 14 a: *In vitro* evaluation of *Bacillus* spp. against *Fusarium oxysporum* (isolate 4)

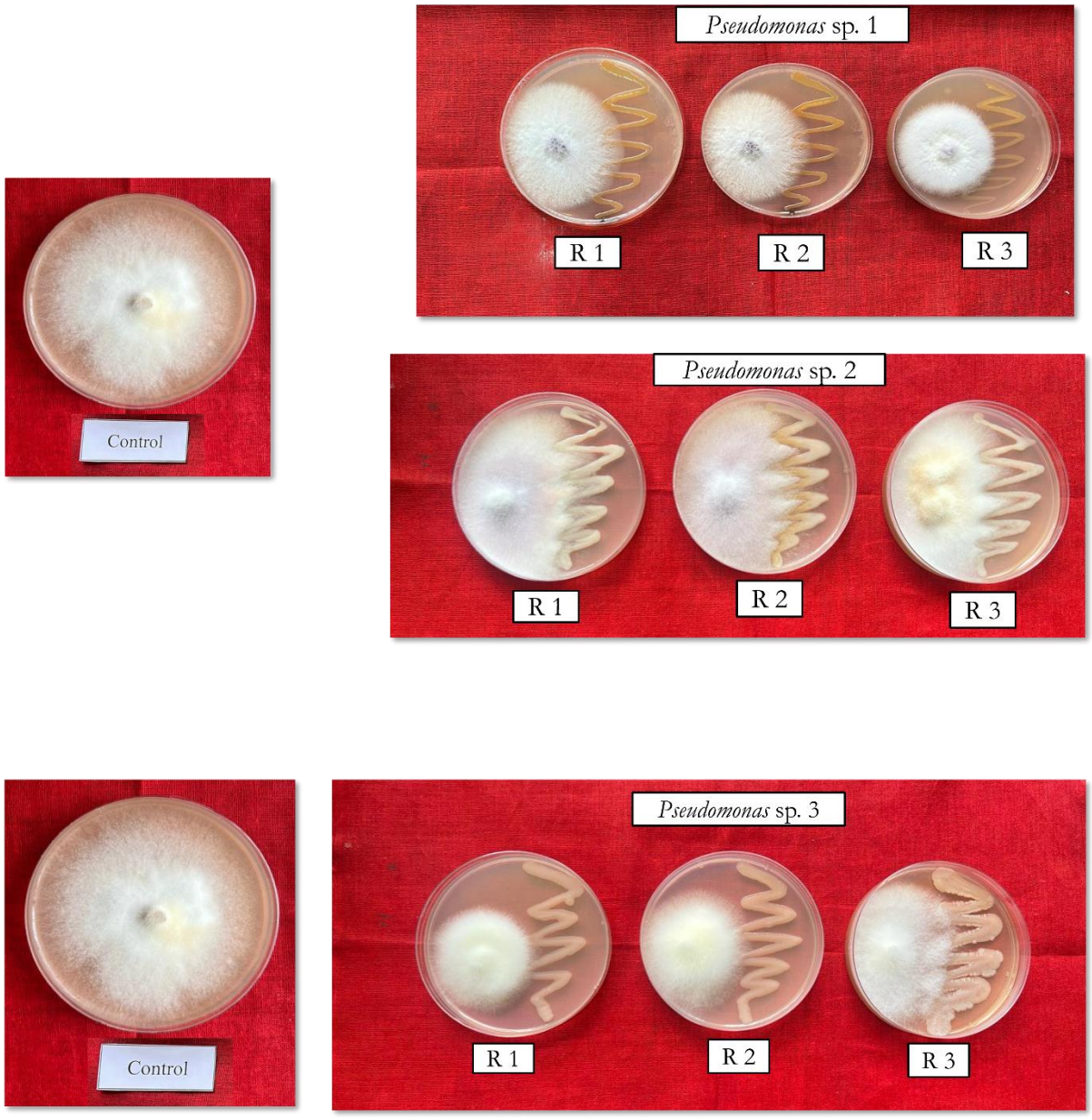


Plate 14 b: *In vitro* evaluation of *Pseudomonas* spp. against *Fusarium oxysporum* (isolate 4)

Antagonistic strains of *Bacillus subtilis, B. amyloliquefaciens, B. liquefaciens,* and *B. pumilus* have been found effective to combat *Fusarium verticilliodes*, *F*. *oxysporuim*, *F. acuminatum, F. solani, F. proliferatum, and F. oxysporum f. sp. melonis*, which were isolated from garlic (*Allium sativum*), melon (*Cucumis melo*), and pepper plants (*Capsicum annuum*) (Morales et al. 2016).

Similar results have been found by Singh et al. (2021) that in dual culture bioassays of biocontrol agents *T. harzianum* demonstrated the highest level of growth inhibition, with a remarkable 66 per cent inhibition against *F. oxysporum*. This was followed by *P. fluorescens*, which displayed inhibition rates of 40 per cent against the same pathogens, and *B. subtilis*, with inhibition rates of 41 per cent.

The effectiveness of *Bacillus* spp. and *Pseudomonas* spp. against *Fusarium oxysporum* has been well documented in the literature (Sahi, 2012; Sundaramoorthy et al. 2012; Dukare and Paul, 2021; Fentahun G et al. 2022).

Evaluation of biocontrol agents against Fusarium wilt of capsicum under pot culture condition

The efficacy of various fungal and bacterial bio control agents was tested against *Fusarium oxysporum* in pot culture conditions using the methods described previously in chapter 3. Data on disease incidence and disease control (Table 10) were recorded and presented below.

Table 10: Evaluation of biocontrol agents against *Fusarium* wilt of capsicum under pot culture condition

Sr.No.	Bioagents	Disease incidence (%)	Disease control (%)
Fungal bioagents			
1.	*Trichoderma* sp. 1	33.33	66.66 (54.72)
2.	*Trichoderma* sp. 2	66.66	33.33 (35.24)
3.	*Trichoderma* sp. 3	16.67	83.33 (59.97)
Bacterial bioagents			
4.	*Bacillus* sp. 1	50.00	50.00 (44.98)
5.	*Bacillus* sp. 2	83.33	16.66 (24.07)
6.	*Pseudomonas* sp 1	100.00	0.00 (00.00)
7.	*Pseudomonas* sp 2	100.00	0.00 (00.00)
8.	(+) Control	00.00	----
9.	(-) Control	100.00	----
	$CD_{(0.05)}$	3.075	3.13 (1.88)

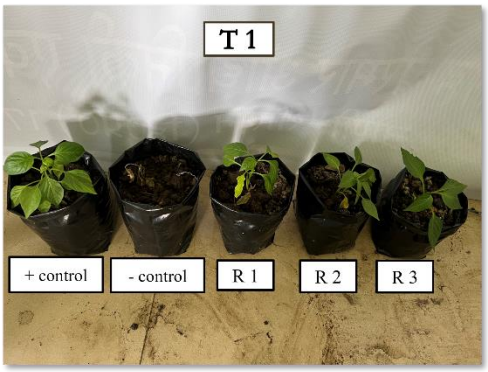
***Figures in the parentheses are arc sine transformed values**

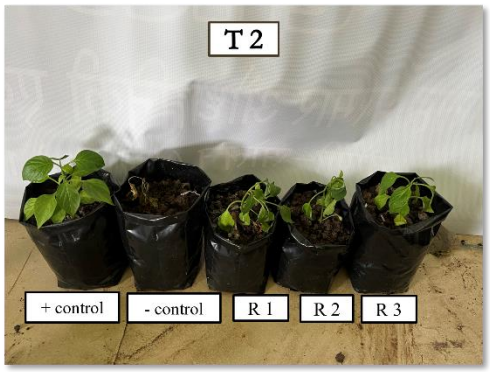
Perusal of the data (Table 10) revealed that among all bioagents, *Trichoderma* sp. were more effective in significantly lowering disease incidence as compared to the control where bacterial bioagents were not so effective. Among the fungal bio control agents tested against *Fusarium oxysporum*, *Trichoderma* sp. 3 proved the most effective with minimum disease incidence of 16.67 per cent and exhibited a disease control of 83.33 per cent which was followed by *Trichoderma* sp. 1, which had a disease incidence of 33.33 per cent and a disease control of 66.66 per cent. Among the bacterial bio control agents, *Bacillus* sp. 1 had exhibited lowest disease incidence (50.00 %) and gave disease control of 50.00 per cent, followed by *Bacillus* sp. 2 with only 16.66 percent disease control. *Pseudomonas* spp. were not found effective against *F. oxysporum* under pot culture condition (Plate 15).

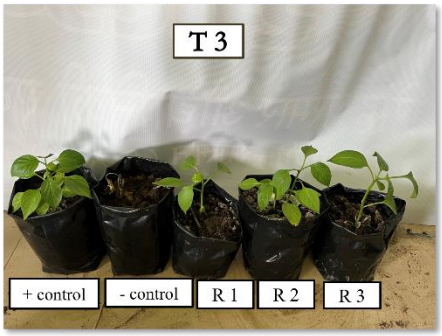
Findings of Anjum et al. (2020) corroborates the results of present study. They assessed the antagonistic capabilities of various *Trichoderma* spp., including *T. atroviride, T. hamatum, T. harzianum, T. longibrachiatum* and *T. viride* against *Fusarium oxysporum* f. spp. *capsici* using the dual culture technique. *In vitro* experiments demonstrated that *T. hamatum* exhibited the highest efficacy in inhibiting the mycelial growth of *F.oxysporum* f. spp. *capsici*, with a substantial 70.15 per cent inhibition rate. Following *T. hamatum, T. atroviride, T. harzianum, T. longibrachiatum*, and *T. viride* also displayed inhibitory effects, with inhibition rates of 67.18 per cent, 68.75 percent, 69.46 per cent, and 66.75 per cent, respectively.

Study conducted by Yu et al. (2011) revealed that *Bacillus subtilis* CAS15 exhibited a notable reduction in the occurrence of Fusarium wilt in bell pepper, ranging from 12.5 % to 56.9 %, suggesting that it effectively stimulated systemic resistance against Fusarium wilt in pepper plants. Whereas, in studies of Ajilogba et al. 2013 they found that during *in vivo* evaluation, *B. cereus* had the lowest disease incidence and higher disease control per cent (18.75% and 81.2%), *B. amyloliquefaciens* (25% and 75%), *B. pumilus* (37.5% and 62.5%) and *B. subtilis* (37.5% and 62.5%), respectively.

The findings of the present investigation are in accordance with other workers (Fridlender et al. 1993; Kishore and Kulkarni , 2008; Ramezani , 2008; Shanmugam and Kanoujia, 2011; Devi et al. 2012; Akrami and Yousefi, 2015; Subash et al. 2014; Cucu et al. 2020; Sharma, 2021).

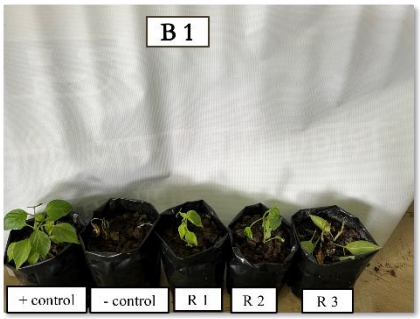


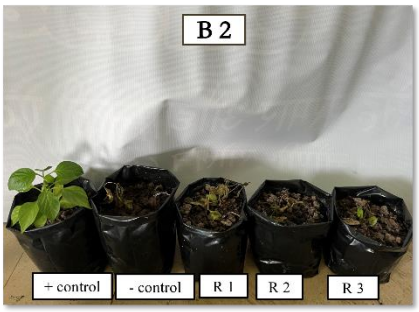


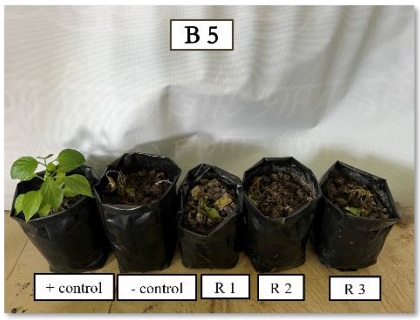


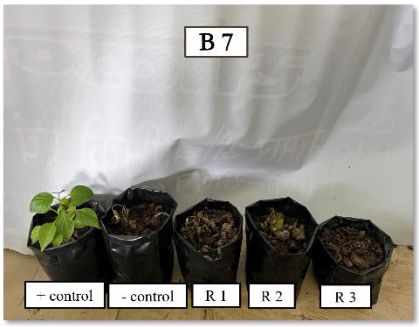
T 1: *Trichoderma* sp. 1
T 2: *Trichoderma* sp. 2
T 3: *Trichoderma* sp. 3

Evaluation of Trichoderma spp. against Fusarium wilt of capsicum under culture house condition









B 1: Bacillus sp.1
B 2: Bacillus sp.2
B 5: Pseudomonas sp.5
B 7: Pseudomonas sp.7

Evaluation of *Bacillus* spp. and *Pseudomonas* spp. against Fusarium wilt of capsicum under culture house condition

Plate 15: Evaluation of biocontrol agents against Fusarium wilt of capsicum under pot culture condition

Chapter-5

SUMMARY AND CONCLUSION

Bell pepper or *capsicum annum* is one of the most important vegetable crops belonging to the family Solanaceae. It is a rich source of vitamins (vitamin A, E and C) and minerals. Capsicum crop is affected by various diseases from germination stage to fruiting stage of which, wilt disease is one of the most devasting diseases leading to complete death of the plant and cause serious economic losses to the farmers.

The present investigations on Fusarium wilt of capsicum was undertaken on various aspects of the disease viz., occurrence, symptomatology, pathogenicity, pathogen cultural and morphological identification, isolation of bio control agents from rhizosphere soil of healthy capsicum plants and evaluation of their efficiency against the pathogen under study. The result obtained are summarized below:

Fusarium wilt of capsicum was observed to occur in moderate to sever from in different capsicum growing areas in Solan district of Himachal Pradesh. The maximum incidence was found at Dadhog (30%) and minimum at Zadari (9%) under open field conditions whereas, under polyhouse conditions maximum disease incidence was recorded at Mansar (38.88 %) while, minimum disease incidence was recorded at Dyarag Bukhar (6.22%).

During surveys, typical symptoms of *Fusarium* wilt were observed as yellowing and drooping of leaves followed by browning of the vascular tissue resulting in complete death of the plant. Infected plants were collected during surveys and associated pathogen(s) was isolated in pure form on PDA and identified based on cultural and morphological characteristics. The culture and microscopic study of nine isolates revealed that the isolates of *Fusarium oxysporum* produced white fluffy cottony to light pink, white colonies. Hyphae were septate, branched and hyaline with diameter ranging from 2.88 to 4.83 µm. *Fusarium oxysporum* produced two types of spores: macroconidia and microconidia.

The pathogenicity tests revealed that all nine isolates of *Fusarium* were pathogenic on bell pepper. Disease symptoms were observed after 20^{th} day of soil inoculation by spore suspension method. Whereas, symptoms were recorded after 18^{th} day of soil inoculation by mass culture method and complete wilting of plant or death observed on 24^{th} day. Out of nine

isolates, isolates from Dadhog, Dedgharat, Top ki Ber and Shatal were found highly pathogenic and for further studies, isolate 4 was used.

Isolation of antagonistic microorganisms was done by using serial dilution method. Three species of *Trichoderma* and seven *Bacterial* species were isolated from the rhizospheric soil of healthy capsicum plants and identified on the basis of the cultural and morphological characteristics.

Out of the three species of *Trichoderma*, two species i.e *Trichoderma* sp.1 and *Trichoderma* sp.3 were identified on the basis of molecular characterization as *Trichoderma brevicompactum* (Accession number: OR733695) and *Trichoderma atroviride* (Accession number: OR733696).

In vitro evaluation of biocontrol agents against *F. oxysporum* was done by dual culture method and streak plate method to know their efficiency against the pathogen. Among fungal antagonists, *Trichoderma* sp.3 (*Trichoderma atroviride,* accession number: OR733696) was found most effective and exhibited 80.37 % inhibition whereas, in case of bacterial antagonists, *Bacillus* sp. 1 was found most effective with 36.46 percent inhibition of *Fusarium oxysporum*.

All the biocontrol agents were evaluated against *Fusarium* wilt of capsicum under pot culture conditions wherein, maximum disease control (83.33%) was recorded in *Trichoderma* sp.3 (*Trichoderma atroviride,* accession number: OR733696).

The following conclusions can be drawn from the findings of the current studies:

- Fusarium wilt of capsicum is most devasting disease in capsicum growing areas of Solan district of Himachal Pradesh under open field conditions as well as under polyhouse conditions with mean disease incidence of 18.31 % and 19.73 %, respectively.
- Cultural, morphological and pathological variability was observed among nine isolates of *F.oxysporum*.
- Under *in vitro* and pot culture conditions, *Trichoderma* sp.3 (*Trichoderma atroviride,* accession number: OR733696) and *Bacillus* sp. 1 were found to be the most effective fungal and bacterial antagonists, respectively.
- *Trichoderma* sp. 3 (*Trichoderma atroviride,* accession number: OR733696) isolate being the most effective against *Fusarium oxysporum* can be used for the biological management of Fusarium wilt of capsicum.

LITERATURE CITED

Abada KA and Ahmed MA. 2014. Management Fusarium wilt of sweet pepper by *Bacillus* strains. *American Journal of Life Sciences*. 2(6-2). pp.19-25.

Abdel MF and Ismail ME. 2010. The use of antioxidants to control root rot and wilt diseases of pepper. *Notulae Scientia Biologicae* 2:46-55.

Agrios GN. 1988. Plant Pathology, 3rd. ed. Academic Press, New York.803p.

Ajilogba CF, Babalola O and Ahmad F. 2013. Antagonistic effects of *Bacillus* species in biocontrol of tomato Fusarium wilt. *Studies on Ethno-Medicine*, 7(3), pp.205-216.

Akrami M and Yousefi Z. 2015. Biological controls of Fusarium wilt of tomato (*Solanum lycopersicum*) by *Trichoderma* spp. as antagonist fungi. Biological Forum—*An International Journal*. 7(1). 887-892.

Altinok HH, Yüksel G and Altinok MA. 2020. Pathogenicity and phylogenetic analysis of *Fusarium oxysporum* f. sp. *capsici* isolates from pepper in Turkey. *Canadian Journal of Plant Pathology* 42:279-91.

Aneja KR and Sharma C. 2010. Antimicrobial potential of fruit extracts of *Elettaria cardamomum maton* (Chhoti elaichi) against the pathogens Causing ear infection. *Pharmacology online*. 3: 750-756.

Anjum N, Shahid AA, Iftikhar S, Mubeen M, Ahmad MH, Jamil Y, Rehan MK, Aziz A, Iqbal S and Abbas A. 2020. Evaluations of *Trichoderma* isolates for biological control of Fusarium wilt of chili. *Plant Cell Biotechnology and Molecular Biology*, 21(59-60). p.42.

Atiq M, Adil S, Rajput NA, Sahi ST, Usman M, Iqbal S, Chand SA, Nawaz A, Arif AM, Akram A and Shahbaz H. 2021. Activation of *Capsicum annuum* L. Defense System against Fusarium Wilt through Plant Activators and their Impact on Horticultural Attributes. *International Journal of Phytopathology*. 10(3). pp.155-166.

Baba ZA, Padder SA, Bhat MA, Wani SA, Mohiddin FA, Hamid B, Rizvi G, Bhat KA, Hamid A and Alam A. 2014. Incidence of Fusarium wilt of chilli (*Capsicum annum L.*) in Kashmir valley and its management by *Trichoderma* spp. *Myco- phytopathological Society of Pakistan.* 12(1).

Bai AT, Ruth C, Gopal K, Arunodhayam K, Priya BT and Ramakrishna M. 2018. Survey and Identification of Fusarium Wilt Disease in Chilli (*Capsicum annuum* L.). *International Journal of Current Microbiology and Applied Sciences* .7.pp.1073-1078.

Black L and Rivelli V. (1990). Fusarium wilt of pepper in Louisiana. In E. K. Delaware & S. A. Johnson (Eds.). Proceeding 10th National Pepper Conference. June 25-27. University of Delaware. Wilmington (p. 44).

Booth C. 1971. The Genus Fusarium (Commonwearth Mycological Institute).

Bose T K, Kabir J, Maity T K, Parthasarthy VA and Som M G. 2002. Vegetable Crops. Naya Prakash Publishers, Calcutta. pp.168-204.

Boseland PW and Votava NP. 2006. Peppers, vegetables,and spices capsicum. P.198. Centre for Agriculture and Bioscience International publishing, New York.

Buurlage M, Budai-Hadrian O, Pan Q, Carmel-Goren L, Vunsch R, Zamir D and Fluhr R.. 2001. Genome-wide dissection of Fusarium resistance in tomato reveals multiple complex loci. *Molecular Genetics and Genomics*. 265. pp.1104-1111.

Cabral CS, Gonçalves AM, Fonseca M, Urben AF, Costa H, Lourenco Jr, Boiteux LS and Reis A. 2020. First detection of *Fusarium oxysporum* f. sp. *radicis–lycopersici* across major tomato–producing regions in Brazil. *Phyto parasitica*. 48(4). pp.545-553.

Carballas T and Acea MJ. 1992. Seasonal fluctuations in microbial populations and available nutrients in forest soil. *Biology Fertility Soils* 16: 198-204.

Cerkauskas RF. 2017. Etiology and management of Fusarium crown and root rot (*Fusarium oxysporum*) on greenhouse pepper in Ontario, Canada. *Canadian Journal of Plant Pathology* 39:121-32.

Cha SD, Jeon YJ, Ahn GR, Han JI, Han KH and Kim SH. 2007. Characterization of *Fusarium oxysporum* isolated from Paprika in Korea. *Mycobiology* 35:91-96.

Chadha KL. 2003. *Capsicum*. Hand book of horticulture. *Indian agriculture research institute* (IARI), New Delhi. pp. 368-71.

Chauhan A, Kumar P and Sood A. 2021. Status of Bacterial Wilt (*Ralstonia Solanacearum*) of Solanaceous Vegetables in Himachal Pradesh. *Himachal Journal of Agricultural Research* 46:216-20.

Chowdhury SK, Majumdar S, Mandal V. 2020. Application of *Bacillus* sp. LBF-01 in *Capsicum annuum* plant reduces the fungicide use against *Fusarium oxysporum*. *Biocatalysis and Agricultural Biotechnology*. 01714

Cucu MA, Gilardi G, Pugliese M, Gullino ML and Garibaldi A. 2020. An assessment of the modulation of the population dynamics of pathogenic *Fusarium oxysporum* f. sp. *lycopersici* in the tomato rhizosphere by means of the application of *Bacillus subtilis QST* 713. *Trichoderma* sp. TW2 and two composts. *Biological Control. 142*. p.104158.

Devi P, Prabhakaran N, Kamil D, Pandey P and Borah JL. 2012. Characterization of Indian native isolates of *Trichoderma* spp. and assessment of their bio-control efficiency against plant pathogens. *African Journal of Biotechnology*. *11*(85). pp.15150-15160.

Directorate of Agriculture. 2021-22. https://www.hpagriculture.nic.in

Dukare A. and Paul S. 2021. Biological control of Fusarium wilt and growth promotion in pigeon pea (*Cajanus cajan*) by antagonistic rhizobacteria, displaying multiple modes of pathogen inhibition. *Rhizosphere*. 17. p.100278.

EARO (Ethiopian agricultural research organization). 2004. Plant protection research strategy. EARO. Addis Ababa.

Endriyas G. 2019. Distribuation of hot pepper fusarium wilt (*Fusarium oxysporum* f.sp. *capsici*) and evaluation of hot resistance and biocontrol agents against the pathogen in the

central rift valley of Ethiopia (Msc thesis, Melkassa Agriculture research Centre, Ethiopian institute of agriculture research, adama, Ethiopia).

Fadhal FA, Abedy AN and Alkhafije DA. 2019. Isolation and molecular identification of *Rhizoctonia solani* and *Fusarium solani* isolated from cucumber (*Cucumis sativus* L.) and their control feasibility by *Pseudomonas fluorescens* and *Bacillus subtilis*. *Egyptian Journal of Biological Pest Control*, *29*. pp.1-11.

Fentahun G, Kibret M, Stotaw B and Asrat A. 2022. Biocontrol effects of endophytic and rhizospheric bacteria against the root pathogen *Fusarium oxysporum* of hot pepper (*Capsicum annum L.*). *Vegetable Science*. 49(1). pp.75-85.

Ferniah RS, Daryono BS, Kasiamdari RS and Priyatmojo A. 2014. Characterization and pathogenicity of *Fusarium oxysporum* as the causal agent of Fusarium wilt in chilli (*Capsicum annuum* L.) *Microbiology Indonesia*; 8(3):121-126.

Food and Agriculture Organization (FAO). 2019. Area Harvested and Production. https://www.fao.org/faostat/en/# data/QC

Fravel D, Olivain C and Alabouvette C. 2003. *Fusarium oxysporum* and its biocontrol. *New phytologist*. 157(3). pp.493-502.

Fridlender M, Inbar J and Chet I. 1993. Biological control of soilborne plant pathogens by a β-1, 3 glucanase-producing *Pseudomonas cepacia*. *Soil Biology and Biochemistry*. *25*(9). pp.1211-1221.

Gabrekiristos E, Teshome D and Ayana G. 2020. Distribution and Relative Importance of Hot Pepper Fusarium Wilt (*Fusarium oxysporium* f. sp. *capsici*) and Associated Agronomic Factors in the Central Rift Valley of Ethiopia. *Advances in Crop Science and Technology*. 8:2p.

Gabrekiristos E and Ayana G. 2020. In vitro evaluation of some fungicides against *Fusarium oxysporum* the causal of wilt disease of hot pepper (*Capsicum annum L.*) in Ethiopia. *Advances in Crop Science and Technology*. 8. pp.443-449.

Girma A. 2022. In vitro biocontrol evaluation of some selected *Trichoderma* strains against the root pathogen *Fusarium oxysporum* of hot pepper (*Capsicum annum L.*) in Bure Woreda. Ethiopia. *International Journal of Microbiology*.

Gomez K A and Gomez A A. 1986. *Statistical Procedure for Agricultural Research*. 2nd ed. Wiley Eastern Ltd. New Delhi. 704p.

Gowda SD, Bhat SA, Kantharaju V, Nishani S, Abdul K and Hadimani HP. 2020. Pathogenic Variability and Fungicial Sensitivity of *Fusarium* spp. causing wilt in chilli in Karnataka. *International journalof current microbiology and applied sciences*. Vol. 9(10): 301-309.

Gupta CP, Dubey RC and Maheshwari DK. 2002. Plant growth enhancement and suppression of Macrophomina phaseolinacausing charcoal rot of peanut by fluorescent *Pseudomonas*. *Biology and Fertility of Soils*.35: 399-405.

Gupta SK and Thind TS. 2006. Disease problems in vegetable production. Scientific publishers(India) Ltd. Jodhpur. India. 335-380.

Hami AR, Khan NA, Mansoor S, Mir MA, Ahmed N and Masoodi KZ. 2021. Morpho-molecular identification and first report of *Fusarium equiseti* in causing chilli wilt from Kashmir (Northern Himalayas). *Scientific Reports* 11:1-14.

Kannan MN, Sethi S, Badoni A, Chamoli V and Bahuguna NC. 2018. Isolation and characterization of *bacterial* isolates from agriculture field soil of Roorkee region. *Journal of Pharmacognosy and Phytochemistry*. *7*(5S). pp.108-110.

Khan MF, Akram S, Khan SM, Khan HU, Tariq A, Umar UU and Gill A. 2018. Antifungal activity of different systemic fungicides against *Fusarium oxysporum F. sp. lycopersici* associated with tomato wilt and emergence of resistance in the pathogen. *Pakistan Journal of Phytopathology*, *30*(2). pp.169-176.

Kim SH, Lee Y, Balaraju K and Jeon Y. 2023. Evaluation of *Trichoderma a*troviride and *Trichoderma longibrachiatum* as biocontrol agents in controlling red pepper anthracnose in Korea. *Frontiers in Plant Science*. 14. p.1201875.

Kishore C and Kulkarni S. 2008. Management of Carnation Wilt Caused by *Fusarium oxysporum* F, Sp. Dianthi. *Journal of Plant Disease Sciences*. *3*(1). pp.17-20.

Kraft KH, Brown CH, Nabhan GP, Luedeling E, Ruiz JDJL, Eeckenbrugge GC, Hijmans RJ and Gepts P. 2014. Multiple lines of evidence for the origin of domesticated chili pepper (*Capsicum annuum*) in Mexico. *Proceedings of the National Academy of Sciences*.111: 6165-6170.

Kumar PR, Hemanth G, Niharika PS and Kolli SK. 2015. Isolation and identification of soil mycoflora in agricultural fields at Tekkali Mandal in Srikakulam District. *International Journal of Advances in Pharmacy, Biology and Chemistry*. *4*(2). pp.484-490.

Leonian LH. 1919. Fusarium wilt of Chilli Pepper. Techanical Bulletin No. 121. New Mexico Agricultural Experiment Station, Las Cruces, USA.

Lugtenberg BJJ, Dekkers L, Bloemberg GV. 2001. Molecular determinants of rhizosphere colonization by Pseudomonas. *Annual review of phytopathology*. 39. 461–490.

Madbouly AK, Boari A, Vurro M, Gebreel HM and Abouzeid MA. 2014. Biocontrol of *Rhizoctonia Solani* causing stem canker disease of potato using rhizosphere mold fungi. *International Journal of Agricultural Research*.4(6): 34-45.

Mamta J, Rashmi S, Anil KS and Anil P. 2012. screening of resistant varieties and antagonistic *Fusarium oxysporum* for biocontrol of fusarium wilt of chilli. *Plant pathology and microbiology.* 3(5). *Microbiology Indonesia*. 2014;8(3):121-126.

Morales GG, Francisco N, Ochoa Fuentes YM, Delgado Ortiz JC. and Hernandez Castillo FD. 2016. In vitro antagonism of *Bacillus* strains against *Fusarium* species. *Mycopath*, 14(1/2). pp.15-19.

Mushtaq M and Hashmi MH.1997. Fungi associated with wilt disease of Capsicum in Sindh, Pakistan. *Pakistan Journal of Botany*. 29(2). pp.217-222.

Naik MK, Devika Rani GS and Madhukar HM. 2008. Identification of resistance sources against wilt of chilli (*Capsicum annuum* L.) resistance caused by *Fusarium Solani* (Mart.) Sacc. *Journal of Mycopathological Research*. 46. 93-96.

Najar AG and Ahmad M. 2012. Spatial distribution of chilli wilt causing pathogens in Kashmir. *SKUAST Journal of Research*.14(1and2). pp.54-58.

Najar AG, Mushtaq A, Bhat MS and Bhat ZZ. 2006. Effect of various soil amendments on chilli wilt (*Fusarium pallidoroseum (Cooke) Sacc*). *Sher-e Kashmir University of Agricultural Sciences and Technology Journal of Research* 8:236-39.

National Horticulture Board (NHB). 2021-22. Area and Production of Horticulture Crops-All India. https://nhb.gov.in/

Nelson PE, Dignani MC and Anaissie EJ. 1994. Taxonomy, biology, and clinical aspects of *Fusarium species*. *Clinical Microbiology Reviews*, *7*(4), 479-504.

Paker CA, Ruvira AD, Moore KJ and Wong P. 1985. Ecology and management of soil-borne plant pathogens. *American Physical Society*, St. MN Paul.

Pandey AK, Samota MK, Tanti AJ and Babu A. 2023. *Trichoderma reesei* induces defense-related biochemical markers associated with resistance to *Fusarium* die back in tea crop. *Biological Control*. 180. p.105200.

Parihar TJ, Bhat ZA, Shah MD, Nehvi FA, Dhekale B, Bhat MA, Shah LR, Khan T and Bashir T. 2022. Studies on Fusarium wilt caused by *Fusarium oxysporum* in Solanaceous crops.

Passari AK, Lalsiamthari PC, Zothanpuia Leo VV, Mishra VK, Yadav MK, Gupta VK and Singh BP. 2018. Biocontrol of Fusarium wilt of *Capsicum annuum* by rhizospheric bacteria isolated from turmeric endowed with plant growth promotion and disease suppression potential. *European Journal of Plant Pathology*. 150. pp.831-846.

Perez-Hernandez A, Serrano-Alonso Y, Aguilar-Pérez MI, Gómez-Uroz R and Gómez-Vázquez J. 2014. Damping-off and root rot of pepper caused by *Fusarium oxysporum* in Almería province. Spain. *Plant disease*. 98(8). pp.1159-1159.

Raghu S, Benagi VI and Nargund VB. 2016. Cultural, morphological and pathogenic variability among the isolates of *Fusarium Solani* causing wilt disease of Chilli (*Capsicum annuum* L.). *Journal of Pure and Applied Microbiology*. Shahjahanabad. 10(1). pp.599-604.

Ramezani H. 2008. Efficacy of some fungal and bacterial bioagents against *Fusarium oxysporum* f. sp. *ciceri* on chickpea. *Journal of Novel Researches on Plant Protection*.1(1). pp.108-113.

Rubio JS, Garcia Sandez F, Florers P, Navarro JM and Martinez V. 2010. Yield and fruit quality of sweet pepper in response to fertilization with Ca^{2+} and K^{+}. *Spanish Journal of Agricultural Research* 8:170-77.

Sahi IY and Khalid AN. 2007. In vitro biological control of *Fusarium oxysporum*, causing wilt in Capsicum annuum. *Myco- phytopathological Society of Pakistan*. 5. 85-88.

Sahi IY. 2012. In vitro biological control of Fusarium oxysporum-causing wilt in Capsicum annuum. *Mycopath*. 5(2).

Sang MK, Oh JY and Kim KD. 2007. Root-dipping application of antagonistic rhizobacteria for the control of phytophthora blight of pepper under field conditions. *The Plant Pathology Journal*. 23. 109–112.

Shaheen N, Khan UM, Azhar MT, Tan DK, Atif RM, Israr M, Yang SH, Chung G and Rana IA. 2021. Genetics and genomics of Fusarium wilt of chilies: A review. *Agronomy*.11(11), p.2162.

Shanmugam V and Kanoujia N. 2011. Biological management of vascular wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycospersici* by plant growth-promoting rhizobacterial mixture. *Biological control*. *57*(2). pp.85-93.

Sharma A, Shukla A and Gupta M. 2023.Effect of bioagents on cucumber seed mycoflora, seed germination and seedling vigour. *Scientific reports*. 13:6053.

Sharma A. 2021. Studies on root rot of cucumber (MSc thesis. Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal pardesh, India.

Sharma A. 2022. Studies on status of wilt diseases in capsicum. MSc Thesis. Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal pardesh, India.

Sharma D. 2019. Studies on Fusarium wilt of cucumber (*Cucumis sativus* L.). MSc Thesis. Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal pardesh, India.

Sharma M, Attri K and Gupta SK. 2018. Influence of edaphic factors on Fusarium wilt of bell pepper. *International Journal of Bio-resource and Stress Management*. 9(5). pp.606-610.

Singh JK. 2016. Pathogenic Variability and Management of Fusarium Wilt of Chilli (*Capsicum annuum* L.) Plant Pathology, CCS HAU Hisar.

Singh S, Balodi R, Meena PN and Singhal S. 2021. Biocontrol activity of *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* against Meloidogyne incognita, *Fusarium oxysporum* and Rhizoctonia Solani. *Indian Phytopathology*, 74(3), pp.703-714.

Singh US and Zaidi NW. 2002. Current Status of formulation and delivery of fungal and bacterial antagonists for disease management in India. In R. J. Rabindra, S. S. Hussaini and B. Ramanujam (Eds.). *Microbial Biopesticide Formulations and Application* (pp. 168-179). Project Directorate of Biological Control, Bangalore.

Subash N, Meenakshisundaram M, Sasikumar C and Unnamalai N. 2014. Mass cultivation of *Trichoderma harzianum* using agricultural waste as a substrate for the management of damping off disease and growth promotion in chilli plants (*Capsicum annuum* L.). *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(5). pp.188-192.

Sundaramoorth S, Raguchander T, Ragupathi N and Samiyappan R. 2012. Combinatorial effect of endophytic and plant growth promoting rhizobacteria against wilt disease of *Capsicum annum L.* caused by *Fusarium Solani*. *Biological Control*. 60(1). pp.59-67.

Swain MR and Ray RC. 2009. Biocontrol and other beneficial activities of *Bacillus* subtilis isolated from cowdung microflora. *Microbiological research*. 164(2). pp.121-130.

Tapwal A, Thakur G, Chandra S and Tyagi A. 2015. In-vitro evaluation of *Trichoderma* species against seed borne pathogens. *International Journal of Chemical and Biological Science research paper*.1(10). pp.14-19.

Teixeira LM. 2015. *Caracterização de isolados de Fusarium oxysporum e resistência de genótipos de Passiflora à fusariose.*

Trotel-Aziz P, Couderchet M, Biagianti, S and Aziz A. 2008. Characterization of new bacterial biocontrol agents *Acinetobacter*, *Bacillus*, *Pantoea* and *Pseudomonas* spp. mediating grapevine resistance against Botrytis cinerea. *Environmental and Experimental Botany*. 64(1). pp.21-32.

Velarde-Felix S, Garzon-Tiznado JA, Hernandez-Verdugo S, Lopez-Orona CA and Retes-Manjarrez JE. 2018. Occurrence of *Fusarium oxysporum* causing wilt on pepper in Mexico. Canadian Journal of Plant Pathology. 40(2). pp.238-247.

Venkataramanamma K, Reddy BV, Jayalakshmi RS, Jayalakshmi V and Rajendran L. 2022. Isolation, in vitro evaluation of Bacillus spp. against *Fusarium oxysporum* f. sp. *ciceris* and their growth promotion activity. *Egyptian Journal of Biological Pest Control*. 32(1). pp.1-8.

Vincent JM. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*. *159*(4051). pp.850-850.

Wongpia A and Lomthaisong K. 2010. Changes in the 2DE protein profiles of chilli pepper (*capsicum annum*) leaves in response to *Fusarium oxysporum* infection. *Science asia*. 36. 259-270.

Yu X, Ai C, Xin L and Zhou G. 2011. The siderophore-producing bacterium, *Bacillus subtilis CAS15*, has a biocontrol effect on Fusarium wilt and promotes the growth of pepper. *European Journal of Soil Biology*, 47(2). pp.138-145.

Zhao D, Zhao H, Zhao D, Zhu X, Wang Y, Duan Y, Xuan Y and Chen L. 2018. Isolation and identification of bacteria from rhizosphere soil and their effect on plant growth promotion and root-knot nematode disease. *Biological control*. 119. pp.12-19.

APPENDIX-I

Culture media used for growing microorganism

1. Potato dextrose agar (PDA) media

Peeled potato	: 250 gm
Dextrose	: 20 gm
Agar	: 20 gm
Distilled water	: 1000 ml

2. Nutrient Broth (NB) Media

Beef extract	: 3 gm
Peptone	: 5 gm
NaCl	: 5 gm
Distilled water	: 1000 ml

3. Nutrient Agar (NA) Media

Beef extract	: 3 gm
Peptone	: 5 gm
NaCl	: 5 gm
Agar	: 15 gm
Distilled water	: 1000 ml

4. Pseudomonas media

Peptone	: 20 gm
Magnesium chloride	: 1.4 gm
Potassium sulfate	:10 gm
Agar-agar	:12.6 gm
Distilled water	: 1000 ml

Also, to be added glycerol : 10.0 ml

5. Bacillus Selective Agar media

Enzymatic Digest of Casein	: 10.0
Meat Extract	: 1.0
D-Mannitol	: 10.0
Sodium Chloride	: 10.0
Phenol Red	: 0.025
Agar	: 14.0
Distilled water	: 1000 ml

Final pH 7.2 ± 0.2

APPENDIX-II

ANOVA 1: Analysis of variance for effect of Trichoderma bioagent against *Fusarium* oxysporum isolates (Table 8)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	8	1,146.731	143.341	12.136	0.00001
Error	18	212.603	11.811		
Total	26	1,359.333			

ANOVA 2: Analysis of variance for effect of Trichoderma bioagent against *Fusarium oxysporum* isolates (Table 8)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	8	1,434.992	179.374	8.022	0.00013
Error	18	402.501	22.361		
Total	26	1,837.493			

ANOVA 3: Analysis of variance for effect of Trichoderma bioagent against *Fusarium oxysporum* isolates (Table 8)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	8	1,905.751	238.219	18.971	0.00000
Error	18	226.026	12.557		
Total	26	2,131.776			

ANOVA 4: Analysis of variance for effect of bacterial bioagent against *Fusarium oxysporum* (Isolate 4) (Table 9)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	6	1,709.242	284.874	4.101	0.01389
Error	14	972.511	69.465		
Total	20	2,681.753			

ANOVA 5: Analysis of variance for evaluation of bacterial bioagent against *Fusarium oxysporum* (Isolate 4) under pot culture condition (Table 10)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	6	17,440.355	2,906.726	810.486	0.00000
Error	14	50.210	3.586		
Total	20	17,490.564			

ANOVA 5: Analysis of variance for evaluation of bacterial bioagent against *Fusarium oxysporum* (Isolate 4) under pot culture condition (Table 10)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	6	9,353.729	1,558.955	3,758.538	0.00000
Error	14	5.807	0.415		
Total	20	9,359.535			

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ABSTRACT

Wilt caused by *Fusarium oxysporum* is one of the devastating diseases of bell pepper in Himachal Pradesh. The present investigation entitled **"Studies on variability and management of Fusarium wilt of capsicum"** was carried out during the cropping season 2022 and 2023 with the objectives to record the status of the disease, cultural and morphological characterization of isolates, isolation of biocontrol agents from the rhizosphere of capsicum, their characterization and to test their efficacy against the pathogen under *in vitro* and pot culture conditions. Survey studies of Solan district of H.P. revealed that the wilt disease occurred in moderate to severe form with disease incidence varying from 9.00 to 24.50 per cent under open field conditions while, under polyhouse conditions it varied from 6.22 to 38.88 percent. Symptoms of disease appeared as yellowing of leaves followed by epinasty (downward drooping) of older leaves and browning of the vascular tissue leading to completely death of plant by wilting. Based on cultural and morphological characterization, the pathogen found associated with the wilt disease was identified as *Fusarium oxysporum*. Among the two methods of proving pathogenicity, soil inoculation by mass culture was found more effective having incubation period of 18 days. Biocontrol agents (*Trichoderma* spp., *Bacillus* spp. and *Pseudomonas* spp.) were isolated from rhizosphere soil of healthy capsicum plants by using serial dilution method. Among fungal bioagents, *Trichoderma* sp. 3 was found the most effective in inhibiting the mycelial growth (73.71%) in dual culture method. Whereas, in case of bacterial biocontrol agents, *Bacillus* sp. 1 was found effective during *in vitro* evaluation. Evaluation of biocontrol agents against Fusarium wilt of capsicum under pot culture condition revealed that *Trichoderma* sp. 3 was most effective and exhibited highest disease control (83.33%), while *Bacillus* sp. 1 showed 50.00 percent disease control against isolate 4 of *Fusarium oxysporum*.

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