

**“STUDIES ON WILT OF GREEN GRAM [*Vigna radiata* (L.)
wilczek] INCITED BY *Fusarium oxysporum*”**

THESIS

Submitted in partial fulfilment of the requirements
for the Degree of

**MASTER OF SCIENCE
IN
AGRICULTURE
(PLANT PATHOLOGY)**

By
DIVKAR PURVA MOHAN
(ADPM/21/2803)

DEPARTMENT OF PLANT PATHOLOGY,
COLLEGE OF AGRICULTURE, DAPOLI



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NOVEMBER, 2023

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Under the Guidance of
Dr. P. D. POTPHODE
Assistant Professor,
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DEPARTMENT OF PLANT PATHOLOGY,
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NOVEMBER, 2023

DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of the Thesis entitled " **STUDIES ON WILT OF GREEN GRAM [*Vigna radiata* (L.) wilczek] INCITED BY *Fusarium oxysporum* "** or part thereof has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/publication of any University or scientific organization. The source of materials used and all assistance received during the course of investigation have been duly acknowledged and that no part of the thesis has been submitted for any other degree or diploma.

Place: Dapoli

Date: 16/02/2024



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CERTIFICATE

This is to certify that the thesis entitled, “**Studies on wilt of green gram [*Vigna radiata* (L.) wilczek] incited by *Fusarium oxysporum*”** submitted for the degree of M.Sc. (Agri.) in Plant Pathology, of the College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli is a bonafide research work carried out by **Ms. Divkar Purva Mohan (ADPM/21/2803)** under my supervision and that no part of this thesis has been submitted for any other degree. The student had completed all the Course and Research as per the norms in regular mode and submitted one research paper from her M.Sc. work.

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

Place: Dapoli

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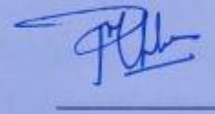
This is to certify that the thesis entitled, "Studies of wilt of green gram [*Vigna radiata* (L.) wilczek] incited by *Fusarium oxysporum*" submitted by Ms. Divkar Purva Mohan (ADPM/21/2803) to the College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, in partial fulfillment of the requirements for the degree of M.Sc.(Plant Pathology) in the subject of Plant Pathology, having Principles of Plant Physiology, Breeding for biotic and abiotic stress resistance and Principles of Biotechnology as minor subject of Department of Agricultural Botany and Department of Biotechnology, respectively have been approved by student's advisory committee, board of studies of the department and evaluated by one External Examiner after an open *Viva voce* examination in the presence of external examiner on the same held on dated 18 / 02 / 2024

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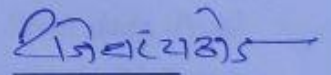


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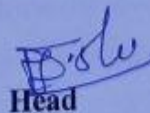
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Place: Dapoli

Date: 16 / 02 / 2024



(Miss. Divkar Purva Mohan)

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ABBREVIATIONS USED

%	:	Per cent
/	:	Per
@	:	At the rate of
<i>Spp.</i>	:	Species
°C	:	Degree Celsius
C.D.	:	Critical difference
CRD	:	Completely Randomized Design
CV	:	Coefficient of variation
cm	:	Centimeter
Conc.	:	Concentration
d.f.	:	Degree of freedom
Dist.	:	District
Dr.B.S.K.K.V.	:	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth.
E.M.S.	:	Error mean sum of squares
et al.	:	And others
etc.	:	Etcetera
hrs.	:	Hour(s)
Fig.	:	Figures
g	:	Gram(s)
i.e.	:	That is
Kg	:	Kilogram
m	:	Meter
M.S.S.	:	Mean sum of squares
mg	:	Milligram
Lit.	:	Liter
P.D.A.	:	Potato Dextrose Agar
ppm	:	Parts per million
S.E.	:	Standard error
Tr./T	:	Treatment
<i>viz.</i>	:	Namely
w/w	:	Weight / weight
Sig.	:	Significant
BE	:	Biological Efficiency
RH	:	Relative Humidity
SS	:	Sum of squares
Sr.	:	Serial
&	:	And
No.	:	Number
PDI	:	Plant Disease Incidence

CHAPTER I : INTRODUCTION

1.1 Background information:

Green gram scientifically known as [*Vigna radiata* (L.) Wilczek] belongs to family leguminaceae and commonly called as mung bean. India is its primary origin and is mainly cultivated in South east Asia. It is the third important pulse crop of India grown in nearly 16 percent of the total pulse area of the country. Green gram is grown in about 4.5 million hectares with the total production of 2.5 million tonnes with a productivity of 548kg/ha and contributing 10% to the total pulse production (Anonymous, 2021). Important green gram producing states in the country are Rajasthan, Maharashtra, Andhra Pradesh, Madhya Pradesh and Bihar.

The mung bean is a warm-season, frost-sensitive plant. Mung bean can be grown in temperate, subtropical, and tropical climates. Mung bean germination and growth are best at a temperature of 15 to 18 °C. It is quite adaptable to several types of soil, and the ideal pH range for soil is between 6.2 and 7.2. Long days will hinder the blossoming and podding of the mung bean plant because it prefers short days. Mung bean is a legume plant that works in symbiosis with Rhizobia to fix atmospheric nitrogen (58–109 kg per hectare mung bean). It may supply the soil with significant levels of nitrogen (30 to 251 kg/ha) and biomass (7.16 t biomass/ha). It can meet its own nitrogen needs as it has its ability to fix nitrogen, and subsequent crops will also have benefit from it. It can be grown as a cover crop either before or after cereal crops in a rotation, making it an excellent green manure.

Mung beans are renowned for having a high nutritive value. They are rich in protein, fat, vitamins, and minerals and comprise between 55% to 65% of their dry weight in carbohydrates (about 630 g/kg). Its protein content ranges from 20% to 50% of its total dry weight, with albumin (25%) and globulin (60%) serving as the two main storage proteins. The mung bean is regarded as a significant source of dietary proteins. Even more of these proteins are cleaved by proteases during sprouting. Comparatively to other types of legumes, the carbohydrates in mung beans are easily absorbed by humans, which results in less flatulence. Mung bean seeds and sprouts have fewer calories than other cereals, making them more palatable to overweight and diabetic people. Whole beans are frequently boiled to create a dry dish that is frequently eaten with congee in Karnataka, Maharashtra, Gujarat, Kerala, and Tamil Nadu. Mung beans that have been hulled can be used to make sweet soups in a similar way as whole beans. To prepare mung dal, mung beans are peeled of their outer covering in several Indian regional cuisines. The stripped and split bean is used to make mug dal, a dal that resembles soup, in Bangladesh and West Bengal. Steamed whole beans are seasoned with spices and freshly grated coconut in the South Indian states of Karnataka, Tamil Nadu, Telangana, and Andhra Pradesh, as well as in Maharashtra.

There are hundreds of cultivars of green gram cultivated throughout the world. Some of the important green gram cultivars cultivated in India are MH 2-15 (Sattya), KM 2241 (Sweta), Pant Mung 6, Trombay Pesara (TM 96-2), IPM 4103 (Shikha), Pusa 1371, PKV Green gold (AKM 9911), Jalgaon 781, Kopergaon, Pusa Baisakhi, PS 16, ML 131, Pusa 105, TAP 7, ML 337, PDM 11, TMB-37, Phule M-2, Vaibhav.

1.2 Importance of study:

The crop is known to be infected with various fungal, bacterial and viral diseases as mentioned below:

Table no. 1: Diseases of Green gram

Sr.No	Disease	Causal Organism
Fungal Diseases		
1.	Powdery Mildew	<i>Erysiphe polygoni</i>
2.	Anthracnose	<i>Colletotrichum lindemuthianum</i>
3.	Dry Root Rot	<i>Rhizoctonia bataticola</i>
4.	Rust	<i>Uromyces phaseoli</i>
5.	<i>Cercospora</i> leaf spot	<i>Cercospora canascens</i>
6.	Wilt	<i>Fusarium oxysporum</i>
Viral Diseases		
1.	Yellow mosaic disease	<i>Mungbean yellow mosaic virus</i> (MYMV)
2.	Leaf crinkle disease	<i>Urdbean leaf crinkle virus</i> (ULCV)
Bacterial Diseases		
1.	Bacterial leaf blight	<i>Xanthomonas campestris pv. phaseoli</i>

Among these various diseases *Fusarium* wilt is also one of the diseases which cause losses in the crop. The symptoms of the disease are slight vein clearing on the outer portion of the young leaves, followed by epinasty (downward drooping) of leaves. At seedling stage, plant get infected by *Fusarium oxysporum* may wilt and die soon after symptoms appear. In older plants, vein clearing and leaf epinasty are often followed by stunting, yellowing of the older leaves, formation of adventitious roots, wilting of leaves and drying of young stems, defoliation, marginal necrosis of remaining leaves and death of the plant. Often these symptoms appear on only one side of the stem and progress upward until the foliage is killed and the stem dies. Pods may occasionally become infected. Roots also become infected after an initial period of stunting.

In cross sections near the base of the infected plant stem, a brown ring is evident in the area of the vascular bundles (Agrios, 1988). The upward extent of the discoloration depends upon the severity of the disease. In Konkan region of Maharashtra, farmers are growing this crop under field conditions for many years. The crop is found to be affected by various diseases like powdery mildew, anthracnose, *Cercospora* leaf spot, MYMV. However, wilt caused by *Fusarium* spp. is the minor disease triggering hurdle in cultivation of green gram in Konkan region. Considering the losses and regular incidence of *Fusarium* in recent past years created interest to conduct this study with following objectives

1.3 Objectives of study:

1. Isolation and pathogenicity of the fungus causing wilt disease in green gram.
2. To study cultural and morphological characteristics of fungus causing wilt disease
3. To study *in vivo* (pot) cross infectivity potential of test fungus.
4. Integrated management of *Fusarium* wilt disease.

1.4 Hypothesis:

The premise of the current study is that green gram, the most significant commercial pulse crop, is what farmers in Maharashtra are growing. However, a significant barrier to successful green gram cultivation is the wilt disease brought on by *Fusarium*. Therefore, it is essential to search the best methods for managing the disease through an integrated approach. The usage of fungicides in plant disease control will be reduced to the absolute minimum through an integrated approach. This will assist farmers in promoting the adoption of environment friendly and sustainable disease management techniques, reduce losses brought on by the severe prevalence of the wilt disease, and ultimately increase the production of the green gram crop.

1.5 Scope and limitations:

Among the fungal diseases, wilt caused by *Fusarium*, is a significant barrier to the effective production of green gram in the Konkan region of Maharashtra as well as other parts of India. According to a review of the literature, there are many different control methods used to prevent *Fusarium* wilt of green gram, including fungicides, botanicals, resistance sources against pathogens, bio-agents and integrated management strategies. The study was carried out in the Department of Plant Pathology and at Experimental farm, Department of Agronomy, College of Agriculture, Dr. B. S. K. V., Dapoli, using all the accepted practices and methodology. This study will give the farmer the most trustworthy, genuine and effective techniques needed for

controlling and minimizing the occurrence of the wilt disease on green gram and the significant losses it causes.

CHAPTER II : REVIEW OF LITERATURE

1. Isolation and pathogenicity of the fungus causing wilt disease in green gram.

Ferniah *et al.* (2014) isolated *Fusarium oxysporum* from vascular browning of chilli plants. The stem was cut on the border of brown vascular and healthy vascular. Five millimeter slice of the stems were surface sterilized in 70% ethanol for one minute, rinsed in sterile water and dried with tissue, and grown on the Potato Dextrose Agar (PDA). The cultures were incubated at 25 °C with 12 h dark and 12 h light cycle for 2 days. Each colony was transferred into new PDA and incubated for 5 days to get pure culture of isolate. The fungus was grown in Potato Dextrose Broth (PDB) for 4 days. Conidia densities were calculated with haemocytometer and adjusted to 10 conidia/ml. The inoculum was inoculated in chilli plant by root dip method. One month old of healthy chilli plants were taken from the soil. The roots were rinsed in water, soaked in 1% Chlorox for 1 minute, rinsed with sterilized water, and then soaked in fungal suspension for 30 minutes. This treatment was done for 10 plants. Roots of healthy plant were soaked in sterilized water was done as control. Each plant was planted in sterile soil in polybags. Disease symptoms were observed every odd day after inoculation (DAI).

Wani *et al.* (2014) carried out isolation of *Fusarium* from infected root and shoot samples of chilli. The chilli samples were disinfected by dipping in sterilized distilled water until all the dust particles were removed and dried with sterilized blotting paper. Diseased pieces of chilli samples were separated and cut into small pieces (2-5 mm), surface sterilized in 1% mercuric chloride up to 40 seconds, rinsed three times in sterilized distilled water and dried on sterilized blotting paper. The pieces were placed aseptically to a Petri-plates containing solidified potato dextrose agar (PDA) with 5% lactic acid to inhibit bacterial growth. The inoculated plates were incubated in an incubator at 25 ± 2 °C. The fungal colonies developed in the plates were sub cultured on PDA slants. The fungi developed on slants were examined microscopically to identify and isolate the pure culture of *Fusarium* spp. The established pure cultures of *Fusarium* spp. were multiplied on maize seeds mixed with sand. First the seeds were transferred to conical flasks of 500 mL capacity. The flasks were autoclaved twice for 15-20 minutes. Thereafter, the flasks were inoculated with the pure culture of *Fusarium* spp. and incubated for 8-10 days in an incubator at 25 ± 2 °C. During incubation, the flasks were shaken manually for a few minutes daily for uniform colonization of seeds. The inoculum so prepared was incorporated in pots containing sterilized soil mixed with vermicompost. The pots were mixed thoroughly. Three replicates were maintained and in each pot surface sterilized plantlets were grown. The pots were irrigated with tap water

regularly to maintain adequate moisture. After 15 days plants were observed for wilt disease symptoms.

Soliman *et al.* (2016) isolated *Fusarium oxysporum* from diseased mung bean plants showing different degrees of wilt disease. The affected roots were washed and cut into small portions. The cut pieces were immersed in Sodium hypochlorite 5% for 1 min and washed with distilled water. The surface sterilized pieces were dried between two sterilized filter papers and were directly placed into Petri dishes containing PDA medium and incubated at 28⁰C for 3-5 days. They also proved the pathogenicity test, firstly earthen pots were sterilized by immersing them in 5% formalin and covered overnight with plastic sheets, then left to dry in open air. Soil sterilization was carried out by autoclaving. Susceptible mung bean seeds were surface sterilized with 1% sodium hypochlorite which were washed with several changes of distilled water and dried. Such seeds were used for sowing. Bottles containing sand-barley medium were autoclaved and were inoculated with the fungal isolate. After incubation at 28⁰C for 2 weeks the fungus was thoroughly mixed with sterilized soil @1g inoculum per 100g of soil. Inoculated pots were watered for a week before planting. Pots used for control were filled with same soil without inoculum. Disease incidence was recorded, after 30 days of inoculation.

Choudhary *et al.* (2017) using taxonomic and morphological references identified isolated pathogen as *Fusarium oxysporum f.sp. vigni*. The infected diseased samples along with healthy tissues were cut into small pieces and were surface sterilized by dipping in 0.1% sodium hypochloride solution for two minutes, the treated plant tissues were washed three times with sterilized distilled water. Excess water was decanted by soaking with sterilized blotting paper. The cut pieces were then placed onto sterilized dextrose agar (PDA) medium in Petri dish (20ml/Petri dish) and incubated at 28_±1⁰C for three days for mycelium formation in Petri dish.

Golakiya *et al.* (2018) Fifteen isolates of *Fusarium oxysporum f. sp. ciceri* causing chickpea wilt were screened to test the pathogenicity. Inoculum of each isolate was prepared on half boiled sorghum media and incubated at 28⁰C for 10 days. These inoculums were used for soil inoculation @40g/kg soil. One set of pot was filled with only sterilized uninoculated soil as a control. Wilt susceptible variety seeds were sown and observed until development of symptoms. As symptoms appeared, fungus was re-isolated from the roots.

Aslam *et al.* (2019) isolated wilt causing pathogen *Fusarium oxysporum* from root samples showing vascular discoloration. Root sections (1-2 cm long) of pea plant were rinsed with distilled water. After surface sterilization with 5% sodium hypochlorite (for 2 min), pieces were thoroughly rinsed two times with double distilled water. Root sections were dried on sterilized filter paper and transferred on potato dextrose agar containing Petri plates. The plates were incubated for 7 days at 24-25⁰C. Pure fungal isolates were obtained by transferring agar plugs to freshly prepared PDA

plates. Pathogenicity test: A susceptible pea variety (Alina) was selected for further experiments. The seeds of pea were surface sterilized with 2% sodium hypochlorite (for 2 min) following by two washings with sterilized distilled water. *In vitro* and *in vivo* experiments were conducted to perform pathogenicity assay. In plate assay, seeds were grown on moistened sterilized filter paper. After seedling germination, 2 mm mycelia plug of pathogen was provided as inoculum. Disease incidence was recorded, after 7 days of inoculation. During pot assay, sterilized pea seeds were sown in sterilized soil. When plants reached at 3-4 leaflets, 5 mm mycelia plug of 7 days old pathogen culture was inoculated at two opposite corners of pot. The disease incidence was scored after fifteen days of inoculation.

Avinash *et al.* (2019) collected wilt affected plants from Zonal Agricultural Research Station, GKVK, Bengaluru. Plant samples were collected based on visual observation of wilt symptoms (yellowing of leaves, partial or complete drying of plant and dark coloration of xylem of plant when stem/root slit into half vertically). Wilted red gram samples were collected in a paper envelope and brought to the laboratory. The pathogen was isolated by direct culturing of infected parts with minor modifications. Briefly, the infected roots were washed with running tap water to remove all adhering soil particles, and then cut into small pieces prior to surface sterilization using 96% ethanol for 30s. All the sterilized pieces were placed onto potato dextrose agar (PDA) plates. Plates were incubated under the standard incubation conditions for 48h and the resulting single-spores of *Fusarium* colonies were transferred to fresh potato dextrose agar (PDA) plates for further studies. The species were identified on the basis of macroscopic and microscopic characteristics such as, pigmentations of colony, types of conidiogenous cells, shape and size of conidia.

K. Venkataramanamma *et al.* (2019) conducted an experiment on *Fusarium* wilt of chickpea. *Fusarium* wilt affected chickpea plants collected from six districts of Andhra Pradesh and three districts of Telangana were used for isolation of pathogen by using tissue segment method and pure cultures were obtained by single spore culture method. The isolates were preliminarily identified as *Fusarium* by a key provided by Booth (1971), which included microscopic studies. After isolation, the isolates were tested for pathogenicity test to prove Koch's postulates on susceptible cultivar JG-62 by using soil infestation method to confirmed it as *Fusarium oxysporum f.sp. ciceris*. Three replications were maintained within plants per pot. In each pot number of plants wilted and days required for wilting was recorded. Based on wilting percentage, they were divided into three pathogenic groups. These groups were, weakly pathogenic (upto 30% wilt incidence), moderately pathogenic (>30 to 50% wilt incidence) and highly pathogenic (>50% wilt incidence).

Sun *et al.* (2019) reported *Fusarium oxysporum* as a causal agent of mung bean wilt in China. The stems of the diseased plants were dissected to inspect the vascular discoloration. The discoloured stems were washed under tap water and cut into several 2-3 mm thick segments. After being surface sterilized in 2% Sodium hypochloride solution for 2 min, tissues were rinsed with sterilized distilled water three times before being placed on potato dextrose agar (PDA)

supplemented with lactic acid. Colonies resembling *Fusarium oxysporum* were purified by single spore isolation.

Vani *et al.* (2019) selected mung bean samples showing distinct wilt characteristics for isolation of pathogen. The selected roots, collar region and stem were washed and sterilized with 0.1% mercuric chloride solution in laminar air flow followed by washing 3 to 4 times with sterilized water and were placed in blotting paper to remove excess moisture. The pieces were transferred to Petri plates containing potato dextrose agar medium with the help of sterilized needle. Three to four pieces of diseased parts of mung bean were placed equidistant from each other and plates were kept at $28 \pm 2^{\circ}\text{C}$ for 7 days in an incubator for further mycelial growth. Colonies resembling *Fusarium oxysporum* were purified by single spore isolation.

Biswal *et al.* (2020) isolated fungus *Fusarium oxysporum* causing wilt of black gram using PDA medium taking affected tissue of cut piece measuring 0.5-1 cm. Profuse mycelial growth of fungus was recorded on potato dextrose agar medium and sporulation was also found maximum with 8 days of incubation at $25 \pm 2^{\circ}\text{C}$ in the BOD incubator.

Ayele *et al.* (2021) isolated *Fusarium* wilt pathogen following the tissue transplanting technique. The tomato plant parts were washed with running tap water to remove soil and dust from the tissues; followed by the stem and root samples cut into 5 mm-10 mm sized slices using sterile scissors and surface sterilized using 96% ethanol for 1 minute. The surface disinfected tissues are then washed with three successive changes of sterilized distilled water to washout the ethanol and were blot dried using Whatman Number 1 filter papers. Blot dried tissues were placed onto 10 cm glass Petri dishes containing the solidified potato dextrose agar (PDA) medium. The plates were then incubated at $25 \pm 2^{\circ}\text{C}$ for seven days with periodic checks for the emergence of fungal mycelia from the transplanted sample of tomato tissues. Fungal hyphae emerging on the transplanted tissues were transferred onto the fresh PDA medium by means of the hyphal tip transfer technique. The cultured fungal isolates were purified by three subsequent sub-culturing on to PDA. Eight pure fungal cultures inspected to be *Fusarium*, were maintained in the form of slant culture at 4°C for further study. The pathogenicity of the isolated *Fusarium* species was tested through bioassay on detached leaves of the tomato cultivar-Melka oda grown in pots following standard procedure. Healthy looking vigorous tomato leaves and autoclaved sterilized soil were used for the bioassay. Sixty (60) days old leaves were cut from the parent plant and transported to the laboratory. The leaves were surface sterilized with 70% ethanol for 30 seconds, rinsed with three successive changes of sterile distilled water and blot dried. The leaves were placed in sterile plates and were inoculated with 500 μl spore suspension of *Fusarium oxysporum*. The leaves in control were inoculated with the same amount of sterilized distilled water and the treatments were

in three replicates. The plates were incubated at $25 \pm 2^\circ \text{C}$ until the emergence of mould growth was observed on the surface of the leaves. Observations were taken starting from the second day where the lesion diameter was recorded.

Manju kumari *et al.* (2021) collected and isolated ten samples of *F. oxysporum f. sp. lentis* (FOL-01, FOL-02, FOL-03, FOL-04, FOL-05, FOL-06, FOL-07, FOL-08, FOL-09 and FOL-10) from different lentil growing areas of Rajasthan. Infected plants were washed carefully to remove the adhering soil particles. The washed plants were cut into small pieces and surface sterilized by immersing the root pieces in 1% NaOCl solution for 5 min and then transferred on to potato dextrose agar (PDA) in Petri dishes and incubated at $25 \pm 1^\circ \text{C}$ for 7 days. The growing fungi were individually transferred to the PDA medium. Pure cultures of fungi were obtained using a single spore or hyphal tip technique. Pure culture was kept in a refrigerator for further studies. Pathogenicity test of different isolates of *F. oxysporum f. sp. lentis* (FOL-01 to FOL-10) were tested during Rabi 2017-2018 in cage house condition at Rajasthan Agricultural Research Institute, Durgapura (Jaipur) by seed and soil inoculation. Observation of diseased and healthy plants was recorded 7 to 60 days and PDI was calculated by $\text{PDI} = [(\text{number of diseased plants}/\text{total number of plants}) \times 100]$.

Metsena *et al.* (2021) carried out isolation from the infected roots and stems tissues of cowpea plant. Roots were washed with the running tap water to remove the solids adhering particles and were dried under laboratory air. Roots and stems surfaces were sterilized in 95% alcohol for five minutes. Each plant was then dissected longitudinally in two parts with sterilized scalpel following the median plane from root to stem. Two to three pieces of tissues showing chlorosis were cut and surface disinfected in sodium hypochloride 1% for 15 to 30 seconds. They were washed thrice in distilled water. Tissues pieces were dried under two filter papers and aseptically transferred on potato dextrose agar (PDA) in the Petri dishes of 90 mm diameter. Colonies developed after 2 to 4 days were purified by three successive subcultures. Pathogenicity test was done using soil inoculation technique in a greenhouse on susceptible and resistant varieties in completely randomized design replicated thrice.

Shweta (2022) collected samples from infected roots of lentil plants and brought to laboratory for isolation and identification of pathogen causing root rot. The samples were first surface sterilized twice with distilled sterilized water and then were treated with 0.5% NaOCl (Sodium hypochlorite) for 2 minutes. After surface sterilization, the samples were dried on sterilized blotter paper and placed in Petri plates containing sterilized potato dextrose agar

medium. All the Petri dishes were incubated at $25 \pm 1^{\circ}\text{C}$ for about seven days. After seven days of inoculation, the fungi isolated, were then identified with the help of colony character and microscopic observation.

2. To study morphological and cultural characteristics of fungus causing wilt disease.

Ferniah *et al.* (2014) isolated fungus from the stems of chilli and identified as *Fusarium oxysporum* causing wilt in chilli. They studied the morphological characters of the fungi and reported the colony with white cottony aerial mycelium while purple on the reverse with 4 - 5 cm diameter at 5 days incubation on PDA. Conidia were grown from short phialide with a false head. Macroconidia were straight fusiform, pedicellate basal cell, $27 - 46 \times 3 - 4, 5 \mu\text{m}$, with 3 - 5 septates. Microconidia were abundant, ellipsoid or fusiform without or with 1 - 2 septates, $5 - 15 \times 2, 2 - 3, 5 \mu\text{m}$. Chlamydospore was formed terminally or intercallary, single or in pairs.

Sanjeev Kumar and Upadhyay (2014) studied the cultural, morphological and pathogenic variability in fifteen isolates of *Fusarium udum* causal agent of pigeon pea wilt. The isolates were procured from four major pigeon pea growing states viz., Bihar, Jharkhand, Orissa, West Bengal. These isolates exhibited considerable variations in cultural and morphological characters on PDA medium viz., colony colour, pigmentation, sporulation, mycelial growth and septation of conidia.

Singh and Kumar (2016) conducted laboratory studies to study the effect of different culture media, and pH on mycelia growth, sporulation and dry mycelia weight of *Fusarium oxysporum f.sp. lentis*. The fungus grew the best on PDA and Richard's agar media among five culture media tested. The most suitable pH level for growth of fungus was 6.5 and 7.0 with excellent sporulation.

Maina *et al.* (2017) studied cultural and morphological variability among *Fusarium oxysporum f.sp. phaseoli* strains, isolated from French beans. Isolates showed luxuriant, moderately luxuriant and scanty aerial mycelial growth. Mycelial texture was either fluffy or fibrous. Isolate Fop8 had the highest growth (85 mm) at 7th day followed by Fop3 (84 mm) and Fop6 (84 mm) while Fop7 had the least growth (71 mm). The colony colour was purple, pink or white. Microconidia sizes ranged from 8×3.0 to $10 \times 3.4 \mu\text{m}$ while macroconidia ranged from 28×3.8 to $42 \times 4.2 \mu\text{m}$. Macroconidia had 3 septa. The microconidia were abundant and aseptate. Chlamydospore formation was terminal and intercalary, occurring in singles and in pairs. Although all the 8 isolates were pathogenic on 'Amy' French bean variety, their pathogenic potential was significantly different ($P < 0.01$). The most pathogenic isolate was Fop03, followed

by Fop06 and Fop07 at means of 97.0, 92.4 and 92.0%, respectively. The least pathogenic isolate was Fop05 with a mean of 65.9% pathogenicity.

Nath *et al.* (2017) conducted studies on nine isolates of *Fusarium oxysporum f. sp. ciceri* infecting chickpea collected from major chickpea growing areas of Bangladesh and their cultural, morphological, physiological and pathogenic characteristics were described. The isolates varied significantly in their cultural, morphological and physiological traits i.e., colony color, shape, margin and texture, mycelial radial growth and spore production.

Santram S. (2017) Collected eight isolates of *Fusarium oxysporum f.sp. udum* and studied cultural and morphological variability The colony diameter ranged from 80 to 90 mm after nine days of incubation at $28 \pm 1^\circ\text{C}$. The size of macroconidia ranged from 16.5 to 20.6 μm (18.47 μm) \times 2.1 to 4.1 μm (2.98 μm) in RFU to 24.0 to 37.0 μm (30.24 μm) \times 2.6 to 4.0 μm (3.52 μm) in BeFU. The size of microconidia varied from 4.9 to 11.8 μm (8.10 μm) \times 2.2 to 4.0 μm (2.98 μm) in BeFU to 8.3 to 17.4 μm (12.94 μm) \times 2.5 to 5.8 μm (4.0 μm) in RFU. The macroconidia were 1 to 4 septate, fusaroid to sickle shaped. The shape of chlamydospores varied from round in KoFU, RFU, RjFU and BeFU isolates to oval in isolate RgFU and long oval in KFU, MFU and DFU. The size of round chlamydospores ranged from 3.8 to 4.7 μm in RFU to 6.4 to 8.7 μm in diameter in RjFU and long oval chlamydospores were 11 to 13 \times 3 to 4 μm in MFU to 19 to 21 \times 4 to 5 μm (Length \times Breadth) in DFU.

Chaithra *et al.* (2018) collected twelve *Fusarium oxysporum f.sp. ciceri* isolates and grown on PDA medium in Petri plate and observed for morphological characters such as length and width of micro and macroconidia, diameter of chlamydospores, shape, color, number of spores and number of septation per macro and microconidia were measured under a light microscope.

Ghante (2018) studied cultural, morphological and molecular characteristics of *Fusarium oxysporum f. sp. udum* in ten isolates. Observations on cultural characteristics viz., colony diameter, colony shape, colony margin, zonation, substrate pigmentation were recorded at 10 days of incubation and the spores were observed under microscope. The morphological characteristics viz., length and breadth, septation of microconidia and macroconidia of each isolate were recorded by using J image software, TS view and with help of compound microscope.

Golakiya *et al.* (2018) isolated fifteen isolates of *Fusarium oxysporum f. sp. ciceri* and were separately grown on PDA in Petri plates and incubated at $28 \pm 2^\circ\text{C}$ for 7 days. Observations on cultural characteristics viz., colony colour and type, growth and pigmentation were recorded a week after inoculation. Morphological characters of spores of different isolates were studied by

observing in cotton blue stained slides under microscope. Measurements of macro- micro conidia and chlamydo spores were made with the help of imaging microscope which shows size of conidia.

Khushboo and Singh (2018) studied *Fusarium* wilt of lentil caused by *Fusarium oxysporum f.sp. lentis* and reported the most serious disease of lentil. Ten isolates of *F. oxysporum f.sp. lentis* were studied for its cultural, morphological and pathogenic variability. Pigmentation is varied among the isolates. Out of 10 isolates, 4 isolates FOLR3, FOL-V8, FOL-M6 and FOL-R9 were purple dull yellowish while isolates FOL-B7 and FOL-S10, four isolates like FOL-A2, FOL-L4, FOL-S5 and FOL-S10 were white. FOL-F1 was light buff in colour. The size of microconidia varied between 7.30×3.43 (FOLL4) to $13.25 \times 2.68 \mu\text{m}$ (FOL-S10) and macroconidia varied from 24.11×4.67 (FOL-A2) to $40.05 \times 4.71 \mu\text{m}$ (FOL-V8). The number of septa in macroconidia was mostly 3-6 and microconidia are mostly no septum and some are 0-1. Chlamydo spores are terminal and intercalary. Pathogenic variability revealed that most of the isolates were highly pathogenic.

Avinash *et al.* (2019) isolated *Fusarium* pathogen from red gram plants showing wilt symptoms and fungal isolates were sub-cultured to study morphological and cultural characteristics. Cultural characters viz., colony color, nature of mycelia, pigmentation on the media were observed. Microscopic observations like conidial characters such as shape and color of conidia were done to confirm the *Fusarium* isolates. Confirmed *Fusarium* isolates were purified and further used for pathogenicity studies.

K. Venkataramanamma *et al.* (2019) collected thirty-two isolates of *Fusarium oxysporum f.sp. ciceris* (Foc). Based on wilting % in pathogenicity test, the isolates were grouped into three categories. All the isolates differed in cultural characters like mycelium colour, margin, texture, pigmentation and morphological characters like macro and micro conidial length, width and chlamydo spores diameter. There was no correlation between cultural and morphological characters of Foc isolates except three isolates (Foc-6, Foc-12 and Foc-17). These three isolates have white mycelium, cream colour pigmentation, slow radial growth and highly pathogenic (100% wilt incidence) in nature. Sporulation ability and radial growth of all isolates were also measured. All the isolates exhibited genetic variation in RAPD banding pattern and three major clusters (I, II and III) were found in the dendrogram. Maximum genetic similarity of 80.3% was observed between Foc-27 and Foc-28 isolates.

Saxena *et al.* (2019) described morphological and cultural variability in *F. oxysporum f.sp. lentis* isolated from the wilted lentil plants collected from Madhya Pradesh, Chhattisgarh, Jharkhand and Rajasthan State's, in India. Total 146 *F. oxysporum f.sp. lentis* (Fol) isolates were obtained after confirmation of pathogenicity tests on susceptible variety L9–12. The colonies of Fol isolates were white, dull white, pinkish white, yellowish white and brownish white. The

pigmentations on standard Hi-media potato dextrose agar medium were yellowish brown, dark brown, creamy and dark tan. The isolates exhibited three types of growth pattern as oppressed, partially oppressed and fluffy. The rate of mycelia growth of the Fol isolates was slow (< 4 cm/week), medium (4–7 cm/week) and fast (>7 cm/week). Based on the morphological and cultural characteristics the isolates were grouped into fifty groups. The growth rate was considered as the main criterion for grouping of the isolates. Other important parameter for grouping the Fol isolates was the size of macroconidia; size more than 15 µm was considered as large, whereas, less than that was grouped in to small. Fast growing isolates comprised fourteen groups, those having large macroconidia formed six groups, while small size conidia were in eight groups. In medium growth rate there were seventeen groups, out of these eight groups were having large macroconidia, while in nine groups macroconidia were small. In slow growth rate there were nineteen groups, those having large macro conidia were eight, while in eleven groups small macroconidia were present. The phylogenetic tree prepared involving different morphological and cultural characteristics of the isolates depict two major groups of *F. oxysporum f.sp. lentis* isolates.

Sun *et al.* (2019) studied morphological characteristics of *Fusarium oxysporum* causal agent of mung bean wilt from the collected isolates by sub-culturing at 25⁰C for 13-15 days on PDA medium and carnation leaf agar plate CLA under 12hr light/ 12hr dark cycle. The microconidia and macroconidia produced on PDA and CLA medium were observed and measured under a light microscope.

Poorvasandhya *et al.* (2020) studied effect of media, temperature and pH on growth and sporulation of *Fusarium oxysporum f. sp. udum* under *in vitro* condition and revealed that among the seven culture media tested the fungus grew best on PDA and Czapeks Dox agar media with suitable pH level of 6.0 and 7.0 and growth was maximum at 30⁰C after seven days of inoculation.

Ayele *et al.* (2021) studied the cultural characters of the isolates on the PDA medium by culturing each isolate in three replicates at 25+2⁰C. Cultural characteristics (colony appearance, growth pattern, marginal shape, surface colony colour, reverse colony colour/pigmentation, radial growth) were examined seven days after inoculation (7 DAI) and the growth rate was measured. Radial growth was evaluated by measuring the radius of the Petri dish covered by the colony culture using an mm-calibrated glass ruler. Growth rate data was recorded by measuring the radius that the colony covered 24-hour intervals for seven days. Morphological characters were studied using slide culture technique. A slide culture was prepared for each of the eight isolates by taking a 5mm bit from a seven day old pure culture and inoculating on to a rectangular cut of the PDA medium. It was then placed on sterilized glass slides and covered with coverslips. The slides were then placed inside a sterilized Petri dish underlay with moistened filter paper to provide moisture

for the fungi, and incubated at $25 \pm 2^\circ\text{C}$. The mycelia developed on the slide culture are then subjected to microscopic examination to morphologically differentiate the identity of the isolates to species level at 40X magnification using an Olympus Microscope (Carl Zeiss Microscopy, Germany).

Deepti *et al.* (2021) carried out study about wilt disease in Pigeon pea which was found to be infected with *Fusarium oxysporum f.sp. udum* at all ten locations surveyed in 2015-16 and 2016-17 in Birsa Agricultural University, Kanke, Ranchi. Morphological studies of *Fusarium oxysporum f.sp. udum* revealed that the dimensions of macroconidia, microconidia and chlamydospores shows larger variations among different isolates. The cultural characters observed on PDA medium and the mycelium colour was white and pinkish at the center with dense to fluffy growth. The size of mycelial width varied from $3 \mu\text{m}$ (Fou-Bor -4) to $6.32 \mu\text{m}$ (Fou – Ran - 4). Whereas microconidia were small, oval in shape and hyaline in colour, unicellular or with one or two septa, and measures in the range of size between $6.00 \times 2.10 \mu\text{m}$ (Fou-Ran- 4) to $10.80 \times 3.00 \mu\text{m}$ (Fou- Kok-1). The macroconidia were long, curved, sickle shaped, pointed at the tip, hyaline in colour and knotted at the base, septate (2-4 septa) and measured between $19.05 \times 3.25 \mu\text{m}$ (Fou- Hoc-4) to $28.70 \times 2.50 \mu\text{m}$ (Fou- Kok-1). Chlamydospores with spherical in shape and hyaline in colour and its dimensions also varied in all 40 isolates of the pathogen. Chlamydospores dimension varied from $8.20 \times 7.92 \mu\text{m}$ (Fou- Buk – 2) to $11.35 \times 8.20 \mu\text{m}$ (Fou-Nag-3). Spore density also varied between conidia per ml of culture under 10 X microscopic field, it is varied for macroconidia in the range of 6 to 13, micro conidia it is between 20 to 42 for chlamydospores, it is between 2 to 8 number per ml.

Manju kumari *et al.* (2021) identified ten isolates of *Fusarium oxysporum f.sp. lentis* based on their morphological and cultural characters. For the morphological identification, from a pure culture of each isolate, temporary slide mounts were prepared in lactophenol solution and were examined under the light microscope (Nikon YS100) at 100 X magnification. The number of observations were taken for the size of macroconidia, the number of conidia per microscopic field belonging to each isolate. For the cultural identification, twenty ml of sterilized PDA was poured aseptically in each Petri-plate after that plates were inoculated with 5 mm dia. mycelial disc obtained from 5-day-old cultures of different isolates and then incubated at $25 \pm 1^\circ\text{C}$. One observation regarding mycelium color (Pink, purple, white, and dull white), Pigmentation (pinkish, dark tan, light buff) were recorded. Three replications were maintained for each isolate in a completely randomized design (CRD).

3. To study *in vivo* (pot) cross infectivity potential of test fungus.

Armstrong *et al.* (1978) studied *Fusarium oxysporum f. sp. cucumerinum* from U.S., Germany and Japan; *f. sp. melonis* from Canada, France, Israel, Japan, and the U.S.; *f. sp. niveum* from Florida and South Carolina; *f. sp. lagenariae* and *luffae* from Japan were used, respectively, in inoculations of 16 cultivars or breeding lines of cucumber, 49 of muskmelon, 23 of watermelon, eight of gourds, and one each of citron and colocynth. Variations in virulence for a specific host occurred, but each forma specialis showed sufficient selective pathogenicity for the host from which it was derived to be retained as a valid forma specialis. No races were identified with *f. sp. cucumerinum*, *lagenariae*, or *luffae*, nor could the races of *f. sp. niveum* reported from the U.S. be clearly separated. In addition to the four clearly defined races of *f. sp. melonis* from France, three new races; viz., race 5 from the U.S.-Canada, race 6 from Israel, and race 7 from Japan, were found among the 24 isolates in the collection of *f. sp. melonis*. Fifty other different wilt *fusaria* were non-pathogenic on a cultivar of cucumber, muskmelon, watermelon, and dishrag gourd, each of which was susceptible to its respective forma specialis. A forma specialis causing wilt of cucumber, muskmelon (race 5), watermelon, and dishrag gourd, respectively, was non-pathogenic on plants of 46 other species and cultivars that have been helpful in differentiating formae speciales and races of *F. oxysporum*.

Rowe (1980) collected forty-two isolates from crown rot and root rot affected tomato plants and were inoculated to four differential tomato lines. The symptoms and pattern of infection on these lines were similar for all isolates tested and differed from that of *Fusarium oxysporum f. sp. lycopersici* races 1 and 2. Results from inoculation of 15 hosts representing five botanical families indicated that many legumes were moderately susceptible to the crown and root rot isolates tested while cucurbits, crucifers and cereals are unaffected. *Fusarium oxysporum f. sp. lycopersici* isolates were host specific to tomato.

McMillan (1986) studied isolates of *Fusarium oxysporum* from Abaco, the Bahamas, obtained from wilted plants of cucumber (*Cucumis sativus*) or watermelon (*Citrullus lanatus*). They were found pathogenic to cucumber, watermelon, and cantaloupe (*Cucumis melo* var. *reticulatus*). The West Indian gherkin (*Cucumis anguria*), pumpkin (*Cucurbita pepo*), and three cultivars of summer squash (*C. pepo* var. *melopepo*) were not susceptible. Strains of *F. oxysporum* from wilted cucumber or watermelon plants from Florida were highly pathogenic only to their original host species and are regarded as different formae speciales.

Gerlagh and Blok (1988) studied isolates of *Fusarium oxysporum* from wilted muskmelons, watermelons, cucumbers and from the muskmelon rootstock *Benincasa hispida* were screened for pathogenicity on seedlings and adult plants of these crops and related species. In seedling tests, the isolates were not typically species-specific, contrary to what might be expected as an implication of their characterization as forma specialis. They often attacked species

of several genera of plants, but not beyond the family of the Cucurbitaceae. In the adult stage, plants were much more exclusively attacked by their corresponding formae speciales, but essential exceptions occurred. Isolates from cucumber were highly pathogenic to muskmelons, in the adult stage even causing more wilt of the latter than of cucumber. Comparing the results of these experiments with data from the literature, it is argued that the proposed *f.sp. cucurbitacearum*, embracing all formae speciales which specialize on plants within the family of the Cucurbitaceae

Cafri *et al.* (2005) studied the population structure of *Fusarium oxysporum f. sp. cucumerinum* using the vegetative compatibility grouping (VCG) approach. All 37 of the examined isolates from Israel were assigned to VCG 0180, the major VCG found in North America and the Mediterranean region. Approximately two-thirds of the tested isolates were pathogenic to both cucumber and melon, but cumulatively they were more aggressive on cucumber, their major host, than on melon. Disease symptoms on melon plants were less destructive and often expressed as growth retardation.

Zhou and Everts (2007) collected eighty-eight isolates of *Fusarium oxysporum f. sp. niveum*, from wilted watermelon plants and, were characterized by cross pathogenicity to muskmelon race, and vegetative compatibility. Four isolates (4.5%) were moderately pathogenic to ≥ 2 of 18 muskmelon cultivars in a greenhouse test, and one representative isolate also was slightly pathogenic in field microplots.

Case (2009) studied isolates of *F. oxysporum* and tested for pathogenicity only on the host they were isolated from; therefore, it is not known whether isolates of *f.sp. betae* can be pathogenic on common bean, or *f. sp. phaseoli* pathogenic on sugar beet; which has implications regarding common bean and sugar beet crop rotations. The objective was to determine if isolates of *F. oxysporum f. sp. betae* are cross-pathogenic to common bean. They inoculated common bean with 52 isolates of *F. oxysporum f. sp. betae* along with 4 isolates of *F. oxysporum f. sp. phaseoli*, (used as controls) to common bean (Viva) and sugar beet (FC716) in the greenhouse using a root dip assay and assessed disease severity based on a disease index scale (1-9 for common bean; 0-5 for sugarbeet). Neither *F. oxysporum f. sp. betae* isolates showed cross-pathogenicity to common bean, nor the *F. oxysporum f. sp. phaseoli* controls cross pathogenic to sugar beet. These results suggest that common bean does not serve as a host for *F. oxysporum f. sp. betae*, and that crop rotation with bean and sugar beet can be a useful method of control.

Mousa and Sharma (2009) studied cross pathogenicity among the isolates of *Fusarium oxysporum* causing wilt in cucumber and muskmelon. Some isolates of *F. oxysporum* from India, obtained from wilted plants of cucumber (*Cucumis sativus* L) or melon (*C. melo* L), were pathogenic to cucumber (cultivars Long Green and Poinsette) melon and watermelon. One cultivar

of sponge gourd, two cultivars of bitter melon (local and Long Coimbatore), Tomato were immune to all the 30 isolates of *F.oxysporum* tested. Strains of *F. oxysporum* from wilted cucumber or melon plants were highly pathogenic only to their original host species and were regarded as different formae speciales. Five of the tested isolates were pathogenic to both cucumber and melon, but cumulatively they were more aggressive on their original host, compared to other hosts tested. Disease symptoms of cross pathogenicity on melon plants were apparently less harmful and often expressed as growth retardation, but disease symptoms on cucumber plants were more destructive.

Wim *et al.* (2011) conducted a study on the host range of *Fusarium oxysporum f.sp. asparagi* (Foa) in inoculation experiments with 21 plant species. Typical root rot symptoms were incited only in asparagus, in all experiments; lupin and pea were susceptible under *in vitro* conditions but showed only mild symptoms occasionally when tested in soil; none of the other species showed external disease symptoms. Root colonization by Foa was studied for 14 plant species. The pathogen was detected in externally disinfested roots of all species except leek and onion, with asparagus the most extensively colonized species. Asparagus was not susceptible to isolates of *F. oxysporum f.sp. pisi*, *F. oxysporum f. sp. lupini*, *cepa*, *lini* and *gladioli* and *Fusarium sacchari* var. *elongatum*. Naturally infested field soil was planted twice for 11 – 13 weeks with 11 plant species, including asparagus and several symptomless hosts, and subsequently with asparagus as a biotest plant. Of these crops, only asparagus greatly increased the severity of Foa root rot. It was concluded that Foa has a narrow host range as a pathogen but a broad host range as a parasite. The consequences of the latter for the epidemiology of Foa are discussed. Twenty-four Foa isolates were assigned to 18 different vegetative compatibility groups (VCGs); three additional *F. oxysporum* isolates, which were not pathogenic on asparagus, each belonged to a unique VCG. These findings indicate that the Dutch Foa population is very diverse genetically, as was found previously for the Foa population in the United States.

Webb *et al.* (2013) collected thirty-eight *F. oxysporum* isolates from symptomatic sugar beet throughout the United States to investigate diversity of the *F. oxysporum* population and the influence of crop rotation on pathogenic variation. These isolates were characterized for pathogenicity to sugar beet, dry edible bean, and onion, as well as vegetative compatibility. Pathogenicity testing indicated that some *F. oxysporum* isolates from sugar beet may cause disease on onion and dry edible bean. Furthermore, vegetative compatibility testing supported previous reports that *F. oxysporum f. sp. betae* is polyphyletic and that pathogenic isolates cannot be differentiated from non-pathogenic *F. oxysporum* using vegetative compatibility.

Yu and Babadoost (2013) conducted pathogenicity tests of *F. commune* and *F. oxysporum* on two horseradish cultivars, '1573' and 'Big Top Western', in a greenhouse. Root segments were inoculated by dipping them in a conidial suspension and then growing them in pots in a greenhouse for 4 months. For plants inoculated with *F. commune*, internal root discoloration and root rot developed 1 month after inoculation and almost all roots of the plant were completely rotten 4 months after inoculation. Inoculation of the plants with *F. oxysporum* resulted in only internal root discoloration but not root rot symptoms.

Carlos *et al.* (2019) studied the host specificity of *Fusarium oxysporum*. To achieve that the host range of 10 strains of *Fusarium oxysporum* collected from diseased pepper plants were checked on the following species: *Capsicum annuum*, *Solanum lycopersicum*, *Nicotiana tabacum*, *Nicotiana bethamiana*, *Solanum melongena*, *Physalis ixocarpa*, *Cucurbita pepo*, *Cucumis sativus*, *Cucumis melo*. These 10 strains were also tested on four differential tomato cultivars for *F. oxysporum* sp. *lycopersici* races identification. All 10 strains of *F. oxysporum* caused wilting symptoms on each of the species mentioned above.

Okello and Mathew (2019) conducted a survey across South Dakota on soybean and corn fields in 2014 and 2015, respectively, to assess the prevalence of species of *Fusarium* causing root rot. *Fusarium acuminatum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. subglutinans* were identified common to soybean and corn. A total of 21 isolates, representing these seven species, were evaluated for their pathogenicity on soybean ('Williams 82') and corn ('B73') using the inoculum layer inoculation method in the greenhouse. Two *F. proliferatum* isolates and one *F. graminearum* isolate from corn caused significantly greater RTE than the other treatments (including the noninoculated control) on soybean and corn. Results indicate that soybean and corn can serve as inoculum sources of the seven species of *Fusarium* that are pathogenic to both crops.

Sun *et al.* (2019) carried out host range test of seven representative pathogenic isolates on eight other crops, only two isolates caused mild symptoms on one of two adzuki bean cultivars, and no visual symptoms were observed on chickpea, common bean, cowpea, pea, soybean, or cotton, indicating the isolates were host-specific to mung bean.

Manju kumari *et al.* (2021) carried out study using ten isolates FOL-01 to FOL-10 of *F. oxysporum* f. sp. *lentis* (Fol) isolated from wilted lentil plants that were collected from different major lentil growing parts of Rajasthan. During 2017-18 a pathogenicity test was tested in pot house condition by seed and soil inoculation techniques for all isolates and epidemiological factors evaluated *in vitro* conditions. Results indicated that the Fol isolates represent a single race but differ in their aggressiveness on the susceptible cultivar L9-12. Pathogenicity test revealed clearly

that Fol was associated with wilt symptoms and were pathogenic to lentil plants. A maximum percent disease incidence of 70.00 was showed by isolate FOL-02 in soil inoculation technique. In the morphological and cultural characterization, all the ten isolates showed various character in conidial frequency, colony color and growth pattern. Twenty plant species were tested to know the host range of Fol, out of these lentil, chickpea and pea show positive reaction with the pathogen. The influence of temperature, relative humidity and pH on the growth and sporulation of Fol was studied under *in vitro* conditions. Maximum mycelial growth and sporulation of the Fol were observed at 30°C, 6.0 pH and 60% relative humidity.

Moparthy S. (2021) studied *Fusarium* isolates (n = 112) were obtained from seeds and roots of chickpea, dry pea, and lentil. Isolates were identified by comparing the sequences of the internal transcribed spacer region and the translation elongation factor 1- α in *Fusarium*-ID database. *Fusarium avenaceum* was the most abundant species (28%), followed by *F. acuminatum* (21%), *F. poae* (13%), *F. oxysporum* (8%), *F. culmorum* (6%), *F. redolens* (6%), *F. sporotrichioides* (6%), *F. solani* (4%), *F. graminearum* (2%), *F. torulosum* (2%), and *F. tricinctum* (0.9%). The aggressiveness of a subset of 50 isolates that represent various sources of isolation was tested on three pulse crops and two cereal crops. Nonparametric analysis of variance conducted on ranks of disease severity indicated that *F. avenaceum* and *F. solani* isolates were highly aggressive on pea and chickpea. In lentil, *F. avenaceum* and *F. culmorum* were highly aggressive. In barley, *F. avenaceum*, *F. solani*, *F. culmorum*, and *F. graminearum* were highly aggressive. In wheat, *F. avenaceum*, *F. graminearum*, and *F. culmorum* were highly aggressive. Two *F. avenaceum* isolates were highly aggressive across all the crops tested and found to be cross-pathogenic. One isolate of *F. culmorum* and an isolate of *F. graminearum* obtained from chickpea and lentil seed were highly aggressive on barley and wheat. The results indicate that multiple *Fusarium spp.* from seeds and roots can cause root rot on both pulse and cereal crops.

Soleha *et al.* (2022) carried out host range studies of *Fusarium oxysporum*, causal agent of seedling wilt disease of *Acacia mangium*. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several forests and industrial plants. Three isolates of *F. oxysporum* with different translation elongation factor (tef 1- α) sequences were tested for pathogenicity on different Fabaceae family plants and the growth of population was also observed. The results showed that all three isolates were able to infect all the tested plants with different reactions to wilt disease. *Acacia crassicarpa* and *Falcataria moluccana* were highly susceptible; *Archidendron pauciflorum*, *Leucaena leucocephala*, and *Parkia speciosa* were moderately vulnerable and *Acacia auriculiformis* was moderately resistant. The pathogen population in *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease

scores, while in *L. leucocephala* it was moderate, and slow in *A. pauciflorum*, *P. speciosa* and *A. auriculiformis* plants. In conclusion, *F. oxysporum* pathogen, which was isolated from *A. mangium*, has a wide range of hosts in the Fabaceae family.

4. Integrated management of Fusarium wilt disease.

Chauhan *et al.* (1988) carried out field tests on the effects of soil solarization on pigeonpea (*Cajanus cajan* (L.) Millsp.) and chickpea (*Cicer arietinum* L.) crops through a multidisciplinary team effort, at ICRISAT Center during 1984-87. The studies were conducted in fields infested with *Fusarium* wilt. Solarization was done by covering the soil with transparent polythene sheet (100 μ m thick) for 6-8 weeks during summer (April/May). This increased soil temperatures by 6-10°C in the 0-20 cm soil profile. Other changes recorded were increased mineralization of soil nitrogen to nitrate, a decline in populations of *Fusarium* propagules and plant parasitic nematodes, and decreased weed infestation. When the crops were grown. Effective control of *Fusarium* wilt disease in the susceptible genotypes of pigeonpea and chickpea was observed along with improved plant growth and yield.

Prasad and Anuroop (2002) checked the efficacy of the biological control agent *Trichoderma harzianum* on various levels of *Fusarium udum* the pigeon pea wilt pathogen. *Trichoderma harzianum* was applied as a seed treatment (10 to 20g/kg of seed) and as the soil amendment (10 to 20g/9m²). Seed treatments resulted in less than 30% disease control when compared to the check.

Khan *et al.* (2004) studied the effect of treating seed of chickpea (*Cicer arietinum* L.) cv. BG 256 with commercial formulations (2 g kg⁻¹ seed) of *Trichoderma harzianum* and *Pseudomonas fluorescens*, singly and jointly, to control wilt caused by *Fusarium oxysporum* f. sp. *ciceri* was examined in chickpea plants growing in microplots under field conditions. On untreated control plants, the wilt fungus caused the characteristic symptoms of wilt and significantly (P=0.05) decreased dry weight and the yield of chickpea by 20 and 18% respectively (significant at P=0.05). On chickpea without wilt, treatment with *P. fluorescens* improved the yield by 36% and *T. harzianum* + *P. fluorescens* by 25%. Both biofungicides suppressed wilt severity (P=0.05), the most effective being *T. harzianum* + *P. fluorescens* (66%). Carbendazim reduced wilt severity by 51%. On inoculated chickpea, yield increased by 39% with *P. fluorescens*, by 33% with *T. harzianum*+*P. fluorescens*, by 44% with *T. harzianum*, and by 20% with carbendazim as compared with the inoculated control. The soil population of the wilt fungus (cfu g⁻¹ soil) in untreated plots increased during the first two months (P=0.05), but in the biofungicide/fungicide treated plots it gradually and significantly (P=0.05) decreased during the four months of the crop season. The greatest decrease in the soil population of the wilt fungus occurred with *T. harzianum*

or *T. harzianum*+*P. fluorescens*, followed by *P. fluorescens* and carbendazim. The rhizosphere population of the bioagents increased significantly in those plots where wilt populations decreased. The greatest increase in the population of the bioagents was recorded for *T. harzianum* (108-120%), followed by *P. fluorescens* (65-119%) in the combined treatment, compared with the pre-plant control (December). When the bioagents were applied alone, the population of *T. harzianum* increased by 71-96% and that of *P. fluorescens* was by 46-103%.

Haseeb *et al.* (2005) conducted studies under pot conditions to determine the comparative efficacy of carbofuran at 1 mg a.i./kg soil, bavistin at 1 mg a.i./kg soil, neem (*Azadirachta indica*) seed powder at 50 mg/kg soil, green mould (*Trichoderma harzianum*) at 50.0 ml/kg soil, rhizobacteria (*Pseudomonas fluorescens*) at 50.0 ml/kg soil against root-knot nematode - *Meloidogyne incognita*, Wilt fungus - *Fusarium oxysporum* disease complex on green gram, *Vigna radiata* cv ML-1108. All the treatments significantly improved the growth of the plants as compared to untreated inoculated plants. Analysis of data showed that carbofuran and *A. indica* seed powder increased plant growth and yield significantly more in comparison to bavistin and *P. fluorescens*. Carbofuran was highly effective against nematode, bavistin against fungus, *A. indica* seed powder against both the pathogens and both the bioagents were moderately effective against both the pathogens.

Nikam *et al.* (2007) studied Chemical seed treatment with Thiram (0.15%) + Carbendazim (0.1%) is proved to be the most effective against *Fusarium oxysporium f. sp. ciceri*. *In vitro* evaluation of *Trichoderma sp.* against *F. oxysporium f. sp. ciceri* revealed the positive cumulative effect of *Trichoderma viride* + *Trichoderma harzianum* + *Trichoderma hamatum* in respect to the percent inhibition of the test fungus. Pot culture studies revealed that the soil application of *T. viride* (@ 25 kg/ha) as the most effective in reducing the incidence of chickpea wilt. Soil amendment with groundnut cake is proved to be effective against *F. oxysporium f. sp. ciceri* followed by neem cake. Genetic diversity already existing in pigeon pea germplasm lines can be exploited for breeding wilt resistant chickpea varieties. Thus, chickpea wilt incited by *F. oxysporium f. sp. ciceri* being soil borne disease could be managed by the integration of various practices like using resistant varieties, seed treatment with chemicals, seed and soil application of bioagents and amendment of soils with oilseeds cakes

Maheshwari *et al.* (2008) tested seven fungitoxicants against *Fusarium oxysporium f. sp. lentis in vitro*. All these significantly checked the growth of the pathogen as compared to control. Carbendazim proved most effective fungitoxicant for checking the fungal growth (5.6 mm) followed by captan (9.9 mm), hexaconazole 12.5 mm) and difenconazole (16.4 mm). Seed

treatment + soil drenching with carbendazim was most effective to minimize the wilt incidence (10.6%), plant mortality (4.5%) and gave highest grain yield (7.48q/ha) followed by captan (15.9%, 6.3% & 6.10 q/ ha). Hexaconazole and difenconazole were the next best fungitoxicants in order of superiority in case of disease incidence, plant mortality and grain yield.

Animisha *et al.* (2012) conducted an investigation to diminish wilt of chickpea by use of integrated disease management. It was observed that *in vitro* condition (dual culture technique) *Trichoderma viride* was highest inhibiting the growth of *Fusarium oxysporum f. sp. ciceris* at the ratio 1:4 followed by 1:2 and 1:1 in poisoned food technique. Maximum inhibition under field condition was obtained by 0.3% followed by 0.2 and 0.1%, doses of carbendazim. After that carbendazim it was neem cake at concentrations 7% followed by 5 and 3% in that order which give maximum inhibition of test pathogen under field condition. Lowest percentage of incidence of wilt (19.0%) was found with *T. viride* (T₂) followed by carbendazim (21.0%), neem cake (42.6%), carbendazim+neem cake (45.2%), carbendazim+*T. viride* (47.2%), neem cake+*T. viride* (48.2%). Pot culture studies revealed that the soil application of *T. viride* (4 g kg⁻¹) was found most effective treatment in reducing the incidence of chickpea wilt. Thus, chickpea wilt could be managed by the integration of various practices like, seed treatment with chemicals, seed and soil application of bioagents and amendment of soils with neem cakes.

Kamdi *et al.* (2012) studied two antagonists and two fungicides against *Fusarium oxysporum f. sp. ciceri* causing chickpea wilt. Field studies found that carbendazim seed treatment (2g/kg of seed) gave minimum wilt incidence (26.38%) and maximum yield (13.47qt/ha) followed by *T. viride* + carbendazim, *T. viride* + thiram and *Trichoderma viride* alone.

Pandey *et al.* (2014) carried out field experiment to compare the effect of organic soil amendment (FYM, vermi-compost, green manure, Neem cake) and Soil solarization against *Fusarium* wilt of chickpea. Results revealed that among all the treatments with soil solarization were more effective in controlling wilt disease of chickpea (54.4%) as compared to Neem cake (46.7%), Farm Yard Manure (39.9%), vermi compost (39.3%) and green manure (26.2%). Shoot and Root growth also increased in above treatments as compare to control. Yield percent is highest in soil solarization i.e., (15.90 q/ha) however CBR shows that Vermicompost @ 10 ton/h economically cost benefit treatment (1:1:54).

Raj *et al.* (2016) conducted studies to integrate the treatment of soil solarization with soil amendments of neem cake, neem granules and biofumigants for management of carnation wilt caused by *Fusarium oxysporum f. sp. dianthi*. Combination of soil solarization with soil amendment with neem cakes or *T. viride* formulation was more effective than soil drenching with chemical fungicides in improving the plant growth. Among all the treatments, integration of soil

solarization with soil application of *Trichoderma viride* followed by amendment of neem cake was found effective in improving shoot length.

Jat *et al.* (2017) conducted trials with various organic amendments i.e neem oil cake, groundnut cake, mustard cake and poultry manure for management of coriander wilt caused by *Fusarium oxysporum*. Studies on the relative efficacy of organic cakes in field conditions showed neem oil cakes was most effective followed by mustard cake. Neem oil cake recorded 21.45 % disease incidence with 52.25% disease control.

Pandey *et al.* (2017) To find out the effective management of the chickpea diseases through seed biopriming and soil application of biocontrol agents, the field studies were conducted during Rabi season of 2013-14 and 2014-15. Seed biopriming checked the incidence of wilt and root rot in the range of 45-60% and increased the yield of chickpea by 10-20%. However, combined applications of seed biopriming as well as soil application significantly checked the disease incidence in the range of 46-78% and increased the grain yield by 13-27%. The disease control and yield enhancement were highest with *T. viride* followed by *T. harzianum*. The pooled result of two years revealed that soil application of *Trichoderma viride* or *T. harzianum* (2×10^8 cfu/g) enriched FYM (10 kg bioagent/ ton FYM) in furrow @ 1 ton/ ha, followed by seed biopriming at the time of sowing i.e., soaking of chickpea seeds for 10 hrs in suspension of talc based formulation 1% WP (2×10^8 cfu/g) of *T. viride* or *T. harzianum*, respectively @ 50 g product/ 250 ml of water/ kg seed and shade dried for the effective management of wilt and root rot complex.

Chudasama and Pithia (2018) conducted field studies for effective management of chickpea wilt caused by *Fusarium oxysporum f. sp. ciceri*. Seed treatment checked the wilt incidence and increase the yield of chickpea. Seed treatment with *Trichoderma harzianum* checked the wilt incidence (3.80%) and increased the grain yield (1352kg/ha).

Kripalini *et al.* (2019) conducted a detailed survey for disease incidence of pea wilt at seven pea growing districts of Manipur viz., Imphal East, Imphal West, Thoubal, Senapati, Ukhrul, Tamenglong and Bishnupur during 2014-2015. Under pot experiment, efficacy of four native *Trichoderma* isolates i.e., TIE-1 TBI-2, TIW-1, TSE-1 and two commercial *Trichoderma spp.* viz., *T. viride* and *T. hamatum* of seed, soil and seed plus soil treatment against wilt of pea showed reduction in disease incidence. Among the different treatments, seed+soil treatment was found better than the soil and seed treatment alone. The highest germination percentage was observed in isolate TIE-1 of seed (60%), soil (66.60%) and seed+soil treatment (80.20%) and lowest germination percentage was observed in commercial *T. hamatum* of seed (53.60%), soil (60.00%) and seed+soil treatment (65.00%). Disease incidence of four native *Trichoderma* isolates and two commercial *Trichoderma* were found nil in 30 DAS. In 60 DAS, the highest disease incidence was

found in commercial *T. hamatum* in seed treatment (20.00%) and lowest (2.50%) disease incidence was found in native *Trichoderma* TSE-1 of seed+soil treatment whereas in 90 DAS (40.00%) highest disease incidence was also found in *T. hamatum* in soil treatment and lowest (10.66%) disease incidence was found in native *Trichoderma* isolate TIE-1 of seed+soil treatment. The highest plant height (70.28 cm) was recorded in seed+soil treatment of isolate TBI-2 at 90 DAS. However, in seed+soil treatment of *Trichoderma* isolate TIE-1 recorded the highest plant canopy (2085.03 cm²) at 90 DAS, no. of pods plant⁻¹ (5.34) and yield (6.91 g pot⁻¹).

Anjum N. *et al.* (2020) collected *Trichoderma* species *T. atroviride*, *T. hamatum*, *T. harzianum*, *T. longibrachiatum*, and *T. viride* and were evaluated for *in vitro* antagonistic potential against *Fusarium oxysporum f. spp. capsici* using the dual culture technique. *In vitro* results revealed that *T. hamatum* was the most potent in reducing the mycelial growth of *Fusarium oxysporum f. spp. capsici* with 70.15% inhibition followed by *T. atroviride*, *T. harzianum*, *T. longibrachiatum* and *T. viride* with 67.18%, 68.75%, 69.46% and 66.75% inhibition, respectively. The antifungal potential of five *Trichoderma* species was further evaluated under *in-vivo* conditions by applying spore suspension (1×10⁶ conidia/ml). *T. longibrachiatum* treatment showed the minimum disease severity of 24.7% under greenhouse conditions. *T. longibrachiatum* also promoted the length and weight of root and shoot. The other *Trichoderma* species showed moderated control of *Fusarium* wilt. Overall, *T. hamatum* and *T. longibrachiatum* were the most effective treatment for the control of *Fusarium* wilt under *in vitro* and *in vivo* conditions and generally, it depends on plant species tolerating disease.

Jamil *et al.* (2021) studied *Fusarium* wilt of tomato (*Solanum lycopersicum* L) incited by *Fusarium oxysporum f.sp. lycopersici* causes heavy yield loss due to the lack of a long-term management options for this pathogen. The study was undertaken to assess the efficacy of biocontrol agents *Trichoderma harzianum*, *T. viride* and *Aspergillus niger* against wilt fungus. All biocontrol agents produced significant inhibitory effect on pathogen development. *T. viride* caused maximum reduction (83.56%) in growth and fresh (332.49 mg) and dry weight (52.19 mg) of the pathogen. In pot trial, seed treatment with *T. viride* caused minimum disease severity (1.2 %) and maximum significant increase in plant fresh (60.23 g) and dry weight (23.82 g), plant length (63.91 cm) and yield/plant (258.05 g). *T. harzianum* seed treatment showed highest increase in chlorophyll (3.04 mg/g fresh weight) and lycopene content (75.43 mg/kg fresh weight) whereas *T. viride* seed treatment showed maximum total phenols (93.58 µg catechol/g fresh weight).

Bawane *et al.* (2022) carried out tests for the management of *Fusarium* wilt using botanicals, organic amendments, bio-agent and a fungicide (treated check). Treatments were evaluated *in-vitro* and *in-vivo* in plants against *Fusarium* wilt and readings were taken at 30, 60

and 90 DAS for growth parameters and the percentage of disease incidence were calculated. Among the treatments, soil application with *Trichoderma viride* were found most effective in the percentage of disease control in chickpea followed by Neem cake as compared to Carbendazim (treated check) and control. The maximum plant height, dry shoot weight, dry root weight and yield were found in *Trichoderma viride* treated plants. Thus, *Fusarium* wilt could be managed by the integrated of various approach like, soil application of bio-agent and soil amendment.

CHAPTER III : MATERIAL AND METHODS

All the experiments on the present research work “**Studies on wilt of green gram [*Vigna radiata* (L.) wilczek] incited by *Fusarium* spp.**” was carried out in the Department of Plant Pathology and Experimental farm of Department of Agronomy, College of Agriculture, Dr, Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, District. Ratnagiri (M.S).

A detail description about various material, techniques and methods used during the pursuing of research work is given in the succeeding pages.

3.1 MATERIALS REQUIRED

3.1.1 Collection of disease samples

Naturally infected green gram plants showing typical wilt symptoms were collected from research field at Department of Agronomy, College of Agriculture, Dapoli. The Disease sample was wrapped in a separate paper bag and brought to the laboratory at Department of Plant Pathology, College of Agriculture, Dr. B. S. K. K. V, Dapoli for further investigations.

3.1.2 Glassware

The common glassware (Make: Borosil) viz., Petri plates, test tube, conical flasks, measuring cylinders, beakers, glass slides, glass rods, pipettes were obtained from the Department of Plant Pathology during the course of investigation.

3.1.3 Equipments

The common laboratory equipment's viz., Autoclave, Hot air oven, Laminar air flow cabinet, BOD incubator, Refrigerator, pH meter, Binocular research microscope, compound microscope, electronic balance and other equipment's available at the Department of Plant Pathology, Dapoli were used for research work.

3.1.4 Culture media

Potato Dextrose Agar (PDA) was used for isolation, purification, multiplication and maintenance of pure culture of the test pathogen and also for various *in-vitro* studies of organism. The culture media such as Potato dextrose agar (PDA), Oat meal agar (OMA), Martin's agar (MA), Czapek's dox agar (CDA), Richard's agar (RA), Sabouraud's dextrose agar (SA), Conn's agar (CA) and Asthana and Hawker's agar (AHM) medium were used for morphological and cultural studies of the isolated pathogen.

3.1.5 Chemicals

All the chemicals viz., Sodium hypochlorite, Formaldehyde used in different experiments were of scientific grade. The antibiotic Streptomycin was used to avoid the bacterial contamination in the media.

3.1.6 Fungicides

For the purpose of seed treatment in integrated management of the disease, fungicides from different fungicidal groups were obtained from the Department of Plant Pathology, College of Agriculture, Dr. B. S. K. K. V, Dapoli.

Table 2: List of Fungicides

Sr. No.	Common Name	Formulation	Manufacturer/Marketed
1.	Carbendazim	50% WP	Gharda Chemicals Limited
2.	Captan	50% WP	Indofil Chemicals Limited

3.1.7 Bio-agents

Three different strains of bio control agent of *Trichoderma* were obtained from the Department of Plant Pathology, College of Agriculture, Dr. B. S. K. K. V, Dapoli were used for seed treatment in integrated management of the disease.

Table 3: List of Bioagents

Sr. No.	Name of Bioagent
1.	<i>Trichoderma harzianum</i>
2.	<i>Trichoderma longibrachiatum</i>
3.	<i>Trichoderma koningii</i>

3.1.8 Miscellaneous material

Earthen pots, plant protection appliances, Transparent polyethene sheet, Neem cake, blotter paper, inoculating needles, forceps, polythene bags, staple, spirit lamp, sodium hypochlorite, labels, scales, marker, nylon rope, sand, soil and FYM etc. were used during the course of present investigation.

3.2 Methodology

3.2.1 Cleaning of glassware

The glassware's were suspended in cleaning solution containing 60 g potassium dichromate ($K_2Cr_2O_7$) and 60 ml concentrated sulphuric acid (H_2SO_4) in 1000 ml water for 24 hrs followed by washing with tap water and then finally rinsed with distilled water.

3.2.2 Sterilization

All the glassware were sterilized in hot air oven at 160^0 C for 1 hr and media used were sterilized in an autoclave at 1.054 kg/cm² (121^0 C) pressure for 20 minutes. Surface disinfection of plant tissue was done by using 0.1% mercuric chloride, followed by washing in three sequential changes with sterile distilled water. Under aseptic conditions of laminar air-flow cabinet, isolation or inoculation of fungus was performed. By using denatured spirit or 70 % ethanol, the working hands and working bench were also disinfected. Minor metallic tools like blades, inoculation needles, cork borer, forceps, etc. were frequently sterilized by ethanol or heating on flame of the spirit lamp.

3.2.3 Preparation of culture media

Potato dextrose agar medium was used for isolation, pathogenicity test, biocontrol agents and preserving stock culture of test pathogen.

Table 4: Composition of PDA medium

Sr. No.	Ingredients	Composition g/ml
1	Peeled Potato	200.0 g
2	Dextrose	20.0 g
3	Agar	20.0 g
4	Distilled Water	1000.0 ml

Potato Dextrose Agar (PDA) medium was used as basal medium for *in vitro* studies of fungus and maintenance of fungal cultures. For making 1 litre of PDA, 200g of potatoes were peeled, chopped and boiled in 500 ml of water. Agar-agar (20 gm) was dispensed and boiled to melt in remaining 500 ml of distilled water. The potato extract was collected by filtering through muslin cloth and filtrate was added to the molten agar. By adding 20 g of dextrose, the final volume was made upto 1000ml using distilled water. The pH of the medium was adjusted to 6.5. Later the media was sterilized in an autoclave at 1.054 kg/cm² (121^0 C) pressure for 20 minutes.

3.2.4. Isolation and Pathogenicity of the fungus causing wilt disease

3.2.4.1. Isolation

The pathogen was isolated by following standard Tissue Isolation Technique. For isolation of the pathogen, naturally infected plant sample of green gram showing typical symptoms of wilt was collected from green gram research field at Department of Agronomy College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, and brought to the laboratory for further isolation.

Diseased sample were washed thoroughly in running tap water, blot dried and cut with sharp sterilized blade into small bits (5mm), having half healthy and half diseased portion intact. Disinfection of bits of sample was done by dipping in 0.1% solution of mercuric chloride for one and half minute. Later such bits were passed through three Petri plates containing distilled sterile water to remove traces of 0.1% HgCl₂. These bits were dried on dry sterilized blotter paper and inoculated on solidified Potato dextrose agar (PDA) medium in already poured Petri plates under aseptic conditions. These inoculated plates were then incubated at room temperature ($27 \pm 2^{\circ}\text{C}$) for seven days and observed for the growth of associated organism. The slants with pure fungal growth were maintained in pure form by preserving in refrigerator at 4°C for further use.

3.2.4.2 Proving pathogenicity of fungus.

Pathogenicity test of the isolated fungus was carried out on highly wilt disease susceptible green gram cultivar 'TMB-37' using standard Sick soil method of inoculation. For this purpose, autoclaved potting mixture of soil: sand: FYM (2:1:1) were filled into disinfected pots. Pure sporulating culture of test fungus isolated from infected green gram plant was used to prove its pathogenicity. Seven days old pure culture of test fungus was used to make spore suspension through homogenization of mycelial mass in sterile distilled water followed by filtration through muslin cloth. The spore cum mycelial suspension was incorporated in top 5-6 cm layers of the pots containing potting mixture, watered lightly and maintained in green house for two weeks, so as to proliferate the test pathogen and make the potting mixture sick with *Fusarium oxysporum*. Surface sterilized healthy seeds of susceptible green gram cultivar were sown (10 seeds/pot) in the pots, watered lightly and maintained. The plants were observed regularly for the appearance and development of disease symptoms. As the symptoms of the disease appeared, the fungus was re-isolated from these plants and re-isolation of pathogen was done aseptically on PDA medium. After a week of incubation at $27 \pm 2^{\circ}\text{C}$, the cultural and morphological characteristics of the reisolated fungus were observed and it was compared with the characteristics (cultural and morphological) of the original fungus.

3.2.4.3 Identification of the organism

The morphological characteristics of the causal fungus were studied on the host and in the culture grown on PDA. Identification of isolated fungus was carried out on the basis of colony characteristics, spore shape and size with the help of illustrated books and CMI description. Further, the culture identification of the fungus was confirmed by the mycologist, Department of Plant Pathology, College of Agriculture, Dapoli.

3.2.5 Cultural and morphological characteristics of the fungus causing wilt disease.

3.2.5.1 Cultural Characters of causal fungus

Eight different culture media were used in the present investigation. Media were prepared with given ingredient as per the required quantity. Other procedure was same as mentioned earlier as preparation of culture media.

Media used and their Ingredients

1. Oat meal agar medium

1.	Oat meal	:	60 g
2.	Agar- agar	:	20 g
3.	Distilled water	:	1000 ml

2. Conn's agar medium

1.	Potassium nitrate	:	2 g
2.	Magnesium sulphate	:	1.2 g
3.	Monopotassium dihydrogen phosphate	:	2.7 g
4.	Sucrose	:	7.2 g
5.	Potato starch	:	10 g
6.	Agar-agar	:	20 g
7.	Distilled water	:	1000 ml

3. Sabouraud's dextrose agar medium

1.	Sucrose	:	40 g
2.	Peptic digest of animal tissue	:	10 g
3.	Agar-agar	:	20 g

4. Distilled water : 1000 ml

4. Asthana and Hawker's medium

1. Potassium nitrate : 3.5 g

2. Monopotassium dihydrogen phosphate : 1.75 g

3. Magnesium sulphate : 0.75 g

4. Glucose : 5 g

5. Agar-agar : 20 g

6. Distilled water : 1000 ml

5. Czapek's dox agar medium

1. Sucrose : 30 g

2. Sodium nitrate : 2 g

3. Dipotassium hydrogen phosphate : 1 g

4. Magnesium sulphate : 0.5 g

5. Potassium chloride : 0.1 g

6. Ferric sulphate : 0.01 g

7. Agar-agar : 20 g

8. Distilled water : 1000 ml

6. Richard's agar medium

1. Potassium nitrate : 10 g

2. Monopotassium dihydrogen phosphate : 5 g

3. Magnesium sulphate : 2.5 g

4. Ferric chloride : 0.02 g

5. Sucrose : 50 g

6. Agar-agar : 20 g

7. Distilled water : 1000 ml

7. Martin's agar medium

1. Peptic digest of animal tissue : 23 g

2.	Starch	:	1 g
3.	Sodium chloride	:	5 g
4.	Dextrose	:	2.5 g
5.	Agar-agar	:	20 g
6.	Distilled water	:	1000 ml

8. Potato dextrose agar medium

1.	Peeled potato	:	200 g
2.	Dextrose	:	20 g
3.	Agar-agar	:	20 g
4.	Distilled water	:	1000 ml

Above listed media were prepared as per the requirement and were poured into sterilized Petri plates, separately. After solidification, 5 mm mycelium disc of the test fungus from actively growing 7 days old culture were cut with the help of sterilized Cork borer and single disc was placed at the center of each Petri plate and incubated at $27 \pm 2^{\circ}$ C. Each treatment was replicated three times. The measurement of the colony diameter was taken when the maximum mycelium growth was achieved in any of the media tested. The cultural character such as colony diameter, colony colour and colony character were recorded.

3.2.5.2 Morphological characters of causal fungus

The slide cultures techniques were followed (Booth, 1971) to study the morphological character of the test fungus. The pathogen grown on PDA for seven days at room temperature $27 \pm 2^{\circ}$ C with 90 per cent humidity. Temporary mounts of fungal structures viz., mycelium and conidia were prepared and observed under light microscope at magnification of 45 x or 100 x. The filar micrometre was calibrated with the help of stage micrometre prior to measurements. Measurements of each structure were recorded from five to eight slides at three microscopic fields randomly. For conidial measurements, 20 observations were taken while for all other structures, 15 observations were recorded.

3.2.6 To study *in vivo* (pot) cross infectivity potential of test fungus.

Cross infectivity of *Fusarium oxysporum* isolated from the green gram causing wilt disease is tested with different pulses viz., chickpea (*Cicer arietinum*), cowpea (*Vigna unguiculata*), black

gram (*Vigna mungo*), dolichos bean (*Lablab purpureus*), horse gram (*Macrotyloma uniflorum*) and moth bean (*Vigna aconitifolia*).

The test was carried out using sick soil inoculation technique. For this purpose, autoclaved potting mixture of soil: sand: FYM (2:1:1) were filled into disinfected pots. Pure sporulating culture of test fungus isolated from infected green gram plant was used to check its cross infectivity potential. Seven days old pure culture of test fungus was used to make spore suspension through homogenization of mycelial mass in sterile distilled water followed by filtration through muslin cloth. The spore cum mycelial suspension was incorporated in top 5-6 cm layers of these pots containing potting mixture, watered lightly and maintained in green house for two weeks, so as to proliferate the test pathogen and make the potting mixture sick with *Fusarium oxysporum*. Surface sterilized healthy seeds of various pulses mentioned above were sown (8seeds/pot) in these pots, watered lightly and maintained. The plants were observed regularly for the appearance and development of disease symptoms and checked whether the fungus shows cross pathogenicity or not.

3.2.7 Integrated management of *Fusarium* wilt disease.

A field experiment was laid out in randomized block design with three replications. Soil solarization treatment was given for 45 days (March - April) to entire plot using transparent polyethene sheet (0.05mm) except control which was left opened (without sheet). Fungicides and bioagents were selected and used along with soil solarization treatment for integrated management of *Fusarium* wilt disease of green gram. The fungicides and bioagents were applied as seed treatment to the healthy green gram seeds which were used for sowing after soil solarization was done. For each treatment 3 replications were maintained. The plot without any treatment was maintained as untreated control.

Experiment details:

Design : Randomized Block Design (RBD)

Treatments : Eight

Replications : Three

Table 5 : Treatments details for Integrated management of *Fusarium* wilt

Tr No.	Treatments
T ₁	Soil Solarization
T ₂	Soil Solarization + Soil application of neem cake @ 1.5 tonnes/ha
T ₃	Soil Solarization + Seed treatment with <i>Trichoderma harzianum</i> @ 6g/kg
T ₄	Soil Solarization + Seed treatment with <i>Trichoderma koningii</i> @ 6g/kg
T ₅	Soil Solarization + Seed treatment with <i>Trichoderma longibrachiatum</i> @ 6g/kg
T ₆	Soil Solarization + Seed treatment with captan 50% WP@ 2g/kg
T ₇	Soil Solarization + Seed treatment with carbendazim 50% WP @ 2g/kg
T ₈	Control

Germination (%), wilt incidence (%) and % Reduction over control were calculated by applying following formulae.

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds sown}} \times 100$$

$$\text{Wilt incidence (\%)} = \frac{\text{Total number of wilted plants}}{\text{Total number of plants observed}} \times 100$$

$$\text{Reduction (\%)} = \frac{C - T}{C} \times 100$$

Where,

T = Wilt Incidence (%) in treatments plot.

C = Wilt Incidence (%) in untreated control plot.

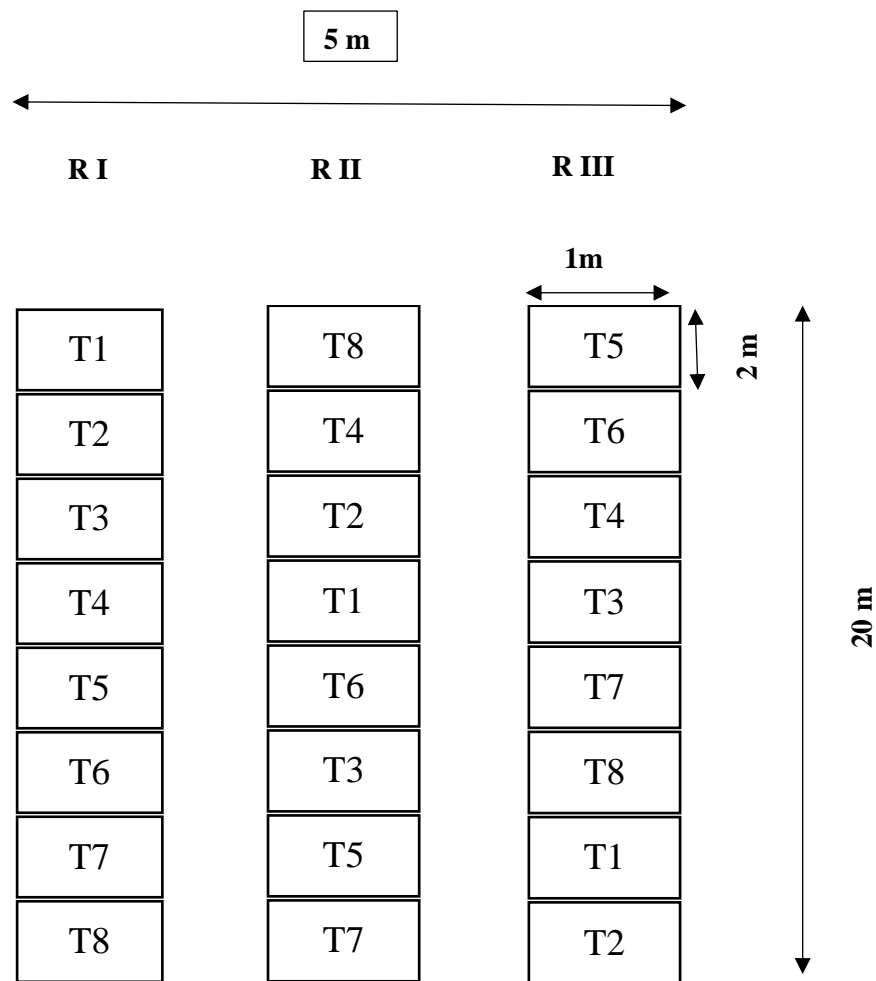


Fig 1: Layout of the Experimental Field

3.2.7.1 Seed yield

The crop was harvested at its physiological maturity, sun dried and threshed manually and seed yield was taken.

3.3. Statistical analysis

The data generated of all the experiments was statistically analyzed (Panse and Sukhatme, 1967) and results thereof will be interpreted. The per cent values were transformed into arsine values.

3.4. Collaboration (if any)

Nil.

CHAPTER IV: RESULTS AND DISCUSSION

The present investigation entitled, “Studies on wilt of green gram [*Vigna radiata* (L.) wilczek] incited by *Fusarium oxysporum*”, was undertaken during 2022-23 at Department of Plant Pathology and Department of Agronomy, College of Agriculture, DBSKKV, Dapoli. As per the objectives defined, the results are emphasised and summarized in this chapter, below various sub-heads.

4.1 Isolation and pathogenicity of fungus causing wilt disease in green gram.

4.1.1 Isolation of causal organism

The plant showing typical symptoms of wilt disease on green gram were collected from the field and brought to the laboratory in paper bag. To confirm the presence of associated fungus, microscopic examination of disease samples were made under compound microscope. The fungus associated with the diseased plant tissue was isolated by following standard tissue isolation technique on potato dextrose agar medium under aseptic conditions. After 7 days, cottony white fungal mycelium growth was observed around the inoculated plant tissue in a Petri plate. For the presence of conidia in a culture of isolated fungus, a loopful of pure fungal culture was mounted on glass slide in lactophenol cotton blue and observed under microscope. For maintaining pure culture of the isolated fungus, periodic transfer at the interval of every 15 days on potato dextrose agar medium in Petri plates and agar slants was done for further study.

Earlier work was carried out by Sun *et al.*, (2019) who isolated *Fusarium oxysporum* from stems of diseased plants of green gram using standard tissue isolation method. Vani *et al.*, (2019) reported mung bean samples showing distinct wilt symptoms and isolated *Fusarium oxysporum* using isolation technique. Soliman *et al.*, (2016) also carried out isolation of *Fusarium oxysporum* from diseased mung bean plants showing different degrees of wilt disease. Choudhary *et al.*, (2017) using taxonomic and morphological references identified the isolated pathogen as *Fusarium oxysporum f.sp. vigni* from the infected disease samples of mung bean plants.

4.1.2 Proving of Pathogenicity

4.1.2.1 Inoculation

The pathogenicity of the isolated fungus, proved by sick soil technique in pots, by growing plants of susceptible green gram cultivar TMB-37. After 20-25 days of fungus inoculation initial symptoms appeared were yellowing or a slight wilting and drooping of the lower leaves on a stem. Leaves turn yellow, dried and remain still attached to the plant (yellow-flagging appearance). At advanced stage, browning of the vascular system and finally death of entire plant. These symptoms were similar to that of wilted green gram plants. The pathogen was reisolated

from these artificially wilted plants on PDA plates, which after incubation exhibited cottony white mycelial growth on the PDA medium.

The microscopic observations showed branched, septate and hyaline hyphae. The fungus produced microconidia, macroconidia and chlamydospores. Microconidia were round to cylindrical shaped and with 0-1 septa. Macroconidia were hyaline and sickle shaped with 3-5 septa. Chlamydospores were spherical in shape and were present singly or in chains. These morpho-cultural characteristics were similar to those of the pathogen isolated from naturally wilted green gram plants. Thus, proved the pathogenicity, by applying Koch's postulates.

4.1.2.2 Re-isolation

The similar type fungus was repeatedly isolated on potato dextrose agar medium from artificially inoculated green gram plants showing typical wilt symptoms. The morphological and cultural characteristics of the reisolated test fungus observed were exactly identical to that of the pure culture isolated from naturally wilt infected green gram plants tissues. Thus, the pathogenicity of isolated fungus was proved on green gram (cv: TMB-37) following Koch's postulates and the pathogen was confirmed as *Fusarium oxysporum* on the basis of colony and morphological characters with those described in literature as well as information available on various websites.

The results of present study are in accordance with Soliman *et al.*, (2016) who proved pathogenicity of *Fusarium Oxysporum* on Green gram by sick soil method. Pathogenicity of *Fusarium oxysporum f. sp. ciceri* also proved on chickpea by following sick soil technique Golakiya *et al.*, (2018) and Metsena *et al.*, (2021) also carried out pathogenicity test of *Fusarium oxysporum* on cowpea plant using soil inoculation technique.

4.2. To study cultural and morphological characteristics of the fungus causing wilt disease.

4.2.1 Cultural characteristics

The cultural characters were studied on eight different solid media. The radial growth of *F. oxysporum* was measured and colony characters such as colony color, elevation, margin were recorded.

4.2.1.1 Colony radial growth

The data represented in the table 6, revealed that *F. oxysporum* exhibited variation in growth on eight different media under study. All the treatments were statistically significant. In terms of radial growth, the growth on potato dextrose agar was the maximum (90.00 mm) followed by richard's agar (85.30 mm), oat meal agar (79.10 mm), czapek's dox agar (78.10 mm), martin's agar (74.90 mm), conn's agar (74.50 mm), Asthana and hawker's medium (64.60 mm) and sabouraud's agar medium (54.90 mm).



PLATE II: Pathogenicity of *F. oxysporum* on green gram (cv. TMB-37)



Front view



Rear view

PLATE I: Pure culture of *Fusarium oxysporum* on PDA medium

4.2.1.2 Colony Color

The growth on oat meal agar, conn's agar, asthana and hawker's medium, czapek's dox agar, richard's agar and potato dextrose agar was dense cottony white while growth on sabouraud's agar and martin's agar was sparse white.

In rear view, the growth of *F. oxysporum* on oat meal agar, conn's agar, asthana and hawker's medium, czapek's dox agar, richard's agar showed whitish pigmentation while pale yellow pigmentation was observed on sabouraud's agar and potato dextrose agar. Light brown pigmentation was recorded on martin's agar in rear view.

4.2.1.3 Elevation

Raised elevation of growth was observed on oat meal agar, conn's agar, asthana and hawker's medium, czapek's dox agar, richard's agar and potato dextrose agar were having raised elevation whereas flat elevation on sabouraud's agar and martin's agar.

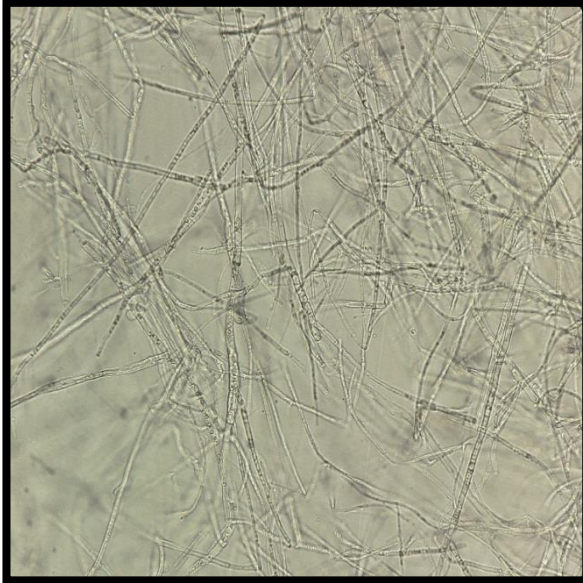
4.2.1.4 Margin

Variation in margin of growth of *F. oxysporum* was observed on various media. The margin of growth was regular found on oat meal agar, conn's agar, czapek's dox agar, richard's agar and potato dextrose agar, while it was irregular on sabouraud's agar, asthana and hawker's medium and martin's agar.

Table 6: Cultural characters of *Fusarium oxysporum* on different solid media

Tr. No.	Medium Used	Mean Colony Dia.*(mm)	Colony Color		Elevation	Margin
			Front	Rear		
T1	OMA	79.10	Dense cottony white	Whitish	Raised	Regular
T2	CA	74.50	Dense Cottony white	Whitish	Raised	Regular
T3	SA	54.90	Sparse white	Pale yellow	Flat	Irregular
T4	AHM	64.60	Dense Cottony white	Whitish	Raised	Irregular
T5	CDA	78.10	Dense Cottony white	Whitish	Raised	Regular
T6	RA	85.30	Dense Cottony white	Whitish	Raised	Regular
T7	MA	74.90	Sparse white	Light brown	Flat	Irregular
T8	PDA	90.00	Dense Cottony white	Pale yellow	Raised	Regular
SE(m) ±		0.11				
C.D at 1%		0.34				

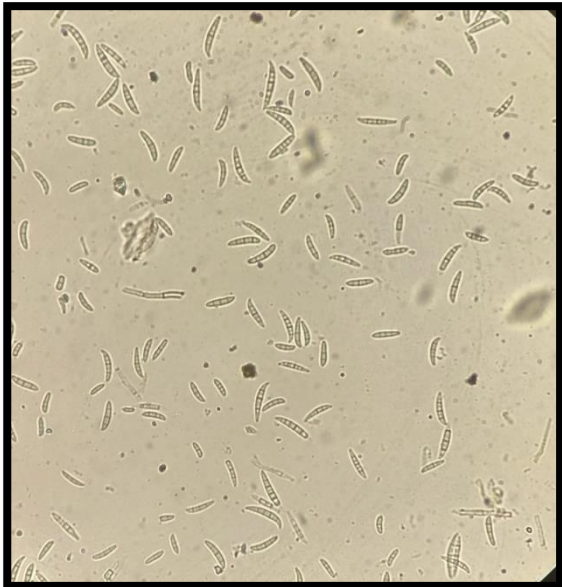
* Mean of three replications



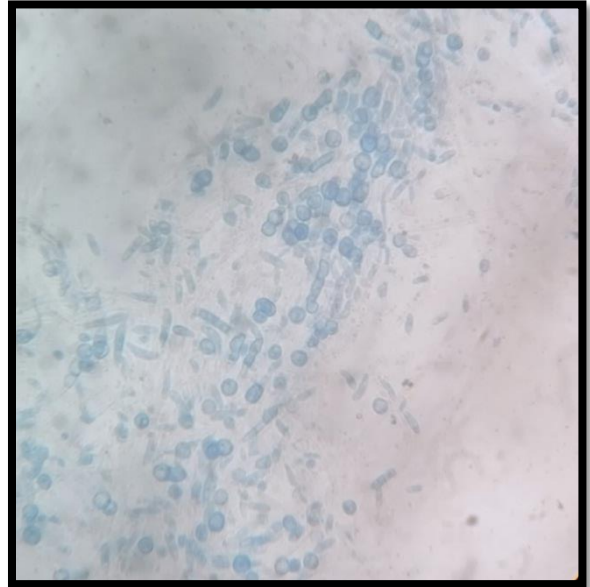
Mycelium



Micro conidia



Macro conidia



Chlamydospores

PLATE III: Microscopic view of *F. oxysporum*

TREATMENT DETAILS

(For Plate IV; Fig. 2)

Cultural characters of *F. oxysporum* on different solid media

Tr. No.	Medium used
T ₁	Oat meal agar (OMA)
T ₂	Conn's agar (CA)
T ₃	Sabouraud's dextrose agar (SA)
T ₄	Asthana and Hawker's medium (AHM)
T ₅	Czapek's dox agar (CDA)
T ₆	Richard's agar (RA)
T ₇	Martin's agar (MA)
T ₈	Potato dextrose agar (PDA)

LEGENDS

(For Fig. 2)

Cultural characters of *F. oxysporum* on different solid media

Tr. No.	Medium Used	Mean Colony Dia.*(mm)
T1	OMA	79.10
T2	CA	74.50
T3	SA	54.90
T4	AHM	64.60
T5	CDA	78.10
T6	RA	85.30
T7	MA	74.90
T8	PDA	90.00



PLATE IV: Cultural characters of *F. oxysporum* on different solid

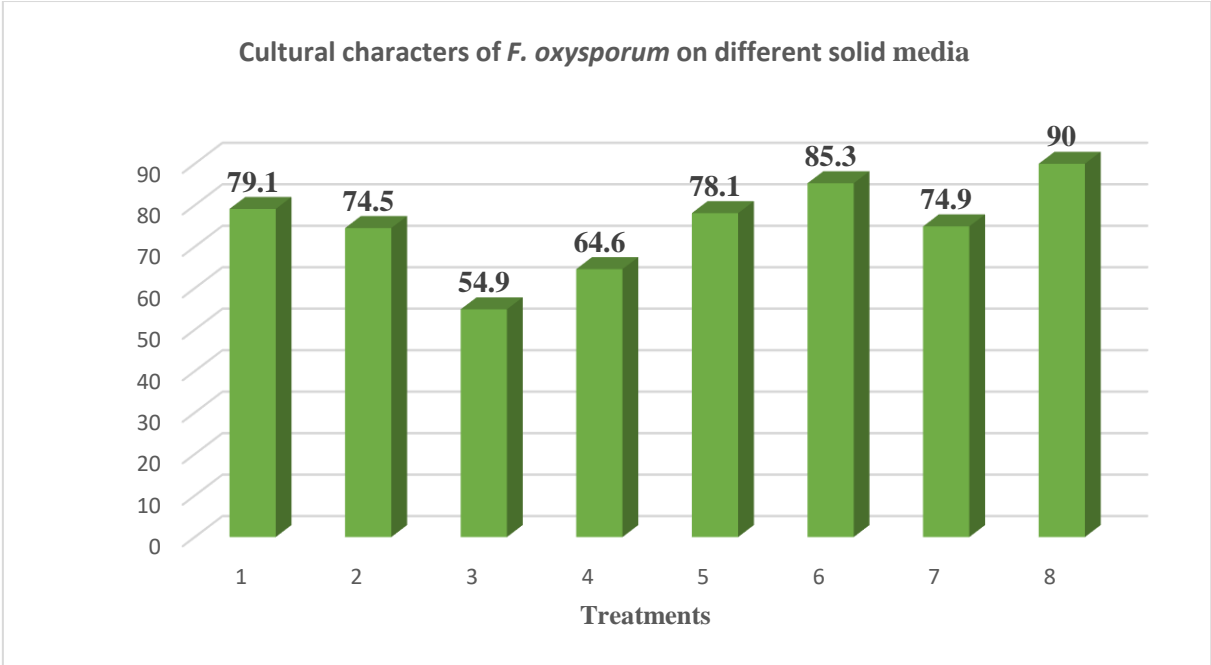


FIG 2: Cultural characters of *F. oxysporum* on different solid media

Golakiya *et al.*, (2018) reported that mycelial colour varied between dense cottony white to sparse cottony white on upper side of the culture and they also reported pigmentation varied from pale yellow to light brown on the lower side of the culture plate.

Nath *et al.*, (2017) studied physiological and morphological variation of *F. oxysporum f. sp. ciceri* isolates causing wilt disease in chickpea. They observed purplish white, whitish orange, creamy white, cottony white colour while irregular, regular, regular with sector, regular without sector shapes. They revealed variations in margins of colony like irregular, entire and wavy and in textures were fluffy, flat/velvet.

The inference drawn by Poorvasandhya *et al.*, (2020) who studied cultural characters *Fusarium oxysporum f. sp. ciceri* on seven different media also stated that maximum radial growth was on PDA (8.77mm) and on Czapek's Dox agar medium (7.80 mm). These conclusions are similar with present results in terms of Potato Dextrose Agar.

As per the results drawn by Singh and Kumar (2016) the best growth of *Fusarium oxysporum f. sp. lentis* was noticed on PDA and Richards agar media among the five culture media tested and least growth was on Asthana and Hawkera agar media. These results are similar with current results in reference to PDA and Richards agar media.

4.2.2 Morphological characters

Morphological characters were used for the identification of fungus. The morphological characters of the *Fusarium oxysporum* were as follows:

4.2.2.1 Mycelium

Microscopic observations revealed that the mycelium was hyaline and septate.

4.2.2.2 Conidia

Microconidia of the fungus were small, cylindrical in shape having 0-1 septa. Conidial size ranges from 9.88 μm x 2.35 μm .

Macroconidia were long, curved, sickle shaped, pointed at tip consisting of 3-5 septa and size ranged from 29.8 μm x 3.66 μm .

Chlamydoconidia were spherical in shape with size of 8.6 μm .

Characteristics were in close conformity to the characters reported for *F. oxysporum* by Golakiya *et al.*, (2018), who observed microconidia with size ranging from 15.52 μm x 4.82 μm , Macroconidia with size ranging from 22.46 μm x 5.84 μm and spherical shaped chlamydoconidia with size of m 7-14 μm .

Patra and Biswas (2016) also studied cultural and morphological characters of *Fusarium oxysporum f. sp. ciceri*. The size of macro-conidia was ranged from 13-15 x 2-3 μm to 15-19 x 3-4 μm and micro-conidia was from 3-4 x 1-2 μm to 5-6 x 2-3 μm . The number of septa in macroconidia was mostly 2-3 and micro-conidia were mostly no septum and some are 0-1.

Sun *et al.*, (2019) studied morphological characters of *Fusarium oxysporum* of mung bean wilt. The microconidia of the isolates on PDA were usually hyaline and elliptical, having a single spore or one septum, with an average size of $10.27 \times 3.14 \mu\text{m}$. The macroconidia were hyaline and sickle-shaped. They usually had three to four septa and were an average size of $29.91 \times 3.96 \mu\text{m}$.

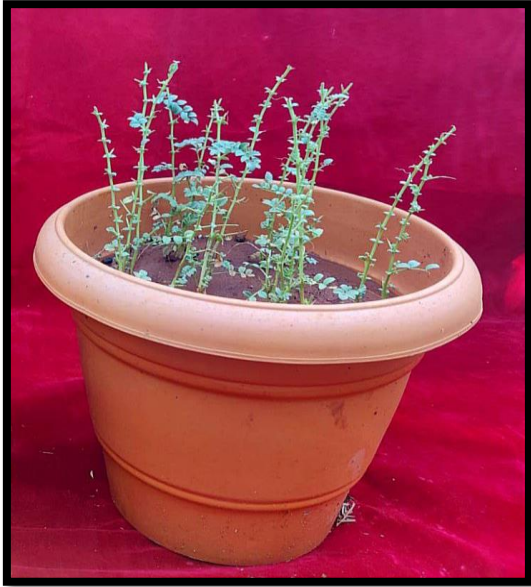
4.3 To study *in vivo* (pot) cross infectivity potential of test fungus.

The cross infectivity potential test was carried out using sick soil inoculation technique. For this purpose, autoclaved potting mixture of Soil: Sand: FYM (2:1:1) were filled into disinfected pots. Pure sporulating culture of *Fusarium oxysporum*, isolated from infected green gram plant was used to check its cross infectivity potential on six different pulses. Seven days old pure culture of *Fusarium oxysporum* was used to make spore suspension through homogenization of mycelial mass in sterile distilled water followed by filtration through muslin cloth. The spore cum mycelial suspension was incorporated in top 5-6 cm layers of these pots containing potting mixture, watered lightly and maintained in green house for two weeks, so as to proliferate the pathogen and make the potting mixture sick with *Fusarium oxysporum*. Surface sterilized healthy seeds of various pulses viz. chickpea (*Cicer arietinum*), cowpea (*Vigna unguiculata*), black gram (*Vigna mungo*), dolichos bean (*Lablab purpureus*), horse gram (*Macrotyloma uniflorum*) and moth bean (*Vigna aconitifolia*) were sown (8 seeds/pot) in these pots, watered lightly and maintained. The plants were observed regularly for the appearance and development of disease symptoms. From the observations taken it was revealed that the pathogen proved to be non-pathogenic to various different pulses inoculated with the *Fusarium oxysporum* as plants shown no wilt symptoms.

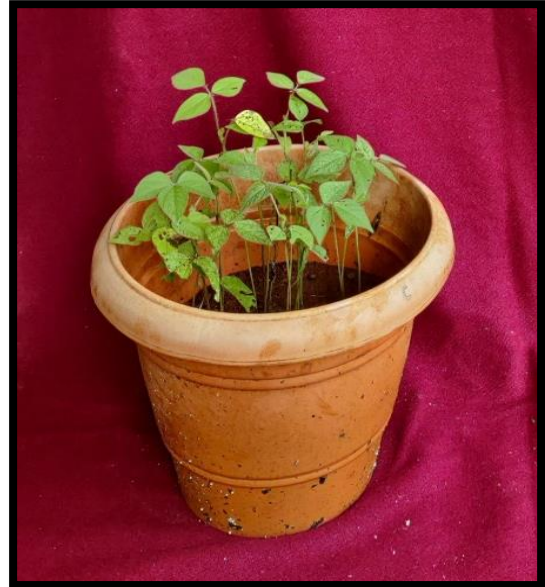
Table 7: Cross infectivity potential of *Fusarium oxysporum* on pulses

Sr No.	Host	Cross infectivity
1	Chickpea (<i>Cicer arietinum</i>)	Non- pathogenic
2	Cowpea (<i>Vigna unguiculata</i>)	Non- pathogenic
3	Black gram (<i>Vigna mungo</i>)	Non- pathogenic
4	Dolichos bean (<i>Lablab purpureus</i>)	Non- pathogenic
5	Horse gram (<i>Macrotyloma uniflorum</i>)	Non- pathogenic
6	Moth bean (<i>Vigna aconitifolia</i>)	Non- pathogenic

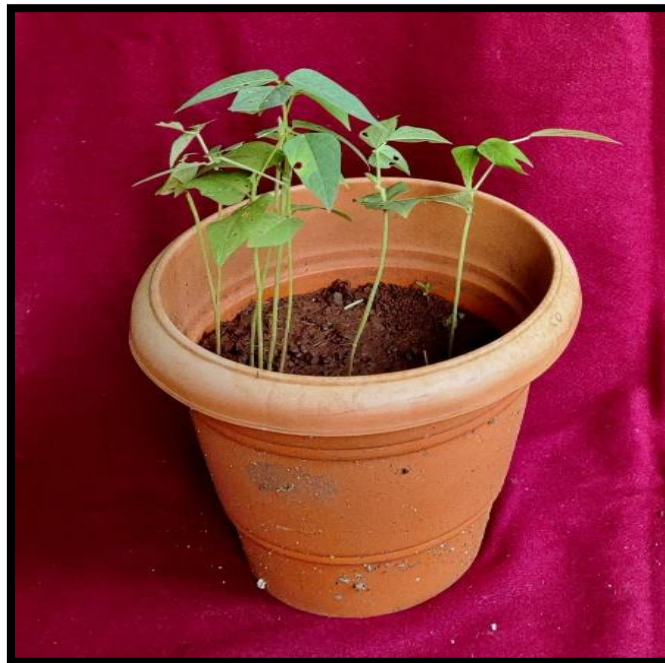
Same outcomes were recorded by Sun *et al.* (2019) who carried out the host range test of green gram against various other crops including chickpea, common bean, cowpea, pea, rice bean, soybean and cotton.



CHICKPEA



BLACK GRAM

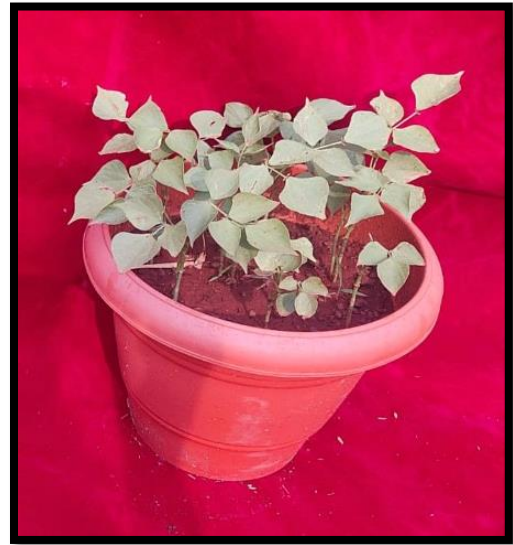


COWPEA

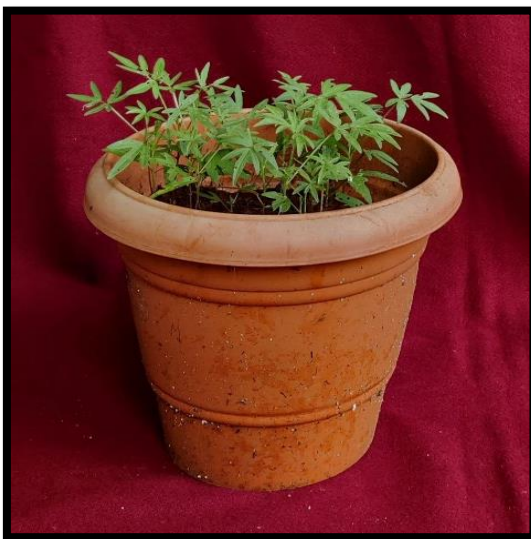
PLATE V: Cross infectivity on different pulses



HORSE GRAM



DOLICHOS BEAN



MOTH BEAN



GREEN GRAM

PLATE V : Cross infectivity on different pulses

4.4 Integrated management of *Fusarium* wilt disease.

An integrated approach for the management of the wilt of green gram caused by *Fusarium oxysporum* using various fungicides, bioagents, soil amendments along with soil solarization and soil solarization alone was carried out under field conditions during *Rabi* 2022-23.

Fungicides such as Carbendazim 50% WP and Captan 50% WP, Bio-agents such as *Trichoderma harzianum*, *T. koningii*, *T. longibrachiatum* and one organic amendment viz., Neem cake (soil application) were selected and integrated along with soil solarization to manage green gram wilt (*Fusarium oxysporum*).

Soil solarization treatment was done using transparent polyethylene mulch of 0.05 mm. Soil temperature was recorded using soil thermometer at 5cm soil depth. The average maximum temperature (morning) recorded in solarized plot was 26.9°C i.e., there was 2.9°C increase in temperature over non-solarized plot which was 24.0°C. The average maximum temperature (afternoon) recorded in solarized plot was 40.4°C i.e., there was 9.1°C increase in temperature over non-solarized plot which was 31.3°C. The average maximum temperature (evening) recorded in solarized plot was 38.3°C i.e., there was 8.2°C increase in temperature over non-solarized plot which was 30.1°C.

The seeds of susceptible green gram cv. TMB 37 were treated before sowing with the fungicides viz., carbendazim 50% WP, captan 50% WP, bioagents viz., *T. harzianum*, *T. koningii*, *T. longibrachiatum* and soil application of organic amendment viz., Neem cake

Fungicide and bio-agent treated seeds of green gram cv. TMB-37 were sown (30 cm x 10 cm) in randomized plots, block size per treatment: 2 m x 1m. The crop was grown by applying all recommended package of practices and irrigated as and when required.

4.4.1 Per cent germination

Results revealed that there was increased per cent germination by integrating treatments with various fungicides, bioagents and soil amendment along with soil solarization. The per cent seed germination significantly improved and it was observed in the range of 94.11 to 68.62. Highest seed germination was recorded in seed treatment with carbendazim 50% WP (94.11%) followed by captan 50% WP (90.19%), *T. harzianum* (88.23%), *T. longibrachiatum* (84.31%), soil application of neem cake (82.35%), seed treatment with *T. koningii* (80.39%) and least germination per cent was observed in soil solarization alone (74.50%) of green gram cv. TMB-37.

4.4.2 Per cent wilt incidence

All treatments significantly influenced the disease incidence which was recorded against untreated control. The wilt incidence recorded ranged from 8.33 per cent in carbendazim 50% WP to 26.32 per cent in solarization alone and 62.86 per cent in untreated control. The lowest wilt incidence recorded with carbendazim 50% WP (8.33%) followed by captan 50% WP (10.87%), *T. harzianum* (13.33%), *T. longibrachiatum* (16.28%), soil application of neem cake (19.05%), seed treatment with *T. koningii* (21.95%) and highest the wilt incidence observed in soil solarization alone (26.32%) which was found least effective.

4.4.3 Per cent disease control

All treatments significantly improved the disease control per cent as compared untreated control. The disease control per cent recorded ranged from 58.13 to 86.74 per cent over untreated control. The highest disease control per cent recorded with carbendazim 50% WP (86.74%) followed by captan 50% WP (82.71%), *T. harzianum* (78.79%), *T. longibrachiatum* (74.10%), soil application of neem cake (69.70%), seed treatment with *T. koningii* (65.08%) and the lowest disease control percentage was observed in soil solarization alone (58.13%).

Table 8: Daily observations of soil temperature during soil solarization

Date	Morning (° c)		Afternoon (° c)		Evening (° c)	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
22-02-2023	24.50	21.50	36.50	28.00	35.00	26.50
23-02-2023	24.00	21.00	37.00	27.50	34.00	25.00
24-02-2023	25.00	21.50	36.50	27.50	34.00	25.50
25-02-2023	24.00	21.00	36.50	27.00	34.50	25.00
26-02-2023	24.50	22.00	36.00	27.50	34.00	26.00
27-02-2023	25.00	21.50	37.00	28.00	35.50	27.00
28-02-2023	25.00	21.00	36.00	27.00	35.00	25.00
01-03-2023	24.50	21.50	36.50	27.50	35.00	26.00
02-03-2023	25.00	21.00	36.00	27.00	34.00	25.00
03-03-2023	25.00	21.50	36.50	27.00	35.50	26.50
04-03-2023	24.50	22.00	37.00	28.00	34.50	27.00
05-03-2023	24.00	21.00	38.00	27.50	35.00	25.00
06-03-2023	24.50	21.50	37.00	27.50	35.50	25.50
07-03-2023	24.50	22.00	38.50	28.00	36.00	26.50
08-03-2023	24.00	22.50	38.00	31.00	36.00	29.00
09-03-2023	24.50	22.00	39.00	34.00	37.00	30.00
10-03-2023	25.00	22.00	40.00	36.00	38.50	34.50
11-03-2023	27.00	25.00	40.50	36.00	39.00	35.00
12-03-2023	28.50	25.50	39.50	35.50	38.00	34.50
13-03-2023	28.00	26.00	41.00	36.50	39.00	34.00
14-03-2023	27.50	25.00	42.00	32.50	40.50	31.50
15-03-2023	29.00	26.00	40.00	32.50	39.00	32.00
16-03-2023	28.50	26.00	39.50	31.50	38.00	31.00
17-03-2023	28.00	26.00	42.00	33.00	40.50	32.50
18-03-2023	29.00	26.50	43.00	32.00	41.00	31.00
19-03-2023	28.50	25.50	42.50	32.50	40.50	31.50
20-03-2023	29.00	26.00	44.00	34.00	43.00	32.00
21-03-2023	29.00	25.00	38.00	29.50	37.00	29.00
22-03-2023	27.00	24.00	39.50	30.00	38.50	29.00
23-03-2023	28.00	25.00	43.00	33.00	42.50	32.00
24-03-2023	29.00	26.00	42.00	32.00	45.00	32.00
25-03-2023	28.00	25.00	42.50	33.00	42.00	32.50
26-03-2023	28.50	25.00	42.00	32.50	41.50	32.00
27-03-2023	28.00	24.00	43.00	32.50	41.50	31.00
28-03-2023	29.00	25.50	44.40	32.50	43.00	32.00
29-03-2023	28.00	25.00	44.00	33.50	42.50	32.00
30-03-2023	29.50	26.50	44.00	33.50	42.00	32.50
31-03-2023	29.50	26.00	45.50	34.00	45.00	33.50
01-04-2023	29.00	26.50	45.00	33.00	44.00	32.50
02-04-2023	28.50	26.50	45.50	34.00	44.50	33.00
03-04-2023	29.50	26.50	45.50	34.50	44.00	34.00
04-04-2023	29.00	26.50	45.00	34.50	44.50	34.00
05-04-2023	29.00	26.00	45.50	34.50	44.50	34.50



PLATE VI: Soil Solarization

Table 9: Integrated management of *Fusarium* wilt disease

Tr No.	Treatments	Germination (%)	Wilt Incidence (%)	Percent Reduction over control	Yield (q/ha)
T1	Soil Solarization	74.50 (59.67)	26.32 (30.86)	58.13	4.92
T2	Soil Solarization + Soil application of neem cake @ 1.5 tonnes/ha	82.35 (65.15)	19.05 (25.87)	69.70	6.30
T3	Soil Solarization + Seed treatment with <i>T. harzianum</i> @ 6g/kg	88.23 (69.93)	13.33 (21.41)	78.79	7.80
T4	Soil Solarization + Seed treatment with <i>T. koningii</i> @ 6g/kg	80.39 (63.71)	21.95 (27.93)	65.08	5.35
T5	Soil Solarization + Seed treatment with <i>T. longibrachiatum</i> @ 6g/kg	84.31 (66.66)	16.28 (23.79)	74.10	6.76
T6	Soil Solarization + Seed treatment with captan 50 % WP @ 2g/kg	90.19 (71.74)	10.87 (19.25)	82.71	8.42
T7	Soil Solarization + Seed treatment with carbendazim 50 % WP @ 2g/kg	94.11 (75.95)	8.33 (16.77)	86.74	9.85
T8	Control	68.82 (55.93)	62.86 (52.45)	-	3.85
SE(m) ±		0.63	0.50		2.62
C.D at 5%		1.82	1.53		7.97

The results of present investigation are in close consonance with Harender raj *et al.* (2016) who studied integrated management of carnation wilt caused by *Fusarium oxysporum f. sp. dianthi* using soil solarization and soil amendments.

Same results were recorded by Maheshwari *et al.* (2008) who tested the seven fungitoxicants against *Fusarium oxysporum f. sp. lentis* in which carbendazim was proved effective in checking the fungal growth followed by captan.

Same inferences were also drawn by Chauhan *et al.* (1988) who carried out field tests of soil solarization on pigeonpea (*Cajanus cajan* (L.) Millsp.) and chickpea (*Cicer arietinum* L.) against *Fusarium* wilt.

TREATMENT DETAILS
(For Plate VII)
Integrated management of *Fusarium* wilt

Tr No.	Treatments
T ₁	Soil Solarization
T ₂	Soil Solarization + Soil application of neem cake @ 1.5 tonnes/ha
T ₃	Soil Solarization + Seed treatment with <i>Trichoderma harzianum</i> @ 6g/kg
T ₄	Soil Solarization + Seed treatment with <i>Trichoderma koningii</i> @ 6g/kg
T ₅	Soil Solarization + Seed treatment with <i>Trichoderma longibrachiatum</i> @ 6g/kg
T ₆	Soil Solarization + Seed treatment with captan 50% WP@ 2g/kg
T ₇	Soil Solarization + Seed treatment with carbendazim 50% WP @ 2g/kg
T ₈	Control

LEGENDS
(For Fig. 3)
Integrated management of *Fusarium* wilt

Tr No.	Germination (%)	Wilt Incidence (%)	Percent Reduction over control (%)
T ₁	74.50 (59.67)	26.32 (30.86)	58.13
T ₂	82.35 (65.15)	19.05 (25.87)	69.70
T ₃	88.23 (69.93)	13.33 (21.41)	78.79
T ₄	80.39 (63.71)	21.95 (27.93)	65.08
T ₅	84.31 (66.66)	16.28 (23.79)	74.10
T ₆	90.19 (71.74)	10.87 (19.25)	82.71
T ₇	94.11 (75.95)	8.33 (16.77)	86.74
T ₈	68.82 (55.93)	62.86 (52.45)	-



PLATE VII: Experimental plot general view

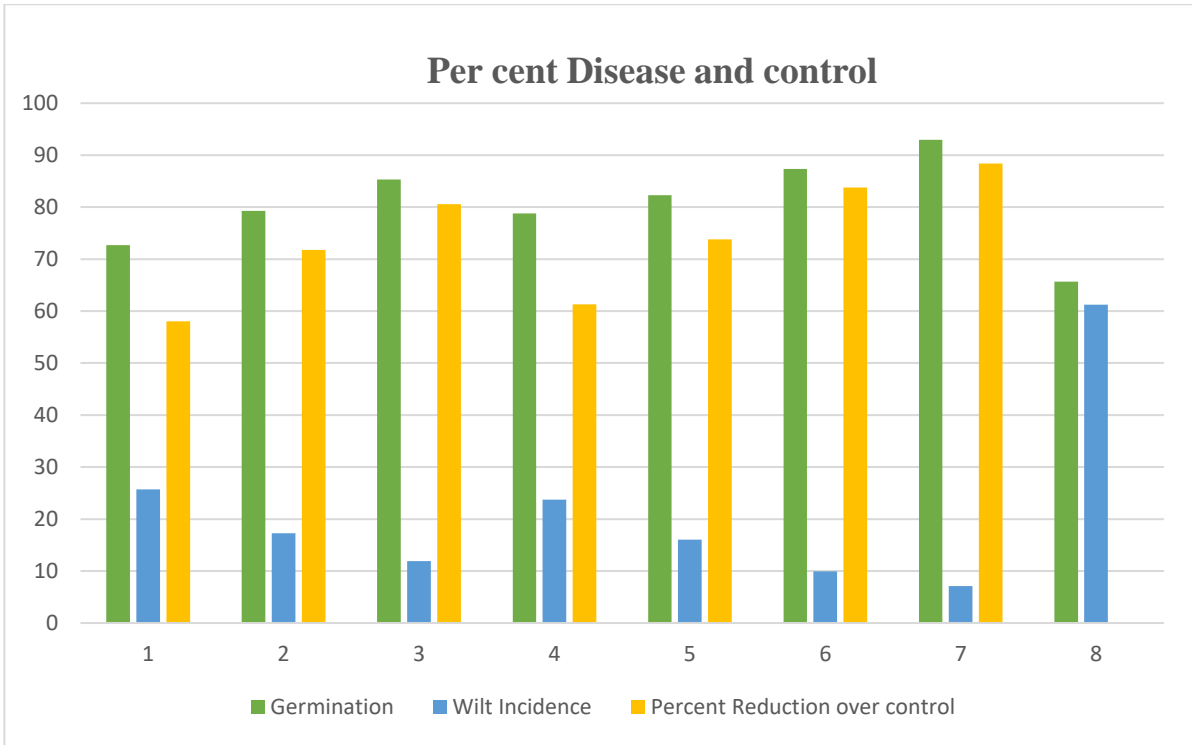


FIG 3: Per cent Disease and control

CHAPTER V : SUMMARY AND CONCLUSION

Green gram, one of the most economically important pulse crop growing in different pockets of India is attacked by many pathogens leading to cause serious diseases. Among the various diseases of green gram, wilt caused by *Fusarium oxysporum* is one of the most devastating, catastrophic and ruinous disease that causes accountable quantitative loss. The present investigation on wilt of green gram caused by *Fusarium oxysporum* was conducted at Department of Plant Pathology and Department of Agronomy, College of Agriculture, Dr. B.S.K.K.V., Dapoli during the year 2022-2023 on the aspects viz., isolation and pathogenicity, cultural and morphological characteristics, cross infectivity potential of the fungus and integrated management of *Fusarium* wilt disease.

The fungus associated with naturally infected plant was isolated on potato dextrose agar medium and pathogenicity of the test pathogen was proved on healthy and vigorously growing plant of green gram (cv.TMB-37) by following Koch's postulates and the fungus was identified as *Fusarium oxysporum*.

Green gram plant naturally infected with the *Fusarium* wilt disease, manifested typical symptoms such as yellowing or a slight wilting and drooping of the lower leaves on a stem. Leaves turn yellow, dried and remain still attached to the plant (yellow-flagging appearance). At advanced stage, browning of the vascular system and finally death of entire plant was recorded.

Healthy Green gram plants when inoculated artificially by sick soil technique of fungal mycelium-cum-spore suspension of the test fungus, typical symptoms of wilt disease were began to appear after 20-25 days of inoculation. Initially, lower leaves started yellowing followed by drying and still remain attached to the stem. In advanced stage, vascular system started browning and finally death of the plant was noticed after 40-45 days of the inoculation.

Cultural characters of *Fusarium oxysporum* on various different media were studied and revealed that the fungus grew well on potato dextrose agar medium followed by Richard's agar medium while on oat meal agar, Conn's agar, Asthana and Hawker's agar, Czapek's dox agar, Martin's agar it was fair and the least growth was observed on Sabouraud's dextrose agar. Fungus produced white cottony to sparse white mycelial growth. The fungal colonies were whitish, pale yellow to light brown in colour. The fungal colonies were having raised to flat elevation with regular or irregular margin.

Microscopic observation of pure culture of *Fusarium oxysporum* revealed the presence of hyaline and septate mycelium. Microconidia of the fungus were small, cylindrical in shape having 0-1 septa. Conidia size ranged from 9.88 μ x 2.35 μ . Macroconidia were long, curved, sickle

shaped, pointed at tip consisting of 3-5 septa and size ranged from 29.8 μ x 3.66 μ . Chlamydospores were spherical in shape with size of 8.6 μ .

For studying cross infectivity potential of the pathogen, different pulses were grown in the pots viz., chickpea (*Cicer arietinum*), cowpea (*Vigna unguiculata*), black gram (*Vigna mungo*), dolichos bean (*Lablab purpureus*), horse gram (*Macrotyloma uniflorum*) and moth bean (*Vigna aconitifolia*). Results revealed that the pathogen was non-pathogenic to various different pulses which were inoculated with the pathogen using sick soil technique. No wilt symptoms appeared on the plants.

Integration of various treatments like soil solarization alone and in combinations with various fungicides, bio-agents and organic soil amendment were found effective in reduction of the disease. All the treatments used in this were significantly involved in reduction of disease incidence.

Soil solarization treatment was done using transparent polyethylene mulch of 0.05 mm. Soil temperature was recorded using soil thermometer at 5cm soil depth. The average maximum temperature (morning) recorded in solarized plot was 26.9°C i.e., there was 2.9°C increase in temperature over non-solarized plot which was 24.0°C. The average maximum temperature (afternoon) recorded in solarized plot was 40.4°C i.e., there was 9.1°C increase in temperature over non-solarized plot which was 31.3°C. The average maximum temperature (evening) recorded in solarized plot was 38.3°C i.e., there was 8.2°C increase in temperature over non-solarized plot which was 30.1°C.

Among all the eight treatments, soil solarization along with seed treatment with carbendazim 50% WP @ 2g/kg resulted in effective reduction of the disease (86.74 %) followed by Soil solarization along with seed treatment with captan 50 % WP @ 2g/kg (82.71%), Soil solarization along with seed treatment with *T. harzianum* @ 6g/kg (78.79%), soil solarization along with seed treatment with *T. longibrachiatum* @ 6g/kg (74.10%), soil Solarization along with soil application of neem cake @ 1.5 tonnes/ha(69.70%), soil solarization along with seed treatment with *T. koningii* (65.08%) and soil solarization alone (58.13%) which was found the least effective.

From the present investigation the following conclusions can be drawn:

1. Green gram wilt caused by *Fusarium oxysporum* is the most devastating disease of green gram and one of the major biotic constraints in the production of green gram, responsible for heavy yield losses in konkan region.

2. The pathogen, *F. oxysporum* thrives best on Potato Dextrose Agar Medium with dense cottony white mycelial growth having pale yellow colour and having raised elevation with regular margin.
3. The pathogen lacks the cross infectivity potential on different pulses viz., chickpea, cowpea, black gram, dolichos bean, horse gram, moth bean.
4. The wilt disease in field can be effectively managed by using integrated management technique of soil solarization along with seed treatment with carbendazim 50 % WP @ 2g/kg.

LITERATURE CITED

- Agrios G. N. 1936. Plant Pathology 5th Edition. Elsevier Academic press, USA.523
- Alwathnani H. A., K. Perveen, R. Tahmaz and S. Alhaqbani 2012. Evaluation of biological control potential of locally isolated antagonist fungi against *Fusarium oxysporum* under *in vitro* and pot conditions. African Journal of Microbiology Research. 6(2):312-319.
- Animisha, S. Zacharia, K.K. Jaiswal and P. Pandey 2012. Integrated management of chickpea wilt incited by *Fusarium oxysporum f. sp. ciceris*. International Journal of Agricultural Research. 7(5): 284-290.
- Anjum N., Shahid A. A., Iftikhar S., Mubeen M., Ahmad M, M., Jamil Y., Rehan K., Aziz A., Iqbal S. and Abbas 2020. Evaluations of *Trichoderma* isolates for biological control of *Fusarium* wilt of chili. *Plant Cell Biotechnology and Molecular Biology*. 21:42-57.
- Anonymus 2021. Green gram outlook report January to May 2021. <https://angrau.ac.in>
- Armstrong G. M., Armstrong J. K., 1978. Formae speciales and races of *Fusarium oxysporum* causing wilts of the Cucurbitaceae. *Phytopathology*. 68:19-28.
- Aslam, S., Ghazanfar, M. U., Munir, N., & Hamid, M. I. 2019. Managing *Fusarium* wilt of pea by utilizing different application methods of fungicides. *Pakistan Journal of Phytopathology*. 31(1): 81-88.
- Avinash, M., Nagaraju, K., Muthuraju, R., & Asha, N. N. 2019. Pigeon pea *Fusarium* wilt: *In vitro* pathogenicity test of *Fusarium* isolates and its biological control. *Journal of Pharmacognosy and Phytochemistry* 8(6): 1600-1604.
- Ayele T. M., Gebremariam G. D. and Patharajan S. 2021. Isolation, identification and *in vitro* test for the biocontrol potential of *Trichoderma viride* on *Fusarium oxysporum f. sp. lycopersici*. *The open agriculture journal* .14:10-20.
- Bawane A. S., Zacharia S. and Seyoun R. K. 2022. Eco-friendly management of chickpea wilt *Fusarium oxysporum f. sp. ciceri* (Schlecht & Emend Synd. & Hans.) *Pharma Innovation*. 11(3):1284-1288.
- Biswal K., Sahu K.C., Ranasingh N. and Moharana R. L. 2020. *Fusarium oxysporum* causing black gram wilt, a new threat in the western undulated zones of odisha. *International Journal of Current Microbiology and Applied Sciences*. 9(6): 3027-3031.
- Booth C. (1971) Introduction to general methods. In: Booth C (ed), *Methods in Microbiology*. Vol 4. Academic Press, London, 1-48.
- Cafri D., Katan J., and Katan T. 2005. Cross-pathogenicity between Formae Speciales of *Fusarium oxysporum*, the pathogens of cucumber and melon. *Journal of Phytopathology*. 53(10):615-622.

- Carlos Alfonso López- Orona, Sergio Hernández-Verdug, Sixto Velarde-Félix and José Antonio Garzón-Tiznado & Ousmane Sy and Jesús Enrique Retes-Manjarrez. 2019. Cross pathogenicity of *Fusarium oxysporum* isolated from peppers. *European Journal of Plant Pathology*. 154: 1111–1112.
- Case A. 2009. Cross Pathogenicity of *Fusarium oxysporum f. sp. betae* on sugar beet and common bean. *Footprints in the Landscape: sustainability through plant and soil sciences*.
- Chaithra H. R., Manjunatha H., Saifulla M., and Pagala D. 2018. Pathogenic and morphological variability among *Fusarium oxysporum f. sp. ciceri* isolates causing wilt in chickpea. *Legume Research an International Journal*. 1-5.
- Chauhan, Y.S., Nene Y.L., Johansen, C, Haware, M P. Saxena, N.P., Sardar Singh, Sharma, S., Sahrawat, K. I., Burford, J R., Rupela, O P., Kumar Rao, J.V.D.K. and Sithanatham, S. 1988. Effects of soil solarization on pigeonpea and chickpea. India: International Crops Research Institute for the Semi-Arid Tropics. Research Bulletin no. 11. Patancheru, A.P. 502 324.
- Choudhary A., Shabbir A. and Musheer N. 2017. Screening of phytoextracts to control of *Fusarium oxysporum f. sp. vigni* incitant of mungbean (*Vigna radiata*) wilt. *International Journal of Academic Research and Development*. 2: 1181-1184.
- Chudasama M. K. and Pithia M. S. 2018. Integrated management of wilt disease in chickpea. *International Journal of Current Microbiology and Applied Sciences*. 7(2):951-956.
- Deepti S., Mudadla Hareesh and Dr. HC Lal. 2021. Study on variations in morphological and cultural characters of *Fusarium oxysporum f.sp. udum*. *The Pharma Innovation*. 10(6): 1070-1073.
- Ferniah R., Daryono B., Kasiamdari R. and Priyatmojo A. 2014. Characterization and pathogenicity of *Fusarium oxysporum* as the causal agent of *Fusarium* wilt in chilli. *Microbiology Indonesia*. 8:121-126.
- Gerlagh M. and Blok W. J. 1988. *Fusarium oxysporum f.sp. cucurbitacearum* embracing all formae speciales of *F. oxysporum* attacking Cucurbitaceous crops. *Netherlands Journal of Plant Pathology*. 94:17–31.
- Ghante P. H, Kanase K. M., Kale S. P., Chavan R. L., Sharma K. M., and Ghuge S. B. 2018. Cultural, morphological and molecular variability of *Fusarium oxysporum f. sp. udum* isolates by RAPD method. *International Journal of Current Microbiology and Applied Sciences*. 7(10) 2109-2122.
- Golakiya B. B., Bhimani M. D. and Akbari L. F. 2018. Characterization of Indian isolates of *Fusarium oxysporum f. sp. ciceri* causing chickpea wilt. *International Journal of Current Microbiology and Applied Sciences*. 7(3):1152-1162.

- Haseeb A., Sharma A., and Shukla P.K. 2005. Studies on the management of root-knot nematode, *Meloidogyne incognita*-wilt fungus, *Fusarium oxysporum* disease complex of green gram, *Vigna radiata* cv ML-1108. Journal of Zhejiang University Sciences. 6(8):73-742.
- Jamil A., Musheer N. and Manish kumar 2021. Evaluation of biocontrol agents for management of wilt disease of tomato incited by *Fusarium oxysporum f. sp. lycopersici*. Archives of Phytopathology and Plant Protection. 54:1722-1737.
- Jat M. K., Ahir R. R., Choudhary S. and Kakaraliya G. L. 2017. Management of coriander wilt (*Fusarium oxysporum*) through cultural practices as organic amendments and date of sowing. Journal of Pharmacognosy and Phytochemistry. 6(5):31-33.
- K. Venkataramanamma, Bhaskara Reddy B. V., Sarada Jayalakshmi R., V. Jayalakshmi, Prasad K. V. and Naidu Mohan G. 2019. Cultural, morphological and molecular characterization of *Fusarium Oxysporum f.sp. ciceris* inciting wilt of chickpea. Legume Research - An International Journal. LR-4178 :1-9.
- Kamdi D. R., Mondhe M.K., Jadesha G., Kshirsagar D. N. and Thakur K. D. 2012. Efficacy of botanicals, bio-agents and fungicides against *Fusarium oxysporum f. sp. ciceri* in chickpea wilt sick plot. Annals of Biological Research. 3(11):5390-5392.
- Khan M. R., Khan S. M. and Mohiddin F. 2004. Biological control of *Fusarium* wilt of chickpea through seed treatment with the commercial formulation of *Trichoderma harzianum* and/or *Pseudomonas fluorescens*. Phytopathology Mediterrean. 43:20–25.
- Khushboo Dubey and Singh S. 2018. Study cultural, morphological and pathogenic variation among different isolates of *Fusarium oxysporum f. sp. lentis*. International Journal of Current Microbiology and Applied Sciences. 7(9): 170-175.
- Kripalini N., Biswas M. K., Sobita Devi and Sinha B.2019. Studies on survey of *Fusarium* wilt of Pea (*Pisum sativum* L.) and its management by native *Trichoderma* Isolates and commercial *Trichoderma* under pot condition in manipur. International Journal of Bio-resource and Stress Management. 10(1):001-008.
- Maheshwari, S.K., Bhat, Nazir A., Masoodi, S.D. and Beig M.A.2008. Chemical control of lentil wilt caused by *Fusarium oxysporum f. sp. lentis*. Annals of Plant Protection Sciences. 16(2):419-421.
- Maina, P. K., Wachira, P. M., Okoth, S. A., & Kimenju, J. W. (2017). Cultural, morphological and pathogenic variability among *Fusarium oxysporum f. sp. phaseoli* causing wilt in French bean (*Phaseolus vulgaris* L.). Journal of Advances in Microbiology. 2(4): 1-9.
- Manju Kumari, Sharma, O.P., Nathawat B.D.S. (2021). Pathogenicity, host Range and influence of temperature, humidity and pH Levels on the Growth of *Fusarium*

- oxysporum f.sp. lentis*. Legume Research-An international journal. DOI: 10.18805/LR-4509.
- McMillan R. T. 1986. Cross pathogenicity studies with isolates of *Fusarium oxysporum* from either cucumber or watermelon pathogenic to both crop species. *Annals of Applied Biology*. 109:101-105.
- Metsena P., Sonda G., Kosma P., Fankou D. and Ndouvhad L. 2021. Identification of *Fusarium oxysporum f. sp. tracheiphilum* strains responsible of cowpea wilt in far-north region of Cameroon. *Journal of Applied Biosciences*. 164: 17001 – 17011.
- Morpathi S., Burrows M., Mgbechi Ezeri J., Agindotan B. 2021. *Fusarium* spp. associated with root rot of pulse crops and their cross-pathogenicity to cereal crops in montana. *Plant Diseases*.105(3):548-557.
- Mousa Najafinia and Sharma P. 2009. Cross pathogenicity among isolates of *Fusarium oxysporum* causing wilt in cucumber and muskmelon. *Indian phytopathology*. 62(1): 9-13.
- Nath N., Ahmed A. U. and Aminuzzaman F. M. 2017. Morphological and physiological variation of *Fusarium oxysporum f. sp. ciceri* isolates causing wilt disease in chickpea. *International Journal of Environment, Agriculture and Biotechnology (IJEAB)*. 2:202-212.
- Nikam P. S., Jagtap G. P. and Sontakke P. L. 2017. Management of chickpea wilt caused by *Fusarium oxysporium f. sp. ciceri*. *African Journal of Agricultural Research*. 2(12):692-697.
- Okello P. N. and Mathew F. N. 2019. Cross Pathogenicity Studies Show South Dakota Isolates of *Fusarium acuminatum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. subglutinans* from either soybean or corn are pathogenic to both Crops. *Plant Health Progress*. 20:44–49.
- Pandey M., Simon S. and Neelam.2014. Effect of organic soil amendment and soil solarization on wilt of chickpea. *International Journal of Botany and Research*. 4:61-66.
- Pandey R. N., Gohel N. M. and Jaisani P. 2017. Management of wilt and root rot of chickpea caused by *Fusarium oxysporum f. sp. ciceri* and *Macrophomina phaseolina* through seed biopriming and soil application of bio-agent. *International Journal of Current Microbiology and Applied Sciences*. 6(5):2516-2522.
- Panse V. G. and Sukhatme P. V. 1967. *Statistical Methods for agricultural workers*, ICAR, New Delhi.
- Patra S. And Biswas Mohan Kumar. 2016. Studies on cultural, morphological and pathogenic variability among the isolates of *Fusarium oxysporum f. sp. ciceri* causing wilt of

- chickpea. International Journal of Plant, Animal and Environmental Sciences. 7: 11-16.
- Poorvasandhya R., Sinha B. and Sobita Devi 2020. Effect of media, temperature and pH on growth and sporulation of *Fusarium oxysporum f. sp. udum* under *in vitro* condition. International Journal of Current Microbiology and Applied Sciences. 9(4): 2406-2412.
- Prasad R. D. and Anuroop C. P. 2002. Effect of soil and seed application of *Trichoderma harzianum* on pigeon pea wilt caused by *Fusarium udum* under field conditions. Crop Protection. 21 :293-297.
- Priyanka Singh and Sanjeev Kumar 2016. Effect of different media and pH on growth and sporulation of *Fusarium oxysporum f.sp. lentis* causing wilt of lentil. Indian Phytopathology. 69 (4s): 80-82.
- Raj Harender, Chauhan P. and Chandel S. 2016. Integrated management of carnation wilt caused by *Fusarium oxysporum f. sp. dianthi* using soil solarization and soil amendments. Indian Phytopathology. 69(4): 396-399.
- Rowe R. C. 1980. Comparative pathogenicity and host ranges of *Fusarium oxysporum* isolates causing crown rot and root rot of green house and field-grown tomatoes in North America and Japan. Phytopathology. 70:1143-1148.
- Sanjeev Kumar and Upadhyay J. P. 2004. Studies on cultural, morphological and pathogenic variability in isolates of *Fusarium udum* causing wilt of pigeon pea. Indian Phytopathological Society. 67(1): 165-178.
- Santram S. (2017). Variability among isolates of *Fusarium oxysporum f. sp. udum* causing pigeon pea wilt. International Journal of Microbiology Research. 9: 850-853.
- Saxena, D. R., Saxena, M., & Tiwari, N. (2019). Morphological and cultural variability in *Fusarium oxysporum f. sp. lentis* causing wilt of lentil. Indian Phytopathology. 72(4): 665-673.
- Shweta Deshpande 2022. Isolation of *Fusarium* species from the infected plant. International Journal of Science and Research (IJSR). 11:797-799.
- Siddiqui S. A., Bajwa R., Khan S. M. and Farhat Fatima jamil 2011. Effect of single or double soil solarization on density of *Fusarium oxysporum f. sp. ciceri* in field. Pakistan Journal of Phytopathology. 23(1):78-85.
- Singh and Kumar 2016. Effect of different media and pH on growth and sporulation of *Fusarium oxysporum f. sp. lentis* causing wilt of lentil. Indian Phytopathology. 69:4s 80-82.
- Soleha S, Muslim A, Suwandi S, Kadir S, Pratama R. 2022. Host range studies of *Fusarium oxysporum*, causal agent of seedling wilt disease of *Acacia mangium*. Biodiversitas. 23: 25-32.

- Soliman H. M., Musa F. H., Elmetwally Mohamed A. and Haroun S.A. 2016. Improving the performance, productivity and resistance of *Vigna radiata* to *Fusarium oxysporum* by cytokinins and beta-sitosterol. *Research Journal of Microbiology*. 11:186-193.
- Song W., Zhou L., Yang C., Cao X., Zhang L., Liu X. 2004. Tomato *Fusarium* wilt and its chemical control strategies in a hydroponic system. *Crop Protection*. 23: 243–247
- Sun F., Sun S., Zhu L., Duan N. and Zhu Z. 2019. Confirmation of *Fusarium oxysporum* as a agent of mung bean wilt in China. *Crop Protection*. 117: 77-85.
- Vani Sowmya M, Kumar S. and Gulya R. 2019. *In vitro* evaluation of fungicides and plant extracts against *Fusarium oxysporum* causing wilt of mung bean. *The Pharma Innovation Journal*. 8(8):297-302.
- Wani S. A., Mohiddin F. A., Hamid B., Gazala Rizvi 2014. Incidence of *Fusarium* wilt of chilli (*Capsicum annum* L.) in kashmir valley and its management by *Trichoderma* spp. *Mycopathology*. 12(1): 1-8.
- Webb, K. M., Case, A. J., Brick, M. A., Otto, K., and Schwartz, H. F. 2013. Cross pathogenicity and vegetative compatibility of *Fusarium oxysporum* isolate sugar beet. *Plant Diseases*. 97:1200-1206.
- Wim J. and Gerrit J. Bollen 2011. Host specificity and vegetative compatibility of Dutch isolates of *Fusarium oxysporum f. sp. asparagi*. *Canadian Journal of Botany*. 75(3):383-393.
- Yu, J. M., and Babadoost, M. 2013. Occurrence of *Fusarium commune* and *F. oxysporum* in horseradish roots. *Plant Diseases*. 97:453-460.
- Zhou, X. G., and Everts, K. L. 2007. Characterization of a regional population of *Fusarium oxysporum f. sp. niveum* by race, cross pathogenicity, and vegetative compatibility. *Phytopathology*. 97:461-469.

APPENDIX- I

ANOVA for growth of cultural characters on PDA medium						
Source	DF	SS	MSS	FCAL	FTAB	RESULT
TREATMENT	7	2609.074	372.725	9631.705	2.66	Sig.
ERROR	16	0.619	0.039	-	-	-
TOTAL	23	2609.693	-	-	-	-

S.E(m) ± 0.11
 CD at 1% 0.34

APPENDIX- II

ANOVA for integrated management of <i>Fusarium</i> wilt disease (Germination)						
Source	DF	SS	MSS	FCAL	FTAB	RESULT
TREATMENT	7	389.625	55.6607	51.10	2.76	Sig.
REPLICATION	2	0.75	0.375	0.34	3.74	Non- sig.
ERROR	14	15.25	1.08929	-	-	-
TOTAL	23	405.625	-	-	-	-

S.E(m) ± 0.63
 CD at 5% 1.82

APPENDIX- III

ANOVA for integrated management of <i>Fusarium</i> wilt disease (Wilt incidence)						
Source	DF	SS	MSS	FCAL	FTAB	RESULT
TREATMENT	7	598.5	85.5	111.35	2.76	Sig.
REPLICATION	2	5.25	2.625	3.42	3.74	Non- sig.
ERROR	14	10.75	0.76786	-	-	-
TOTAL	23	614.5	-	-	-	-



S.E(m) ± 0.50
 CD at 5% 1.53

APPENDIX- IV

ANOVA for integrated management of <i>Fusarium</i> wilt disease (Yield)						
Source	DF	SS	MSS	FCAL	FTAB	RESULT
TREATMENT	7	820573	117225	5654.25	2.76	Sig.
REPLICATION	2	49.0833	24.5417	1.18	3.74	Non- sig.
ERROR	14	290.25	20.7321	-	-	-
TOTAL	23	820912	-	-	-	-

S.E(m) ± 2.62
 CD at 5% 7.97

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE, DAPOLI

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ABSTARCT

Fusarium wilt of green gram [*Vigna radiata* (L.) wilczek] caused by *Fusarium oxysporum* has been emerging as one of the major threats to profitable cultivation of green gram crop. Therefore, present study was undertaken to emphasize the efficacy of plant protection based on following objectives viz., isolation and pathogenicity, morphological and cultural characteristics, cross infectivity and integrated management of the *Fusarium oxysporum* wilt disease of green, during 2022-23, at the Department of Plant Pathology and Department of Agronomy, College of Agriculture, Dapoli.

Initially, the plant showing typical wilt symptoms was brought to the laboratory and isolation of the associated fungus was carried out using standard tissue isolation technique on PDA medium. The pathogenicity of the fungus was carried out using sick soil inoculation technique following the Koch's postulates and the pathogen was confirmed as *Fusarium oxysporum*.

The fungus produced dense cottony septate mycelium which after seven days turned from white to pale yellow on PDA. Microscopic observations revealed that the microconidia of the fungus were small, cylindrical in shape having 0-1 septa. Conidia size ranges from $9.88\mu\text{m} \times 2.35\mu\text{m}$. Macroconidia were long, curved, sickle shaped, pointed at tip consisting of 3-5 septa and size ranged from $29.8\mu\text{m} \times 3.66\mu\text{m}$. Chlamydospores were spherical in shape with size of $8.6\mu\text{m}$. Among the eight different culture media evaluated against *F. oxysporum in vitro*, exhibited variation in growth. Potato dextrose agar was found most suitable and was significantly superior over other media tested. On Richard's agar, Oat meal agar, Conn's agar, Asthana and Hawkers agar, Martin's agar, and Czapek's dox agar fungal growth was fair and it was poor on Sabouraud's dextrose agar.

Cross infectivity test of *Fusarium oxysporum* on various different pulses viz., chickpea, cowpea, black gram, dolichos bean, horse gram and moth bean revealed that the pathogen is non-pathogenic.

Fusarium wilt disease of green gram can be effectively managed by integrating treatment of soil solarization along with seed treatment with carbendazim 50% WP @ 2g/kg.

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