

**“STUDIES ON WATERMELON (*Citrullus lanatus*) WILT INCITED BY
Fusarium oxysporum f. sp. *niveum*”**

by

Miss. Chaitra G V

(Reg. No. 2020/240)

A thesis submitted to the

**MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA**

In partial fulfilment of the requirements for the degree
of

MASTER OF SCIENCE (AGRICULTURE)

In

PLANT PATHOLOGY



**DEPARTMENT OF PLANT PATHOLOGY AND
AGRICULTURAL MICROBIOLOGY**

**POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH
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RAHURI - 413 722, DIST. AHMEDNAGAR
MAHARASHTRA, INDIA**

2023

CANDIDATE'S DECLARATION

I hereby declared that this thesis or part
there of has not been submitted
by me or other person to any
other University or Institute
for a Degree or
Diploma

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This is to certify that the thesis “**STUDIES ON WATERMELON (*Citrullus lanatus*) WILT INCITED BY *Fusarium oxysporum* f. sp. *niveum***”, submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar in partial fulfilment of the requirement for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY**, is a record of bonafide research work carried out by **Miss. CHAITRA G V**, under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

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

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LIST OF ABBREVIATIONS AND SYMBOLS

@	: At the rate of
&	: And
BOD	: Biological Oxygen Demand
CD	: Critical Difference
CV	: Coefficient of Variation
CRD	: Completely randomized design
cm	: Centimeter
<i>et al</i>	: Co-workers (<i>et alli</i>)
<i>cv.</i>	: Cultivar
d. wt.	: Dry weight
↓	: Decrease
Dia.	: Diameter
DNA	: Deoxyribonucleic acid
etc.	: Et cetera
EC	: Emulsifiable concentrate
EC (dSm ⁻¹)	: Electrical conductivity
e.g.,	: For example
Fig.	: Figure(s)
f. sp.	: <i>formae specialis</i>
g	: Gram
hr	: Hours
ha	: Hectare
i.e.,	: That is
IU	: International Unit
kg	: Kilo gram
Lit.	: Litre
mm	: Millimeter
µm	: Micro meter
µg	: Micro gram
ml	: Milli litre
MT	: Metric tonne(s)
mg	: Milligram

Mha	:	Million Hectare (s)
MMT	:	Million Metric Tonne (s)
MT	:	Metric Tonne (s)
Min.	:	Minutes
>	:	More than
<i>Viz.,</i>	:	Namely
No.	:	Number
PDA	:	Potato Dextrose Agar
PDB	:	Potato Dextrose Broth
pH	:	Potential of hydrogen
ppm	:	Parts per million
psi	:	Pressure per square inch
%	:	Per cent
/	:	Per
SE(m)	:	Standard Error
<i>spp</i>	:	Species
NaOCl	:	Sodium Hypochlorite
SL	:	Soluble Liquid
SC	:	Suspension concentrate
Sr. No.	:	Serial number
Temp	:	Temperature
Tr.	:	Treatment
V8	:	Vegetable-8
w/v	:	Weight by volume
w/w	:	Wight by weight
WP	:	Wettable powder

ABSTRACT

**“STUDIES ON WATERMELON (*Citrullus lanatus*) WILT INCITED BY
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**POST GRADUATE INSTITUTE,
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RAHURI-413 722
2023**

Research Guide	:	Dr. S.V. Kolase
Department	:	Plant Pathology and Agril. Microbiology

The current work entitled on the “Studies on Watermelon (*Citrullus lanatus*) wilt incited by *Fusarium oxysporum* f. sp. *niveum*” was carried out to see the cultural and morphological properties of pathogen, effect of soil properties such as soil types, soil pH and intervals of irrigated days on wilt incidence and host range studies under glass house condition, evaluation of fungicides, botanicals and bioagents on pathogen under *in vitro* condition as *Fusarium* wilt is one of biggest challenge to watermelon production which affects both quality and quantity of this vegetable fruit which quenches summer thirst.

The visual observations in field revealed the symptoms such as vascular browning, necrotic lesions on roots, chlorosis, cortical rot and damping off of seedlings. Microscopic observations from the isolated pure culture showed the presence of septate, hyaline, profusely branched mycelium and asexual spores such as microconidia, macroconidia and chlamydospores. Different ten media were selected to see the cultural and morphological properties of pathogen, among those media used Potato dextrose agar media showed maximum mean mycelial diameter of 86.3 mm and cultural characteristics of whitish to pale yellow colour excellent mycelial growth and recorded sporulation as excellent (++++).

The study on the effect of soil properties on wilt incidence under glass house condition revealed that clay loam soil showed the least incidence of 20 per cent, soil with pH range of 8.5-9.5 showed least wilt incidence of 24 per cent and the disease incidence was observed lower at 2 and 4 days of irrigated intervals such as 32 and 36 per cent respectively. While conducting the host range studies under glass house condition, host plants were inoculated with test pathogen, wilt symptoms were not observed on any other host plants except watermelon which was used as control.

The study on *in vitro* evaluation of different fungicides on mycelial inhibition of test pathogen at 500 and 1000 ppm concentrations showed that treatment with fungicide carbendazim 12 % EC+ mancozeb 63 % WP given significantly maximum mycelial inhibition of 99.74 and 99.81 per cents respectively. In addition to fungicides, evaluation of botanicals was also studied under *in vitro* condition showed that *Allium cepa* (Onion) showed maximum inhibition of 80.32 and 82.62 per cent at 5 and 10 per cent concentrations respectively than other botanicals used for study.

Evaluation of bioagents were also studied under *in vitro* condition which showed that *T. harzianum* (83.03%) showed maximum per cent inhibition on test pathogen followed by *B. subtilis*, *A. flavus*, *P. fluorescence*, *T. viride* and *A. niger*.

Considering the initial *in vitro* findings from these investigations it's need of time to use eco-friendly combinations of botanicals, bio-agents for *in vivo* testing on field at large level in the future for reducing and management of watermelon wilt disease.

1. INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai)] is a popular summer vegetable fruit belonging to the gourd family Cucurbitaceae with a chromosome number of $2n=2x=22$ (Edwards *et al.*, 2003). The crop originated in the Kalahari deserts of tropical Africa; fruit was depicted four thousand years back ago in ancient Egyptian art which reflects the antiquity of agriculture (Strauss, 2015).

Watermelon crop is cultivated in all tropical and subtropical regions of the globe. The major watermelon growing countries are China, Turkey, India, Iran, Algeria and Brazil. China alone accounts for 60 per cent of the total production of the world, by producing 60.1 million tonnes per year (Anon, 2019-20). Among the Asian countries, India stands second in the production of watermelon (Tiamiyu *et al.*, 2015). In India, major watermelon producing states are Uttar Pradesh, Andhra Pradesh, Karnataka, West Bengal, Orissa, Maharashtra and Tamil Nadu. In this case, Maharashtra shares 3.16 per cent area with the production of 101.91 tonnes per year (Anon, 2020-2021).

The plant is an annual trailing creeper with pinnately-lobed leaves and yellow flowers, and it has a special kind of berry, which is botanically called a 'pepo'. Fruit is deep green in color with thick rind having grey or light green vertical stripes. Black seeds are embedded inside a red color fleshy fruit (Levi *et al.*, 2001). Watermelon is a warm season crop preferably grown in a temperature range of 24-27⁰C. For better accumulation of sugars in fruits, cool nights and warm days are needed. Crop is grown in sandy loam soil with pH range of 6.5-7.5 (Kumar *et al.*, 2013).

The nutritional profile of the crop consists of mostly 91 per cent water and 7.5 per cent carbohydrates. The 100 g of raw watermelon contains protein 0.6 g, carbohydrates 8 g, sugar 6.2 g, fiber 0.4 g, fat 0.2 g, vitamin C-13 per cent, vitamin A-865 IU (Anon, 2021). The fruit is an excellent source of the carotenoid lycopene and phenolic antioxidants, and it is rich at immuno-supportive vitamin C and vitamin A. It is good source of potassium and magnesium. The chemical nature of fruit shows that it has phytochemicals which reduces the risk of Cardio Vascular Diseases (CVD) and weight loss issues (Aderiye *et al.*, 2020). The watermelon juice has diuretic properties and it is an alternative to drinking water in desert areas (Kamanna *et al.* 2010).

Diseases are more common in watermelon, which deteriorates quality and productivity. Diseases can emerge at any point in the crop development cycle, damaging all parts of the plant from roots to fruit. Diseases are most severe when vines are attacked and killed prematurely. Pathogens, which include fungi, bacteria, viruses and nematodes can cause watermelon diseases. Diseases such as Anthracnose (*Colletotrichum orbiculare*), Bacterial fruit blotch (*Acidovorax citrulli*), Cercospora leaf spot (*Cercospora citrullina*), Downy mildew (*Pseudoperonospora cubensis*), *Fusarium* wilt (*Fusarium oxysporum* f. sp. *niveum*), Gummy stem blight (*Stagonosporopsis cucurbitacearum*), Powdery mildew (*Podosphaera xanthii*), Viral diseases such as Watermelon Mosaic Virus (WMV), Zucchini Yellow Mosaic Virus (ZYMV), Cucumber Mosaic Virus (CMV), Watermelon Chlorotic Stunt Virus and Root-knot nematode (*Meloidogyne* spp.). Among this the most destructive soil-inhabiting fungal pathogen limiting watermelon production is *F. oxysporum* f. sp. *niveum*.

Fusarium oxysporum is an anamorphic fungus that includes a large number of pathogenic variants that cause diseases in more than hundred economically important agricultural and horticultural host plants (Boughalleb and El Mahjoub, 2006). This is a species complex ubiquitous to soil found in wider ecosystems. Based on scientific and economic importance, international community of fungal pathologists ranked *Fusarium oxysporum* as fifth in a list of ten recently published pathogens (Dean *et al.*, 2012). The isolates which are pathogenic are categorized based on their host range into *formae specialis* (ff. spp., plural: *forma specialis*, f. sp., singular) (Gordan and Martyn, 1997).

Fusarium oxysporum f. sp. *niveum* incites a widespread wilt of watermelon. This was the second described *Fusarium* wilt after cotton wilt by Erwin F Smith (Martyn, 2012). They suggested that the asexual stage of pathogen was *Neocosmospora vasinfectum* Atk., a cotton wilt causing pathogen. However, this was proved wrong later. Snyder and Hansen (1940) revised the *Fusarium* section and reduced ten species into one, as *Fusarium oxysporum*. Thus, watermelon wilt fungi became *Fusarium oxysporum* f. sp. *niveum*.

Major symptoms of the disease include chlorosis, defoliation, cortical rot, damping off of seedlings, sudden progressive wilt of older plants, necrotic lesions on the

roots, vascular browning, tyloses formations (Agrios, 2005). The pathogen is transmitted through seeds, animal manure, fertilizer, compost, tools, irrigation, soil, wind and rain. The chlamydospores can survive in plant debris and infected roots. The severity of the disease is determined by the type of soil, irrigation, application of fertilizer, crop management practices and seedling preparation. *Fusarium* wilt disease is more common in monocropping systems, where watermelon crop is cultivated continuously in a shorter rotation for a longer period such as in China (Everts and Himmelstein, 2015).

Importance of *Fusarium* wilt of watermelon is increasing throughout the world because production practices for watermelon have changed dramatically over the past two decades. Application of fungicides is general management tactic's for diseases. However, disease is soil borne in nature, which can survive for longer time in soil. Hence fungicides are less effective (King *et al.*, 2010). The disease is best managed with resistant cultivars. However, new virulent physiological races may develop in specific locations. Biological control of soil borne pathogens by microorganisms has been considered as a good alternate solution which is safer, ideal, cheap, eco-friendly when compared with chemicals (Eziashi *et al.* 2007).

Keeping all above points, it is necessary to combat the disease in early stages with suitable management practices. Hence, the present research is conducted with following objectives in mind,

1. To isolate and identify the pathogen.
2. To study the pathogenicity for *F. oxysporum* f. sp. *niveum*
3. To study the cultural and morphological characters of *F. oxysporum* f. sp. *niveum*.
4. To study the influence of soil properties on wilt incidence of watermelon in glass house condition
5. To study the host range of *F. oxysporum* f. sp. *niveum* in glass house condition
6. To study the efficiency of different bioagents, botanicals and fungicides on inhibition of *F. oxysporum* f. sp. *niveum* under *in vitro* condition

2. REVIEW OF LITERATURE

Watermelon wilt is one of the most destructive diseases of crop causes greater economic losses. Smith (1899) was the first who described the fungi *F. oxysporum*, f. sp. *niveum* which was associated with watermelon wilted plants, studied its life cycle, inoculation, host specificity (Martyn, 2012). This chapter consists of recent and important literature citations which are related to isolation, identification, symptomatology, pathogenicity, cultural and morphological characterization, *in vitro* evaluation of fungicides, bioagents, botanicals and integrated disease management, influence of soil properties on wilt incidence and host range study of wilt of watermelon.

2.1 Isolation, identification and pathogenicity of pathogen

2.1.1 Isolation of pathogen

Smith (1899) first isolated *F. oxysporum* f. sp. *niveum* from the infected watermelon plants in United States. Lakshminarayanan (1953) reported in *F. oxysporum* f. sp. *lycopersici*, wilting of plants are caused by plugging the vascular vessels, the plugging theory states that wilt of *Fusarium* occurs because of blocking of xylem vessels through the formation of callose, tyloses or gels, as the hosts tries to escape from the pathogen.

Larkin *et al.* (1993) isolated the pathogen from infected watermelon plants and soil samples of infected fields. They proved the pathogenicity on watermelon and determine the vegetative compatibility groups (VCG) within *F. oxysporum* f. sp. *niveum*. Punja *et al.* (2001) isolated fungi from diseased root, crown, stem tissues and fruits of infected muskmelon plants grown under field conditions and studied the symptoms associated with seedlings of crop.

Boughalleb and Mahjoub (2006) isolated fungi complex from infected watermelon samples such as *Fusarium oxysporum*, *F. solani*, *Rhizoctonia bataticola*, *R. solani*, *Monosporascus cannonballus*, *Pythium ultimum* and *P. echinulatum*. They confirmed that wilt of watermelon was caused by two fungi, one is *F. oxysporum* f. sp. *niveum* and other one is collar rot caused by *F. solani* f. sp. *cucurbitae*.

Srinon *et al.* (2006) isolated the pathogens *Fusarium oxysporum* f. sp. *cucumerinum* and *F. oxysporum* f. sp. *lycopersici* from wilt of cucumber and tomato

respectively. Pathogenicity test for all isolates of cucumber and tomato was done by Koch's Postulates.

Tziros *et al.* (2007) isolated *F. oxysporum* f. sp. *niveum* from infected watermelon plant and reported that it is a devastating soil-borne disease that causes extensive losses throughout the world. Wechter *et al.* (2012) isolated *F. oxysporum* f. sp. *niveum* from wilted watermelon plants in Clemson University's Coastal Research and Educational Center in Charleston, SC.

Zhao *et al.* (2013) isolated 25 *Fusarium* spp. from greenhouse melon soils in Liaoning province, China. They studied the pathogenicity of 13 strains of *F. oxysporum* to muskmelon, cucumber and watermelon seedlings by infecting the seedlings with a spore suspension after cutting the root and results showed that the *F. oxysporum* strains were pathogenic to all three melon types.

Tran-Nguyen *et al.* (2013) first time detected watermelon wilt caused by *F. oxysporum* f. sp. *niveum* in Northern Territory, Australia in May 2011. The causal agent was isolated from symptomatic watermelon seedlings by excising stem sections close to the crown of the plant surface sterilizing in 70 % ethanol for 1 min, then rinsed for 1 min in sterile distilled water. These tissue pieces were then sliced into smaller pieces and embedded into potato dextrose agar plates supplemented with 1 % lactic acid. Isolated plates were incubated at 25⁰C for 2-3 days, after which *Fusarium* like spores were observed. Single spore *Fusarium* cultures were used for all subsequent pathogenicity tests and DNA analysis. Pathogenicity tests were conducted and the fungus was shown to be pathogenic. This outbreak is the first report of *Fusarium* wilt in triploid seedless watermelon with symptoms similar to bacterial leaf blotch and typical wilt in Australia.

Majdah and Tuwajri (2015) isolated *F. oxysporum* f. sp. *cucumerinum* from wilted cucumber plant samples collected from eight different localities in Egypt. They found that *F. oxysporum* f. sp. *cucumerinum* is the most common pathogen on cucumber plants causing *Fusarium* wilt on cucumber and reduced the yield and also observed that all tested isolates of fungi were pathogenic to cucumber Beit alpha cultivar.

2.1.2 Identification of pathogen

Vakalounakis *et al.* (2004) observed that thirty-four isolates of *Fusarium oxysporum* obtained in China from cucumber plants showing either *Fusarium* wilt (*F. oxysporum* f. sp. *cucumerinum*) or root and stem rot (*F. oxysporum* f. sp. *radices-cucumerinum*) symptoms, were characterized by pathogenicity, vegetative compatibility and random amplified polymorphic DNA (RAPD) of these, 23 isolates were identified by pathogenicity as *F. oxysporum* f. sp. *cucumerinum* and one as *F. oxysporum* f. sp. *radices-cucumerinum*, while 10 isolates were avirulent on cucumber, melon, sponge gourd and pumpkin.

Gargouri *et al.* (2000) identified the various *Fusarium* spp. which were isolated from watermelon seeds based on their morphological criteria and rDNA analysis. The species are *F. oxysporum*, *F. solani*, *F. moniliformae* (*Gibberella fujikuroi*) and *F. equiseti*.

Naveenkumar *et al.* (2017) observed that *F. oxysporum* f. sp. *niveum* produces cottony white or creamy white or pale yellow with white or pinkish white mycelial growth on PDA medium while studying morphological and cultural characterization of the *Fusarium*. Zhong *et al.* (2022) detected and quantified *F. oxysporum* f. sp. *niveum* race 1 by using real time polymerase chain reaction (PCR) assay using FONRT-18 specific primer.

2.1.3 Pathogenicity of pathogen

Radhakrishnan and Sen (1986) proved the pathogenicity of *F. oxysporum* f. sp. *melonis* and *F. solani* (Mart) causing muskmelon wilt by seed and soil inoculation among which seed inoculation shows highest disease incidence. Sumitra (2006) proved the pathogenicity of *F. oxysporum* f. sp. *gladioli* in gladiolus by sick soil method.

Egel *et al.* (2005) observed symptoms such as partial wilting and vascular discoloration, which were typical *Fusarium* wilt symptoms and the suspected causal agent was *F. oxysporum* f. sp. *niveum* in watermelon from Knox and Gibson countries of south western Indiana in USA.

Zhou and Everts (2007) were performed cross pathogenicity tests by using root-dip technique in greenhouse and reported that no stem colonization was observed where the incompatible interaction between muskmelon and *F. oxysporum* f. sp. *niveum*

isolate occurred. However, high levels of stem colonization were observed where compatible interactions took place. This indicated that isolates of *F. oxysporum* f. sp. *niveum* that were not cross pathogenic to muskmelon and *F. oxysporum* f. sp. *niveum* causes the symptoms of *Fusarium* wilt and internal stem colonization in watermelon.

Dau *et al.* (2009) reported *Fusarium* wilt of watermelon caused by *F. oxysporum* f. sp. *niveum* caused seedling in nurseries as well as severe losses in many crops in Nghe and province, Vietnam in 2008. Isolates of the fungus were shown to be pathogenic. All 20 cultivars grown in the province in 2008 were susceptible. This is the first formal of this disease in Vietnam.

Perez *et al.* (2009) conducted detailed study about the pathogenicity of 6 isolates of *F. oxysporum* f. sp. *radices-cucumerinum* is presented. Three isolates were obtained from Almeria (Spain) and three from Crete (Greece). Pathogenicity was studied on four Cucurbitaceae species and one pumpkin hybrid (*Cucurbita maxima* × *C. moschata*) and on eight species belonging to different botanical families (parsley, carrot, bean, beet, chinese cabbage, aubergine, tomato, sweet pepper). All six *F. oxysporum* isolates showed pathogenicity on three Cucurbitaceae, but didn't on squash and pumpkin hybrid and didn't on each of eight other species different to Cucurbitaceae.

Cumagun *et al.* (2010) studied pathogenicity and aggressiveness of *F. oxysporum* *schl.* in bottle guard and bitter guard. They reported that all bitter guard isolates produce typical symptoms of vein clearing and wilting after 21 days after inoculation.

Nikam *et al.* (2011) confirmed the pathogenicity of the *F. oxysporum* f. sp. *ciceris* by sick soil inoculation technique in earthen pots under greenhouse condition by using susceptible cultivar JG-62.

Raghu *et al.* (2016) observed pathogenicity of *Fusarium* spp on three different varieties of chilli under artificially inoculated condition under glasshouse condition. The isolates of *Fusarium* were multiplied by using sand corn media. The twenty-day old giant culture was used to inoculate into sterilized soil at 10 w/w. The surface sterilized seeds of three varieties of chilli were sown at 20 seeds pot with two replications. The pots were maintained with optimum moisture at field capacity by

watering regularly. The *Fusarium* caused the pre-emergence and post emergence seedling death due to artificial inoculation of different isolates of pathogen.

2.2 Symptomatology

Smith (1899) reported the formation of “embolisms in the vascular systems called as tyloses” in the infected watermelon plants. They also gave detailed description on fungi, its host specificity. Palodhi and Sen (1980) observed the symptoms of *Fusarium* on cucurbit plants that severe stunting of the veins, yellowing and necrosis of the leaves followed by complete wilting.

Vakalounakis and Fragkiadakis (1999) characterized the total 106 isolates of *F. oxysporum* obtained from diseased cucumber plants and observed typical root and stem rot or *Fusarium* wilt symptoms by pathogenicity and vegetative compatibility.

Cerkauskas *et al.* (2001) observed foliar chlorosis on the lower foliage of *Fusarium* stem and root rot affected cucumber plants. The basal stem tissue developed a yellow buff discoloration with superficial rot, followed by advanced stages of stem disintegration accompanied by the production of white buff fungus mycelium and orange spore masses externally and yellowish- or reddish-brown discoloration of vascular tissue extended up to 5-6 cm.

Egel and Martyn (2007) reported that initial symptoms of diseases are dull, gray green appearance of leaves, loss of turgor pressure, yellowing of leaves and finally necrosis. Wilting starts from older leaves to younger ones.

Zhou and Everts (2004) reported that severity of disease directly depends on degree of colonization by the fungus in vascular system. Perez *et al.* (2009) observed the symptoms while studying the pathogenicity tests on various isolates of *F. oxysporum* f. sp. *radices-cucumerinum* on Cucurbitaceae family that inoculated plants showed mainly root and crown rot, xylem necrosis and reduced emergence of watermelon seeds. They also observed that severity of disease was higher at 17⁰C than 25⁰C.

Shankar *et al.* (2014) reported that fungal infection may occur at any age of the plant. For young seedlings damping off may occur and rot in the soil, where the hypocotyls are surrounded by a watery and soft rot causing the plants to become stunted. Later wilting occurs in more mature plants causing the plant to die. A one-sided wilting and others remaining healthy, flaccid, withered and brown leaves, as well as vascular

discoloration are common disease symptoms. The roots of infected plants may be healthy, but the vascular tissue is brown and discolored. Thus, rotten roots are not necessarily associated with *Fusarium* wilt disease.

Egel Dan (2018) observed first symptoms of wilt in the field as a one-sided wilt of a plant or the wilt of one runner. Infected plants have white, healthy roots while the vascular tissue is brown and discolored. Symptoms of *Fusarium* wilt may also be observed on mature plants, in which case a portion of the vines at the base of the plants may have died and the older leaves of living vines may have turned brown.

2.3 Cultural and Morphological characters of *F. oxysporum* f. sp. *niveum*

Jamaria (1972) recorded maximum growth and sporulation of *F. oxysporum* f. sp. *niveum* on Potato Dextrose Agar, Richard's agar and Czapek's agar. Larkin *et al.* (1993) studied population dynamics and chlamyospore germination of *F. oxysporum* f. sp. *niveum* as well as colonization of watermelon roots by *F. oxysporum* were monitored in relation to other micro-organism populations and the incidence of *Fusarium* wilt in four soils representing different suppressive soils.

Smith (2007) reported that members of *Fusarium* species produce both microconidia and macroconidia. They observed that macroconidia are typically 25-35 μ long 3-5 μ wide, dorsi-ventrally curved, sickle shaped 3-5 septated, with thin walls and tapering toward the ends which are quite pointed. The microconidia are abundantly, which tend to be 5-12 \times 3-5 μ in size, oval to ellipsoidal, occurring singly or in aggregates as false heads held together by moisture which dries out to become a powdery substance dispersed in the mycelium.

Nikam *et al.* (2011) studied morphological characters of *F. oxysporum* f. sp. *ciceri* on various media and revealed that maximum mycelial growth was obtained on host leaf extract of susceptible cultivar JG-62 (90 mm) followed by Richard's agar (86 mm) and Potato dextrose agar (83 mm). All three media showed significantly good mycelial growth and sporulation. The least growth was observed on Oat meal agar (71 mm).

Ingle and Rai (2011) reported that ten isolates of *F. semitectum* recovered from different hosts. Identification of these isolates was determined by morphological and cultural characteristics and confirmed by RAPD-PCR analysis, these isolates can be

categorized in three groups depending upon similarity co-efficient. Genetic similarity co-efficient between pair wise isolates varied from 0.00 to 1.95 based on an unweighted paired group method of arithmetic average (UPGMA) cluster analysis. RAPD-PCR technique can be used as an important tool for the genetic differentiation among isolates of *F. semitectum*.

Kleczewski and Egel (2011) reported that *F. oxysporum* will produce banana-shaped, often 3-septate macroconidia on sporodochia, which are located at the center of the Petri plate and small oval to kidney shaped, often aseptate, microconidia are produced on false heads on short monophialids formed on aerial mycelia.

Scarlett *et al.* (2015) reported that *F. oxysporum* f. sp. *cucumerinum* is the fungal pathogen responsible for *Fusarium* vascular wilt of cucumber and identified both macroconidia and microconidia as airborne propagules.

Dutta *et al.* (2017) revealed that *F. oxysporum* f. sp. *niveum* reproduces by microconidia and macroconidia. Microconidia are produced on short conidiophores and are small, single celled and kidney shaped. Macroconidia are fusiform (boat shaped) and often have three to five cells. Macroconidia have the ability to form chlamydospores. Chlamydospores may arise from macroconidia or mycelia and are formed under unfavorable environmental conditions.

2.4 *In vitro* evaluation of bioagents, botanicals and fungicides against pathogen

2.4.1 *In vitro* evaluation of bioagents

Kudryavtseva (1980) reported that in culture *T. viride* was highly antagonistic to *F. solani* and *Fusarium sp.* isolated from cucumber. Jee and Kim (1987) reported that *T. harzianum* was the best antagonist under *in vitro* against *F. oxysporum* f. sp. *cucumerinum* causing wilt of cucumber. Kaur and Mukhopadhyay (1992) observed that *T. harzianum* significantly inhibited the growth of *F. oxysporum*.

Mukherjee and Sen (1998) reported that *Aspergillus niger* (A 27) was shown to be a promising biocontrol agent against *F. oxysporum* f. sp. *melonis* which causes muskmelon wilt. Hamed (1999) evaluated the inhibitory effect of two beneficial microbes *Bacillus subtilis*, *Gliocardium reseau* and six *Trichoderma spp.* against *Pythium ultimum* and *F. oxysporum* f. sp. *cucumerinum* under *in vitro*, among them *T.*

viride (isolate 2) and *T. harzianum* (isolate 5 and 6) suppressed *Fusarium* wilt of cucumber by 33.30, 33.30 and 26.30 per cent, respectively.

Ozaktan and Bora (2000) studied *P. fluorescens* isolates obtained from the rhizosphere of more than 300 healthy muskmelon, 64 isolates showed inhibitory activity against *F. oxysporum* f. sp. *melonis*. Out of 64 isolates, 28 showed more than 50 per cent inhibition and these were used for control of *F. oxysporum* f. sp. *melonis* in muskmelon pots.

Patibanda and Sen (2004) found *A. niger* more useful antagonist against *F. oxysporum* f. sp. *melonis* in the *in vitro* condition. Seven isolates of the antagonist *A. niger* and three isolates of the muskmelon wilt pathogen *F. oxysporum* f. sp. *melonis* were assayed for their *in vitro* interaction. Isolates AN 27 were found promising based on its bio control capabilities.

Bharath *et al.* (2006) used chemicals and antagonists for their efficacy in management of seed borne fungal pathogens of watermelon and reported that *Fusarium* species was effectively suppressed due to Bavistin followed by the topsin and seed treatment with antagonists like *T. harzianum* and *T. viride* improved the seed germination, seedling vigor and reduced the incidence of seed-borne fungal pathogens. They also reported that *T. harzianum* showed its efficacy against all *Fusarium* species.

Srinon *et al.* (2006) studied the efficacy of antagonistic fungi on growth of plant pathogens by dual culture test and results shown that *T. harzianum* WS01 was inhibited highest spore production against *F. oxysporum* f. sp. *cucumerinum* whereas *Penicillium* sp. WS01 was inhibited highest spore production against *F. oxysporum* f. sp. *lycopersici*.

Al-Tuwaigri (2008) studied the biological control of *Fusarium* root-rot of cucumber by rhizosphere isolates of *Bacillus subtilis* and *T. viride* and results showed that *T. viride* MT15 and *Bacillus subtilis* MB10 can be employed as soil treatment or as seed treatment to control root rot disease of cucumber caused by *F. oxysporum* and *F. solani*

Hamed *et al.* (2009) studied on suppression of *Fusarium* wilt of watermelon by biological and chemical control. They tested four antagonistic microbes as *Pseudomonas ceparica* (B1), *Bacillus polymyxa* (B2), *B. subtilis* (B3) and *P. fluorescens*

(B4), reported that *P. fluorescens* (B4) gave the highest reduction to the wilt incidence after 4, 8 weeks of application when compared with the control treatment.

Ahmed (2010) studied that effect of four *Pseudomonas* and two *Serratia* isolates on growth of *F. oxysporum* showed that, *P. fluorescens* No. 2 and No.3 and *Serratia marcescens* No. 2 gave highest inhibition zones which were 37.33, 35.00 and 31.33 mm, respectively and also inhibit the spore germination of *Fusarium*. Akila *et al.* (2011) reported that among eight antagonistic micro-organisms of tested for their efficacy in reducing *F. oxysporum* f. sp. *cubense* causing wilt of banana; *T. viride* and *P. fluorescens* were found equally effective.

Cao *et al.* (2011) used several strategies to control the causative pathogen, *F. oxysporum* f. sp. *cucumerinum* including soil solarization, fungicide seed treatment and biological control. In this study, *F. oxysporum* f. sp. *cucumerinum* was successfully controlled by a newly isolated strain *Bacillus subtilis* SQR 9, *in vitro* and *in vivo*.

Sundarmoorthy and Balabaskar (2013) evaluated the efficacy of the native isolates of *Trichoderma* species to promote the growth and yield parameters of tomato and to manage *Fusarium* wilt disease under *in vitro* and *in vivo* conditions. Under *in vitro* conditions, the results revealed that *T. harzianum* (ANR-1) isolate was found to effectively inhibit the radial mycelial growth of the pathogen (by 53 %) when compared to all other isolates.

Pagoche and Raina (2013) studied the effect of *Trichoderma* isolate on growth of *F. oxysporum* f. sp. *cucumerinum* causing wilt in cucumber under dual culture technique and by the release of volatile metabolites. Among the four *Trichoderma* species, *T. viride* restricted the growth of the test pathogen in the range of 53.80 to 70.75 per cent and the inhibition percentage reached in the range of 72.73 to 83.99 per cent on 7th day. Similarly, among *T. aureoviride* isolates, the growth restriction of test pathogen on the 7th day of incubation ranged between 74.30 to 85.77 per cent *T. harzianum* isolates restricted the growth of the test pathogen in the range of 74.71 to 82.21 per cent on 7th day. *T. atroviride* recorded growth inhibition of 81.23 per cent on 7th day of incubation.

Singh *et al.* (2014) studied efficacy of bioagents against *Fusarium* spp. *in vitro*. Six bioagents *viz.*, *Trichoderma viride*, *Gliocladium virens*, *T. harzianum*, *T. hamatum*, *Aspergillus niger*, and *Pseudomonas fluorescens* were assessed for their

efficacy against *Fusarium* spp. by using dual culture technique. It was concluded that seed treatment with *T. viride* and *T. harzianum* gave significantly better results in respect to seed germination, shoot length, root length and wilt incidence and the minimum inhibition was seen with *P. fluorescens*.

Majdah and Tuwajri (2015) studied the effect of some biological control agents such as *T. harzianum* 1 (Th1), *T. harzianum* 2 (Th2), *T. hamatum*, *T. glaucum*, *T. reesei* and *T. viride* on growth of three isolates of *F. oxysporum* f. sp. *cucumerinum* under laboratory conditions by using dual culture technique and observations on linear growth, reduction of fungal growth under bioagents stress and over growth and/or inhibition zone were recorded. The results showed that *T. reesei* greatly affected linear growth and reduction of fungal growth of *F. oxysporum* f. sp. *cucumerinum* followed by *T. viride* whereas the least effect of the bioagents was noticed by *T. glaucum*.

Barari (2016) studied efficacy of the native isolates of *Trichoderma* species to promote the growth and yield parameters of tomato and to manage *Fusarium* wilt disease under *in vitro* and *in vivo* conditions. Under *in vitro* condition, the results revealed that *Trichoderma harzianum*, isolate N-8, was found to inhibit effectively the radial mycelial growth of the pathogen (by 68.22 %).

2.4.2 In vitro evaluation of botanicals

Benkeblia (2004) studied antimicrobial activity of different concentrations (50, 100, 200, 300 and 500 ml/l) of essential oil extracts of three type of onions (green, yellow and red) and garlic against two bacteria, *Staphylococcus aureus*, *Salmonella enteritidis* and three fungi, *Aspergillus niger*, *Penicillium cyclopium* and *Fusarium oxysporum*. The essential oil extracts of these *Allium* plants exhibited marked antibacterial activity, with garlic showing the highest per cent inhibition and marked antibacterial activity and green onion showed the lowest inhibition per cent.

Taskeen-Un-Nisa and Mir (2010) evaluated plant extracts of *Allium sativum*, *Allium cepa* and *Mentha arvensis* for their effect on the inhibition of mycelial growth and spore germination of *Fusarium oxysporum* and reported that the extract of *A. sativum* at highest concentrations was found to be most effective in reducing the spore germination followed by highest concentrations of extract of *A. cepa* and *M. arvensis*. The inhibition in spore germination varies from 43.94 to 90.7 per cent in different

concentrations of *A. sativum* and *A. cepa*, the inhibition in spore germination ranges from 60.51 to 17.58 per cent, whereas inhibition of spore germination ranges from 60.39 to 20.44 per cent in different concentrations of extract of *Mentha arvensis* respectively as compared to untreated control which showed least inhibition in spore germination.

Rongai *et al.* (2012) described the antifungal activity of plant extracts on the development of *F. oxysporum* f. sp. *lycopersici* *in vitro*. The results of a study showed inhibitory activity of extracts from Kenyan medicinal plants *Warburia ugandensis*, *Azadirachta indica*, *Tagetes minuta* and *Urtica massaica* were against three soil pathogens *Fusarium oxysporum*, *Alternaria passiflorae* and *Aspergillus niger*.

Farrag *et al.* (2013) studied the efficacy of plant extracts such as Common walnut, Cowslip, Garlic, Golden rod, Mugword, Nettle, Peppermint, Rheum, Salvia and Soapwort on spore germination and mycelial growth of *F. oxysporum* under *in vitro* conditions and revealed that Peppermint extract was the most effective and completely inhibited spore germination and mycelial growth at concentrations of 2 per cent, whereas garlic and rheum extracts were completely suppressed spore germination and mycelial growth at rate of 3 per cent.

Hadi *et al.* (2013) studied antifungal activity of *Mentha piperita*, *Cinnamomum zeylanicum*, *Allium hirtifolium* and *Allium sativum* were investigated against *Fusarium oxysporum* *sclecht*. Inhibition of the growth rate and spore germination was evaluated after 8 and 10 days in PDA and PDB media. The results showed that plant extracts and their main components had inhibitory activities on the growth rate and spore germination of *Fusarium*. The results exhibited *Cinnamomum zeylanicum*, *Mentha piprita*, *Allium hirtifolium* and *Allium sativum* showed maximum inhibition of the spore germination at 1000 and 500 ppm after 8 days, while the least spore germination was occurred at 100 and 25 ppm after 10 days in PDB media.

Singh *et al.* (2014) studied efficacy of plant leaf extracts against *Fusarium* spp. *in vitro*. Five plant extracts *viz.*, *Azadirachta indica*, *Pongamia pinnata*, *Parthenium hysterophorus*, *Calotropis gigantia* and *Annona squamosa* were assessed for their efficacy against *Fusarium* spp. The growth of pathogen was significantly lowest on *Azadirachta indica* followed by *Pongamia pinnata*, *Parthenium hysterophorus*, *Calotropis gigantia* and *Annona squamosa* leaf extracts.

Awad (2016) used six plant materials for preparations of extracts, *i.e.*, Garlic, Black pepper, Clove bud, Black cumin, Cinnamon and Ginger as environmentally safe alternative method of fungal control and indicated that all plant extracts were effective on inhibiting the linear growth in Petri dishes for all tested isolates. All plant extract showed a high effect at concentrations (5 and 10 %), there was no significant differences between the effects of two concentrations. The most effective plant extracts at 1 % on linear growth were Garlic (0.2 cm) and Clove bud (0.1 cm), while the lowest effective plant extract was Cinnamon (3.4 cm).

2.4.3 *In vitro* evaluation of fungicides

Hopkins and Elmstrom (1975) evaluated soil fungicides and fumigants for the control of *Fusarium* wilt (*F. oxysporum* f. sp. *niveum*) of watermelon cultivars with different levels of tolerance to the disease. Only the fumigant type materials DD-MENCS and sodium azide provided good, consistent control while benomyl and thiabendazole reduced wilt. Maraitte and Meyer (1971) reported that muskmelon plants treated with benomyl as a soil drench reduced infection by *F. oxysporum* f. sp. *melonis*.

Kalra and Sohi (1984) studied the efficacy of different fungicides against wilt of vegetable crop caused by *Fusarium oxysporum* under *in vitro* and reported that fungicides *viz.*, benomyl, carbendazim, thiophanate-methyl, mancozeb and thiram inhibited the growth considerably. Gaikwad *et al.* (1987) observed that carbendazim and cercobin were most effective of six systemic fungicides tested in inhibiting spore germination and growth of *F. oxysporum* under *in vitro* and *in vivo* conditions causing cucumber wilt.

Mukherjee and Tripathi (2000) evaluated eight fungicides of different concentrations *viz.*, 2.5, 5, 10, 25, 50 and 100 mg/ml under the *in vitro* condition against *Sclerotium rolfsii*, *R. solani* and *F. oxysporum* f. sp. *phaseoli*. Out of these fungicides bavistin and contaf (10-20 mg/ml) inhibited 100 per cent radial growth of *F. oxysporum* f. sp. *phaseoli*.

Gupta and Bansal (2003) tested carbendazim, mancozeb, captan, thiram and topsin M at 0.2 % concentration against *F. oxysporum* inducing fenugreek wilt under pot conditions and reported that carbendazim was found significantly effective fungicide followed by mancozeb.

Bharath *et al.* (2006) used chemicals and antagonists for their efficacy in management of seed fungal pathogens of watermelon and reported that *Fusarium* species was effectively suppressed due to bavistin followed by the topsin and seed germination, seedling vigor and reduced the incidence of seed borne fungal pathogens. They also reported that *Trichoderma harzianum* showed its efficacy against all *Fusarium* species. Nel *et al.* (2007) reported that benomyl was also partly effective as a root dip treatment and soil drench against *F. oxysporum* f. sp. *cubense*.

Amipara (2008) recorded that fungicides combination *viz.*, carbendazim + mancozeb, carbendazim + thiram at 500 ppm completely inhibited growth of *F. moniliformae* var. *subglutinans* and *F. oxysporum* causal agents of mango malformation. Atta *et al.* (2009) studied the efficacy of four fungicides *viz.*, Topsin M-70 WP (thiophanate) @ 3 g per 1 kg seed, Derosol 60 WP (carbendazim) @ 3 g/ kg seed against gram wilt. All the tested fungicides controlled the gram wilt effectively, however, Topsin M-70 WP (thiophanate) @ 3 gm/kg seed treatment resulted in better control of wilt.

Amini and Sidovich (2010) studied the effect of fungicides such as benomyl, carbendazim, prochloraz, fludioxonil, bromuconazole and azoxystrobin on *Fusarium oxysporum* f. sp. *lycopersici* associated with *Fusarium* wilt of tomato at different concentrations (0.0001, 0.001, 0.01, 0.1, 1, 10, 100 µg/ml) under *in vitro* and *in vivo*, followed by benomyl and carbendazim. All other fungicides were less effective.

Taskeen-Un-Nisa and Mir (2010) evaluated fungicides such as carbendazim, hexaconazole, bitertanol, myclobutanil, mancozeb, captan and zineb for their effect on the inhibition of mycelial growth and spore germination of *F. oxysporum* and reported that maximum inhibition in mycelial growth was observed in the hexaconazole at 1000 ppm followed by other fungicides at the same concentration.

Vatchev and Maneva (2012) studied chemical control of root rot complex and stem rot of greenhouse cucumber in straw-bale culture by using three fungicides, either alone or as tank-mixed combinations of 0.1 % Topsin M 70 WP (thiophanate-methyl 700 g/kg) +0.15 % Previcur 607 SL or 0.1 % benomyl 50 WP + 0.15 % Previcur 607 SL and results revealed that the potential of two mixture, comprising either benomyl or thiophanate-methyl (Topsin M 70 WP), combined with propamocarb -hydrochloride

(Previcur 607SL) protect straw-bale cultivated cucumber plant against the dominant pathogen *F. oxysporum* f. sp. *cucumerinum* and other associated fungi and oomycetes.

Everts *et al.* (2014) conducted greenhouse trials in Maryland, Indiana and Georgia to test the efficacy of 14 chemicals on *Fusarium* wilt and reported that prothioconazole and thiophanate-methyl can reduce the severity of *Fusarium* wilt of watermelon. Parmer (2014) studied the efficacy of six different combination fungicides at different concentrations against *F. oxysporum* f. sp. *niveum* using poisoned food technique and results revealed that cymoxanil 8 % + mancozeb 64 % and carbendazim 50 WP + mancozeb 75 WP were most effective in inhibiting growth of test fungus (100 %) at lowest concentration of 250 ppm whereas carbendazim 12 % + mancozeb 63 % also inhibited growth of *F. oxysporum* f. sp. *niveum* (100 %) at 1000 and 2000 ppm concentrations.

Miller (2017) studied new fungicides for the management of *F. oxysporum* f. sp. *niveum* (FON). This study examined the *in vitro* sensitivity of *F. oxysporum* f. sp. *niveum* to fungicides and for control of *Fusarium* wilt of watermelon in the field. Fungicides prothioconazole, pydiflumetofen, tebuconazole and propiconazole reduced *F. oxysporum* mycelia growth *in vitro*. They reported that prothioconazole and pydiflumetofen reduced *Fusarium* wilt incidence and severity in field experiments.

Petkar *et al.* (2017) studied the effects of prothioconazole and thiophanate-methyl on mycelial growth and spore germination of *F. oxysporum* f. sp. *niveum* isolates collected in watermelon fields in Georgia. *In vitro* mycelium growth studies indicated that all 100 isolates evaluated were sensitive to prothioconazole, the effective concentration that suppressed mycelium growth by 50 per cent ranged from 0.75 to 5.69 mg/ml (averaged 1.62 mg/ml). In contrast, 3 and 4 per cent of the isolates were resistant that prothioconazole may be a viable option for management of *Fusarium* wilt of watermelon where as Thiophanate-methyl should be used judiciously due to the existence of isolates resistant to the fungicide. Karki *et al.* (2022) reported that watermelon wilt would be managed by soil fumigation with 40:60 mixture of 1, 3-D and chloropicrin (Piclor 60) along with root knot nematodes.

2.5 Influence of soil properties on wilt incidence in glasshouse condition

Taya *et al.* (1988) reported that incidence of *Macrophomina phaseolina* on Chickpea was highest on sandy soil than on clay soil while studying the influence of soil type, soil moisture and fertilizers on the severity of chickpea dry root- rot. They reported that low soil moisture was conducive to disease development.

Kuldhar *et al.* (2013) studied the effect of soil types on the incidence of pea wilt incited by *Fusarium oxysporum* in a pot culture by using five different types of soils such as black, red, sandy, sandy loam and clay soils. They concluded that disease incidence was highest in black soil followed by clay soil.

Singh *et al.* (2017) reported that sandy soil supported highest wilt incidence in case of watermelon, tomato and marigold and least was found in case of silt clay soil while carrying out effect of different edaphic factors on wilt incidence. They also concluded that plants which were irrigated after the interval of two days showed less disease incidence.

2.6 Host range studies of *F. oxysporum* f. sp. *niveum* in glasshouse condition

Romberg and Davis (2007) studied host range of *F. solani* f. sp. *eumartii*, causal organism of foot rot of potato. They conducted host range studies on tomato, eggplant, pepper. Isolates from both potato and tomato caused dry rot symptoms on potato tubers and root or collar rot all four host species in the greenhouse. In field trials also isolates from both tomato and potato were pathogenic on tomato, potato and pepper.

Goswami *et al.* (2008) reported that *Fusarium equiseti* causes reddish brown discoloration on ginseng roots, was inoculated and studied on hosts like tomato, pepper, carrot, cucumber did not exhibit any visible symptoms. However, seed decay and reddish brown to black lesions were observed on hypocotyls and roots of kidney bean, bush bean, broad bean, chickpea and pea.

Zarafi and Abdulkadir (2014) conducted host range studies on *F. oxysporum* f. sp. *strigae* on twenty-six economically important crops by recording plant vigour, plant height, plant fresh and dry weight before sowing/planting, out of total ten crops were statistically differed.

3. MATERIALS AND METHODS

The present work entitled “**Studies on watermelon (*Citrullus lanatus*) wilt incited by *F. oxysporum* f. sp. *niveum***” was carried out at laboratory and glasshouse of Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra. The materials and methods used for various experiments were as follows.

3.1 Materials

3.1.1 Experimental Site

The experiment was conducted at the Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri.

3.1.2 Collection of diseased samples

Naturally infected watermelon plants (*Citrullus lanatus*) showing the typical symptoms of wilt were collected from All India Coordinated Research Project on Vegetables, Mahatma Phule Krishi Vidyapeeth, Rahuri. The diseased samples were brought into laboratory for further studies.

3.1.3 Cultural media

The growth characteristics of *F. oxysporum* f. sp. *niveum* were studied on ten different solid media. The following are the media used for studying the morphological and cultural variability of the pathogen.

Potato Dextrose Agar (1 lit.)

Potato extract	200.0 g
Dextrose	20.00 g
Agar-agar	20.00 g
Distilled water	1000 ml

Richard's Agar (1 lit.)

Sucrose (C ₁₂ H ₂₂ O ₁₁)	20.00 g
Potassium nitrate (KNO ₃)	10.00 g
Potassium dihydrogen phosphate (KH ₂ PO ₄)	05.00 g
Magnesium sulphate (MgSO ₄ .7H ₂ O)	02.50 g
Ferric chloride (FeCl ₃ .6H ₂ O)	00.02 g
Agar-agar	20.00 g
Distilled water	1000 ml

Czapek's Dox Agar (1 lit.)

Sucrose (C ₁₂ H ₂₂ O ₁₁)	30.0 g
Sodium nitrate (NaNO ₃)	2.00 g
Dipotassium phosphate (K ₂ HPO ₃)	1.00 g
Magnesium sulphate (MgSO ₄ .2H ₂ O)	0.50 g
Potassium chloride (KCl)	0.50 g
Ferrous sulphate (FeSO ₄)	0.01 g
Agar-agar	15.0 g
Distilled water	1000 ml

Corn Meal Agar (1 lit.)

Maize extract powder	30.0 g
Agar-agar	20.0 g
Distilled water	1000 ml

Oat Meal Agar (1 lit.)

Oat meal powder	30.0 g
Agar- agar	20.0 g
Distilled water	1000 ml

Sabouraud's Dextrose Agar (1 lit.)

Dextrose	40.0 g
Peptone	10.00 g
Agar-agar	20.00 g
Distilled water	1000 ml

V8 juice Agar (1 lit.)

V8 juice	8.30 g
L-Asparagine	10.00 g
Yeast extract	2.00 g
Calcium carbonate	2.00 g
Glucose	2.00 g
Agar- agar	20.0 g

Host Leaf extract Agar (1 lit.)

Watermelon leaf extract	100.0 g
Dextrose	20.00 g
Agar-agar	20.00 g
Distilled water	1000.0 ml

Conn's Agar (1 lit.)

Potassium nitrate	2.00 g
Magnesium sulphate	1.20 g
Potassium dihydrogen phosphate	2.70 g
Maltose	7.20 g
Potato starch	10.00 g
Agar	15.00 g
Distilled water	1000.0 ml

Ashby's Mannitol Agar (1 lit.)

Mannitol	20.00 g
Dipotassium hydrogen phosphate	0.200 g
Magnesium sulphate	0.200 g

Sodium chloride	0.200 g
Potassium sulphate	0.100 g
Calcium carbonate	5.00 g
Agar-agar	15.00 g
Distilled water	1000 ml

3.1.4 Chemicals

Standard chemicals, reagents, fungicides, culture media *etc.* required for the experiment were obtained from Department of Plant Pathology and Agricultural Microbiology, MPKV, Rahuri.

3.1.5 Glass wares

The common glass wares *viz.*, Petri plates, test tubes, glass slides, conical flasks, measuring cylinder, glass rods, beakers, funnels, pipettes *etc.* were used.

3.1.6 Laboratory Instruments and Equipments

Different laboratory instruments which include autoclave, hot air oven, laminar-airflow unit, biological oxygen demand incubator (BOD), refrigerator, binocular research microscope, electronic balance, mixture-cum-grinder, pH meter *etc.* were used during the course of investigation were used.

3.1.7 Miscellaneous materials

Other materials used during experimentation like earthen pots (10 cm and 30 cm dia.), inoculation needle, non-absorbent cotton, forceps, micropipette, test tube stands, cork and borer, blotter paper, filter paper, scissors, polythene bags, spirit lamp, stock solution of mercuric chloride (0.1 %), labels, muslin cloth, rubber bands, glass marking pens, sticky labels, etc.

3.1.8 Fungicides

The following fungicides were used for *in vitro* experiments conducted during present studies,

Sr. No.	Common name	Formulations @ 500 and 1000 ppm
1.	Carbendazim	50 % WP
2.	Mancozeb	75 % WP
3.	Hexaconazole	5 % SC
4.	Propiconazole	25 % EC
5.	Chlorothalonil	75 % WP
6.	Carbendazim 12 % + Mancozeb 63 %	75 % WP

3.1.9 Seeds

Seeds of watermelon cultivar Sugar Baby were purchased from local market of Rahuri and were used for pot culture experiments to prove pathogenicity, host range analysis and for soil properties studies.

3.1.10 Biocontrol agents

Pure cultures of biocontrol agents viz., *Trichoderma viride*, *T. harzianum*, *Aspergillus niger*, *A. flavus*, *Bacillus subtilis* and *Pseudomonas fluorescens* were obtained from the Department of Plant Pathology, MPKV, Rahuri; maintained and multiplied on appropriate culture media and used for further studies.

3.1.11 Botanicals

The various plant extracts used for the management of *Fusarium* wilt of watermelon were collected from the farms of MPKV, Rahuri. Following locally available seven plant species were used during present studies.

Sr. No.	Common Names	Scientific Names	Plant parts used
1.	Neem	<i>Azadirachta indica</i>	Leaf
2.	Karanj	<i>Pongamia pinnata</i>	Leaf
3.	Parthenium	<i>Parthenium hysterophorus</i>	Leaf
4.	Gaint milkweed	<i>Calotropis gigantea</i>	Leaf
5	Custard apple	<i>Annona squamosa</i>	Leaf
6.	Basil	<i>Ocimum sanctum</i>	Leaf
7.	Onion	<i>Allium cepa</i>	Bulb

3.2 Methods

3.2.1 Collection of disease samples and isolation of the pathogen

Diseased sample of watermelon showing typical symptoms of wilt were collected from the field, brought into the laboratory and washed thoroughly in running tap water. These diseased specimens (stems and roots) were blot dried and cut with sharp sterilized blade into small bits (5 mm) keeping half healthy and half diseased portion intact. These pieces were surfaces sterilized with 0.1 % aqueous solution of sodium hypochlorite (NaClO_2) for two minutes and then washed with sterile distilled water to remove traces of chemical and again blot dried. The surface sterilized diseased pieces were inoculated on the solidified and cooled PDA medium in Petri plates under aseptic

conditions of laminar-air-flow cabinet. Inoculated plates were then incubated in BOD incubator at $27 \pm 2^{\circ}\text{C}$ temperature. After a week of incubation, the well-developed mycelial growth free from any contamination was obtained. Following single hyphal-tip isolation technique, the fungus was sub-cultured aseptically on the PDA slant in test tubes. Through frequent sub-culturing, the fungus was purified and pure culture was maintained on agar slants in test tubes and stored in refrigerator for further studies.

3.2.2 Identification of the isolated pathogen

Identification of the pathogen was done based on pathogenicity test, microscopic examination and by comparing cultural and morphological characteristics of the pathogen with those described by previous workers.

3.2.2.1 Pathogenicity test

3.2.2.1.1 Mass multiplication of pathogen

Mass multiplication of the pathogen was done on sand maize meal medium by placing 90 g of sand, 10 g of maize meal and 20 ml of distilled water in each 250 ml conical flask. The medium was autoclaved at 121°C for 15 minutes. These flasks were inoculated with a bit of actively growing fungal culture and incubated at 25°C for 15 days. Fungal soil mixture was prepared by hand mixing contents of each flask with the required quantity of autoclaved field soil under hygienic conditions.

3.2.2.1.2 Soil inoculation technique

Pathogenicity test was conducted by soil inoculation technique under pot conditions. Pots were filled with sterilized soil. Inoculum from mass multiplied culture of pathogen was inoculated in pots. Healthy and sterilized watermelon seeds of cv. Sugar Baby were sown in pots. Surface sterilized seeds sown in un inoculated sterilized soil, served as check. These pots were watered regularly. Observations on disease incidence were taken. Re isolation was made from the infected parts of the watermelon plants and the culture obtained was compared with original one.

3.2.2.1.3 Re Isolation

To fulfill Koch's postulates the organism was reisolated from the roots of artificially inoculated and diseased plants showing the typical symptoms of wilting. The fungus growth obtained was transferred on potato dextrose agar medium for comparison with original culture of the test pathogen.

3.2.2.2 Microscopic examination

The pure culture of pathogen was mounted on clean glass slide in 0.1 % lactophenol cotton blue, covered with cover slip and observed under compound microscope.

3.2.3 Cultural and Morphological characters of pathogen

A total of ten media *viz.*, Ashby's mannitol agar, Corn meal agar, Czapek's dox agar, Potato dextrose agar, Conn's agar, V8 juice agar, Richard's agar, Oat meal agar, Sabouraud maltose agar and Watermelon leaf extract agars were used to study their effect on growth and sporulation of the test pathogen. All the media were sterilized in autoclave at 121⁰C for 15 min.

Autoclaved and cooled media were poured (@ 20 ml / plate) in sterilized glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. On solidification of the media, Petri plates of each culture medium were inoculated by placing 5 mm mycelial disc of actively growing 7 days old pure culture of test pathogen in center. Each culture medium was replicated thrice. Plates were then incubated in BOD incubator at 27 ± 2⁰C temperature. Observations on mycelial growth, colony colour and sporulation were recorded after 7 days of incubation.

3.2.3.1 Spore Morphology

A loopful of the culture of *F. oxysporum* f. sp. *niveum* obtained from each of 12 days old culture media was placed on the slide and mixed thoroughly with lactophenol to obtain uniform spread and cover slip was placed to observe under microscope. The sporulation was graded as follows.

Sporulation = No of Conidia/ microscopic field (400 X)

Sporulation	Remark	Range
++++	Excellent	>75
+++	Good	50-75
++	Moderate	25-50
+	Poor	1-25
-	No sporulation	00

3.2.4 Study of influence of soil properties on wilt incidence of watermelon in glass house condition

To study the influence of soil properties such as effect of soil type, soil pH and number of irrigated days on the wilt incidence of watermelon, five types of soil *viz.*, clay, clay loam, sandy clay loam, silt clay loam and silt loam were collected from the

various fields of Mahatma Phule Krishi Vidyapeeth, Rahuri under the guidance of soil chemist. Before using, these soils were sterilized in autoclave for two consecutive days at 1.1 kg/cm² pressure for two hours and mixed with the inoculum of *Fusarium* spp. at 5 per cent weight of soil in pot. These inoculated soils were put in pots and moisten with water. Surface sterilized five seeds were sown in each pot and regularly observed for the appearance of the wilt, so that effect of soil types on per cent disease incidence was recorded. Following are the physical properties of soils at the time of collection.

Sr. No	Soil type	pH	EC (dSm ⁻¹)
1.	Clay	8.12	0.23
2.	Clay loam	7.76	0.39
3.	Sandy clay loam	7.12	0.18
4.	Silt clay loam	7.59	0.21
5.	Silt loam	7.82	0.42

To study the influence of pH, soils of five different pH ranges such as 8.5-9.5, 7.5-8.5, 6.5-7.5, 5.5-6.5 and 4.5-5.5 were selected from fields of Mahatma Phule Krishi Vidyapeeth, Rahuri, calcium hydroxide was used for adjustment of pH. Pots were allowed to weather for seven days before sowing and inoculated with *Fusarium* spp. at 5 per cent weight of soil in pot. For each pH level, five surface sterilized seeds were sown and observed regularly for the appearance of the wilt. To study the influence of number of irrigated days on wilt incidence, clay loam soil was filled and inoculated with *Fusarium* spp. at 5 per cent weight of soil in pot. These pots were irrigated at the intervals of 2, 4, 6, 8 and 10 days to maintain different moisture regimes. Surface sterilized five seeds were sown in each pot and per cent disease incidence was recorded. During each experiment three replications were maintained along with control and seeds were purchased from local market of Rahuri town. Per cent disease incidence was calculated by using formula given by Mayee and Datar (1985).

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

3.2.5 Host range study of *F. oxysporum* f. sp. *niveum* in pot culture under glasshouse condition

Plants from different families as mentioned below were used in host range study of watermelon wilt. Pots of 10 and 30 cm in diameter were filled with sterilized clay loam soil, each pot was inoculated with *Fusarium* spp. at 5 per cent weight of soil in pot and each pot was sown with 3 seeds or seedlings with 3 replicates of each mentioned hosts. Plants were incubated under glasshouse condition watered regularly for the appearance of per cent disease incidence.

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

Sr. No.	Family	Host plants	Scientific Names
1.	Alliaceae	Onion	<i>Allium cepa</i>
2.	Cucurbitaceae	Muskmelon	<i>Cucumis melo</i>
		Bitter gourd	<i>Momordica charantia</i>
3.	Cruciferaeae	Cabbage	<i>Brassica oleracea</i> var. <i>capitata</i>
		Radish	<i>Raphanus sativus</i>
4.	Leguminaceae	Dolichos bean	<i>Lablab purpureus</i>
		Chick pea	<i>Cicer arietinum</i>
		Soyabean	<i>Glycine max</i>
5.	Solanaceae	Tomato	<i>Solanum lycopersicum</i>
		Chilli	<i>Capsicum annum</i>

3.2.6 Evaluation of fungicides on inhibition of *F. oxysporum* f. sp. *niveum* under *in vitro* condition

Five systemic fungicides and one combination fungicide were evaluated at 500 and 1000 ppm concentrations against *F. oxysporum* f. sp. *niveum* on the potato dextrose agar medium by using poison food technique (Nene and Thapliyal, 1993) under *in vitro* conditions.

Details of experiment

Design : Completely Randomized Design
 Replications : Three
 Treatments : Seven

Observations on radial mycelial growth/colony diameter of the test fungus were recorded at an interval of 24 hrs. and continued till growth of the test pathogen in

untreated control plate was fully covered. Per cent inhibition of the test pathogen was calculated by applying the formula given by Vincent (1927).

Tr. No.	Chemical name	Formulations (@ 500 and 1000 ppm)
T ₁	Carbendazim	50 % WP
T ₂	Mancozeb	75 % WP
T ₃	Hexaconazole	5 % SC
T ₄	Propiconazole	25 % EC
T ₅	Chlorothalonil	75 % WP
T ₆	Carbendazim 12 % WP + Mancozeb 63 % WP	75 % WP
T ₇	Control	-

$$\text{Per cent inhibition} = \frac{\text{Growth of test fungus in control plates} - \text{Growth in treated plates}}{\text{Growth of test fungus in control plates}} \times 100$$

3.2.7 Evaluation of bio-agents on inhibition of *F. oxysporum* f. sp. *niveum* under *in vitro* condition

Four fungal antagonists viz., *Trichoderma viride*, *T. harzianum*, *Aspergillus niger*, *A. flavus* and bacterial antagonist *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated under *in vitro* condition against *F. oxysporum* f. sp. *niveum* by applying dual culture technique (Dennis and Webster, 1971). Seven days old cultures of the test bio-agents and test fungus *F. oxysporum* f. sp. *niveum* grown on PDA were used for the study. Discs of 5 mm diameter of PDA along with culture growth of the test fungus and bio-agents were cut out with sterilized cork borer. Then two culture discs, one each of the test fungus and bio-agents were placed at equidistance and exactly opposite with each other on solidified PDA medium in Petri plates aseptically and plates were incubated at $27 \pm 2^{\circ}\text{C}$. PDA plates inoculated only with culture disc of the test fungus were maintained as control.

Details of the experiment;

Design : Completely Randomized Design

Replications : Three

Treatments : Seven

Observations on linear mycelial growth of the test fungus and bio-agents were recorded at an interval of 24 hours and continued till untreated control plates were

fully covered with mycelial growth of the test fungus. Per cent inhibition of the test fungus by the bio-agents over untreated control was calculated by applying formula (Arora and Upadhyay, 1978).

$$\text{Per cent Growth Initiation} = \frac{\text{Colony growth in control plate} - \text{Colony growth in Intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

3.2.8 Evaluation of botanicals on inhibition of *F. oxysporum* f. sp. *niveum* under *in vitro* condition

Locally available plant extracts were evaluated *in vitro* against *F. oxysporum* f. sp. *niveum* by applying poisoned food techniques (Nene and Thapliyal, 1993) and using PDA as basal medium. Fresh healthy plant parts (100 g of leaves/bulbs) collected from fields were washed with distilled water, air-dried and crushed in 100 ml of distilled water (w/v). The crushed product was tied in muslin cloth and collected the filtrate. The prepared solution gave 100 per cent concentration, which was further diluted to required concentrations of 5 and 10 per cent. A quantity of 12.5 and 25 ml of each plant extract was separately mixed thoroughly with autoclaved and cooled PDA medium in conical flasks of 250 ml capacity to obtain desired concentrations of 5 and 10 per cent respectively. The PDA medium with separate plant extract was then poured (20 ml/plate) into sterile Petri plates (90 mm dia.) and allowed to solidify at room temperature. Upon solidification of PDA, all the treatments and control plates were aseptically inoculated by placing a 5 mm mycelial disc obtained from a week old actively growing pure culture of *F. oxysporum* f. sp. *niveum* at the center of plate. For each test plant extract and their respective concentrations, three replications were maintained. All these plates were then incubated at $27 \pm 2^{\circ}\text{C}$ temperature for a week or till the untreated control plates were fully covered with mycelial growth of the test fungus.

Details of the experiment:

Design : Completely Randomized Design

Replications : Three

Treatments : Eight

Tr. No.	Name of plant extracts	Scientific names	Plant parts used at 5 and 10 % concentrations
T ₁	Neem	<i>Azadirachta indica</i>	Leaf extract
T ₂	Karanj	<i>Pongamia pinnata</i>	Leaf extract
T ₃	Parthenium	<i>Parthenium hysterophorus</i>	Leaf extract
T ₄	Gaint milkweed	<i>Calotropis gigantea</i>	Leaf extract
T ₅	Custard apple	<i>Annona squamosa</i>	Leaf extract
T ₆	Basil	<i>Ocimum santum</i>	Leaf extract
T ₇	Onion	<i>Allium cepa</i>	Bulb extract
T ₈	Control	-	-

Observation on radial mycelial growth of the test fungus were recorded at 24 hrs. interval and continued till growth of the test pathogen in control plate was fully covered. Per cent inhibition of the test pathogen was calculated by applying the formula given by Vincent (1927).

$$\text{Per cent Growth} = \frac{\text{Growth of test fungus in control plates} - \text{Growth of test fungus in treated plates}}{\text{Growth of test fungus in treated plates}} \times 100$$

3.2.9 Statistical Analysis

The data was analyzed statistically by using completely randomized design. Per cent data was first transformed to the arc sine values before analysis and the treatments were compared to the means of critical difference at 1 per cent level of significance (Panse and Sukhatme, 1967).

$$S. E = \sqrt{2 \text{ EMSS} / r}$$

Where,

S. E. = Standard error

EMSS = Error means of sum of square

r = Number of replications

C. D. = $t_{\text{error}} * S. E.$

Where,

C. D. = Critical difference

t = t value at error degrees of freedom

4. RESULT AND DISCUSSION

The present research work entitled “Studies on watermelon (*Citrullus lanatus*) wilt incited by *Fusarium oxysporum* f. sp. *niveum*” was undertaken during 2021-22 at Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, MPKV, Rahuri. The results of research aspects are described and explained in this chapter.

4.1 Isolation and identification of the pathogen

The wilt infected watermelon samples showing typical symptoms of the disease were collected from All India Coordinated Research Project on Vegetables, MPKV, Rahuri. The disease was observed from seedling to maturity stage of watermelon plant. Symptoms recorded from seedling stage were chlorosis, wilting of leaves, cortical decay, blackish grey discolouration of stem near to the soil base, softening of roots. In older plants, brownish vascular discolouration was observed when section of root was kept under microscope as xylem vessels were blocked by hyphal clumps. In field, infected plants shown complete wilting and defoliation as showed in Plate 1. The most common symptom observed in field condition is one sided wilting of a plant. Defoliation of the leaves was also seen before entire plant wilted.

Kurt *et al.* (2008) described similar symptoms of wilted watermelon plants at seedling stage these were damping off, adult plant wilting, yellowing of the older leaves, stunting followed by rotting of roots and eventually death of plants. Egel Dan (2018) also observed similar type of symptoms of wilt in watermelon filed as one-sided wilting and also vascular brownish discolouration in roots. Further, they observed defoliation of leaves and decay of portion of the vines at the base of mature plants.

The pathogen was isolated by using standard procedures on PDA medium which produced a pale yellow to whitish cottony colour growth, microscopic view showed profusely branched hyaline mycelium with microconidia, macroconidia and chlamydospores. Further isolated pathogen was brought into pure culture by hyphal tip method and sub cultured periodically on PDA slants. They were preserved for future studies as showed in Plate 2.

Boughalleb and Mahjoub (2006) isolated *F. oxysporum* f. sp. *niveum* from infected watermelon plants on PDA medium similarly. Tziros *et al.* (2007) carried out

similar studies on isolation of *F. oxysporum* f. sp. *niveum*. Naveenkumar *et al.* (2017) recorded *F. oxysporum* f. sp. *niveum* produced cottony white or creamy white or pale yellow with white or pinkish white mycelial growth on PDA medium in a similar way while studying morphological and physiological characteristics of the fungi.

Microscopic examination of mounts prepared from the isolated pure culture revealed that *F. oxysporum* f. sp. *niveum* produced septate, hyaline profusely branched mycelium and asexual spores as microconidia, macroconidia and chlamydospores as shown in Plate 3.

Microconidia were one celled, hyaline elliptic to ovoid in shape. Macroconidia were sickle-shaped, hyaline, slightly curved, septate (1-5 septa) and varying in size. Both macro and microconidia were formed from conidiophores on PDA. Chlamydospores were spherical in shape borne terminally and intercalary, hyaline to light brown colour was observed.

Kleczewski and Egel (2011) observed that *F. oxysporum* f. sp. *niveum* produced banana shaped often 3 septate macroconidia at the center of the Petri plate and small oval to kidney shaped, often aseptate, microconidia were produced on false heads on short monophialdes formed on aerial mycelia. Dutta *et al.* (2017) recorded morphological characteristics of *F. oxysporum* f. sp. *niveum* in a similar way that pathogen produced asexual spores such as microconidia, macroconidia and chlamydospores. They described macroconidia were fusiform (boat- shaped) and often have three to five cells. Chlamydospores were small, single-celled and kidney shaped.

4.2 Pathogenicity test for test pathogen

Pathogenicity was confirmed by proving Koch's postulates for test pathogen. The test was conducted by using soil inoculation technique under pot conditions. The pathogen was mass multiplied on sand maize meal medium at 25°C for 15 days for a soil inoculum. Fungal inoculum was mixed with sterilized soil in pots. Healthy and sterilized watermelon seeds of cv. Sugar baby were sown in pots. After seedling growth, results showed that typical wilt symptoms were observed first after three weeks of incubation in glass house. Leaves showed interveinal chlorosis as initial symptoms. Later stages symptoms were observed such as shrivelled leaves, loss of turgor pressure in vines and finally plant showed complete wilting. Whatever the symptoms

observed on plants in inoculated pots were similar to diseased plants which were seen in field. However, uninoculated pot did not show any symptoms of wilt as showed in Plate 4.

The test pathogen was reisolated from artificially diseased plant of cv. Sugar Baby and cultural characters were observed after a week of incubation. The results showed similar characters of the pathogen which were isolated and incubated earlier from naturally infected diseased tissue of cv. Sugar Baby. Thus, the characteristics symptoms of the test pathogen were similar in both artificial and naturally infected plants of cv. Sugar baby which confirms the pathogenicity for test pathogen as *F. oxysporum* f. sp. *niveum* and hence proved Koch's postulates.

Radhakrishnan and Sen (1986) proved pathogenicity of *F. oxysporum* f. sp. *melonis* on muskmelon in a similar way under controlled conditions of screen house by sick soil method. Zhou and Everts (2007) also successfully proved pathogenicity of *F. oxysporum* f. sp. *niveum* in a similar way on susceptible watermelon cultivar under controlled conditions. Sumitra (2006) also studied pathogenicity of *F. oxysporum* f. sp. *gladioli* similarly by sick soil method.

4.3 Cultural and Morphological characters of pathogen

The cultural and morphological characters were studied on ten different solid media as described earlier in 3.1.3 section. The characters such as colony colour, shape, margin and meal colony diameter in mm were recorded. The colony colour observed on all media were mostly white and it was varied from pale yellow to pinkish colour in some media also. Mycelial growth was observed puffy, profuse, loose cottony white and circular to irregular. There was a good growth of the pathogen on most of the media however no growth was observed on Ashby's mannitol agar. In 5th and 7th day, Potato Dextrose Agar medium showed significantly highest mean mycelial growth of 54.28 mm and 86.32 mm respectively. Least growth was observed in case of Oat meal agar as 22.60 mm and 34.12 mm. The colony growth in case of other media was as follows Corn meal agar- 48.32 mm and 50.23 mm, Richard's agar- 44.28 mm and 57.34 mm, Czapek's dox agar-43.08 mm and 53.41 mm, Watermelon leaf extract agar-42.60 mm and 58.22mm, Conn's agar- 40.22 mm and 50.63 mm, V8 agar-34.04 mm and 45.65 mm, Sabouraud maltose agar- 28.80 mm and 36.84 mm respectively in 5th and 7th days.

Sporulation was observed on all media except Ashby's mannitol agar. Excellent (++++) sporulation was found on media such as Potato dextrose agar, Richard's agar, Oat meal agar, Sabouraud maltose agar. Media such as Czapek's dox agar and Conns agar showed good (+++) sporulation. V8 agar and Watermelon leaf extract agar showed moderate (++) sporulation. Whereas, poor (+) sporulation was observed on Corn meal agar. The detailed morphological and cultural characters were described in Table 1, depicted in Plate 5 and showed in Fig.1

Jamaria (1972) conducted research on cultural characters of *F. oxysporum* f. sp. *niveum* on different media in a similar way and reported that Potato dextrose agar, Richard's agar and Czapek's agar showed maximum growth and sporulation.

Smith (2007) reported that members of *Fusarium* sp. produce both microconidia and macroconidia. Macroconidia were sickle shaped 3-5 septate with thin walls and tapering toward the ends which were pointed. The microconidia were oval to ellipsoidal shape.

Nikam *et al.* (2011) studied in a similar way that morphological characters of *F. oxysporum* f. sp. *ciceri* on various media and revealed that maximum mycelial growth was obtained on host leaf extract medium of susceptible cultivar JG-62 (90mm) followed by Richard's agar (86mm) and Potato dextrose agar (83mm). All three media showed significantly good mycelial growth and sporulation. The least growth was observed on Oat meal agar (71mm). Shinde (2019) reported that Potato dextrose agar showed maximum mycelial growth of 86.33 mm with excellent sporulation while conducting similar research on cultural characters of *F. oxysporum* f. sp. *niveum* on ten different solid media.

All the above-described investigations by various workers on pathogenicity test, symptomatology, microscopic examination, morphological and cultural characteristics of isolated *Fusarium* sp. showed close identity with isolated pathogen. Thus, *Fusarium* sp. isolated and used under study was identified and confirmed as *F. oxysporum* f. sp. *niveum*.

Table 1. Evaluation of different culture media on growth and sporulation of *F. oxysporum* f. sp. *niveum*

Tr. No.	Growth Media	Mean Colony diameter (mm) After 5 & 7 days		Cultural characteristics	Sporulation
		5	7		
T ₁	Ashby's mannitol agar	0	0	No growth of fungi	-
T ₂	Corn meal agar	48.32	50.23	White colony colour, medium mycelial growth not touching to lid	+
T ₃	Czapek's dox agar	43.08	53.41	White cottony circular growth with mycelia touching to lid	+++
T ₄	V8 agar	34.04	45.65	White growth with concentric rings of mycelia	++
T ₅	Conn's agar	40.22	50.63	White cottony growth slightly touching to lid	+++
T ₆	Potato dextrose agar	54.28	86.32	Pale yellow to white cottony growth with excellent growth of mycelia	++++
T ₇	Richard's agar	44.28	57.34	White irregular growth	++++
T ₈	Oat meal agar	22.60	34.12	Initially pinkish growth later turns to white cottony with excellent mycelium	++++
T ₉	Sabouraud maltose agar	28.80	36.84	White growth not touching to lid	++++
T ₁₀	Watermelon leaf extract agar	42.60	58.22	White puffy growth later turns o dirty white	++

4.4 Study of influence of soil properties on wilt incidence of watermelon in glass house condition

The effect of soil properties such as soil type, soil pH and number of irrigated days on the wilt incidence of watermelon was studied in the glass house of Department of Plant pathology in pot culture on Sugar Baby variety. The soils were collected from fields of Mahatma Phule Krishi Vidyapeeth, Rahuri and methodology was followed as described in section 3.2.4, per cent disease incidence was recorded. Among

the five types of soils used, clay loam soil showed least wilt incidence of 20 per cent. Maximum per cent disease incidence of 76 per cent was observed in case of sandy clay loam soil. Per cent disease incidence showed by other treatments was mentioned in Table 2. For studying the influence of pH on wilt incidence, clay loam soil of different pH range was collected and modified as per the methodology mentioned in section 3.2.4.

Per cent disease incidence was recorded from each pH range showed that pH range of 8.5-9.5 recorded lower incidence of 24 per cent, other treatments as 7.5-8.5 as 44 per cent, 6.5-7.5 as 52 per cent, 5.5- 6.5 as 92 per cent and 4.5-5.5 as 64 per cent as depicted in Table 3.

The per cent disease incidence was significantly highest at higher intervals of 6, 8, 10 days as 44, 56 and 64 while lower at 2,4 days interval as 32, 36 respectively as shown in Table 4. And also, results were shown in Plate 6.

Chand and Thakur (1969) reported that sandy soil showed highest wilt incidence in case of *F. oxysporum* f. sp. *lycopersici* where as clay soil showed least incidence. They reported that percentage of infection appeared to increase with the increase of sand content in soil and they also mentioned that soil pH and per cent infections had direct correlations.

Table 2. Influence of soil types on disease incidence of wilt caused by *F. oxysporum* f. sp. *niveum*

Treatments	Soil types	Per cent Disease Incidence
T ₁	Clay	30
T ₂	Clay loam	20
T ₃	Sandy clay loam	76
T ₄	Silt clay loam	36
T ₅	Silt loam	28

Table 3. Influence of pH ranges on disease incidence of wilt caused by of *F. oxysporum* f. sp. *niveum* in clay loam soil

Treatments	Soil pH range	Per cent Disease Incidence
T ₁	8.5-9.5	24
T ₂	7.5-8.5	44
T ₃	6.5-7.5	52
T ₄	5.5-6.5	92
T ₅	4.5-5.5	64

Table 4. Influence of intervals of irrigated days on disease incidence of wilt caused by *F. oxysporum* f. sp. *niveum* in clay loam soil

Treatments	Number of intervals of irrigated days	Per cent Disease Incidence
T ₁	2	32
T ₂	4	36
T ₃	6	44
T ₄	8	56
T ₅	10	64

Singh *et al.* (2017) conducted similar research on influence of soil properties on watermelon, tomato and marigold wilt and reported that sandy soil had highest wilt incidence and least in silt clay soil, soils with lower pH range encouraged pathogen growth. And plants which were irrigated at ten days interval showed highest wilt incidence.

4.5 Host range studies of *F. oxysporum* f. sp. *niveum* in glasshouse condition

The study of incidence of *F. oxysporum* f. sp. *niveum* on different host plants such as muskmelon, dolichos bean, chick pea, soya bean, bitter gourd, tomato, onion, chilli, cabbage and radish was conducted in glasshouse of Department of Plant Pathology, MPKV, Rahuri as per the methodology mentioned in section 3.2.5. The results showed in Plate 7 revealed that watermelon crop was found infected severely under glasshouse conditions and all other host plants from diverse families selected for study were found disease free and showed no infection of wilt under the glasshouse conditions.

Hamed *et al.* (2009) studied host range for *F. oxysporum* f. sp. *niveum* under glasshouse with plants like cucumber, squash, watermelon (Cucurbitaceae), trifolium and bean (Leguminaceae), strawberry (Rosaceae) and cabbage (Cruciferceae) and found infection only on watermelon. No other plant was infected with pathogen and concluded that watermelon was only susceptible host to the pathogen *F. oxysporum* f. sp. *niveum*.

Romberg and Davis (2007) in a similar way studied host range of *F. solani* f. sp. *eumartii* causal organism of foot rot of potato on host plants like tomato,

eggplant, pepper. Isolates from both potato and tomato showed dry rot symptoms and root or collar rot on all four host species in the glasshouse conditions.

Goswami *et al.* (2008) reported that *F. equiseti*, causes reddish brown discoloration on ginseng roots, not showed any symptoms on host plants like tomato, pepper, carrot, cucumber whereas seed decay and reddish brown to black lesions were observed on hypocotyls and roots of kidney bean, bush bean, broad bean, chickpea and pea under glasshouse conditions.

4.6 *In vitro* evaluation of fungicides against inhibition of *F. oxysporum* f. sp. *niveum*

Different fungicides were evaluated *in vitro* at 500 ppm and 1000 ppm levels against *F. oxysporum* f. sp. *niveum* by poison food technique (Arora and Upadhyaya, 1978). The results are interpreted in Table 5 and depicted in Plate 8 and Fig. 2.

Table 5. *In vitro* evaluation of fungicides against inhibition of *F. oxysporum* f. sp. *niveum*.

Tr. No.	Treatments details	Per cent inhibition (ppm)*	
		500	1000
T ₁	Carbendazim 50 % WP	83.51 (66.04)	84.44 (66.76)
T ₂	Mancozeb 75 % WP	60.92 (51.30)	66.29 (54.50)
T ₃	Hexaconazole 5 % SC	62.22 (52.07)	66.66 (54.73)
T ₄	Propiconazole 25 % EC	51.11 (45.63)	56.11 (48.50)
T ₅	Chlorothalonil 75 % WP	83.07 (65.70)	84.25 (66.61)
T ₆	Carbendazim 12 % WP + Mancozeb 63 % WP	99.74 (87.07)	99.81 (87.50)
T ₇	Control	0.00 (0.00)	0.00 (0.00)
	S.Em. ±	0.18	0.17
	CD at 1 %	0.78	0.71

*: Figures in parenthesis are arc sine transformed values

It was observed that at 500 ppm, per cent inhibition of test pathogen was ranged from 51.11 to 99.74 per cent. Fungicide carbendazim 12 % WP + mancozeb 63 %

WP was statistically found best which inhibited 99.74 per cent mycelial growth followed by fungicide carbendazim 50 % WP (83.51 %), chlorothalonil 75 % WP (83.07 %), hexaconazole 5 % SC (62.22 %), mancozeb 75 % WP (60.92 %) over untreated control. Propiconazole 25 % EC was found less effective with minimum mycelial inhibition per cent of 51.11.

At 1000 ppm, per cent inhibition of test pathogen *F. oxysporum* f. sp. *niveum* was ranged from 56.11 to 99.81 per cent. The treatment of fungicide carbendazim 12 % EC + mancozeb 63 % WP was again found significantly highest per cent inhibition of 99.81 per cent among all other fungicides used, which was followed by treatment of carbendazim 50 % WP (84.44 %), chlorothalonil 75 % WP (84.25 %), hexaconazole 5 % EC (66.66 %), mancozeb 75 % WP (66.29 %) and propiconazole 25 % EC (56.11 %) against per cent inhibition of test pathogen over untreated control. The least per cent inhibition was in the treatment of propiconazole 25 % EC (56.11 %). The observations are showed in Table 5, Plate 8 and Fig. 2. Thus, all fungicidal treatments used *in vitro* against test pathogen were fungistatic and they significantly inhibited mycelial growth of *F. oxysporum* f. sp. *niveum*.

Mukherjee and Tripathi (2000) conducted the same work on *in vitro* evaluation of different fungicides against *F. oxysporum* f. sp. *phaseoli* found carbendazim and hexaconazole best which inhibited 100 per cent growth of test pathogen.

Parmar (2014) did same work on efficacy of fungicides on *F. oxysporum* f. sp. *niveum* and reported that cymoxanil 8 % + mancozeb 64 % (100 %) and carbendazim 12 % + mancozeb 63 % (100 %) inhibited mycelial growth of test pathogen.

4.7 *In vitro* evaluation of botanicals against inhibition of *F. oxysporum* f. sp. *niveum*

In addition to the routine fungicides, the locally available botanicals were used against *F. oxysporum* f. sp. *niveum* at 5 and 10 per cents by poisoned food techniques (Nene and Thapliyal, 1993) and the results obtained on mycelial growth inhibition was recorded in the Table 6, Fig.3, Plate 9. The results revealed that at 5 per cent concentration fungal per cent inhibition was ranged from 45.89 per cent to 80.32 per cent. Significantly maximum per cent inhibition was recorded in *Allium cepa* (80.32 %),

which was followed by *Parthenium hysterophorus* (79.72 %), *Calotropis gigantea* (69.92 %), *Azadirachta indica* (64.82 %), *Ocimum santum* (62.64 %), *Annona squamosa* (61.55 %). Least inhibition was observed in *Pongamia pinnata* (45.89 %) against the test pathogen over untreated.

At 10 % concentration, pattern of botanical efficacy was found same as in case of 5 % concentrations, the mycelial growth inhibition of the test pathogen was ranged from 60.25 to 82.62 per cent. Onion (*Allium cepa*) showed significantly maximum per cent inhibition of 82.62 per cent followed by *Parthenium hysterophorus* (80.81 %), *Calotropis gigantea* (72.88 %), *Azadirachta indica* (68.40 %), *Annona squamosa* (62.44 %), *Ocimum santum* (60.55 %). Least inhibition was observed at *Pongamia pinnata* (60.25 %) against the test pathogen untreated.

Table 6. *In vitro* evaluation of botanicals against inhibition of *F. oxysporum* f. *sp. niveum*

Tr. No.	Treatments details	Per cent inhibition*	
		5 %	10 %
T ₁	<i>Azadirachta indica</i>	64.82 (53.60)	68.40 (85.31)
T ₂	<i>Pongamia pinnata</i>	45.89 (49.87)	60.25 (50.91)
T ₃	<i>Parthenium hysterophorus</i>	79.72 (58.26)	80.81 (64.01)
T ₄	<i>Calotropis gigantea</i>	69.92 (56.92)	72.88 (58.61)
T ₅	<i>Annona squamosa</i>	61.55 (51.67)	62.44 (52.2)
T ₆	<i>Ocimum santum</i>	62.64 (52.61)	60.55 (51.09)
T ₇	<i>Allium cepa</i>	80.32 (63.60)	82.62 (65.36)
T ₈	Control	0.00 (0.00)	0.00 (0.00)
	S.Em. ±	0.20	0.17
	CD at 1 %	0.85	0.69

*: Figures in parenthesis are arc sine transformed values

Farrag *et al.* (2013) conducted similar *in vitro* evaluation of plant extracts, they concluded that peppermint extract 2 % was the most effective and completely inhibited spore germination and mycelial growth where as garlic and rheum extracts at 3

% showed complete spore suppression and mycelial growth. Hadi *et al.* (2013) did same work on *in vitro* evaluation of plant extracts against *F. oxysporum* schlecht and recorded garlic (*Allium sativum*) showed maximum inhibition percentage of the test pathogen.

Bharath *et al.* (2006) carried out similar study on efficacy of chemicals in management of seed borne fungal pathogens of watermelon and reported that *Fusarium* species was effectively suppressed due to carbendazim (Bavistin) followed by the Thiophanate methyl.

4.8 *In vitro* evaluation of bioagents against inhibition of *F. oxysporum* f. sp. *niveum*

In addition to fungicides and botanicals both fungal and bacterial bioagents were also tried against *F. oxysporum* f. sp. *niveum* using dual culture technique, results obtained are depicted in Table 7, Fig 4, Plate 10. The results revealed that, mycelial inhibition of the test pathogen was ranged from 41.48 to 83.03 per cent. Among the all antagonists used *Trichoderma harzianum* showed significantly highest per cent inhibition of 83.03 which was followed by bacterial antagonists *Bacillus subtilis* (76.48 %), *Aspergillus flavus* (76.29 %), *Pseudomonas fluorescence* (75.37 %) and *Trichoderma viride* (66.85 %). The least per cent inhibition 41.48 per cent was showed by *Aspergillus niger*. Thus, results revealed that all bioagents which were evaluated *in vitro* against the test pathogen were antagonistic in action and significantly inhibited the growth of *F. oxysporum* f. sp. *niveum*.

Hamed *et al.* (2009) reported that *P. fluorescens* (B4) gave the highest per cent inhibition with control treatment while conducting work on *Pseudomonas ceparica* (B1), *Bacillus polymyxa* (B2), *B. subtilis* (B3) and *P. fluorescens* (B4).

Cao *et al.* (2011) revealed that use of bacterial antagonist *Bacillus subtilis* SQR 9 under *in vivo* and *in vitro* conditions against *F. oxysporum* f. sp. *cucumerinum* gave successful results. Singh *et al.* (2014) conducted similar work on *in vitro* efficacy of bioagents viz., *T. viride*, *Gliocladium virens*, *T. harzianum*, *T. hamatum*, *Aspergillus niger*, *P. fluorescens* against *Fusarium* spp. by using dual culture technique. The results revealed that seed treatment with *T. viride* and *T. harzianum* gave significantly better results.

Table 7. *In vitro* evaluation of bioagents against inhibition of *F. oxysporum* f. *sp. niveum*

Tr. No.	Bioagents	Per cent inhibition*
T ₁	<i>Trichoderma viride</i>	66.85 (54.8)
T ₂	<i>Trichoderma harzianum</i>	83.03 (65.7)
T ₃	<i>Aspergillus niger</i>	41.48 (40.1)
T ₄	<i>Aspergillus flavus</i>	76.29 (60.9)
T ₅	<i>Bacillus subtilis</i>	76.48 (60.98)
T ₆	<i>Pseudomonas fluorescens</i>	75.37 (60.24)
T ₇	Control	00 (0)
	S.Em. ±	0.15
	CD at 1 %	0.78

*: Figures in parenthesis are arc sine transformed values

5. SUMMARY AND CONCLUSION

The current research work “Studies on Watermelon (*Citrullus lanatus*) wilt incited by *F. oxysporum* f. sp. *niveum*” was carried out on various aspects viz., isolation, identification, pathogenicity, cultural and morphological characteristics of the test pathogen, *in vitro* evaluation of fungicides, botanicals, bioagents and influence of soil properties such as soil types, soil pH, number of irrigated days on wilt incidence and host range studies on test pathogen under glasshouse condition. The significant results and findings of these works are summarized below.

Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai] is an economically significant vegetable fruit farmed worldwide. The crop belongs to runner family Cucurbitaceae which is an annual creeper. The fruit is rich in water content (91 %) and good source of potassium and magnesium. However, fruit quality and productivity are reduced by number of diseases, among them *Fusarium* wilt is one of the major threats as disease was noticed as soil borne in nature.

The watermelon wilted samples which showed typical symptoms of the disease such as chlorosis, defoliation, cortical rot and damping off of seedlings, sudden progressive wilt of older plants, necrotic lesions on the roots, vascular browning, tyloses formations were collected from the infected fields. The samples were washed, surface sterilized and subjected to isolation. The pathogen *F. oxysporum* f. sp. *niveum* was isolated successfully on Potato Dextrose Agar medium by placing a bits of surface sterilized samples (roots and stems), isolated pathogen was purified and maintained for future studies.

The pathogenicity test of the pathogen was proved successfully by using Koch's postulates on watermelon cv. Sugar Baby in pot culture. The wilt symptoms on watermelon crop in glasshouse condition were found similar to field conditions while performing pathogenicity tests that is appearance of damping off, yellowing of the older leaves, stunting, necrotic lesions on roots and collar regions followed by rotting of roots, vascular brownish discoloration in roots and eventually death of plants. By proving the pathogenicity test and also observing morphological and cultural properties on inoculated PDA medium, microscopic observation of spores viz., microconidia, macroconidia and

chlamydospores, test pathogen was identified as *F. oxysporum* f. sp. *niveum* (E. F. Smith) Snyd. & Hans.

Studies on morphological and cultural characters of *F. oxysporum* f. sp. *niveum* on ten different solid media showed that mean colony diameter was maximum in PDA medium with 86.32 mm growth and colony characters were observed as pale yellow to whitish colour cottony growth with excellent sporulation (++++) was observed. Other media used for studies also showed significantly good mycelial growth of the pathogen. However, on Ashby's mannitol agar medium growth of the pathogen was not observed.

The effect of soil properties under glasshouse condition such as soil types showed that among the five types of soils used, least wilt incidence of 20 per cent in case of clay loam soil while highest of 76 per cent in case of sandy clay soil was observed. Effect of soil pH on wilt incidence showed that pH 5.5-6.5 showed significantly highest wilt incidence of 92 per cent and least was observed at pH of 8.5-9.5 of 24 per cent. The role of soil moisture was studied on wilt incidence by altering the intervals of irrigation days, per cent disease incidence was observed highest at higher intervals like 6, 8, 10 days as 44, 56 and 64 per cents respectively, while lower at 2- and 4-days interval as 32 and 36 per cents respectively. Host range studies on *F. oxysporum* f. sp. *niveum* under glasshouse condition revealed that inoculation of test pathogen on crops from diverse families like muskmelon, bitter melon, onion, cabbage, radish, dolichos bean, chick pea, soyabean, tomato and chilli didn't show any disease symptoms. Thus, proved that watermelon is the only host plant for *F. oxysporum* f. sp. *niveum* under glasshouse conditions.

The studies on *in vitro* evaluation of different fungicides on mycelial inhibition of *F. oxysporum* f. sp. *niveum* showed that carbendazim 12 % WP + mancozeb 63 % WP found best among seven treatments used, which inhibited 99.74 per cent and 99.81 per cent of mycelial growth at 500 and 1000 ppm concentrations respectively. Other treatments also showed significant inhibition in fungal mycelial growth. propiconazole 25 % EC was found less effective among the all treatments used with minimum mycelial inhibition per cent of 51.11 and 56.11 at 500 and 1000 ppm concentrations respectively.

Studies on evaluation of seven botanicals against mycelial growth of *F. oxysporum* f. sp. *niveum* under *in vitro* conditions revealed that *Allium cepa* (Onion) showed 80.32 per cent and 82.62 per cent of mycelial inhibition at 5 and 10 per cent concentrations respectively. *Pongamia pinnata* (Karanj) showed least inhibition of 45.89 and 60.25 per cents at 5 and 10 per cent concentrations respectively. All other seven plant extracts used as treatments were also reported significant mycelial inhibition over untreated control.

While studying the *in vitro* effect of bioagents on mycelial inhibition of *F. oxysporum* f. sp. *niveum* revealed that *T. harzianum* showed significantly highest per cent inhibition of 83.03 per cent with least mean mycelial growth of 15.7mm and *Aspergillus niger* showed 41.48 per cent of inhibition with a mean mycelial growth of 32.5mm. Remaining four bioagents such as *B. subtilis* (76.48 %), *P. fluorescence* (75.37 %), *A. flavus* (76.29 %), *A. niger* (41.48 %), *T. viride* (66.85 %) used for study are also antagonistic in action and good at significantly inhibiting the growth of the pathogen.

Conclusion

The watermelon wilt was caused by *F. oxysporum* f. sp. *niveum*. The disease is becoming a major threat in all watermelon growing areas as the disease is soil borne in nature which can retain in soil as chlamydospores for several years. Hence, present study was focused on morphological and cultural characters of pathogen, influence of soil properties such as soil type, soil pH and interval of irrigated days on wilt incidence, host range study for the pathogen and also on integrated disease management such as evaluation of efficacy of fungicides, botanicals and bioagents under *in vitro* conditions. The conclusions from these studies are as below:

1. The *F. oxysporum* f. sp. *niveum* was isolated from diseased sample and proved the Koch's postulates. The symptoms of disease were seen from seedling stage to older plants. Major symptoms such as chlorosis, defoliation, cortical rot and damping off of seedlings, vascular browning, tyloses formation were noticed.
2. Growth of test pathogen on ten different media showed that, the Potato dextrose agar was superior culture medium for *in vitro* growth with excellent sporulation.
3. The clay loam type of soils and soils with higher pH range such as 8.5-9.5 showed least per cent disease inhibition of pathogen. Soils with 2- and 4-days irrigated

intervals showed least per cent inhibition. The host plants inoculated with pathogen didn't show any symptoms of the disease revealed that watermelon is the only host to the pathogen *F. oxysporum* f. sp. *niveum*.

4. The *in vitro* evaluation of six fungicides showed that carbendazim 12 % WP + mancozeb 63 % WP at both 500 and 1000 ppm concentrations was most effective than other five fungicidal treatments.
5. The *in vitro* evaluation of seven botanicals showed that *Allium cepa* (Onion) at both 5 and 10 per cent concentrations was most effective in mycelial inhibition of test pathogen than other six botanicals used.
6. The *in vitro* evaluation of six bioagents showed that *T. harzianum* showed significantly highest per cent inhibition than other bioagents used for study.
7. Considering the initial *in vitro* findings from these investigations it's need of time to use eco-friendly combinations of botanicals, bio-agents for *in vivo* testing on field at large level in the future for reducing and management of watermelon wilt disease.

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7. VITAE

Miss. CHAITRA G V
MASTER OF SCIENCE (AGRICULTURE)
in
PLANT PATHOLOGY
2023

Title of thesis		:	“Studies on Watermelon (<i>Citrullus lanatus</i>) wilt incited by <i>Fusarium oxysporum</i> f. sp. <i>niveum</i> ”
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