

STUDIES ON CONTROL OF *FUSARIUM* WILT OF CHILLI  
(*Capsicum annuum* L.) BY BIO-CONTROL AGENTS

By

**JITENDRA NANASAHEB THORAT**

(Regd.No.95082)

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

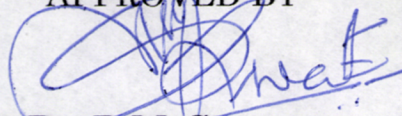
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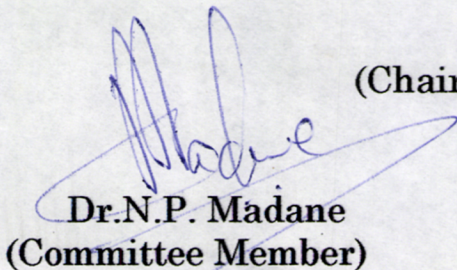
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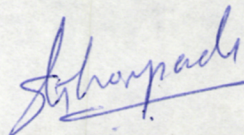
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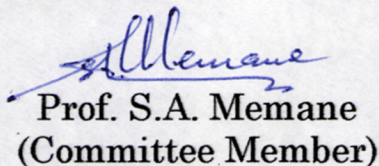
Dr. N.P. Madane

(Committee Member)



Dr. S.A. Ghorpade

(Committee Member)



Prof. S.A. Memane

(Committee Member)

DEPARTMENT PLANT PATHOLOGY AND AGRIL. MICRO BIOLOGY

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Place : MPKV,Rahuri.

Dated : 31 / 12 /1997

Thorat  
(J.N. Thorat)

Dr. D.M. Sawant  
Head,  
Department of Plant Pathology  
and Agril. Microbiology  
Mahatma Phule Krishi Vidyapeeth,  
Rahuri - 413 722. Dist. Ahmednagar,  
Maharashtra State (INDIA)


### C E R T I F I C A T E

This is to certify that the thesis entitled "STUDIES ON CONTROL OF FUSARIUM WILT OF CHILLI (Capsicum annum L.) BY BIO-CONTROL AGENTS", submitted to the Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra State, India for the award of the degree of MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY, embodies the results of a bonafide research work carried out by SHRI. JITENDRA NANASAHEB THORAT, 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 acknowledged.

Place : Rahuri

Dated : 31 / 12 / 1997.

  
(D.M. Sawant)  
Research Guide

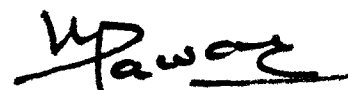
Dr. V.M. Pawar  
Associate Dean,  
Post Graduate Institute,  
Mahatma Phule Krishi Vidyapeeth,  
Rahuri - 413 722. Dist. Ahmednagar,  
Maharashtra State (INDIA)

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Place : Rahuri

Dated : 14 / 1 / 1997.



(V.M. Pawar)  
Associate Dean

### ACKNOWLEDGEMENTS

I have this opportunity to express my deep sense of gratitude and indebtedness to my research guide Dr. D.M. Sawant, Head, Department of Plant Pathology and Agril. Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri, for his inspiring guidance constructive criticism, prompt suggestions, valuable counsel, constant encouragement and immense sympathy during the preparation of this manuscript.

I am also grateful to Dr. N.P. Madane, Prof. S.A. Memane, Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri, for their continued suggestions, helpful comments, encouragement and facilities extended during the period of investigations.

I am equally indebted to Dr. S.A. Ghorpade, Prof. of Seed Technology, M.P.K.V., Rahuri for his constant encouragement and valuable suggestions during the preparation of manuscript.

My sincere thanks are also to all the staff members of Department of Plant Pathology and Agricultural Microbiology, M.P.K.V., Rahuri who provided necessary help during the course of these investigations.

I express my sincere gratitude to Dr. V.G. Rao, Mycologist, Agharkar Research Institute, Pune-4, for rendering the help in identification of the organisms under study.

It is really beyond my capacity to express my feelings for the overwhelming affection and cooperation that I received from Anil, Shivnath, Taru, Tanaji, Bangar, Gulab, Ullhas, Suresh, Gorakh and all my friends and colleagues.

At last but no least, no words are enough to express my heartfelt gratitude to my parents (Aai and Kaka) elder brother Chandrakant and sister Miss. Varsha, younger brother Virendra and all my relatives for their love, sacrifice and encouragment.

I am grateful to all authors, past and present whose literature has been cited. I extend my thanks to 'SUJOG COMPUTERS', Shri. Vishawanath Kadam, Farm Quarter, Rahuri, for neat and tidy typing of my thesis.

Place : Rahuri

Jitendra  
(Jitendra N. Thorat)

Dated : 31 / 12 / 1997.

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## LIST OF ABBREVIATIONS USED

cm	:	Centimeter
°C	:	Degree centigrade
etc	:	Et cetera
g	:	Gram (s)
i.e.	:	That is
kg	:	Kilogram (s)
Ltd	:	Limited
m	:	Meter (s)
Mg	:	Miligram (s)
ml	:	Millilitre (s)
mm	:	Millimeter (s)
No	:	Number
%	:	Per cent
um	:	Micrometer
viz.,	:	Namely
Vit.	:	Vitamin
E.C.	:	Emulsifiable concentrates
kg/cm <sup>2</sup>	:	Kilogram (s) per centimeter square

## ABSTRACT

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STUDIES ON CONTROL OF FUSARIUM WILT OF CHILLI  
(Capsicum annuum L.) BY BIO-CONTROL AGENTS

By

Jitendra Nanasaheb Thorat

Mahatma Phule Krishi Vidyapeeth,

Rahuri - 413 722

1997

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Research Guide : Dr. D.M. Sawant  
Department : Plant Pathology and Agril.  
Microbiology

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Chilli is one of the important spice crop grown in Maharashtra. Amongst the diseases of chilli Fusarium wilt is the most damaging one to the chilli crop in the field causing great losses. In view of the commercial importance of the crop, the present study was undertaken on chemical and biological control of the disease and other allied aspects.

The common symptoms of Fusarium wilt were loss of turgidity of leaves, severe leaf rolling, stunting of plant, flower drop, browning of stem and death of twig. The symptoms on roots were rotting of roots, peeling of epidermis and vascular browning.

Patogenicity was tested by soil inoculation and root dip method. The wilting symptoms were observed 25 days

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Abstract contd....

J.N. Thorat

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after transplanting. The pathogen was identified as Fusarium solani (Mart.) App. and Wollenw., on the basis of morphological characters.

Morphological, cultural and physiological characters were studied. Armstrong Fusarium medium was the best medium for growth and sporulation. Good source of carbon for the fungus was maltose, glucose, sucrose. Among the nitrogen sources potassium, nitrate, sodium nitrate were good nitrogen sources.

The optimum, minimum and maximum temperature requirement of fungus were found to be 28, 10 and 35°C respectively. The thermal death point was 54°C.

In in vitro evaluation the most effective fungicides were carbendazim and mancozeb followed by copperoxychloride.

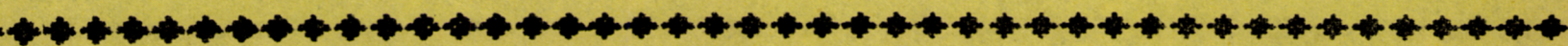
In in vitro evaluation of antagonists such as Trichoderma viride, Trichoderma longiform, Gliocladium virens were found most effective against Fusarium solani.

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Chapter Opener Page



Introduction



## 1 . INTRODUCTION

Chilli (Capsicum annum L.) is the most important spice and solanaceous crop grown for its pungent fruits, which is consumed as green, ripe, fresh or ripe dried. It is also called as 'red pepper', 'hot pepper', 'cayenne pepper', 'sweet pepper' etc. It is an important cash crop. It is also used medicinally and in chutnies and pickles. It is a major ingredient of curry powder in culinary preparations. Five species of Capsicum are under cultivation though a number of wild species have been identified. In India only two species viz., Capsicum annum L. and C. frutescens L. are known and most of the cultivated varieties belong to C. annum. Its origin is considered to be from tropical America, especially Brazil (Thompson and Kelly, 1957). In India, it has been reported to be introduced by Portuguese in 17th century and since then it has become an indispensable part of Indian diet. Chilli is rich in both vit. A and C. Its pungency is due to the capsin (18 H<sub>27</sub>ON<sub>3</sub>) volatile alkaloid. Its red colour at ripening stage is due to pigment capsanthin (C<sub>40</sub>H<sub>58</sub>O<sub>3</sub>). According to Aykroyd (1941), green chilli fruits contain 82.6 per cent water, 6.7 per cent fibre, 0.91 per cent mineral matter, 0.6 per cent fat, 111 mg vitamin C and vitamin A 454 I.U. per 100 g. It forms an essential ingredient for housewives in day to day preparations of spicy and tasty food, because it alone gives the necessary colour, flavour, taste and peculiar aroma to various food dishes.

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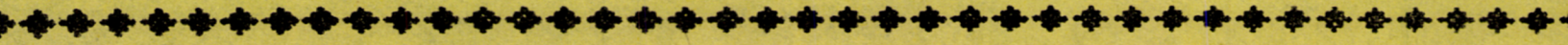
Besides India, the major chilli growing countries are China, Pakistan, Italy, Spain, Indonesia, Japan, Southern United States, Hungary, Mexico, Uganda, Kenya, Nigeria, Sudan, Thailand, Turkey and other African countries. In India, during 1990-91, the area under chilli was 11 lakh hectares with a production of 15,94,000 tonnes of dry chillies (Anonymous, 1995). The major chilli producing states are Maharashtra, Andhra Pradesh, Karnataka, collectively contributing to about 75 per cent of total country's production (Rose and Som, 1985). About 2 to 4 per cent of its produce is exported, while rest is consumed locally. In Maharashtra, in 1993-94, the area under chilli was 70070 hectares with annual production of 679000 tonnes of dry chillies (Anonymous, 1995). The major chilli growing districts in Maharashtra are Akola, Amravati, Yavatmal, Buldhana, Nanded, Parbhani, Beed, Pune, Satara, Dhule, Sangli, Solapur, Thane, Nashik and Jalgaon (Anonymous, 1990-91).

Virtually chilli is grown in wide range of climatic conditions from almost the sea level upto an altitude of 1500 mts in tropical and subtropical regions and like many other crops. Chilli is subjected to attack by many diseases caused by fungi, bacteria, viruses, nematodes, physiological disorders. Annual losses due to these diseases are at the tune of 12 to 25 per cent of the total production, fungal diseases alone cause damage at the rate of 12 to 20 per cent.

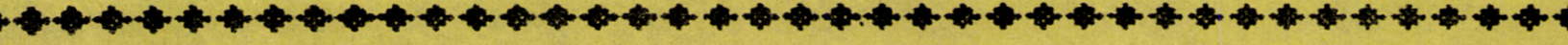
Fifty one different pathogens have been recorded to cause diseases on the various plant parts of chilli. Out of these thirty nine are caused by fungi, two by bacteria, six by viruses and four by nematodes. The important diseases of chilli crop are wilt (Fusarium solani (Mart.) App. and Wollenw.), blight (Phytophthora capsici Leonian), anthracnose (Colletotrichum capsici (Syd.) Butl. and Bisby), leaf spot (Cercospora capsici Heald and Wolf), fruit rot (Colletotrichum gloeosporoides Penz), bacterial spot (Xanthomonas campestris pv. vesicatoria (Doidge) Dye), bacterial soft rot Erwinia carotovora (Jones) Bergey et al. and common viral diseases viz., Tobacco Mosaic Virus (TMV), Tomato Mosaic Virus (TomMV) and Potato Virus X (PVX). All these diseases cause damage to the crop and reduce its market quality and yield. Among these diseases, wilt disease was found to cause severe loss in chilli crop grown in many chilli fields of this university during kharif and rabi 1991. The losses of chilli due to wilt diseases are particularly high in tropical countries such as India, which show the symptoms of loss of turgidity of leaves, stunting of plant and severe leaf fall and flower drop followed by death of twigs. The diseased plants are observed in patches and the yield of entire crop was reduced. Considering the commercial importance of chilli fruits in Maharashtra, it was felt necessary to study, in detail, the causal, organism (s) of wilt disease of chilli with the following objectives in view.

1. To collect disease samples of chilli plant from different fields.
2. To isolate the organism (s) responsible for wilt disease.
3. To prove the pathogenicity of isolated organism (s) and to study symptomatology.
4. To study morphological and cultural characters of pathogen associated and their identification.
5. To study physiological characters.
6. To study the efficacy of different fungicides in vitro.
7. To study the efficacy of different bio-control agents for control of isolated pathogen.

Chapter Opener Page



Review of Literature



## 2. REVIEW OF LITERATURE

Wilt disease of chilli (Capsicum annuum L.) is caused due to Fusarium solani (Mart.) App. and Wollenw. have been reported by few research workers from India and abroad, from the published literature, it is revealed that a very little work has been carried out on wilt disease of chilli. Therefore, literature regarding Fusarium solani on related species of chillies and other hosts are also reviewed.

The wilt disease of pepper was first observed in New Mexico during 1908, since it has been reported in most of the Southern states and as far north as Colorado and New Jersey (U.S.A.). It has also been described in South America and Europe (Sherf and Macnab, 1986). Booth (1971) reported that Fusarium solani is associated with chilli.

### 2.1 Isolation, symptomatology and pathogenicity

Sahni et al. (1967) isolated the seed borne pathogens, Pythium spp., Rhizoctonia spp and Fusarium spp. from the diseased chilli seedlings. From chillies (Capsicum annuum), Fusarium solani is often isolated from rotten roots, rotten stem bases, black coloured stems and fruits since 1974 (Steekelenburg and Paternotte, 1979). Fusarium solani causes the chilli wilt with stem browning above the ground, peeling of epidermis and vascular browning. It also occurs mostly in roots and lower stem, where there is vascular browning. It can also infect fruits (Lukacs and Szarka, 1988).

Seedlings of 19 Capsicum varieties at 2-3 leaf stage were submerged in conidial suspension of Fusarium solani and transplanted in soil. After three weeks, Keystone variety was resistant and California Wonder and export varieties were susceptible to Fusarium solani (Camino et al., 1987).

Gangopadhyay (1984) reported Fusarium solani on chilli showing symptoms of drying and browning of underground stem followed by cortical decay, appearance of water soaked spots on roots and softening of roots, stunting of plant and withering of immature fruits, dropping of apical portion and ultimate drying of whole plant.

Samples of chilli seeds were examined from 30 countries for fungal infection in pathogenicity test. Fusarium solani and Fusarium oxysporum caused seed rot and wilting of capsicum seedlings (Hasmi, 1989).

Growth characteristics of cultures of Fusarium solani from sweet pepper and Fusarium solani f. sp. cucurbitae from courgette (Cucurbita pepo) have been compared and pathogenicity has been tested. Plants were inoculating by dipping roots in conidial suspension. Suspension was also applied around the stem base. Plants showed symptoms within one week and died within two weeks (Paternotte, 1987).

## 2.2 Morphology of fungus

The measurement of conidia from fungus Fusarium solani martii, Var. Pisi was given as 27 - 40 x 4 - 4.5  $\mu\text{m}$  (Jones, 1923).

The average measurement of 3 septate conidia taken from sporodochia produced in culture of Fusarium solani var. martii may be 30 x 3.9  $\mu\text{m}$  (Snyder and Hanson, 1941).

Booth (1971) reported that microconidia of Fusarium solani are septate, abundant having size 16 - 18 x 2 - 4  $\mu\text{m}$ . While macroconidia are 1 - 5 septate, fusiform and curved at tip having size 35 x 4.5  $\mu\text{m}$ .

Agarwal and Sarbhoy (1975) reported that microconidia are numerous, hyaline, thick walled, 8.16 x 2.4  $\mu\text{m}$  in diameter while macroconidia are fusoid with thick wall, 3.5 septate having size.

One septate	-	15 x 3.3 $\mu\text{m}$
Three septate	-	35 x 3.5 $\mu\text{m}$ and
Five septate	-	44 x 4.5 $\mu\text{m}$

## 2.3 Cultural characters of fungus

Booth (1971) studied the growth of Fusarium on different media and reported that Armstrong Fusarium medium was best medium to increase inoculum. Thakur and Singh (1973)

studied the cultural characters of Fusarium solani on six different synthetic and common laboratory media. They observed that the growth was better on Richards medium. Kore and Patil (1984) observed the growth of Fusarium solani (Mart.) Sacc. on potato dextrose agar medium which was cottony white and later changed to pinkish colour. Gonzalez and Jimenez (1988) studied the effectiveness of four different culture media for Fusarium solani (Mart.) Sacc. and observed the best growth on Czapek's Dox agar medium with aerial mycelium and whitish colour.

## 2.4 Physiological studies of fungus

### 2.4.1 Utilization of carbon compounds

Satyavir and Grewal (1973) studied the physiology of Fusarium causing guar wilt. They reported that out of ten carbon sources tested maximum growth was recorded on maltose while lactose was utilized slowly.

Bhatnagar et al. (1968) studied the effect of different carbon sources on growth of Fusarium solani and reported that fungus could utilize carbon sources from variety of compounds. In general, monosaccharides proved to be better than other sources tested for growth and sporulation. Among the disaccharides maltose proved best carbon source and among polysaccharides dextrin and starch proved better sources.

Gaur and Agnihotri (1979) studied the growth of Fusarium solani in relation to carbon sources and observed

that Pathogen utilized variety of compounds as carbon source. Galactose proved to be the best source for growth of fungus followed by lactose. Among monosaccharides xylose supported good growth as compared to glucose and fructose. Among disaccharides maximum growth was obtained on lactose followed by maltose and sucrose. Among polysaccharides dextrin was good carbon source, while among hexahydric alcohols maximum growth was observed on sorbitol followed by mannitol.

#### 2.4.2 Utilization of nitrogen compounds

Bhatnagar *et al.* (1968) studied the nitrogen requirement of Fusarium solani and observed that nitrates of potassium and sodium were good sources of nitrogen. Sporulation was excellent on potassium nitrate and good sporulation on sodium nitrate. Abundant large sized chlamydospores were formed in ammonium sulphate.

Satyavir and Grewal (1973) observed that maximum growth of the fungus was observed on DL aspartic acid and DL glutamic acid followed by sodium nitrate. Gaur and Agnihotri (1978) reported that among the nitrate nitrogen compounds, sodium nitrate gave good growth followed by potassium nitrate and sporulation was good on potassium nitrate followed by sodium nitrate. Among the ammonical nitrogen compounds ammonium oxalate gave maximum dry mycelial weight followed by ammonium nitrate and sporulation was maximum on ammonium nitrate.

#### 2.4.3 Effect of temperature on growth and sporulation

Tandon and Bhargava (1963) studied the effect of temperature on growth and sporulation of Fusarium solani and they reported that the organism was capable to grow between 10 and 36°C, the optimum temperature was 25°C while no growth was observed at 6°C temperature.

Paternotte (1987) compared the effect of temperature on growth of Fusarium solani and F. solani f. sp. cucurbitae. Maximum colony diameter was observed at 27-29°C temperature.

#### 2.4.4 Thermal death point of Fusarium solani

Tandon and Bhargava (1963) reported that the growth of Fusarium solani was upto 56°C, but failed to grow above 56°C. Hence thermal death point was 55°C.

#### 2.5 Chemical control

Sahni et al. (1967) studied the efficacy of fungicides against Fusarium. Among 13 fungicides captan was found most effective in controlling Fusarium spp.

Nedelnik (1986) examined the effect of fungicides on growth of Fusarium solani in vitro and reported that thiobendazole and benomyl controlled the fungi most effectively.

Satiya and Indra Hooda (1987) reported that Fusarium solani causing chilli wilt can be effectively controlled by captafol and copper oxychloride.

Gaur (1990) reported that out of six fungicides tested, the best control of Fusarium solani under pot condition was with benomyl followed by carbendazim.

Kapoor and Kumar (1991) studied the efficacy of systemic and non-systemic fungicides against Fusarium solani and Fusarium oxysporum. They reported that carbendazim and benomyl were most toxic. The decreasing order of overall efficacy of different fungicides was carbendazim, benomyl, captafol, thirum and captan.

Monga and Grover (1991) tested 16 fungitoxicants in vitro and reported that captafol and mancozeb were most toxic and also inhibited sporulation and mycelial growth of Fusarium solani.

Singh et al. (1991) tested fungitoxicity against Fusarium solani and reported that the activity of this fungi was effectively reduced by mancozeb (Indofil M-45).

## 2.6 Biological control

Brain and McGowan (1945) reported that Trichoderma viride produces an antibiotic 'Viridin' which suppresses the growth of soil borne pathogens.

Chohan and Singh (1993) studied the control of Aspergillus niger, a seed borne pathogen of groundnut in in vitro by the antagonist Trichoderma polysporum. The antagonist Trichoderma polysporum and the test fungus Aspergillus niger were plated opposite to each other near the edges of petriplate so that the microbes grow towards each other. After incubation of the petriplates, it was seen that Trichoderma polysporum was parasitic over the colonies of Aspergillus niger and suppressed its growth.

Marois et al. (1981) studied conidial suspensions of five fungal antagonistic of Fusarium oxysporum f sp. radicis lycopersici which were applied to roots and crowns of tomato transplanted at the time of planting. The suspension contained  $5 \times 10^5$  conidia of each of three isolates of Trichoderma harzianum, one isolate of Aspergillus ochraceus and one isolate of Penicillium funiculosum. The pathogen was added at the time of planting in the soil 10 cm from the transplanted plants as 0, 50, 500 and 5000 chlamydo spores per plant in 20 ml of water. The incidence of disease increased as the inoculum density of the pathogen was increased. In fumigated soil not augmented with the antagonists, disease incidence did not increase, while the pathogen population decreased from 600 to 200 propagules per gram in soil augmented with antagonists but increased from 1000 to over  $5 \times 10^4$  propagules per gram in

non augmented soils. Yield was not affected significantly by treatment or planting date.

Tarunina (1982) observed Trichoderma lignorum (T. viride) str. A-1 showed antagonism to Fusarium oxysporum Str.1 on an agar medium but not in sterile soil, whereas, all tested strains of Trichoderma viride antagonistic to Fusarium culmorum str. 34 on agar retained this activity in soil.

Ebben and Budge (1983) studied the carnation wilt pathogen Fusarium oxysporum f. sp. dianthi and its antagonists using peat and peat loam composts. Number of Fusarium and Pseudomonas spp. decreased in both composts but Gliocladium and Trichoderma populations remained steady.

Rai and Upadhyay (1987) observed that colonization of tur substrate by Fusarium udum was highly suppressed by Trichoderma viride where these were used in inoculum mixtures with Fusarium udum.

Ercole et al. (1984) studied 60 isolates of Trichoderma viride, T. harzianum, T. koningii, T. hamatum, T. polysporum and Trichoderma spp. on PDA against Rhizoctonia solani, R. fragariae, Fusarium moniliforme, Pythium ultimum and Verticillium dahliae. Four types of antagonism found were relief formation in the contact zone between the fungi, lytic phenomena, complete covering of the pathogen by the antagonistic and variable behaviour.

Morshed (1985) demonstrated antagonism between Trichoderma spp. and Fusarium oxysporum f. sp. culmorum, Alternaria lindemuthianum. Growth of Trichoderma viride was vigorous in dual culture and it was an effective hyper parasite, penetrating and coiling its hyphae around the host hyphae. T. glaucum produced effective metabolites, while T. album caused lysis and inhibited the pathogens.

Sivan and Chet (1986) conducted the screening tests on 35 isolates of Trichoderma harzianum from the rhizosphere of cotton seedlings grown in a field with Fusarium oxysporum f. sp. vasinfectum and indicated the potentiality of one isolate as an antagonist. It was shown to be effective against Fusarium oxysporum f. sp. vasinfectum on cotton, F. oxysporum f. sp. melonis on melon and F. roseum, F. culmorum on wheat, reducing incidence and the Fusarium population in soil.

Ercole and Nipoti (1986) studied the isolates of Trichoderma species viz., T. viride, T. harzianum and T. koningii and inoculated these into the growing medium at a concentration of  $4.6 \times 10^8$  propagules per gram of dry soil to assess for the control of Verticillium dahliae and Fusarium oxysporum f. sp. lycopersici on tomato seedling cv. Marmande in a greenhouse.

Sesan (1986) reported the good results with biopreparations of T. viride applied in soil for seed

treatment against Pythium debaryanum, Rhizoctonia solani and Fusarium spp. on sunflower and leguminous plants.

Windham et al. (1986) reported that, addition of Trichoderma harzianum and Trichoderma koningii increased rate of emergence of tobacco and tomato seedlings over that of control. The tomato root and shoot dry weight in soil treated with Trichoderma spp. increased by 213 to 275 per cent and 259 - 318 per cent respectively over the controls and that tobacco root and top weights increased by 266-291 per cent.

Sesan (1987) listed 120 fungal spp. against which Trichoderma viride has an inhibitory effect determining its use as a means of biological control.

Dula et al. (1987) reported that the field infection by Fusarium oxysporum was reduced by 44.4 per cent using Trichoderma viride.

Sesan (1988) reported the literature on the use of species of Trichoderma, Aspergillus, Gliocladium, Penicillium and Verticillium to control fungal pathogen of soybean, beans, pea, fababean and lentil. Good results were obtained in Romania with preparations of Trichoderma viride for seed treatment of bean and pea against pathogens such as Collectotrichum lindemuthianum, Fusarium culmorum, Rhizoctonia solani, Pythium debaryanum and Sclerotinia sclerotiorum.

Ercole et al. (1988) reported that the good results were obtained with Trichoderma spp. in the control of seed rot and early stem rot in maize and sorghum caused by Fusarium moniliforme, Rhizoctonia solani. Use Trichoderma spp. led to a decrease in symptoms caused by Verticillium dahliae and Fusarium oxysporum f.sp. lycopersici on green house tomato and a corresponding increase in yield.

Samiyappan et al. (1989) reported that Fusarium wilt of pigeonpea was reduced by Trichoderma viride under greenhouse conditions. When fungal biomass consisting of conidia, mycelium and chlamydospores multiplied on groundnut shells were added.

Lynch (1989) outlined the mechanisms by which fungi can antagonize plant pathogens in vitro and in vivo. A brief analysis opportunities for gaining a better understanding of fungal systems was presented with emphasis on Trichoderma spp. as antagonists.

Chet (1990) reviewed the control of plant pathogens with Trichoderma spp. and other antagonists in combination with chemical or physical soil treatments under the headings. Large scale production of fungal antagonists and methods of application, Trichoderma activity in the greenhouse and the field, disease control with combination of Trichoderma and

chemical and physical treatments and Trichoderma as a plant growth promoting micro organisms.

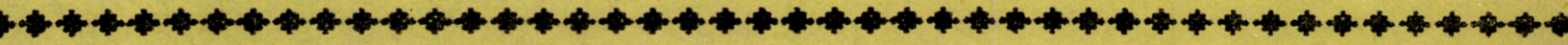
Chung and Choi (1990) reported that, the incidence of sesame damping off caused by Rhizoctonia solani and Fusarium oxysporum f.sp. sesami was reduced by coating seed with 3 isolates of Trichoderma viride or Benlate, benomyl in pot and field trials using naturally infested soils. When sesame seeds were treated with benomyl or conidia ( $10^7$ /ml) of the antagonists, seedlings emergence was significantly increased compared with the untreated control.

Deb and Dutta (1991) isolated the eleven spp. of fungi from the rhizosphere of soybean plants grown in soil infested with Sclerotium rolfsii. Most were antagonists of the pathogen in vitro, a clear inhibition zone was produced by Trichoderma viride and to some extent by Fusarium solani, with other species e.g. Aspergillus flavus, suppressed growth of the pathogen by overgrowing it. Addition of T. harzianum and T. koningii to pathogen infested soil gave 45 and 38 per cent control of foot rot disease of soybeans respectively and yield increases of 1.5 - 1.5 g/plant. This decreases in disease severity was associated with a 50 per cent reduction in pathogen population.

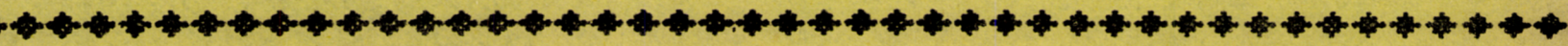
Mukhopadhyay et al. (1992) reported that the antagonists Trichoderma and Gliocladium species had shown

considerable potential in controlling several plant pathogenic fungi, particularly soil borne ones, several plant diseases were successfully reduced by the application of these antagonists. Treatment of seed with a spore suspension of these antagonists was a relatively recent and more feasible way to deliver them for seed and soil borne disease management. The integration of biological and fungicidal seed treatment was found to improve the disease control potential to a great extent. A new treatment applying this strategy developed which involved testing seeds first with the antagonists Gliocladium virens ( $10^7$  conida/ml) and then with fungicide carboxin (0.1 %). This method demonstrated in research trials and farmers fields had proved to be highly effective in managing several soil borne plant pathogens including Sclerotium rolfsii, Rhizoctonia solani, Fusarium oxysporum in chickpea, lentil and groundnut.

Chapter Opener Page



Material and Methods



### 3. MATERIAL AND METHODS

The material used and methods followed during the present investigation were as follows.

#### 3.1 Materials

##### 3.1.1 Collection of diseased samples

Several chilli plants showing typical symptoms of wilting were collected from experimental area of plant pathology and All India Coordinated Vegetable Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri.

##### 3.1.2 Culture media

Potato dextrose agar, a common laboratory medium was used for the isolation of fungus, Armstrong Fusarium medium was used for poison food technique studies, and also for carbon and nitrogen studies. In cultural studies, the media used were Czapek's agar medium, Sach's agar medium, Coon's medium, Armstrong Fusarium medium, Sabourd's medium and Nutrient agar medium, Asthma Hawkers Medium, Richards medium.

##### 3.1.3 Glasswares

Different types of glasswares were used for the experimental work. The common glasswares used were test tubes, petriplates, conical flasks, measuring cylinders, glass rods, microslides, coverslips etc.

##### 3.1.4 Equipments

Common laboratory equipments used were autoclave, oven, BOD incubator, laminar flow cabinet, refrigerator, water

bath, research microscope, ocular micrometer, stage micrometer, chemical balance, thermometer, pH meter etc.

### 3.1.5 Miscellaneous material

Blotter papers, filter papers, inoculating needles, forceps, spirit lamp, scale, earthen pots, mercuric chloride, cork borer, chemicals etc., were used during the investigation.

### 3.1.6 The following fungicides were included in the investigation programme

#### 1. Copperoxy chloride

- a. Chemical name : Copper oxychloride
- b. Trade name : Blitox-50
- c. Formulation : Wettable powder
- D. Concentration : 0.25 %
- E. Manufacturer : Rallis India Ltd., Bombay

#### 2. Carbendazim

- a. Chemical name : Methyl 2-benzimidazole carbamate
- b. Trade name : Bavistin
- c. Formulation : Wettable powder
- D. Concentration : 0.1 %
- E. Manufacturer : BASF, India Ltd., Bombay

#### 3. Captan

- a. Chemical name : N-(trichloromethyl-thio)4 tetra cyclohexane-1,2-dicarboximide

- b. Trade name : Captaf
- c. Formulation : Wettable powder
- D. Concentration : 0.2 %
- E. Manufacturer : Rallis India Ltd., Bombay
4. Mancozeb
- a. Chemical name : Zinc ion manganese ethylene bisdithiocarbamate
- b. Trade name : Indofil M-45
- c. Formulation : Wettable powder
- D. Concentration : 0.20 %
- E. Source : Indofil Chemicals Ltd.,  
Bombay
5. Thirum
- a. Chemical name : Tetramethyl Thirum disulphide
- b. Trade name : Thiride
- c. Formulation : WDP
- D. Concentration : 0.20 %
- E. Source : The Alkali and Chemical  
Corporation of India Ltd.,  
Calcutta

### 3.2 Methods

#### 3.2.1 Symptomatology

The symptoms of the disease were recorded under field condition and during pathogenicity test.

#### 3.2.2 Isolation

The samples of wilted chilli plants were collected from the experimental site of Plant Pathology and All India

Coordinated Vegetable Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri. The affected portions of the stems and roots of the samples were cut into suitable pieces, washed thoroughly in tap water so as to remove soil particles. The pieces were then disinfected by 0.1 per cent mercury chloride solution for two minutes followed by washing in three changes of sterilized water to remove the traces of mercury chloride solution. Three to four such pieces were then plated aseptically on sterilized potato dextrose agar in each petriplate. The petriplates were incubated at room temperature ( $28 \pm 1^{\circ}\text{C}$ ). Well isolated fungal growth free from contamination was transferred to agar slants by hyphal tip method. By single spore isolation technique, the fungus Fusarium solani was purified and agar slants showing pure fungal growth were maintained for further studies.

### 3.2.3 Pathogenicity

Soil inoculation method for Fusarium solani

Pure culture of Fusarium solani was multiplied separately on sand maize meal medium (sieved fine river sand 100 g, maize meal 80 g and water 150 ml) for ten days and were uniformly mixed with sterilized soil which was already mixed with F.Y.M. in the proportion of 3:1.

Eight earthen pots of 15 cm diameter were treated with 5 per cent copper sulphate ( $\text{CuSO}_4$ ) solution. Out of eight

pots, six pots were filled in with Fusarium inoculated soil and remaining two pots kept as control filled in with sterilized uninoculated soil. The inoculated soil in the pots was incubated for 15 days at room temperature, frequently stirred and watered so that the fungus could colonise in the soil. Then surface disinfected ten seeds of Phule Jyoti variety of chilli were sown in each pot. Observations were recorded from germination upto complete wilting of the plants.

#### 3.2.4 Reisolation

Reisolation was made from roots of infected plants showing typical symptoms. The culture obtained was transferred on PDA slants and compared with original culture.

#### 3.2.5 Maintenance of pure culture

Fusarium solani fungus isolated from chilli wilted plant was subcultured on potato dextrose agar medium and kept at  $28 \pm 1^{\circ}\text{C}$  for eight days for growth. Such slants were preserved in refrigerator at 5 to  $10^{\circ}\text{C}$ . These cultures were sub-cultured once in a month and maintained for further studies.

#### 3.2.6 Identification of culture

The isolated pathogenic culture was identified with the help of morphological characters and confirmed from Dr. V.G. Rao, Mycologist, Division of Plant Sciences, Agharkar Research Institute (M.A.C.S.), Pune.

### 3.2.7 Morphology of fungus

Morphology of fungus was studied on potato dextrose agar medium and from slide culture techniques, morphological characters viz., size, shape and septation were recorded from seven days old culture grown on potato dextrose agar medium at 28°C. The conidial measurements were recorded with the help of stage and ocular micrometer.

### 3.3 Cultural characters of fungus

The isolates of Fusarium solani were grown on following agar media in order to study its growth characters and ability of sporulation on different media.

1. Czapek's medium
2. Armstrong Fusarium medium
3. Coon's medium
4. Nutrient agar
5. Sabourd's medium
6. Asthma Hawkers medium
7. Richards medium

The above media were prepared as per their composition and were sterilized in autoclave at 1.05 kg/cm<sup>2</sup> pressure for 15 minutes. Twenty ml of each of the above sterilized media were poured into 100 mm diameter petriplate. Each medium was replicated three time. These plates were

inoculated with fungal disc of 5 mm diameter and incubated at  $28 \pm 1^{\circ}\text{C}$  for 7 days. Seven days old cultures were used for inoculation. Colony diameter was recorded by averaging linear growth of colony in two directions for each plate. Cultural characters viz., colour of the colony, sporulation and rate of growth on different media were recorded.

### 3.4 Physiological characters

#### 3.4.1 Utilization of carbon compounds

The ability of fungus to utilize different carbon compounds were judged by replacing sucrose of Armstrong Fusarium medium with different carbon sources. The carbon sources used in the present studies were

- |                               |   |               |
|-------------------------------|---|---------------|
| 1. Monosaccharides            | : | i. Glucose    |
|                               |   | ii. Galactose |
| 2. Dis <sup>a</sup> ccharides | : | i. Maltose    |
|                               |   | ii. Lactose   |
|                               |   | iii. Sucrose  |
| 3. Polysaccharides            | : | Dextrin       |
| 4. Polyhydric alcohol         | : | Mannitol      |



Armstrong Fusarium medium without sucrose as a basal medium was prepared and distributed in 100 ml quantities in 250 ml Erlenmeyer flasks. The required quantity of different carbon compounds on molecular weight basis were

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added to each flask separately. The media were sterilized at  $1.05 \text{ kg/cm}^2$  pressure for 15 minutes and triplicate plates were poured. The plates were then inoculated as usual and incubated at  $28^\circ\text{C}$  for 7 days. Observations on colony diameter and sporulation were recorded.

For mycelial dry weight stock solution of Armstrong Fusarium medium without sucrose was prepared and distributed 100 ml lots in 250 ml flasks in triplicate. The required quantity of different carbon compounds on molecular weight basis were added to each flask separately. The media were sterilized at  $1.05 \text{ kg/cm}^2$  pressure for 15 minutes and inoculated with Fusarium isolate. The flask were incubated with  $28^\circ\text{C}$ . The observations on mycelial dry weight were recorded 7 days after inoculation. Mycelial mat was oven dried at  $60^\circ\text{C}$  for 24 hrs and dry weight was taken.

#### 3.4.2 Utilization of nitrogenous compounds

The ability of the fungus to utilize different nitrogenous compounds were judged by replacing calcium nitrate of Armstrong Fusarium medium with different nitrogen sources. The nitrogen sources used in present studies were.

1. Nitrate nitrogen :
  - a. Potassium nitrate
  - b. Sodium nitrate
  - c. Calcium nitrate

2. Ammonical nitrogen : a. Ammonium nitrate  
b. Ammonium sulphate
3. Amide nitrogen : Urea

Armstrong Fusarium medium without calcium nitrate as a basal medium was prepared and distributed in 100ml Erlenmeyer flasks. The required quantity of different nitrogenous compounds on the molecular weight basis were added to each flask separately. The media were sterilized at 1.05 kg/cm<sup>2</sup> pressure at 15 minutes and triplicate plates were poured. Plates were then inoculated as usual and incubated at 28°C for 7 days. Observations on colony diameter and sporulation were recorded.

For mycelial dry weight stock solution of Armstrong Fusarium medium without calcium nitrate was prepared and distributed 100 ml lots in 250 ml flasks in triplicate. The required quantity of different nitrogenous compounds on molecular weight basis were added to each flask separately. The media were sterilized at 1.05 kg/cm<sup>2</sup> pressure for 15 minutes and inoculated with Fusarium isolate. The flask were incubated at 28°C. The observations on mycelial dry weight were recorded seven days after inoculation.

#### 3.4.3 Effect of temperature on growth and sporulation of Fusarium solani

The effect of temperature on growth and sporulation was studied on Armstrong Fusarium medium. Triplicate plates were poured for each temperature studies and inoculated with Fusarium solani. The plates were incubated at different temperatures viz., 5, 10, 20, 28°C and 30, 35 and 40°C for seven days. The observations on colony diameter and sporulation were recorded seven days after inoculation.

#### 3.4.4 Thermal death point of Fusarium solani

For the determination of thermal death point, sterile test tubes of uniform size and thickness were filled in with 10 ml of Armstrong Fusarium medium without agar, sterilized and inoculated with uniform mycelial bits of fungus in duplicate tubes. These tubes were subjected to different temperatures (51, 52, 53, 54, 55, 56, 57, 58, 59 and 60°C) in water bath for 10 minutes. The tubes were immediately placed in cold water and incubated at 28°C temperature. The growth of the fungus in the tube was recorded.

#### 3.5 Fungicidal control of Fusarium solani

Evaluation of different fungicides in vitro. In absence of resistant varieties, the next best alternative is to resort to chemical method of control, which is being followed on an extensive scale in a number of crops all over

the world. Different fungicides were evaluated in vitro to find out their efficacy for controlling wilt disease of chilli. Armstrong Fusarium medium was prepared and distributed in 100 ml aliquot in 250 ml Erlenmeyer flasks and sterilized. After cooling at 40°C a measured quantity of following fungicides to each of the flasks at recommended concentrations. The flasks devoid of fungicides served as control.

Sr. No.	Name of chemical	Concentration (%)	Concentration mg/100 ml of water (mg)
1.	Blitox-50	0.25	250
2.	Bavistin	0.10	100
3.	Captan	0.20	200
4.	Mancozeb	0.20	200
5.	Thiram	0.20	200
6.	Control	-	-

Flasks were shaken thoroughly and then poured in to petriplates separately for each of the chemicals. Each fungicide was replicated four times. These plates were inoculated with 5 mm fungal disc of seven days old culture grown on Armstrong Fusarium medium and incubated at 27 ± 1°C for 7 days. Plates with Armstrong Fusarium medium without fungicide served as control. Observations on colony diameter

and sporulation were recorded on seventh day after inoculation. Radial growth of fungus was measured and result were expressed as per cent inhibition of mycelial growth over control by using the following formula (Padule and Shinde, 1986).

$$I = \frac{100 (C - T)}{C}$$

Where,

- I = Per cent inhibition of fungal growth  
 C = Radial growth of fungus in control  
 T = Radial growth of the fungus in treatment

### 3.6 Biological control

#### Efficacy of antagonists in in vitro

This study was followed as per the procedure given by Chohan and Singh (1973).

Sterilized potato dextrose agar medium was poured in sterilized petriplates and replicated four times for each antagonist. The antagonists used in this study were Trichoderma viride, T. harzianum, T. longiformum, T. hamatum, T. koningii, Gliocladium virens.

The antagonist and the test fungus were inoculated opposite to each other in the petriplates near the edges so that they could grow towards each other. The plates were


incubated at  $28 \pm 1^{\circ}\text{C}$  for a week. Growth of the test fungus was measured and the result was expressed in per cent inhibition of mycelial growth over the control using the formula of Padule and Shinde (1986).

$$I = \frac{100 (C - T)}{C}$$

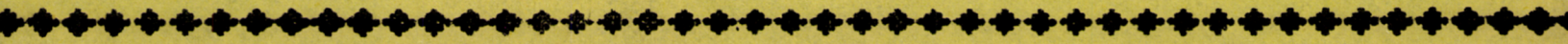
Where,

- I = Per cent inhibition of the growth of the test fungus
- C = Growth of the test fungus in control
- T = Growth of the test fungus in treatment.

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Experimental Results



## 4. EXPERIMENTAL RESULTS

The results of the experiment are presented as under.

### 4.1 Symptomatology

The symptoms of the disease were observed periodically under field conditions and after artificial inoculation of the fungus. Under field conditions, first symptom of the disease in infested plants appeared as loss of turgidity of leaves followed by severe leaf rolling, stunting of plant, flower drop, death of twig and browning of stem. The diseased plants were observed in patches in the field (Plate 1). The symptoms observed on roots were rotting of roots, peeling of epidermis, whitish brown cortical lesions on tap roots and vascular browning (Plate 2).

### 4.2 Isolation, Pathogenicity and Reisolation

Isolation made from infected roots, showing typical symptoms of wilting, yielded the fungus Fusarium solani (Mart.) App. and Wollenw. The growth of fungus from infected tissue was distinctly visible after three to four days in petriplate containing the potato dextrose agar medium, pure culture obtained was maintained for further studies.

Pathogenicity test was carried out by soil inoculation and by dipping the roots of chilli seedlings into spore suspension ( $10^4$  spores/ml). Typical symptoms observed



Plate 1. Symptoms produced by Fusarium solani on chilli under field conditions

H : Healthy plant

D : Diseased plant

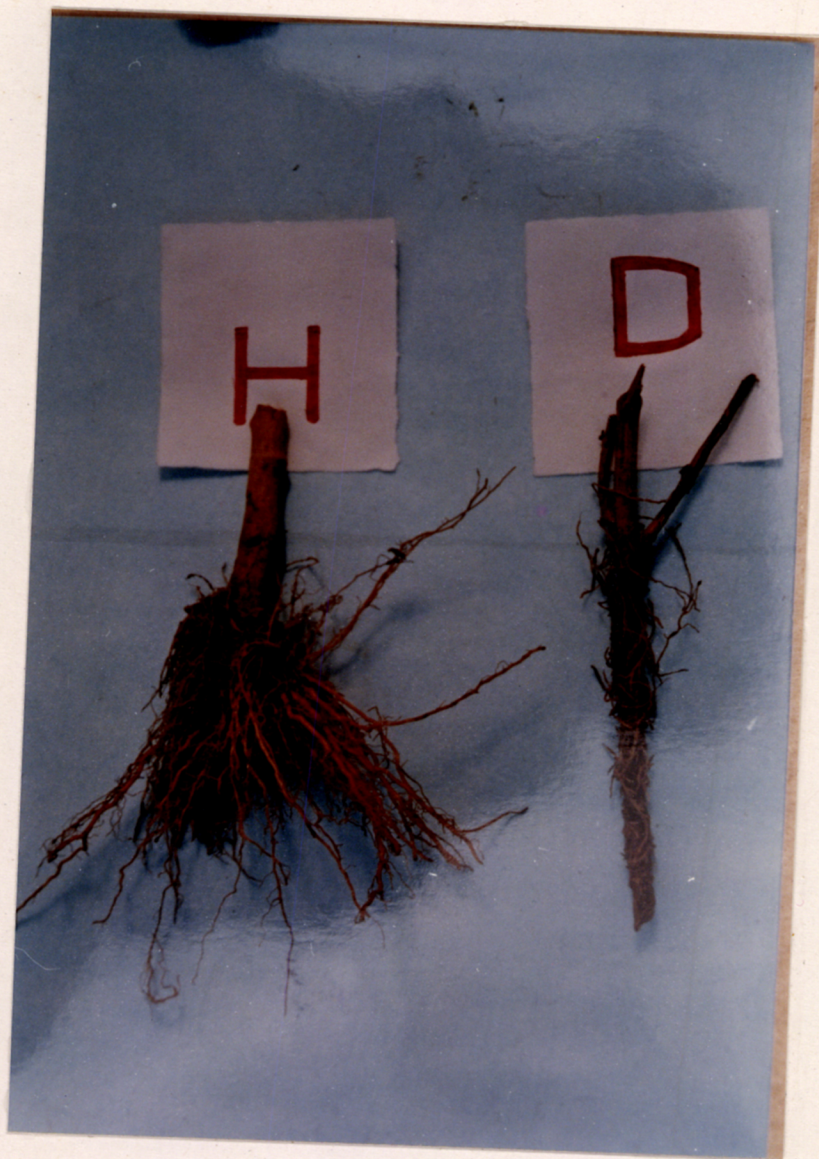


Plate 2. Symptoms produced by Fusarium solani on roots of chilli plant

H : Healthy roots

D : Diseased roots



Plate 3. Symptoms produced by Fusarium solani on  
artificially inoculated chilli plants

C : Control plant

I : Inoculated plants

consisted of leaves becoming pale, drying of leaves browning of stem and complete wilting of plant, which was noticed 25 days after transplanting of seedlings (Plate 3).

Reisolations made from artificially inoculated plants showing typical symptoms of wilting and compared with original culture.

#### 4.3 Identification of culture

The pathogenic culture isolated from diseased plants was identified as Fusarium solani (Mart.) App. and Wollenw. and confirmed from Dr. V.G. Rao, Mycologist, Division of Plant Sciences, Agharkar Research Institute (M.A.C.S.) Pune.

#### 4.4 Morphological characters

Morphological characters of fungus Fusarium solani were recorded from eight days old culture grown on potato dextrose agar medium. Mean length, breadth, septation of conidia was measured with the help of ocular micrometer.

The mycelium was hyaline, septate and profusely branched. Mycelial growth of the fungus was cottony white in colour. The conidia were variable in shape and size. Both microconidia and macroconidia were noticed. Microconidia were oval, mostly 1-2 septate and hyaline. Length and breadth of microconidia was 8-10 um and 3 um, respectively. Macroconidia

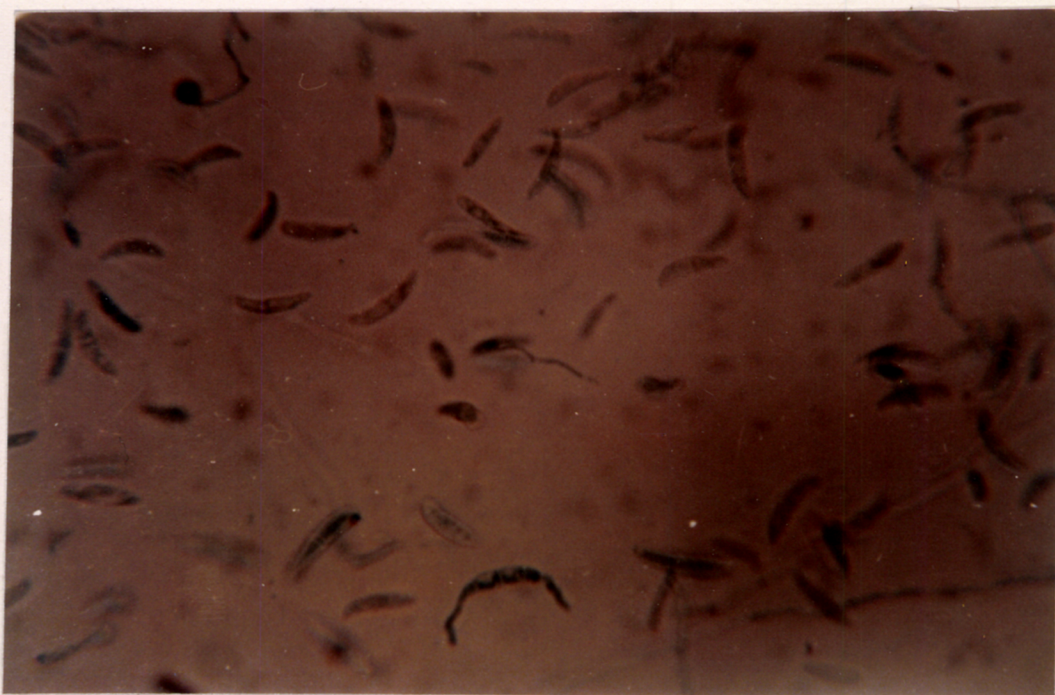


Plate 4. Morphology of Fusarium solani

were 1-2-3 septate, fusiform and slightly curved at the tip. Abundant chlamydospores were formed by the fungus after 10 days. The size of macroconidia was as under.

- |            |                |
|------------|----------------|
| 1. Septate | 10.00 x 3.0 um |
| 2. Septate | 16.20 x 4.0 um |
| 3. Septate | 33.20 x 4.5 um |

#### 4.5 Cultural characters of Fusarium solani

The colony diameter, sporulation and growth characters of Fusarium solani on seven different solid media were studied and the results are represented in Table 1.

The results presented in Table 1 indicated that the fungus could grow on variety of media. It produced significantly maximum growth on Armstrong Fusarium medium, Czapeks medium, Coons medium followed by Richards agar medium, Sabour's medium and nutrient agar. No statistical variation among Armstrong Fusarium medium, Czapek's medium and Coon's medium and also among Richards agar medium, Sabour's medium and Nutrient agar was observed. Among these media under study, Armstrong Fusarium medium was comparatively superior and it was used as a basal medium for further studies.

As regards the sporulation, very good sporulation was observed on Armstrong Fusarium medium, Czapeks medium and Richards medium, moderate sporulation was observed on Coon's medium and nutrient agar medium.

Table 1. Cultural characters of *Fusarium solani* on different media

Sr. No.	Medium	Mean colony diameter (mm)	Sporulation	Growth characters
1.	Czapeks medium	83	+++	Flat circular colony with entire margin, slightly raised at centre
2.	Armstrong <i>Fusarium</i> medium	86	+++	Circular colony with entire margin and whitish in colour
3.	Coon's medium	82	+	Circular colony with entire margin and whitish growth
4.	Nutrient agar	75	+	Circular colony with entire margin and yellowish in colour
5.	Sabour'd's medium	78	++	Flat colony with entire margin and brownish in colour
6.	Asthma Hawkers medium	67	++	Circular colony with entire margin
7.	Richard's agar medium	77	+++	Colony circular to oval

\* Sporulations + = Poor, ++ = Moderate, +++ = Good, ++++ = Excellent



Plate 5. Growth characters of Fusarium solani on different media

- |                      |                                     |
|----------------------|-------------------------------------|
| 1. Czapek's medium   | 2. Armstrong <u>Fusarium</u> medium |
| 3. Coon's medium     | 4. Nutrient agar                    |
| 5. Sabour'd's medium | 6. Asthma hawker's medium           |
| 7. Richards medium   |                                     |



Plate 6. Growth characters of Fusarium solani on different carbon sources

1. Maltose
3. Sucrose
5. Lactose

2. Glucose
4. Dextrin
6. Control

#### 4.6 Physiological studies

##### 4.6.1 Utilization of carbon compounds

The information on the effect of carbon compounds on growth and sporulation is represented in Table 2.

Table 2. Utilization of carbon compounds by Fusarium solani

Sr. No.	Carbon source	Mean colony diameter (mm)	Sporulation of <u>Fusarium solani</u> after 7 days of incubation
1.	Maltose	67.8	+++
2.	Glucose	67.4	++
3.	Sucrose	72.5	+++
4.	Dextrin	64.8	++
5.	Lactose	53.4	+++
6.	Control	30.4	-

+++ = Good sporulation

++ = Poor sporulation

- = No sporulation

It can be seen from Table 2 that the fungus could utilize all the carbon sources. Good growth was observed on sucrose, maltose, glucose whereas moderate growth was noticed on dextrin growth was poor on lactose.

Regarding sporulation, good sporulation was noticed on maltose, sucrose and lactose, while moderate sporulation was observed on glucose and dextrin.

#### 4.6.2 Utilization of nitrogenous compounds

The ability of the fungus to utilize different nitrogenous compounds was studied on Czapek's agar medium and results are given in Table 3.

Table 3. Utilization of nitrogenous compounds by F. solani

Sr. Nitrogen No. source	Mean colony diameter of <u>Fusarium solani</u> after 7 days of incubation	Sporulation of <u>Fusarium solani</u> after 7 days of incubation
1. Potassium nitrate	75.0	++++
2. Sodium nitrate	70.3	+++
3. Asparagine	64.3	+++
4. Ammonium nitrate	60.2	+++
5. Urea	50.0	++
6. Control	39.5	-

++++ = Abundant sporulation  
 +++ = Good sporulation  
 ++ = Moderate sporulation  
 + = Poor sporulation  
 - = No sporulation

It is observed that from Table 3 that the fungus Fusarium solani could grow on variety of nitrogenous compounds. Excellent growth was noticed on potassium nitrate. Good growth was observed on sodium nitrate and asparagine; whereas it was moderate on ammonium nitrate and urea.

Regarding sporulation, abundant sporulation was observed on potassium nitrate. Good sporulation was noticed on

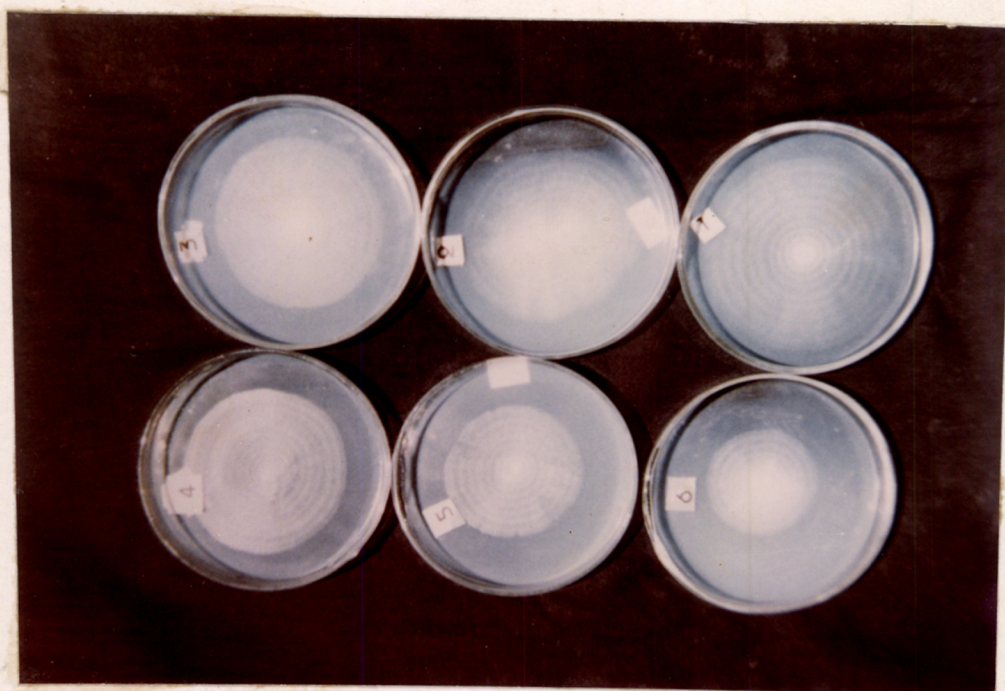


Plate 7. Growth characters of Fusarium solani on different nitrogenous sources

- |                      |                     |
|----------------------|---------------------|
| 1. Potassium nitrate | 2. Sodium nitrate   |
| 3. Asparagine        | 4. Ammonium nitrate |
| 5. Urea              | 6. Control          |

sodium nitrate, asparagine and ammonium nitrate. The sporulation was moderate on urea.

#### 4.6.3 Effect of temperature on growth and sporulation of Fusarium solani

The data in respect of growth and sporulation of Fusarium solani as influenced by different temperatures are presented in Table 4.

The data indicated that the fungus could grow between temperatures 10 to 30°C. Maximum growth was observed at 28°C. No growth was observed at 5 and 40°C temperature.

With regard to sporulation, excellent sporulation was observed at 30°C, good sporulation at 28°C and poor sporulation at 20°C and 35°C. No sporulation noticed below 10°C temperature.

Table 4. Effect of temperature on growth and sporulation of Fusarium solani

Sr. No.	Incubation temperature (°C)	Mean colony diameter (mm)	Sporulation
1.	5	0	-
2.	10	5	-
3.	20	23	+
4.	28	86	+++
5.	30	70	++++
6.	35	28	+
7.	40	0	-

- = Nil, + = Poor, ++ = Moderate, +++ = Good  
++++ = Abundant

#### 4.6.4 Thermal death point of Fusarium solani

The results of studies on thermal death point are presented in Table 5.

Table 5. Thermal death point of Fusarium solani

Sr. No.	Temperature (°C)	Growth of culture after 7 days
1.	51	+
2.	52	+
3.	53	+
4.	54	+
5.	55	-
6.	56	-
7.	57	-
8.	58	-
9.	59	-
10	60	-

+ = Growth      - = No growth

The data showed that the fungus could grow from 51 to 54°C. No growth was noticed at 55°C indicating, that thermal death point of Fusarium solani is slightly above 54°C temperature.

#### 4.7 In vitro evaluation of fungicides against Fusarium solani

It can be seen from the results presented in Table 6 that all the fungicides showed inhibitory effect of the

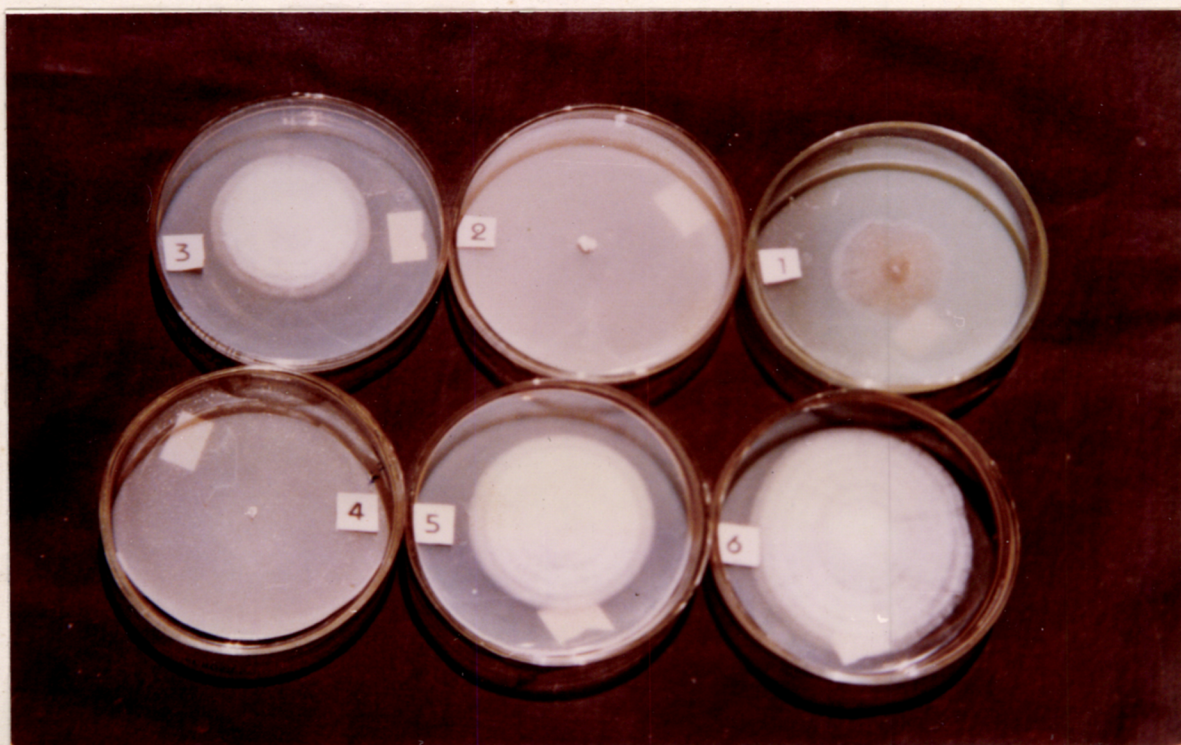


Plate 8. Efficacy of different fungicides on Fusarium solani  
in in vitro

1. Copper oxychloride  
3. Captan  
5. Thirum

2. Carbendazim  
4. Mancozeb  
6. Control

growth and sporulation of the fungus as compared to control. Bavistin and mancozeb completely inhibited the growth and sporulation of the fungus. The least inhibition was observed in thiram. Sporulation was abundant in the control.

Table 6. Effect of fungicides on the growth and sporulation of Fusarium solani.

Sr. No.	Fungicide	Concentration (%)	Concen. mg/100 ml of water	Mean colony diameter (mm)	Sporulation	Per cent inhibition
1.	Copperoxy chloride	0.25	250	34	-	59.18
2.	Carbendazim	0.10	100	-	-	100.00
3.	Captan	0.20	200	40	-	51.98
4.	Mancozeb	0.20	200	-	-	100.00
5.	Thiram	0.20	200	50.7	+	39.14
6.	Control	-	-	83.3	++++	0

++++ = Abundant sporulation  
 + = Poor sporulation  
 - = No sporulation

#### 4.8 Evaluation of antagonists against Fusarium solani in vitro

The inhibitory growth study of the test fungus, Fusarium solani by the antagonists, Trichoderma viride, Trichoderma harzianum, Trichoderma longiformum, Trichoderma hamatum, Trichoderma koningii and Gliocladium virens were

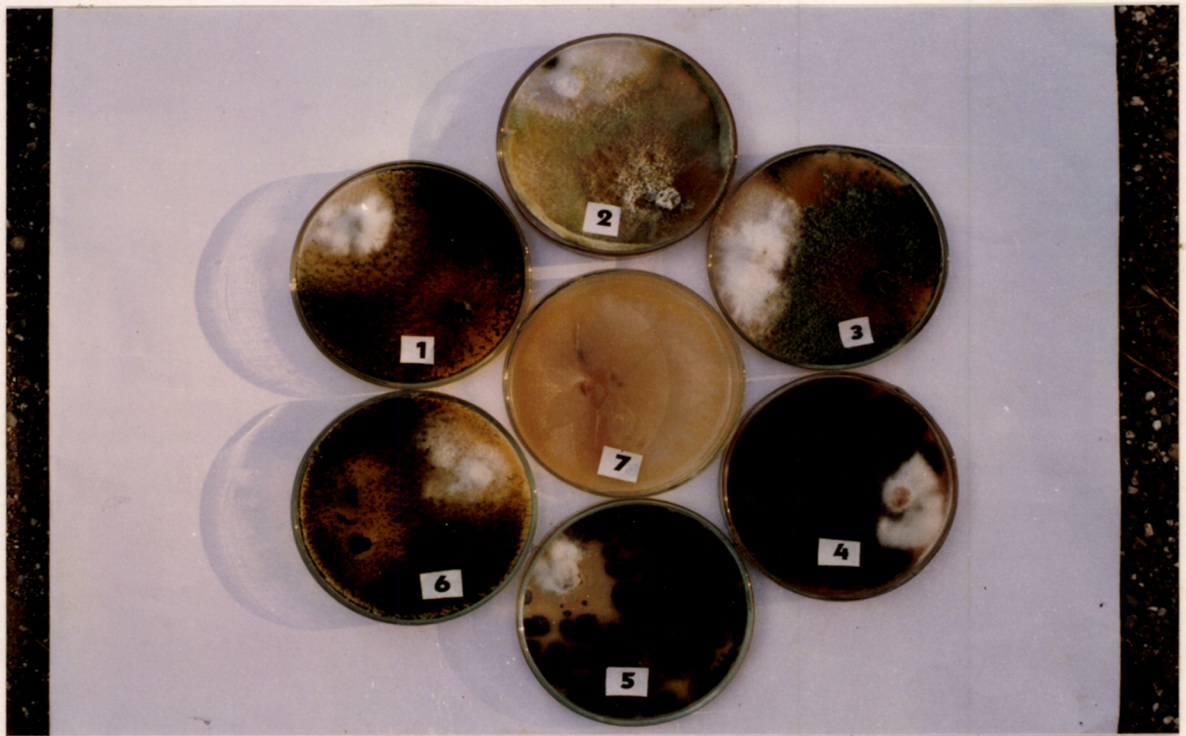


Plate 9. Efficacy of different antagonists on Fusarium solani in in vitro

- |   |                              |
|---|------------------------------|
| 1. <u>T. hamatum</u>                    | 2. <u>T. harzianum</u>       |
| 3. <u>T. koningii</u>                   | 4. <u>T. longiformum</u>     |
| 5. <u>T. viride</u>                     | 6. <u>Gliocladium virens</u> |
| 7. <u>Fusarium solani</u> alone control |                              |

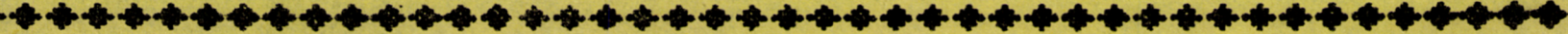
carried out in laboratory and the results are presented in Table 7.

Table 7. Growth of Fusarium solani as influenced by antagonistic organisms

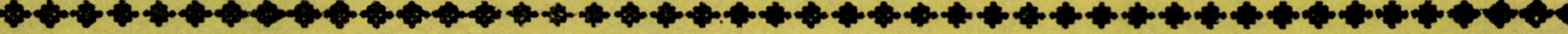
Sr. No.	Treatment	Per cent growth of <u>Fusarium Solani</u>	Per cent inhibition of <u>Fusarium solani</u> by the antagonist
1.	<u>T. hamatum</u>	25.60	74.40
2.	<u>T. harzianum</u>	26.40	73.60
3.	<u>T. koningii</u>	30.50	69.50
4.	<u>T. longiformum</u>	18.80	81.20
5.	<u>T. viride</u>	16.00	84.00
6.	<u>Gliocladium virens</u>	25.00	75.00
7.	<u>Fusarium solani</u> alone control	100	-

It is seen from Table 7 that the different antagonists showed inhibitory effect on growth of the test fungus and were effective in controlling the growth of the test fungus to the extent of 74.40, 73.60, 69.50, 81.20, 84.00, 75.00 per cent, respectively.

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**Discussion**



## 5. DISCUSSION

Chilli is one of the most important vegetable crops grown in Maharashtra. Amongst the various disease of chilli, Fusarium wilt is economically important as it causes heavy crop losses. The disease is caused by Fusarium solani (Mart.) App. and Wollenw, a soil borne pathogen.

It affects the plant growth of chilli and reduces the yield. The studies on the occurrence of the disease, cultural, morphological, chemical and biological control in vitro were undertaken. The results obtained from the investigations are discussed below.

### 5.1 Symptomatology

The symptoms observed under field conditions were loss of turgidity of leaves followed by leaf rolling, stunting of plant, death of twigs, flower drop and browning of stem. The symptoms on roots consisted of rotting of roots, peeling of epidermis and on tap roots whitish brown cortical lesions and vascular discolouration.

The symptoms observed in the present study were more or less similar to those described by Steekelenburg and Paternotte (1979), Gangopadhyay (1984) and Lukacs and Szarka (1988). The symptoms under study were some what similar to those symptoms described by Cucuza et al. (1992) for wilt of tomato caused by F. Solani.

## 5.2 Isolation, pathogenicity and Identification

The isolation of pathogen was made from infected roots and brown coloured stem bases of chilli plant collected from experimental area of Plant Pathology and All India Coordinated Vegetable Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri. The causal fungus was identified as Fusarium solani (Mart.) App. and Wollenw. on the basis of morphological characters. Pathogenicity was carried out by artificial soil inoculation and dipping roots of chilli seedlings in spore suspension before transplanting. The same pathogen was found responsible for developing symptoms on leaves, stem and roots 25 days after transplanting of seedlings. Reisolations made from the affected plant parts yielded F. solani identical to the original one.

Sahni et al. (1967) isolated the Fusarium spp. from the diseased chilli seedlings.

Camino et al. (1987) examined the fungal infection and pathogenicity tests were carried out, chili plants showed wilting symptoms three weeks after transplanting.

## 5.3 Morphology of the fungus

The mycelium of Fusarium solani (Mart.) App. and Wollenw was hyaline, septate and profusely branched. Both microconidia and macroconidia were observed. Microconidia were

oval and hyaline having size 8-10 x 3 um, while macroconidia were 1-3 septate, fusiform and slightly curved at the tip having size 10.00 x 3. um (one septate), 16.20 x 4.0 um (two septate), 33.20 x 4.5 um (three septate).

These results are in agreement with those reported by Snyder and Hanson (1941), Booth (1971) and Agarwal and Sarbhoy (1975).

#### 5.4 Cultural studies

While studying the cultural characters, it was observed that the fungus produced maximum growth on Armstrong Fusarium medium, Czapeks medium, Coons medium followed by Richards medium, with regard to sporulation, excellent sporulation was observed on Armstrong Fusarium medium, Czapeks medium and Richards medium. Poor sporulation was observed on Coon's medium and nutrient agar.

The results are much similar to those reported by Booth (1971). Thakur and Singh (1973) observed better growth on Richards medium, while Gonzalez and Jumenez (1988) observed best growth on Czapek's Dox agar medium.

#### 5.5 Physiological studies

##### 5.5.1 Utilization of carbon compounds

Fusarium solani could utilize all carbon compounds tested, good growth was observed on sucrose, maltose, glucose

whereas, moderate growth was noticed on dextrin. Growth was poor on lactose.

The present results are in conformity with those reported by Gaur and Agnihotri (1979). Among the disaccharides, maltose was the best source of carbon (Bhatnagar et al., 1968; Satyavir and Grewal, 1973; While lactose was utilized slowly (Satyavir and Grewal, 1973). In the present study, among disaccharides Maltose was the best source of carbon.

#### 5.5.2 Utilization of nitrogenous compounds

It was noticed that maximum growth was observed on potassium nitrate followed by sodium nitrate and asparagine, whereas it was moderate on ammonium nitrate and urea.

Above findings are similar to the results of Bhatnagar et al. (1968), Satyavir and Grewal (1973) and Gaur and Agnihotri (1978).

#### 5.5.3 Effect of temperature on growth and sporulation

The optimum temperature for growth of Fusarium solani was found to be 28°C, no growth was observed at 5 and 40°C. Excellent sporulation was observed and good sporulation at 28°C temperature.

These results were in agreement with the findings obtained by Paternotte (1987). Tandon and Bhargava (1963)

reported that the optimum temperature for growth of Fusarium solani was 25°C, while Monga and Grover (1991) found maximum mycelial growth at 30°C temperature.

#### 5.5.4 Thermal death point of Fusarium solani

No growth was noticed at 55°C which indicated that thermal death point was slightly above 54°C.

Tandon and Bhargava (1963) reported that the thermal death point was slightly above 55°C, but in the present study, it was found to be above 54°C.

#### 5.6 Evaluation of fungicides against Fusarium solani

In vitro studies on efficacy of different fungicides were carried out to find out the effective fungicide which can inhibit the growth and sporulation of Fusarium solani.

The fungicides carbendazim and mancozeb were found highly effective in inhibition of the fungal growth and sporulation followed by copperoxychloride.

The results obtained in the present investigation are in confirmity with the results obtained by Satija and Indra Hood (1987), Gaur (1990), Kapoor and Kumar (1991), Monga and Grover (1991) and Singh et al. (1991).

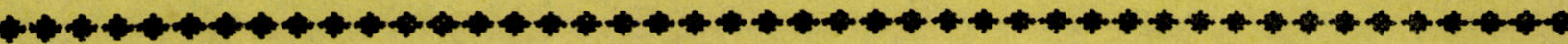
5.7 Evaluation of antagonists against Fusarium solani in in vitro

The results of the present studies revealed that the antagonists viz., Trichoderma hamatum, T. harzianum, T. koningii, T. longiformum, T. viride, Gliocladium virens showed inhibitory effect on the growth of fungus Fusarium solani.

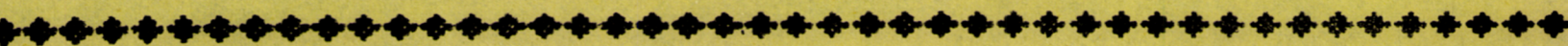
The results indicated that Trichoderma viride, T. longiformum, Gliocladium virens, T. hamatum, T. harzianum were most efficient biocontrol agents against wilt pathogen of chilli.

Similar results were reported by Upadhyay and Rai (1987) F. udum, T. viride and A. niger were antagonists against Fusarium solani and Rhizoctonia solani diseases of egg plant in in vitro (Bora, 1977).

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Summary and Conclusions



## 6. SUMMARY AND CONCLUSION

Wilt of chilli caused by Fusarium solani is generally found in the chilli growing area of the Maharashtra state. The present investigation was undertaken to find out suitable chemical and biological control of the wilt disease and to study other allied aspects.

The pathogen showed symptoms as loss of turgidity of leaves followed by severe leaf rolling, stunting of plant, flower drop and browning of stem, death of twig. The symptoms on roots are rotting of roots, peeling of epidermis and vascular browning.

Pathogenicity of Fusarium solani was proved by soil inoculation and by dipping the roots of chilli seedlings into spore suspension. The pathogen was identified as Fusarium solani (Mart.) App. and Wollenw. on the basis of morphological characters.

The mycelium of fungus was hyaline, septate, profusely branched with microconidia and macroconidia. Cultural characters were studied and it was noticed that good growth of fungus and sporulation was observed on Armstrong Fusarium medium.

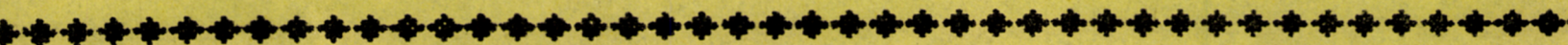
The test fungus could grow well on maltose, glucose and sucrose. It grows best on different nitrogen sources such as potassium nitrate, sodium nitrate and asparagine.

The optimum, maximum, minimum temperature for growth was 28, 35 and 10°C respectively. The thermal death point was 54°C temperature.

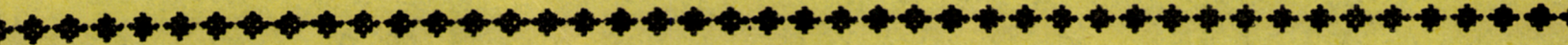
In in vitro evaluation, the most effective fungicides were carbendazim and mancozeb followed by copper oxychloride.

All the six antagonists showed antagonistic activity against Fusarium solani in vitro. Five antagonists viz., Trichoderma viride, T. longiform, Gliocladium virens, T. hamatum, T. harzianum were found most effective in inhibiting the growth of the test fungus Fusarium solani by 84.20, 81.20, 75.00, 74.40 and 73.60 per cent, respectively followed by T. koningii 69.50 per cent.

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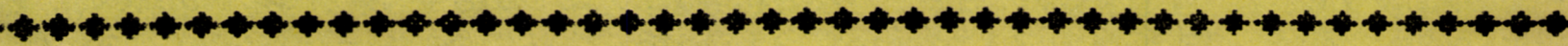
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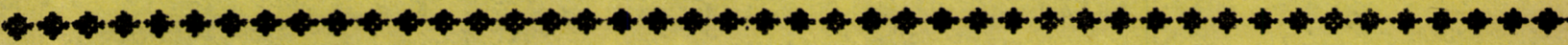
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Chapter Opener Page



Vita



## 8. VITA

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JITENDRA NANASAHEB THORAT

A candidate for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

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- Thesis title : Studies on control of Fusarium wilt of Chilli (Capsicum annuum L.) by Bio-control agents
- Major field : Plant Pathology
- Biographical information :
- Personal data : Born at Adavad, Tahsil Chopda, Dist. Jalgaon, on 9th December, 1973, Son of Shri. Nanasaheb Govindrao Thorat of Virgaon, Tahsil Akole, Dist. Ahmednagar.
- Education : Attended Primary School at Virgaon, Secondary School at Chhatrapati Shivaji Vidyalaya, Ganore, Tahsil Akole, Dist. Ahmednagar.
- Higher Secondary School at Dnyanmata Junior College, Sangamner, Dist. Ahmednagar. Passed S.S.C. and H.S.C. with First class.
- Received the Bachelor of Science (Agriculture) degree with First class in 1995 from College of Agriculture, Dhule, under Mahatma Phule Krishi Vidyapeeth, Rahuri.
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