# "STUDIES ON WILT DISEASE OF GLADIOLUS"

by

**Joshi Sayali Sunil**
**(Reg. No. 016/237)**

**DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL MICROBIOLOGY**
**COLLEGE OF AGRICULTURE, PUNE**

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

**2018**

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In partial fulfillment of the requirements for the degree

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**MASTER OF SCIENCE (AGRICULTURE)**

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APPROVED BY

**Dr. Sunita J. Waghmare**
Chairman and Research Guide

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**DEPARTMENT OF PLANT PATHOLOGYAND AGRICULTURAL MICROBIOLOGY**
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**MAHATMA PHULE KRISHI VIDYAPEETH,**
**RAHURI- 413 722, DIST- AHMEDNAGAR**
**MAHARASHTRA STATE (INDIA)**

**2018**

# CANDIDATE'S DECLARATION

I hereby declare 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

Place : Pune

Date : / /2018 **(Joshi Sayali Sunil)**

**Dr. Sunita J. Waghmare**
Assistant Plant Pathologist
Ergot of Bajra Scheme
Plant Pathology Section
College of Agriculture, Pune

# CERTIFICATE

This is to certify that the thesis entitled, **"STUDIES ON WILT DISEASE OF GLADIOLUS"** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (Maharashtra) in partial fulfillment of the requirement for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY**, embodies the result of a piece of bonafide research work carried out by **JOSHI SAYALI SUNIL** 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.

Place : Pune
Date : / /2018

**(Sunita J. Waghmare)**
Research Guide

**Dr. A. P. Gaikwad**
Professor of Plant Pathology and
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Maharashtra state, India

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Place : Pune

Date : / /2018 **(A. P. Gaikwad)**

**Dr. S. D. Masalkar**
Associate Dean,
College of Agriculture, Pune.
Maharashtra (India)

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Place : Pune

Date : / /2018 **(S. D. Masalkar)**

# APPENDIX

## Composition of Media

**Potato Dextrose Agar**

| | |
|---|---|
| Peeled potatoes | : 200 g |
| Dextrose | : 20 g |
| Agar agar | : 20 g |
| Distilled water | : 1000 ml |

**Potato Dextrose Broth**

| | |
|---|---|
| Peeled potatoes | : 200 g |
| Dextrose | : 20 g |
| Distilled water | : 1000 ml |

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# LIST OF ABBREVIATIONS AND SYMBOLS

| | | |
|---|---|---|
| @ | : | at the rate of |
| BOD | : | Biological Oxygen Demand |
| $^{o}$C | : | Degree Celsius |
| C.D | : | Critical Difference |
| cm | : | Centimeter |
| DAI | : | Days After Incubation |
| *et al.* | : | et alli (and other) |
| etc | : | et cetera |
| Fig. | : | Figure |
| g | : | Gram |
| hr | : | Hour (s) |
| i.e. | : | id edges (that is) |
| kg | : | Kilogram |
| m | : | Meter |
| min. | : | Minute |
| mm | : | Millimeter |
| ml | : | Milliliter |
| No. | : | Number |
| PDA | : | Potato Dextrose Agar |
| PDC | : | Per cent Disease Control |
| PDI | : | Per cent Disease Incidence |
| P.I. | : | Per cent inhibition |
| pH | : | Potential of Hydrogen |
| rpm | : | Revolution per minute |
| S.E. | : | Standard error |
| sp | : | Species |
| spp. | : | More than one species |
| Sq. | : | Square |
| *Viz.* | : | Videlicet (namely) |

# ABSTRACT

**"STUDIES ON WILT DISEASE OF GLADIOLUS"**

By

**Joshi Sayali Sunil**

A candidate for the degree

Of

**MASTER OF SCIENCE (AGRICULTURE)**

In

**PLANT PATHOLOGY**

**MAHATMA PHULE KRISHI VIDYAPEETH, RAHURI - 413 722**

**2018**

**Research Guide : Dr. SUNITA J. WAGHMARE**

**Department : Plant Pathology and Agricultural Microbiology**
**College of Agriculture, Pune - 411 005**

Bulbous flowering plants are one of the most wonderful creations of nature. Of the various bulbous flowering plants gladiolus, "Queen of bulbous flower crop" grown in many parts of the world. Wilt of Gladiolus caused by *Fusarium solani* is an important soil borne disease leading to symptoms on corms with slight discoloration on the base to complete rotting and on leaves in the form of partial and complete yellowing. The diseased samples were collected from NARP, Ganeshkhind, Pune were subjected to isolation, purification and pathogenicity. One pathogenic isolate was selected for further studies. On the basis of colony character, colour of mycelium etc. the fungus was identified as *Fusarium solani* and confirmed the pathogenicity. To develop integrated management strategy by using resistant varieties, fungicides and bioagents present study was carried out. Among twenty five varieties and thirty five hybrids lines, IIHR-77-59-32 variety of gladiolus and 07-7 and 07-23 hybrid lines were free from wilt disease of gladiolus. The hybrid line 94-58 was showed resistant reaction while the Psitacinus hybrid and Sancerre showed were showed susceptible reaction to wilt disease of gladiolus.

Seven fungitoxicants *viz.* Captan, Copper oxy chloride, Carbendazim, Bordeaux Mixture, Azoxystrobin, Benomyl and Hexaconazole were evaluated against *Fusarium solani* by poison food technique. It was observed that, Benomyl was best fungicide for inhibiting the growth of the *Fusarium solani* followed by Carbendazim and Captan.

The effect of biocontrol agents *viz. Trichoderma viride, Trichoderma virens, Trichoderma harzianum, Trichoderma hamatum, Bacilus subtilis, Pseudomonas fluorescence* were studied by dual culture technique. It was found that, the maximum growth reduction of *Fusarium solani* by *Trichoderma viride* (68.51 per cent) and proved to be best bioagent followed by *Bacillus subtilis* (68.55 per cent) and *Trichodrma virens* (62.96 per cent) respectively.

In *in vivo* most effective fungicides *viz.* Captan, Benomyl and Carbendazim and bio agents *Trichoderma viride, Trichoderma virens* and *Bacillus subtilis* were evaluated in glass

house. It was observed that wilt disease of gladiolus significantly controlled by the most effective fungicides Captan and Benomyl (87.67 per cent) and which was at par with each other. They were followed by Carbendazim and the bioagent *Trichoderma viride* (62.96 per cent) respectively. The *Bacillus subtilis* and *Trichoderma virens* (25.93 per cent) found less effective to control wilt disease of gladiolus.

# 1. INTRODUCTION

World would not have been as beautiful, charming, and cherishing as it is today, without flowers. Bulbous flowering plants are one of the most wonderful creations of nature. Of the various bulbous flowering plants, Gladiolus (*Gladiolus hybridus* Hort.) easily tops the list and can rightly be called the "Queen of bulbous flower crops" grown in many parts of the world which provide glamour, perfection and colour (Kaikal and Nauriyal, 1964). The word Gladiolus was originally coined by Pling the Elderr (AD 23 – 79) from the Latin word 'Gladius' meaning 'Sword'. It is also called as 'Sword lily' on account of the shape of its leaves (Randhawa and Mukhopadhya, 1986). In Europe it is commonly called as 'Corn flag' due to its infestation as a weed (Bose and Yadav, 1989).

*Gladiolus* spp. recognized over 2000 years ago, growing in the fields of Asia minor and were called as 'corn lilies' (Larson, 1980). It was introduced into cultivation towards the end of sixteenth century while, it is of comparatively a recent introduction to India. Gladiolus, belonging to the family *Iridiaceae* is represented by 250 species, of which 103 species are native to South Africa and the rest to tropical Africa (Anon, 1976). Latest hybrids have been derived from at least 12 species (Ewart, 1981) which are now called as *Gladiolus grandiflorus* (Wilfret, 1980). Gladiolus is a tender herbaceous perennial. It is popular for its attractive spikes having florets of huge form, dazzling colors and spikes with long keeping quality. Gladiolus is one of the most popular cut flowers, at both national and international level. In the International cut flower trade, gladiolus occupies fourth place (Bose and Yadav, 1989). It is commercially cultivated in all parts of the world. In India, it is grown on an area of 289 ha with production of 459 lakh spikes. In Karnataka it was grown in an area of 97 ha with production 239 ton (Anon., 2003). Major Gladiolus growing states are Karnataka, Maharashtra, Tamil Nadu, Punjab, Haryana, Delhi, Uttar Pradesh, West Bengal and Nagaland. Gladiolus is commonly propagated by corms and cormels. The corms are bulb like globes or ovoid having series of nodes and are wholle covered with tunic or husks.

The total production of top ten producer states was 174.63 thousand tonnes during the year 2015-16 out of which 42.60 and 132.46 thousand tonnes quantity contributed by loose and cut flowers value respectively. West Bengal has the largest share contributing 52.66 thousand tonnes followed by Madhya Pradesh (39.00 thousand tonnes), Maharashtra (26.29 thousand tonnes) and Chattisgarh (25.08 thousand tonnes) annual production.

The climatic conditions of Maharashtra are quite congenial for growth and production of quality flowers of gladiolus. However, these conditions are also suitable for the development of several diseases, which adversely affect quality and quantity of flowers.

In India, following fungi, bacteria, virus and nematodes severely infect gladiolus and adversely affect growth and flower production. *Fusarium* wilt or corm rot (*Fusarium oxysporum* f. sp. *gladioli, Fusarium solani),* Fusarium rot and yellowing (*Fusarium orthoceras* f. sp. *gladioli),* Core or spongi rot (*Botrytis gladiolorum*), Dry or neck rot (*Sclerotiana gladioli*), Curvularia blight (*Curvularia trifolii* f. sp. *gladioli*, *Curvularia brachyspora* ), Storage corm rot (*Fusarium solani, Fusarium oxysporum* f. sp. *gladioli* ), Nematodes (*Meloidogyne, Pratylenchus, Trichodorus*, *Belonolaimus*), Scab (*Pseudomonas marginata),* Viruses (Bean yellow mosaic virus, Cucumber masaic virus, Tomato ringspot virus).

Among all these, wilt caused by *Fusarium solani* is an important soil borne disease. It is spreading widely and causing yield losses up to 60-70 % threatening the crop cultivation. (Vlasova and Shitan, 1974).

In Maharashtra, Pune, Satara, Sangali, Kolhapur, Nasik and some areas of Thane and Ahmednagar districts are severely affected by wilt disease caused by *Fusarium solani* leading to rotting of corms and the death of plant. *Fusarium* disease of gladiolus is commonly known as yellows, wilt or corm rot. Many farmers are scared now a days to grow gladiolus because of this wilt disease. Although disease has become very severe now a days and also not much work is carried out on wilt disease. The common methods of disease management are use of resistant varieties, chemicals and biocontrol agents. Thus with the help of these strategies we can reduce the losses caused in gladiolus due to wilt disease and grow gladiolus successfully.

Even though, resistant varieties have been evolved against wilt, yet all varieties are not performing well in all the areas. The variety which is resistant in one locality is susceptible in other localities due to variability in pathogen, but information on this aspect is meager. Species of *Fusarium* are widely distribution both in temperate and tropical regions of the world and are mainly soil inhabitants fungi. Many species causes globally economically significant disease on most of the world's important crop plant affecting heavily qualitative and quantitative losses. (Booth, 1971). Cultivation of resistant varieties is an effective and cheaper method to combat the disease. Hence, identification of resistant varieties will be useful for development of resistant cultivar where the disease is prevalent and causing heavy losses. Resistance in cultivar 'Mentee

White' while four cultivars 'Wood Pecker', 'Lilac Wonder', 'True Love' and 'White Friendship' were reported moderately resistant to the disease (Tarabeih *et al*., 1981)

Various disease management methods have been implemented to control and eradicated pathogen. These include cultural, regulatory, physical chemical and biological methods. All these methods are effective only when employed well in advance as precautionary measures. Once a disease has appeared, these methods become impractical or ineffective. In that situation, chemical control of plant pathogens has been considered as an effective tool to eradicate the pathogen from the site of infection. Chemical pesticides have been in use of since long and they provide quick, effective and economic management of plant disease.

Biological control of phytopathogens by micro-organisms is an important method of plant disease management. It has been used as an alternative in the fight against pathogens in plants (Baker and Paulitz, 1996). Fungi and bacterial bioagents actively suppress the growth of plant pathogens. Several *Trichoderma* spp. reduce the incidence of soil borne plant pathogenic fungi under natural conditions. (Benitez *et al*. 2004)

Keeping in view the importance of wilt disease in Gladiolus with reference to management, the present study was carried out with the following objectives.

**OBJECTIVES**

1) To isolate the fungus pathogen associated with wilt of gladiolus.
2) To prove Koch's postulates.
3) To screen the gladiolus genotypes for resistance against wilt disease.
4) To study the effect of fungicides and bioagents against wilt pathogen in *in vitro* and *in vivo*.

# 2. REVIEW OF LITERATURE

The wilt disease of gladiolus caused by *Fusarium solani* is known to occur in almost all gladiolus growing areas. Not much research work is being carried out. The available literature to the present study is reviewed.

## 2.1 Distribution of the Disease

Pryal (1909) reported for the first time the fungal pathogen *Fusarium oxysporum* f .sp. *gladioli* (Massey) Snyd. & Hans. causing *Fusarium* wilt on Gladiolus from California.

Massey (1926) reported *Fusarium* wilt disease was from other gladiolus growing areas of the USA.

Singh (1969) first time reported the existence of wilt of gladiolus in India. The disease has also been reported from Pakistan, Italy, Germany, Russia and China.

Sorbhoy A. K. and Agarwal, D. K. (1983) recorded corm rot of gladiolus caused by *Fusarium solani* for the first time in India.

Mirza and Shakir (1991); Infantino and Rumine (1993); Chen *et al*. (1994) reported *Fusarium* wit disease of gladiolus from Pakistan, Italy, Germany, Russia and China.

Kosiak *et al*. (2004)) reported that temperature and humidity normally affect the distribution of *Fusarium solani* causing wilt disease of Gladiolus.

Schollenberger *et al*. (2005)) reported that *Fusarium solani* comprise a complex group of species that are widely distributed worldwide in soils. It causes tuber, root, and stem rots of plants. It includes at least 50 subspecies lineages.

## 2.2 Economic Importance of the Disease

Bruhn (1955) and Protsenko (1958) estimated about 30% annual loss in Germany and 60-80% annual loss in Russia due to *Fusarium* wilt caused by pathogen *Fusarium* spp.in gladiolus.

Valasova and Shitan (1974) *Fusarium* yellows is considered a serious and highly devastating disease which causes 60-70% plant mortality in gladiolus.

Essmat (1992) reported that *Fusarium oxysporum* f. sp. *gladioli* caused heavy annual losses to flowers, corms and cormels production which destroyed the gladiolus plantations in Egypt.

Pathania and Misra (2000) reported that *Fusarium oxysporum* f. sp. *gladioli* caused 60-100% damage to gladiolus depending on varietal response.

Armitage (1993); Remotti *et al*. (1997); Chandel and Bhardwaj (2000) reported that the caused by *Fusarium* wilt is *Fusarium oxysporum* Schlecht. f. sp. *gladioli* (L. Masey) Snyder & Hans., which deteriorates its quality and market value.

Nazir and Riazuddin (2008) reported that *Fusarium oxysporum* f. sp. *gladioli* is an important pathogen that can reduce corm and flower production of Gladiolus in the world.

**2.3 Symptomatology**

Massey (1926) reported that the fungus produces yellowing on the leaves which starts from the tip downwards in *Fusarium* wilt disease of gladiolus.

McCulloch (1944) discussed the disease symptoms on inflorescences, florets and corms of gladiolus caused due to *Fusarium solani*.

Buxton and Robertson (1953); Singh (1969); Tomar *et al.* (1997) studied that, in storage the *Fusarium* produces brown lesions on corms which later turn into hard, dry, brownish-black structures called mummies in gladiolus.

Chen *et al.* (1994) *Fusarium* wilt disease is a soil-borne that spreads through infected corms from one place to another also studied in proving the pathogenic nature of *Fusarium oxysporum* f. sp. *gladioli* and *Fusarium solani* under *in vitro* conditions in gladiolus.

Tomar *et al.* (1997) studied the effect of edaphic factors such as soil temperature, moisture, pH and soil type on the development of *Fusarium* yellows of gladiolus. Soil temperature of 27-33 °C, soil moisture of 60 percent and soil pH 6.5 was most conducive for the development and spread of wilt or yellows disease of gladiolus. Loam and sandy loam soils were rated as the most suitable soils for the development of the disease.

Lund *et al*. (1998) studied plants affected with *Fusarium* wilt and observed that it first develops stunted seedlings and yellowing of the lowest leaves that is often restricted to one side of the plant or a single shoot and later show the defoliation of older leaves. The affected leaves wilt and die. Wilting progresses up the stem until the foliage is killed and the stem decays.

Khan and Khan (2002) reported that wilting is seen on the lower leaves first and this later extends to the upper leaves. Leaves, twigs or even the whole plant turns brown and later dies and dries up due to *Fusarium solani* in plants.

Burgess *et al*. (2008) stated that the general symptoms of many diseases of the root and stems are yellowing, wilting and stunting while a key symptom of pathogens which cause

vascular wilt disease, including *Fusarium* wilt pathogens, is the browning of the internal stem (vascular) tissue.

**2.4 Pathogen**

Massey (1926) opined that *Fusarium oxysporum* f. sp. *gladioli* was primarily a storage disease of gladiolus.

Tandon and Bhargava (1963) reported that *Fusarium solani* was the causal agent to be infesting the corms of gladiolus.

The Genus *Fusarium* was erected by Link in 1809 for the species with fusiform, nonseptate spores borne on a stroma (Booth, 1971).

Bald *et al*. (1971) suggested that advancement of hyphae by penetration between the cells of the vascular parenchyma which is common in isolates, causing rot in bulbs and corms, represents a helpful stage in the evaluation of the truly vascular habit among *Fusarium* spp. in gladiolus.

Chen *et al.* (1994) studied the gladiolus root rot in Shanghai (China) and they confirmed the pathogen as *Fusarium* spp.

Fry (2004) reported taxonomically *Fusarium solani* belongs to the kingdom Fungi, phylum Ascomycota, subphylum Pezizomycota, class Sordariomycetes, subclass Hypocromycetidae, order Hypocreales, family *Nectriaceae* and genus *Fusarium*.

Thangavelu *et al*. (2003) reported that *Fusarium solani* is destructive to plants and progresses swiftly, and the plant wilts and dies.

**2.5 Host**

Infantino and Rumine (1993) reported that the *Fusarium solani* infected other members of the *Iridiaceae* family.

Walid *et al*. (2011) observed that *Fusarium solani* causes leaf yellowing in Gladiolus and also infects corms of *Crocus*, *Freesia*, bulbous *Iris*, *Ixia*, and some other iridaceous plants.

**2.6 Isolation, purification of pathogen and establishment of pathogenicity**

Porta and Varase (1985) isolated *Fusarium oxysporum* f. sp. *gladioli* from wilt affected gladiolus plant and observed the presence of *F*. *oxysporum*. f. sp. *gladioli* in gladiolus corms and confirmed the pathogenicity by inoculating plants with two isolates.

Garibaldi *et al*. (2004) isolated *Fusarium* spp. consistently and readily from symptomatic vascular tissues on a *Fusarium*-selective medium from the wilt affected plants of Gladiolus.

Chen *et al.* (2005) developed selective media for isolation of *Fusarium oxysporum* by amending Komada Medium (KM) with 0.1% Benlate that could support good growth and high spore germination rate.

Pande *et al*. (2007) proved the pathogenicity of *F. oxysporum* f. sp. *ciceri*, isolated from wilted seeds of the cultivar K 850. The isolates were separately multiplied on sterilized potato dextrose broth in 250 ml conical flask and incubated for 7 days at 25°C and 124 rpm. This culture was then homogenized in sterile distilled water and adjusted to 5 x 105 conidia/ml and used as inoculum. Eight-day-old seedling of a susceptible cultivar JG 62, grown in sterile sand were uprooted, sand particles were removed from the root by washing with distilled water, the root inoculated by dipping in the inoculum for 30 seconds to enable conidia to adhere to the roots. Root inoculated seedlings were transplanted in pre-irrigated sterile vertisol and sand (3:1) pot mix filled in 15 cm plastic pots and kept in a green house at 25+ 3°C. Inoculated seedlings were observed for wilt symptom upto 30 days after inoculation.

Riaz *et al.* (2008) isolated *Fusarium oxysporum* f. sp. *gladioli* from the diseased portions of infected corms by surface sterilizing the corms with 1 percent sodium hypochlorite solution followed by transfer to plates into Malt Extract Agar (MEA) media. The plates were kept for incubation at $25^0$ C for 7 days.

Sharma and Tripathi (2008) maintained the cultures of *F. oxysporum* f. sp. *gladioli* on PDA medium containing plates at 25±1 $^0$C by periodic culture.

**2.7 Identification of Pathogen**

Pryal (1909) identified for the first time the fungal pathogen *Fusarium oxysporum* f .sp. *gladioli* (Massey) Snyd. & Hans. causing *Fusarium* wilt of Gladiolus from California.

Buxton (1953); Dallavalle *et al.* (2002) reported the soil borne fungus *Fusarium oxysporum* f. sp. *gladioli* is a major causal organism of yellowing and corm rot in gladiolus.

Tomar *et al*. (1997) reported *F. moniliforme* in gladiolus as an additional fungus in causing wilt under the sub-temperate zone of Himachal Pradesh of India.

Singh (1969) studied the pathogenic nature of particular fungus by reisolation method and confirmed its identity.

**2.8 Morphological and Cultural Characteristics**

Arya and Jain (1964) recorded the morphology of *Fusarium solani*, which caused corm rot. He observed that macroconidia are slender, straight to slightly curved, slight tapering

towards both ends, mainly hyaline and sometimes light coloured, mostly three septate and varied in size.

Major (1923) studied the cultural characteristics of *Fusarium solani* isolated from lupin and further from asters on various natural and synthetic media and observed that the growth varied on different media from complete absence to profuse production of aerial mycelium with color variation.

Massey (1926) studied morphological characters of fungus *Fusarium* spp. isolated from gladiolus in detailed and observed sickle shaped macro-conidia which were curved at the top, weakly pedicellate, and dominantly three septate. The micro-conidia were oval, rarely septate, numerous and hyaline.

McCulloch (1944) observed that in cultures, the pathogen *F. oxysporum* f .sp. *gladioli* produced white to peach pale salmon or purple mycelium. Microconidia were abundant, hyaline and ovoid to ovate. Macroconidia were scarce, often lacking and variable, 3 septate. Chlamydospores were hyaline, usually vacuolate and spherical.

Prasad (1949) studied thirty strains of *F. solani* f .sp. *cucurbitae* which were found to differ from each other in culture type, rate of growth, pigmentation and size of macroconidia.

Buxton (1955b); Chen *et al.* (1994) recorded the morphological characters of *Fusarium oxysporum* f. sp. *gladioli*. The fungus *Fusarium oxysporum* f. sp. *gladioli* (Massey) Synder and Hansen produces aerial mycelium which is hyaline, branched, septate, well-developed and cottony in appearance. The culture is slightly purple or pinkish white in colour on Potato Dextrose Agar (PDA). The fungus produces abundant conidia in culture, and conidia are of two types; micro-and macroconidia.

Subramaniam (1955) observed considerable variation in cultural characters of *Fusarium udum* causing wilt disease in Chickpea.

Venkataraman (1955) showed that culture of *Fusarium* cause wilt disease of muskmelon produced fluffy mycelium with sparse number of conidia differing with ‘wild type’ strain having abundant sporulation.

Prasad and Patel (1964) studied the growth of *Fusarium* on different media and observed the best growth on Richard's medium.

Sharma and Mathur (1971) showed that monoconidial lines of the linseed wilt caused by *F. oxysporum* f .sp. *lini* isolated from different linseed growing regions and observe variation differed in their cultural and morphological characters with marked diversity in virulence.

Jamaria (1972) reported that Potato Dextrose Agar, Richards's Agar and Czapek's agar provided maximum growth and sporulation of *F. oxysporum* f .sp. *niveum.*

Booth *et al.* (1978) gave a detailed account of *Fusarium oxysporum, Fusarium solani, and Fusarium moniliforme*.

Reddy and Chaudhari (1985) observed that *Fusarium solani* growth was maximum in Richard's medium.

Sowmya (1993) studied four isolates of banana panama wilt pathogen on different nutrient media and observed maximum growth and sporulation of the pathogen on Potato sucrose agar and Richards's agar, respectively.

**2.9 Varietal Screening**

McCulloch (1944); Bajaj *et al.* (1989) Screening programme undertaken by several workers and reported resistance in many cultivars against *Fusarium oxysporum* f. sp. *gladioli* causing yellows disease in gladiolus. Cultivars like 'Albana', 'Apricot', 'Souvenir', 'Hopman's Glory', 'Sylvia', 'White Friendship' and 'White Prosperity' are reported to be resistant while 'Australian Fair' and 'Mansoor' were reported as tolerant to the disease.

Palmer and Pryal (1958) reported that out of 160 gladiolus cultivars, 10 per cent cultivars were resistant to wilt disease of gladiolus caused by *Fusarium oxysporum* f. sp. *gladioli*.

Ronald *et al.* (1974) had screened 211 cultivars for resistance to *F. oxysporum* f. sp. *gladioli* by inoculating dormant corms and they found that selections 66-109 -5 and 63-5-1 were found most resistant.

Chandra *et al.* (1985) screened 41 cultivars and 14 hybrids for resistance to *Fusarium oxysporum* f. sp. *gladioli* and they found that Australian fair and Monsoer were tolerant.

Rai *et al*. (2000) evaluated 16 varieties of gladiolus under sodic waste land based on different characters such as plant height and number of tillers per plant, spike length, number of florets per spike, the varieties like White Prosperity, White Goddess, Red Beauty, Friendship, Venetei, Aldebran, First Lady were found superior in comparison to others.

Tarabeih *et al*. (1981) Screened the cultivars against *Fusarium* causes wilt disease in Gladiolus and found that, one cultivar 'Mentee White' was resistance while four cultivars 'Wood

Pecker', 'Lilac Wonder', 'True Love' and 'White Friendship' were reported moderately resistant to the disease.

**2.10 *In vitro* and *In vivo* Evaluation of Fungicides**

Falck (1907) reported that poisoned food technique is the most common practice for evaluating fungicides under laboratory condition.

Gould and Miller (1970) reported that benzimidazole fungicide increased yield of healthy bulbs better than mercurial fungicides without causing phytotoxic effect when used to treat *Fusarium* infected stocks of Narcissus and Iris.

Forsberg (1970) reported that Benomyl was more effective than Thiram to control corm rot of gladiolus resulted in more economic yield.

Magie (1971) reported that Benomyl to be more effective to controlling *Fusarium* rot diseasse of gladiolus.

Magie and Wilfret (1974) observed that Benomyl was found to be effective to control wilt disease caused by *Fusarium*, but when used twice yearly on mother and daughter corms during a two year period, against *Fusarium* wilt disease.

Georgieva and Peikova (1976) reported that effective and excellent protection of *Fusarium oxysporum* f. sp. *gladioli* was provided by Captan Chlorothalonil, Benomyl, Thiobendazole, Zinc, Ion-Maneb Complex and Zineb.

Wani *et al.* (1982) reported that the combination of Bavistin (0.1%) and Difoltan (0.5%) found best to control wilt disease of gladiolus caused by *Fusarium oxysporum* f. sp. *gladioli* than other fungicides individually.

Shah *et al.* (1983) observed the effective control and a high sprouting percentage in gladiolus with Benomyl (0.2 per cent), Bavistin (0.2 per cent) or Calixin at 0.1 per cent against *Fusarium* spp.

Sharma and Jain (1984) found that, Bavistin, a systemic fungicide inhibited the growth of *Fusarium* spp.

Valaskova (1985) carried out field trials against *Fusarium* spp. and found effective with Benomyl + Polyram + Ultra (thiram) or Benomyl + Liro-maneb, each at 0.25 per cent in gladiolus and Liro-maneb (0.5 per cent) and Benlate T (Benomy+Thuram) (0.5 per cent) to be effective in tulip.

Chauhan *et al.* (1988) suggested that a pre-sowing drenching with Carbendazim or Carboxin could be used to reduce losses, due to wilt and root rot diseases, caused by *Fusarium*.

Kaur *et al.* (1989) reported that the use of resistant cultivars and soil drenches with Bavistin (0.3 %), which gave best control of *Fusarium* wilt after 45 days planting of gladiolus

Singh (1992) reported preplanting corm and cormel dip in 0.05 % Benomyl or Thiocur for 15 minutes followed by drenching with the same fungicide 15 days after planting controlling corm rot of gladiolus sucessfully.

Hanks *et al.* (1996) reported that double treatment with fungicides such as Carbendazim, Chlorothalonil and Benomyl were very effective against *Fusarium* spp.

Sud *et al.* (1999) evaluated six fungicides against saffron corm rot caused by *Fusarium solani*. Out of the six fungicides tested, Bavistin and Thiabendazole @ 0.2 per cent as a dip or drench gave complete disease control. In all other treatments, including the use of healthy corms, the disease levels increased each year. The use of healthy corms followed by application of Bavistin or Thiabendazole as a drench in subsequent years appeared to be the best management strategy.

Chandel and Bhardwaj (2000) studied the management of *Fusarium* wilt of gladiolus caused by *Fusarium oxysporum* f. sp. *gladioli* with Carbendazim treated corms, which were found effective in disease control than Chlorothalonil and Mercuric chloride also higher corm yield. Dipping of corms in Carbendazim prior to sowing was found effective irrespective of date of sowing.

Elmer (2006) evaluated efficacy of preplant treatments of gladiolus corms with combinations of Acibenzolar-S-Methyl (ASM), biological and chemical fungicides for suppression of *Fusarium* corm rot. Corms treated with ASM produced 48% more marketable flower spikes than untreated corms and the value of the area under the disease progress curve (AUDPC) was reduced by 12%. However, chemical fungicides Medallion Reg 50WP (fludiozonil) and Terranguard TM 50WP (triflumizole) reduced AUDPC by 27% and 23% respectively and none of the bioagents found effective.

Fulsundar *et al.* (2009) found Carbendazim treatment was most effective in disease control as well as improving the plant height, spike length, corm weight and cormels per plant as compare to other fungicides.

**2.11 Biological Management**

Morton and Stroube (1955) developed dual culture technique for testing of antagonism of *Trichoderma* spp. against broad range of common plant pathogens.

Dennis and Webster (1971) observed coiling of isolates of *Trichoderma* spp. immediately on contact with *Fusarium oxysporum* as well as delayed coiling with other isolates of *Fusarium* spp. and no interaction with them by some other isolates of *Trichoderma* spp.

Gasanov (1977) reported that wilt disease of watermelon caused by *Fusarium* spp. was effectively controlled (61 per cent) control with *Trichoderma* spp.

Chet *et al.* (1980) observed *Trichoderma harzianum* a biocontrol agent has been effective against *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii* under *in vitro* and *in vivo* conditions.

Morshed (1985) studied antagonism between *Trichoderma* spp. and *Fusarium oxysporum, Fusarium culmorum, Alternaria tenuis, Botrytis cinnera* and *Colletotrichum lindemuthianam*. He observed that growth of *Trichoderma viride* was vigorous in dual culture and it was an effective hyper-parasite in which penetrating and coiling its hyphae around the host hyphae.

Konde and Haral (1986) showed that culture filtrates of *Pseudomonas fluorescens* strain obtained from the rhizosphere of *Cicer arietinum* plants which to reduced the mycelial weight of *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani.*

Bhardwaj and Gupta (1987) studied in *in vitro* evaluationof antagonistic potential of different *Trichoderma* spp. *Pythium aphanidermatum, Fusarium equiseti, Fusarium solani, Cladosporium cladosporioides* and *Mucor hiemmalis.* Among the *Trichoderma* spp. *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma hamatum* were found most effective.

Kumar and Dube (1992) reported that seed bacterization with *Pseudomonas fluorescens* in chickpea seeds and reduced the number of chickpea wilted plants in wilt sick soil by 52 per cent observed and also increased seed germination, growth [in terms of length, weight of root and shoot and yield of plants.

Roebroeck and Mes (1992) obtained reduction in wilt incidence with non-pathogenic *Fusarium* isolates before corms were dipped in a spore suspension of nonpathogenic *Fusarium* isolate and incubated under moist conditions at 20°C and achieved good biological control agent results.

Deshmukh *et al.* (1994) observed that *Trichoderma harzianum* and *Trichoderma viride* were the most efficient mycoparasites on a wide range of seed and soil borne fungi.

Sheela *et al.* (1995) worked on biological control of *Fusarium* causes wilt disease in eggplant. *Trichoderma hamatum* when multiplied in F.Y. M. and coconut coir pith and applied to soil recorded the least incidence followed by *Trichoderma viride* as compared to the control.

Vidyasekaran and Muthiamilan (1995) observed the control of chickpea by antagonist like *Pseudomonas fluorescens*. Seed treatment with this antagonist formulation controlled chickpea wilt and when seed treatment was followed by root zone applications the efficacy of *Pseudomonas fluroscensens* formulations increased.

Karampour and Okhovvat (1996) showed antagonistic effect of *Trichoderma harzianum, Trichoderma viride Trichoderma koningii* and *Gliocladium virens* against *Fusarium solani* and *Fusarium oxysporum* f. sp. *ciceri* in chickpea and bioagent found effective.

Padmodaya and Reddy (1996) screened ten isolates of *Trichoderma* spp. *in vitro* for their efficacy in suppressing the growth of *Fusarium oxysporum* f. sp. *lycopersici.* In dual culture *Trichoderma viride* was found highly inhibitory to *Fusarium oxysporum* f. sp. *lycopersici* in dual culture, followed by *Trichoderma harzianum.*

Nautiyal (1997) showed on chickpea rhizosphere competent *Pseudomonas fluorescens*, NBRI 1303, to be antagonistic to *Fusarium bataticola* and *Pythium* spp.

Satyaparasad *et al.* (1998); Ali Anwar *et al*. (2008) reported that *Trichoderma harzianum* and *T. viride* are deleterious bioagents against wilt pathogen of brinjal. It has been established that *Trichoderma* spp. inhibit pathogenic invasion through phenomena of mycoparasitism, antibiosis and competition against *Fusarium solani*.

Pandey and Upadhyay (1999) reported that *Trichoderma viride* and *Trichoderma* harzianum C isolates were found best biological agents in comparison to study of chemical, which gave integrated approach for management of *Fusarium* wilt of Pigeon pea.

Siddiqui *et al*. (1999) reported root dip treatment with *Pseudomonas aeruginosa* with or without *Trichoderma harzianum, Trichoderma koningii* and *Trichoderma hamatum* which significantly controlled infection of roots by *Fusarium solani, Rhizoctonia solani* and *Meloidogyne javanica* on chilli. He also reported that *P. aeruginosa* when mixed with *Trichoderma* spp. increases plant growth.

Hassanein *et al.* (2000) reported that *Trichoderma harzianum, Trichoderma viride* overlapped with pathogen and suppressed the growth by 53 and 48 per cent for *Rhizoctonia solani* and 46.3 per cent and 72.8 per cent for *Fusarium oxysporum* under greenhouse conditions against damping off and root rot /wilt diseases of Lucern.

Misra and Prasad (2003) noted the success of *Trichoderma* as biocontrol agents (BCAs) is due to their high reproductive capacity, ability to survive under unfavorable conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi and efficiency in promoting plant growth and defense mechanisms. These properties have made *Trichoderma* a ubiquitous genus present in any habitat and at high population density.

Sharma and Chandel (2003) reported that growth inhibition of pathogen by *Trichoderma harzianum, T. viride* and *T. virens*. This inhibition can be attributed to antibiosis. A similar antagonistic activity of *Trichoderma* spp. was reported by Dennis and Webster (1971) and Mukhopadhyay and Mukherjee (1996).

Harman *et al.* (2004) showed *Trichoderma* spp. as opportunistic, avirulent, plant symbionts. *Trichoderma* spp. are known to produce a number of antibiotics, such as trichodermin, trichodermol, polyketides and steroids and frequently associated with both biocontrol activity and promotion of plant and root growth.

Gardener (2004) studied Multiple *Bacillus* and *Paenibacillus* spp. can promote crop health in a variety of ways. Some populations suppress plant pathogens and pests by producing antibiotic metabolites, while others may directly stimulate nutrient uptake by plants, either promoting rhizobial and mycorrhizal symbiosis or by fixing atmospheric nitrogen directly.

Mishra *et al.* (2005) reported integrated and biological control of gladiolus corm rot and wilt caused by *Fusarium oxysporum* f. sp. *gladioli*.

Harman (2006) stated that *Trichoderma* spp. are known as potential biocontrol agents because they can reduce the incidence of diseases caused by plant pathogenic fungi such *Fusarium* spp. through mechanisms such as mycoparasitism, antibiosis, competitive saprophytic ability and the metabolites secretions.

Sharma and Chandel (2006) reported biological control of gladiolus wilt caused by *Fusarium oxysporum* f. sp. *gladioli* using different methods. The soil placement method proved effective compared to the corm dip method. *Trichoderma harzianum* in comparison to

*Trichoderma viride* performed superior against wilt pathogen and resulted in minimum disease incidence in addition to improvement in growth parameters of gladiolus

Kulkarni *et al.* (2007) reported screening of biocontrol agents and cultivars where maximum reduction in colony diameter was observed in *Trichoderma harzianum* (76.08) which was significantly superior over all other bioagents tested. The second best was *Trichoderma koningii* (72.48%) followed by *Trichoderma viriens* (66.30%) and *Trichoderma viride* (61.44%) while *B. subtilis* and *Pseudomonas fluorescens* were least effective in inhibiting mycelial growth of *Fusarium* spp.

# 3. MATERIALS AND METHODS

The experiments described in this chapter were conducted during *kharif* 2016-18 in Plant Pathology Section, College of Agriculture, Pune. The general laboratory techniques were followed for the present study which are described by Nene and Thapliyal, Dhingra and Sinclair (1993) and Aneja (2004) for preparation of media, sterilization, isolation and maintenance of fungus cultures with slight modification whenever necessary.

## 3.1 Glasswares

The standard Borocil brand glasswares were used for laboratory study *viz*. Petri plates, Test tubes; Moisture chambers (Desiccators), Beakers, Conical flasks, Measuring cylinders, Slides, Pipette, Spirit jar etc.

## 3.2 Miscellaneous Materials

Miscellaneous materials includes inoculating needle, scalpel, spirit lamp, spirit, cork borer, sodium hypochloride, rubber bands, thread, pins, racks, formalin, *Streptomycin* sulphate, non-absorbent cotton, rough papers etc. available in Plant Pathology Section, College of Agriculture, Pune were used during the study.

## 3.3 Sterilization of Media and Glassware's

Liquid materials such as media and distilled water were sterilized in an autoclave at 15 psi at 121 ºC for 15 minutes then the medium was cooled at room temperature. Glassware's were sterilized in hot air oven at 180 ºC for two hours.

For surface sterilization 0.1 per cent sodium hypochloride was used and rectified spirit for other materials like inoculation needle, forceps and hands.

## 3.4 Collection of Wilt Disease Samples

Wilt affected gladiolus plant samples were collected from National Agricultural Research Project Ganeshkhind, Pune – 411 007 and High tech Project, College of Agriculture, Pune-05 during 2016-17. These diseased samples were preserved at $4^0$c in refrigerator and used for further studies.

## 3.5 Isolation and Purification of the Pathogen

### 3.5.1 Culture Media

The common laboratory culture medium Potato dextrose agar (PDA) was used for isolation of the pathogen responsible for wilt disease of gladiolus and maintained its pure culture on PDA slant tubes in refrigerator for further study. PDA was also used for Poison food technique and Dual culture technique.

Sand Maize Flour Medium was used for mass multiplication of pathogen.

**Potato Dextrose Agar**

The basic culture medium Potato dextrose agar (PDA), comprising following ingredients was used.

| | |
|---|---|
| Peeled potatoes | : 200 g |
| Dextrose | : 20g |
| Agar agar | : 20g |
| Distilled water | : 1000ml |

For the purpose, 200 g peeled and sliced potatoes were boiled in one liter of water until potatoes became soft. Then it was filtered through muslin cloth and adjusted its final volume to one liter. To this dextrose 20 g and agar agar 20 g were added, boiled again, filled into glass conical flasks (250 ml cap), plugged with non-absorbent cotton and sterilized in an Autoclave at 15 pounds per square inch (psi) (1.54 kg/cm$^2$) pressure and corresponding 121$^0$ C temperature, for 15 minutes.

**Potato Dextrose Broth**

The basic culture medium Potato Dextrose Broth (PDB), comprising following ingredients was used.

| | |
|---|---|
| Peeled potatoes | : 200 g |
| Dextrose | : 20g |
| Distilled water | : 1000ml |

For the purpose, 200 g peeled and sliced potatoes were boiled in one liter of water until potatoes became soft. Then it was filtered through muslin cloth and adjusted its final volume to one liter. To this dextrose 20 g was added, boiled again, filled into glass conical flasks (250 ml cap), plugged with non-absorbent cotton and sterilized in an Autoclave at15 pounds per square inch (psi) (1.54 kg/cm$^2$) pressure and corresponding 121$^0$ C temperature, for 15 minutes.

**Sand Maize Flour Medium**

The culture of *Fusarium solani* was multiplied on sand maize flour medium (1:1). 15 g of maize flour was mixed in 85 g of river bed sand and was filled in the conical flasks of 250 ml capacity (50 g/flask) and sterilized in autoclave at 15 pounds per square inch pressure for 30 min. Then, the flasks were inoculated aseptically with pure culture of *Fusarium solani* and incubated at room temperature for 15 days. After 15 days of incubation, the inoculum was taken out from the flask and mixed thoroughly with sterilized sand plus soil mixture (1:1) at 100 g inoculums per kg soil.

**3.5.2 Isolation**

Isolation was made from wilt affected gladiolus plant parts i.e. corms which were selected for isolation of pathogen. Five corms were selected for isolation.

The infected corms were washed thoroughly under running water and transferred to blotting paper. They were cut into small pieces and surface sterilized in 0.1 per cent mercuric chloride solution for one min. followed by three washings with sterile distilled water and were plated on Potato Dextrose Agar (PDA) medium under aseptic conditions and incubated at the temperature 25±1$^0$C. Out of the five isolates one isolate showed presence of *Fusarium solani* while, four of them exhibited contaminants (other than *Fusarium solani*).

**3.5.3 Purification of Pathogen**

The isolate was purified by hyphal tip method described by Dohroo and Sharma (1992b).

The colony of fungus was grown on the Potato Dextrose Agar (PDA) medium. Selected single growing hyphal tip of mycelium with the help of microscope and marked it with glass marking pencil. The marked out portion was transferred with the help of cork borer and to PDA slant medium and incubate at the temperature 27±1 ºC for 7 days in Biological Oxygen Demand (BOD) and observed for pure growth of culture.

Sufficient care was taken to maintain the purity of the isolate throughout the study.

**3.5.4 Identification and Maintenance of the Pathogen**

The isolate of pathogen was identified based on colony characters, Microconidia, Macroconidia and Chlymadospores etc. by using monograph (Booth, 1971). Isolate was maintained on PDA medium at 4$^0$ C and subcultured every three months.

**3.5.5 Pathogenicity Test**

Pathogenicity of isolate of *Fusarium solani* was proved by Soil Inoculation Method.

**3.5.5.1 Soil Inoculation Method**

The isolate of *Fusarium solani* was multiplied on Sand Maize Flour Media (Dohroo and Sharma, 1992 b).

Four pots were used with sterile soil keeping one pot as control. In pots sterile soil and mixture of sand +soil + inoculum was added .One pot filled with only sterilized soil as control. To allow maximum multiplication of inoculum the pots were adequately watered with sterile water daily. After 8 days, the corms of susceptible gladiolus Sancerre were sown per pot. Plants emerged out of soil within 7 days. The observations were recorded after one month. Few plants were found wilted. Some wilted plants from isolate were collected separately. They were subjected to isolation by 0.1 percent $HgCl_2$ surface sterilization and there change of sterile water and finally one isolate was recovered from artificially inoculated plants and the isolated fungus was reisolated on PDA medium, on the basis of morphological characteristics of pathogen developed were compared with those of the pathogen isolated from naturally wilted gladiolus plants and confirming the pathogenicity of *Fusarium solani*. The recovered isolate was maintained on PDA slants for further study.

### 3.6 Varietal Screening for Disease Resistance

To find out the source of resistance in gladiolus varieties and hybrid lines, screening of gladiolus were done in field. Twenty five varieties and thirty five hybrid lines of gladiolus were selected for screening.

#### 3.6.1 Disease Reaction

Virulence of pathogen and disease reaction against twenty five varieties and thirty five hybrid lines of gladiolus were recorded by (C. D. Mayee and Datar, 1986).

| **Reactions** | | | **% Wilt Incidence** |
|---|---|---|---|
| Immune | (I) | : | 00 |
| Resistant | (R) | : | < 1 % |
| Moderately resistant | (MR) | : | 1.1 - 10 % |
| Moderately susceptible | (MS) | : | 10.1 - 20 % |
| Susceptible | (S) | : | 20.1 - 50 % |
| Highly susceptible | (HS) | : | 50.1 % and above |

#### 3.6.2 Varieties of Gladiolus

Twenty five varieties of gladiolus were selected for screening against *Fusarium solani* (Table 1).

**Table 1. Varieties of gladiolus selected for screening**

| **Sr.No.** | **Variety** | **Sr.No.** | **Variety** |
|---|---|---|---|
| 1 | Pricilla | 14 | Summer Sunshine |
| 2 | Arka Keshar | 15 | Psitacinus hybrid |
| 3 | Rose Supreme | 16 | IIHR-77-59-32 |
| 4 | Sapana | 17 | Yellow Stone |
| 5 | Frienship | 18 | White Prosperity |
| 6 | Fedelio | 19 | Sancerre |
| 7 | IARI-77-86-26 | 20 | IARI-Sel-3 |
| 8 | Sel.fromSel-1 | 21 | Chaubattia6/4 |
| 9 | Jag-G-7 | 22 | DHN-86-1 |
| 10 | Good White | 23 | Phule Ganesh |
| 11 | Jackson Villa Gold | 24 | Big Time Supreme |
| 12 | Red Beauty | 25 | Snow Princes |
| 13 | Chandani | | |

### 3.6.3 Hybrid Lines of gladiolus

Thirty five hybrids of gladiolus were selected for screening against *Fusarium solani* (Table 2).

**Table 2. Hybrid lines of gladiolus selected for screening**

| Sr.No. | Hybrid Lines | Sr.No. | Hybrids Lines |
|---|---|---|---|
| 1 | 94-2 | 19 | 07-17 |
| 2 | 94-58 | 20 | 07-18 |
| 3 | 07-1 | 21 | 07-19 |
| 4 | 07-2 | 22 | 07-20 |
| 5 | 07-3 | 23 | 07-21 |
| 6 | 07-4 | 24 | 07-22 |
| 7 | 07-5 | 25 | 07-23 |
| 8 | 07-6 | 26 | 07-24 |
| 9 | 07-7 | 27 | 07-25 |
| 10 | 07-8 | 28 | 07-26 |
| 11 | 07-9 | 29 | 07-27 |
| 12 | 07-10 | 30 | 07-28 |
| 13 | 07-11 | 31 | 07-29 |
| 14 | 07-12 | 32 | 07-30 |
| 15 | 07-13 | 33 | 07-31 |
| 16 | 07-14 | 34 | 07-32 |
| 17 | 07-15 | 35 | 07-33 |
| 18 | 07-16 | | |

Gladiolus corms of varieties and hybrid lines were stored in refrigerator for one month. After this, corms were surface sterilization with 0.1percent Mercuric chloride. Then corms were washed with sterile water and dried at room temperature before sowing.

In the wilt sick soil, corms of gladiolus varieties and hybrid lines were sown. Observation on wilt percent incidence was recorded after 15 days. Percent wilt incidence was calculated by using following formulae.

$$\% \text{ Incidence} = \frac{\text{Number of wilted plants}}{\text{Total number of sown corms}} \times 100$$

### 3.7 *In Vitro* Evaluation of Different Fungicides Against *Fusarium solani*

The fungicides used in the present study along with particulars of trade name, ingredient of the chemical in formation and sources of supply represented in (Table 3).

**Table. 3 Particulars of Fungicides employed in the investigation / study.**

| Sr. No. | Chemical name | Trade name | Active ingredient | Conc. (%) | Manufacturer |
|---|---|---|---|---|---|
| 1 | Captan | Captaf | 50% WP | 0.3% | Rallis India Ltd. |
| 2 | Carbendazim | Fungiguard | 50% WP | 0.2% | Gharde Chemicals Ltd. |
| 3 | Hexaconazole | Contaf | 5% EC | 0.1% | Dhanuka Agriteh Ltd. |
| 4 | Benomyl | Benlate | 50 % WP | 0.2% | E.I. du Pont de Nemours and company |
| 5 | Azoxystrobin | Godiwa | 23 % SC | 0.2% | Dhanuka Agriteh Ltd. |
| 6 | Copper oxychloride | TopGun-DF | 50% WG | 0.3% | Sumil Chemical Industry Pvt. Ltd. |
| 7 | Bordeaux Mixture | | | 1% | |

For evaluation of different fungicides poisoned food technique (Nene and Thapliyal, 1993) was adapted to determine the sensitivity of the isolate of *Fusarium solani*.

**3.7.1 Poison Food Technique**

The required quantity of fungicides was added to the PDA medium at luke warm stage to 100 ml concentration on active ingredient basis. The stock solution was prepared on whole chemical basis from this stock serial dilutions were made by adding required quantity of PDA. Three replication were maintained in respect of each fungicides.

Five mm discs of the test fungal i.e. isolate of *Fusarium solani* were cut with sterile crock borer and transferred aseptically to the center of poisoned medium. Similarly control was maintained by placing five mm discs of the test isolate i.e. *Fusarium solani* in the center of the non-poisoned PDA medium. All the plates were incubated at $25\pm1^{0}$c in BOD incubated for nine days. The diameter of fungal colony was measured in each treatment.

Percent mycelial growth inhibition of the test pathogen with treatments over untreated control was calculated by applying following formulae (Vincent, 1927)

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

Where, I = Per cent Inhibition of fungal growth.

C = Growth/colony diameter (mm) of the fungus in control plate.

T = Growth / colony diameter (mm) of the fungus in treatment plate

### 3.8 *In vitro* Evaluation of Different Bioagents Against *Fusarium solani*

The potential antagonistic activity of bicontrol agents viz. *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma hamatum* and *Trichoderma virens* were collected from Biological Nitrogen Fixation Scheme, College of Agriculture, Pune – 05 and *Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from the National Collection of Industrial Microorganisms (NCIM), Pune.

The antagonist potential of bioagents was assessed against *Fusarium solani* by dual culture technique on PDA medium as per procedure described by Stack *et al*. (1986).

#### 3.8.1 Dual Culture Technique

For this, 20 ml of sterilized and cooled medium (PDA) was poured in each petri plate allowed to solidify. Cut discs of pathogen and bioagents isolates with the help of cork borer. A 5 mm disc of pathogens was placed at one end of the medium with the help of sterilized inoculating needle. Just opposite to it, 5 mm disc of bioagents isolate was placed. Control i.e. without inoculation of the bioagents isolates fungus were maintained. Petri plates were incubated at 25 ±1 ºC temperature.

Percent mycelial growth inhibition of the test pathogen with treatments over untreated control was calculated by applying following formulae (Vincent, 1927).

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

Where, I = Per cent Inhibition of fungal growth.

C = Growth/colony diameter (mm) of the fungus in control plate.

T = Growth/colony diameter (mm) of the fungus in treatment plate.

### 3.9 *In Vivo* Evaluation of Effective Fungicides and Bioagents Against *Fusarium solani.*

Most effective fungicides and bioagents were evaluated in pot culture against *Fusarium solani.* The experiment was conducted in glasshouse of Plant Pathology Section, College of Agriculture, Pune.

For this, the isolate of *Fusarium solani* was multiply on Sand Maize Flour Medium. Then mixed in sterilized soil and made wilt sick soil.

Potato Dextrose Broth was prepared in colonial flask. Mycelial discs of five mm diameter were cut from the margin of the seven day old culture of each *Trichoderma* spp. and transformed to the conical flasks containing the sterilized PDB medium under aseptic conditions. Nutrient Broth (NB) was prepared in conical flask. Seven days old Bacterial culture of each bacteria and transformed to the conical flask containing sterilized NA broth medium under aseptic condition. All flasks were incubated at 25 ±1 ºC in an incubator for ten days.

For evaluation of effective bioagents the culture filtrate effective of bio agents (each @0.5%) was added in the earthen pots containing wilt sick soil. After eight days the corms of susceptible Gladiolus Sancerre were sown per plot. For evaluation of the effective fungicides

solution at their recommended dose were separately drenched twice i.e. seven and fifteen days after sowing of the gladiolus corms of gladiolus Sancerre in the earthen pot containing wilt sick soli . One pot filled with only wilt sick soil kept as control. Three pots per treatment were maintained. The observations on the number of wilted plants were recorded after fifteen days. Percent wilt incidence was calculated by using following formulae.

$$\% \text{ Incidence} = \frac{\text{Number of wilted plants}}{\text{Total number of sown corms}} \times 100$$

**3.10 Statistical analysis**

The data obtained in different experiments was statistically analyzed following Completely Randomized Block Design (CRD) as per procedure suggested by Panse and Sukhatme (1969). The data pertaining to percentage was angularly transformed whenever necessary.

# 4. RESULTS AND DISCUSSION

The present study was undertaken to isolate causal organism of wilt of gladiolus and study their identification, pathogenicity, varietal screening for disease resistance, chemical and biological management in laboratory and glasshouse. The studies were conducted during *Kharif* season in the year 2016-18. The results of these experiments are compiled and statistically analyzed wherever necessary and the inferences drawn are presented on the following pages.

## 4.1. Collection of Disease Samples

The diseases samples of gladiolus were collected form National Agricultural Research Project, Ganeshkhind and High Tech Project, College of Agriculture, Pune in the year 2016-18. The diseased sample exhibited typical symptoms of gladiolus wilt. (Plate 1)

### A. Symptoms on corms

In corms the disease symptoms varied from a slight discoloration at the base to complete rotting. The center of bulb turned black and was rotten. Lesions on corm were reddish to brown with well-defined margin round to oval, depressed, leading to hard shrunken and mummified corms. Extensive interior rot was seen in some cases without any outward sign of disease. In Severe infection, especially if the soil is moist, the parent corm completely rotted during harvesting. In partially infected plants, size of the corm was reduced and black discoloration was seen.

### B. Symptoms on Leaves

Symptoms started on lower leaves in the form of yellowing. Later the whole leaf turned brown with reduction in size resulting in narrowing of leaves. (Some cases, bending of leaves was also observed). In severe cases, infected plants showed complete wilting or premature drying of leaves.

### C. Symptoms on Flowers and Spike

Symptoms were also observed at flowering stage. In severe cases infected plants failed to produce flowers. In other cases spike became very small with unopened flowers. Whenever flowers were produced they were malformed. In some cases bending of flower spike was observed and their size and number was reduced.

Similar typical symptoms were noticed by McCulloch (1944) reported the disease symptoms observed on inflorescences, florets and corms. Buxton and Robertson (1953); Singh (1969); Tomar (1997) and Khan and Khan (2002) reported that wilting symptoms was seen on the lower leaves, twigs or even the whole plant turns brown and later dies.

**A. Partial yellowing**

**B. Complete yellowing**

**C. Brown to black discolouration of Corms**

**D. Transverse section of corms showing brown discolouration**

**E. Rotting of corms**

**Plate 1. Typical symptoms of gladiolus wilt**

### 4.2 Isolation and Identification of Pathogen

The causal organism was isolated by tissue isolation method (corm infected) from diseased samples of gladiolus wilt. From 5 wilted samples of gladiolus, one isolate was obtained and identified as *Fusarium solani.* Growth of fungus was observed 3-4 days after incubation at $25 \pm 1^{o}$ C. The maximum colony growth was obtained in 10 days after plating. Thereafter the growth of culture ceased. The culture was raised fluffy in its growth. While, the colony colour was dull white and pinkish. (Plate 2)The fungus produced macroconidia, microconidia and chlamydospores. Microconidia were abundant, hyaline, continuous or 1-septate, ovoid to ovate. Macroconidia were scarce, often lacking and variable, 3–septate or rarely 4–5 septate (Plate 3). Chlamydospores were hyaline, usually vacuolated and spherical. On the basis of above morphological and cultural characters, the isolate was identified as *Fusarium solani* which was similar mentioned in monograph by Booth (1971). The isolate was purified and mass multiplied for further studies.

Similar study was carried out by Garibaldi *et al*. (2004) isolated *Fusarium* spp. consistently and readily from symptomatic vascular tissues on *Fusarium*-selective medium from the wilt affected plants. Also Sharma and Tripathi (2008) isolated and maintained the cultures of *Fusarium* spp. on PDA medium. The identification was confirmed by compared with monograph by Booth (1971).

### 4.3. Pathogenicity Test

The pathogenicity test was proved by soil inoculation method.

#### 4.3.1. Soil Inoculation Method

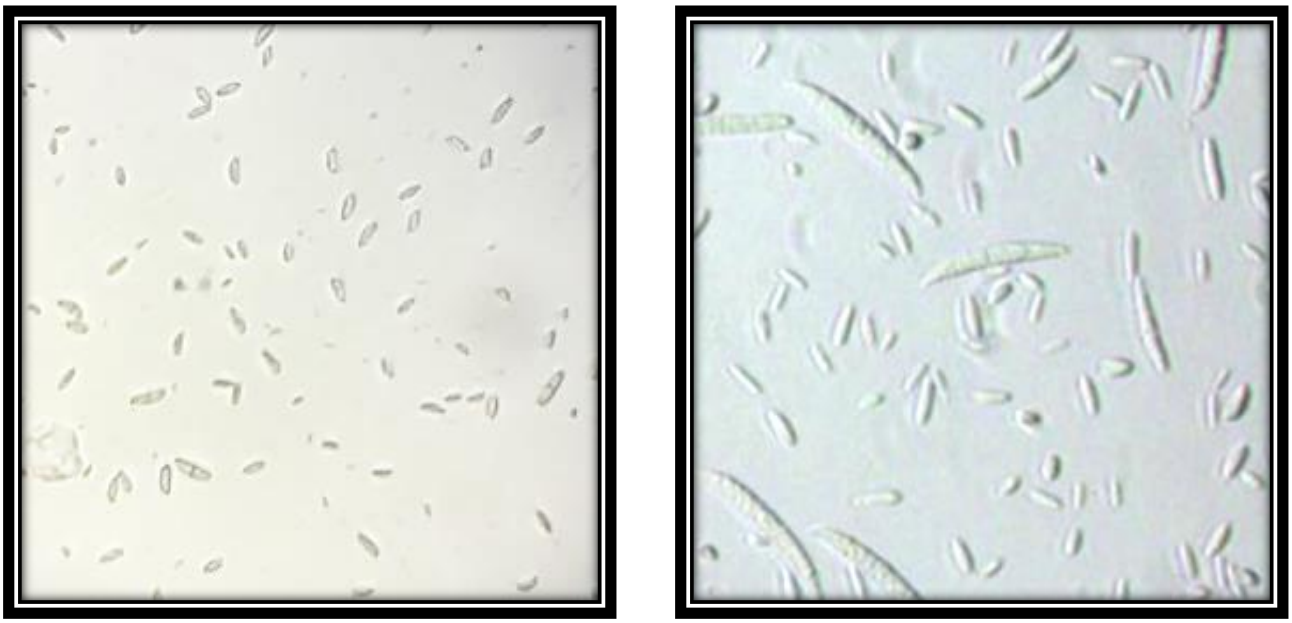
The isolate of *Fusarium solani* was multiplied on Sand Maize Flour Media. Four pots were used. In pots, mixture of sand + sterile soil+ inoculum was added. One pot filled with sterilized soil was kept as a control. To allow maximum multiplication of inoculum, the pots were adequately watered with sterile water daily. After 8 days, the corms of susceptible Gladiolus Sancerre were planted per pot under glasshouse conditions.

The observations on germination and per cent wilting were recorded on 7 days of sowing respectively. The results indicated that the isolate of *Fusarium solani* was pathogenic resulting 100 per cent wilting (Plate 4). The wilt symptoms induced were similar to naturally wilted gladiolus plant.

#### 4.3.2. Reisolation

From artificially inoculated and wilted gladiolus corms, the resiolations were undertaken. This reisolated culture was morphologically studied and compared with original isolate and used for inoculation in pathogenicity test.

**Plate 2. Isolate of *Fusarium solani***



**A. Microconidia** **B. Macroconidia**

**Plate 3. Microphotograph of *Fusarium solani* showing Microconidia and Macroconidia**

**Healthy** **Wilted**

**Plate 4. Pathogenicity test on gladiolus variety Sancerre**

**Plate 5. Mass multiplication of *Fusarium solani* on Sand Maize Flour Media (SMFM)**

The comparison revealed that original isolate and reisolate culture was identical thus proved Koch's postulates. The reisolated culture was thereafter maintained on PDA slants.

Results are in conformity with those reported by Porta and Varase (1985) isolated *Fusarium oxysporum* f. sp. *gladioli* from wilt affected gladiolus plant and observed the presence of *F. oxysporum*. f. sp. *gladioli* in gladiolus corms and they confirmed the pathogenicity by soil inoculation method. Also Pande *et al*. (2007) proved the pathogenicity of *F. oxysporum* f. sp. *ciceri*, isolated from wilted seeds of the cultivar K 850. The isolates were separately multiplied on sterilized potato dextrose broth in 250 ml conical flask and incubated for 7 days at 25°C and 124 rpm. Eight-day-old seedling of a susceptible cultivar JG 62, grown in sterile sand were uprooted, sand particles were removed from the roots by washing with distilled water, the roots inoculated by dipping in the inoculum for 30 seconds to enable conidia to adhere to the roots. Root inoculated seedlings were transplanted in pre-irrigated sterile vertisol and sand (3:1) pot mix filled in 15 cm plastic pots and kept in a green house at 25+ 3°C. Inoculated seedlings were observed for wilt symptom upto 30 days after inoculation.

**4.4 Maintenance of Isolate of *Fusarium solani***

The culture of isolate of *Fusarium solani* was maintained on PDA medium by frequent subculturing and storing at $4^{o}$C in refrigerator. The culture was revived on PDA medium whenever required.

**4.5 Varietal Screening for Disease Resistance**

Results (Table 4 and 6) indicated that the gladiolus varieties and hybrid lines have variable reaction against *Fusarium solani*. Gladiolus varieties and hybrid lines continued exhibiting wilt symptoms over an observation period. Within first fifteen to twenty days wilting plant appears and after some days of observations majority of the gynotypes exhibited wilting symptoms.

The reaction of genotypes was worked out as per C. D. Mayee and Datar (1986) per cent wilt incidence scale with slightly modifications.

| **Reactions** | | **% Wilt Incidence** |
|---|---|---|
| Immune | (I) | : 00 |
| Resistant | (R) | : < 1 % |
| Moderately resistant | (MR) | : 1.1-10% |
| Moderately susceptible | (MS) | : 10.1-20% |
| Susceptible | (S) | : 20.1-50 |
| Highly susceptible | (HS) | : 50.1 and above |

### 4.5.1 Screening of Gladiolus Varieties Against Wilt Disease

Out of twenty five gladiolus varieties, only one variety IIHR-77-59-32 was found free from wilt disease and nine varieties showed between 2.00 to 5.00 %. Twelve varieties showed between 5.01 to 10 % disease incidence. Two varieties *viz*. Psitacinus hybrid and Sancerre showed maximum disease incidence 32.5 % and 30.50 % respectively (Table 4).

**Table 5. Reaction for Disease Resistance of Gladiolus Varieties against Wilt Disease**

| Disease rating | Reaction | No. of Varieties | Name of varieties |
|---|---|---|---|
| 00 % | Immune | 01 | IIHR-77-59-32 |
| <1 % | Resistant | 00 | -- |
| 1.1-10 % | Moderately resistant | 22 | Pricilla, Arka Keshar, Rose, Supreme, Sapana, Frienship, Fedelio, IARI-77-86-26, Sel.fromSel-1,Jag-G-7, Good White, Jackson Villa Gold, Red Beauty, Chandani, Summer Sunshine, Yellow Stone, White Prosperity, IARI-Sel-3, Chaubattia6/4, DHN-86-1,Phule Ganesh, Big Time Supreme, Snow Princes |
| 10.1-20 % | Moderately susceptible | 00 | -- |
| 20.1-50 % | Susceptible | 02 | Psitacinus hybrid, Sancerre |
| Above 50 % | Highly susceptible | 00 | -- |

It is seen from results depicted in Table 5 indicated that, the only one variety was noticed zero per cent wilt. Moderately resistant reaction (1.1-10 % wilt incidence) was noticed in twenty two cultivars while two varieties showed susceptible reaction.

### 4.5.2 Screening of Gladiolus Hybrid Lines against Wilt Disease

The result presented Table 6 indicated that, out of thirty five gladiolus hybrid lines, two hybrid lines *viz*. 07-7 and 07-23 were found free from wilt disease. Only one hybrid 94-58 showed 1.00 % disease incidence whereas twenty five hybrid lines showed between 2.0 to 5.0 % disease incidence. Six hybrid lines showed between 5.01 to 6.25 % disease incidence whereas maximum disease incidence 6.50% was shown by one hybrid line *viz.* 07-24.

It is seen from results depicted in Table 7, that two hybrid lines had showed no wilting. Resistant reaction (wilt incidence upto 1 %) was noticed in one hybrid line and thirty two hybrid lines were found moderately resistant (wilt incidence 1 to 10%).

**Table 4. Virulence Reaction of *Fusarium solani* on Different Gladiolus Varieties**

| Sr.No. | Name of Varieties | Per cent Disease Incidence | Disease Scale |
|---|---|---|---|
| 1 | Pricilla | 9.75 | 3 |
| 2 | Arka Keshar | 7.25 | 3 |
| 3 | Rose Supreme | 6.0 | 3 |
| 4 | Sapana | 5.70 | 3 |
| 5 | Frienship | 3.25 | 3 |
| 6 | Fedelio | 5.15 | 3 |
| 7 | IARI-77-86-26 | 10.15 | 3 |
| 8 | Sel.fromSel-1 | 3.20 | 3 |
| 9 | Jag-G-7 | 6.95 | 3 |
| 10 | Good White | 3.75 | 3 |
| 11 | Jackson Villa Gold | 3.50 | 3 |
| 12 | Red Beauty | 5.15 | 3 |
| 13 | Chandani | 4.00 | 3 |
| 14 | Summer Sunshine | 4.50 | 3 |
| 15 | Psitacinus hybrid | 32.50 | 7 |
| 16 | IIHR-77-59-32 | 0.00 | 0 |
| 17 | Yellow Stone | 5.5 | 3 |
| 18 | White Prosperity | 9.50 | 3 |
| 19 | Sancerre | 30.50 | 7 |
| 20 | IARI-Sel-3 | 2.50 | 3 |
| 21 | Chaubattia6/4 | 9.75 | 3 |
| 22 | DHN-86-1 | 3.15 | 3 |
| 23 | Phule Ganesh | 3.00 | 3 |
| 24 | Big Time Supreme | 5.25 | 3 |
| 25 | Snow Princes | 6.00 | 3 |

**Table 6. Reactions of gladiolus Hybrids Lines against *Fusarium solani***

| Sr.No. | Name of Hybrid Lines | Per cent Disease Incidence | Disease scale |
|---|---|---|---|
| 1 | 94-2 | 3.50 | 3 |
| 2 | 94-58 | 1.0 | 1 |
| 3 | 07-1 | 4.50 | 3 |
| 4 | 07-2 | 4.15 | 3 |
| 5 | 07-3 | 3.15 | 3 |
| 6 | 07-4 | 2.00 | 3 |
| 7 | 07-5 | 2.00 | 3 |
| 8 | 07-6 | 4.15 | 3 |
| 9 | 07-7 | 0.00 | 0 |
| 10 | 07-8 | 3.15 | 3 |
| 11 | 07-9 | 3.15 | 3 |
| 12 | 07-10 | 6.25 | 3 |
| 13 | 07-11 | 3.95 | 3 |
| 14 | 07-12 | 4.0 | 3 |
| 15 | 07-13 | 3.50 | 3 |
| 16 | 07-14 | 5.15 | 3 |
| 17 | 07-15 | 4.15 | 3 |
| 18 | 07-16 | 4.00 | 3 |
| 19 | 07-17 | 3.65 | 3 |
| 20 | 07-18 | 5.15 | 3 |
| 21 | 07-19 | 4.50 | 3 |
| 22 | 07-20 | 4.10 | 3 |
| 23 | 07-21 | 5.10 | 3 |
| 24 | 07-22 | 5.15 | 3 |
| 25 | 07-23 | 0.00 | 0 |
| 26 | 07-24 | 6.50 | 3 |
| 27 | 07-25 | 3.75 | 3 |
| 28 | 07-26 | 4.85 | 3 |
| 29 | 07-27 | 3.15 | 3 |
| 30 | 07-28 | 4.50 | 3 |
| 31 | 07-29 | 3.50 | 3 |
| 32 | 07-30 | 4.25 | 3 |
| 33 | 07-31 | 2.50 | 3 |
| 34 | 07-32 | 275 | 3 |
| 35 | 07-33 | 5.25 | 3 |

**Table 7. Reaction for Disease Resistance of Gladiolus Hybrid Lines against Wilt disease**

| Disease rating | Reaction | No. of Hybrids | Name of Hybrids |
|---|---|---|---|
| 00 % | Immune | 02 | 07-23, 07-7 |
| <1 % | Resistant | 01 | 94-58 |
| 1.1-10 % | Moderately resistant | 32 | 94-2, 07-1,07-2, 07-3, 07-4, 07-5, 07-6, 07-8, 07-9, 07-10, 07-11, 07-12, 07-13,07-14, 07-15,07-16, 07-17, 07-18 ,07-19, 07-2007-21, 07-22, 07-24, 07-25, 07-26, 07-27, 07-28, 07-29, 07-30, 07-31, 07-32, 07-33 |
| 10.1-20 % | Moderately susceptible | 00 | -- |
| 20.1-50 % | Susceptible | 00 | -- |
| Above 50 % | Highly susceptible | 00 | -- |

Other workers have also made efforts to search sources of resistance to *Fusarium* wilt of gladiolus. Similar results were reported by Palmer and Pryal (1958), out of 160 gladiolus cultivars, 10 per cent cultivars were resistant to wilt disease of gladiolus caused by *Fusarium spp.,* Ronald *et al*. (1974), had screened 211 cultivars for resistance to *F*. *oxysporum* f. sp. *gladioli* by inoculating dormant corms and they found that selections 66-109 -5 and 63-5-1 were found most resistant. Tarabeih *et al*. (1981) found resistance in cultivar 'Mentee White' while four cultivars 'Wood Pecker', 'Lilac Wonder', 'True Love' and 'White Friendship' were reported moderately resistant to the disease.

**4.6 *In vitro* Evaluation of Fungicides**

Seven fungitoxicants namely Captan 50 % WP, Copper oxy chloride 50 % WG, Carbendazim 50 % WP, Bordeaux Mixture, Azoxystrobin 23 SC , Benomyl 50 % WP and Hexaconazole 5 % EC were evaluated against *Fusarium solani* by poison food technique and per cent inhibition in mycelia growth is presented in Table 8.

**Table 8. Efficacy of fungicides against *Fusarium solani* in *In vitro***

| Sr. No. | Treatment | Conc. (%) | Mean Colony Diameter * (mm) | Percent Inhibition |
|---|---|---|---|---|
| 1 | Captan 50 % WP | 0.15 | 25.33 | 71.85 |
| 2 | Copper oxychloride 50 % WG | 0.15 | 71.00 | 21.11 |
| 3 | Carbendazim 50 % WP | 0.1 | 7.67 | 91.48 |
| 4 | Bordeaux Mixture | 1 | 67.66 | 24.81 |
| 5 | Azoxystrobin 23 SC | 0.1 | 73.33 | 18.51 |
| 6 | Benomyl 50 % WP | 0.1 | 3.67 | 95.92 |
| 7 | Hexaconazole 5 % EC | 0.05 | 42.33 | 52.96 |
| 8 | Control | - | 90.00 | |
| | | **S.E. (m)±**<br>**C.D. (5%)** | **1.63**<br>**4.92** | |

*** = Mean of three replications**

Perusals of result from Table 8 and Fig. 1 indicated that, all the test fungicides significantly inhibited the growth of *Fusarium solani* over untreated control. The Benomyl 50 % WP @ 0.1 % and Carbendazium 50 % WP @ 0.1 % showed minimum growth colony diameter (3.67 mm and 7.67mm respectively.) and were found significantly superior over all the test fungicides. It was followed by Captan 50 % WP @ 0.15 % (25.33 mm). The Bordeaux Mixture @ 1 % showed growth colony diameter 67.66 mm which was at par with Copper oxy chloride 50 % WG @ 0.15 % (71.00mm). The Hexaconazole 5 % EC @ 0.05 % showed growth colony diameter of 42.33 mm whereas the Azoxystrobin 23 SC @ 0.1 % showed maximum growth colony diameter 73.33 mm and found to be inferior over all the test fungicides.

All the test fungicides significantly reduced growth of *Fusarium solani* than control. Inhibition of growth varied from 18.51 to 95.92 per cent in different test fungicides. Highest inhibition was observed by Benomyl 50% WP @ 0.1 % (95.92 per cent) and Carbendazim 50% WP @ 0.1 % (91.48 per cent) found effective to control wilt disease of gladiolus over all the test fungicides followed by and Captan 50 % WP @ 0.15 % (71.85 per cent). Rest of the test fungicides ranged between 18.51 percent to 52.96 per cent inhibition. The Azoxystrobin 23 SC @ 0.1 % was found least effective to control wilt disease of gladiolus (Plate 6 and Fig. 2).

| | | | |
|---|---|---|---|
| T1 - | Captan 50 % WP | T5 - Bordeaux Mixture |
| T2 - | Copper oxycholoride 50 % WP | T6 – Benomyl 50 % WG |
| T3 - | Carbendazim 50 % WP | T7 – Hexaconazole 5 % EC |
| T4 - | Azoxystrobin 23 SC | T8 – Control |

**Plate 6. Effect of Different Fungicides Against Mycelial Growth of *F. solani***

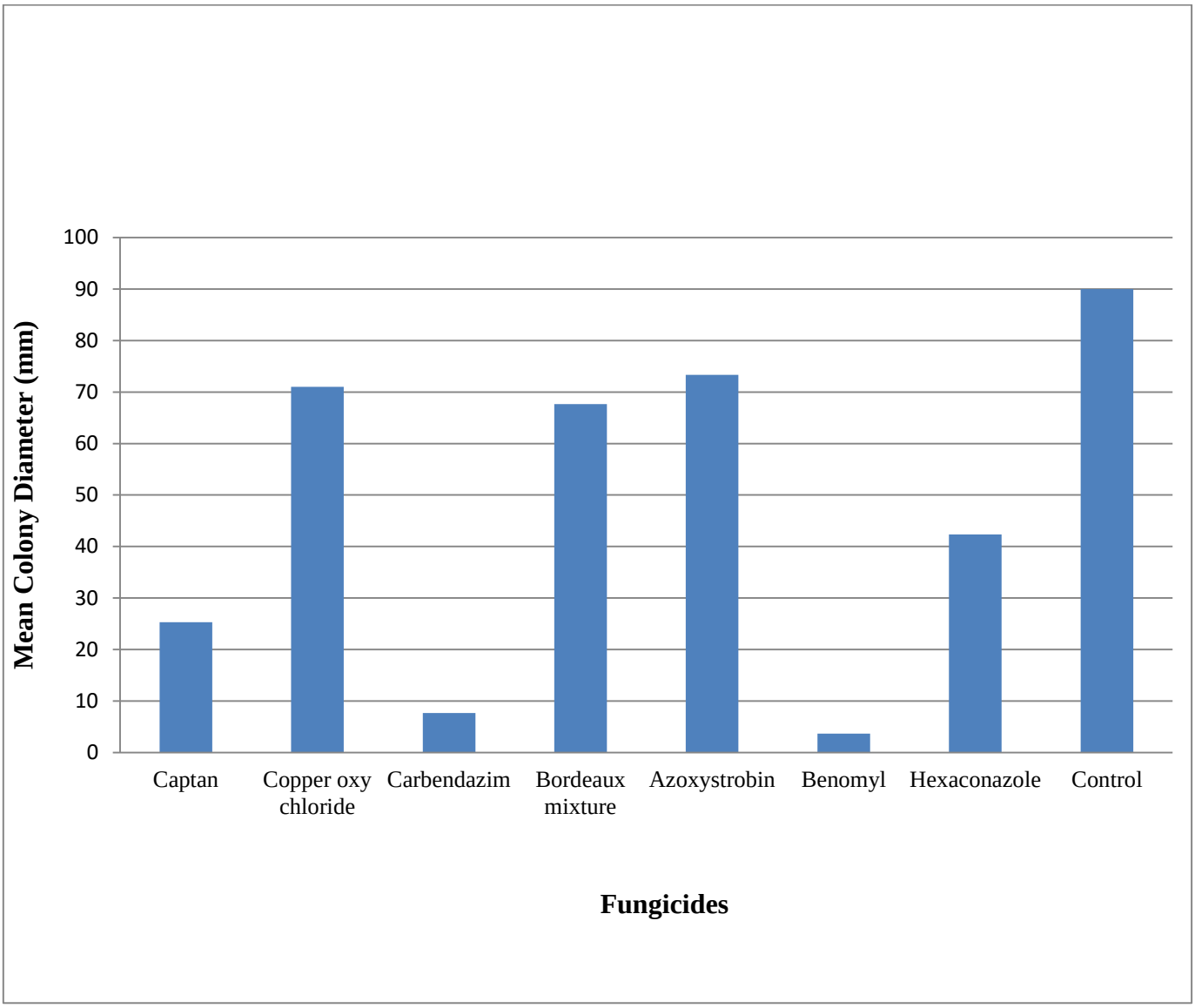



**Fig. 1. Effect of fungicides on mycelia growth of *Fusarium solani* in *in vitro***

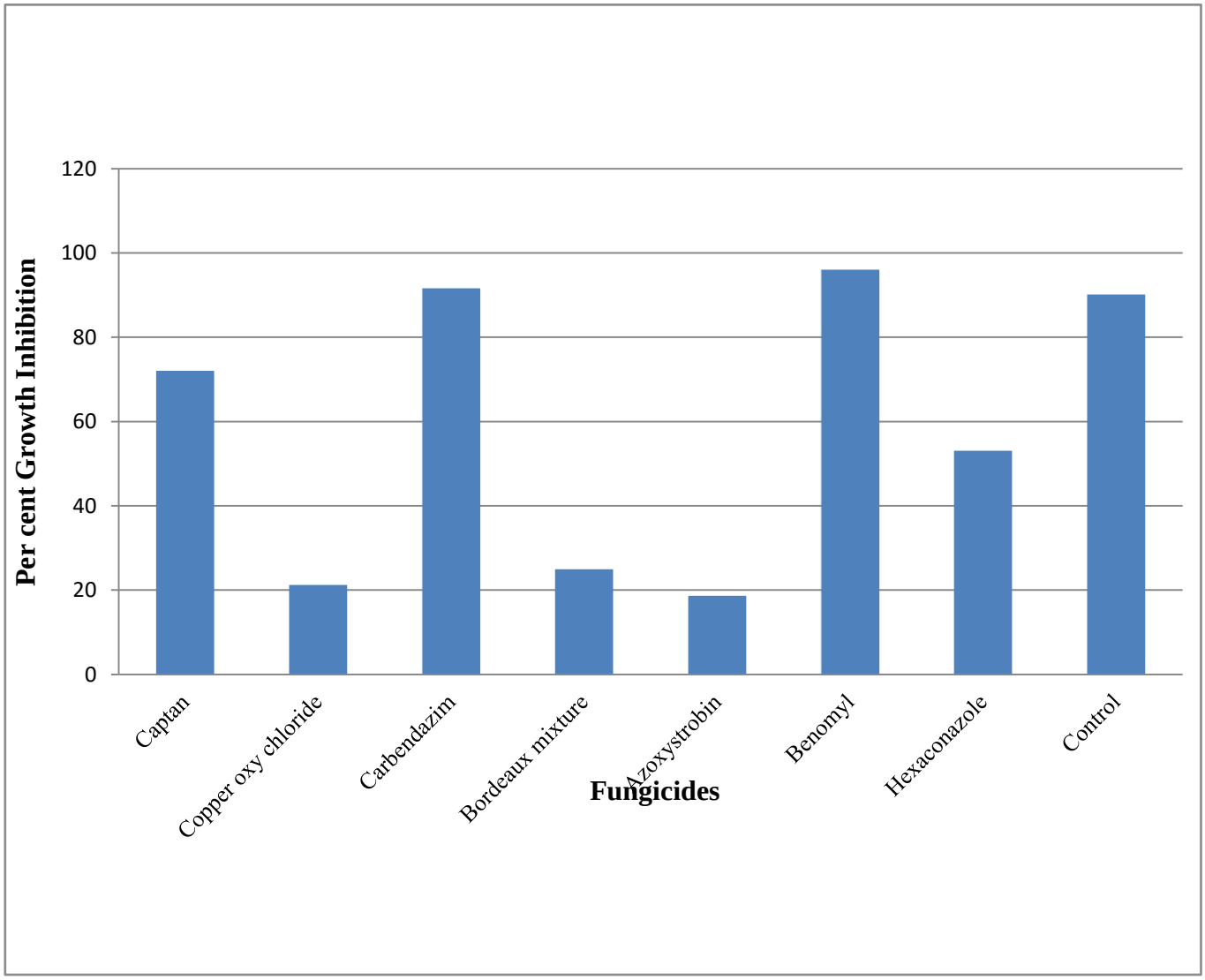



**Fig. 2. Per cent growth inhibition of *Fusarium solani* by different fungicides**

Results are in accordance with Falck (1907) reported that poisoned food technique is the most common practice for evaluating fungicides under laboratory condition. Georgieva and Peikova (1976) reported that effective and excellent protection of *Fusarium oxysporum* f. sp. *gladioli* was provided by Captan, Chlorothalonil, Benomyl, Thiobendazole, Zinc ion-maneb complex and Zineb. Wani *et al.* (1982) reported that the combination of Bavistin (0.1%) and Difoltan (0.5%) gave best control of wilt of gladiolus caused by *Fusarium oxysporum* f. sp. *gladioli* than other fungicides individually.

**4.7 Biological Management**

**4.7.1 *In vitro* evaluation of bioagents against *Fusarium solani***

The effect of biocontrol agents viz. *Trichoderma viride, Trichoderma virens, Trichoderma harzianum, Trichoderma hamatum, Bacilus subtilis* and *Pseudomonas flurosence* were tested against the isolate of *Fusarium solani* by dual culture technique.

The result presented in Table 9 revealed that, the growth of *Fusarium solani* was significantly influenced by all bioagents under study at all the observation period. *Trichoderma viride* (28.33 mm) was significantly superior over all the bioagents and it was at par with *Bacilus subtilis (3*1.00 mm) and *Trichoderma virens* (33.33 mm). Rest of the bioagents ranged between 40.00 mm to 48.00 mm. Inhibition of growth ranged from 46.66 to 68.51 per cent in different bio agents (Plate 7 and Fig. 3).

Highest inhibition was observed in *Trichoderma viride* (68.51 per cent) and proved to be best followed by *Bacilus subtilis* (68.55 per cent) and *Trichoderma virens* (62.96 per cent), *Trichoderma hamatum* (55.55 per cent), *Trichoderma harzianum* (46.66 per cent) and *Pseudomonas flurosence* (46.28 per cent). (Fig. 4)

Simillar results were reported by Morton and Stroube (1955) developed dual culture technique for testing of antagonism of *Trichoderma* spp. against broad range of common plant pathogens, Morshed (1985) studied antagonism between *Trichoderma* spp. and *Fusarium oxysporum*, *Fusarium culmorum*, *Alternaria tenuis, Botrytis cinnera* and *Colletotrichum lindemuthianam*. He observed that 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, Bhardwaj and Gupta (1987) studied *in vitro* antagonism against inhibition of *Pythium aphanidermatum, Fusarium equiseti, Fusarium solani, Cladosporium cladosporioides* and *Mucor hiemmalis* by *Trichoderma viride, Trichoderma harzianum* and *Trichoderma hamatum*, Karampour and Okhovvat (1996) shown antagonistic effect of *Trichoderma harzianum, Trichoderma viride Trichoderma koningii* and *Gliocladium virens* against *Fusarium solani* and *Fusarium oxysporum* f. sp. *ciceri* in chickpea.

Padmodaya and Reddy (1996) screened ten isolates of *Trichoderma* spp. *in vitro* for their efficacy in suppressing the growth of *Fusarium oxysporum* f. sp. *lycopersici*. In dual culture *T. viride* was found highly inhibitory to *Fusarium oxysporum* f. sp. *lycopersici*, followed by *T. harzianum* and Kulkarni *et al.* (2007) evaluated biocontrol agents and maximum reduction in colony diameter was observed in *T. harzianum* (76.08 %) which was significantly superior over all other bioagents tested. The second best was *T. koningii* (72.48 %) followed by *T. viriens* (66.30 %) and *T. viride* (61.44 %) while *B. subtilis* and *Pseudomonas fluorescens* were least effective in inhibiting mycelial growth of *Fusarium* spp.

**Table 9. *In vitro* efficacy of bio-agents against *Fusarium solani***

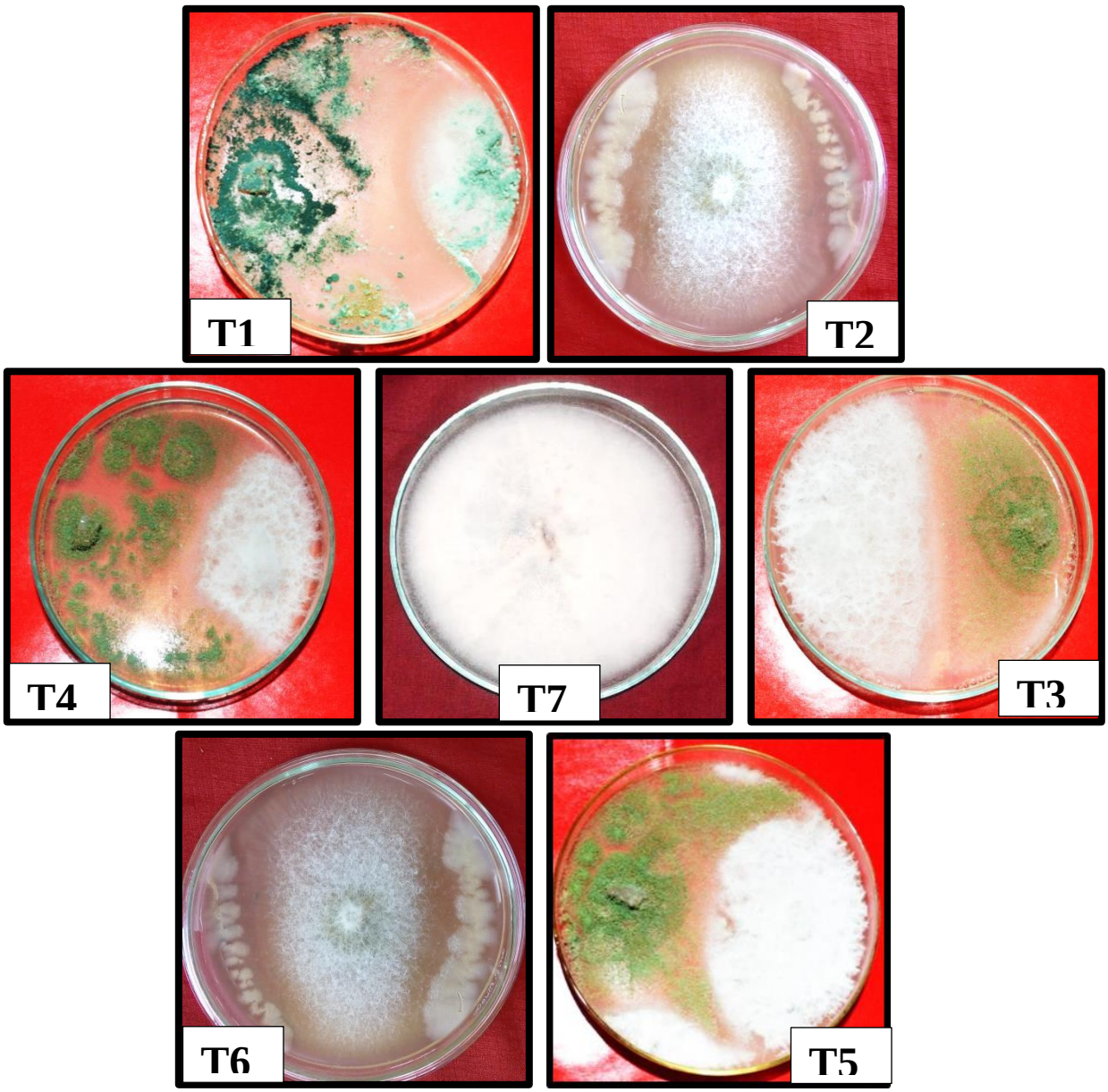
| Sr.No. | Treatment | Mean Colony Diameter * (mm) | Percent Inhibition |
|---|---|---|---|
| 1 | *Trichoderma viride* | 28.33 | 68.51 |
| 2 | *Bacilus subtilis* | 31.00 | 65.55 |
| 3 | *Trichoderma harzianum* | 48.00 | 46.66 |
| 4 | *Trichoderma hamatum* | 40.00 | 55.55 |
| 5 | *Trichoderma virens* | 33.33 | 62.96 |
| 6 | *Pseudomonas flurosence* | 48.33 | 46.29 |
| 7 | Control | 90.00 | - |
| | **S.E. (m)±**<br>**C.D. (1%)** | **3.11**<br>**9.36** | |

*** = Mean of three replications**

**4.8 *In vivo* evaluation of effective fungicides and bioagents against *Fusarium solani*.**

Most effective fungicide *viz*. Captan 50 % WP, Benomyl 50 % WP and Carbendazim 50% WP and bio agents *Trichoderma viride, Trichoderma virens* and *Bacillus subtilis* were evaluated in pot culture in glass house of Plant Pathology Section, College of Agriculture, Pune and per cent disease incidence and per cent disease control is presented in Table 10.

The results represented in Table 10, Plate 8 and fig. 5 indicated that, the effective fungicides and bioagents were significantly inhibited the growth of *Fusarium solani*. The effective fungicides and bioagents treatments showed significantly less incidence than control (90.00%). Minimum incidence (11.11 %) was shown by Captan 50 % WP @ 0.3 % and Benomyl 50 % WP @ 0.2 % followed by Carbendazim 50 % WP @ 0.2 per cent (33.33%) and *Trichoderma viride* @ 0.5 per cent (33.33 %). Rest of the bio agents *viz. Trichoderma virens* @ 0.5 % and *Bacillus subtilis* @ 0.5 % showed 66.66 % disease incidence.




T1 – *Trichoderma viride*
T2- *Bacillus subtilis*
T3- *Trichoderma hamatum*
T4- *Trichoderma harzianum*
T5 - *Trichoderma virens*
T6 - *Pseudomonas fluorscence*
T7 - Control

**Plate 7. Inhibition of *Fusarium solani* by different bio control agents**

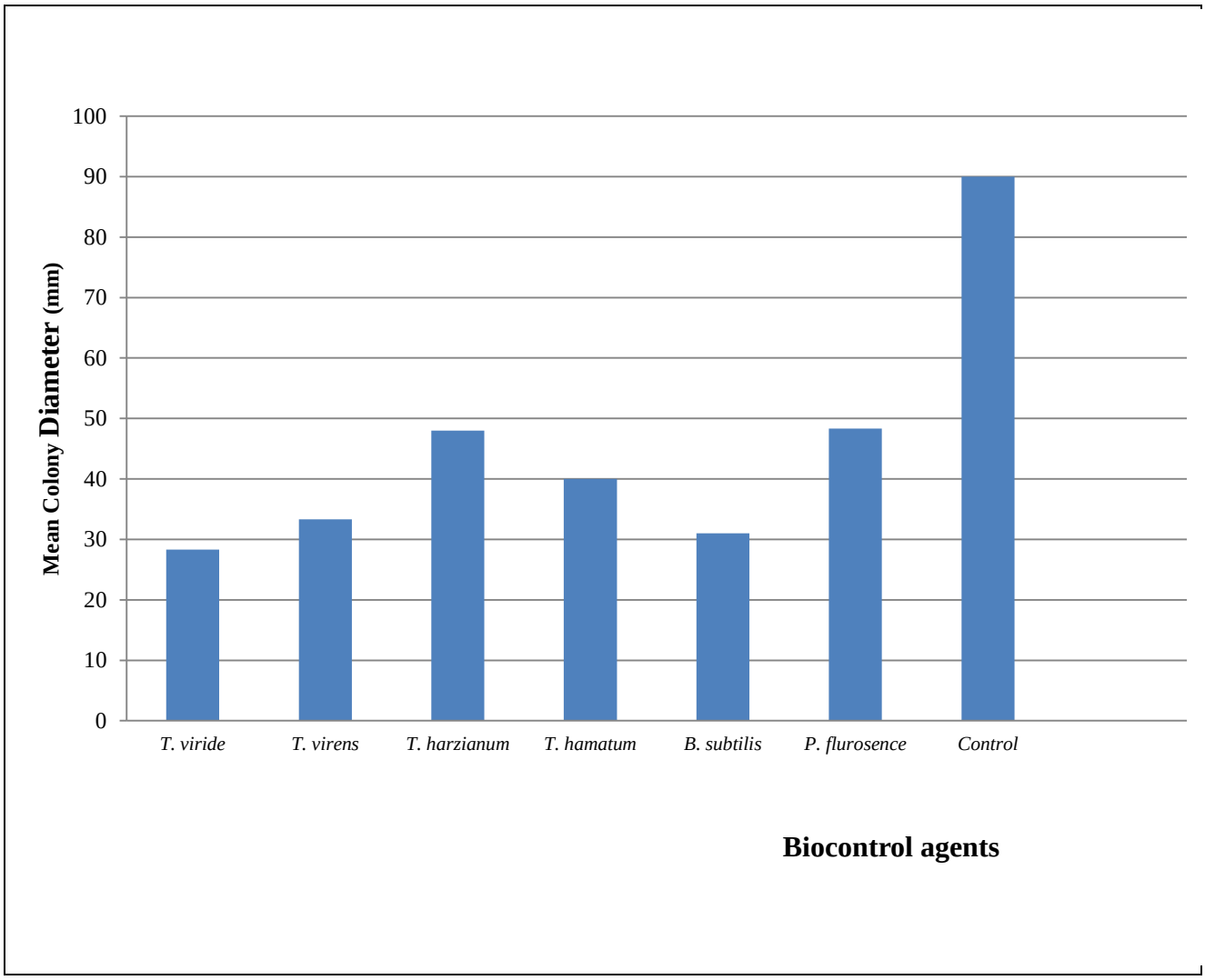



**Fig. 3. Effect different of bio-agents on mycelia growth of *Fusarium solani* in *in vitro***

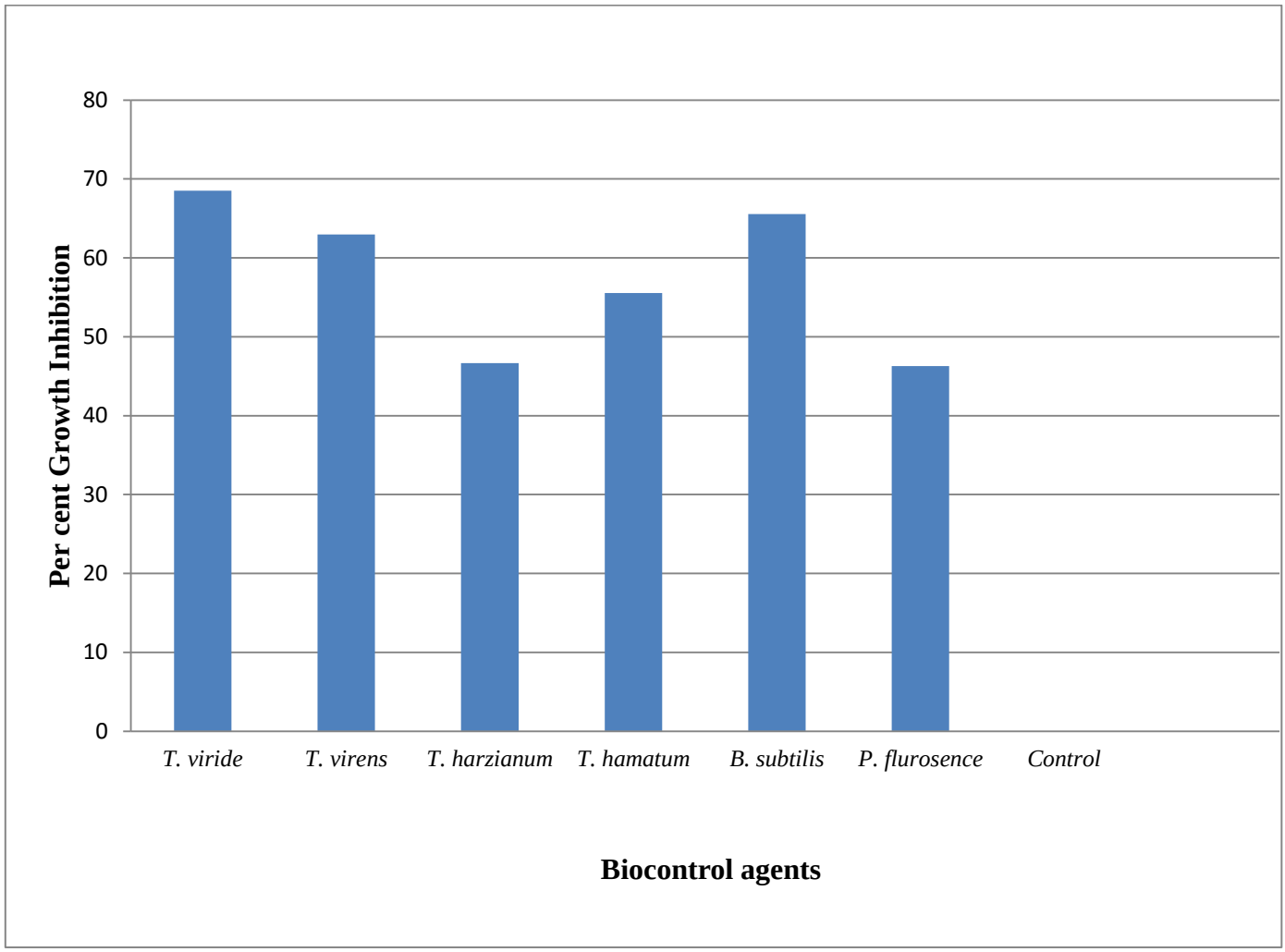



**Fig. 4. Per cent growth inhibition of *Fusarium solani* by different bio-agents.**

**Table 10. *In vivo* evaluation of effective fungicides and bio-agents against *Fusarium solani***

| Sr. No. | Treatment | Conc. (%) | Per cent Disease Incidence * | Per cent Disease Control |
|---|---|---|---|---|
| 1 | Captan 50 % WP | 0.3 | 11.11 (11.74) | 87.67 |
| 2 | *Trichoderma viride* | 0.5 | 33.33 (35.26) | 62.96 |
| 3 | Benomyl 50 % WP | 0.2 | 11.11 (11.74) | 87.67 |
| 4 | *Bacillus subtilis* | 0.5 | 66.66 (54.73) | 25.93 |
| 5 | Carbendazium 50 % WP | 0.2 | 33.33 (35.26) | 62.96 |
| 6 | *Trichoderma virens* | 0.5 | 66.66 (54.73) | 25.93 |
| 7 | Control | - | 90.00 (71.57) | - |
| | | **S.E. (m) ± C.D. (5%)** | **6.28 19.06** | - |

*** = Mean of three replications**

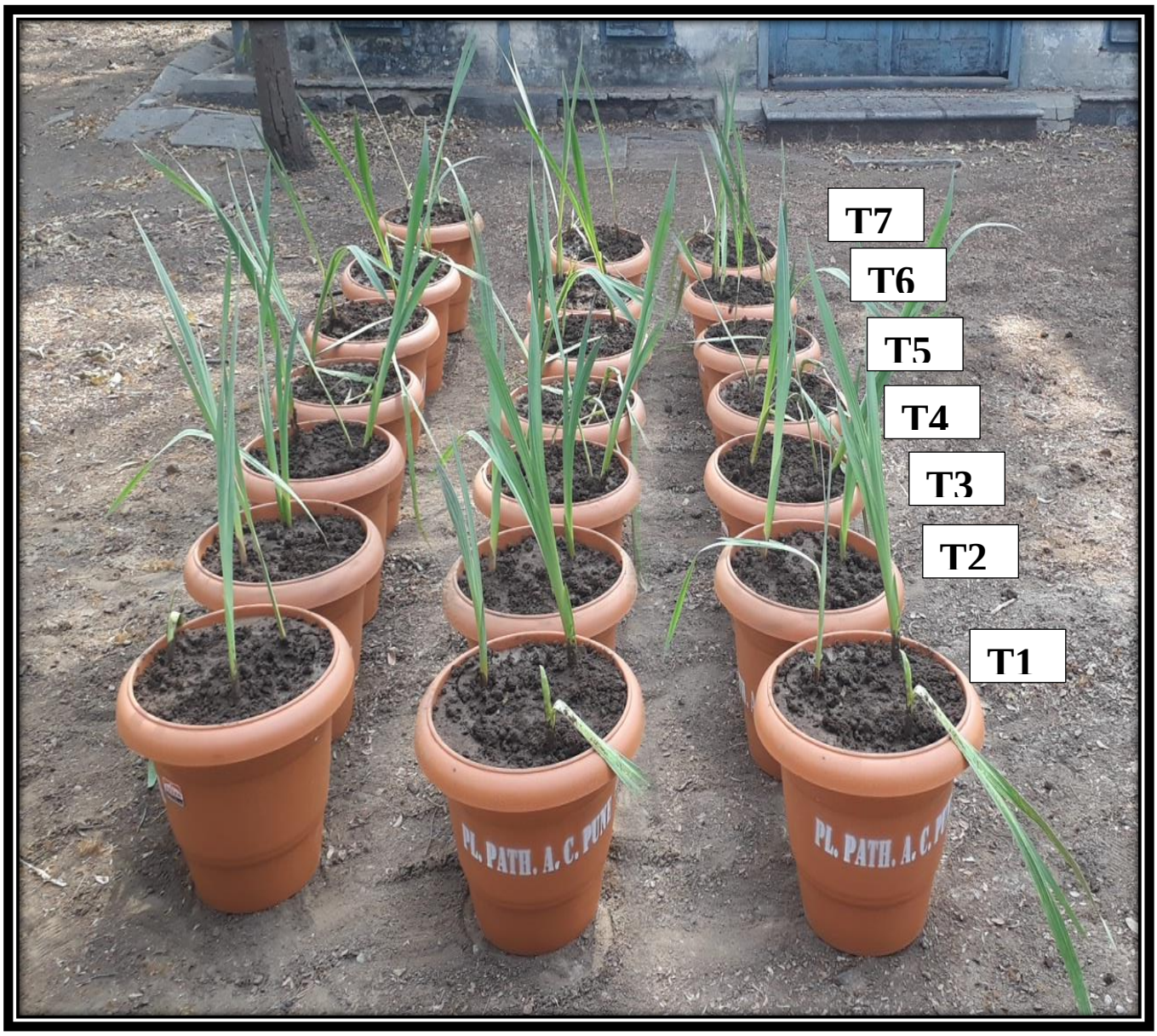
**Figures in the parentheses are arcsine transformed values.**

It was observed that the wilt disease significantly controlled by the effective fungicides *viz.* Captan 50 % WP @ 0.3 % and Benomyl 50 % WP @ 0.2 % (87.65 %) respectively followed by Carbendazim 50 % WP @ 0.2 % and *Trichoderma viride* @ 0.5 % (62.96 %) respectively. Rest of the bioagents *Bacillus subtilis* and *Trichoderma virens* found to be less effective to control the wilt disease (25.93 %) respectively (Fig. 6).

Results are in conformity with those reported by Sud (1999) used six fungicides against saffron corm rot caused by *Fusarium solani*. Out of the six fungicides tested. Bavistin and Thiabendazole @ 0.2 per cent as a dip or drench gave complete disease control. In all other treatments, including the use of healthy corms, the disease levels increased each year. The use of healthy corms followed by application of Bavistin or Thiabendazole as a drench in subsequent years appeared to be the best management strategy.

Forsberg (1970) reported that Benomyl was more effective than Thiram in efficiently controlling corm rot of gladiolus resulting in more economic yield, Magie (1971) reported Benomyl

to be much more effective in controlling *Fusarium* rot of gladiolus, Pandey and Upadhyay (1999) reported that *Trichoderma viride* and *Trichoderma* harzianum C isolate were found best among biological agents in comparative study of chemical, biological und integrated approach for management of *Fusarium* wilt of Pigeon pea, Hassanein *et al.* (2000) reported that *Trichoderma harzianum, Trichoderma viride* overlapped with pathogen and suppressed the growth by 53 and 48 per cent for *Rhizoctonia solani* and 46.3 per cent and 72.8 per cent for *Fusarium oxysporum* under greenhouse conditions of damping off and root rot /wilt diseases of Lucern. Sharma and Chandel (2003) growth inhibition of pathogen by *Trichoderma harzianum, T. viride* and *T. virens* has been reported. This inhibition can be attributed to antibiosis. A similar antagonistic activity of *Trichoderma* spp. was reported by Dennis and Webster (1971) and Mukhopadhyay and Mukherjee (1996).




**T1 - Captan 50 % WP**
**T2 - *Trichoderma viride***
**T3 - *Benomyl 50 % WP***
**T4- Carbendazim 50 % WP**
**T5 - *Trichoderma virens***
**T6 - *Bacillus subtilis***
**T7 - Control**

**Plate 8. Evaluation of effective fungicides and biocontrol agents against wilt disease of gladiolus**

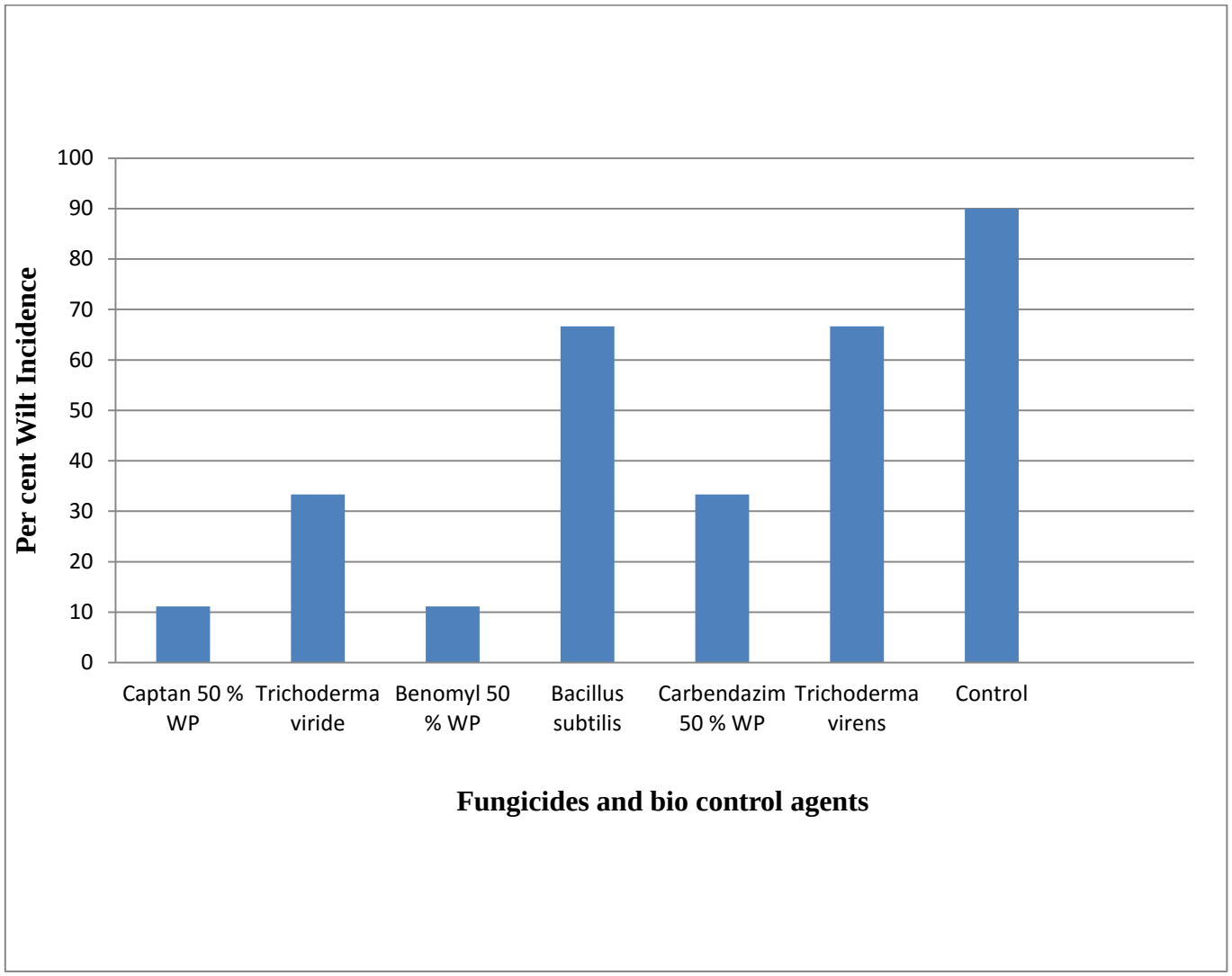



**Fig. 5. *In vivo* efficacy of fungicides and bio-agents against *Fusarium solani* causes wilt disease in gladiolus**

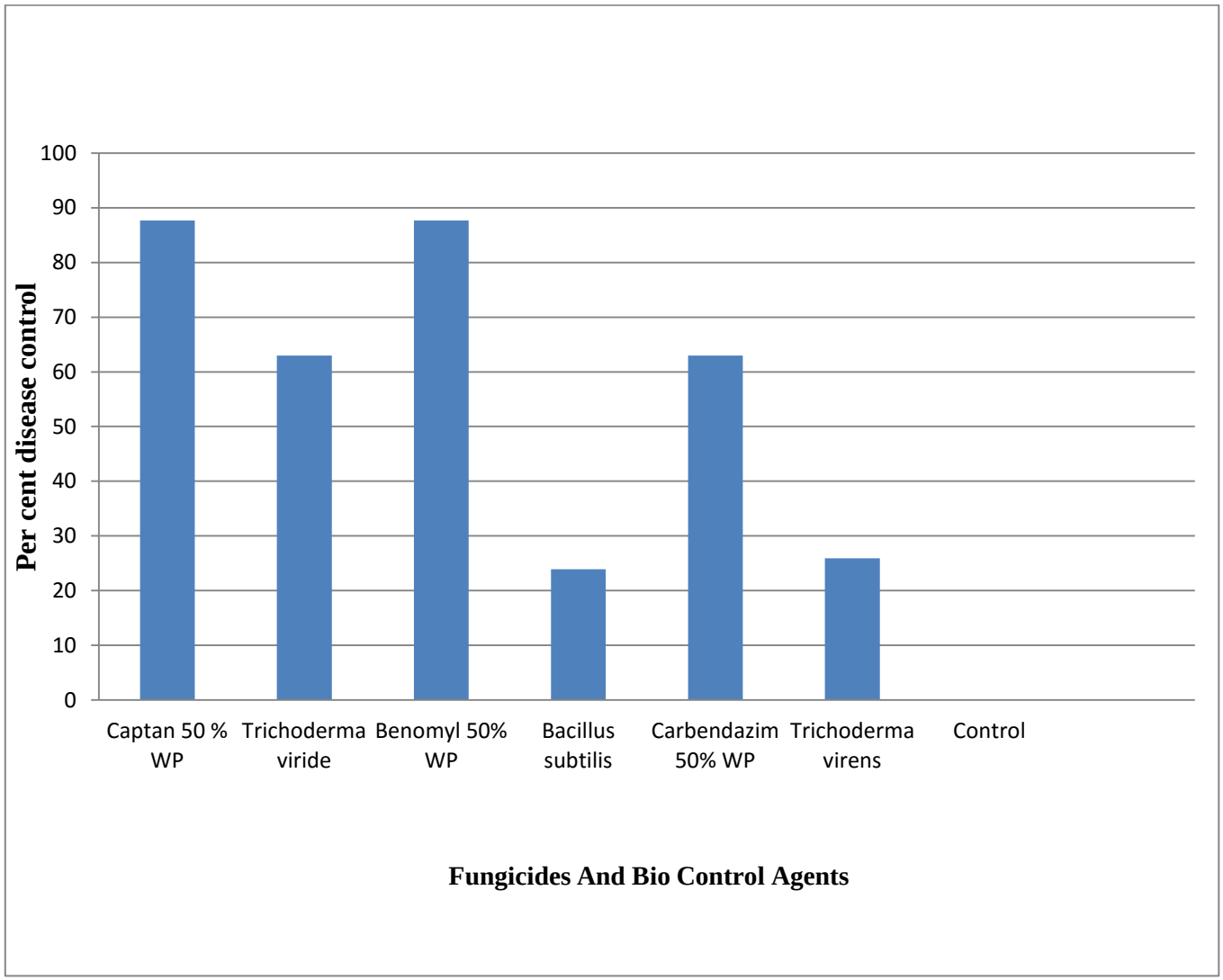



**Fig. 6. Per cent Disease Control of *Fusarium solani* by effective fungicides and bio-agents**

# 5. SUMMARY AND CONCLUSIONS

The present study was undertaken on “Studies on Wilt Disease of Gladiolus”, which is wide spread in all the gladiolus growing areas. The information available on this disease as well as pathogen is very limited. Hence it was essential to study various aspects of the pathogen and also the disease.

The main objectives were to isolate the fungus pathogen, prove Koch’s postulates, screen the gladiolus genotypes for resistance against wilt disease and study the effect of fungicides and bioagents against wilt pathogen in *In vitro* and *In vivo*. Characteristic symptoms of wilt disease noticed were yellowing of leaves, stunting of plants and in severe cases infected plants failed to produce flowers and corms.

The culture of the pathogen was isolated from the samples collected from diseased plants. The wilt affected gladiolus plant samples were collected from National Agricultural Research Project, Ganeshkhind and High tech Project, College of Agriculture, Pune. The pure culture of pathogen was obtained by hyphal tip method. On the basis of morphological and cultural studies, the pathogen was identified as *Fusarium solani* and further its pathogenicity was confirmed.

Among twenty five varieties and thirty five hybrid lines, the results revealed that twenty five IIHR-77-59-32 variety of gladiolus and 07-7 and 07-23 hybrid lines of gladiolus showed immune reaction to *Fusarium solani;* 94-58 hybrid shows resistant reaction; while Psitacinus hybrid and Sancerre showed susceptible reaction. Other all varieties and hybrid lines shows moderately resistant reaction.

To develop effective management strategies, attempts were made by evaluating seven fungitoxicants and six biocontrol agents against *Fusarium solani*, first in *in vitro* and then in *in vivo*. Benomyl and Carbendazim followed by Captan were found most effective in inhibiting growth of *Fusarium solani* in *in vitro*. The maximum per cent inhibition of mycelial growth (95.92 per cent) at 0.2 per cent of *Fusarium solani* was observed in Benomyl 50% WP. Among the six biocontrol agents *Trichoderma viride* was superior over all other followed by *Trichoderma virens* and *Bacilus subtilis*. The maximum per cent inhibition of mycelial growth (68.51 per cent) observed by *Trichoderma viride*.

Under pot culture in glass house, the effective Benomyl, Carbendazim and Captan fungicides and *Trichoderma viride*, *Trichoderma virens* and *Bacilus subtilis* biocontrol agents used as corm treatment for management of *Fusarium* wilt of gladiolus in cultivar “Sancerre”.

Among all these fungicides Captan, Benomyl fungicides and biocontrol agent *Trichoderma viride* were proved to be best to control the *Fusarium* wilt of gladiolus.

**Conclusions**

Results indicated that, IIHR -77 -59 -32 and Hybrid lines 07-7 and 07-23 found immune to wilt disease. Captan and Benomyl found superior fungicides and *Trichoderma viride* were proved to be best control wilt disease of gladiolus. Finally adaption of integrated disease management was an ecologically sound management approach for effective control of wilt disease of gladiolus.

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# VITAE

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**MASTER OF SCIENCE (AGRICULTURE)**

**AGRICULTURAL MICROBIOLOGY**

**2018**

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