INVESTIGATIONS ON WILT [*Fusarium solani* (Mart.) Sacc.] OF GERBERA [*Gerbera jamesonii* L.] UNDER SOUTH GUJARAT CONDITIONS

A

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ABSTRACT

Gerbera (*Gerbera jamesonii* L.) is one of the economically most important flower crop grown in India. Gerbera crop was found to be severely affected by wilt disease resulting in complete loss of crop in the college garden of Navsari Agricultural University, Navsari and commercial green houses. Looking to the seriousness of the disease, present investigation was undertaken to study the cause of disease and to find out suitable control measures for minimizing the crop losses.

The repeated isolation from infected plants revealed the association of *Fusarium* sp. which was identified after cultural and morphological studies as *Fusarium solani* (I.T.C.C. No. 6148-05). The pathogenicity was proved by artificial inoculation methods with positive results. Among the various methods tried, root dip cum soil and root dip inoculation methods

were most quick and effective methods which were followed by soil inoculation.

Out of seven different media tested, Richards', potato dextrose and Czapek's (Dox) proved superior for the growth and sporulation of *F. solani.*

Studies on different pH regimes revealed that the *fungus produced maximum growth and sporulation in pH ranging* from 6.0 to 6.5.

Out of seven different nitrogenous sources tested, sodium nitrate, calcium nitrate and potassium nitrate stimulated while all ammonical nitrogenous sources suppressed the growth and sporulation of *F. solani.*

On the basis of varietal screening against the disease, Primrose proved resistant while Avant grade, Zingaro and Savannah were moderately resistant, and rest of the varieties showed moderately susceptible to susceptible reactions.

Studies on interaction of known antagonist by dual culture, pathogen at the periphery and pathogen at the centre methods revealed strong antagonism of *Trichoderma viride* against *F. solani* whereas *Trichoderma harzianum, Aspergillus niger, Trichoderma longibrachyatum* and *Aspergillus flavus* appeared as potential antagonists.

The result of organic extracts evaluated against *F. solani* revealed that extract of neem cake was strongly inhibitory to fungal growth followed by poultry manure, pressmud, mustard cake, FYM and castor cake.

The phytoextracts of seven plant species evaluated *in vitro* against *F. solani* revealed that significantly lower mycelial growth of pathogen was recorded in the extracts of garlic and was found superior over the rest. Next best extract was onion and ginger followed by tulsi, turmeric and nilgiri.

All the fungicides tested *in vitro* effectively inhibited the growth of *F. solani* as compared to control. Among these fungicides, carbendazim and M.E.M.C. followed by thiophanate methyl and hexaconazole proved to be the most effective in suppressing the growth of *F. solani*.

Six herbicides were screened *in vitro* to evaluate their fungitoxic effect on *F. solani* at three different concentrations by poisoned food technique. Among different herbicides tested, atrazine and glyphosate showed maximum growth inhibition and proved to be the most effective in suppressing the growth of *F. solani*.

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CERTIFICATE

This is to certify that the thesis entitled "**INVESTIGATIONS ON WILT DISEASE [*Fusarium solani* (Mart.) Sacc.] OF GERBERA [*Gerbera jamesonii* L.] UNDER SOUTH GUJARAT CONDITIONS.**", submitted by Mr. **HITEN KUMAR RAMESH BHAI DHAMSANIA** in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY** of the **NAVSARI AGRICULTURAL UNIVERSITY** is a record of bonafied research work carried out by him under my guidance and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

Place : Navsari
Date: November, 2006.

(Dr. D. M. JOSHI)

Major Advisor

DECLARATION

This is to declare that the whole of the research work reported in the thesis in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY** by the undersigned is the result of investigations done by me under direct guidance and supervision of **Dr. D. M. Joshi,** Professor and Head, Dept. of Plant Pathology, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari and no part of the work has been submitted for any other degree so far.

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Place : Navsari

Date : Nov. 2006

H.R.Dhamsania

(Dhamsania Hiten R.)

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INTRODUCTION

I INTRODUCTION

The importance of flower crops has uninterruptively continued since the very dawn of civilization. The diverse agroclimatic conditions of India are very conducive for growing a large variety of flower crops throughout the year. The enigma of nature with its infinite variety, unique colours and unmatchable patterns has kept man attached with it, in this modern age.

Floriculture has considerable potential for export looking towards the commercial enterprise. In India, total area under flower crops is 80,000 hectares with the production of 3.0 lac tones of loose flowers and 600 millions cut flowers (Sengupta and Rajkamal, 2006). In Gujarat, the area under flower crops is 5000 hectares with the production of 30187 metric tons (MT). The area under floriculture has been increasing at the rate of 5 to 6 per cent every year because of increased demand and more use of flower. As a cash crop, flower cultivation gives higher net return. Six districts of south Gujarat having 1318 hectares area with a production of 8266 MT of flowers which accounts for 27 per cent of total area and production of flower in Gujarat (Dhaduk and Panj, 2006).

Gerbera is an important commercial cut flower, suitable for export and domestic markets. It is commonly known as Transvaal Daisy, Barberton Daisy or African daisy and belongs to the family compositae (Bose and Yadav, 1989). The genus Gerbera

consist of about forty species of hardy and perennial flowering plants, out of which only *Gerbera jamensonni* L is under cultivation. It is native to Natal and Transavaal and an important flower grown throughout the world under wide range of climatic conditions.

The genus *Gerbera* was named in the honour of German naturalist, Traugott Gerber. It is a beautiful flower with remarkable geometrical regularity of its form and is ideal for flower beds, borders, pots and rock gardens. The large daisy like bloom certainly does give the best impression. Plants are tender perennials herbs. Flower head is solitary, many flowered and with conspicuous ray florets are in wide range of colours, such as yellow, orange, cream-white, pink, brick red, scarlet, salmon and various other intermediate shades. Based on flower head types or forms they are grouped in to single, double and semi double cultivars. The flower stalks are long, thin and leafless. This characteristic made gerbera very popular and is of great demand in market for preparation of bouquets. Because of its graceful appearance, hardiness and its long lasting flower quality, gerbera is in great demand as cut flower in market and also having very good export potential (Gardiner, 1967).

In India, gerbera is grown in temperate Himalayas from Nepal at an altitude of 1300-3200m (Bose and Yadav, 1989). It is also commercially grown in Kalim pong in West Bengal, Banglore in Karnataka and Pune, Nagpur, Nasik and Thane in Maharastra. In

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Gujarat, particularly Ahamdabad, Vadodara, Surat, Navsari and Valsad district have tremendous scope for the cultivation of gerbera.

Like other crops, many diseases caused by fungi, bacteria and viruses, which are reported by different workers, also attack gerbera.

The gerbera crop is known to be infected by a number of fungal diseases viz., wilt (*Fusarium oxysporum* f. sp. dianthi); foot rot (*Fusarium oxysporum* Snyder and Hansen and *Phytophthora cryptogea*); blight and gray mold (*Botrytis cinerea*); root rot (*Pythium* sp., *Sclerotium rolfsii* and *Rhizoctonia solani*); powdery mildew (*Erysiphe cichoracearum* and *Oidium crysiphoides* f. sp. gerbera); and phyllody caused by *Phytoplasmas* (Chadha, 2002). In addition, the crop is also affected by root rot (*Pythium irregularae* and *Rhizoctonia solani* Kuhn.); foot rot (*Fusarium oxysporum* Snyder and Hansen and *Phytophthora cryptogea*); sclerotium rot (*Sclerotium rolfsii* Sacc.); blight (*Botrytis cinerea*); powdery mildew (*Erysiphe cichoracearum* DC. and Oidium *crysiphoides* f. sp.gerbera); leaf spot (*Phyllosticta gerbarae*, *Alternaria* sp.); downy mildew (*Bremia lactucae*); bacterial disease viz., bacterial blight (*Xanthomonas* sp.); and viral disease viz., tobacco rattle virus and mosaic (gerbera mosaic virus) diseases (Bose and Yadav, 1989).

Wilt of gerbera is reported to be caused by several species of *Fusarium* (Jacob and Folk, 1986; Kaewruang *et al.*, 1987; Chadha, 2002; Garibaldi, 2004; Pardikovic *et al.*, 2000 and Minuto

et al., 2006). In south Gujarat region, wilt is a severe disease of gerbera caused by *Fusarium solani* and is also observed to be causing considerable losses to the crop. Little is known about Fusarium wilt diseases on gerbera.

Looking to the seriousness of the disease and economic importance of this crop in this area, the present investigations were under taken to study the disease and to generate necessary information for suitable disease management measures to minimize crop losses and to develop scientific information on the following aspects:

1. Isolation and identification of causal organism associated with wilt disease of Gerbera.
2. Morphology of pathogen
3. Pathogenicity test and Symptomatology
4. Physiological investigation *in vitro* studies
    - 4.1 Superior culture medium for the growth and sporulation of *F. solani*
    - 4.2 Effect of different pH regimes on growth and sporulation of *F. solani*
5. Effect of nitrogenous fertilizers on the growth and sporulation of *F. solani in vitro*
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8.1 Bio-efficacy of fungicides against *F. solani*

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REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Plant diseases are one of the major constraints in crop production, resulting in drastic losses by reducing the quality and yield. More than 34 diseases have been reported in gerbera at various stages of crop growth. There are 22 fungal, 2 bacterial and 4 nematode, 5 viral and viroids and 1 phytoplasmal disease (Wick and Dicklow, 2000). Among them, Fusarium wilt is very important causing severe yield loss. Wilt disease of gerbera (*Gerbera jamesonii* L.) caused by *Fusarium solani* has been reported by few research workers from abroad posing a serious threat to its commercial cultivation.

During the survey, gerbera crop was found severely affected by wilt in college garden and in greenhouse at N.A.U. Navsari. The available information regarding gerbera wilt is reviewed and presented here as under.

2.1 Isolation

Jacob and Folk (1986) reported *F. oxysporum* f. sp. gerbarae, the main fungal pathogen infecting *Gerbera jamesonii* from German democratic republic.

Kaewruang *et al.* (1987) isolated *F. oxysporum*, *F. solani* and *F. equiseti* from rotted roots of gerbera from Australia.

Pardikovic *et al.* (2000) isolated *F. oxysporum* f.sp. gerberae from affected plants of Elly-May (825 plants) and Fironija (546 plants) from Croatia.

F. solani was isolated from symptomatic vascular tissue of infected plants of gerbera from Italy (Garibaldi *et al.*, 2004).

2.2 Symptomatology

Arthur *et al.* (1976) reported *F. solani* as incited of a stem rot disease of chrysanthemum, the symptoms included red to brown discolouration and decay of pith and vascular tissues of cutting, death of growing stem and sprouting of plants with diseased or dead stems.

Talieva *et al.* (1979) reported that, the wilt of Iris caused by *F. oxysporum; F. solani* and *F. macroceras* were characterized by destructive rot of rhizome, followed by yellowing wilt and in general under development of aerial parts.

Murkar *et al.* (1994) reported that wilt of chrysanthemum (*F. oxysporum* f. sp. *chrysanthemi*) is characterized by loss off turgidity of leaves and apical portion followed by drooping, withering, wilting and on the stem, necrotic black streaks and vascular discolouration of roots and stem were observed on the infected plant.

Chadha (2002) described the symptoms of gerbera wilt caused by *F. oxysporum* f. sp. *dianthi*, and the infected plants show

yellowing of lower leaves. The collar portion of infected plants displayed vascular discoloration.

Garibaldi *et al.* (2004) observed the symptoms in gerbera as yellowing of leaves with initially brown and eventually black streaks in the vascular system and stunting of the plants affected with *F. oxysporum*. From these affected plants, *F. oxysporum* was readily isolated from symptomatic vascular tissue.

2.3 Pathogenicity

Many workers have proved the pathogenicity of wilt disease on various plant species successfully.

Wellman (1939) proved the pathogenic nature of *F. bulbigenum* var. *lycopersici* by dipping the roots of tomato seedling in inoculum made from *F. bulbigenum* culture.

Ghosh and Singh (1982) conducted the pathogenicity tests by growing the plants in 6 inches diameter earthen pots in sterilized soil. They inoculated the fungus on the injured roots, symptoms developed after about twenty days of inoculation.

Radhakrishnan and Sen (1985) proved the pathogenicity of *F. solani* causing wilt of muskmelon by three different methods of inoculation viz., seed inoculation, soil inoculation and root dip inoculation. Seed inoculation resulted in a higher disease incidence as compared to soil inoculation. In case of root dip treatment wilting symptoms developed quickly and it required much less labour and materials.

Patel (1987) proved the pathogenicity of *F. solani* causing wilt of okra by three different methods of inoculation viz., soil inoculation, seed cum-soil inoculation and sick soil inoculation and found that seed-cum-soil inoculation proved as a better method for proving the pathogenicity.

Kaewruang *et al.* (1987) reported the pathogenic nature of *F. solani* by blotter test method and in glasshouse test with pasteurized soil and found *F. solani* as highly pathogenic (100%) in blotter test method.

Paternotte (1987) proved the pathogenicity of *F.solani* causing wilt of *Capsicum annuum* L. by root dipping in spore suspension and adding the suspension to the soil around the stem base.

Pandav (2002) proved pathogenicity of *Fusarium solani* causing wilt of cowpea by four different method of inoculation *viz.*, soil inoculation, seed inoculation, seed-cum-soil inoculation and root cutting + soil inoculation and found that soil inoculation after root cutting method is quick and effective for cowpea wilt.

Garibaldi *et al.* (2004) inoculated 30 days old gerbera plants by dipping healthy roots in to conidial suspension ($5x10^7$ conidia/ml) of *F. oxysporum*. Wilt symptoms and vascular discoloration in the roots,crown and vein were developed within 30 days on inoculated plants and *F. oxysporum* was consistently reisolated from infected plants.

2.4 Taxonomy and identification of the pathogen

2.4.1 Morphological and cultural characters of *Fusarium Sp.*

Chattopadhyay and Basu (1957), reported *F. solani*, the causal agent of okra wilt produced oval shaped thick walled micro conidia with rounded ends or straight with pointed ends measuring 2.8-5.5 x 5.5-6.5 µm; macro conidia with 1-3 septa and measuring 10.9-36.3 x 3.3-6.5 µm while chlamydospores were round to spherical, intercalary as well as terminal, single or in chain and measuring 10.1-11.1 µm.

Booth (1977) have described the morphology of *Fusarium solani* (Mart.) Sacc. The fungus is known to form three types of spores i.e. micro conidia, macro conidia and chlamydospores. Microconidia are 8-16 x 2-4 µm in size, cylindrical to oval, sometimes one septate, produced from long lateral phialides, laterally borne on the branched conidiophores. Macroconidia are fusoid with widest point above the center, one to five septate and measuring 35-55 x 4.5-8.0 µm while chlamydospores are globose, smooth and rough walled, measuring 9-12 x 8-10 µm, borne singly or in chain or short lateral branches intercalary or terminal.

Singh and Khare (1977) reported that the fungus *F. solani* causing wilt of pointed gourd produced both micro and macro conidia as well as intercalary chlamydospores in culture. The micro conidia were hyaline, one celled; straight, measuring 4.5-12.0 x 2.6

µm and macro conidia were thick walled, curved and rounded at the tip, 1-6 septate measuring 15.85-17.13 x 3.5-5.5 µm while chlamydospores formed intercalary measuring 5.5-12 µm in diameter.

Sen and Palodhi (1979) reported that the fungus *F. solani* causing wilt of muskmelon produced micro conida and macro conidia and terminal or intercalary chlamydospores in culture. Micro conidia were usually aseptate, sometimes one septate and measured 7.5-12.5 µm × 3.5-5 µm. Macro conidia were slightly curved, in equilaterally fusoid wider in the upper half, thick walled, 1-3 septate and measured 32.5-35 µm × 4.2-5.5 µm, and the chlamydospores developed abundantly in old cultures. These were globose, rough walled, 7-9.5 µm and formed either terminally or on short lateral braches or were intercalary, sometimes in chains.

Gupta and Mathew (1999) reported that *F. solani* f. sp. *phaseoli* causing root rot of bean produced sparse to greyish white mycelium. Macro conidia were hyaline and fusiform with a pointed, slightly-beaked apical cell, measuring 44-55 x 5.1-5.3 µm and 3 to 5 septa depending upon culture medium used and incubation conditions. Microconidia were rare and developed from sparsely branched conidiophores. They were broad, oval and with one septation. Chlamydospores were measuring 6-16 µm in diameter and formed terminally or short lateral branched and also were

intercalary. They formed singly, in pairs or occasionally in short chains and round to subglobular to pear shaped.

According to Pandav (2002) F. solani, the causal agent of cowpea wilt produces micro conidia as hyaline, elliptical, unicellular, measuring 9.64–16.67 x 3.62–4.82 µm, macro conidia varied from fusiforme to nearly straight with rounded or pointed ends, hyaline, measuring 24.10–45.97 x 3.6–4.82 µm having 1–4 septa while chlamydospores were produced abundantly in aged culture which were spherical to globose, rough walled, light brownish, terminal and intercalary measuring from 9.64–12.05 µm in diameter.

2.5 The physiological investigations

2.5.1 Effect of different media on the growth and sporulation of *F. solani in vitro*

Papavizas (1967) recommended 18 media for isolation and enumeration of *Fusarium* spp. from soil. Out of them, peptone pentachloronitrobenzene (PCNB) and V-8 juice dextrose-yeast-extract agar (VDYA) was found the most useful in soil dilution plate method.

Thakur and Singh (1973) tried 6 different synthetic and common laboratory media for the growth of *F. solani* causing wilt in *Kochia trichophyla* Stapf. and found that Richards' medium provided better growth.

Booth (1977) suggested potato dextrose agar, potato sucrose agar and oat meal agar as good media for the growth of *F. solani* Bilay's medium modified by Joffe for sporulation and Armstrong Fusarium to increase inoculum potential of *Fusarium sp.*

Gaur and Agnihotri (1980) tested different media and observed maximum growth of *F. solani* on Richards' medium. Sporulation on potato-dextrose- agar in case of solid media was maximum while in case of liquid media it was maximum on sabouraud's medium.

Raghuwanshi (1995) carried out cultural studies of *F. oxysporum* f. sp. *sesami* with six different media viz, Asthana and Hawker's medium, Czapek Dox's agar medium, Potato Dextrose Agar, Richards' medium and Waksman's medium. Out of these, the fungus showed luxurient growth and maximum sporulation on Potato Dextrose Agar medium.

Tripathi *et al.* (1999) cultured 5 different *Fusarium* spp. including *F. solani* on 10 different media to study growth and development of fungi and found that modified Asthana and Hawker's medium provided fair growth and excellent sporulation.

Cho *et al.* (2001) utilized modified Nash and Snyder's medium for isolation and identification of *Fusarium solani* f. sp. *glycines* causing soybean wilt isolated from soil.

2.5.2 Effect of pH regimes on the growth and sporulation of *F. solani in vitro.*

Chi and Handson (1964) reported that, *F. solani* (wilt of red clover) could be grown well with maximum spore germination (96-97%) at pH 5.0-5.5.

Agarwal and Sarbhoy (1978) reported that, acidic pH favours the growth of all *Fusarium* spp., *F. oxysporum* and *F. solani* grew best at pH 4.5 and 6.0 while *F. graminearum* and *F. equiseti* at pH 3.5 and 5.5, respectively.

Ghosh and Singh (1982) reported that *F. oxysporum* grew well on PDA with acidic pH (4.4-5.6), with optimum temperature 28°C $\pm$ 2°C.

Monga and Grover (1991) recorded maximum growth and spore germination of *Fusarium solani*, the cause of root rot of cowpea at 6.0 – 7.0 pH.

Farkya *et al.* (1996) observed maximum growth and sporulation of *F. solani* at 5.5 pH.

The fungus could grow and sporulate under wide range of pH from 4.0-8.0. However, pH 6.5 and 7.0 proved optimum for growth and sporulation of the fungus *F. solani* (Chauhan, 1997).

The organism *F. solani* causing wilt of cowpea, grew and sporulated well at pH 6.5 (Pandav, 2002).

2.6 Effect of various 'N' sources on the growth and sporulation of *F. solani in vitro.*

Woltz and Engelhard (1973) recorded that nitrate nitrogen (NO_3N) together with $Ca(OH)_2$ decreased Fusarium wilt of chrysanthemum additatively.

Agarwal and Sarbhoy (1978) tested different inorganic and organic nitrogenous sources against four species of *Fusarium* viz, *F. oxysporum, F. solani, F. equiseti* and *F. graminearum in vitro.* Among inorganic nitrogen sources, ammonium salts suppressed the growth of *Fusaria*. Among organic nitrogen sources, DL-alanine, leucine and DL-valine suppressed the mycelial growth. In case of amines, urea significantly reduced the growth of *F. oxysporum* and *F. graminearum.*

Guerra and Anderson (1985) reported the increased lesion size of *F. solani* affected bean in iron and boron deficient soil.

Mohamed *et al.* (1987) observed least infection by *F. solani* when N was added in field and green house test for pelargonium.

Anggraeni and Suharti (1996) observed urea inhibiting the growth of *Fusarium spp.* in *in vitro* conditions.

Pandav (2002) tested different seven nitrogenous fertilizers, and among them ammonical nitrogenous fertilizers inhibited the growth and sporulation of *F. solani* isolated from cowpea.

2.7 Control measures

2.7.1 Varietal screening in polyethylene bags

There are no other previous reports for the varietal screening of gerbera cultivars against wilt disease.

2.7.2 Biological control

2.7.2.1 Effects of antagonists on growth of *Fusarium* sp.

Ushamalini *et al.* (1997 a) found inhibitory effect of the antagonist viz., *Trichoderma viride, T. harzianum, T. hamatum, T. koningii, T. pseudokoningii, Bacillus subtilis* and *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *tracheiphilum* causing cowpea wilt. Growth of the pathogen was minimum with *Bacillus subtilis* followed by *T. harzianum* and *Pseudomonas fluorescens.*

Sychew and Shaposhnik (1982) found inhibitory effects of *T. viride* to *Rhizoctonia solani, F. solani* and *F. oxysporum* in culture.

Locke *et al.* (1985) reported that, *T. viride* alone or in combination with *Aspergillus ochraceus* reduced wilt of chrysanthemum by least 50 per cent.

Alippi and Monaco (1994) observed *Bacillus subtilis, B. pumilus* and *B. licheniformis* inhibiting the growth of *Fusarium solani* and *Sclerotium rolfsii.*

Patel (1995) tested three promising fungal antagonists namely *T. harzianum, T. viride* and *A. niger* in pot culture trial as

soil amendment and found that *T. harzianum* + carbendazim seed treatment proved most effective in checking pigeon pea wilt caused by *F. solani*

Various antagonists like *Trichoderma viride*, *Trichoderma hamatum*, *Trichoderma koningii* and *Trichoderma piluliferum* showed the strongest antagonistic effect in limiting the growth of *Fusarium oxysporum* f.sp. *gerberae* (Kurzawinska and Klima, 1997).

Mathur and Gurjar (2002) observed *T. viride*, *T. harzianum* and two isolates of *F. solani* isolated from the rhizosphere of chilli which significantly inhibited the mycelial growth and sclerotial production by *R. solani* causing stem rot of chilli.

Gurjar *et al.* (2004) reported that *Trichoderma harzianum* and *T. viride* gave effective management of *Fusarium* sp. in okra.

Patibanda and Sen (2004) observed *Aspergillus niger* as a useful antagonist against *F. oxysporum* f.sp. *melonis* causing wilt in muskmelon.

Jha and Jalali (2006) reported that under *in vitro* conditions, isolate *T. viride* showed strongest antagonistic activity towards *F. solani* f.sp. *pisi* in dual culture followed by *A. niger*, *A. terreus*, *A. sydowi*, *A. flavus* and *Spicaria sylavatica*.

2.7.2.2 Evaluation of organic extracts on the growth and sporulation of *F. solani.*

Champawat and Pathak (1988) showed poultry manure and mustard cake giving good control of cumin wilt caused by *F. oxysporum* f. sp. *cumini.*

Chakrabarti and Sen (1991) observed that neem cake, mustard cake, saw dust and groundnut cake reduced the wilt incidence in muskmelon caused by *F. solani in vitro* up to 80, 65, 45, and 37 per cent respectively. Srivastava and Singh (1991) reported, neem cake effectively reduced the infection of *F. solani* and *Meloidogyne incognita* in *Phaseolus vulgaris.*

Srivastava and Singh (1991) reported that neem cake effectively reduced the damage done by *Fusarium solani* and *Meloidogyne incognita* on *Phaseolus vulgaris.*

Pandey *et al.* (1996) reported soil solarization in combination with neem exhibited maximum reduction in population of *F. oxysporum* f. sp. *ciceri* and completely eliminated the chickpea wilt.

Ushamalini *et al.* (1997 b) suggested soil application of neem cake @ 150 kg/ha for the control of cowpea wilt caused by *Fusarium oxysporum.*

Padmodaya and Reddy (1999) reported FYM and neem cake as the most effective against *F.oxysporum* f. sp. *lycopersici* causing seedling disease of tomato under green house condition.

Padmodaya (2003) reported that minimum of 0.1% oil cakes, 1.5% dry leaves and FYM and 3% green leaves were required to achieve maximum protection against *Fusarium* wilt of tomato.

Pandav (2002) reported, maximum inhibition of the growth of *F. solani* in neem cake (52.7%) followed by poultry manures (51.5%) and pressmud (49.4%).

2.7.2.3 Evaluation of botanicals against *F. solani* for their antifungal activity.

Patel (1995) tested 29 phytoextracts in *in vitro* condition for their biological efficacy against *F. solani*., among which garlic clove (*Allium sativum* L.) proved strongly inhibitory to the growth of *F. solani*.

Chauhan (1997) evaluated that garlic bulb have strong inhibitory effect against *F. solani* followed by neem, onion bulb, and turmeric rhizome where as the extract of lantana, kadvi mehndi and tulsi were found to promote growth of the pathogen.

Thiribhuvanamala and Narasimhan (1998) reported that leaf extracts of *Delonix regia*, *Pongamia pinnata* and *Acacia nilotica* inhibited the spore germination, mycelial growth and spore production of *Fusarium solani*, significantly.

Among seven leaf extracts, *Azadirachta indica* at 100% concentration proved highly toxic to *F. oxysporum* with complete inhibition of mycelial growth and spore germination in the *in vitro* conditions (Bansal and Gupta, 2000).

Pandav (2002) screened the phytoextracts of 13 plant species and evaluated *in vitro* against *F. solani* and revealed that significantly lower mycelial growth of pathogen was recorded in extract of Acalypha and was found superior over the rest.

Mamatha and Rai (2004) reported that, leaf extracts of *Lantana camara* followed by *Azadirachta indica*, *Acalypha indica* and *Bacopa monnieri* were found to be equally effective in inhibiting the growth of *F. solani* under *in vitro* conditions.

Patel and Vala (2004) observed maximum growth inhibition of *F. solani* causing wilt of okra by unsterilized garlic extract under *in vitro* conditions.

Singh and Chand (2004) reported that leaf extract of neem completely checked the spore germination of *F. oxysporum* f. sp. *ciceri* causing wilt of chickpea.

2.7.3 Chemical control *in vitro*

2.7.3.1 Bio-efficacy of fungicides against *F. solani*

Kulova and Petkova (1975) reported that, Thiophanate methyl (0.1%), Kriptanol (0.02%), Benomyl (0.1%) and Carbendazim (0.18%) gave good control of *F. oxysporum* var. *dianthi*, causing wilt disease of carnation and *F. avenaceum*, *F. solani* var. *redolens*, and *F. culmorum* causing root and stem rotting of carnation.

Agarwal and Sarbhoy (1976) screened 22 fungicides *in vitro* against four species of *Fusarium* causing seedling rot of

soybean and found that Ceresan, Agrosan GN, Agrosan 5W and Thiram-72 were completely effective in checking the pathogen at 5 ppm concentration and above.

Patel (1987) observed that Derosal, Bavistin, Agrozim and Dithane-M were proved highly fungitoxic to *F. solani* causing wilt of okra.

Singh and Saxena (1990) tested twelve fungicides *in vitro*. Out of this, seven viz., Aureofungin, Bavistin WP, Captan, Emisan-6, Hexaferb, Vitavax and Ziram were variably effective, while Blitox-50 w, Captafol, Dithane M-45; Streptocyclin, Sulfex-80 % WP were ineffective against the cauliflower wilt pathogen *F. solani*.

Kapoor and Kumar (1991) studied the efficacy of systemic and non-systemic fungicides against *F. solani* responsible for pre and post emergence damping off of tomato under *in vitro* condition and reported that carbendazim was most effective followed by benomyl.

Monga and Grover (1991) evaluated 16 fungitoxicants against *F. solani* causing cowpea root rot under *in vitro* condition and observed that Captafol was most effective to inhibit the spore germination and mycelium growth of *F. solani* followed by thiram, mancozeb and M.E.M.C. at less than 10 µg ml^{-1} concentration.

Mukherjee and Tripathi (2000) *in vitro* evaluated eight fungicides at 2.5, 5, 10, 25, 50 and 100 µg /ml concentrations

against *S. rolfsii, R. solani* and *F. oxysporum* f. sp. *phaseoli*. Out of these fungicides, Bavistin, Contaf, Tilt, Opus and Antracol were found most effective for inhibiting the growth of *F. oxysporum* f. sp. *phaseoli* even @ of 25 µg /ml.

Pandav (2002) tested ten fungicides *in vitro*. Out of these, he found M.E.M.C. was more effective for control of *Fusarium solani* causing wilt of cowpea.

2.7.3.2 Bio-efficacy of herbicides against *F. solani*

Kaufman (1964) observed that number of *Fusarium sp.* were lower in atrazine treated soil than in simazine treated soil.

Desai *et al.* (1985) evaluated the relative efficacy of five herbicides viz., 2-4-D, atrazine, ametryne, simazine, and alachlor at different concentrations against *F. moniliformae* under *in vitro* condition and reported 71.72 and 48.24 per cent growth inhibition at 4000 ppm of ametryne and atrazine respectively, while least inhibition (20.54 %) was noticed in case of 2-4-D.

Patel and Patel (1993) observed good control of *F. oxysporum* f. sp. *cumini*. the causal agent of cumin wilt with metribuzine at 1000, 2000 and 3000 ppm.

El-Shanshoury *et al.* (1996) recorded that pendimethalin and metribuzin inhibit the growth of *Pseudomonas solanacerum* and *F. oxysporum in vitro* causing bacterial and fungal wilt in tomato respectively.

Chauhan (1997) observed significant reduction in mycelial growth of *F. solani*, the isolate of bottle gourd, in 2,4-D at all concentrations tested followed by metribuzine and fluchloralin.

Sanogo *et al.* (2000) studied three herbicides (Lactofen, glyphosate and imazethapyr) against *F. solani* f. sp. *glycines* in the *in vitro* conditions, and reported that conidial germination, mycelial growth and sporulation were reduced by glyphosate and lactofen herbicides.

MATERIALS AND METHODS

III MATERIALS AND METHODS

3.1 Pathological investigations

3.1.1 Collection of samples and isolation of causal agent

Gerbera plants showing typical wilt symptoms were collected from farmer's poly house and green house and college garden, Aspee College of Horticulture and Forestry, N.A.U., Navsari, brought to the laboratory and subjected to microscopic examination and isolation.

The samples were subjected to tissue isolation by cutting the infected tissue of root in to small bits. The bits were surface sterilized with 1:1000(w/v) mercuric chloride ($HgCl_2$) solution for a minute followed by three subsequent washings of sterile distilled water and these bits were then aseptically transferred to sterile petriplates containing 20 ml Potato Dextrose Agar (PDA) medium (peeled potato 200g, dextrose 20g and agar agar 20g in 1000ml distilled water) and incubated at room temperature (out side temperature: minimum 17.97°C and maximum 33.25°C). The growth of the fungus developed after 48 hrs. incubation was subcultured to obtain pure culture which was further purified by single spore isolation technique and the isolate was maintained for further studies.

3.1.2 Pathogenicity test

The pathogenicity test of *Fusarium* sp. isolated from wilt

infected roots of gerbera plant was carried out in net house in pots using highly susceptible gerbera cv. local (Deshi) by three methods viz., Root dip inoculation, soil inoculation, root dip-cum-soil inoculation. The spore suspension of the fungus having 4.0 x 10^5 spores/ml was used as inoculum.

The healthy plants planted in sterilized soil in the pots. Such three replications were kept in each case with suitable uninoculated control. The pots were labeled, watered as and when required and left undisturbed in net house for development of the symptoms.

3.1.2.1 Soil inoculation

The healthy plants transplanted in pots containing sterilized soil were allowed to set for 30 days and after that 200 ml spore suspension (4.0 x 10^5 spores/ml) was applied in soil around each plant.

3.1.2.2 Root dip inoculation

The healthy 30 days old gerbera plants in pots were carefully uprooted and their roots were washed with sterilized distilled water. These plants were inoculated by dipping roots in to the spore suspension (4.0 x 10^5 spores/ml) for 24 hrs. and then were replanted in to pots containing sterilized soil.

3.1.2.3 Root dip-cum-soil inoculation

The healthy 30 days old gerbera plants in pots were

carefully uprooted and their roots were washed with sterilized distilled water. These plants were inoculated by dipping roots in to a spore suspension (4.0 x 10^5 spores/ml) for 24 hrs. and were replanted in the pots and 200 ml spore suspension was poured on around the plants in each pot.

3.2 Identification of the pathogen

Identification of the pathogen was done with the help of morphological and cultural character studies and compared with the literature. The pure culture of the fungus was also sent to Indian Type Culture Collection (I.T.C.C.), Division of Mycology and Plant Pathology, I.A.R.I., New Delhi-110 012 for identification.

3.2.1 Studies on morphological characters of the pathogen

Culture of *Fusarium solani* was incubated for ten days at room temperature (out side temperature: minimum 27.84°C and maximum 33.93°C). The fungal growth was stained and observed under the microscope (450x magnifications). Observations on size and shape of micro conidia, macro conidia and chlamydospores were recorded.

3.3 Physiological studies

3.3.1 Effect of different media on the growth and sporulation of *F. solani* in the *in vitro* condition

For finding out the suitable media for better growth and sporulation of *F. solani* under study, the following seven different

media of semi-synthetic and synthetic types in solid and liquid state were tested.

(A) Semi-synthetic media

(1) Potato Dextrose Agar (PDA)

Peeled potato	: 250.0 g
Dextrose ($C_6H_{12}O_6$)	: 20.0 g
Agar agar	: 20.0 g
Distilled water	: 1000.0 ml

(2) Potato Carrot Sucrose Agar (PCSA)

Peeled potato	: 100.0 g
Carrot	: 100.0 g
Sucrose ($C_6H_{22}O_{11}$)	: 20.0 g
Agar agar	: 15.0 g
Distilled water	: 1000.0 ml

(3) Wheat Meal Agar (WMA)

Wheat (flour)	: 50.0 g
Agar agar	: 20.0 g
Distilled water	: 1000.0 ml

(B) Synthetic media

(1) Czapek's (Dox) Agar (CzDA)

Sucrose ($C_6H_{22}O_{11}$)	: 30.0 g
Sodium nitrate ($NaNO_3$)	: 2.0 g
Dipotassium hydrogen orthophosphate (K_2HPO_4)	: 1.0 g

Magnesium sulphate ($MgSO_4$-$7H_2O$)	: 0.50 g
Potassium chloride (KCl)	: 0.50 g
Ferrous sulphate ($FeSO_4$-$7H_2O$)	: 0.01 g
Agar agar	: 20.0 g
Distilled water	: 1000.0 ml

(2) Richards' Agar (RA)

Potassium nitrate (KNO_3)	: 10.0 g
Potassium dihydrogen orthophosphate (KH_2PO_4)	: 5.0 g
Magnesium sulphate ($MgSO_4$-$7H_2O$)	: 2.5 g
Ferric chloride ($FeCl_3$-$6H_2O$)	: 0.02 g
Sucrose ($C_6H_{22}O_{11}$)	: 50.0 g
Agar agar	: 20.0 g
Distilled water	: 1000.0 ml

(3) Asthana and Hawker's Agar (A & HA)

Glucose ($C_6H_{12}O_6$)	: 5.0 g
Potassium nitrate (KNO_3)	: 3.5 g
Potassium dihydrogen orthophosphate (KH_2PO_4)	: 1.75 g
Magnesium sulphate ($MgSO_4$-$7H_2O$)	: 0.75 g
Agar agar	: 20.0 g
Distilled water	: 1000.0 ml

(4) Elliot's Agar (EA)

Sodium carbonate (Na_2CO_3)	: 1.05 g
Magnesium sulphate ($MgSO_4$-$7H_2O$)	: 0.6 g

Asparagin	: 3.0 g
Dextrose ($C_6H_{12}O_6$)	: 3.0 g
Potassium dihydrogen orthophosphate (KH_2PO_4)	: 1.36 g
Agar agar	: 20.0 g
Distilled water	: 1000.0 ml

3.3.1.1 Solid media

Twenty ml of the agar agar based sterilized medium was poured aseptically in each sterilized petriplate (9.0 cm diameter). Each medium was adjusted to 7.0 pH and were inoculated by placing a 5 mm mycelial disc at the centre of petriplate. Ten days old pure culture of the fungus maintained on PDA and incubated at room temperature (out side temperature: minimum 17.97°C and maximum 33.25°C) was used for inoculation. Each treatment was replicated four times. Linear growth of the fungal colony was measured at 24 hrs interval and the colony cultural characters were also recorded.

3.3.1.2 Liquid media

The compositions of the liquid media were kept similar as in solid media except addition of agar agar. Each medium was adjusted to 7.0 pH by addition of 0.1 N NaOH or 0.1 N HCl and using Backman pH meter. Twenty ml of the liquid medium was poured in each 150-ml conical flask and plugged with non-absorbent cotton. These flasks were subjected for sterilization at 1.2kg cm^{-2} pressure for 20 minutes in the autoclave. A pure culture block of 5

mm diameter was cut with the help of a cork borer from 10 days old culture of *F. solani* and was placed aseptically in the medium, replicating four times. Flasks were incubated at room temperature (out side temperature: minimum 17.97°C and maximum 33.25°C). Out of these flasks, three were used for recording growth and fourth one for sporulation. After 15 days, the mycelial mat was harvested on previously weighed and oven dried Whatman's filter paper no. 42 and final pH of the medium was also recorded. The filter paper with mycelial mats were dried in oven for 3 days at 60°C temperature untill the constant weights obtained and the dry weight of the mycelium was recorded by deducting the weight of filter paper.

The spore count was recorded from fourth replication. At the end of incubation period, the whole of the fungal growth was transfered to beaker containing 50 ml sterile distilled water, stirred thoroughly, filtered through muslin cloth, and a drop of it was examined under microscope. The numbers of conidia per low power microfield (10x) were recorded at random from three microfields and were averaged. The data were subjected to statistical analysis.

3.3.2 Effect of different pH regimes on growth and sporulation of *F. solani in vitro*

To study the effect of pH, the fungus was grown in sets of eight different pH adjusted Richards' medium, ranging from 4.0 to 8.0. The pH was maintained by addition of 0.1 N NaOH or 0.1 N HCl with the help of a Backman pH meter.

Twenty ml of liquid Richards' medium was poured in to 150 ml conical flasks. Each treatment was replicated four times. After sterilizing at 1.2kg cm^{-2} pressure for 20 minutes in the autoclave, these flasks were inoculated with 5 mm diameter disc of mycelial mat obtained from margin of 10 days old actively growing cultures with the help of a sterile 5 mm cork borer under aseptic condition. Inoculated flasks were incubated at room temperature (out side temperature: minimum 14.42°C and maximum 30.60°C) for 15 days. Mycelial mats were collected from three replications in each case on previously weighed Whatman's filter paper no. 42 and dried in oven at 60°C for 3 consecutive days untill the constant weight was received.

The sporulation was recorded from fourth replication at the end of incubation period as described earlier. The data were subjected to statistically analysis.

3.4 Effect of nitrogenous fertilizers on the growth and sporulation of *F. solani in vitro*

Twenty ml of liquid Czapek's (Dox) medium was poured in to 150 ml conical flasks. Sodium nitrate in the basal medium was replaced by nitrogen sources viz., urea (46 % N), potassium nitrate (13 % N), sodium nitrate (16% N), calcium nitrate (15% N), ammonium nitrate (33.5% N), ammonium sulphate (20.6% N) and ammonium chloride (25 % N). Nitrogen sources were added singly to furnish 240 mg of nitrogen per litre of basal medium. Medium

without nitrogen source served as control. Each treatment was replicated four times. Then pH of the medium was adjusted to 5.5 by adding 0.1 N CH_3COOH or 0.1 N NaOH with the help of Backman pH meter. After sterilizing at 1.2kg cm^{-2} pressure for 20 minutes in the autoclave, these flasks were inoculated aseptically with 5 mm diameter disc of mycelial mat obtained from the margin of 10 days old actively growing culture with the help of a sterile cork borer. Inoculated flasks were incubated at room temperature (out side temperature: minimum 16.85°C and maximum 36.63°C) for 15 days. Mycelial mats were collected from three replications in each case after 15 days on previously weighed Whatman's filter paper no. 42 and dried in an oven at 60°C for 3 consecutive days untill the constant weight were received. The average dry weights of the mats were statistically analysed.

Sporulation of the fungus was recorded from fourth replication as described earlier. The data were subjected to statistical analysis.

The data on the growth and sporulation of *F. solani* on different nitrogen sources were classified as described by Agarwal and Sarbhoy (1978).

Grading	Growth (Dry mycelium weight in mg)	Sporulation [conidia per high power micro field (45x)]
E = Excellent	>150	>150
G = Good	100-150	100-150
M = Moderate	50-100	50-100
P = Poor	0-50	0-50

3.5 Control measures

3.5.1 Varietal screening in polyethylene bags

Seven gerbera varieties viz., Double dutch, Goliath, Primrose, Avant grade, Zingaro, Savannah and Local (Deshi) were screened for their resistance against *F. solani* (Mart.) Sacc. in polyethylene bags. The healthy plants were planted in a polyethylene bags containing autoclaved soil and were labeled for each variety. Ten plants were used for each variety with suitable uninoculated control. The plants in the polyethylene bags were watered as and when required and left undisturbed in net house for development. After 30 days, 200 ml spore suspension (4.0×10^5 spores/ml) was poured around the plants and left undisturbed in net house for development of the symptoms.

The observations on wilt incidence were recorded. The wilt incidence was calculated by following formula as suggested by Mandhare and Patil (1993).

Grading	Growth (Dry mycelium weight in mg)	Sporulation [conidia per high power micro field (45x)]
E = Excellent	>150	>150
G = Good	100-150	100-150
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The observations on wilt incidence were recorded. The wilt incidence was calculated by following formula as suggested by Mandhare and Patil (1993).

$$\% \text{ wilt incidence} = \frac{\text{No. of infected (diseased) plants}}{\text{Total no. of plants}} \times 100$$

These varieties were grouped under different degrees of resistance on the basis of disease grades suggested by Mandhare and Patil (1993) for the screening of brinjal wilt caused by *F. oxysporum* f. sp. *melongenae* under artificial as well as field conditions and on this basis, the grading of varietal reaction were made as under.

Grading

Per cent wilt	Reaction
0 to 24	Resistant (R)
25 to 49	Moderately Resistant (MR)
50 to 74	Moderately Susceptible (MS)
75 and above	Susceptible (S)

3.5.2 *in vitro* studies on Biological control

3.5.2.1 Testing efficacy of antagonists against *F. solani in vitro*

Nine known antagonist's *viz.*, *Trichoderma viride, T. harzianum, T. longibrachyatum, Gliocladium virens, Chaetomium globosum, Aspergillus niger, A. flavus, Bacillus subtilis* and *Pseudomonas fluorescens* were tested *in vitro* against *F. solani*. Different methods employed in the study are as under:

3.5.2.1.1 Dual culture technique (Dennis and Webster, 1971)

The test organism and the pathogen were grown on PDA and from 10 days old culture, a 5 mm disc of the test organism (antagonist) was cut aseptically from the periphery of the colony and placed at one end of the petriplate containing 20 ml PDA. In the opposite place and approximately 70 mm away from the first, a similar disc of the pathogen was aseptically placed. Three repetitions of each were kept and the plates with only pathogen served as control. The plates were incubated at room temperature (out side temperature: minimum 23.92°C and maximum 34.36°C) and the radial growth of the test organism and pathogen was measured after 10 days and the per cent inhibition was calculated.

3.5.2.1.2 Pathogen at periphery (Asalmol *et al.*, 1990)

The test organisms and pathogen were grown on PDA and from 6 days old culture, 5 mm disc of test organism were cut and placed aseptically in the centre of the plates containing 20 ml PDA. Four similar disc of the pathogen were cut and placed at periphery *i.e.* 35 mm away from test organism, in the same plate. Three repetitions of each were kept. The plates with only culture disc of pathogen placed at periphery served as control. The plates were incubated at room temperature (out side temperature: minimum 23.92°C and maximum 34.36°C) and the radial growth of the test organism and pathogen was measured after 10 days and the per cent inhibition was calculated as per the following formula suggested by Asalmol *et al.* (1990).

$$\% \text{ Growth Inhibition} = \frac{C-T}{C} \times 100$$

Where, C = Growth of pathogen in control after incubation

T = Growth of pathogen in treatment after incubation

3.5.2.1.3 Pathogen at centre (Asalmol *et al.*, 1990)

In this method, 5 mm culture disc of pathogen was cut from the periphery of the colony and placed aseptically in the centre of the plates containing 20 ml PDA. Four similar discs of the test organism were placed at periphery *i.e.* 35 mm away from the pathogen in the same plate. Three repetitions of each were kept and the plates with only pathogen at centre served as control. The plates were incubated at room temperature (out side temperature: minimum 23.92°C and maximum 34.36°C) and the radial growth of the test organism and pathogen was measured after 10 days and the per cent inhibition was calculated as mentioned earlier.

3.5.2.2 Testing of organic extracts against *F. solani in vitro*

The aqueous extracts of different organic materials *viz.*, FYM, pressmud, poultry manure, castor cake, mustard cake and neem cake were prepared by suspending 30 g of each organic material in 150 ml sterile distilled water in flask and left for 25 days. The flasks were shaken on alternate day for thorough mixing and dissolution of the content. After 25 days, the flasks were

thoroughly shaken and content were filtered through double layered muslin cloth and autoclaved at 1.2 kg cm^{-2} pressure for 20 minutes. The sterile extracts were used for testing their inhibitory effect on *F. solani in vitro* by poisoned food technique (Grover and Moore, 1962). The autoclaved extracts were individually added in previously sterilized melted and cooled potato dextrose agar medium @ 10 per cent (v/v) at the time of pouring in petriplates and mixed thoroughly. All the plates were incubated at room temperature (out side temperature: minimum 19.91°C and maximum 33.72°C) after placing the 5 mm disc of actively growing 10 days old pure culture of *F. solani*. Four repetitions were kept for each treatment. Medium without organic extract served as control.

The observations on colony diameter were recorded periodically and per cent growth inhibition was calculated as mentioned earlier.

3.5.2.3 Bio-efficacy of botanicals against *F. solani in vitro*

The effect of plant extracts of various plant species *viz*., onion, neem, nilgiri, garlic, ginger, tulsi and turmeric were tested *in vitro* by poisoned food technique to know their inhibitory effect on the growth of *F. solani*.

Healthy fresh leaves, bulbs and rhizomes were taken, washed thoroughly with fresh water and finally rinsed with sterile distilled water. Fifty grams of either leaves or bulbs or rhizomes were cut in to small pieces and mixed in a grinder by adding 50 ml

distilled sterile water. Extracts thus obtained were filtered through double layered muslin cloth in 150 ml flasks and plugged. The extracts were then autoclaved at 1.2 kg cm^{-2} pressure for 20 minutes.

Potato Dextrose Agar (PDA) medium was prepared and 100 ml was taken in 150 ml conical flasks, plugged and sterilized at 1.2 kg cm^{-2} for 20 minutes. The autoclaved extracts were individually added in melted, cooled and sterilized PDA @ 10 per cent (v/v) at the time of pouring in the petriplates and mixed thoroughly. All the plates containing phyto-extracts were incubated at room temperature (out side temperature: minimum 19.91°C and maximum 33.72°C) after placing the 5 mm disc of actively growing 10 days old pure culture of *F. solani*. Four repetitions were made for each treatment. Medium without phyto-extract served as control.

The observations on colony diameter were recorded and statistically analysed and per cent growth inhibition was also worked out as mentioned earlier.

3.5.3 Chemical control

3.5.3.1 Bio-efficacy of fungicides against *F. solani in vitro*

The poisoned food technique was employed to test the *in vitro* efficacy of ten fungicides with their three different concentrations viz., 250, 500 and 1000 ppm (Table-3.1) against *F. solani*.

Table-3.1: Fungicides tested against *F. solani in vitro*

Sr. No.	Trade name	Technical name	Concentration (ppm)		
1.	Emisan 6% W.P.	M.E.M.C.	250	500	1000
2.	Dithane M-45 75% W.P.	Mancozeb	250	500	1000
3.	Sheathmar 3% L	Validamycin	250	500	1000
4.	Tilt 25% EC	Propiconazole	250	500	1000
5.	Kasu-B 3% SL	Kasugamycin	250	500	1000
6.	Bavistin 50% W.P.	Carbendazim	250	500	1000
7.	Beam 75% W.P.	Tricyclazole	250	500	1000
8.	Topsin-M 70% W.P.	Thiophanate-methyl	250	500	1000
9.	Contaf 5 EC	Hexaconazole	250	500	1000
10.	Blitox 50% W.P.	Copper oxychloride	250	500	1000

The desired quantity of test fungicides was diluted with autoclaved lukewarm PDA medium in conical flask. The flask containing fungicidal medium was shaken well to facilitate uniform mixture and 20 ml was distributed to each sterile petriplate. The inoculum mycelial disc of 5 mm diameter was cut with the help of sterile cork borer from 10 days old pure culture and placed at the centre on petriplate containing solidified fungicidal medium. Three repetitions of each treatment were kept. The medium without fungicide served as control. The inoculated plates were incubated at room temperature (out side temperature: minimum 27.84°C and maximum 33.93°C). The colony diameter of the fungus was recorded from three replications periodically. The per cent growth inhibition over control was calculated by using formulae as mentioned earlier.

3.5.3.2 Bio-efficacy of herbicides against *F. solani in vitro*

Six herbicides with three different concentrations (Table-3.2) were evaluated for their efficacy against *F. solani* by poisoned food technique *in vitro*.

Required quantity of each herbicidal concentration (ppm) was added aseptically at the time of pouring the medium (PDA).

The desired quantity of test herbicides was diluted with autoclaved PDA medium in conical flask. The flask containing herbicidal medium was shaken well to facilitate uniform mixture and 20 ml was distributed to each sterile petriplate. The inoculum

Table-3.2: Herbicides tested against *F. solani in vitro*

Sr. No.	Trade name	Common name	Concentration (ppm)		
1.	Atrataf 50% W.P.	Atrazine	1000	2000	3000
2.	Basalin 45% EC	Fluchloralin	1000	2000	3000
3.	Fernoxon 80% W.S.P.	2,4-D	1000	2000	3000
4.	Glycel 41% S.L.	Glyphosate	1000	2000	3000
5.	Stomp 30% EC	Pendimethalin	1000	2000	3000
6.	Gold 23.5% EC	Oxyflourfen	1000	2000	3000

disc of 5 mm diameter was cut with the help of sterile cork borer from 10 days old pure culture and placed at the centre on petriplate containing solidified herbicidal medium. Three repetitions of each treatment were kept. The medium without herbicide served as control. The inoculated plates were incubated at room temperature (out side temperature: minimum 27.84°C and maximum 33.93°C). The colony diameter of the fungus was recorded periodically. The per cent growth inhibition over control was calculated by using formula as mentioned earlier.

RESULTS AND DISCUSSION

IV RESULTS AND DISCUSSION

Gerbera (*Gerbera jamesonni* L.) is one of the most economically important flower crops grown in India. Plant diseases are major constraint in economic crop production as they inflict heavy losses. Wilt is one of the major threat in gerbera cultivation and has been reported to be incited by *Fusarium* sp. *viz.*, *F. solani* and *F. oxysporum*.

In present investigation, *F. solani* was found constantly associated with gerbera causing wilt disease under south Gujarat agroclimatic conditions The scientific information on this disease is presently lacking. Hence, the investigation on this problem was under taken for generating more scientific information and suitable management strategies to prevent crop losses.

4.1 Pathological investigation

4.1.1 Collection of specimen and isolation of pathogen

The samples of infected gerbera plants cv. local (Deshi) showing wilting symptoms were collected from College garden and green house, N.A.U., Navsari, were subjected to tissue isolation that yielded pure culture of *Fusarium* sp.; which was further purified by single spore isolation technique and maintained on potato dextrose agar (PDA) slants for further investigation.

4.1.2 Symptomatology

Under natural and green house conditions the symptoms were observed during the visit and symptoms on sample collected were as under.

The symptoms were recorded from cv. local (Deshi) grown in the college garden and green house. Most of the plants were found infected and showing varying degree of infection indicating their susceptibility to the pathogen at almost all stages of crop growth. Leaves became yellow with initially brown and eventually black streaks in the vascular system. Plants showing severe stunting and initiation of wilting symptoms and at later stage found to be completely wilted. (Plate-I)

On critical examination, the fungus growth was observed on the roots of the wilting plants. Microscopic examination revealed the presence of micro and macro conidia of *Fusarium* sp. with dirty white mycelium.

Garibaldi *et al.* (2004) reported that yellowing of leaves with initially brown and eventually black streaks in the vascular system and stunting of the gerbera plants affected with *F. oxysporum*. The present results were in confirmation with earlier workers.

4.1.3 Pathogenicity test

The pathogenicity test of the isolate was carried out by three different methods *viz.*, soil inoculation, root dip inoculation and root dip cum soil inoculation. The results presented in Table-

Plate-I : Wilted plant of Gerbera Var. Local (Deshi) naturally infected with *F. solani*

4.1 Plate-II showed that the disease was produced successfully in all the methods of inoculation.

On the standing plants, wilt symptoms were initiated earlier (18.00 DAI) in root dip cum soil inoculation method. In other methods, the symptom development was initiated comparatively later. In soil inoculation and root dip inoculation methods, the first wilt symptom was observed after 38.33 and 22.67 days of inoculation, respectively. Cent per cent plants were showing typical wilt symptoms in soil inoculation, root dip and root dip cum soil inoculation methods.

With the above results root dip cum soil inoculation method was found to be the quickest and most effective method for proving the pathogenicity of gerbera wilt caused by *F. solani* followed by root dip and soil inoculation method.

Various workers have also proved the pathogenicity of *Fusarium* sp. causing wilt in different crops by these methods. Wellman (1939) proved the pathogenic nature of *F. bulbigenum* var. *lycopersici* (Brushi) by dipping the roots of tomato seedling in inoculum made from *F. bulbigenum* culture. Radhakrishnan and Sen (1985) proved the pathogenicity of *F. solani* (Mart.) Sacc. causing wilt of muskmelon by three different methods of inoculation *viz*., seed inoculation, soil inoculation and root dip inoculation. Seed inoculation resulted in a higher disease incidence as compared to soil inoculation. In case of root dip treatment wilting symptoms developed quickly and it required

Table 4.1: Pathogenicity of *Fusarium* sp. on gerbera cv. Local (Deshi) in pots by different methods

Sr. No.	Inoculation method	Total no. of plants inoculated	Wilting symptoms produced days after inoculation (DAI)	Total no. of wilted plants	Wilt (%)	Disease phenology
1.	Soil inoculation	3	38.33	3	100	Yellowing of lower leaves, complete wilting of the plant at later stage, vascular discoloration
2.	Root dip inoculation	3	22.67	3	100	Leaves became yellow with initially brown, stunting, vascular discoloration, plant wilted later
3.	Root dip cum Soil inoculation	3	18.00	3	100	Leaves became yellow with initially brown, stunting, vascular discoloration, plant wilted later
4.	Control	3	Not wilted	0	0.00	-

A. Soil inoculation

B. Root dip inoculation

C. Root dip cum soil inoculation

C = Control I = Inoculated

Plate-II : Wilting symptoms proudced under different methods of inoculation in pathogenicity test of *Fusarium* sp.

much less labour and materials. Patel (1987) proved the pathogenicity of *F. solani* causing wilt of okra by 3 different methods of inoculation *viz.*, soil inoculation, seed-cum-soil inoculation and sick soil inoculation. Seed-cum-soil inoculation proved as a better method for proving the pathogenicity. Paternotte (1987) proved pathogenicity of *F. solani* causing wilt of *Capsicum annuum* by root dipping in spore suspension and adding the suspension to the soil around the stem base. Pandav (2002) proved pathogenicity of *F. solani* causing wilt of cowpea by four different methods of inoculation *viz.*, soil inoculation, seed inoculation, seed-cum-soil inoculation and root cutting + soil inoculation and found that soil inoculation after root cutting method was quick and effective for cowpea wilt. The present results are in conformity with these findings.

4.2 Taxonomy and identification of pathogen

The isolates of *Fusarium* sp. obtained by tissue isolation from the infected plants of gerbera were purified by single spore technique. The morphological and cultural characters of the pure culture grown on PDA were studied and compared with those mentioned in literature. The pure culture was also sent to Indian Type Culture Collection (I.T.C.C.), Division of Mycology and Plant Pathology, I.A.R.I., New Delhi-110 012 and was identified as *Fusarium solani* (I.T.C.C. No.- 6148.05). The fungus also produced wilt symptoms under pathogenicity test. Thus, *Fusarium* sp. under study was identified and confirmed as *Fusarium solani*.

4.2.1 Studies on morphological characters of pathogen

In all cases, mycelium was dirty white and with hyaline conidia [Plate-III (a)]. Macro conidia were thick-walled, straight with rounded ends, having 1-3 septa and measured 26.24-39.74 x 3.16-5.00 µm, micro conidia were oval and measured 9.24-15.28 x 2.96-4.92 µm [Plate-III (b)] and chlamydospores were terminal and intercalary, single, round and measured 8.97-12.93 µm in size [Plate-III (c)].

4.2.2 Studies on cultural characters of pathogen

Colony grew fairly rapid on PDA medium and covered the whole petriplate within 8 days at room temperature (out side temperature: minimum 27.84°C and maximum 33.93°C). The mycelium was profuse hyaline when young and later turned dirty white (Plate-IV).

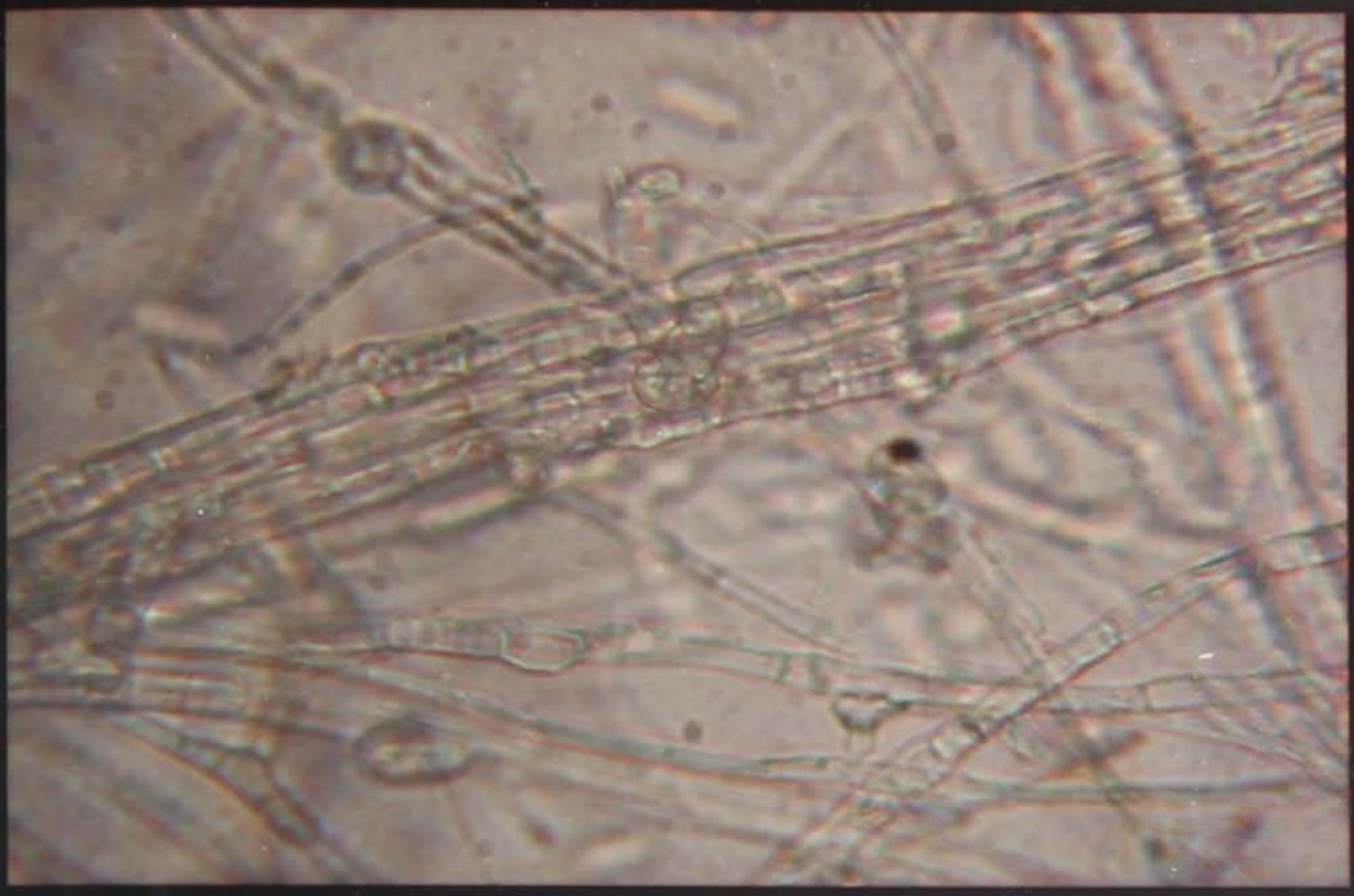
These studies on the morphological and cultural characters of isolated *Fusarium* sp. showed its close identity with *Fusarium solani* described by Chattopadhyay and Basu (1957), Booth (1977), Singh and Khare (1977), Sen and Palodhi (1979) and Gupta and Mathew (1999).

4.3 Physiological studies

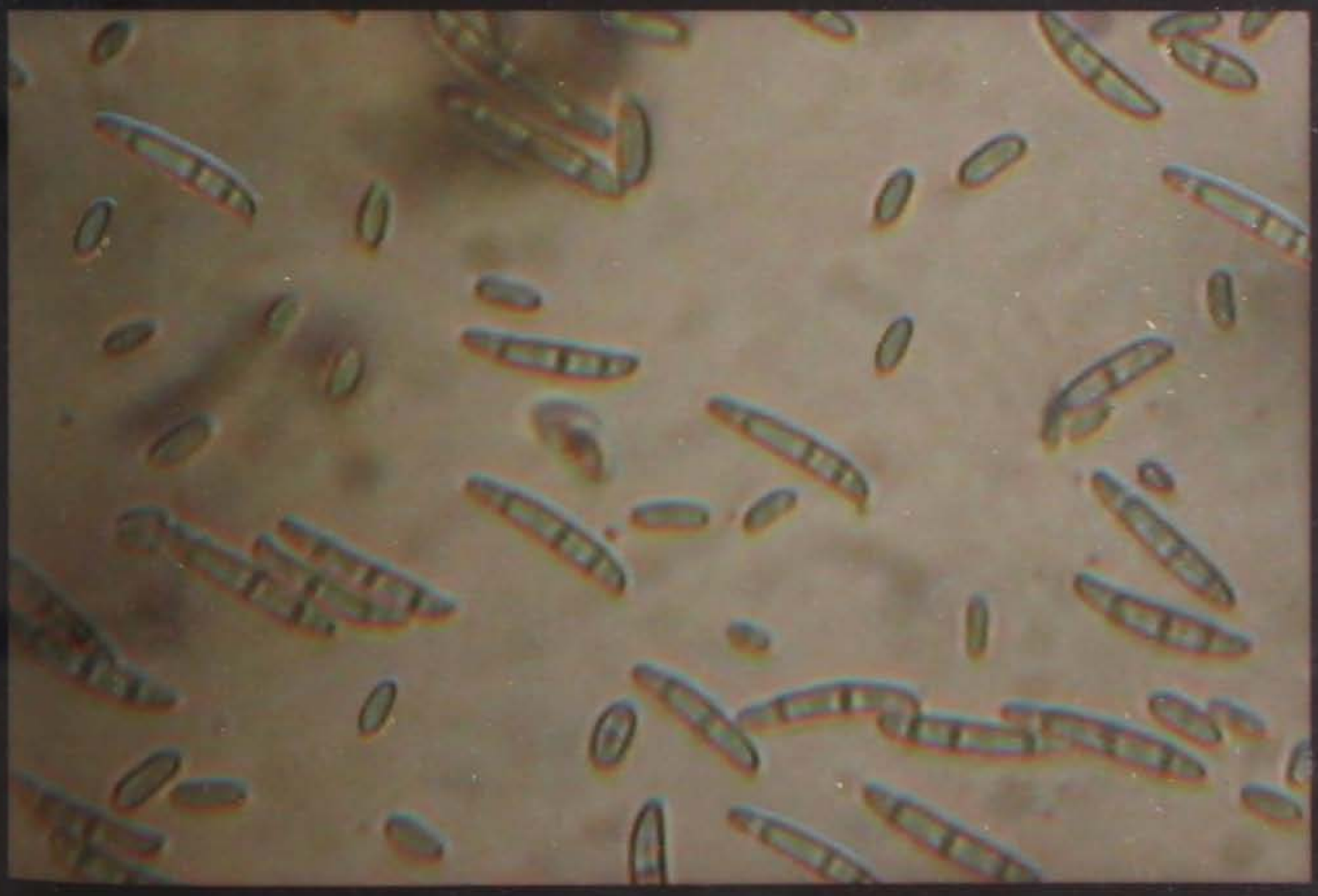
4.3.1 Effect of different media on the growth and sporulation of *F. solani in vitro*

Seven different media including synthetic and semi-synthetic in solid and liquid states were tested for their suitability

Plate-III : Microphotograph of *F. solani* (A, B and C)

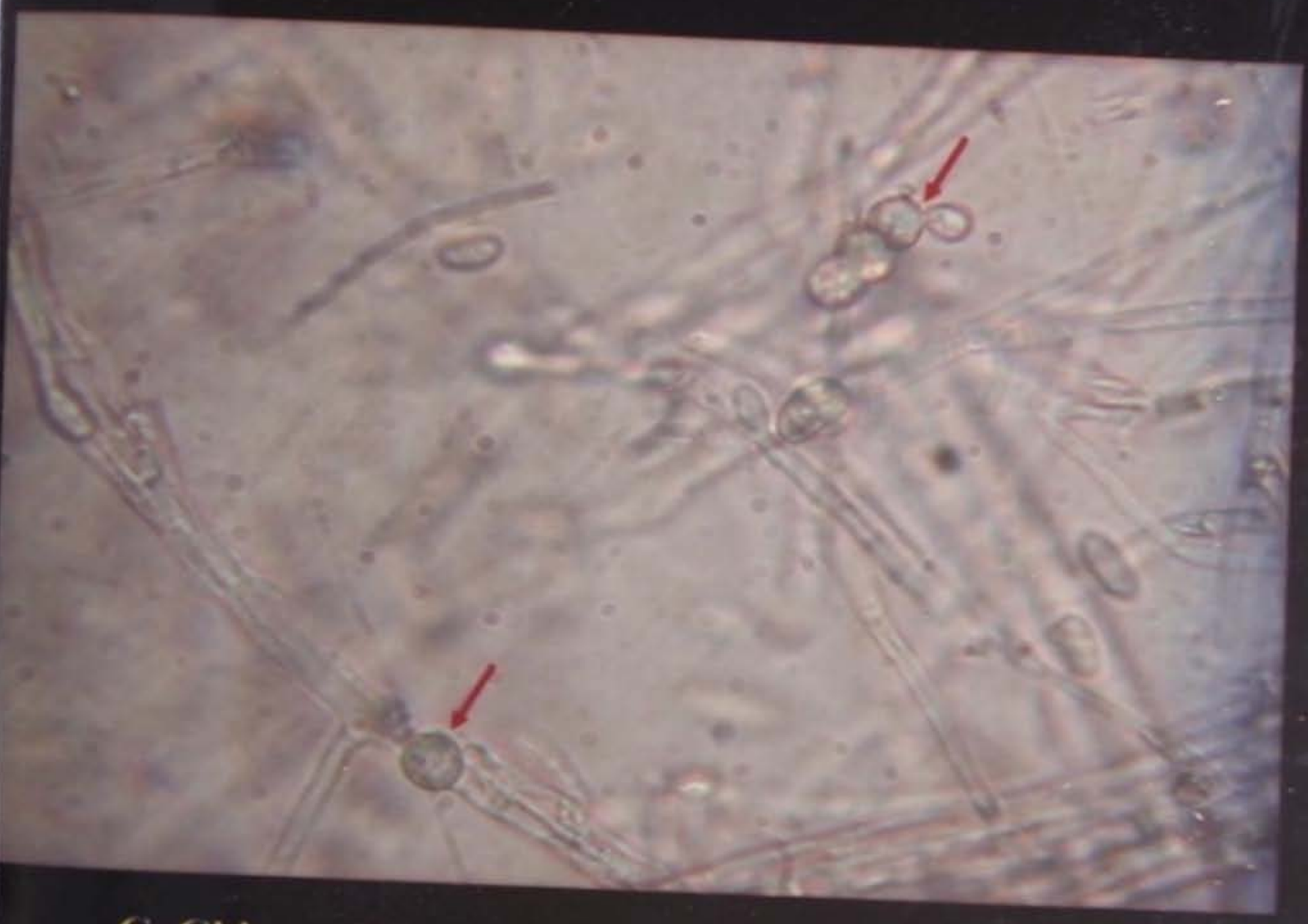


A. Fungal mycelium



B. Micro and Macro conidia

Contd... Plate-III



C. Chlamydospores of *F. solani* (Marked by arrow)

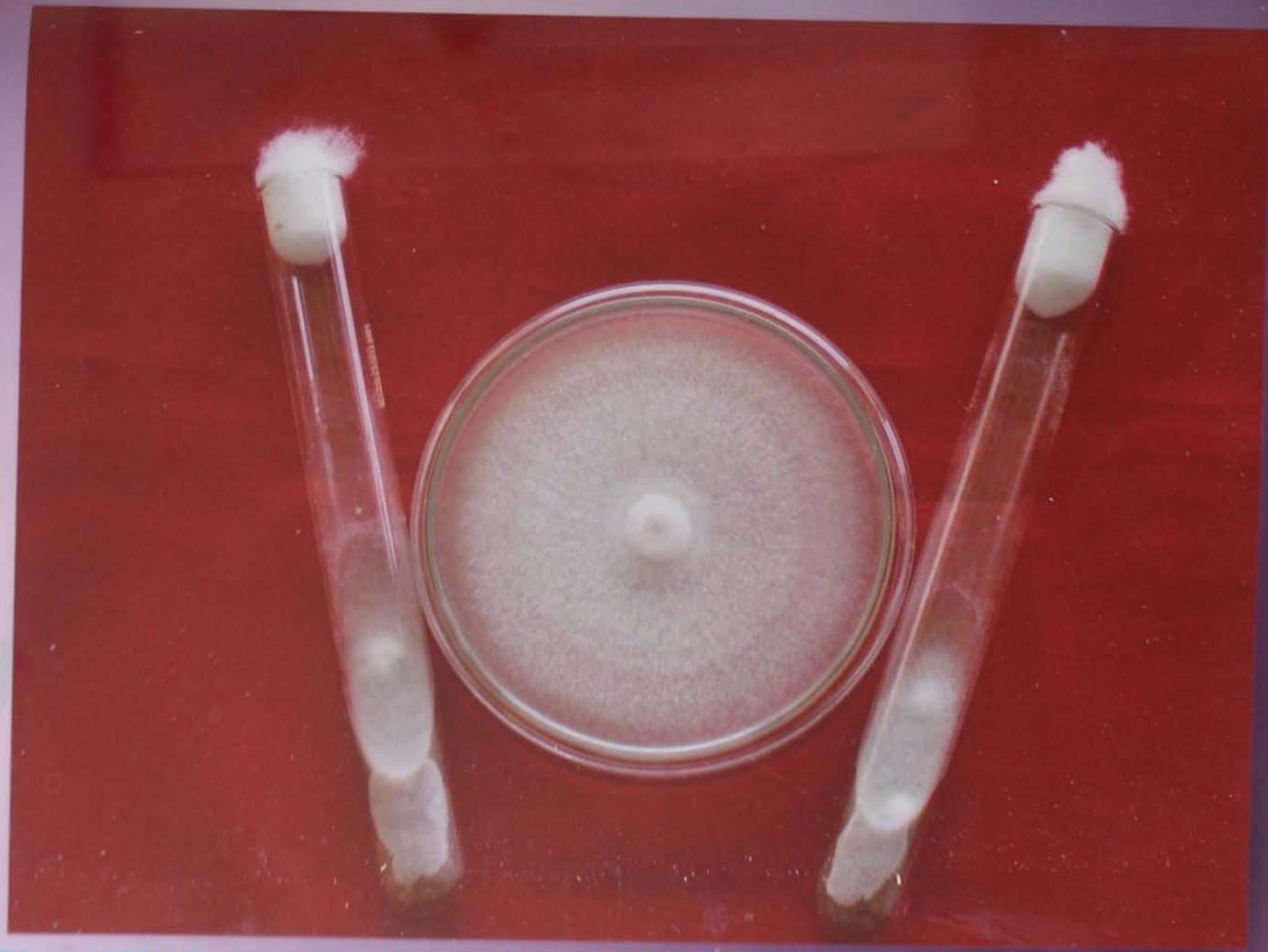


Plate-IV : Pure culture of *F. solani* isolated from wilted gerbera plant

on the growth and sporulation of the fungus, *F. solani*. The results on the growth and sporulation are presented in Table-4.2 (Fig.-1&2). The colony/cultural characters of the fungus recorded in different solid media are presented in Table-4.3 and Plate-V.

The results revealed that among all solid media tested, maximum mycelial growth was obtained in Richards' agar medium (87.50 mm) which was statistically at par with potato dextrose agar (86.17 mm). The next best in order of merit was Czapek's Dox agar medium (84.67 mm) followed by wheat meal agar (77.83 mm), potato carrot sucrose agar (67.33 mm) media while, Asthana and Hawkers agar (57.00 mm) and Elliots agar (47.83 mm) media had least mycelial growth.

In the liquid media, maximum dry mycelial weight was obtained in Richards' medium (530.47 mg) which was statistically at par with potato dextrose medium (491.35 mg), Czapek's medium (466.26 mg) and wheat meal medium (443.58 mg) while, potato carrot sucrose (351.92 mg), Asthana and Hawkers (203.23) and Elliot's (149.95 mg) media had least dry mycelial weight.

Significantly more sporulation was recorded in Richard's [420.77 spores/low power microfield (LPM)] which was statistically at par with potato dextrose (392.33 spores/LPM) and Czapek's (Dox) (354.33 spores/LPM). Next best in order of merit was wheat meal (298.66 spores/LPM) which was statistically at par with potato carrot sucrose (258.89 spores/LPM) followed by Asthana and Hawker's (144.55 spores/LPM) and Elliot's (96.55

Table 4.2: Effect of different solid and liquid media on growth and sporulation of *F. solani in vitro*

Sr. No.	Media	Solid media	Liquid media	
		Av. colony dia. of pathogen (mm)	Av. dry mycelial wt. (mg)	Av. no. of conidia /low power microfield (10x)
1.	Potato dextrose	86.17	2.69* (491.35)**	2.59* (392.33)**
2.	Richards'	87.50	2.72 (530.47)	2.62 (420.77)
3.	Wheat meal	77.83	2.65 (443.58)	2.47 (298.66)
4.	Czapek's (Dox)	84.67	2.67 (466.26)	2.55 (354.33)
5.	Potato carrot sucrose	67.33	2.54 (351.92)	2.41 (258.89)
6.	Elliot's	47.83	2.17 (149.95)	1.98 (96.55)
7.	Asthana and Hawker's	57.00	2.30 (203.23)	2.16 (144.55)
	S.Em. ±	0.8637	0.0305	0.0259
	C.D. at 5%	2.6201	0.0925	0.0785
	C.V. %	2.06	2.08	1.87

* Figures indicate logarithmic transformed values

** Figures in parentheses are retransformed values

Table 4.3: Colony/cultural characteristics of *F. solani* under different solid media

Sr. No.	Media	Cultural characters
1.	Potato dextrose agar	Circular colony, Flat growth with zonation , Dull white mycelium ,abundant sporulation.
2.	Richards' agar	Circular colony, Highly fluffy and fully raised growth with slight zonation, Mycelium milky white, abundant sporulation
3.	Wheat meal agar	Circular colony, Flat growth without zonation ,dirty white mycelium, abundant sporulation
4.	Czapek's (Dox) agar	Circular colony, Moderate fluffy growth with zonation and raised at center, Cottony white mycelium, abundant sporulation
5.	Potato carrot sucrose agar	Circular colony, Flat growth with zonation, Dull white mycelium, poor sporulation
6.	Elliot's agar	Circular colony, Sparse flat growth with zonation, fully white mycelium, poor sporulation
7.	Asthana and Hawker's agar	Circular colony, Highly fluffy growth with zonation and raised at center, milky white mycelium, moderate sporulation

Fig.-1 : Effect of different solid media on growth of *F. solani in vitro*

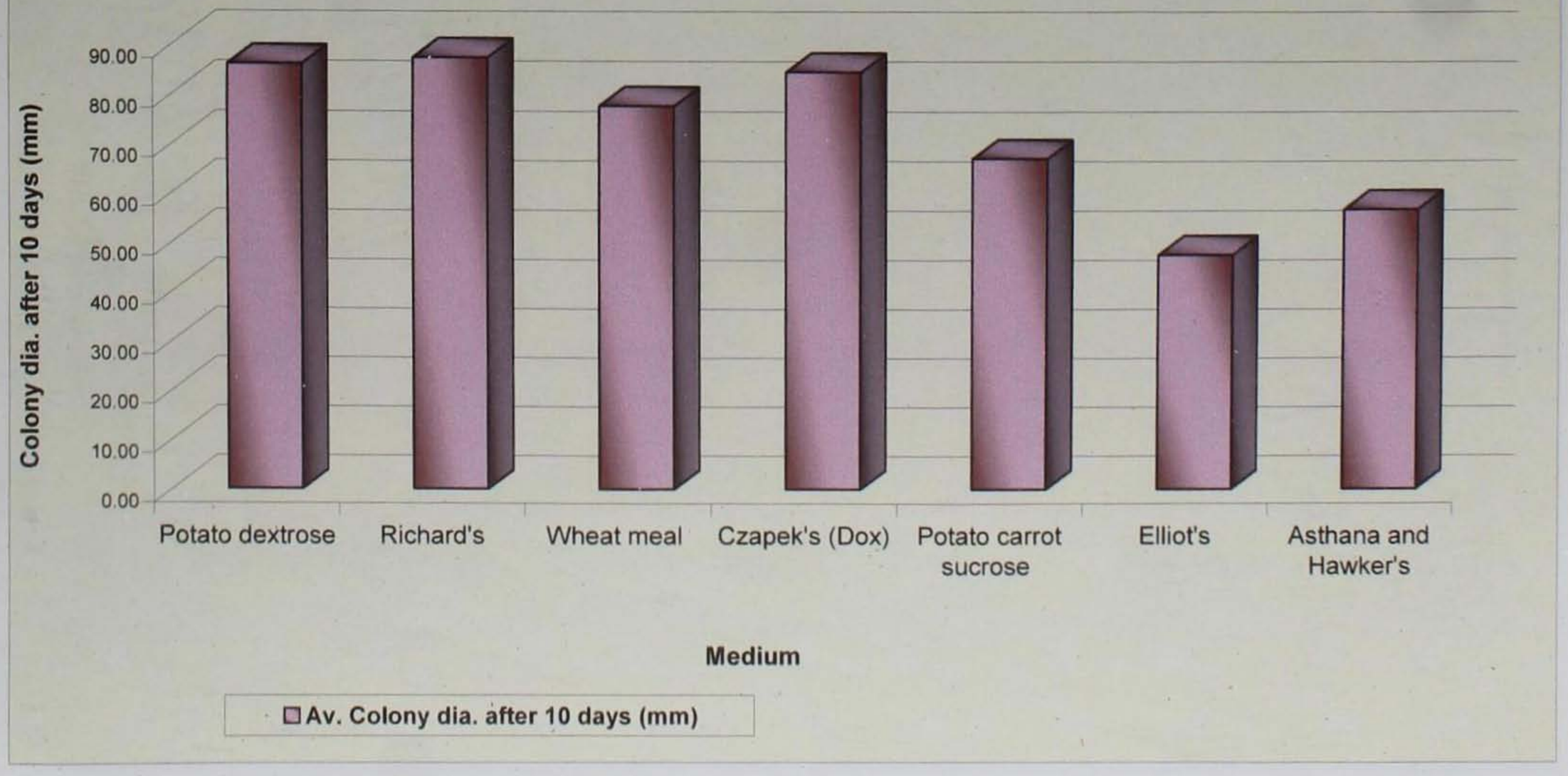


Fig.-2 : Effect of different liquid media on growth and sporulation of *F. solani in vitro*

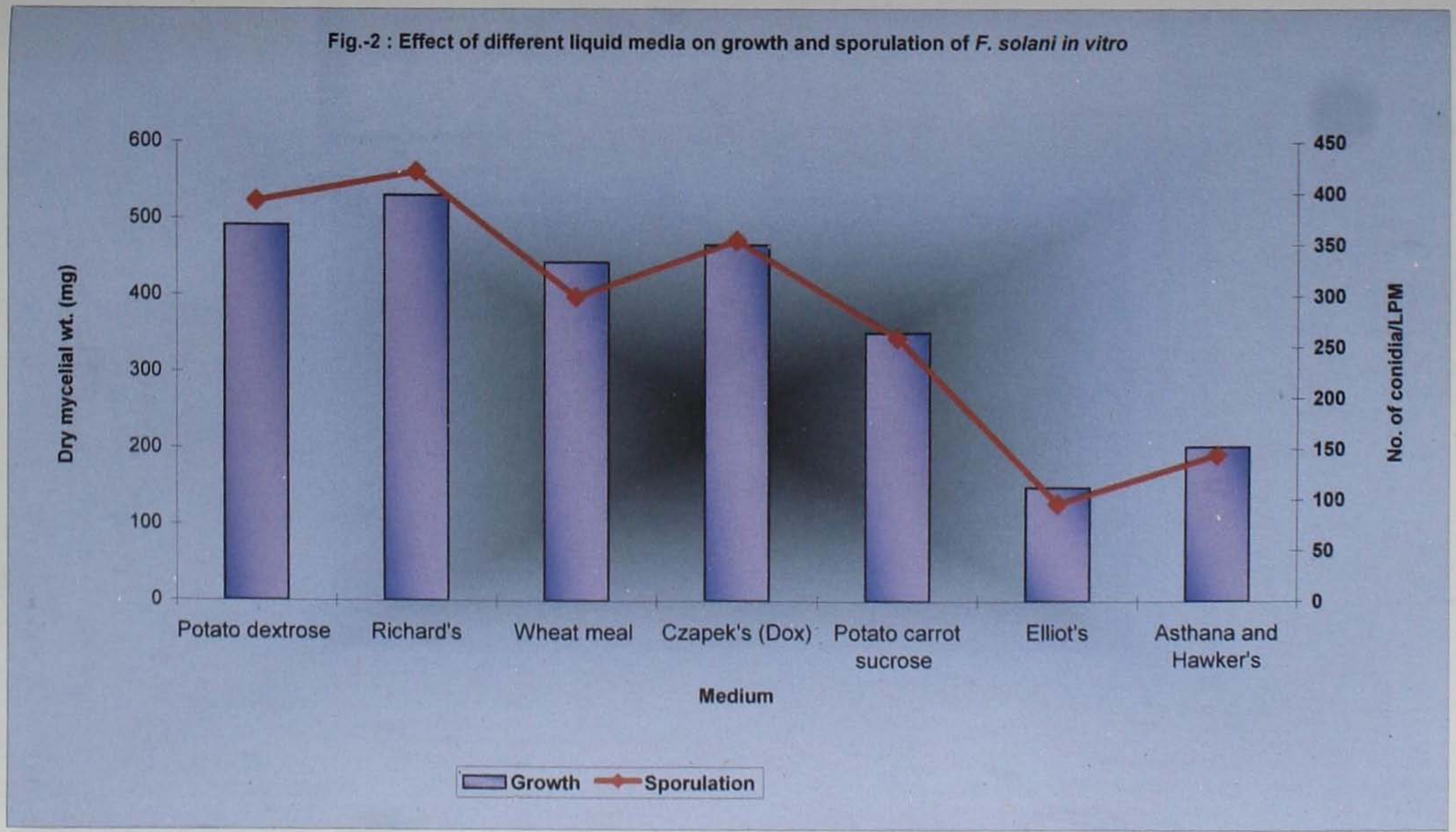
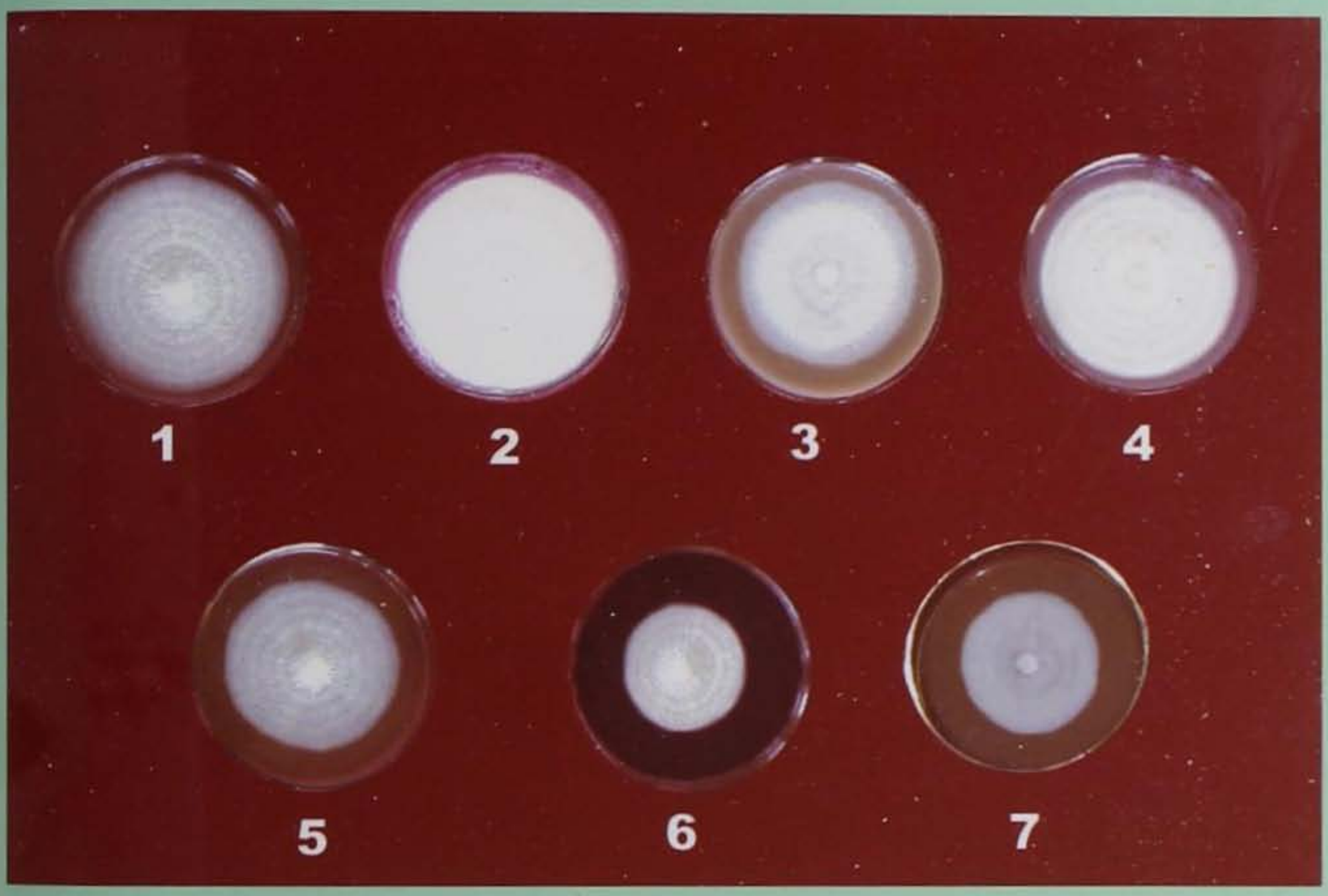


Plate-V : The growth of *F. solani* on different solid media *in vitro*



1. Potato dextrose agar
2. Richards' agar
3. Wheat meal agar
4. Czapek's (Dox) agar
5. Potato carrot sucrose agar
6. Elliot's agar
7. Asthana and Hawker's agar

spores/LPM) broths had poor sporulation.

Considering the over all performance of different media in solid as well as liquid state, Richards', potato dextrose and Czapek's (Dox) were found useful.

These media were found better for the growth and sporulation of *F. solani*, They were also reported better by earlier workers (Thakur and Singh, 1973; Booth, 1977; Gaur and Agnihotri, 1980 and Raghuwanshi, 1995). The present results confirmed with these findings.

4.3.2 Effect of different pH regimes on growth and sporulation of *F. solani in vitro*

The Richard's liquid medium was taken as a basal medium in this study. The dry mycelial weight and spore count were recorded. The data were statistically analysed and presented in Table-4.4 and Fig.-3.

The results obtained have clearly indicated that the fungus can grow and sporulate in a wide pH range form 4.0 to 8.0. Dry mycelial weight was significantly higher at 6.5 pH (455.83 mg) which was statistically at par with 6.0 (425.56 mg). Next best in order of merit was 5.5 (340.22 mg) which was statistically at par with 7.0 (293.78 mg) followed by 5.0 (202.02 mg) and 7.5 (142.07 mg). The least growth of the fungus was recorded at 4.0 (112.64 mg) and 8.0 (98.62 mg).

The sporulation was significantly superior at 6.5 pH (484.88 spores/LPM) which was statistically at par with 6.0

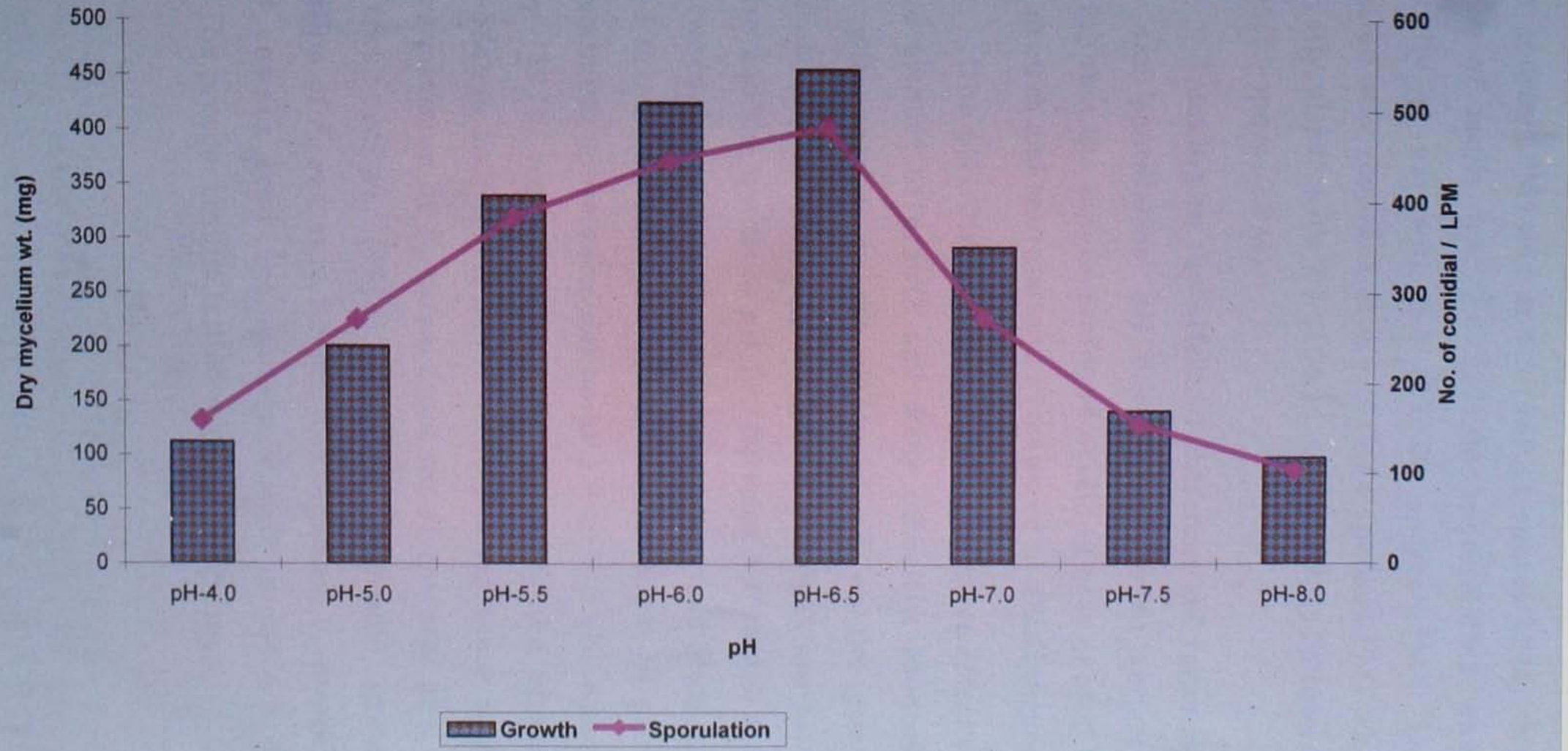
Table 4.4: Effect of different pH regimes on growth and sporulation of *F. solani in vitro*

Sr. No.	pH	Liquid medium (after 15 days)			
		Av. dry wt. of mycelium (mg)		No. of conidia/low power microfield (10x)	
1.	4.0	2.05*	(112.64) **	2.20*	(159.44) **
2.	5.0	2.30	(202.02)	2.43	(272.00)
3.	5.5	2.53	(340.22)	2.58	(385.33)
4.	6.0	2.63	(425.56)	2.65	(447.55)
5.	6.5	2.66	(455.83)	2.68	(484.88)
6.	7.0	2.47	(293.78)	2.44	(275.00)
7.	7.5	2.15	(142.07)	2.19	(156.11)
8.	8.0	1.99	(98.62)	2.01	(105.11)
S.Em. ±		0.027		0.033	
C.D. at 5%		0.081		0.099	
C.V. %		2.00		2.39	

* Figures indicate logarithmic transformed values

** Figures in parentheses are retransformed values

Fig.-3 : Effect of different pH regimes on growth and sporulation of *F. solani in vitro*



(447.55 spores/LPM) and at 5.5 (385.33 spores/LPM). Next best in order of merit was 7.0 (275.00 spores/LPM) which was statistically at par with 5.0 (272.00 spores/LPM). The least sporulation was recorded at 4.0 (159.44 spores/LPM) which was statistically at par with 7.5 (156.11 spores/LPM) followed by pH 8.0 (105.11 spores/LPM).

Looking to the effect of different pH regimes on the growth and sporulation, pH 6.0 to 6.5 proved very effective indicating that the fungus preferred acidic to near neutral medium for the growth and sporulation as compared to alkaline medium.

The pH of the medium exerts a decisive effect upon the rate and amount of growth and many other life processes. The studies with regards to the effect of pH on the growth and sporulation of *F. solani* suggested that the fungus was acidophilic in nature and the optimum pH lies between 6.0 to 6.5 for growth and between 5.5 to 6.5 for sporulation. Chi and Hanson (1964) reported that *F. solani* (wilt of red clover) could be grown well with maximum spore germination at 5.0 to 6.0 pH. Agarwal and Sarbhoy (1978) reported acidic pH favouring the growth of *F. solani*. Monga and Grover (1991) recorded maximum growth and spore germination of *F. solani* (root rot of cowpea) at 6.0 to 7.0 pH. Farkya *et al.* (1996) observed maximum growth and sporulation of *F. solani* at 5.5 pH. The organism *F. solani* causing wilt of cowpea grew and sporulates well in pH 6.5 (Pandav, 2002). The present results tallied with these findings.

4.4 Effect of nitrogenous fertilizers on the growth and sporulation of *F. solani in vitro*

Seven different nitrogenous fertilizers in liquid Richard's medium were tested to know their effect on the growth and sporulation of the fungus. The results are presented in Table-4.5 and Fig-4.

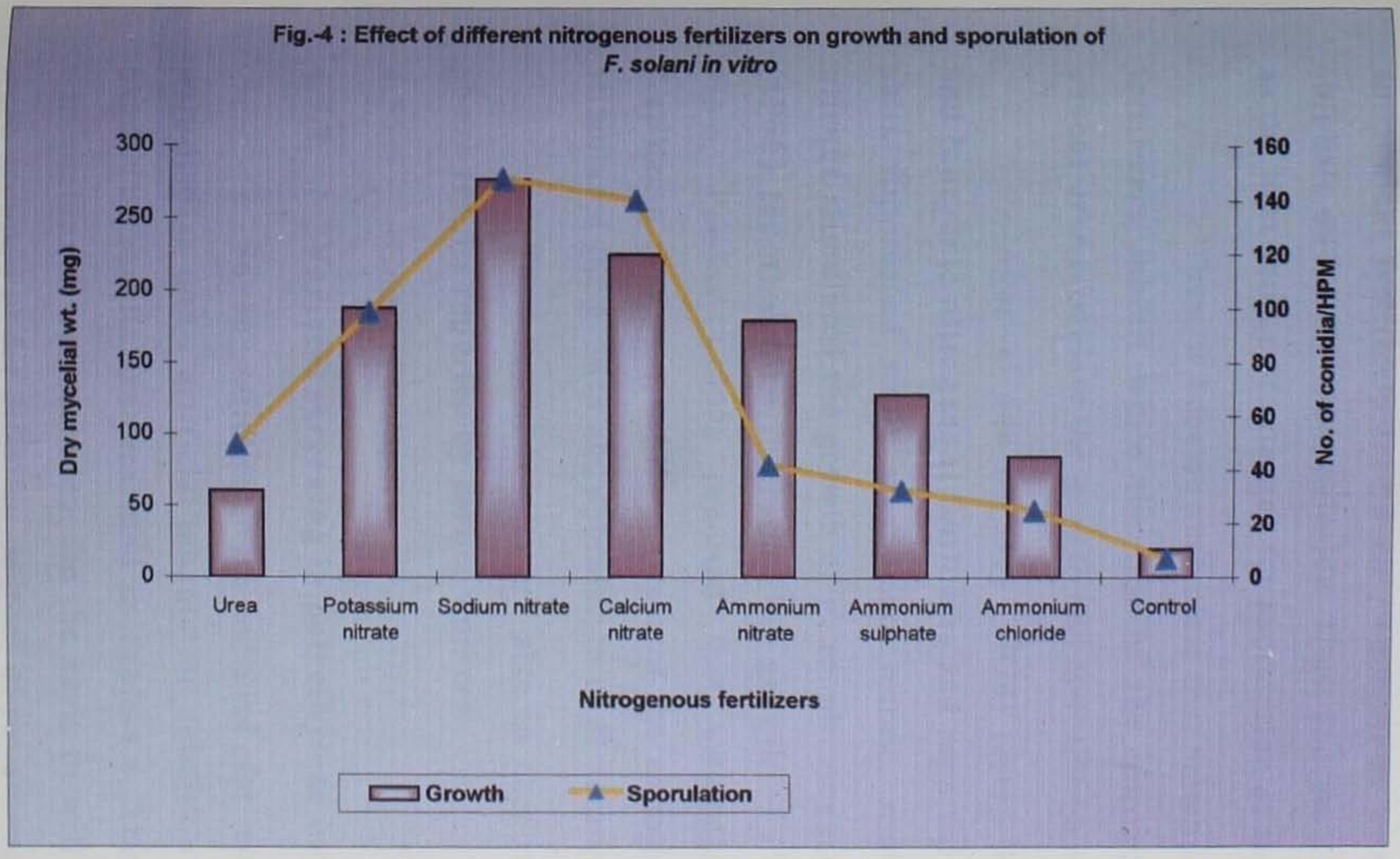
It is observed from the results that among seven nitrogenous fertilizers tested, sodium nitrate was found significantly better, giving maximum growth of the fungus (276.80 mg). Next best in order of merit was calcium nitrate (224.95 mg) followed by potassium nitrate (187.81 mg) which was statistically at par with ammonium nitrate (179.93 mg). The least growth of the fungus was observed in ammonium sulphate (128.03 mg), ammonium chloride (84.99 mg) and urea (60.41 mg). These all nitrate fertilizers except urea produced excellent mycelial growth while remaining ammonical fertilizers *viz.*, ammonium nitrate, ammonium sulphate and ammonium chloride showed poor growth of the fungus

The sporulation was significantly superior in sodium nitrate [148.00 spores/high power microfield (HPM)] which was statistically at par with calcium nitrate (140.11 spores/HPM) as compared to the rest. Next best in order of merit was potassium nitrate (98.88 spores/HPM). Sporulation was found poor in urea (49.77 spores/HPM) which was statistically at par with ammonium nitrate (42.33 spores/HPM) followed by ammonium

Table 4.5: Effect of different nitrogenous fertilizers on growth and sporulation of *F. solani in vitro*

Sr. No.	Nitrogenous Fertilizer	Av. dry mycelial wt. (mg)	Av. no. of conidia/ high power microfield (45x)
1	Urea	60.41	49.77
2	Potassium nitrate	187.81	98.88
3	Sodium nitrate	276.80	148.00
4	Calcium nitrate	224.95	140.11
5	Ammonium nitrate	179.93	42.33
6	Ammonium sulphate	128.03	32.77
7	Ammonium chloride	84.99	25.11
8	Control	19.77	7.00
	S.Em. ±	6.37	3.35
	C.D. at 5%	19.10	10.04
	C.V. %	7.59	8.53

Fig.-4 : Effect of different nitrogenous fertilizers on growth and sporulation of *F. solani in vitro*



sulphate (32.77 spores/HPM) and ammonium chloride (25.11 spores/HPM). Sporulation was found to be very poor in all the ammonical fertilizers tested *viz*., ammonium nitrate, ammonium sulphate and ammonium chloride. Thus, sodium nitrate was found as the best source of nitrogen for sporulation of the fungus.

Thus, it became very clear that ammonical nitrogenous fertilizers inhibit while the rest of the nitrogenous fertilizers except urea stimulate the growth and sporulation of *F. solani*.

Agarwal and Sarbhoy (1978) tested different organic and inorganic nitrogenous fertilizers and reported that ammonium salts suppressed while magnesium nitrate, sodium nitrate and nitrites stimulated the growth of *F. solani*. Anggraeni and Suharti (1996) reported urea inhibiting the growth of *Fusarium* spp. *in vitro*. Pandav (2002) reported that ammonical nitrogenous fertilizers inhibited, while the rest of the nitrogenous fertilizers stimulated the growth and sporulation of *F. solani*.

The present results are also in confirmation with the above results reported by earlier workers.

4.5 Control measures

4.5.1 Varietal screening in polyethylene bags

Seven varieties were selected for screening against wilt disease under artificial inoculation condition in polyethylene bags. The results are presented in Table-4.6. The observation on wilt incidence was recorded. On the basis of wilt incidence, they were grouped under different degrees of resistance.

Table 4.6: Evaluation of different gerbera varieties against *F. solani* under artificial inoculation condition in polyethylene bag

Sr. No.	Variety	Total no. of plants inoculated	Total no. of wilted plants	Av. wilt incidence (%)	Reaction
1.	Double dutch	9	5	55.55	MS
2.	Goliath	9	5	55.55	MS
3.	Primrose	9	2	22.22	R
4.	Avant grade	9	4	44.44	MR
5.	Zingaro	9	3	33.33	MR
6.	Savannah	9	4	44.44	MR
7.	Local (Deshi)	9	9	100.00	S

MR : Moderately resistant
MS : Moderately susceptible
S : Susceptible
R : Resistant

Wilt incidence

Cent per cent wilt incidence was recorded in gerbera cv. local (Deshi), this cultivar recorded more than 74 per cent wilt incidence and proved to be susceptible to wilt. Double dutch (55.55%) and Goliath (55.55%) cultivars showed wilt incidence between 50 to 74 per cent and proved to be moderately susceptible, while Avant grade (44.44%), Zingaro (33.33%) and Savannah (44.44%) exhibited wilt incidence between 25 to 49 per cent and proved to be moderately resistant. Primrose (22.22%) cultivar showed wilt incidence between 0 to 24 per cent and proved to be resistant.

4.5.2 Bio-control

4.5.2.1 Testing efficacy of antagonists against *F. solani in vitro*

Eight known antagonists were evaluated for their antagonism against *F. solani* by three different methods *viz*., dual culture, pathogen at the periphery and pathogen at the center.

4.5.2.1.1 Dual culture method

The results presented in Table-4.7 [Plate-VI (a)] revealed that all the antagonists screened against *F. solani* were significantly superior over control. Out of these, significantly the least growth of the pathogen was recorded in *Trichoderma viride* (15.00 mm) which was statistically at par with *Trichoderma harzianum* (16.33 mm). Next best in order of merit was *Aspergillus niger* (19.00 mm) which was statistically at par with

Table 4.7: Testing of antagonists against *F. solani in vitro* under dual culture method

Sr. No.	Test organism	Av. colony dia. of pathogen (mm)	Growth inhibition (%)
1.	*Trichoderma viride*	15.00	65.64
2.	*Trichoderma harzianum*	16.33	62.59
3.	*Trichoderma longibrachyatum*	20.33	53.43
4.	*Aspergillus niger*	19.00	56.48
5.	*Aspergillus flavus*	24.33	44.27
6.	*Gliocladium virens*	21.67	50.37
7.	*Chaetomium globosum*	24.00	45.04
8.	*Bacillus subtilis*	25.67	41.21
9.	Control	43.67	-
	S.Em. ±	0.47	
	C.D. at 5%	1.40	
	C.V. %	3.50	

Table 4.7: Testing of antagonists against *F. solani in vitro* under dual culture method

Sr. No.	Test organism	Av. colony dia. of pathogen (mm)	Growth inhibition (%)
1.	*Trichoderma viride*	15.00	65.64
2.	*Trichoderma harzianum*	16.33	62.59
3.	*Trichoderma longibrachyatum*	20.33	53.43
4.	*Aspergillus niger*	19.00	56.48
5.	*Aspergillus flavus*	24.33	44.27
6.	*Gliocladium virens*	21.67	50.37
7.	*Chaetomium globosum*	24.00	45.04
8.	*Bacillus subtilis*	25.67	41.21
9.	Control	43.67	-
	S.Em. ±	0.47	
	C.D. at 5%	1.40	
	C.V. %	3.50	

Trichoderma longibrachyatum (20.33 mm) followed by *Gliocladium virens* (21.67 mm), *Chaetomium globosum* (24.00 mm), *Aspergillus flavus* (24.33 mm) and *Bacillus subtilis* (25.67 mm).

T. viride and *T. harzianum* showed maximum growth inhibition (65.64% and 62.59%) and appeared to be the most superior over all the antagonists tested. Next best in order of merit was *A. niger* (56.48%) and *T. longibrachyatum* (53.43%) followed by *G. virens* (50.37%), *C. globosum* (45.02%), *A. flavus* (44.27%) and *B. subtilis* (41.21%).

4.5.2.1.2 Pathogen at the periphery

The results presented in Table-4.8 [Plate-VI (b)] revealed that all the antagonists screened against *F. solani* were significantly superior over control. Out of these, significantly least growth of the pathogen was recorded in *Trichoderma viride* (14.25 mm) which was statistically at par with *Trichoderma harzianum* (15.00 mm). Next best in order of merit was *Aspergillus niger* (20.00 mm) which was statistically at par with *Trichoderma longibrachyatum* (21.08 mm) followed by *Aspergillus flavus* (22.50 mm), *G. virens* (24.42 mm), *Chaetomium globosum* (26.17 mm) and *Bacillus subtilis* (28.25 mm).

T. viride and *T. harzianum* showed maximum growth inhibition (66.86% and 65.11%) and appeared to be the most superior over all the antagonists tested. Next best in order of

Table 4.8: Testing of antagonists against *F. solani in vitro* under pathogen at the periphery method

Sr. No.	Test organism	Av. Colony dia. of pathogen (mm)	Growth inhibition (%)
1.	*Trichoderma viride*	14.25	66.86
2.	*Trichoderma harzianum*	15.00	65.11
3.	*Trichoderma longibrachyatum*	21.08	50.96
4.	*Aspergillus niger*	20.00	53.48
5.	*Aspergillus flavus*	22.50	47.67
6.	*Gliocladium virens*	24.42	43.21
7.	*Chaetomium globosum*	26.17	39.14
8.	*Bacillus subtilis*	28.25	34.30
9.	Control	43.00	-
	S.Em. ±	0.61	
	C.D. at 5%	1.82	
	C.V. %	4.45	

A. Dual culture method

B. Pathogen at periphery

Plate-VI : Efficacy of antagonists on the growth of *F. solani in vitro* (A, B and C)

merit was *A. niger* (53.48%) and *T longibrachyatum* (50.96%) followed by *A. flavus* (47.67%), *G. virens* (43.21%), *C. globosum* (39.14%) and *B. subtilis* (34.30%).

4.5.2.1.3 Pathogen at the centre

The results presented in Table-4.9 [Plate-VI (c)] revealed that all the antagonists screened against *F. solani* were significantly superior over control. Out of these, significantly the least growth of the pathogen was recorded in *Trichoderma viride* (18.83 mm) which was statistically at par with *Trichoderma harzianum* (20.67 mm). Next best in order of merit was *Aspergillus niger* (24.50 mm), *Aspergillus flavus* (26.83 mm) and *Trichoderma longibrachyatum* (30.33 mm) followed by *Gliocladium virens* (35.83 mm), *Chaetomium globosum* (38. mm) and *Bacillus subtilis* (41.83 mm).

T. viride and *T. harzianum* showed maximum growth inhibition (77.03% and 74.80%) and appeared to be the most superior over all the antagonists tested. Next best in order of merit was *A. niger* (70.12%) and *A. flavus* (67.28%) followed by *T. longibrachyatum* (63.01%), *G. virens* (56.30%), *C. globosum* (53.66%) and *B. subtilis* (48.98%).

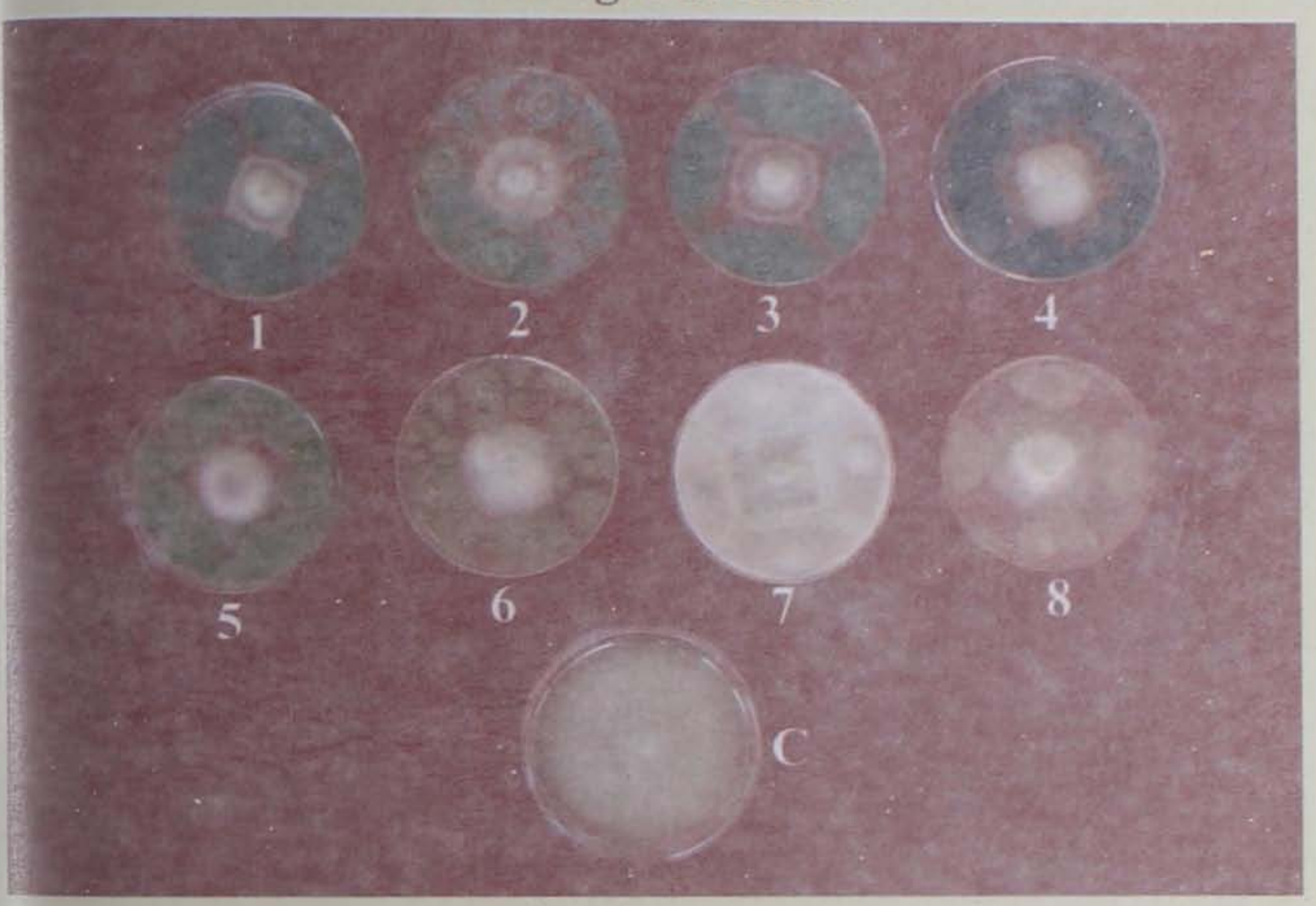
It appeared from this study that all the antagonists tested by three different methods proved effective against *F. solani* and may be very useful as potential biological control agents. Among them, *T. viride* proved highly antagonistic followed by *T. harzianum*, *A. niger*, *T longibrachyatum* and *A.*

Table 4.9: Testing of antagonists against *F. solani in vitro* under pathogen at the centre method

Sr. No.	Test organism	Av. Colony dia. of pathogen (mm)	Growth inhibition (%)
1.	*Trichoderma viride*	18.83	77.03
2.	*Trichoderma harzianum*	20.67	74.80
3.	*Trichoderma longibrachyatum*	30.33	63.01
4.	*Aspergillus niger*	24.50	70.12
5.	*Aspergillus flavus*	26.83	67.28
6.	*Gliocladium virens*	35.83	56.30
7.	*Chaetomium globosum*	38.00	53.66
8.	*Bacillus subtilis*	41.83	48.98
9.	Control	82.00	-
	S.Em. ±	0.77	
	C.D. at 5%	2.29	
	C.V. %	3.76	

Contd... Plate-VI

C. Pathogen at centre



1. ***Trichoderma viride***
2. ***Trichoderma harzianum***
3. ***Trichoderma longibrachyatum***
4. ***Aspergillus niger***
5. ***Aspergillus flavus***
6. ***Gliocladium virens***
7. ***Chaetomium globosum***
8. ***Bacillus subtilis***
9. **C = Control**

flavus. This is in harmony with the finding of earlier workers *viz*., Sychew and Shaposhnik (1982), Locke *et al*. (!985), Kurzawinska and Klima (1997), Mathur and Gurjar (2002), Gurjar *et al*. (2004), Patibanda and Sen (2004) and Jha and Jalali (2006).

4.5.2.2 Testing of organic extracts against *F. solani in vitro*

The aqueous extracts of different organic sources were evaluated for their effect on *F. solani*.

The results presented in Table-4.10 (Fig.-5, Plate-VII) indicated that out of six organic sources tested, all showed significantly more inhibitory effect on the fungus. Among all the organic sources, significantly lower growth was recorded in the extract of neem cake (47.00 mm) which was statistically at par with poultry manure (48.00 mm). Next best in order of merit was pressmud (53.50 mm) followed by mustard cake (59.75 mm), FYM (68.00 mm) and castor cake (76.87 mm).

Maximum inhibition of the growth of *F. solani* was recorded in neem cake (46.59%) and poultry manure (45.45%). Next best in order of merit was pressmud (39.20%) followed by mustard cake (32.10), FYM (22.72%) and castor cake (12.64%).

Poultry manure and mustard cake for *F. oxysporum* f. sp. *cumini*. (Champawat and Pathak, 1988); neem cake for *F. solani* (Srivastava and Singh, 1991); neem cake, mustard cake, saw dust and groundnut cake for *F. solani* (Chakrabarti and Sen., 1991); neem cake for *F. solani* and *Meloidogyne incognita* in *Phaseolus vulgaris* (Srivastava and Singh, 1991); neem for

Table 4.10: Effect of various organic sources on the growth of *F. solani in vitro*

Sr. No.	Organic sources	Av. Colony dia. of pathogen (mm)	Growth inhibition (%)
1.	Farm yard manure	68.00	22.73
2.	Pressmud	53.50	39.20
3.	Poultry manure	48.00	45.45
4.	Castor cake	76.87	12.64
5.	Mustard cake	59.75	32.10
6.	Neem cake	47.00	46.59
7.	Control	88.00	-
	S.Em. ±	0.66	
	C.D. at 5%	1.95	
	C.V. %	2.10	

Fig.-5 : Effect of various organic extracts on the growth of *F. solani in vitro*

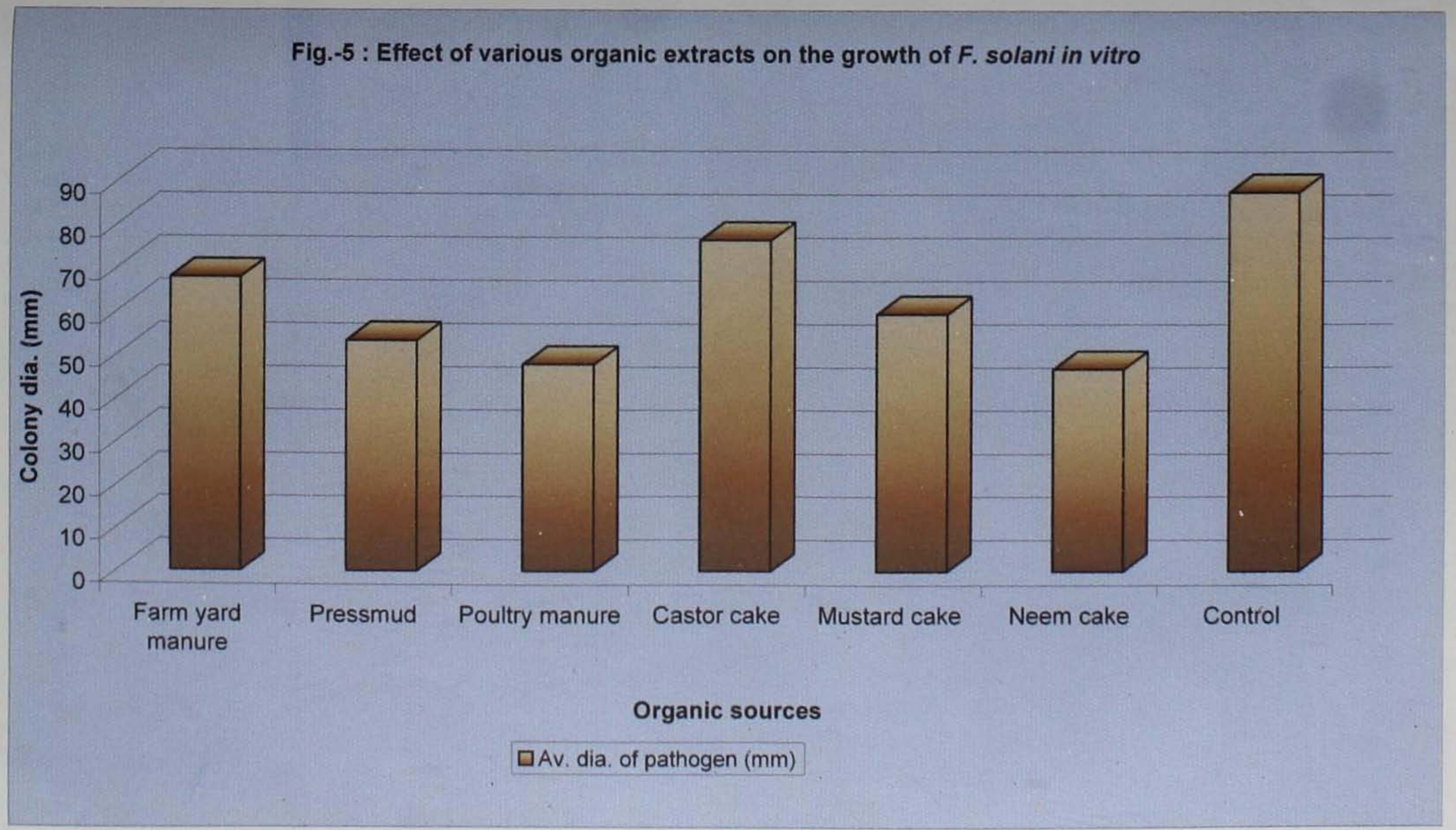
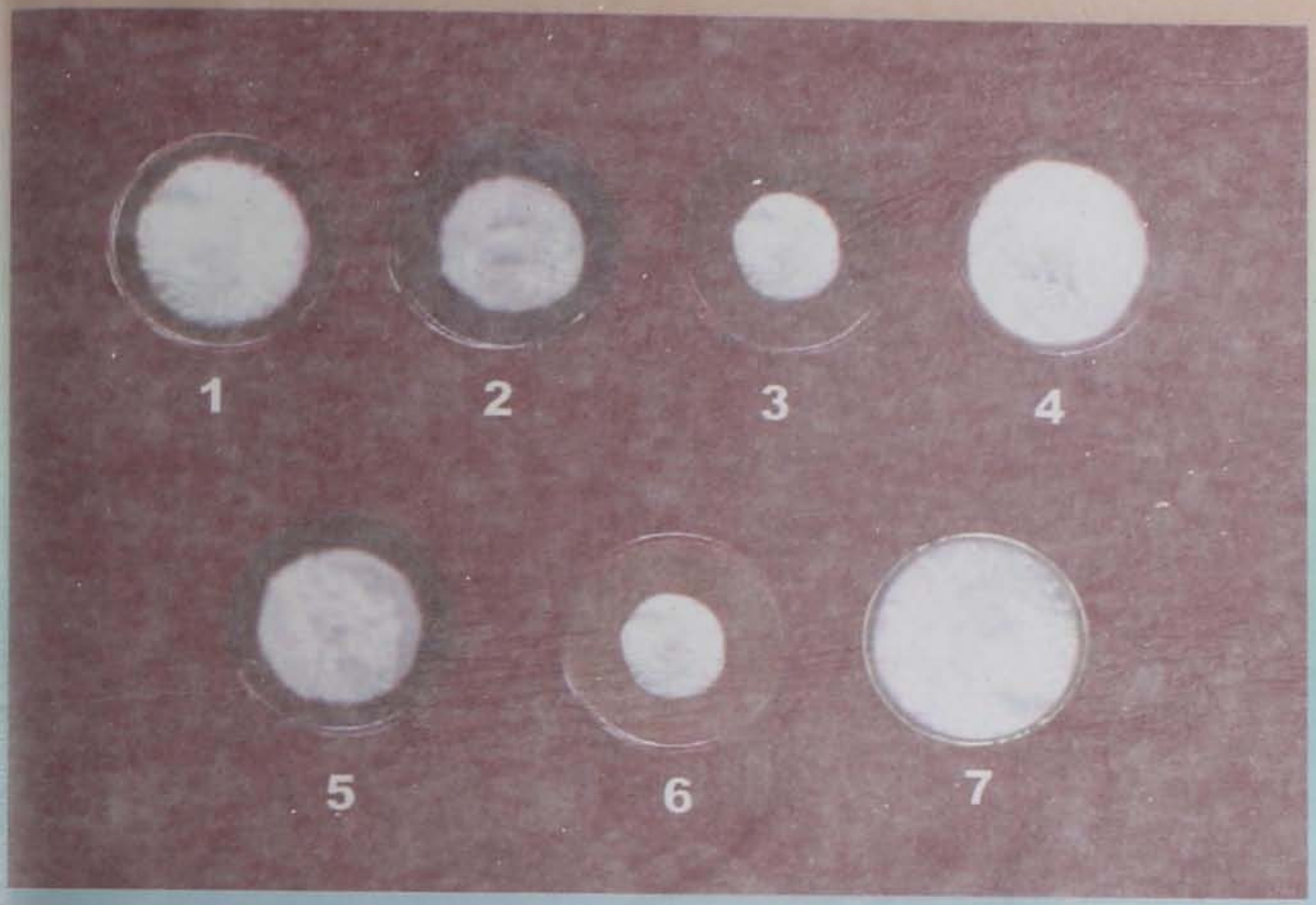


Plate-VII : Effect of various organic extracts on the growth of *F. solani in vitro*



1. Farm yard manure
2. Pressmud
3. Poultry manure
4. Castor cake
5. Mustard cake
6. Neem cake
7. Control

chickpea wilt caused by *F. oxysporum* f. sp. *ciceri* (Pandey *et al.*, 1996); neem cake for cowpea wilt caused by *F. oxysporum* (Ushamalini *et al.*, 1997); farm yard manure (FYM) and neem cake for *F.oxysporum* f. sp. *lycopersici* (Padmodaya and Reddy, 1999) and neem cake followed by poultry manures and pressmud for *F. solani*. (Pandav, 2002) were reported to have been very effective for growth inhibition of respective pathogens by earlier workers. The present results are in agreement with the earlier published results.

4.5.2.3 Bio-efficacy of botanicals against *F. solani in vitro*

Many plant extracts are known to have inhibitory effect on the growth and reproduction of various fungi. This information is certainly useful in exploiting inhibitory principle in plant disease control. In the present investigations, seven botanicals (Phytoextracts) of various plant species with suitable control were screened by poisoned food technique *in vitro* to know their inhibitory effect on the growth of *F. solani*.

The results presented in Table-4.11(Fig.-6, Plate-VIII) showed that all the plant extracts significantly inhibited growth of the fungus. Among the effective phytoextracts, lowest mycelial growth of *F. solani* was recorded in the extract of garlic (43.50 mm) significantly superior which was statistically at par with neem (45.33 mm). Next best in order of merit was onion (55.00 mm) which was statistically at par with ginger (57.17 mm) followed by tulsi (62.83 mm), turmeric (70.83 mm) and nilgiri (75.17 mm).

Table 4.11: Effect of various botanicals on the growth of *F. solani in vitro*

Sr. No.	Local name of Botanicals	Botanical name	Plant part used	Av. colony dia. (mm) after 10 days	Growth inhibition over control (%)
1	Onion	*Allium cepa* L.	Bulb	55.00	37.14
2	Neem	*Azadirachta indica* A. Jass.	Leaves	45.33	48.19
3	Nilgiri	*Eucalyptus citriodora* Hoch.	Leaves	75.17	14.09
4	Garlic	*Allium sativum* L	Bulb	43.50	50.28
5	Ginger	*Zingiber officinals* Rob.	Rhizome	57.17	34.67
6	Tulsi	*Ocimum sanctum* L.	Leaves	62.83	28.19
7	Turmeric	*Curcuma longa* L	Rhizome	70.83	19.04
8	Control	-		87.50	-
S. Em. ±				0.74	
C.D. at 5%				2.22	
C.V.%				2.06	

Fig.-6 : Effects of various botanicals on the growth of *F. solani in vitro*

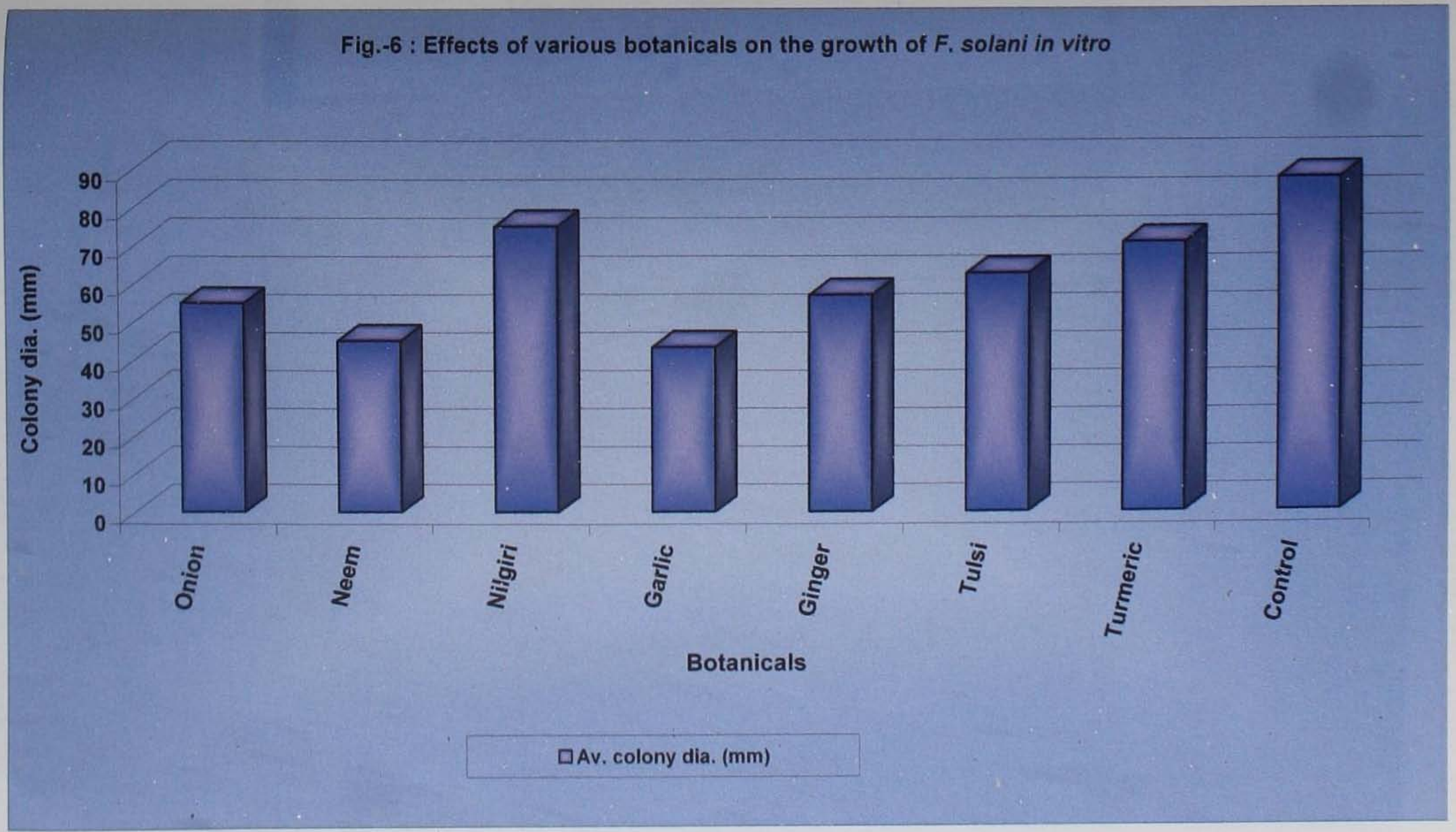
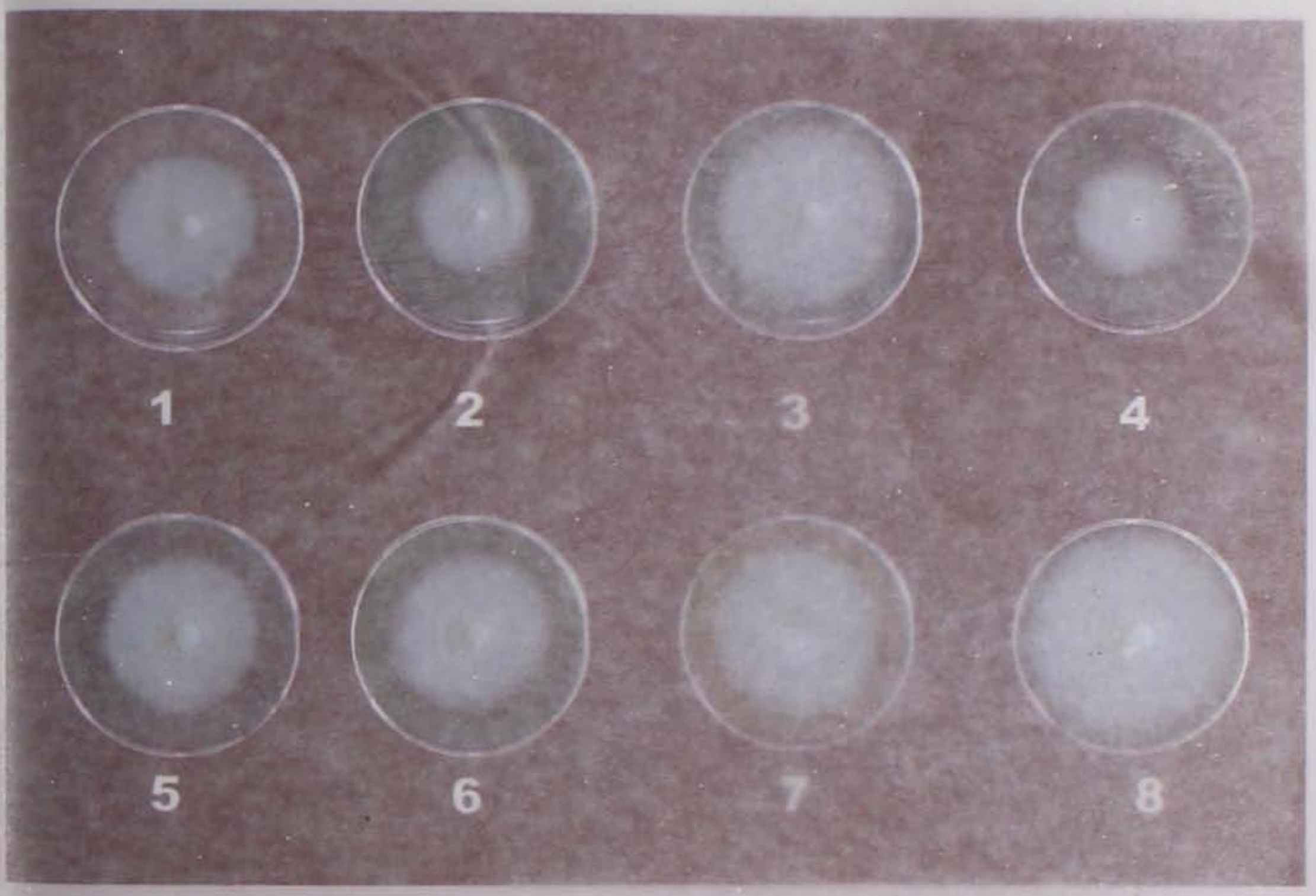


Plate-VIII : Effect of various botanicals on the growth of *F. solani in vitro*



1. Onion
2. Neem
3. Nilgiri
4. Garlic
5. Ginger
6. Tulsi
7. Turmeric
8. Control

The extract of garlic produced maximum inhibition (50.28%). Next best in the order of merit was neem (48.19%) followed by onion (37.14%), ginger (34.67%), tulsi (28.19%) turmeric (19.04%) and nilgiri (14.09%).

From this experiment, it is very clear that extracts of garlic (*Allium sativum* L.), neem (*Azardirachta indica* A. Jass.), onion (*Allium cepa* L) and ginger (*Zingiber officinals* Rab.) may have some strong toxic principle present which directly affect the growth of *F. solani*.

Extracts of garlic clove for *F. solani* (Patel, 1995); garlic bulb extract for *F. solani* (Chauhan, 1997); acalypha and garlic extract for *F. solani* (Pandav, 2002); *Azadirachta indica* for *F. oxysporum* (Bansal and Gupta, 2000); garlic extract for *F. solani* (Patel and Vala, 2004) were reported to be very effective for inhibition of growth of respective pathogens by earlier workers. The present results are in conformity with these findings.

4.5.3 Chemical control

4.5.3.1 Bio-efficacy of fungicides against *F. solani in vitro*

Ten fungicides belonging to different groups with three concentrations *viz*., 250, 500 and 1000 ppm were evaluated *in vitro* by poisoned food technique for their efficacy against *F. solani*.

It is evident from the results presented in Table-4.12 (Fig.-7, Plate-IX), that all the fungicides evaluated significantly

Table 4.12: Evaluation of different fungicides against *Fusarium solani in vitro*

Fungicide	Concentration (ppm)	Av. Colony diameter (mm)		Per cent inhibition over control
M.E.M.C. (Emisan 6% W.P.)	250	3.21*	(9.83)**	88.80
	500	2.91	(8.00)	90.89
	1000	2.80	(7.33)	91.65
Mancozeb (Dithane M-45 75 % W.P.)	250	4.45	(19.33)	77.99
	500	3.81	(14.00)	84.06
	1000	3.56	(12.17)	86.15
Validamycin (Sheathmar 3% L)	250	4.95	(24.00)	72.67
	500	4.41	(19.00)	78.37
	1000	3.85	(14.33)	83.68
Propiconazole (Tilt 25 % E.C.)	250	4.43	(19.17)	78.18
	500	4.08	(16.17)	81.60
	1000	3.48	(11.67)	86.72
Kasugamycin (Kasu-B 3% SL)	250	4.86	(23.17)	73.62
	500	4.58	(20.50)	76.66
	1000	3.96	(15.17)	82.73
Carbendazim (Bavistin 50 % W.P.)	250	0.71	(0.00)	100.00
	500	0.71	(0.00)	100.00
	1000	0.71	(0.00)	100.00
Tricyclazole (Beam 75% W.P.)	250	4.90	(23.50)	73.24
	500	4.53	(20.00)	77.23
	1000	4.00	(15.50)	82.35
Thiophanate methyl (Topsin-M 75 %W.P.)	250	3.60	(12.50)	85.77
	500	3.16	(9.50)	89.18
	1000	2.86	(7.67)	91.27
Hexaconazole (Contaf 5 EC)	250	3.48	(11.67)	86.72
	500	3.29	(10.33)	88.23
	1000	3.13	(9.33)	89.37
Copper Oxychloride (Blitox50 % W.P.)	250	6.40	(40.50)	53.89
	500	5.70	(32.00)	63.56
	1000	5.32	(27.83)	68.31
Control		9.40	(87.83)	-
S.Em. ±		0.06		
C.D. at 5%		0.18		
C.V. %		2.76		

* Figures indicate SQR+0.5 transformed values

** Figures in parentheses are retransformed values

Note-Experimental conclusions were based on SQR+0.5 transformed values

Table 4.12: Evaluation of different fungicides against *Fusarium solani in vitro*

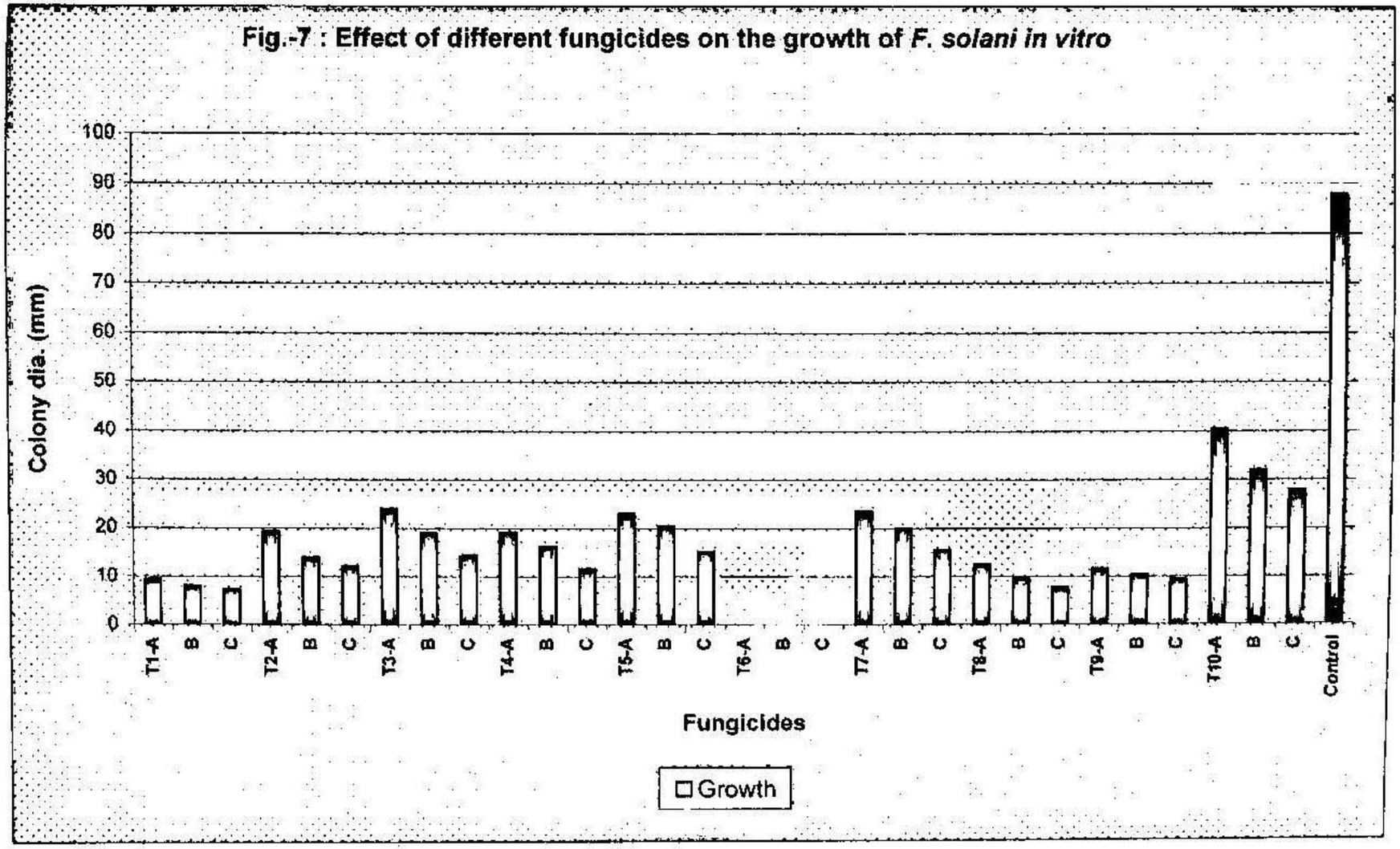
Fungicide	Concentration (ppm)	Av. Colony diameter (mm)		Per cent inhibition over control
M.E.M.C. (Emisan 6% W.P.)	250	3.21*	(9.83)**	88.80
	500	2.91	(8.00)	90.89
	1000	2.80	(7.33)	91.65
Mancozeb (Dithane M-45 75 % W.P.)	250	4.45	(19.33)	77.99
	500	3.81	(14.00)	84.06
	1000	3.56	(12.17)	86.15
Validamycin (Sheathmar 3% L)	250	4.95	(24.00)	72.67
	500	4.41	(19.00)	78.37
	1000	3.85	(14.33)	83.68
Propiconazole (Tilt 25 % E.C.)	250	4.43	(19.17)	78.18
	500	4.08	(16.17)	81.60
	1000	3.48	(11.67)	86.72
Kasugamycin (Kasu-B 3% SL)	250	4.86	(23.17)	73.62
	500	4.58	(20.50)	76.66
	1000	3.96	(15.17)	82.73
Carbendazim (Bavistin 50 % W.P.)	250	0.71	(0.00)	100.00
	500	0.71	(0.00)	100.00
	1000	0.71	(0.00)	100.00
Tricyclazole (Beam 75% W.P.)	250	4.90	(23.50)	73.24
	500	4.53	(20.00)	77.23
	1000	4.00	(15.50)	82.35
Thiophanate methyl (Topsin-M 75 %W.P.)	250	3.60	(12.50)	85.77
	500	3.16	(9.50)	89.18
	1000	2.86	(7.67)	91.27
Hexaconazole (Contaf 5 EC)	250	3.48	(11.67)	86.72
	500	3.29	(10.33)	88.23
	1000	3.13	(9.33)	89.37
Copper Oxychloride (Blitox50 % W.P.)	250	6.40	(40.50)	53.89
	500	5.70	(32.00)	63.56
	1000	5.32	(27.83)	68.31
Control		9.40	(87.83)	-
S.Em. ±		0.06		
C.D. at 5%		0.18		
C.V. %		2.76		

* Figures indicate SQR+0.5 transformed values

** Figures in parentheses are retransformed values

Note-Experimental conclusions were based on SQR+0.5 transformed values

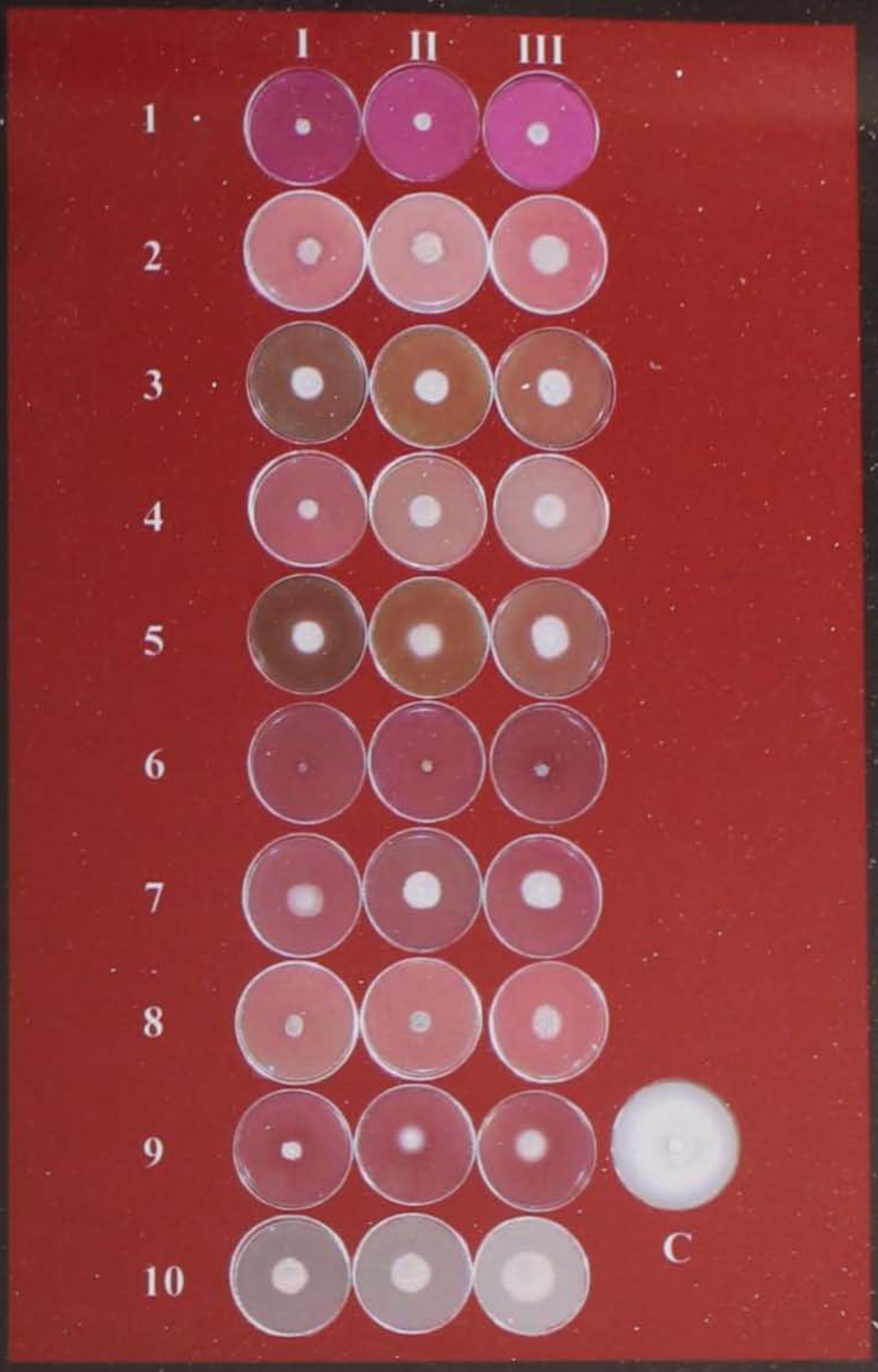
Fig.-7 : Effect of different fungicides on the growth of *F. solani in vitro*



T_1	M.E.M.C.(Emisan 6% W.P.)	T_7	Tricyclazole(Beam 75%WP)
T_2	Mancozeb(Dithane M-45 75 % W.P.)	T_8	Thiophanate methyl (Topsin-M 75 %W.P.)
T_3	Validamycin(Sheathmar 3%L)	T_9	Hexaconazole(Contaf 5 %EC)
T_4	Propiconazole (Tilt 25 % E.C.)	T_{10}	Copper Oxychloride(Blitox50 % W.P.)
T_5	Kasugamycin(Kasu-B 3%SL)	T_{11}	Control
T_6	Carbendazim (Bavistin 50 % W.P.)		

A = 250 ppm **B = 500 ppm** **C = 1000 ppm**

Plate-IX : Effect of various fungicides on the growth of *F. solani in vitro*



1. M.E.M.C. (Emisan 6% W.P.)
2. Mancozeb (Dithane M-45 75 % W.P.)
3. Validamycin (Sheathmar 3% L)
4. Propiconazole (Tilt 25 % E.C.)
5. Kasugamycin (Kasu-B 3% SL)
6. Carbendazim (Bavistin 50 % W.P.)
7. Tricyclazole (Beam 75% W.P.)
8. Thiophanate methyl (Topsin-M 75 %W.P.)
9. Hexaconazole (Contaf 5 EC)
10. Copper Oxychloride (Blitox50 % W.P.)
11. C = Control

I = 1000 ppm

II = 500 ppm

III = 250 ppm

reduced the growth of *F. solani* as compared to control. Out of these, carbendazim suppressed cent per cent growth of the pathogen at all the concentrations tested. Significantly least mycelial growth was recorded in M.E.M.C. at 1000 ppm (7.33 mm) which was at par with thiophanate-methyl at 1000 ppm (7.67 mm) and M.E.M.C. at 500 ppm (8.00 mm). Next best in order of merit was hexaconazole at 1000 ppm (9.33 mm) which was at par with thiophanate-methyl at 500 ppm (9.50 mm), M.E.M.C. at 250 ppm (9.83 mm) and hexaconazole at 500 ppm (10.33 mm) followed by propiconazole at 1000 ppm (11.67 mm) and hexaconazole 250 ppm (11.67 mm) which was at par with dithane M-45 at 1000 ppm (12.17 mm) and thiophanate-methyl at 250 ppm (12.50 mm). Significantly lower mycelial growth was also observed in dithane M-45 at 500 ppm (14.00 mm) which was at par with validamycin and kasugamycin at 1000 ppm (14.33 and 15.17 mm) followed by tricyclazole at 1000 ppm (15.50 mm), propiconazole at 500 ppm (16.17 mm) and validamycin at 500 ppm (19.00 mm). The rest of the fungicides *viz*., propiconazole, dithane M-45 and validamycin at 250 ppm, tricyclazole and kasugamycin at 250 and 500 ppm and copper oxychloride at 250, 500 and 1000 ppm respectively were less effective.

Carbendazim showed cent per cent growth inhibition at all the concentrations tested and appeared as the most effective over all of the fungicides tested followed by M.E.M.C. at 1000 ppm (91.65%), thiophanate-methyl at 1000 ppm (91.27%) and M.E.M.C. at 500 ppm (90.89%). Next best in order of merit was

hexaconazole at 1000 ppm (89.37%) which was at par with thiophanate-methyl at 500 ppm (89.18%), M.E.M.C. at 250 ppm (88.80%) and hexaconazole at 500 ppm (88.23%).

Considering the effect of fungicides on growth of *F. solani*, carbendazim proved to be the most effective followed by M.E.M.C., thiophanate-methyl and hexaconazole. These results are in confirmation with earlier workers (Kutova and Petkova 1975; Patel 1987; Singh and Saxena 1990; Kapoor and Kumar, 1991; Mukherjee and Tripathi 2000; Pandav, 2002).

4.5.3.2 Bio-efficacy of herbicides against *F. solani in vitro*

Six herbicides belonging to different groups with three concentrations *viz.*, 1000, 2000 and 3000 ppm were each evaluated *in vitro* by poisoned food technique for their efficacy against *F. solani*.

It is evident from the results presented in Table-4.13 (Fig.-8, Plate-X) that significantly least mycelial growth was recorded in atrazine at 3000 ppm (21.17 mm) as compared to rest of the herbicides tested at all concentrations. Next best in the order of merit was atrazine at 2000 ppm (26.17 mm) followed by glyphosate at 3000 ppm (27.83 mm), atrazine at 1000 ppm (32.50 mm) and glyphosate at 2000 ppm (38.17 mm) which was statistically at par with 2,4-D at 3000 ppm (38.17 mm) and pendimethalin at 3000 ppm (39.83 mm). Significantly lower mycelial growth was also observed in glyphosate at 1000 ppm (43.50 mm) which was statistically at par with fluchloralin at

Table 4.13: Comparative efficacy of different herbicides against *F. solani in vitro*

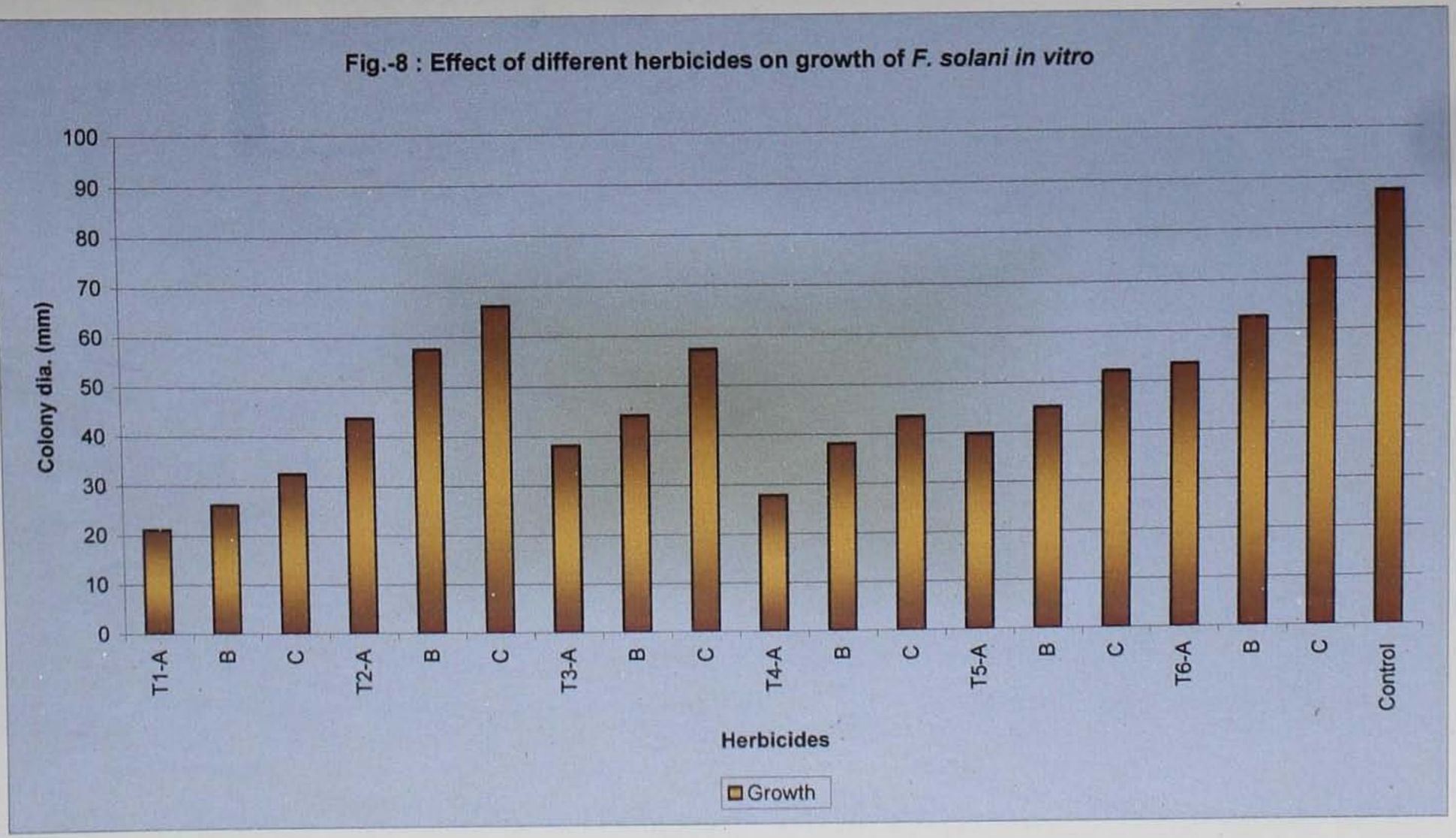
Herbicide	Concentration (ppm)	Av. Colony diameter (mm)		Per cent inhibition over control
Atrazine (Atrataf 50% W.P.)	3000	4.60*	(21.17)**	75.81
	2000	5.11	(26.17)	70.09
	1000	5.70	(32.50)	62.86
Fluchloralin (Basalin 45% E.C.)	3000	6.62	(43.83)	49.90
	2000	7.59	(57.67)	34.09
	1000	8.13	(66.17)	24.38
2,4-D (Fernoxone 80% W.S.P.)	3000	6.18	(38.17)	56.38
	2000	6.64	(44.17)	49.52
	1000	7.56	(57.17)	34.67
Glyphosate (Glycel 41% E.C.)	3000	5.27	(27.83)	68.19
	2000	6.18	(38.17)	56.38
	1000	6.59	(43.50)	50.28
Pendimethalin (Stomp 30% E.C.)	3000	6.31	(39.83)	54.47
	2000	6.72	(45.17)	48.38
	1000	7.22	(52.17)	40.38
Oxyfluorfen (Gold 23.5% E.C.)	3000	7.31	(53.50)	38.86
	2000	7.92	(62.67)	28.38
	1000	8.61	(74.16)	15.24
Control	-	9.35	(87.50)	-
S.Em. ±		0.05		
C.D. at 5%		0.14		
C.V. %		1.27		

* Figures indicate SQR transformed values

** Figures in parentheses are retransformed values

Note-Experimental conclusions were based on SQR transformed values

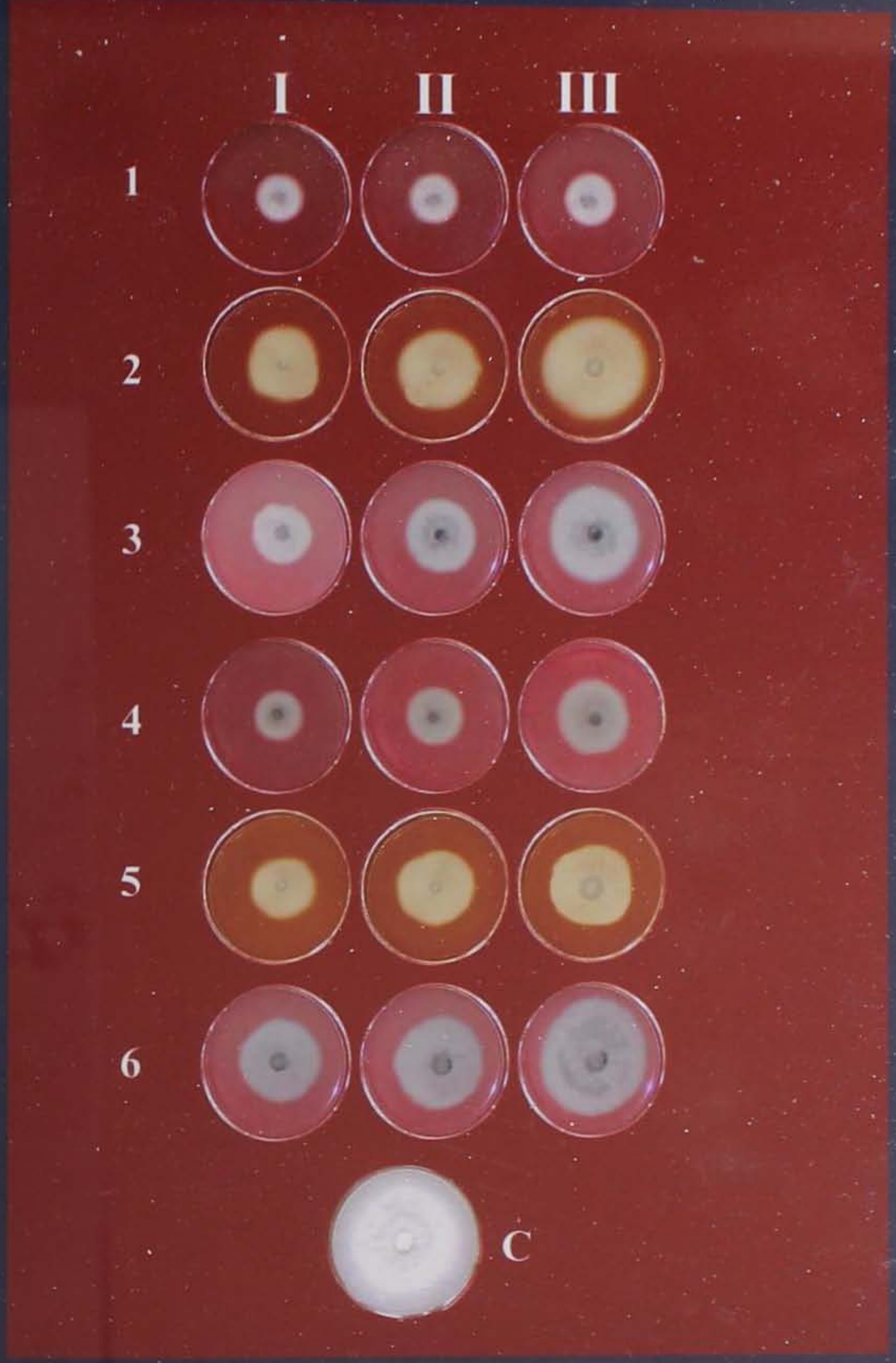
Fig.-8 : Effect of different herbicides on growth of *F. solani in vitro*



T_1	Atrazine (Atrataf 50% W.P.)	T_5	Pendimethalin (Stomp 30% E.C.)
T_2	Fluchloralin (Basalin 45% E.C.)	T_6	Oxyfluorfen (Gold 23.5% E.C.)
T_3	2,4-D (Fernoxone 80% W.S.P.)	T_7	Control
T_4	Glyphosate (Glycel 41% E.C.)		

A = 3000 ppm **B = 2000 ppm** **C = 1000 ppm**

Plate-X : Effect of various herbicides on growth of *F. solani in vitro*



1. Atrazine (Atrataf 50% W.P.)
2. Fluchloralin (Basalin 45% E.C.)
3. 2,4-D (Fernoxone 80% W.S.P.)
4. Glyphosate (Glycel 41% E.C.)
5. Pendimethalin (Stomp 30% E.C.)
6. Oxyfluorfen (Gold 23.5% E.C.)
7. C = Control

I = 3000 ppm
II = 2000 ppm
III = 1000 ppm

3000 ppm (43.83 mm), 2,4-D at 2000 ppm (44.17 mm) and pendimethalin at 2000 ppm (45.17 mm). The rest of the herbicides *viz.*, pendimethalin and 2,4-D at 1000 ppm, oxyfluorfen at 3000, 2000 and 1000 ppm and fluchloralin at 2000 and 1000 ppm were less effective.

Maximum inhibition was recorded in atrazine at 3000 ppm (75.80 %) and 2000 ppm (70.09 %) followed by glyphosate at 3000 ppm (68.19 %) and atrazine at 1000 ppm (62.85 %).

Considering the effect of herbicides on growth of *F. solani*, atrazine proved the most effective followed by glyphosate. These results are similar with findings of Kaufman (1964), Desai *et al.* (1985) and Sanogo *et al.* (2000).

SUMMARY AND CONCLUSION

V SUMMARY AND CONCLUSION

Gerbera (*Gerbera jamensonni* L.) is one of the most important and economic flower crops grown in India. Plant diseases are major constraint in economic crop production as they inflict heavy losses. Wilt is one of the major and economical diseases and have caused approximately 50 to 70 per cent yield loss in gerbera. It has been reported to be caused by different *Fusarium* spp. *viz*., *F. solani* and *F. oxysporum*. In this study *F. solani* was found to be associated with gerbera causing wilt disease. The scientific information on this disease is presently lacking. Hence, the investigation on this problem was under taken for generating more scientific information and suitable management strategies to prevent crop losses.

Microscopic examination and tissue isolation from root and stem of infected plant yielded culture of *Fusarium* sp.. The symptoms were recorded from cv. local (Deshi) grown in the college garden and green house. Most of the plants were found infected and showing varying degree of infection indicating their susceptibility to the pathogen at almost all stages of crop growth. Leaves became yellow with initially brown and eventually black streaks in the vascular system. Plants showing severe stunting and initiation of wilting symptoms and at later stage found to be completely wilted. On critical examination, the fungal growth is observed on the roots of the wilting plants. Microscopic

examination revealed the presence of micro and macro conidia of *Fusarium* sp. with dirty white mycelium.

The morphological and cultural characters of *Fusarium* sp. isolated were studied, which were found closely identical with *Fusarium solani* and this was also confirmed through identification by Indian Type Culture Collection (I.T.C.C No. 6148-05), I.A.R.I., New Delhi. The pathogenicity test was carried out by soil inoculation, root dip cum soil inoculation, and root dip inoculation in the polyethylene bags. All these methods successfully produced typical wilt symptoms similar to those observed under natural condition and described in the literature, confirming pathogenic nature of the fungus. Thus, the causal agent of gerbera wilt was identified and confirmed as *Fusarium solani* (Mart.) Sacc..

Out of seven solid media tested, Richards' agar was found significantly superior giving maximum growth of fungus. Next best solid media were potato dextrose agar and Czapek's (Dox) agar while among the liquid media, Richards' broth supported significantly superior growth which was at par with potato dextrose and wheat meal broths. Significantly more sporulation was recorded in Richards' broth which was at par with potato dextrose broth and Czapek's (Dox) broth.

The pH studies indicated that the fungus could grew and sporulated under wide range of pH from 4.0 to 8.0. However, pH 6.0 to 6.5 proved to be optimum for the growth and sporulation of the fungus.

Among the seven different nitrogenous sources tested for their effect on growth and sporulation of *F. solani*, significantly more growth was recorded in sodium nitrate. Next best in order of merit was calcium nitrate followed by potassium nitrate which was statistically at par with ammonium nitrate. Maximum sporulation was recorded in sodium nitrate which was statistically at par with calcium nitrate. Next best in order of merit was potassium nitrate. All the ammonical nitrogenous sources supported poor growth and sporulation of the fungus.

Seven varieties were selected for screening under artificial inoculation condition in polyethylene bags. Primrose proved resistant and Avant grade, Zingaro and Savannah proved moderately resistant, while rest of the varieties showed moderately susceptible to susceptible reactions.

Eight known antagonists were screened *in vitro* for their antagonism to *F. solani* by three methods *viz*., dual culture. by placing pathogen at the periphery and pathogen at the centre. In all three methods, *T. viride* proved highly antagonistic followed by *T. harzianum*, *A. niger*, *T. longibrachyatum* and *A. flavus*.

The extracts of different organics substrate were tested against *F. solani* by poisoned food technique *in vitro*. Significantly least growth was recorded in the extracts of neem cake which was statistically at par with poultry manure showing excellent inhibitory effect on *F. solani*. Next best in order of

merit was pressmud followed by mustard cake, FYM and castor cake.

Seven phytoextracts of indigenous plants were evaluated *in vitro* against *F. solani* using poisoned food technique. Significantly lower mycelial growth of pathogen was recorded in extracts of garlic which was statistically at par with neem and was found superior over the rest. Next best was onion which was statistically at par with ginger followed by tulsi, turmeric and nilgiri.

Ten fungicides with three different concentrations were screened *in vitro* by poisoned food technique to evaluate their efficacy against *F. solani*. All the fungicides tested, effectively inhibited the growth of *F. solani* as compared to control. Among these fungicides, carbendazim proved to be the most effective fungicide for suppressing the growth and sporulation of *F. solani* at all three concentrations tested followed by M.E.M.C., thiophanate-methyl and hexaconazole.

Six herbicides were screened *in vitro* to evaluate their fungitoxic effect on *F. solani* at three different concentrations by poisoned food technique. Among different herbicides tested, atrazine showed maximum inhibition over growth of the fungus followed by glyphosate.

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