# Efficacy of Partially Purified Plant Product Components *In vitro* and *In vivo* Against Plant Pathogens

# अर्द्धशोषित पादप उत्पादित संघटकों का रोग जनकों पर पात्रे व जीवे क्षमता

**SUNIL ZACHARIA**

**Thesis**

Doctor of Philosophy in Agriculture

(Plant Pathology)

**2002**

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Thesis
Submitted to the
**Maharana Pratap University of Agriculture & Technology, Udaipur**
for the Degree of
***Doctor of Philosophy in Agriculture***
**(Plant Pathology)**

**2002**

by
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This is to certify that **Mr. Sunil Zacharia** had successfully completed the comprehensive/Preliminary Examination held on 24th July, 2000 as required under the regulations for degree of **Doctor of Philosophy in Agriculture**.

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# *Chapter 1*

# *Introduction*

# INTRODUCTION

Plant protection through synthetic chemicals contributed significantly in reducing losses due to diseases and thereby increasing agricultural production. However, chemical's share to environmental pollution through air, water, soil and by induction of resistance amongst pathogens, thereby the need to apply them more with their escalating prices and harmful effect on non-target organisms, currently caused wide spread concern and hence the need to look for an alternative safer means for managing plant diseases are felt greater now than ever before.

Contrary to the problems associated with the use of synthetic chemicals, botanicals are environmentally nonpollutive, renewable inexhaustive, indigenously available, easily accessible, largely non phytotoxic, thus readily biodegradable, relatively cost effective and hence constitute a suitable plant protectants.

Use of botanicals for prevention of plant diseases without the mention of their harmful effect has been recorded in ancient Indian literature. Several botanicals viz., fruits of *Brhati* (*Solanum indicum* L.), Sandal (*Santalum album* L.) and *Mahuva* (*Madhuca indica* L.). seeds of *Mehandi* (*Lawsonia inermis* L.) and *Babool* (*Acacia nilotica* L.) have antimicrobial activities against some plant pathogens (Nene, 1996). Democritus tried plant extracts for controlling disease in 470 BC (Sharville, 1960). Since then there has been several recorded and unrecorded mention of their uses

against a large number of microbial organisms. A number of angiospermic plants have been reported for the presence of antimicrobial activities by several workers (Chopra *et al.*, 1965; Nene, 1996; Daya Ram, 1998; Choudhary *et al.*, 1998; Khan, 1999 and Sharma, 2000). The preparation from several plant parts such as seed, leaves, flowers, fruits, etc. have been used for the presence of antifungal activities. Many angiospermic plants parts such as fruits of Amaltas (*Cassia fistula* L.) contain pectin, hydro-methyl amanthoquinones and sugars while Datura (*Datura stramonium* L.) leaves are known for the presence of alkaloids which possess toxic properties against some plant pathogens. A preparation from bitter Temru (*Diospyros cordifolia* Roxb.) is known for its piscicidal activities (Sharma and Simlot, 1971).

The natural abundance of botanicals, their genetic multiplicity globally and little pollution problems are the key factors for their attractive prospect of application. Considering all these aforementioned facts, the present studies were conducted to find out the efficacy of different partially purified plant product components against various plant pathogens viz., *Fusarium oxysporum* f. sp. *lycopersici* inciting fruit rot of tomato, *Alternaria cyamopsidis* Rangaswami and Rao, causing leaf blight of guar, *Colletotrichum gloeosporioides* causing anthracnose of yam and *Helminthosporium maydis* (Nisikado and Miyake) inciting maydis leaf blight of maize with the following objectives :

1. Collection, isolation and purification of plant pathogens.

2. Pathogenicity test of the isolated plant pathogens.

3. Extraction of the components from plant products of various plants, viz., Sandal (*Santalum album* L.), *Brhati* (*Solanum indicum* L.), Custard apple (*Annona squamosa* L.), Amaltas (*Cassia fistula* L.), *Babool* (*Acacia nilotica* L.), *Satyanashi* (*Argemone mexicana* L.), Datura (*Datura stramonium* L.), bitter Temru (*Diospyros cordifolia* Roxb.) and *Mehandi* (*Lawsonia inermis* L.).

4. Efficacy of components *in vitro* against *Alternaria cyamopsidis, Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Helminthosporium maydis* isolated from guar, yam, tomato and maize, respectively.

5. Efficacy of components *in vivo* against *Helminthosporium maydis* incitant of *maydis* blight of maize leaves.

# *Chapter 2*

# *Review of Literature*

# REVIEW OF LITERATURE

Any scientific work is not complete until it is supported and corroborated by the relevant reports available in the literature and moreover it provides guidelines for future research work.

With this view, efforts have been made to incorporate all the relevant literature and it has been categorised into two distinct parts :

2.1 Review of fruit rot and plant diseases.

2.2 Review of plant extracts.

## 2.1 REVIEW OF FRUIT ROT AND PLANT DISEASES

### 2.1.1 Fusarium Rot of Tomato :

Various fungal plant pathogens are responsible for decaying the tomato fruits under field conditions and storage also. Saccardo (1886) first described *Fusarium* on decaying tomato fruits received from Italy in 1886 and was named as *Fusarium oxysporum* (Schl.) sub spp. *lycopersici* Sacc. In India, Thaxter (1890) reported the fungus *Fusarium* on tomato fruits and identified as *Fusarium lycopersici* Sacc., while Thomas (1994) reported that the tomato fruit rot incited by *Alternaria alternata* (fr.) Keissler is fairly common in all tomato growing areas in India.

Srivastava and Tandon (1966) and subsequently Aulakh and Grover (1968) reported the fusarium rot of tomato caused by *Fusarium roseum*. Aulakh and Grover (1968) also found that the rot could be inhibited by

cotton seed, castor and paraffin oils. Yadav and Thakur (1971) reported that tomato fruit rot incited by *Fusarium nivale* and *Rhizopus stolonifer* were most prevalent in vegetable markets of Haryana.

Khanna and Chandra (1977 a and 1977 b) reported that tomato fruit rot incited by *Fusarium* species is known to occur in many countries including India under field conditions and during storage and after harvesting of fruits, the rot causes serious losses. They made a survey of Allahabad, India, vegetable markets and first reported the fusarium rot is incited by *Fusarium equiseti*. They also found that this rot was most prevalent during the months of January and February.

Garg and Gupta (1979) reported a soft rot of tomato fruits collected from Agra markets and isolated the fungus, *Fusarium solani*.

Kalra and Sohi (1985 a & b) conducted a survey of vegetable markets of Chandigarh and found that *Fusarium* species caused considerable losses of tomato fruits. They also found that alternaria rot was the most prevalent rot in tomato fruits. Arinze (1986) isolated *Fusarium solani* and *F. oxysporum* from tomato fruits collected from the markets of Southern Nigeria and reported to be most pathogenic to tomato fruits.

Okali and Erink (1990) found that *Fusarium equiseti* and *F. oxysporum* causing considerable losses of tomato fruits in laboratory tests. Oladiran and Iwu (1993) reported that the fusarium rot was maximum when relative humidity ranged from 70 to 90 per cent and further found that the rot can be minimised when fruits stored at 0 to 10°C temperature and 60 to 90 per cent relative humidity.

Sharma (1996) reported an average damage of about 0.5 to 19.7 per cent of tomato fruits by post harvest fungal rots from vegetable markets of Himachal Pradesh and found that 81.3 per cent of total spoilage was due to *Fusarium* species. Ramgiry *et al.* (1997) isolated the fruit rot pathogens from 60 different tomato fruit samples collected from Madhya Pradesh and found that most of them were incited by fungi. It was also observed that *Fusarium oxysporum, Alternaria alternata* and *Penicillium notatum* were the most frequent causal agents of tomato decay in field and vegetable markets.

### 2.1.2 Alternaria Blight of Guar :

Butler (1918) described a leaf spot disease of guar caused by the organism identical to *Alternaria brassicae* (Berk.) Sacc. from Pusa (Bihar) and Madras while, Narasimhan (1935) reported an unidentified species of *Alternaria* on guar from Mysore. Uppal *et al.* (1935) found *A. brassicae* on guar along with other fungi in Bombay. Streets (1948) as well as Luttrel (1964) found the occurrence of alternaria leaf spot in U.S.A. and they tentatively identified the pathogen as *A. cucumerina* (Ell. and Eucrh) Elliot. Rangaswami and Rao (1957) observed a severe alternaria blight on guar in Coimbatore and on the basis of cross inoculation and morphological studies of the pathogen, concluded that the fungus was different from *A. brassicae* in possessing much longer beaks than the latter. They identified the organisms as *A. cyamopsidis* Rangaswami and Rao.

Yogendra *et al.* (1995) recorded disease intensity under varying conditions on guar cv. Pusa Navbahar in Kanpur, U.P. Maximum disease

intensity was recorded at 25-31°C, 80 per cent RH and high rainfall. The susceptibility of plants, gradually declined with increasing age. Orellana and Simmons (1966) while surveying in the guar plantations in U.S.A., found maximum infection on leaves, stems and pods in the period between blooming and capsule formation. The disease was found to be destructive in periods of excessive rainfall and humidity. Singh (1970) although mentioned that alternaria blight of guar was widely spread throughout Rajasthan and was responsible for heavy losses, but the exact information on its occurrence and severity in different localities of Udaipur district in Rajasthan are not available.

### 2.1.3 Anthracnose of Yam :

Massee (1908) was first to report anthracnose on the leaves of cultivated yam and identified its causal organism as *Gloeosporium pestis*, subsequently Averna Sacca (1917) described *Colletotrichum dioscoreae*, perhaps as pathogen of the same disease (Chupp and Sherf, 1960). Goto (1929) reported a similar disease, as black dots from Formosa on *Dioscorea alata* and *D. batatas*. Later, Parham (1935) reported leaf and stem wilt of yam from Fiji and recorded failure of tuber production in the crop due to *Gloeosporium pestis* Ward (1938) attributed the leaf spot disease of white and yellow yams, *D. alata* and *D. cayennensis* to the same fungus. Baudin (1956) found anthracnose caused by *Glomerella cingulata* to be a common disease in yam at Ivory coast. In an attempt to revise the genus *Gloeosporium*, Von Arx (1957 a) recognized two species of *Colletotrichum* on *Dioscorea* viz., *C. gloeosporioides* and *C. dematium* which were

understood to be the conidial stages of *Glomerella cingulata* (Stonem) Spaul and Schrenk and *C. capsici* (Syd.) Bulter and Bisby, respectively. He considered *G. pestis*, *C. dioscoreae* and *G. bomplandii* (reported on *Dioscorea*) as synonyms of *C. gloeosporioides* Penz. causing serious disease on *Dioscorea alata*.

Prasad and Singh (1960) described a disease on *Dioscorea alata* from India showing initial brown pin head spots on leaves and stems which later enlarged and coalesced to impart a glazed black colour to stems; leaf spots also coalesced ultimately leading to withering and shedding. These workers found the symptomatology closely resembling the disease symptoms described earlier by Goto (1929) identifying the causal organism as *Colletotrichum gloeosporioides*. Singh *et al.* (1966) reported the pathogen to be the host specific and renamed it as *C. gloeosporioides* f. sp. *alatae*.

### 2.1.4 Maydis Leaf Blight of Maize :

Leaf blight of maize (*Zea mays* L.) also known as southern corn leaf blight because of its being more wide spread in the southern United States, is incited by *Bipolaris* maydis (Nisikado and Miyake) Shoemaker (*Cochliobolus heterostrophus* (Drechsler) Drechsler] commonly known as *Helminthosporium maydis* Nisikado and Miyake.

The disease was first observed in Florida (U.S.A.) on maize and on maize and teosinte from the Philippines by Drechsler (1925). The perfect stage first discovered by Drechsler (1925), was then designated as *Ophiobolus heterostrophus*. Later, Drechsler (1934) erected a new genus, *Cochliobolus* to accommodate the perfect stage of this fungus and

accordingly it came to be known as *Cochliobolus heterostrophus* (Drechsler) Drechsler. The asexual stage of this fungus was described in 1926 from Japan by Nisikado and Miyake as *Helminthosporium maydis* Nisikado and Miyake. Ito (1930) erected the genus *Drechslera* to which this maize pathogen was transferred. Later on, Shoemaker (1959) proposed a new generic name *Bipolaris* for the gramicolous species having fusoid phragmospores with polar germination using *B. maydis* as lactotype. However, Luttrell (1964) disagreed with Shoemaker and recognised *Helminthosporium* as a generic entity and the two broad divisions, cylindro-Helminthosporium (Drechslera) and Eu-Helminthosporium (*Bipolaris*) as subgenera. Subramaniam and Jain (1966) suggested that all the graminicolous Helminthosporia should be accommodated in a single genus *Drechslera* Ito. While Kenneth (1983) suggested genus *Bipolaris* for those species having fusoid conidia with innate hilum, germinating normally from end cells. Under article 50 of the international code of Botanical Nomenclature the valid name of the perfect stage (telomorph) takes precedence over that of the imperfect stage (Anamorph). Accordingly the fungus has been designated as *Bipolaris maydis* (Nisikado and Miyake) Shoemaker anamorph of *Cochliobolus heterostrophus* (Drechsler) Drechsler. It, however, continues to be commonly referred to as *Helminthosporium maydis*.

In India, the disease was first reported by Munjal and Kapoor (1960) on the basis of specimens collected from Malda (W. Bengal) by Butler in 1905. Singh and Singh (1966) also reported it from Kanpur and adjoining

areas where the disease was most severe on variety T-41. The disease was first reported from Udaipur district of Rajasthan by Kothari (1964).

Drechsler (1925) described the symptoms on the basis of material collected from Florida and Philippines as "a destructive disease manifested by the appearence on the leaves of numerous dead cinnamon buff or purplish areas surrounded by a darker reddish brown margin and often delicately varigated with brownish zonate bands, the leisons longitudinally elongated, typically limited to a single intervascular region, often coalescing to form more extensive dead portions."

Orillo (1949) reported that *H. maydis* can infect the leaf sheath, husk of the ears as well as the leaf blade. He also noticed root infection in inoculated soil in green house. Infection on stems and midribs occurred only on wounding under high relative humidities. Robert (1956) observed blackening of silks and ear tips of sweet corn in Florida.

In the year 1970, extreme susceptibility of maize cultivars carrying cytoplasmic male sterility (Texas male sterile, TMS) was reported from the states of Iowa, Illinois, Indiana and Minnesota (Ullstrup, 1970 and 1972) which resulted in an epidemic. It was ascribed to the appearance of a new phenotype designated as race "T" of *H. Maydis* (Hooker *et al., 1970). Nelson* et al. (1970) tested pathogenic behaviour of isolates collected from different countries including USA during 1955 and found that some of them were similar to those characterized as race "T".

Hooker (1971) described the differences between the race "T" and race "O" in detail with respect to symptoms, specificity to cytoplasms, toxin

production, reproductive rate, relation to environment and other respects. The most obvious differences in the two races were in plant parts infected and in symptoms produced. The old common race"O" was primarily a leaf pathogen, producing smaller lesions with parallel sides and little chlorosis. Race "T" however, affected several plant parts i.e., leaves, sheaths, husk-leaves, shank, ear and stalk tissues.

Khehra *et al.* (1976) reported race "T" on maize in India. They observed the disease on leaf sheaths, husks, ear tips and kernels of experimental hybrids 2310 and 2410. Shurtleff *et al.* (1976) reported the lesions caused by race "O" as tan, 0.6 x 1.2 to 1.9 cm long, with parallel sides and buff to brown borders. Those formed on race "T" were longer (0.6 to 1.2 x 0.6 to 2.7 cm) spindle shaped or elliptical, with yellow green or chlorotic halos. Sharma *et al.* (1978) confirmed the findings of Shurtleff *et al.* under glasshouse conditions. Bera and Giri (1979) isolated race "O" and "T" of *H. Maydis* from leaves and kernels of maize collected from different parts of India. Same variation in these symptoms have been reported by different workers (Robert, 1956; Misra and Singh, 1975; Lakshmanan *et al.*, 1988; Sisterna, 1998; Johal and Briggs, 1994 and Isota *et al.*, 1996) Laxmi (1985) studied morphological and cultural variation in the ten isolates of *H. Maydis* collected from different agroclimatic zones of the country and found variation in spore dimensions and cultural characters. Jain (1989) observed that eight isolates of H. Maydis showed difference in cultural and morphological characters.

## 2.2 REVIEW ON PLANT EXTRACTS

A number of plants found in India have been successfully used both for therapeutic as well as poisonous purposes (Chopra *et al.*, 1965).

Shekhawat and Prasad (1971) found that garlic and onion gave good inhibition of spore germination of *Alternaria tenuis, Helminthosporium sativum* and *Curvularia penniseti* due to presence of allincin in garlic and protocatechuic acid and catechol in onion which were responsible for bursting in young hyphae of fungus. Afifi and Dowidar (1977) observed that volatile compounds emitted from extracts of *Ocimum bacillum* and *Origanum majorana* reduced spore germination and spore respiration of number of *Fusarium* species.

Egawa *et al.* (1977) found that antifungal substances extracted from leaves of eleven out of twenty seven *Eucalyptus* species inhibited conidial germination of *Alternaria solani* and *Cochliobolus miyabeanus*. Singh and Sharma (1978) observed anti-fungal activity of crude extracts of thirty four Indian flowering plants, out of these, twenty five strongly inhibited the growth of *Fusarium oxysporum*. Kumar *et al.* (1979) tested aqueous extract of different parts of onion, garlic, parthenium and kalanchoe tested *in vitro* against *Fusarium oxysporum* and other pathogens and observed complete inhibition of spore germination.

Kapoor *et al.* (1981) indicated that extracts from *Convolvulus alsinoids* and *Convolvulus pluriculis* were almost completely fungicidal against spore germination and mycelial growth of *Fusarium oxysporum*. Prasad and Simlot (1983) showed that preparation from the fruit of bitter

Temru was effective in controlling the wilt of arhar caused by *Fusarium udum*. Seed treatment was more effective than root drenching. Chaudhary *et al.* (1998) also reported inhibition of *Fusarium udum*, *Colletotrichum capsici*, *Alternaria tenuis* and *Helminthosporium maydis* from partially purified components extracted from bitter Temru fruits.

Maroon *et al.* (1984) observed that crude extract of *Amaranthus spinous* and *Leucanena leucocephala* reduced development of cercospora leaf spot of mungbean. Babu and Reddy (1986) reported that extracts of *Eucalyptus globulus*, Pomegranate, *Lawsonia inermis* and *Datura stramonium* were effective in checking fruit rot of lemon caused by *Colletotrichum gloeosporioides* and *Botrydiplodia theobromae*. Pre-inoculation treatments were more effective than post-inoculation treatments.

Singh *et al.* (1986) showed that exudate and extract of both young and mature leaves of spinach stimulated linear growth and conidial germination of *Alternaria alternata*, *Cladosporium cladosporioidis*, *Curvularia lunata*, *Drechslera australiensis* and *Fusarium oxysporum* but in *Cercospora bataticola*, growth and germination were inhibited by exudates of young leaves only.

Sinha and Saxena (1987) found that treating of tomato fruits with leaf extract of *Aderocalymma alliacea* protected the fruit from rot. Eswaramurthy *et al.* (1989) observed that extracts of *Azadirachta indica*, *Ipomea canea* and *Acacia arabia* inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *capae* and *Sarocladium oryzae*. Desevedavy *et al.* (1989)

reported that out of 49 crude extracts of higher plants, an ethanol extract of *Bellis perennis* had the greatest antifungal activity against *Ceratocystis ulmi*. Mishra *et al.* (1989) showed that essential oils isolated from leaves of 11 species of higher plants were effective against *Aspergillus flavus* at 2000, 3000, 4000 and 5000 ppm concentrations. The oils of *Chaenopodium ambrosioides, Cinnamomum zeylanicum, Citrus medica, Ocimum canum* and *Ocimum graltissimum* were most effective at 2000 ppm concentration. The others were effective at higher concentrations only.

Singh *et al.* (1990) reported that antifungal activity of ajonene, a compound derived from garlic, inhibited spore germination of *Fusarium lini, F. oxysporum, F. semitectum* and *F. udum* causing serious diseases in some important crop plants in India. Khan and Rishi Kumar (1990) studied the anti-fungal activity of leaf extract of neem (*Azadirachta indica*) on wheat seed mycoflora and found that seed treatment resulted in a marked reduction in seed mycoflora and enhanced seed germination.

Shrivastava *et al.* (1990) evaluated the fungitoxicity of leaves of 12 plants species (*Abutilon indicum, Amaranthus spinosus, Collistemon lancedolus, Cinnamomum camphora, Jatropha curcas, Ranunculus scleratus, Euphorbia hirta, Mussaeand fondosa, Cymbopogon martinii, Santalum album, Zingiber officinalis* and *Zinnia elegans*) for their volatile toxicity against *Fusarium oxysporum* f. sp. *udum*. Out of these *Cymbopogan martinis* exhibited the strongest toxicity. Yasmeen and Saxena (1990) showed that extracts of the ferns, *Adiantum caudatum, Diplazium esculentum* and *Pteris vittata* reduced growth and germination of *Alternaria*

*brassicola* and *Aspergillus niger*. Rhizome extracts were more toxic than leaf extracts. Chauhan and Singh (1991) reported that volatiles from extracts of garlic and onion bulbs inhibited spore germination of *Phytophthora drechsleri* f. sp. *cajani* at 500 and 1000 ppm concentration., respectively.

Singh and Tripathi (1992) reported that the leaf extracts of *Aderocalymma alliacea* and *Artabotrys hexapetalus* were found to be cent per cent fungitoxic out of 40 different plant extracts tried against *Fusarium oxysporum* f. sp. *lentis* causing wilt of lentil. Sarvamangla *et al.* (1993) concluded that *Eucalyptus* species and *Calotropis gigantea* were highly toxic to *Cercospora moricola* at 25% concentration and inhibited conidial germination by 91.5% and 91.3% respectively. Dhanpal *et al.* (1993) experimented with the aqueous extracts of neem leaves, unripened fruit neem seeds and also commercially available neem based formulation such as neem oil, kemisal, Azabin BSB and neem gold against rhizome rot disease of small cardimom. They observed 80-100% growth inhibition of pathogen with all the products tested *in vitro*. Extract from neem seed and neem gold greatly reduced the disease followed by other neem products.

Singh *et al.* (1993) reported that extracts of some medicinal plants including *Calotropis procera*, *Vitex negunds*, *Lantana camara*, *Azadirachta indica*, *Ficus religiosa*, *Ocimum sanctum*, *Datura fastusa* and *Ricinus cummunis* gave good control of *Fusarium oxysporum* and out of these plants, *Azadirachta indica* and *Ocimum sanctum* found to be the most effective against the same.

Chandrasekhar *et al.* (1994) reported that application of potash and *Azadirachta indica* seed kernel extract were effective in controlling tikka leaf spot of groundnut caused by *Cercospora arachidicola* and *Phacoisariopsis personata*. Soil application of 55 kg/ha $K_2O$ plus one per cent foliar application of one per cent *Azadirachta indica* seed kernel extract significantly reduced the incidence of tikka leaf spot disease. Ezhilan *et al.* (1994) reported that extracts of *Caesalpinia pulcherrima*, *Eucalyptus globulus*, sesame oil cake, *Calophllum inophyllum* oil cake, *Prosopis juliflora* and *Thevitia peruviana* (2.5, 5.0 and 10%) were effective against *Rhizoctonia solani*. Maximum inhibition was caused by *Thevitia peruviana* followed by *Prosopis juliflora* and *Eucalyptus globulus* (31.7, 39.7 and 40.3% germination, respectively). Mahapatra and Tewari (1994) showed that ethanol and essential oil extracts of *Ocimum sanctum* inhibited growth and multiplication of *Aspergillus niger* and *A. flavus* and increased seed germination of groundnuts. In green house studies, ethanol extract gave best control of both pathogens.

Ahmed and Prasad (1995) evaluated the aqueous foliar extracts of *Adhatoda varica*, *Azadirachta indica*, *Catharanthus roseus*, *Datura fistulosa*, *Lantana camera*, *Murraya exotica*, *Ocimum sanctum*, *Ricinus cummunis* and *Strychnos nuxvomica* against soft rot disease of *Luffa cylindrica* fruit caused by *Helminthosporium spiciferum* and *Fusarium scirpi*. They observed that conidial germination of *Fusarium scirpi* and *Helminthosporium spicifer* were reduced to 75 per cent when their spores were treated with *Azadirachta indica*, *Catharanthus roseus*, *Datura fistulosa*, *Lantana camera* and *Ocimum sanctum*.

Bist and Khulbe (1995) examined the leaf extract of ten plants against *Drechslera oryzae*. All the tested extracts exhibited fungitoxic properties and suppressed the mycelial growth of fungus. Maximum inhibition of fungal growth (84 %) was recorded in *Juglans regia* and it was at par with *Allium sativum*.

Ganeshan and Krishnaraju (1995) reported that leaf extracts of 23 out of 36 plant species tested against *Drechslera oryzae* exhibited fungitoxic properties. Extract of *Leucas aspera*, *Polygonum chinense* and *Spermacoce articulaus* inhibited spore germination and remaining extracts inhibited the growth of germinated spores.

Sobti *et al.* (1995) examined the effect of three plant extracts and two fungicides on three groundnut pathogens (*Aspergillus niger*, *Macrophomina phaseolina* and *Aspergillus flavus*) and reported that Thiram, Carbendazim and extracts of plants (*Azadirachta indica*, *Polyalthia longifolia*, *Ocimum gratissimum*) significantly inhibited mycelial growth of the fungi. However, fungicides being followed by *Polyalthia longifolia* extract.

Qasem and Abu-blan (1995) reported that aqueous extracts of different weed species reduced the colony growth of three plant pathogens (*Penicillium digitatum*, *Sclerotinia sclerotiorum* and *Verticillium dahliae*), however, the inhibitory effect varied between extracts. *Chenopodium murale*, *Cripis aspera* and *Ranunculus asiaticers* extracts completely inhibited the growth of *Penicillium digitatum* after four days of incubation. Extracts of *Erodium cruciatum*, *Euphorbia helioscopia* and *Ranunculum asiaticers* completely inhibited the growth of *Sclerotinia sclerotiorum* after

four days of incubation. Extracts of *Euphorbia helioscopia*, *Galium tricornutum*, *Sisymbrium irio* and *R. asiaticers* were the most toxic to *Verticillium dahliae* and significantly reduced the colony growth compared with the untreated control for all periods of incubation.

Chatterjee *et al.* (1996) studied the antifungal activities of various fractions extracted from *Coriaria nepalensis* against *Alternaria solani*, *Helminthosporium oryzae* and *Macrophomina phaseolina* and reported total inhibition for some fractions. Dellar *et al.* (1996) reported the antifungal activity of two polyoxygenated fatty acids isolated from aerial parts of *Aedlanthus parvifolius* which inhibited spore germination of *Cladosporium cucumerinum*.

Gohil and Vala (1996) studied the activities of 33 plant extracts against *Fusarium moniliformae in vitro*. They found garlic and *Sopindus trifoliata* were inhibitory, garlic extract being the most effective. Gupta *et al.* (1996) reported that leaf extract of *Pongamia pinnata* was highly fungitoxic to *Fusarium pallidorseum* and *F. moniliformae*, inhibiting mycelial growth on plates by 78.2% and 84.3%, respectively. Further out of five plant extracts tested, *Calotropis gigantea* and *Azadirachta indica* were most effective against *Fusarium oxysporum*.

Kumar *et al.* (1996) showed that leaves of *Lantana camera* possessed six phenolic acids in substantial quantity. These on decomposition possess a potentiality to act against soil fungi, *Fusarium oxysporum* and *Pythium* species. Shekar *et al.* (1996) tested the aqueous extracts of leaves of different plants against *Pythium aphanidermatum*. Out of 50 plants tested,

extracts from *Adenocalymna alliaceum*, *Allium sativum*, *Bougainvillia glabra*, *Carum capticum*, *Lantana indica* and *Moringa oleifera* completely inhibited the growth of the pathogen. Singh *et al.* (1996) investigated the volatile antifungal activity of aromatic plants on the soil microflora of field crops and reported that root extract of *Fimbristytis juncifarmis* significantly reduced radial growth of *Fusarium oxysporum*. Qasem and Abu-blan (1996) studied effect of aqueous extracts of 64 weed species on growth and development of *Alternaria solani*, *Helminthosporium sativum* and *Rhizoctonia solani* in *in vitro*. They reported that extracts varied in strength and persistence of their antifungal effects against the three fungi. Among all species tested, extracts of *Chenopodium murale, Falearia vulgaris, Ramunculus asiaticers* were most toxic to *Alternaria solani* while *Anagallis arvensis*, *Atriplex leucoclada*, *Crepis aspera*, *Runex cripus*, *Vicia norbonensis* were toxic to *Helminthosporium sativum* and *Ramunculus asiaticus*, *Mercurialis annua* to *Rhizoctonia solani*.

Rana *et al.* (1997) reported that essential oil isolated from leaves of *Aegle marmelos* inhibited the spore germination of eight phytopathogenic fungi. Cent per cent inhibition of spore germination was observed for all species at 500 ppm concentration. However, *Fusarium udum* was inhibited by 80 per cent at 400 ppm concentration. Shivpuri *et al.* (1997) reported that ethanol leaf extract of *Azadirachta indica*, *Datura stramonium*, *Ocimum sanctum*, *Polyalthia longifolia* were most toxic against *Alternaria brassicola*, *Colletotrichum capsici*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*.

Suresh *et al.* (1997) reported that two limonoids extracted from neem tree reduced disease intensity considerably in groundnut rust disease (*Puccinia arachidis*). Ushamalini *et al.* (1997) evaluated 21 botanicals and reported that plant extract of *Vitex negundo*, *Adenocalymma alliaceum* and *Ocimum sanctum* inhibited mycelial growth and sclerotial germination of *Macrophomina phaseolina* effectively and the growth and spore germination of *Fusarium oxysporum* f. sp. *trachciphilum* was effectively inhibited by *Abutilon indicum*, *Delonix regia* and *Acalypha indica*. Rodriguez and Valendia (1998) studied the effect of extract of 10 medicinal plants against *Colletotrichum* species *in vitro*. Among them only clove (*Syzygium aromaticum*) inhibited the growth and sporulation of fungus.

Saju *et al.* (1998) studied the antifungal activity of essential oils from turmeric (*Curcuma longa*) against *Colletotrichum gloeosporioides*, *Rhizoctonia solani* and *Aspergillus* species and observed that all concentrations of oil (1-5%) inhibited the growth of *Colletotrichum gloeosporioides* by 100 per cent, *Rhizoctonia solani* by 79 per cent and *Aspergillus* species by 53.2 per cent. Srivastava and Srivastava (1998) tested the antifungal activities of leaf extracts of *Allium cepa*, *Argemone mexicana*, *Calotropis procera*, *Datura metel*, *Ocimum sanctum*, *Rauvolfia serpentina*, *Tagetes erecta* and *Catharanthus roseus* and reported that maximum spore germination of *Alternaria alternata* was inhibited by leaf extracts of *Argemone mexicana*.

Annapurna *et al.* (1999) reported that methanol and aqueous extracts of the leaves of *Saraca asoca* showed good antifungal activity against

*Alternaria alternata*, *Colletotrichum gloeosporioides* and *Drechslera spiciferum*. Chandi Ram *et al.* (1999) tested the seed coat and seed extracts of *Lagenaria siceraria* and *Luffa aegyptica* against spore germination of *Alternaria alternata*, *Aspergillus niger*, *Fusarium oxysporum*, *Gibberella fujikuroi* and *Rhizopus stolonifer* and recorded that maximum spore germination of *Alternaria alternata* was inhibited by *Luffa aegyptica* and *Fusarium oxysporum* by *L. siceraria*. Jain and Singh (1999) reported that Triterpenoids, isolated from the hexane fraction of plants of *Heliotropium subulatum* exhibited antifungal activity against *Aspergillus niger*, *A. flavus*, *Rhizoctonia phaseoli* and *Penicillium crysogenum*.

Karade and Sawant (1999) tested extracts of twelve medicinal and wild plants against *Alternaria alternata* and observed that cent per cent spore germination was inhibited by the extract of *Allium sativum* and minimum spore germination was inhibited with the extract of *Lantana camera*.

Sarma *et al.* (1999) tested 15 extracts of common weed species against *Rhizoctonia solani* and observed that 90 per cent growth inhibited by the extracts of *Solanum nigrum* at 20 per cent concentration.

Saxena and Sharma (1999) reported that essential leaf oil extracts from *Lantana aculeata* showed good antifungal activity against *Penicillium digitatum*, *P. notatum*, *Rhizopus stolonifer* and *Microspermum gypsum*.

Gehlot and Bohra (1999) tested the antifungal activity of root extracts of 25 arid zone plants *in vitro* against *Macrophomina phaseolina*, the causal agent of different diseases in moth bean (*Vigna aconitifolia*). They reported

that alcoholic and aqueous root extracts of *Fagonia cretica*, the aqueous root extract of *Tribulus terrestris*, alcoholic extracts of *Ocimum americanum*, *Calotropis procera* and *Euphorbia antiquorum* roots had severe growth inhibitory effects against *M. phaseolina*.

Bowers and Locke (2000) carried out experiment in soil infested with *Fusarium oxysporum* f. sp. *chrysanthemi* and treated it with 1, 5 and 10 per cent aqueous emulsions of formulated extract of clove (70% clove oil), neem (90% neem oil), pepper mustard (chilli pepper extract and essential oil of mustard), cassia (extract of cassia tree) in separate experiments. The pepper mustard, cassia and clove extracts suppressed disease development in repeated experiments (80-100% healthy plant stand) compared with the untreated infested soil (less than 20% stand). Datar (2000) studied the bioefficacy of different plant extracts *in vitro* against *Macrophomina phaseolina*, the incitant of charcoal rot of sorghum. Among various extracts, leaf extracts of *Polyalthia longifolia* was the most effective in reducing mycelial growth followed by *Parthenium hysterophorus*, *Tabernaemontana coronaria*, *Calotropis procera* and *Ipomea carnea*. Out of four bulb/rhizome extracts, *Allium sativum* was most effective and of the flower extract, *Parthenium hysterophorus* was most effective.

Roat (2000) screened seven partially purified plant product preparations against *Colletotrichum capsici* and *Alternaria alternata* from chilli under *in vitro* conditions and reported the effectivity of bitter Temru fruits and Datura leaves components. Similarly, Sharma (2000) reported the effectiveness of bitter Temru fruits and Datura leaves components against

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*Fusarium oxysporum*, *Alternaria alternata*, *Aspergillus niger* and *Rhizopus stolonifer* isolated from tomato fruits under *in vitro* conditions. Singh *et al.* (2002) evaluated botanicals against seed borne fungus causing root rot disease in chickpea and all of the plant extracts at 20% concentration resulted in significant disease control but maximum control was observed with garlic extract followed by turmeric extract.

Kamalakannan *et al.* (2001) screened leaf extracts of 20 plant species for their inhibitory effect against the rice blast pathogen, *Pyricularia grisea* (*Magnoporthe grisea*). They reported that the mycelial growth was significantly inhibited by *Prosopsis juliflora* followed by *Ziziphus jujuba* and *Abutilon indicum*.

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# Chapter 3

# Materials and Methods

# MATERIALS AND METHODS

## 3.1 COLLECTION OF DISEASE MATERIAL

Infected tomato fruits were collected from vegetable market of Udaipur and brought to laboratory in polythene bags.

Infected plant materials of guar and maize showing symptoms of Alternaria blight and Helminthosporium maydis blight, respectively were collected from fields of nearby area. The potent infected materials were collected carefully and brought to the laboratory in paper bags.

Infected materials of anthracnose of yam were collected from Jhadol area of Udaipur district and brought to laboratory in paper bags.

## 3.2 ISOLATION, PURIFICATION AND IDENTIFICATION

### 3.2.1 Isolation :

Fresh isolations were made from the naturally infected tomato fruit portions collected from the market. The fruits were thoroughly washed in running tap water. The small bits cut from the infected fruit portion were surface sterilized by dipping in 0.1 per cent mercuric chloride ($HgCl_2$) solution for one to two minutes followed by three washings by sterilized distilled water and finally transferred aseptically on PDA (Potato Dextrose Agar) medium in petriplates. These plates were incubated at 28 ± 1°C in incubator chamber for three to four days for the growth of the fungus.

Similarly, fresh isolations were made from the naturally infected leaf of yam, guar and maize collected from different fields. The leaves were thoroughly washed in running tap water. The small bits cut from the infected leaf portion were surface sterilized by dipping in 0.1 per cent mercuric chloride ($HgCl_2$) solution for one to two minutes followed by three washings by sterilized distilled water and finally transferred aseptically on PDA medium in petriplates. These plates were incubated at 28 ± 1°C in incubator chamber for three to four days for the growth of the fungi.

### 3.2.2 Purification :

When adequate colony growth of the fungi were obtained in petri plates, the cultures from uncontaminated peripheral growth were sub cultured on PDA slants. Purification of the fungi were done by single sporing technique. The spore suspension was made in sterile distilled water and the dilution was so adjusted that in one loopful, 25-30 spores could be observed under the low power of microscope. One such loopful was mixed with 25 ml of melted sterilized plain agar (2%) and poured aseptically in sterilized petriplates. After 10-12 hours of incubation, the germinating spores were located under the microscope and one of them was picked and transferred to PDA slants and kept at 28 ± 1°C for growth. After completion of growth, the slants were stored at 10°C in refrigerator for further use.

### 3.2.3 Identification :

After purification each fungus was allowed to sporulate. The sporulating cultures were identified on the basis of morphological characters of somatic and reproductive structures including spores. Fungi isolated were

identified as *Fusarium oxysporum* f. sp. *lycopersici* from tomato, *Alternaria cyamopsidis* from guar, *Colletotrichum gloeosporioides* from yam and *Helminthosporium maydis* from maize. They were further identified and confirmed authentically from Indian type culture, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi.

## 3.3 GLASS WARES USED

In all the experiments, Corning or Borosoil made glass wares were used. Before conducting the experiments, the glass wares were cleaned with 6 per cent chromic acid ($K_2Cr_2O_7$- 60 gm; Conc. $H_2SO_4$ - 60 ml. and distilled water 1000 ml) followed by several washing in running tap water and finally cleaned with distilled water and air dried.

## 3.4 STERILIZATION

The medium used was sterilized at 1.036 kg/cm$^2$ for 30 minutes in an autoclave while all the glass wares were sterilized in the hot air oven at 180°C for 2 hours. The cork borer, inoculation needle, for-ceps, etc were initially dipped in rectified spirit and finally sterilized on flame of spirit lamp and allowed to cool down before use.

## 3.5 PATHOGENICITY TEST

Green mature tomato fruits were bought from market to the laboratory in paper bags and were stored at 28-30°C for three days to become semi ripe. These fruits were then surface sterilized by dipping in mercuric chloride solution (0.1%) for two minutes followed by three

washings in sterilized distilled water and inoculated with *Fusarium oxysporum* f.sp. *lycopersici* which was grown on PDA in petriplates separately for seven days and two mm disc cut out from the periphery of cultural growth were used for inoculation.

For inoculation in fruits, a hole of two mm diameter upto two mm depth was made with the sterilized cork borer. The inoculum was placed in the middle of the hole and the hole was plugged with the host tissue corked out earlier.

Four fruits were inoculated with the fungus and were kept in sterilized polythene bag. A piece of sterilized wet absorbent cotton was placed inside the bag to maintain moisture and the mouth of the bag was loosely tied.

The bagged fruits were kept at 28 ± 1°C and were observed daily for the appearance of the characteristic symptoms. Parallel control was maintained in which fruits were not inoculated with the fungus. The fungus was reisolated and compared with the earlier culture.

To carry out the pathogenicity test of anthracnose of yam, the inoculum was sprayed on one and a half to two months old yam vines grown in earthen pots. Inoculum consisted of conidial suspension (20-25 spores per low power field of microscope) of *Colletotrichum gloeosporioides*, made in sterile distilled water from seven days old growth occurring on PDA medium. Spore suspension was sprayed thoroughly by an atomizer on the plants which were later kept constantly in humid condition for 24 hrs and finally transferred to green house conditions for development of disease

symptoms. Re-isolations of fungus pathogen were made from infected portions and cultures thus isolated were then compared with the original ones. The plants sprayed with distilled water served as control.

In case of pathogenicity test for alternaria blight of guar, the same method as mentioned for yam were adopted and variety Pusa Nav Bahar (PNB) was used.

To carry out the pathogenicity test for leaf blight of maize incited by *Helminthosporium* [illegible] *maydis*, the variety 'Mahi kanchan' was grown in 2 x 3 meters plot in the field. Tests were conducted on about 30 days old plants. For preparation of the inoculum, the pure culture was grown for 8-10 days in petriplates on PDA. A thick conidial suspension was made in water and strained through muslin cloth. Final concentration of the spores was kept at 20-25 spores per low power field of the microscope. Inoculations were made by atomizing 2-5 ml of spore suspension per test plant in evening time. Infection began as small flecks after 48 hrs and symptoms appeared after 4-5 days of inoculations. Re-isolations were made from eight days old flecks and the purified cultures were compared with the original.

## 3.6 COLLECTION OF PLANT MATERIALS FOR EXTRACTION OF COMPONENTS

The following commonly found plant parts were collected and used for extraction of partially purified components :

| S.No. | Name of Plant | Part used as extract |
|---|---|---|
| 1. | Sandal (*Centalum album* L.) | Fruits |
| 2. | Babool (*Acacia nilotica* L.) | Seeds |
| 3. | Custard apple (*Annona squamosa* L.) | Seeds |
| 4. | Amaltas (*Cassia fistula* L.) | Seeds |
| 5. | Satyanashi (*Argemone mexicana* L.) | Seeds |
| 6. | Brhati (*Solanum indicum* L.) | Fruits |
| 7. | Bitter Temru (*Diospyros cordifolia* Roxb.) | Fruits |
| 8. | Datura (*Datura stramonium* L.) | Leaves |
| 9. | Mehandi (*Lawsonia inermis* L.) | Seeds |

Plant materials viz., Sandal, Amaltas, Datura and Mehandi were collected from R.C.A. Farm, Babool, Satyanashi, Brhati and bitter Temru were collected from different localities within Udaipur city whereas Custard apple was bought from market. All of these plant parts were air dried in open till they become wholly dry and then ground in a grinder to a fine powdry mass.

## 3.7 EXTRACTION OF PARTIALLY PURIFIED COMPONENTS FROM PLANT PARTS

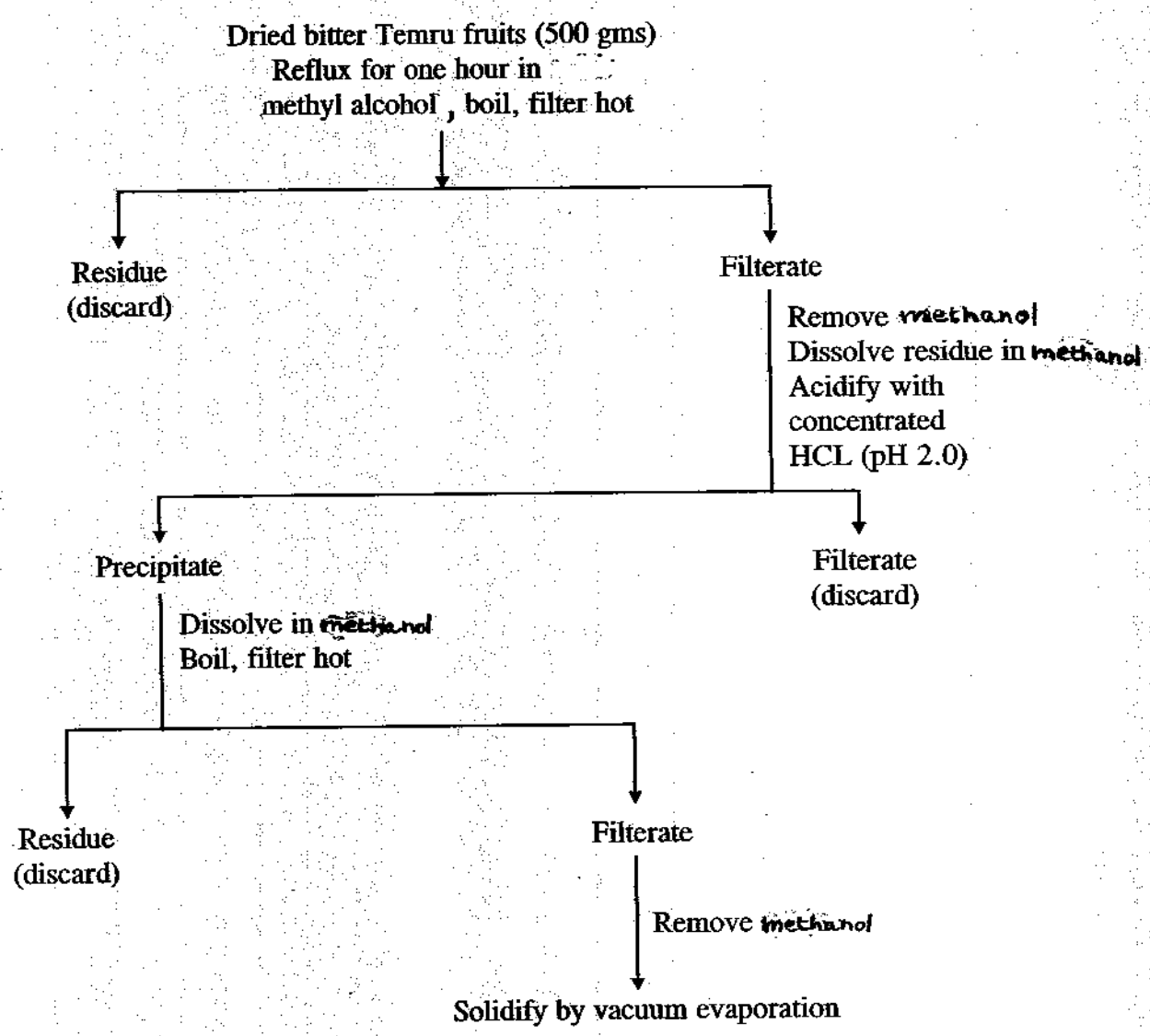
### 3.7.1 Extraction of Partially Purified Component from Bitter Temru Fruits:

Extraction of component from bitter Temru fruits was done in accordance with the method developed by Sharma and Simlot (1971).

Mature bitter Temru fruits at green stage were collected from the plants and air dried in the shade till their colour changed from pale yellow to dark brown to black.

Then finely ground dried fruits powder weighing 500 gms was refluxed with methyl alcohol for anh. and boiled for three hr and filtered through muslin cloth. The residue was discarded and filtrate was kept back for further purification. The which was added earlier in the extract was removed by tion method. When the volume of the filtrate reached one third of total volume, the boiling was stopped. Then acidified with concentrated hydrochloric acid (pH 2.0) and again filtered through filter paper. The filtrate was discarded and precipitate was dissolved in and boiled. After boiling, strained through filter paper and filtrate was taken. The of filtrate was removed to one third of volume and then extract was solidified by vacuum evaporation method.

Fig. : Extraction of partially purified components from bitter Temru fruits :



### 3.7.2 Extraction of Partially Purified Component from Datura Leaves :

Method given by Nicholis (1945) was adopted for the extraction of partially purified component from Datura leaves. It was carried out in following ways :

1. Liberation of free base by treatment of the dried material with alkali viz., $Ca(OH)_2$, $Na_2CO_3$ or $NH_4OH$.
2. Extraction of free base with an organic solvent e.g. chloroform, diethyl ether, benzene, methanol, ethanol by soxhlet extraction.
3. Removal of free base by shaking with dilute HCl or $H_2SO_4$.

Majority of the alkaloids and their salts are soluble in ethanol, this solvent was used without preliminary treatment with alkali. After removal of ethanol, residue was treated with dilute acid and solution was filtered in a weighed beaker. The alkaloids were then extracted from acid solution by making it alkaline and shaking with ether and chloroform separately. The organic solvent were removed by evaporation and the beaker was weighed. The extracted alkaloids were determined by subtracting the weight of empty beaker.

### 3.7.3 Extraction of Partially Purified Components from other Plants :

The procedure adopted for extraction of components of other plant parts viz., Amaltas, Babool, Mehandi, Brhati, Custard apple, Satyanashi, Sandal were similar as mentioned earlier for bitter Temru fruit extraction.

## 3.8 MANAGEMENT OF PLANT PATHOGENS

Partially purified plant products components isolated from different plants were tested at three different concentrations viz., 500, 1000 and 2000 ppm. The following methods were adopted for management of plant pathogens *in vitro* :

1. Poison food technique.
2. Spore germination technique.

### 3.8.1 Poison Food Technique :

The antifungal activity of partially purified plant products components was tested *in vitro* against mycelial growth of various plant pathogens by poison food technique at different concentrations viz., 500, 1000 and 2000.

The different desired amount of partially purified products components were incorporated separately in PDA contained in conical flasks aseptically, thoroughly agitated and 20 ml of medium was poured in each petriplates. These petridishes were inoculated with the mycelial bits (2 mm diameter) taken from the periphery of seven days old culture of the test pathogen and incubated at 28 ± 1°C. All the experiments were replicated thrice. A control was also maintained where no plant product component was added in PDA.

Observations were recorded on radial mycelial growth along the two diagonals through the centre of colony after six days. Growth inhibition percentage was calculated by the following formula.

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

Where,

| | | |
|---|---|---|
| I | = | Inhibition percentage |
| C | = | Average diameter of colony in control. |
| T | = | Average diameter of colony in treatment. |

### 3.8.2 Spore Germination Technique :

To conduct the experiment, on the efficacy of partially purified plant products components from plants, on spore germination of plant pathogens, cavity slide method (Singh *et al.*, 1986) was followed.

The spore suspension was prepared from seven days old culture of pathogens. The spore concentration was adjusted to 10 to 14 spores per low microscope field. The spore suspension was made in sterilized distilled water. A loopfull of spore suspension was put in each cavity slide and one loopful of partially purified products components was mixed with spore suspension. These cavity slides were kept in moist chamber and incubated at 28 ± 1°C for 24 hrs. In addition, a parallel control was also maintained in which no plant products components was added. The observation was calculated on per cent spore germination by observing the number of spores germinated in microscopic field (x 100) on each cavity slide. Per cent spore germination was calculated by using the following formula :

$$\textit{Per cent spore germination} = \frac{\textit{Germinated spores}}{\textit{Total number of spores}} x\ 100$$

## 3.9 EFFICACY OF MOST EFFECTIVE PARTIALLY PURIFIED PLANT PRODUCT COMPONENTS AND FUNGICIDES AGAINST MAYDIS LEAF BLIGHT OF MAIZE *IN VIVO*

The studies were conducted on the maydis leaf blight on maize incited by *Helminthosporium maydis*. The studies were conducted at two different locations, one at Rajasthan College of Agriculture (RCA) Farm, Udaipur and the other at a farmer's field at village Kanpur of Udaipur district.

The field experiments were conducted in Randomized block design for the control of maydis leaf blight, incited by *Helminthosporium maydis*, using "Mahi Kanchan" variety during the kharif season of the year 2001 at two different locations, one at RCA farm and the another at a farmer's field at Kanpur village of Udaipur district. The dimensions of the fields were 11 x 16 meters each. There were ten treatments which included most effective partially purified product component of bitter Temru fruits (1000 and 2000 ppm concentrations), Datura leaves (1000 and 2000 ppm concentrations) and two fungicides viz., Indofil M-45 (1000 and 2000 ppm concentrations) and Bavistin (1000 and 2000 ppm concentrations) including two treatments of control where in one distilled water was sprayed and in another no water was sprayed. The treatments were tested as post inoculation sprays i.e. fungicidal and plant products sprays were given 48 hrs after inoculation with the fungi, *Helminthosporium maydis*.

Inoculation was repeated, four days after the first inoculation. Similarly, the sprays were repeated. Observations after 10 days of last

spray for disease severity/intensity was recorded on randomly selected 10 plants from each plot.

The fields were divided into three blocks and each block had ten plots. The dimension of each plot was 2 x $2^1/_2$ meters. The seeds were sown at 75 cms row to row of distance and 20 cms plant to plant distance. Thus there were thirty six plants in each plots.

### 3.9.1 Inoculation Technique :

Mycelial and conidial suspension prepared as mentioned earlier, in the pathogenicity test of *H. maydis* in maize, with 20-25 spores per low power of the microscope was put in the plastic wash bottles and shaked thoroughly before and while inoculating the plant. The plants were inoculated by pressing out 5-8 ml of spore suspension from bottles in the whorl of each plant at the age of 30 ± 5 days.

### 3.9.2 Procedure of Rating Maydis Leaf Blight Disease Reaction :

To measure the amount of disease, a disease rating scale was developed. The scale consists of six broad categories designated by numerals 0 to 5. The rating scale was developed considering only the inoculated area of the leaf as the disease development was not vigorous as such due to adverse seasonal conditions prevailed during the kharif season of 2001. The rating scale used is as follows :

0. No infection

1. Very slight to slight infection (Minor flecks) - 1 to 10 per cent.

2. Flecks - 11 to 25 per cent

3. Small spots - 26-50 per cent

4. Small to medium spots -51 to 75 per cent

5. Bigger spots and coalescing - more than 76 per cent.

**Infection index of maydis leaf blight :**

It was worked out using formula given by Mckinney, 1923; Chester, 1959 and Wheeler, 1969 which is as follows :

$$\textit{Infection Index (I.I.)} = \frac{\textit{Sum of Numerical rating}}{\textit{Number of plants/leaves assessed}} \times \frac{100}{\textit{maximum disease rating}}$$

**Per cent efficiency of disease control [PEDC (maydis leaf blight)]**

Infection index data were computed to calculate the per cent efficiency in disease control using following formula :

$$P.E.D.C. = \frac{\textit{Infection Index in control} - \textit{Infection index in treatment}}{\textit{Infection index in control}} \times 100$$

# *Chapter 4*

# *Experimental Results*

# EXPERIMENTAL RESULTS

## 4.1 ISOLATION, PURIFICATION AND IDENTIFICATION

Isolations were carried out from infected diseased tissues of tomato (*Lycopersicon esculentum* Mill.) fruits collected from the market of Udaipur and the fungus, *Fusarium oxysporum* f. sp. *lycopersici*, was found to be associated with the infected tissues of tomato fruits causing rot of fruits. After isolation of the pathogen, the culture was purified by adopting single spore culture technique (Plate III-A).

Similarly isolations were made from the infected leaf portions of yam (*Dioscorea alata* L.), guar [*Cyamopsis tetragonoloba* (Linn.) Taub.] and maize (*Zea mays* L.) collected from different fields of Udaipur region and the pathogens viz., *Colletotrichum gloeosporioides* f. sp. *alatae* inciting anthracnose, *Alternaria cyamopsidis* Rangaswami and Rao causing leaf blight and *Helminthosporium maydis* Nisikado and Miyake causing leaf blight were found to be invariably associated with the infected leaf tissues of the aforementioned crops respectively. After isolations, the cultures were purified by adopting single spore culture technique (Plate III-A).

The identification of pure cultures of pathogens were done on the basis of morphological traits of somatic and reproductive structures including spores. The cultures were identified and confirmed authentically

by Indian type culture collection, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.

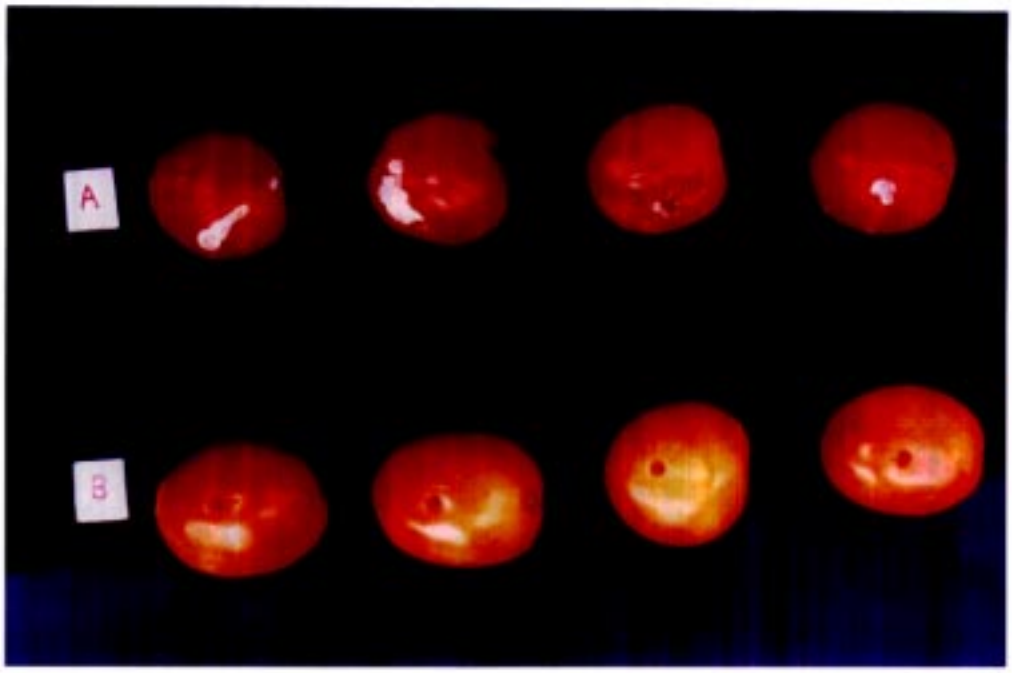
## 4.2 PATHOGENICITY TEST

Green mature tomato fruits were inoculated with the isolated fungus at 28 ± 1°C for conducting the pathogenicity test. It was observed that same symptoms of the fruit rots, as was seen originally, developed on the inoculated fruits, after six days of inoculation (Plate I-A). The fungus was reisolated from the infected fruits which showed the same cultural characteristics as that were observed earlier. The control maintained had no development of infection.

In case of pathogenicity test of anthracnose of yam, the same symptoms developed on leaves, as were observed originally, after four days of inoculation (Plate I-B). The fungus was reisolated from the infected leaves which showed the same cultural characteristics as that were observed earlier. The control showed no development of infection. Similarly, in case of leaf blight of guar, the same symptoms appeared on the leaves, as were seen originally, after five days of inoculation (Plate II-A). The fungus was reisolated from the infected leaves of guar which showed the same cultural characteristics as that of original one. The control maintained had no infection. The same was in case of leaf blight of maize, where identical symptoms appeared on leaves, as was observed earlier, after five days of inoculation (Plate II-B). The fungus was reisolated from the infected

PLATE I. A. Symptoms observed in pathogenicity test of *Fusarium oxysporum* f.sp. *lycopersici* on tomato fruits
A. Inoculated B. Control

B. Symptoms observed in pathogenicity test of *Colletotrichum gloeosporioides* on yam leaves




# I-A

# I-B

PLATE II A. Symptoms observed in pathogenicity test of *Alternaria cyamoposidis* on guar leaves

B. Symptoms observed in pathogenicity test of *Helminthosporium maydis* on maize leaves

II-A

II-B

leaves of maize which showed the same cultural characteristics as that was observed earlier. The control showed no development of infection.

## 4.3 EFFICACY OF PARTIALLY PURIFIED PLANT PRODUCTS COMPONENTS AGAINST DIFFERENT PATHOGENS *IN VITRO*

Efficacy of partially purified plant products components viz., Amaltas (*Cassia fistula*), Babool (*Acacia nilotica*), bitter Temru (*Diospyros cordifolia*), Brhati (*Solanum indicum*), Custard apple (*Annona squamosa*), Datura (*Datura stramonium*), Mehandi (*Lawsonia inermis*), Sandal (*Santalum album*) and Satyanashi (*Argemone mexicana*), were tested against four plant pathogenic fungi viz., *Fusarium oxysporum*, *Colletotrichum gloeosporioides*, *Alternaria cyamopsidis* and *Helminthosporium maydis* isolated from tomato fruits, yam leaves, guar leaves and maize leaves, respectively.

It was observed that different partially purified plant products components were found effective in controlling these plant pathogenic fungi. Two methods were adopted for testing the efficacy of partially purified plant products components *in vitro*.

(1) Poison Food Technique

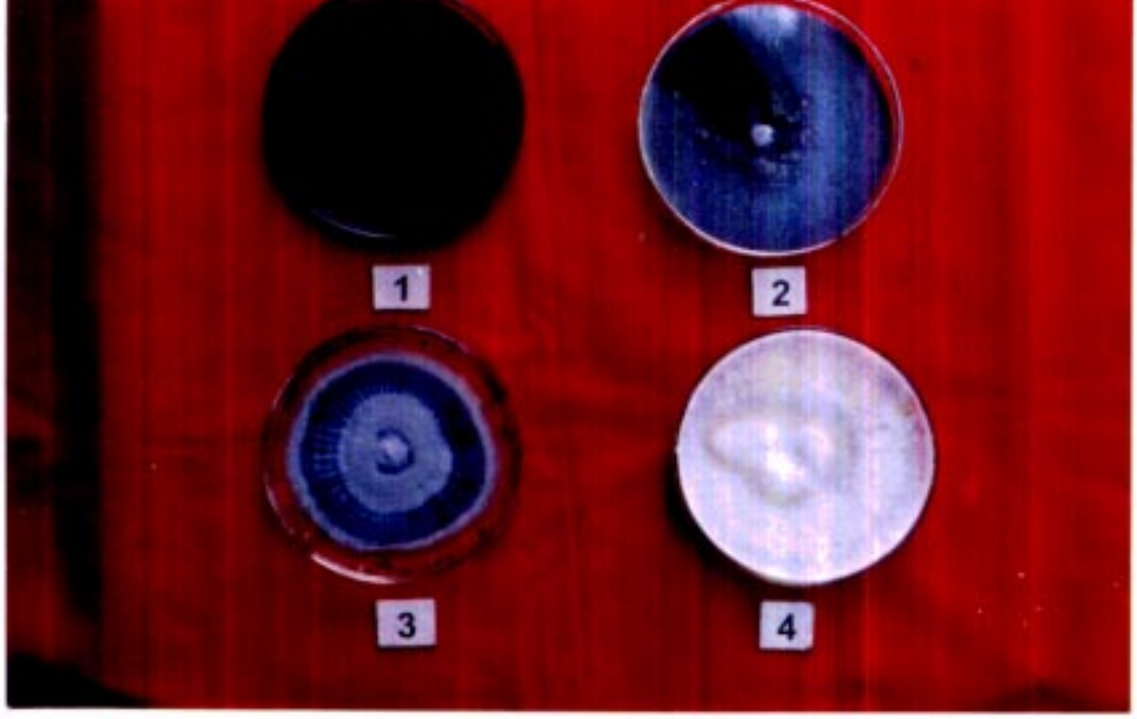
(2) Spore Germination Technique

### 4.3.1 Poison Food Technique :

An experiment was carried out *in vitro* to see the effect of partially purified plant products components isolated from Amaltas seeds, Babool

PLATE III A. *Alternaria cyamoposidis, Helminthosporium (Bipolaris) maydis, Colletotrichum gloeosporioides* and *Fusarium oxysporum f. sp. lycopersici* isolated respectively from guar leaves, maize leaves, yam leaves and tomato fruits.

B. Maize plant showing symptoms of maydis leaf blight in control plot in the field.




III - A

III - B

seeds, bitter Temru fruits, Brhati fruits, Custard apple seeds, Datura leaves, Mehandi seeds, Sandal seeds and Satyanashi seeds, on the mycelial growth of the plant pathogenic fungi, *Alternaria cyamopsidis*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides* and *Helminthosporium maydis* by poison food technique at different concentrations of 500, 1000 and 2000 ppm. Observations were recorded on mycelial growth along the two diagonals through the centre of colony growth after six days.

Poison food technique studies revealed that different partially purified plant products components varied widely in their efficacy to inhibit the mycelial growth of plant pathogenic fungi viz., *Fusarium oxysporum* from tomato, *Colletotrichum gloeosporioides* from yam, *Alternaria cyamopsidis* from guar and *Helminthosporium maydis* from maize.

**4.3.1.1 *Fusarium oxysporum* :**

The results depicted in Table 1, Fig. 1 and Plate IV-A reveal that maximum inhibition of the mycelial growth of the fungus, *Fusarium oxysporum* f. sp. *lycopersici* was observed with partially purified plant product component prepared from bitter Temru fruits (36.20%) after six days of incubation at 28 ± 1°C at 2000 ppm concentration over the control. The next best inhibition of the mycelial growth of above fungus was found with partially purified plant product component prepared from Datura leaves (33.01%) followed by Sandal seeds (22.47%), Satyanashi seeds (21.95%), Babool seeds (19.76%), Custard apple seeds (15.19%), Mehandi seeds (14.63%), Amaltas seeds (14.44%) and Brhati fruits (14.06%) after six days of incubation at 28 ± 1°C at 2000 ppm concentration over the control.

The same partially purified plant products components viz., Amaltas seeds, Babool seeds, bitter Temru fruits, Brhati fruits, Custard apple seeds, Datura leaves, Mehandi seeds, Sandal seeds and Satyanashi seeds were found effective at low concentrations of 1000 and 500 ppm also but were not as much effective as at 2000 ppm concentration.

Sandal seeds and Satyanashi seeds components were at par statistically. Similarly, Custard apple seeds, Mehandi seeds, Amaltas seeds and Brhati fruits components were also at par. But all of these treatments (Sandal seeds, Satyanashi seeds, Custard apple seeds, Mehandi seeds, Amaltas seeds and Brhati fruits) including Babool seeds were significantly different with other treatments, namely bitter Temru fruits and Datura leaves components. Bitter Temru fruits and Datura leaves components showed a significant difference with all the treatments at 2000 ppm concentration.

#### 4.3.1.2 *Colletotrichum gloeosporioides* :

The results presented in Table 2, Fig. 2 and Plate IV-B reveal that maximum growth inhibition of the mycelial growth of the fungus, *Colletotrichum gloeosporioides* was observed with partially purified plant product component prepared from bitter Temru fruits (25.37%) after six days of incubation at 28 ± 1°C at 2000 ppm concentration over the control. The next best inhibition of the mycelial growth of the above fungus was found with partially purified plant product component prepared from Datura leaves (18.70%) followed by Satyanashi seeds (17.77%), Babool seeds (17.59%), Mehandi seeds (13.51%), Amaltas seeds (11.85%), Sandal seeds

**Table 1. Efficacy of partially purified plant products components against tomato fruit rot pathogen, *Fusarium oxysporum* f.sp. *lycopersici* by poison food technique *in vitro***

| Treatments | Percent mycelial growth inhibition | | | |
|---|---|---|---|---|
| | Concentrations | | | Mean |
| | 2000 ppm | 1000 ppm | 500 ppm | |
| Amaltas (*Cassia fistula*) | 14.44 | 14.44 | 2.80 | 10.56 |
| Babool (*Acacia nilotica*) | 19.76 | 13.12 | 13.31 | 15.40 |
| Bitter Temru (*Diospyros cordifolia*) | 36.20 | 30.57 | 20.07 | 28.95 |
| Brhati (*Solanum indicum*) | 14.06 | 16.31 | 15.00 | 15.13 |
| Custard apple (*Annona squamosa*) | 15.19 | 16.03 | 12.94 | 14.72 |
| Datura (*Datura stramonium*) | 33.01 | 17.44 | 8.06 | 19.51 |
| Mehandi (*Lawsonia inermis*) | 14.63 | 16.69 | 15.09 | 15.47 |
| Sandal (*Santalum album*) | 22.47 | 9.07 | 1.86 | 11.14 |
| Satyanashi (*Argemone mexicana*) | 21.95 | 9.94 | 15.38 | 15.76 |
| Control | 0.00 | 0.00 | 0.00 | 0.00 |
| Mean | 21.30 | 15.96 | 11.62 | 16.29 |

| | SEm± | C.D at 5% | CV% |
|---|---|---|---|
| Treatments (T) | 0.21 | 0.60 | 3.94 |
| Concentrations (C) | 0.12 | 0.35 | |
| T X C | 0.37 | 1.04 | |

Fig. 1 **Efficacy of partially purified plant products components against tomato fruit rot pathogen, *Fusarium oxysporum* f.sp. *lycopersici by* poison food technique *in vitro***

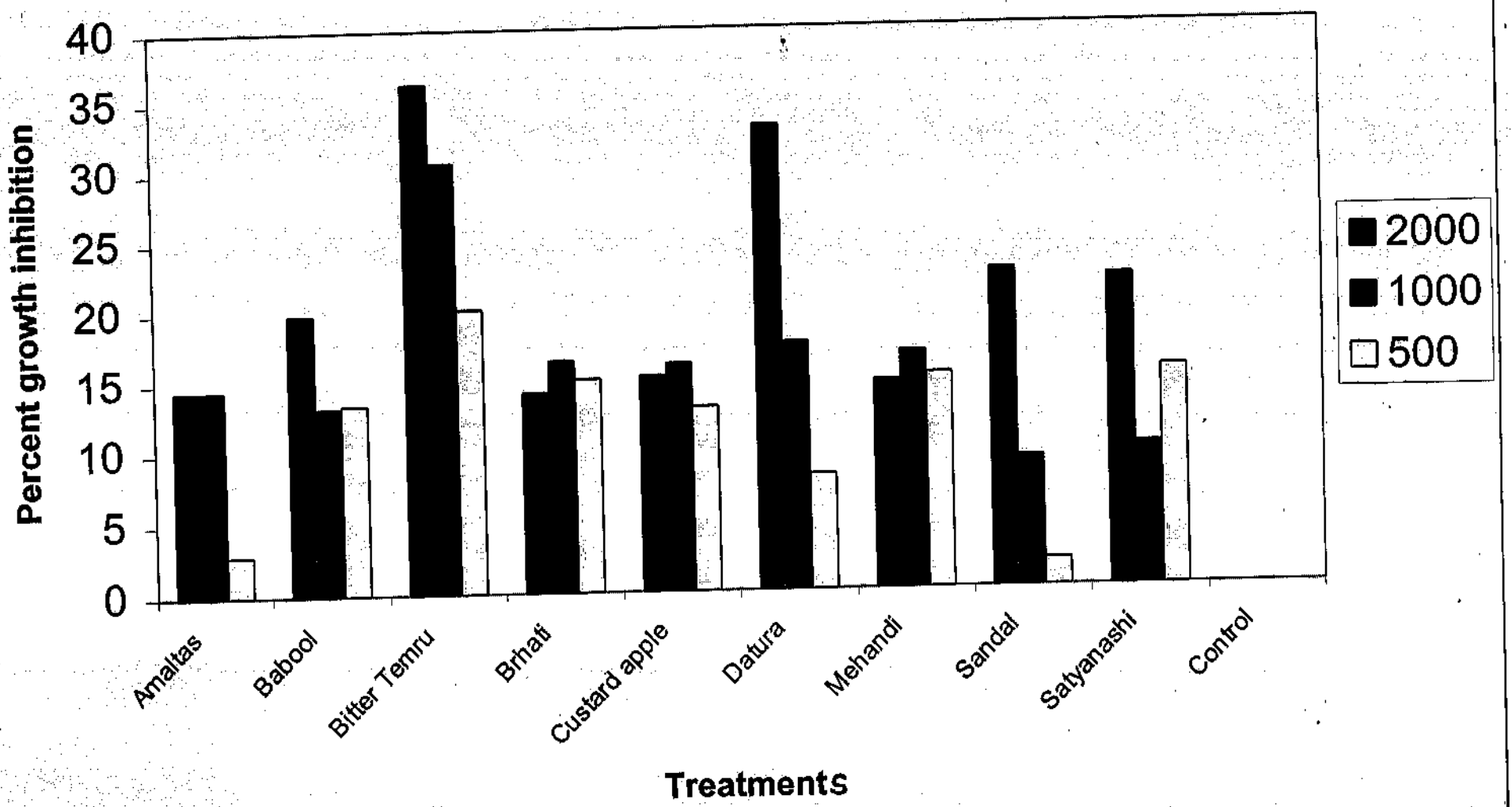


PLATE IV A. Effect of partially purified plant product components against tomato fruit rot pathogen, *Fusarium oxysporum* f.sp. *lycopersici* by poison food technique *in vitro.*

1. Amaltas, 2. Brhati, 3. Babool,
4. Custard apple, 5. Bitter Temru, 6. Mehandi,
7. Satyanashi, 8. Sandal, 9. Datura

D.Control

A. 500 ppm concentration

B. 1000 ppm concentration

C. 2000 ppm concentration

B. Effect of partially purified plant product components against yam anthracnose pathogen, *Colletotrichum gloeosporioides* by poison food technique *in vitro.*

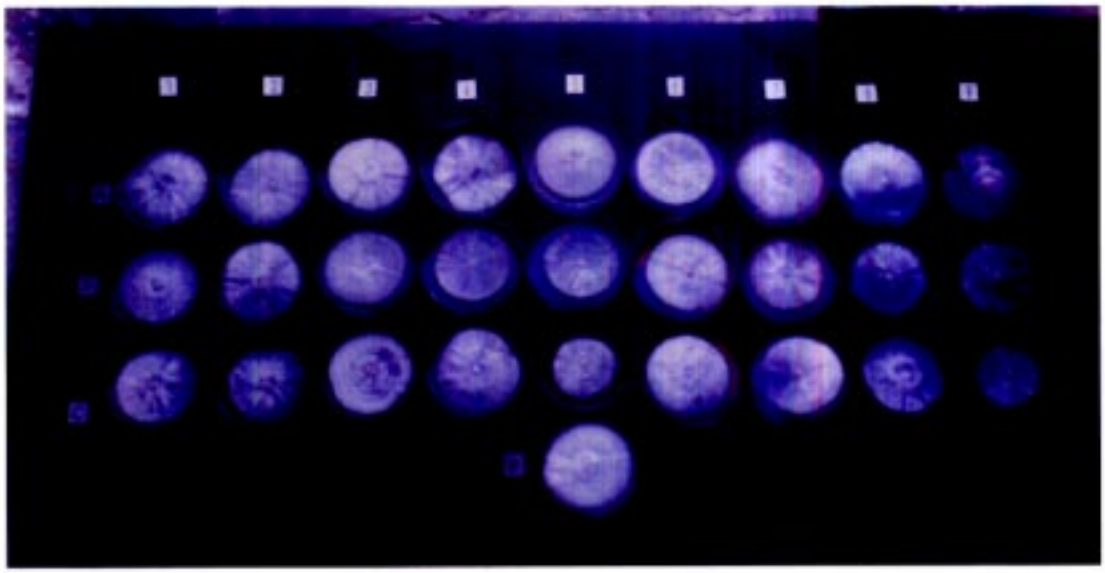
1. Amaltas, 2. Brhati, 3. Babool,
4. Custard apple, 5. Datura, 6. Mehandi,
7. Satyanashi, 8. Sandal, 9. Bitter Temru

D.Control

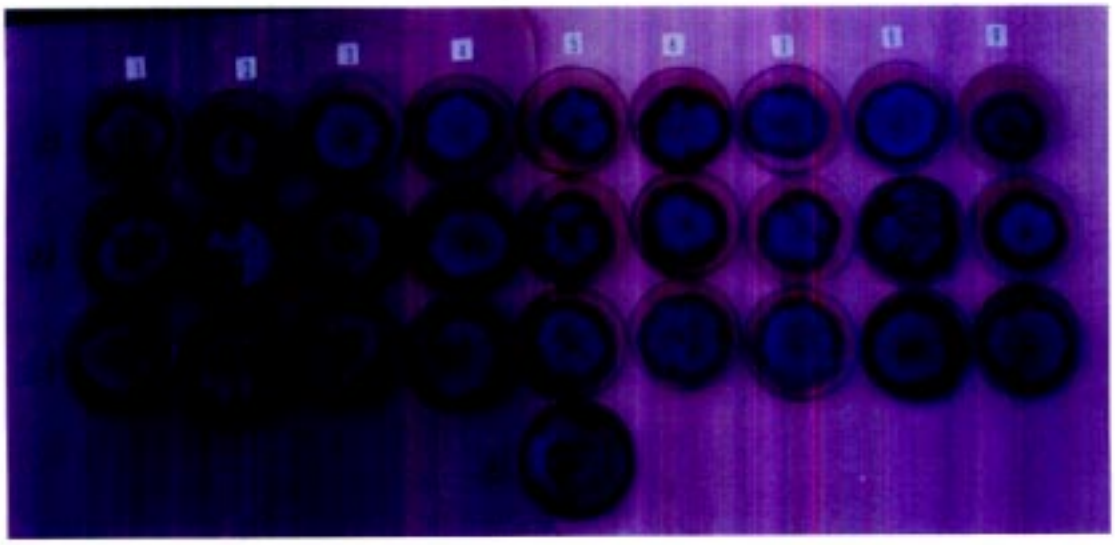
A. 2000 ppm concentration

B. 1000 ppm concentration

C. 500 ppm concentration



# IV-A



# IV-B

(11.66%), Custard apple seeds (11.11%) and Brhati fruits (9.8%) after six days of incubation at 28 ± 1°C at 2000 ppm concentration over the control. The low concentrations (1000 and 500 ppms) of these partially purified plant product components viz., Amaltas seeds, Babool seeds, bitter Temru fruits, Brhati fruits, Custard apple seeds, Datura leaves, Mehandi seeds, Sandal seeds and Satyanashi seeds, were also found somewhat effective.

Both Satyanashi seeds and Babool seeds components were at par statistically. Similarly, Amaltas seeds, Sandal seeds and Custard apple seeds components were found at par. But all these treatments (Satyanashi seeds, Babool seeds, Amaltas seeds, Sandal seeds and Custard apple seeds components) and Mehandi seeds and Brhati fruits components were significantly different with other treatments viz., bitter Temru fruits and Datura leaves components at 2000 ppm concentrations.

**4.3.1.3 *Alternaria cyamopsidis* :**

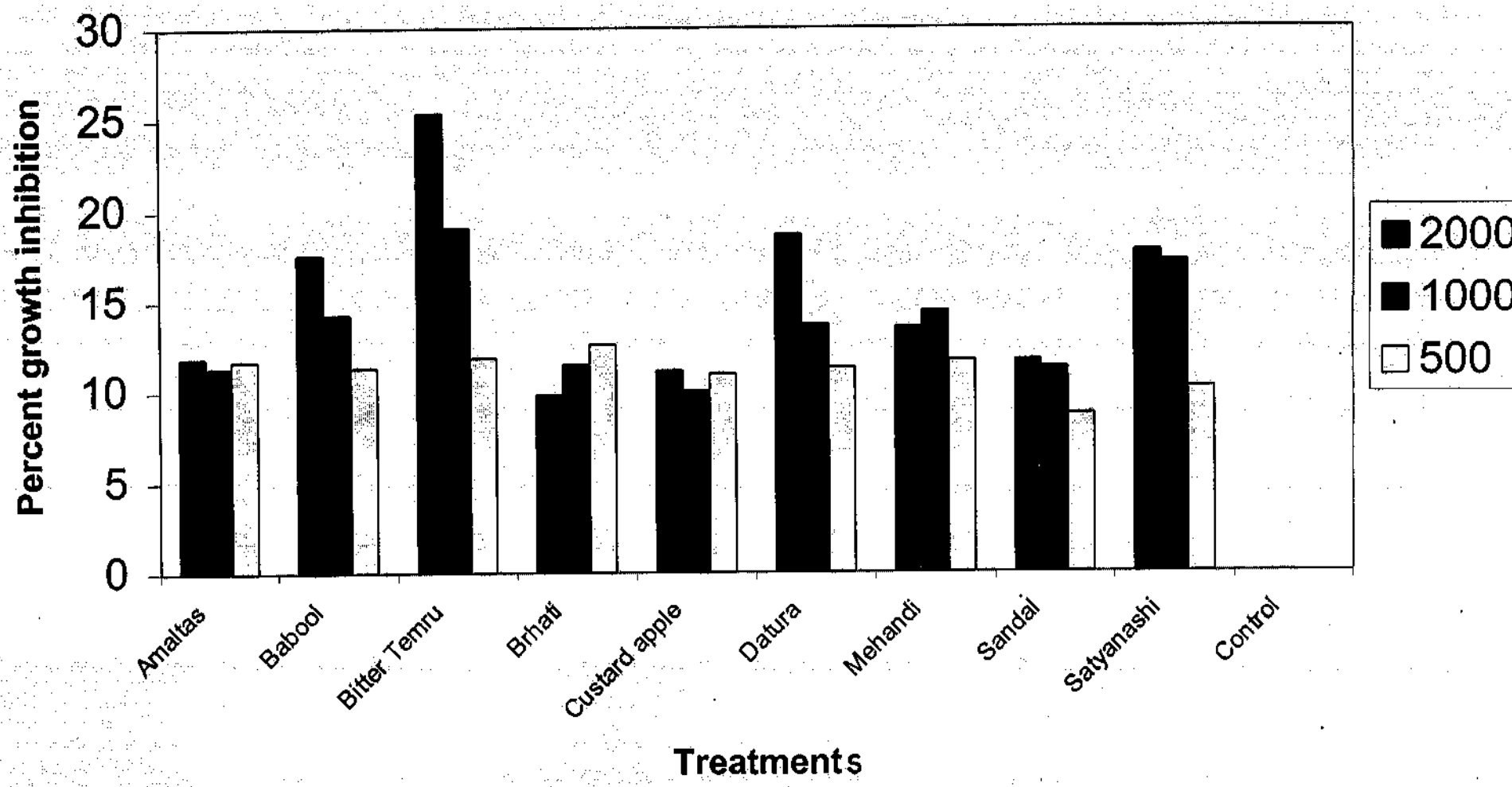
The results showed in Table 3, Fig. 3 and Plate V-A reveal that maximum inhibition of the mycelial growth of the fungus, *Alternaria cyamopsidis* was observed with partially purified plant product components prepared from bitter Temru fruits (69.17%) after six days of incubation at 28 ± 1°C at 2000 ppm concentration over the control. The immediate best inhibition of the above fungus was found with partially purified plant product component prepared from Datura leaves (64.50%) followed by Satyanashi seeds (64.13%), Custard apple seeds (63.75%), Amaltas seeds (60.77%), Babool seeds (58.71%), Sandal seeds (57.59%), Mehandi seeds (57.22%) and Brhati fruits (51.43%) after six days of incubation at 28 ± 1°C

**Table 2. Efficacy of partially purified plant products components against yam anthracnose pathogen, *Colletotrichum gloeosporioides* by poison food technique *in vitro***

| Treatments | Percent mycelial growth inhibition | | | |
|---|---|---|---|---|
| | Concentrations | | | Mean |
| | 2000 ppm | 1000 ppm | 500 ppm | |
| Amaltas (*Cassia fistula*) | 11.85 | 11.29 | 11.66 | 11.60 |
| Babool (*Acacia nilotica*) | 17.59 | 14.25 | 11.29 | 14.38 |
| Bitter Temru (*Diospyros cordifolia*) | 25.37 | 19.06 | 11.84 | 18.76 |
| Brhati (*Solanum indicum*) | 9.81 | 11.48 | 12.58 | 11.29 |
| Custard apple (*Annona squamosa*) | 11.11 | 9.99 | 10.92 | 10.68 |
| Datura (*Datura stramonium*) | 18.70 | 13.69 | 11.29 | 14.56 |
| Mehandi (*Lawsonia inermis*) | 13.51 | 14.44 | 11.66 | 13.21 |
| Sandal (*Santalum album*) | 11.66 | 11.29 | 8.70 | 10.55 |
| Satyanashi (*Argemone mexicana*) | 17.77 | 17.21 | 10.18 | 15.06 |
| Control | 0.00 | 0.00 | 0.00 | 0.00 |
| Mean | 15.26 | 13.64 | 11.13 | 13.34 |

| | SEm± | C.D.at 5% | CV% |
|---|---|---|---|
| Treatments (T) | 0.16 | 0.47 | 3.80 |
| Concentrations (C) | 0.09 | 0.27 | |
| T X C | 0.29 | 0.82 | |

Fig. 2. **Efficacy of partially purified plant products components against yam anthracnose pathogen, *Colletotrichum gloeosporioides* by poison food technique *in vitro***



at 2000 ppm concentration over the control. The same partially purified plant products components viz., Amaltas seeds, Babool seeds, bitter Temru fruits, Brhati fruits, Custard apple seeds, Datura leaves, Mehandi seeds, Sandal seeds and Satyanashi seeds, were found effective at low concentrations of 1000 and 500 ppms also.

Datura leaves and Satyanashi seeds components were at par statistically and Custard apple seeds components were at par with Satyanashi seeds components. Similarly, Sandal seeds and Mehandi seeds components were at par. The bitter Temru fruits component was found significantly different with all other treatments (Amaltas seeds, Babool seeds, Brhati fruits, Custard apple seeds, Datura leaves, Mehandi seeds, Sandal seeds and Satyanashi seeds) at 2000 ppm concentration.

#### 4.3.1.4 *Helminthosporium (Bipolaris) maydis* :

The data depicted in Table 4, Fig. 4 and Plate V-B show that maximum inhibition of the mycelial growth of the fungus, *Helminthosporium maydis* was observed with partially purified plant product component extracted from bitter Temru fruits (73.70%) after six days of incubation at 28±1°C at 2000 ppm concentration over the control. The next best inhibition of mycelial growth of the above fungus was found with partially purified plant product components prepared from Satyanashi seeds (68.14%) followed by Datura leaves (67.03%), Babool seeds (54.81%), Custard apple seeds (51.48%), Mehandi seeds (50.37%), Amaltas seeds (44.07%), Brhati fruits (43.70%) and Sandal seeds (39.25%) after six days of incubation at 28±1°C at 2000 ppm concentration over the control. The

PLATE V A. Effect of partially purified plant product components against guar leaf blight pathogen, *Alternaria cyamopsidis* by poison food technique *in vitro*.

1. Amaltas, 2. Brhati, 3. Babool,
4. Datura, 5. Custard apple, 6. Mehandi,
7. Satyanashi, 8. Sandal, 9. Bitter Temru
D. Control

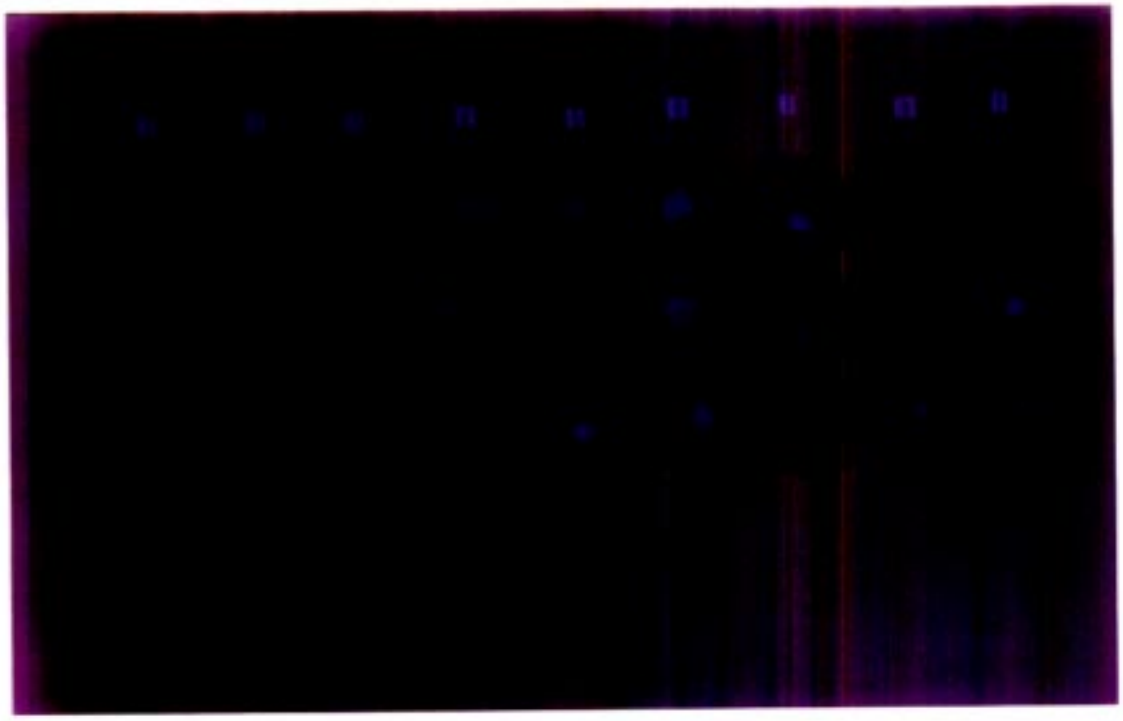
A. 500 ppm concentration
B. 1000 ppm concentration
C. 2000 ppm concentration

B. Effect of partially purified plant product components against maydis leaf blight pathogen, *Helminthosporium maydis* by poison food technique *in vitro*.

1. Amaltas, 2. Brhati, 3. Babool,
4. Custard apple, 5. Satyanashi, 6. Mehandi,
7.Datura, 8. Sandal, 9. Bitter Temru
D.Control

A. 500 ppm concentration
B. 1000 ppm concentration
C. 2000 ppm concentration

# V-A



# V-B

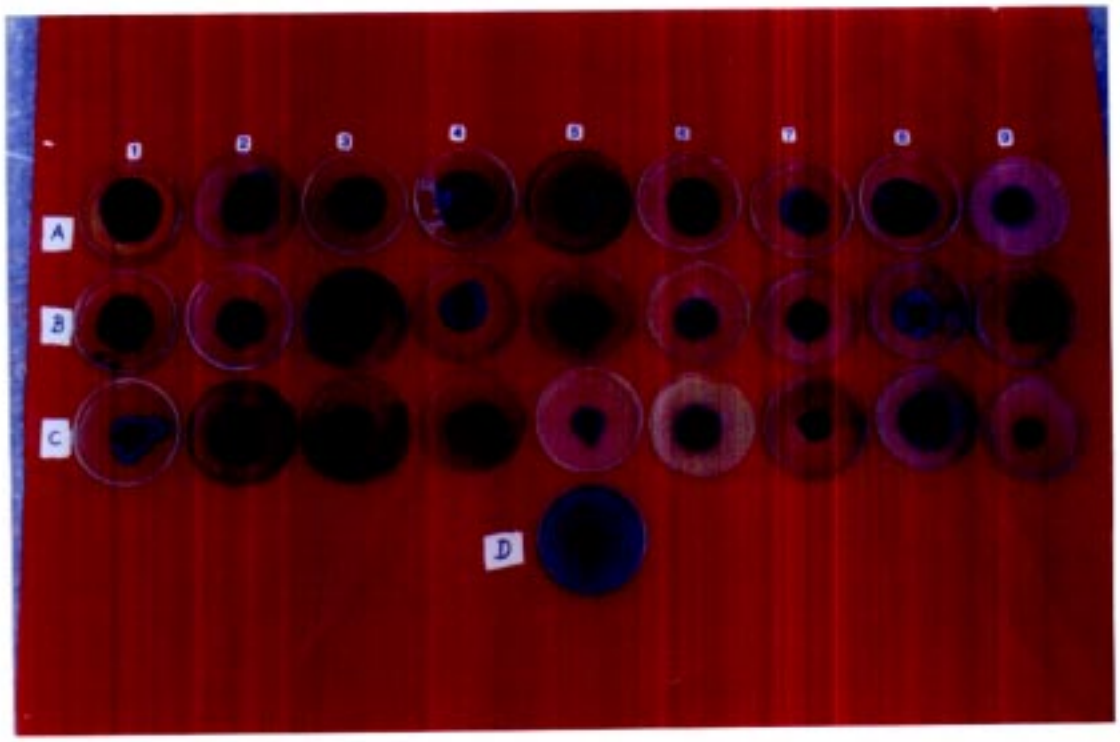
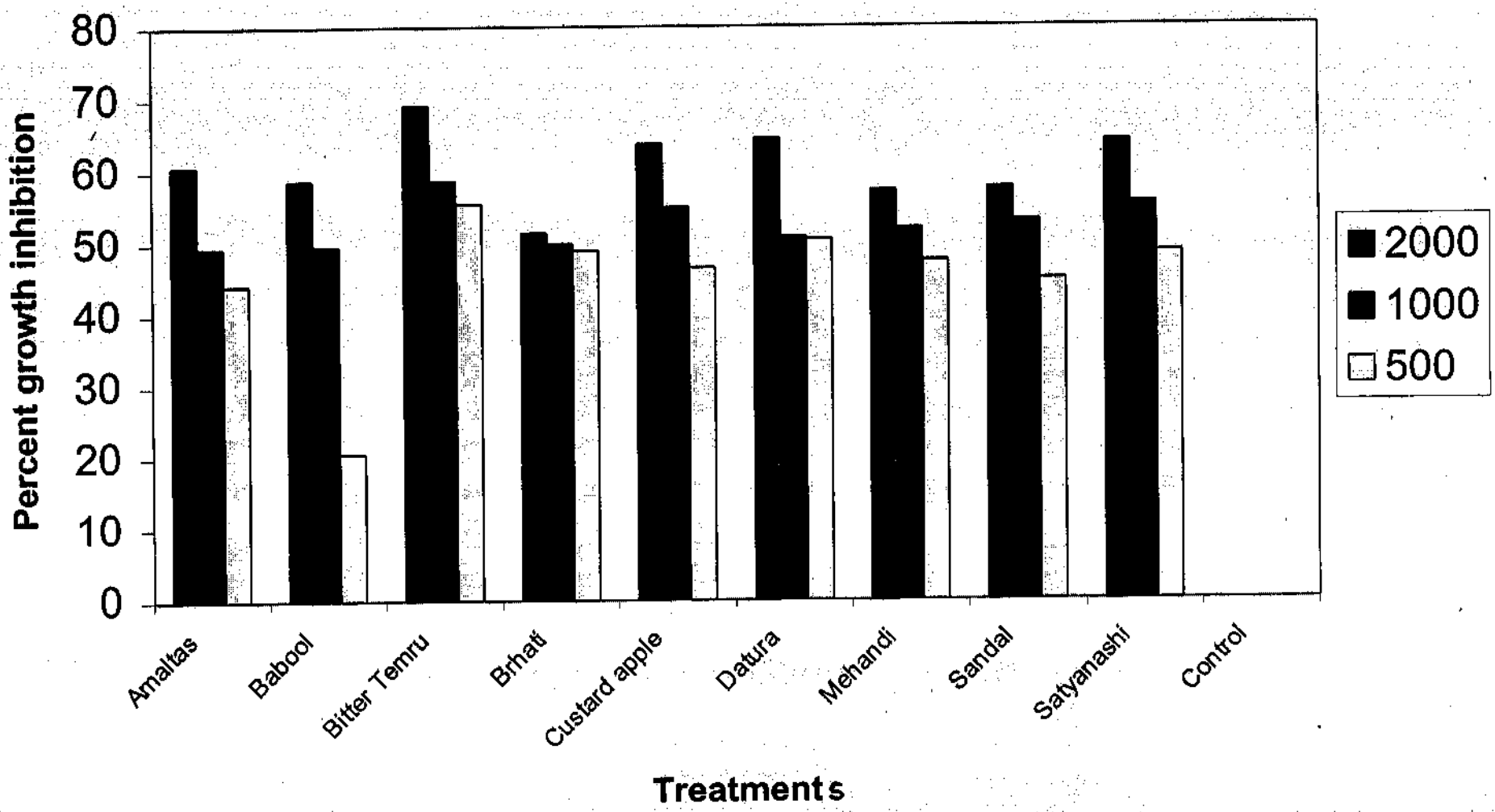


**Table 3. Efficacy of partially purified plant products components against guar leaf blight pathogen, *Alternaria cyamopsidis* by poison food technique *in vitro***

| Treatments | Percent mycelial growth inhibition | | | |
|---|---|---|---|---|
| | Concentrations | | | Mean |
| | 2000 ppm | 1000 ppm | 500 ppm | |
| Amaltas (*Cassia fistula*) | 60.77 | 49.37 | 44.14 | 51.43 |
| Babool (*Acacia nilotica*) | 58.71 | 49.55 | 20.60 | 42.96 |
| Bitter Temru (*Diospyros cordifolia*) | 69.17 | 58.71 | 55.53 | 61.14 |
| Brhati (*Solanum indicum*) | 51.43 | 49.93 | 48.99 | 50.12 |
| Custard apple (*Annona squamosa*) | 63.75 | 54.97 | 46.56 | 55.10 |
| Datura (*Datura stramonium*) | 64.50 | 50.86 | 50.49 | 55.29 |
| Mehandi (*Lawsonia inermis*) | 57.22 | 51.98 | 47.50 | 52.24 |
| Sandal (*Santalum album*) | 57.59 | 53.11 | 44.89 | 51.86 |
| Satyanashi (*Argemone mexicana*) | 64.13 | 55.53 | 48.62 | 56.09 |
| Control | 00.00 | 00.00 | 00.00 | 00.00 |
| Mean | 60.81 | 52.67 | 45.26 | 52.91 |

| | SEm± | C.D.at 5% | CV% |
|---|---|---|---|
| Treatments (T) | 0.13 | 0.39 | 0.78 |
| Concentrations (C) | 0.07 | 0.22 | |
| T X C | 0.23 | 0.67 | |

Fig. 3 **Efficacy of partially purified plant products components against guar leaf blight pathogen, *Alternaria cyamopsidis* by poison food technique *in vitro***



same partially purified plant products components viz., Amaltas seeds, Babool seeds, bitter Temru fruits, Brhati fruits, Custard apple seeds, Datura leaves, Mehandi seeds, Sandal seeds and Satyanashi seeds, were found effective at low concentrations of 1000 and 500 ppms also.

Both Satyanashi seeds and Datura leaves components were found statistically at par. Similarly both Custard apple seeds and Mehandi seeds and also Amaltas seeds and Brhati fruits components were found at par. Bitter Temru fruits component was found significantly different from all the other treatments at 2000 ppm concentration.

### 4.3.2 Spore Germination Technique :

To conduct the experiment, on the efficacy of partially purified components from plants viz., Amaltas seeds, Babool seeds, Brhati fruits, bitter Temru fruits, Custard apple seeds, Datura leaves, Mehandi seeds, Sandal seeds and Satyanashi seeds, on spore germination of plant pathogens viz., *Alternaria cyamopsidis, Fusarium oxysporum, Colletotrichum gloeosporioides* and *Helminthosporium maydis,* cavity slide method (Singh *et al.*,1986) was followed. The observation was calculated on per cent spore germination by observing the number of spores germinated in microscopic field (x 100) on each cavity slide. The experiment revealed that spore germination of the different test fungi were significantly reduced after incubating at 28±1°C for 24 hours with various partially purified plant products components used at different concentrations (2000, 1000 and 500 ppm).

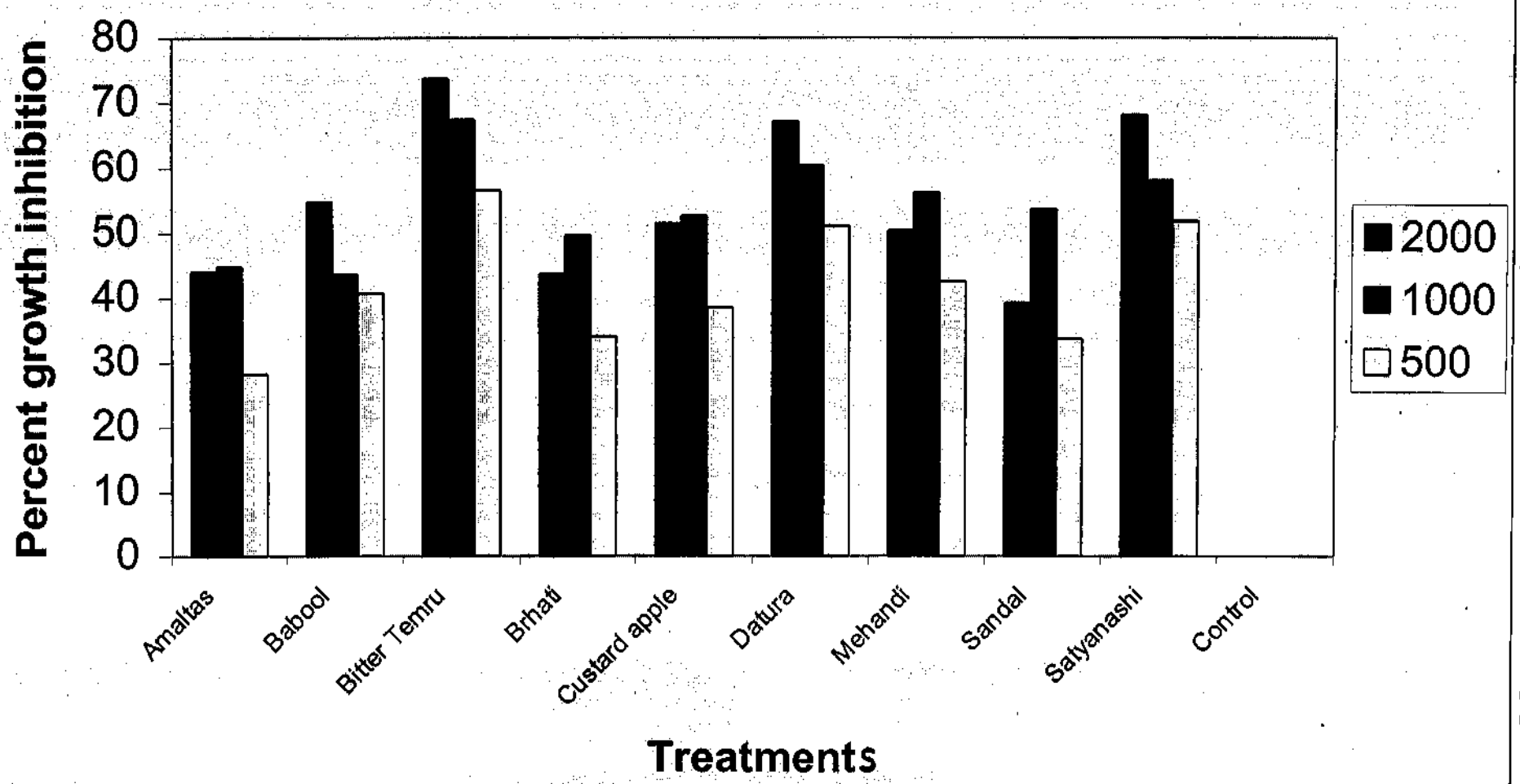
**Table 4. Efficacy of partially purified plant products components against maydis leaf blight pathogen, *Helminthosporium maydis* by poison food technique *in vitro***

| Treatments | Percent mycelial growth inhibition | | | |
|---|---|---|---|---|
| | Concentrations | | | Mean |
| | 2000 ppm | 1000 ppm | 500 ppm | |
| Amaltas (*Cassia fistula*) | 44.07 | 44.81 | 28.14 | 39.01 |
| Babool (*Acacia nilotica*) | 54.81 | 43.70 | 40.74 | 46.42 |
| Bitter Temru (*Diospyros cordifolia*) | 73.70 | 67.40 | 56.66 | 65.92 |
| Brhati (*Solanum indicum*) | 43.70 | 49.62 | 34.07 | 42.47 |
| Custard apple (*Annona squamosa*) | 51.48 | 52.59 | 38.51 | 47.53 |
| Datura (*Datura stramonium*) | 67.03 | 60.37 | 51.11 | 59.50 |
| Mehandi (*Lawsonia inermis*) | 50.37 | 56.29 | 42.59 | 49.75 |
| Sandal (*Santalum album*) | 39.25 | 53.70 | 33.70 | 42.22 |
| Satyanashi (*Argemone mexicana*) | 68.14 | 58.14 | 51.85 | 59.38 |
| Control | 0.00 | 0.00 | 0.00 | 0.00 |
| Mean | 54.73 | 54.07 | 41.93 | 50.24 |

| | SEm± | C.D.at 5% | CV% |
|---|---|---|---|
| Treatments (T) | 0.31 | 0.89 | 1.88 |
| Concentrations (C) | 0.18 | 0.51 | |
| T X C | 0.54 | 1.54 | |

Fig. 4

**Efficacy of partially purified plant products components against maydis leaf blight pathogen, *Helminthosporium maydis* by poison food technique *in vitro***



#### 4.3.2.1 *Fusarium oxysporum* :

The results depicted in Table 5 and Fig. 5 reveal that spore germination of the fungus, *Fusarium oxysporum* was found minimum with partially purified plant product component prepared from bitter Temru fruits (50.00%) after incubating for 24 hours at 28±1°C at 2000 ppm concentration over the control (97.22%). The next most inhibition of spore germination of the above fungus was found with partially purified plant product component extracted from Datura leaves (60.83%) followed by Brhati fruits (65.27%), Satyanashi seeds (67.88%), Sandal seeds (68.89%), Amaltas seeds (68.89%), Mehandi seeds (71.48%), Custard apple seeds (73.50%) and Babool seeds (76.67%), after incubating for 24 hours at 28±1°C at 2000 ppm concentration over the control (97.22%). The low concentrations (1000 and 500 ppms) of these partially purified plant products components viz., Amaltas seeds, Babool seeds, bitter Temru fruits, Brhati fruits, Custard apple seeds, Datura leaves, Mehandi seeds, Sandal seeds and Satyanashi seeds, also reduced the spore germination.

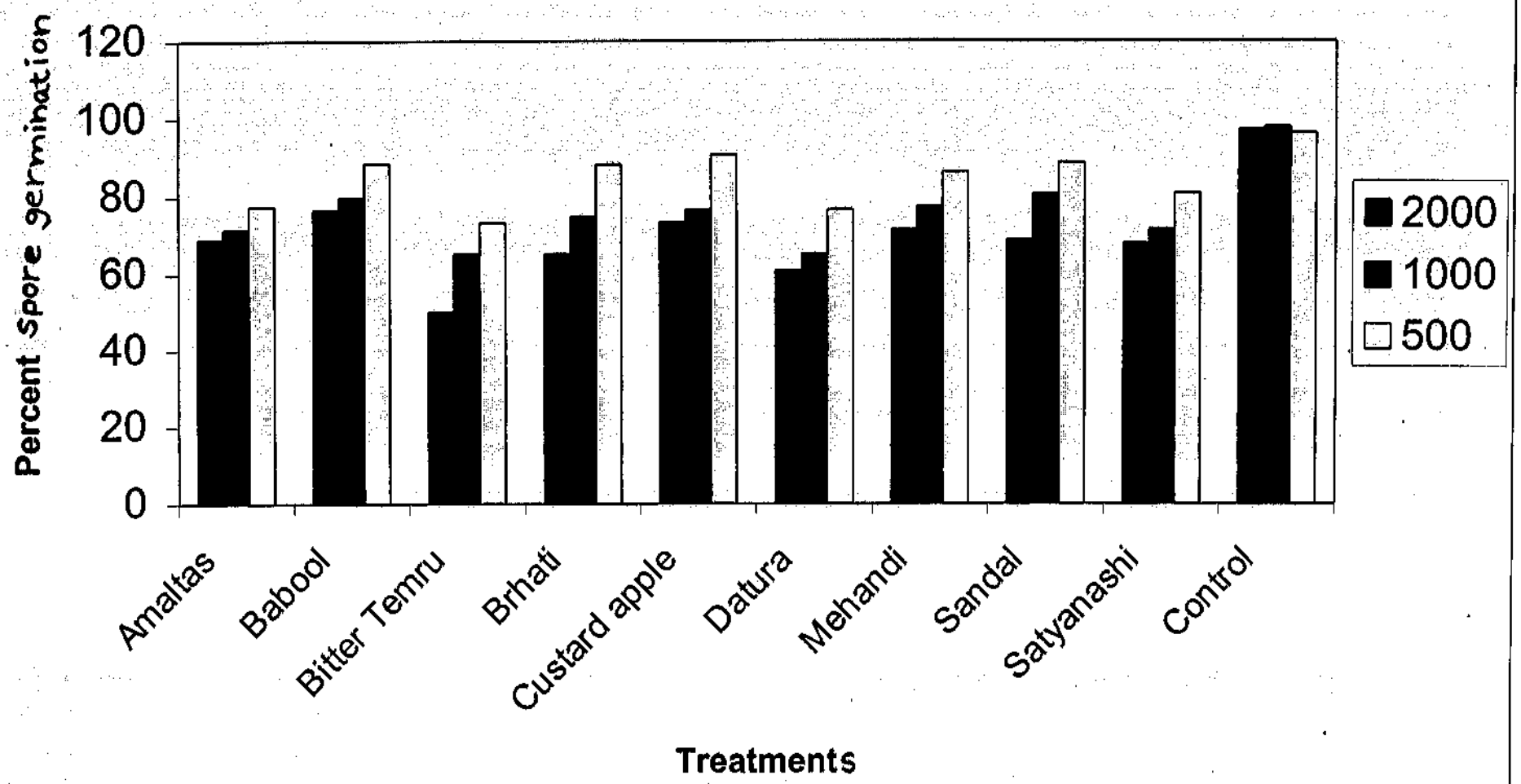
Custard apple seeds and Mehandi seeds components were at par statistically. Also Amaltas seeds, Sandal seeds, Satyanashi seeds and Brhati fruits components were at par. Similarly, Datura leaves and brhati fruits components were found at par statistically. All of these above treatments (Custard apple seeds, Mehandi seeds, Amaltas seeds, Sandal seeds, Satyanashi seeds, Brhati fruits and Datura leaves components) including Babool seeds were significantly different with bitter Temru fruits component at 2000 ppm concentration.

**Table 5. Efficacy of partially purified plant products components on spore germination of tomato fruit rot pathogen, *Fusarium oxysporum* f. sp. *lycopersici in vitro***

| Treatments | Percent spore germination | | | |
|---|---|---|---|---|
| | Concentrations | | | Mean |
| | 2000 ppm | 1000 ppm | 500 ppm | |
| Amaltas (*Cassia fistula*) | 68.89 | 71.67 | 77.59 | 72.71 |
| Babool (*Acacia nilotica*) | 76.67 | 79.86 | 88.63 | 81.72 |
| Bitter Temru (*Diospyros cordifolia*) | 50.00 | 65.27 | 73.33 | 62.87 |
| Brhati (*Solanum indicum*) | 65.27 | 75.00 | 88.33 | 76.20 |
| Custard apple (*Annona squamosa*) | 73.50 | 76.67 | 90.85 | 80.34 |
| Datura (*Datura stramonium*) | 60.83 | 65.27 | 76.67 | 67.59 |
| Mehandi (*Lawsonia inermis*) | 71.48 | 77.59 | 86.44 | 78.50 |
| Sandal (*Santalum album*) | 68.89 | 80.60 | 88.79 | 79.43 |
| Satyanashi (*Argemone mexicana*) | 67.88 | 71.67 | 81.01 | 73.52 |
| Control | 97.22 | 98.00 | 96.45 | 97.22 |
| Mean | 67.04 | 73.73 | 83.52 | 74.76 |

| | SEm± | C.D. at 5% | CV% |
|---|---|---|---|
| Treatments (T) | 1.01 | 2.88 | 4.09 |
| Concentrations (C) | 0.58 | 1.66 | |
| T X C | 1.76 | 5.00 | |

Fig.5 **Efficacy of partially purified plant products components on spore germination of tomato fruit rot pathogen, *Fusarium oxysporum* f. sp. *lycopersici* in vitro**



#### 4.3.2.2 *Colletotrichum gloeosporioides* :

The results presented in Table 6 and Fig. 6 reveal that spore germination of the fungus, *Colletotrichum gloeosporioides* was found minimum with partially purified plant product component prepared from bitter Temru fruits (54.76%) after incubating for 24 hours at 28±1°C at 2000 ppm concentration over the control (96.67%).. The immediate most inhibition of spore germination of the above fungus was found with partially purified plant product component of Datura leaves (61.67%) followed by Satyanashi seeds (68.89%), Brhati fruits (72.59%), Custard apple seeds (73.50%), Mehandi seeds (75.92%), Babool seeds (75.92%), Sandal seeds (78.70%) and lastly by Amaltas seeds (80.60%) after incubating for 24 hours at 28 ± 1°C at 2000 ppm concentration over the control (96.67%). The same partially purified plant products components (Amaltas seeds, Babool seeds, Bitter Temru fruits, Brhati fruits, Custard apple seeds, Datura leaves, Mehandi seeds, Sandal seeds and Satyanashi seeds) were found effective at low concentrations of 1000 and 500 ppm also but not as much as at 2000 ppm.

Amaltas seeds, Sandal seeds, Babool seeds and Mehandi seeds were at par statistically, also Custard apple seeds and Brhati fruits, Mehandi seeds and Babool seeds were at par but all of these treatments viz., Amaltas seeds, Sandal seeds, Mehandi seeds, Custard apple seeds and Brhati fruits components were significantly different with the other treatments. Bitter Temru fruits, Datura leaves and Satyanashi seeds components showed

significant difference from all the other treatments at 2000 ppm concentration.

#### 4.3.2.3. *Alternaria cyamopsidis* :

The results depicted in Table 7 and Fig. 7 indicate that spore germination of the fungus, *Alternaria cyamopsidis* was found minimum with partially purified plant product component extracted from bitter Temru fruits (51.51%) after incubating for 24 hours at 28±1°C at 2000 ppm concentration over the control (100.00%). The next most inhibition of spore germination of the above fungus was found with partially purified plant product component extracted from Datura leaves (57.54%) followed by Satyanashi seeds (58.09%), Amaltas seeds (59.05%), Babool seeds (59.44%) Brhati fruits (60.83%), Custard apple seeds (61.21%), Mehandi seeds (62.38%) and Sandal seeds (70.47%) after incubating for 24 hours at 28±1°C at 2000 ppm concentration over the control (100.00%). The low concentrations of 1000 and 500 ppms of these partially purified plant products components (Amaltas seeds, Babool seeds, bitter Temru fruits, Brhati fruits, Custard apple seeds, Datura leaves, Mehandi seeds, Sandal seeds and Satyanashi seeds) were also found effective in inhibiting the germination of the spores.

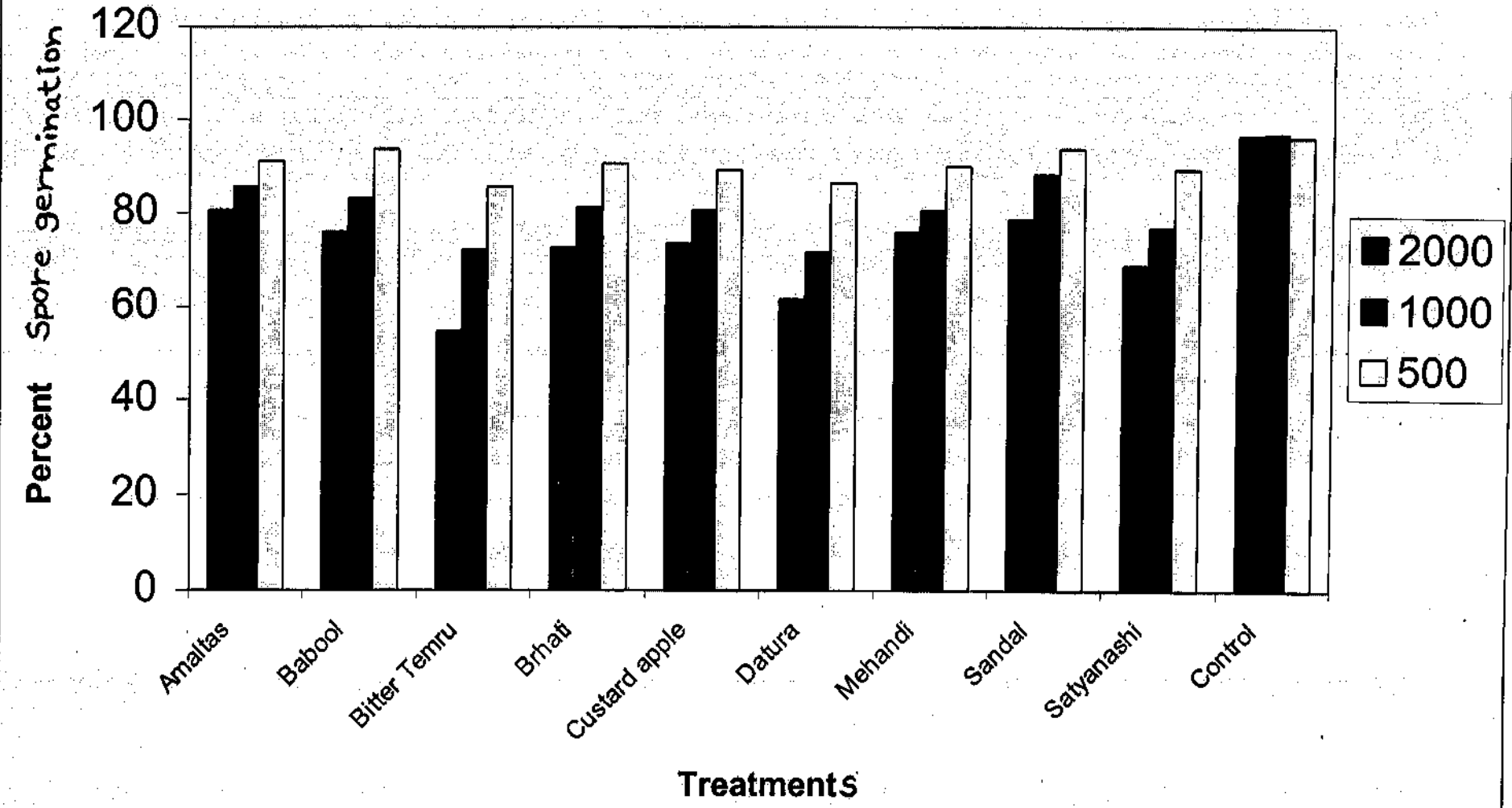
Mehandi seeds, Custard apple seeds, Brhati fruits, Babool seeds and Amaltas seeds components were found statistically at par. Similarly, Satyanashi seeds and Datura leaves were found at par with Custard apple seeds, Brhati fruits, Babool seeds and Amaltas seeds components. All of the above treatments (Mehandi seeds, Custard apple seeds, Brhati fruits,

**Table 6. Efficacy of partially purified plant products components on spore germination of yam anthracnose pathogen, *Colletotrichum gloeosporioides in vitro***

| Treatments | Percent spore germination | | | |
|---|---|---|---|---|
| | Concentrations | | | Mean |
| | 2000 ppm | 1000 ppm | 500 ppm | |
| Amaltas (*Cassia fistula*) | 80.60 | 85.60 | 91.11 | 85.77 |
| Babool (*Acacia nilotica*) | 75.92 | 83.10 | 93.63 | 84.22 |
| Bitter Temru (*Diospyros cordifolia*) | 54.76 | 72.14 | 85.60 | 70.83 |
| Brhati (*Solanum indicum*) | 72.59 | 81.21 | 90.55 | 81.45 |
| Custard apple (*Annona squamosa*) | 73.50 | 80.60 | 89.17 | 81.20 |
| Datura (*Datura stramonium*) | 61.67 | 71.67 | 86.44 | 73.26 |
| Mehandi (*Lawsonia inermis*) | 75.92 | 80.60 | 90.02 | 82.18 |
| Sandal (*Santalum album*) | 78.70 | 88.33 | 93.89 | 86.97 |
| Satyanashi (*Argemone mexicana*) | 68.89 | 76.85 | 89.47 | 78.40 |
| Control | 96.67 | 97.00 | 96.36 | 96.66 |
| Mean | 71.39 | 80.05 | 89.99 | 80.48 |

| | SEm± | C.D. at 5% | CV% |
|---|---|---|---|
| Treatments (T) | 1.02 | 2.90 | 3.82 |
| Concentrations (C) | 0.59 | 1.67 | |
| T X C | 1.77 | 5.02 | |

Fig.6 **Efficacy of partially purified plant products components on spore germination of yam anthracnose pathogen, *Colletotrichum gloeosporioides* *in vitro***



Babool seeds, Amaltas seeds, Satyanashi seeds, Datura leaves and also Sandal seeds components) were found significantly different from other treatment. Bitter Temru fruits component was found significantly different from all the other treatments at 2000 ppm concentration.

#### 4.3.2.4 *Helminthosporium maydis* :

The results presented in Table 8 and Fig. 8 show that spore germination of the fungus, *Helminthosporium maydis* was found minimum with partially purified plant product component extracted from bitter Temru fruits (25.83%) after incubating for 24 hours at 28±1°C at 2000 ppm concentration over the control (100.00%). The immediate most inhibition of spore germination of the above fungus was found with partially purified plant product component extracted from Datura leaves (37.39%) followed by Satyanashi seeds (38.39%), Custard apple seeds (48.33%), Sandal seeds (52.62%), Amaltas seeds (52.62%), Babool seeds (53.33%), Mehandi seeds (55.16%) and Brhati fruits (56.67%) after incubating for 24 hours at 28±1°C at 2000 ppm concentration over the control (100.00%). The same partially purified plant products components viz., Amaltas seeds, Babool seeds, bitter Temru fruits, Brhati fruits, Custard apple seeds, Datura leaves, Mehandi seeds, Sandal seeds and Satyanashi seeds were found effective in inhibiting the germination of spores at low concentrations of 1000 and 500 ppm also.

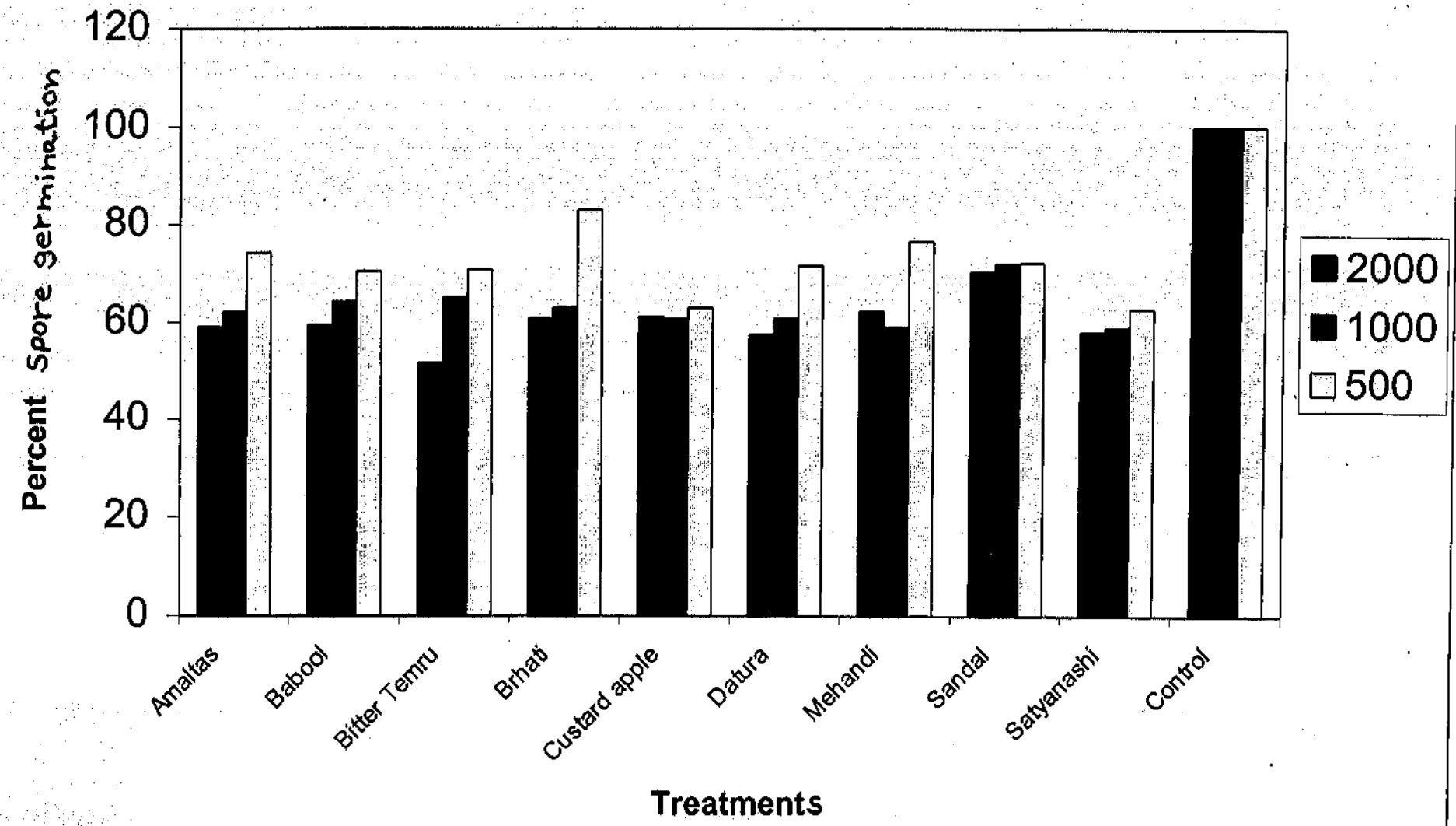
Brhati fruits, Mehandi seeds, Babool seeds, Amaltas seeds and Sandal seeds components were found statistically at par. Similarly, Amaltas seeds, Custard apple seeds and Sandal seeds components were found at par and also Satyanashi seeds and Datura leaves components were found at par

**Table 7. Efficacy of partially purified plant products components on spore germination of guar leaf blight pathogen, *Alternaria cyamopsidis in vitro***

| Treatments | Percent spore germination | | | |
|---|---|---|---|---|
| | Concentrations | | | Mean |
| | 2000 ppm | 1000 ppm | 500 ppm | |
| Amaltas (*Cassia fistula*) | 59.05 | 62.04 | 74.26 | 65.12 |
| Babool (*Acacia nilotica*) | 59.44 | 64.26 | 70.55 | 64.75 |
| Bitter Temru (*Diospyros cordifolia*) | 51.51 | 65.27 | 70.94 | 62.58 |
| Brhati (*Solanum indicum*) | 60.83 | 63.05 | 83.10 | 69.00 |
| Custard apple (*Annona squamosa*) | 61.21 | 60.83 | 63.05 | 61.70 |
| Datura (*Datura stramonium*) | 57.54 | 60.83 | 71.66 | 63.35 |
| Mehandi (*Lawsonia inermis*) | 62.38 | 59.04 | 76.66 | 66.02 |
| Sandal (*Santalum album*) | 70.47 | 72.14 | 72.22 | 71.61 |
| Satyanashi (*Argemone mexicana*) | 58.09 | 59.04 | 62.87 | 60.01 |
| Control | 100.00 | 100.00 | 100.00 | 100.00 |
| Mean | 60.05 | 62.95 | 71.70 | 64.90 |

| | SEm± | C.D. at 5% | CV% |
|---|---|---|---|
| Treatments (T) | 0.80 | 2.28 | 3.72 |
| Concentrations (C) | 0.46 | 1.31 | |
| T X C | 1.39 | 3.95 | |

Fig. 7 **Efficacy of partially purified plant products components on spore germination of guar leaf blight pathogen, *Alternaria cyamopsidis in vitro***



statistically. All of the aforementioned treatments (Brhati fruits, Mehandi seeds, Babool seeds, Amaltas seeds, Sandal seeds, Custard apple seeds, Satyanashi seeds and Datura leaves components) were differing significantly with bitter Temru fruits components at 2000 ppm concentration.

## 4.4 EFFICACY OF MOST EFFECTIVE PARTIALLY PURIFIED PLANT PRODUCTS AND COMMONLY USED FUNGICIDES AGAINST MAYDIS LEAF BLIGHT OF MAIZE *IN VIVO*

Field experiments were carried out for the management of maydis leaf blight on variety 'Mahi kanchan' during kharif season of 2001 at two different locations viz., Agronomy Farm, Rajasthan College of Agriculture, Udaipur and a farmer's field at Kanpur village of Udaipur district.

The partially purified plant product components viz., bitter Temru fruits and Datura leaves performed well under *in vitro* studies, were incorporated with two concentrations of each (1000 and 2000 ppm) alongwith two commonly used fungicides, Indofil M-45 and Bavistin at 1000 and 2000 ppm concentrations for this study. In all, ten treatments were there including two controls (water spray and without any spray) and were tried out as post inoculation sprays i.e. sprays of fungicides (Indofil M-45 and Bavistin) and partially purified plant products components (bitter Temru fruits and Datura leaves) were given 48 hours after inoculation of fungus, *Helminthosporium maydis*. The inoculation of the fungus was repeated on fourth day after first inoculation and similarly the sprays were repeated. Observations were recorded after 10 days of last spray on

**Table 8. Efficacy of partially purified plant products components on spore germination of maydis leaf blight pathogen, *Helminthosporium maydis in vitro***

| Treatments | Percent spore germination | | | |
|---|---|---|---|---|
| | Concentrations | | | Mean |
| | 2000 ppm | 1000 ppm | 500 ppm | |
| Amaltas (*Cassia fistula*) | 52.62 | 52.50 | 62.66 | 55.93 |
| Babool (*Acacia nilotica*) | 53.33 | 56.11 | 56.27 | 55.24 |
| Bitter Temru (*Diospyros cordifolia*) | 25.83 | 39.44 | 49.44 | 38.24 |
| Brhati (*Solanum indicum*) | 56.67 | 54.86 | 63.05 | 58.19 |
| Custard apple (*Annona squamosa*) | 48.33 | 57.06 | 55.55 | 53.65 |
| Datura (*Datura stramonium*) | 37.39 | 40.16 | 46.33 | 41.30 |
| Mehandi (*Lawsonia inermis*) | 55.16 | 55.95 | 56.66 | 55.93 |
| Sandal (*Santalum album*) | 52.62 | 57.06 | 63.05 | 57.58 |
| Satyanashi (*Argemone mexicana*) | 38.89 | 41.66 | 47.66 | 42.74 |
| Control | 100.00 | 100.00 | 100.00 | 100.00 |
| Mean | 46.76 | 50.48 | 55.63 | 50.98 |

| | SEm± | C.D.at 5% | CV% |
|---|---|---|---|
| Treatments (T) | 0.95 | 2.69 | 5.60 |
| Concentrations (C) | 0.54 | 1.55 | |
| T X C | 1.64 | 4.66 | |

Fig. 8 **Efficacy of partially purified plant products components on spore germination of maydis leaf blight pathogen, *Helminthosporium maydis in vitro***

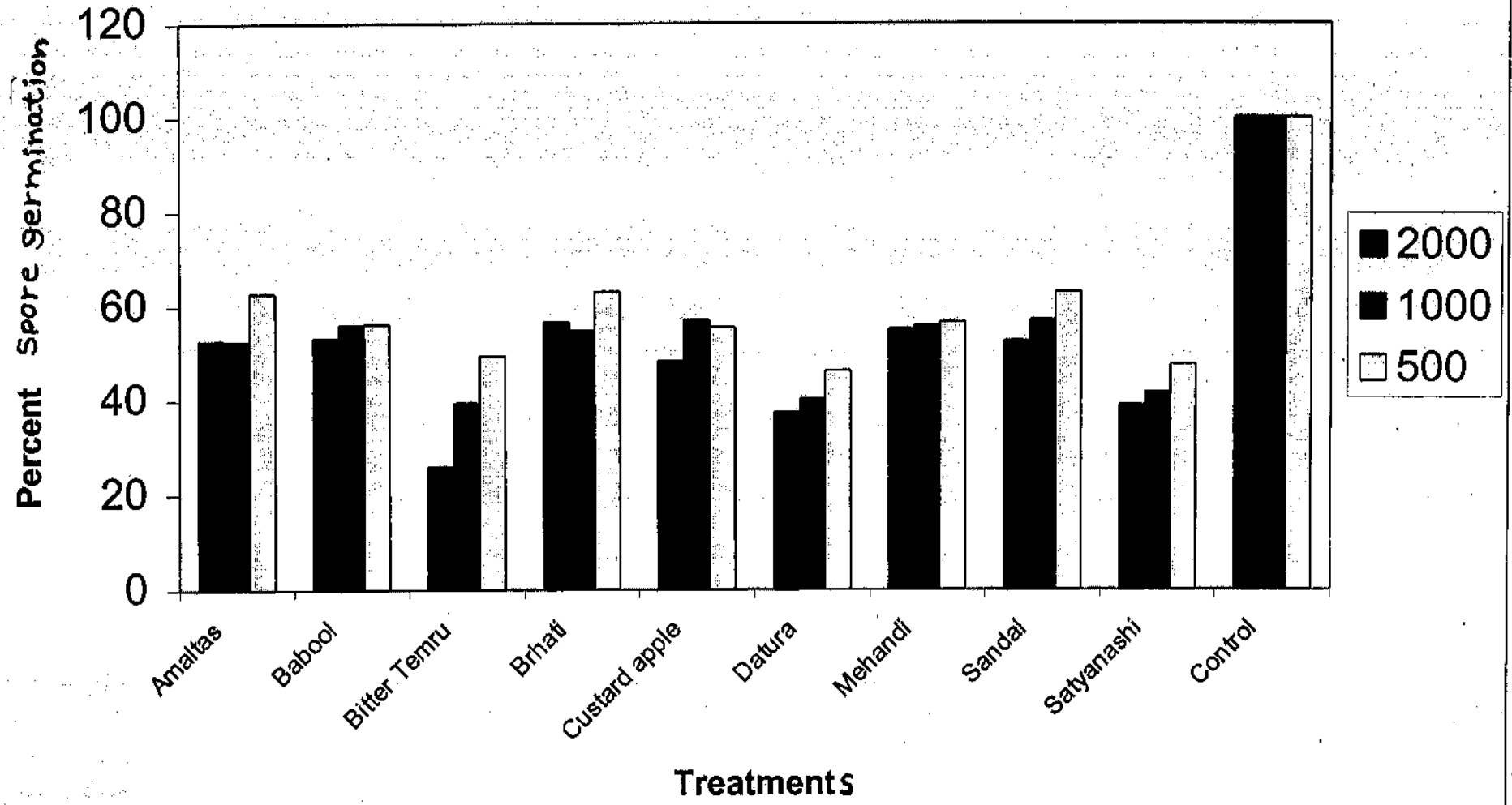


PLATE VI

A. Efficacy of Indofil M-45 at 1000 ppm concentration against maydis leaf blight pathogen, *Helminthosporium maydis in vivo*

B. Efficacy of Indofil M-45 at 2000 ppm concentration against maydis leaf blight pathogen, *Helminthosporium maydis in vivo*

C. Effect of partially purified plant product component of bitter Temru fruits at 1000 ppm concentration against maydis leaf blight pathogen, *Helminthosporium maydis in vivo*

D. Effect of partially purified plant product component of bitter Temru fruits at 2000 ppm concentration against maydis leaf blight pathogen, *Helminthosporium maydis in vivo*

# VI-A

# VI-B

# VI-C

# VI-D

randomly selected 10 plants from each plot and infection index was calculated. Percentage efficiency of disease control was calculated after computing infection index.

The data depicted in Table 9, Fig. 9 and Plates III-B & VI-A to VII-D showed the result observed at the field of Agronomy Farm, Rajasthan College of Agriculture Farm where all the treatments were found effective over both the kinds of controls. The maximum reduction in disease severity was observed with fungicide, Indofil M-45 (67.05%) followed by bitter Temru fruits component (60.39%), Datura leaves components (56.58%) and Bavistin (35.04%) over the control at 2000 ppm concentration. All the treatments at 2000 ppm concentration were significantly different from each other.

Bitter Temru fruits component and Indofil M-45 were at par statistically at 1000 ppm concentration but they were significantly different from other treatments namely Datura leaves component and fungicide, Bavistin.

The data depicted in Table 9 and Fig.9 show the result obtained at the field situated at Kanpur village of Udaipur district where all the treatments were found effective in reducing the disease severity of maydis leaf blight. The maximum reduction in disease severity was observed with fungicide, Indofil M-45 (65.31%) at 2000 ppm concentration followed by Datura leaves component (52.03%), bitter Temru fruits component (51.95%) and Bavistin (20.43%) over both the kinds of controls. The lower concentration of 1000 ppm of these treatments were also found effective in reducing the disease severity.

Bitter Temru fruits component and Datura leaves component were found at par statistically at 2000 ppm concentration. Indofil M-45 and Bavistin were significantly different from other treatments at 2000 ppm concentration.

Bitter Temru fruits components and Indofil M-45 were found at par at 1000 ppm concentration but they were significantly different from other treatments.

PLATE VII

A. Efficacy of Bavistin at 1000 ppm concentration against maydis leaf blight pathogen, *Helminthosporium maydis in vivo*

B. Efficacy of Bavistin at 2000 ppm concentration against maydis leaf blight pathogen, *Helminthosporium maydis in vivo*

C. Effect of partially purified plant product component of Datura leaves at 1000 ppm concentration against maydis leaf blight pathogen, *Helminthosporium maydis in vivo*

D. Effect of partially purified plant product component of Datura leaves at 2000 ppm concentration against maydis leaf blight pathogen, *Helminthosporium maydis in vivo*

# VII-A

# VII-B

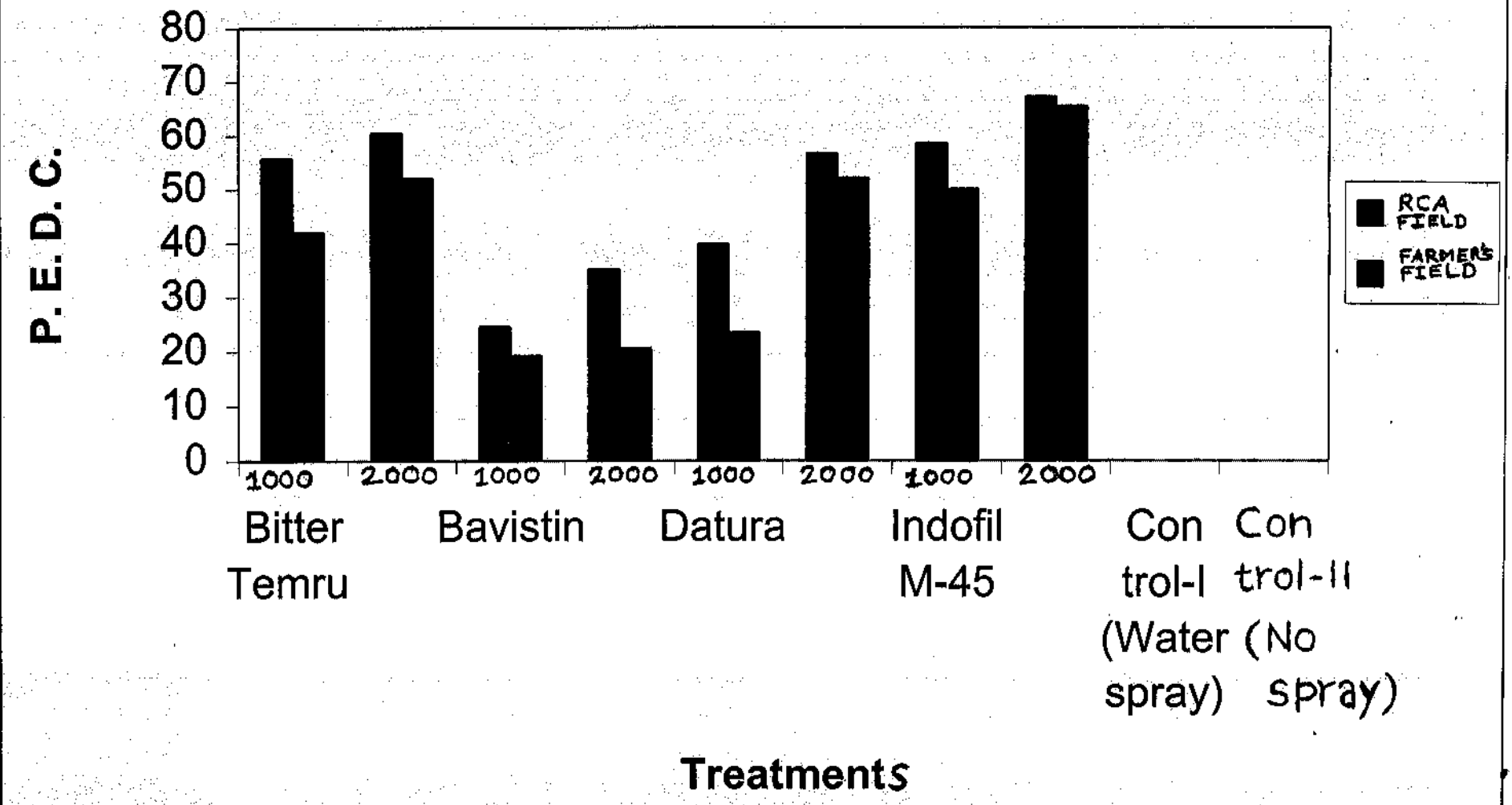
# VII-C

# VII-D

**Table 9. Efficacy of most effective partially purified plant products components and commenly used fungicides against maydis leaf blight pathogen, *Helminthosporium maydis in vivo*.**

| Treatments | Concentrations (ppm) | R.C.A. Field | | Farmer's Field | |
|---|---|---|---|---|---|
| | | Infection Index (I.I) | Percent efficiency of disease control (P.E.D.C) | Infection Index (I.I) | Percent efficiency of disease control (P.E.D.C) |
| Bitter Temru (*Diospyros cordifolia*) | 1000 | 31.33 | 55.66 | 32.66 | 41.91 |
| | 2000 | 28.00 | 60.39 | 31.33 | 51.95 |
| Bavistin | 1000 | 53.33 | 24.49 | 52.81 | 19.10 |
| | 2000 | 45.90 | 35.04 | 51.94 | 20.43 |
| Datura (*Datura stramonium*) | 1000 | 42.61 | 39.67 | 50.00 | 23.34 |
| | 2000 | 30.66 | 56.58 | 31.33 | 52.03 |
| Indofil M-45 | 1000 | 29.33 | 58.46 | 32.66 | 49.91 |
| | 2000 | 23.27 | 67.05 | 22.66 | 65.31 |
| Control-I (Water spray) | | 70.66 | 0.00 | 65.33 | 0.00 |
| Control-II (No spray) | | 71.33 | 0.00 | 66.00 | 0.00 |
| SEm ± | | | | | |
| Treatments (T) | | 0.58 | 0.82 | 0.68 | 0.91 |
| Concentration (C) | | 0.41 | 0.58 | 0.48 | 0.64 |
| T x C | | 0.82 | 1.16 | 0.97 | 1.28 |
| C.D. at 5% | | | | | |
| (T) | | 1.73 | 2.44 | 2.04 | 2.70 |
| (C) | | 1.22 | 1.72 | 1.44 | 1.91 |
| T x C | | 2.45 | 3.45 | 2.89 | 3.83 |
| C.V. % | | 3.35 | 5.05 | 3.86 | 6.73 |

Fig. 9 **Efficacy of most effective partially purified plant products components and commenly used fungicides against maydis leaf blight pathogen, *Helminthosporium maydis in vivo***



# *Chapter 5*

# *Discussion*

# DISCUSSION

After the advent of first chemical to control plant diseases, use of chemicals in controlling/managing plant diseases was being exploited because of its rapid action and quick results. Instead of their excellent effect on plant pathogens they may leave residual toxic effect if used in excess of recommended quantity. Moreover, they also result in development of chemical resistant strains of the pathogens. Most of researchers till date gave main emphasis on controlling plant pathogens by using chemicals, only few researchers used other alternative strategies for controlling plant pathogens including plant product preparations.

The environmental consciousness on the part of scientists and general public has drawn attention to the increasing amount of fungicides and pesticides used on crop plant and their harmful effect on flora and fauna. These resulted in search for bio-degradable products which can be effective in the management of diseases without any toxic effect. Chemical hazards and development of fungicide resistant strain of pathogens call for advent of alternative strategies and natural plant preparations can replace the synthetic fungicides effectively.

Use of botanicals for prevention of plant diseases without the mention of their deleterious effect has been recorded in ancient Indian literature. Nene (1996) reported several botanicals viz., fruits of Brhati (*Solanum indicum* L.), Sandal (*Santalum album* L.) and Mahuva (*Madhuca indica* L.),

seeds of Mehandi (*Lawsonia inermis* L.), Babool (*Acacia nilotica* L.) having antimicrobial activities against some plant disease causing organisms. Democritus (470 BC) tried plant extracts for controlling disease (Sharville, 1960). Since then there has been several recorded and unrecorded mention of their uses against a large number of microbial organisms. A number of angiospermic plants have been reported for the presence of antimicrobial activities by several workers (Chopra et al., 1965; Nene, 1996; Daya Ram, 1998; Chaudhary et al., 1998; Khan, 1999, Sharma, 2000 and Raut, 2000). The preparation from several parts of plants such as seeds, leaves, flowers, fruits, roots, etc. have been used for the presence of antifungal activities.

Several angiospermic plants parts such as fruits of Amaltas (*Cassia fistula* L.) contain pectin, hydro-methyl amanthoquinones and sugars. Datura (*Datura stramonium* L.) leaves are known for the presence of alkaloids which possess toxic properties against some plant pathogens. A preparation from bitter Temru (*Diospyros cordifolia* Roxb.) is known for its piscicidal activities (Sharma and Simlot, 1971).

After going through all of these above mentioned literature and other relevant reports mentioned in review of literature, present studies were conducted to search out commonly available natural plant product components for effective management of plant pathogens and present investigation proved to be effective in managing plant pathogens. Nine plant product components were used in present experiments viz., bitter Temru (*Diospyros cordifolia* Roxb.), Datura (*Datura stramonium* L.), Satyanashi (*Argemone mexicana* L.), Amaltas (*Cassia fistula* L.), Brhati

(*Solanum indicum* L.), Sandal (*Santalum album* L.), Mehandi (*Lawsonia inermis* L.), Babool (*Acacia nilotica* L.) and custard apple (*Annona squamosa* L.) against four plant pathogens viz., *Alternaria cyamopsidis* Rangaswami and Rao inciting leaf blight of guar, *Colletotrichum gloeosporioides* f. sp. *alatae* inciting anthracnose of yam, *Fusarium oxysporum* f. sp. *lycopersici* inciting tomato fruit rot and *Helminthosporium maydis* inciting leaf blight of maize.

The present investigation showed the superiority of partially purified component of bitter Temru fruits against plant pathogens viz., *Fusarium oxysporum*, *Alternaria cyamopsidis*, *Colletotrichum gloeosporioides* and *Helminthosporium maydis* in both spore germination and growth inhibition techniques. In growth inhibition technique, bitter Temru fruits component checked the growth of all the four plant pathogens under test viz., *Fusarium oxysporum* from tomato, *Alternaria cyamopsidis* from guar, *Colletotrichum gloeosporioides* from yam and *Helminthosporium maydis* from maize, at 2000 ppm concentration (36.20, 69.17, 25.37 and 73.70%, respectively) followed by Datura leaves at the same concentration (33.01, 64.50, 18.70 and 67.03 %, respectively).

There was a significant difference between bitter Temru fruits and Datura leaves components amongst *Fusarium oxysporum*, *Alternaria cyamopsidis*, *Colletotrichum gloeosporioides* and *Helminthosporium maydis*. Other treatments which included partially purified components of Brhati fruits, Sandal seeds, Babool seeds, Amaltas seeds, Custard apple seeds, Satyanashi seeds and Mehandi seeds also inhibited the growth of

aforementioned plant pathogens to various degrees. Chaudhary *et al.* (1998) reported the similar result where they recorded the growth inhibition of *Fusarium udum*, *Alternaria alternata*, *Colletotrichum capsici* and *Helminthosporium maydis* by partially purified plant product component of bitter Temru fruits. Babu and Reddy (1986) also reported that extracts of *Lawsonia inermis* and *Datura stramonium* were effective in controlling fruit rot of lemon caused by *Colletotrichum gloeosporioides*. In the present studies also, *D. stramonium* was effective in controlling the plant pathogenic fungi viz., *Alternaria cyamopsidis*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides* and *Helminthosporium maydis*. Similarly Eswaramurthy *et al.* (1989) observed that extracts of *Acacia arabica* inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *capae*. The same was found in present studies where *Acacia nilotica* also checked the growth of fungi to some extent. Shivpuri *et al.* (1997) also reported that ethanol leaf extract of *Azadirachta indica*, *Datura stramonium*, *Ocimum sanctum* and *Polyalthia longifolia* were most toxic against *Alternaria brassicola*, *Colletotrichum capsici*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Similarly, in the present investigation *Datura stramonium* and other plant products components also inhibited the growth of the following fungi viz., *Alternaria cyamopsidis*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides* and *Helminthosporium maydis*.

Gupta et al. (1996) adopted poison food technique and reported that the leaf extract of *Pongamia pinnata* was highly fungitoxic to *Fusarium pallidorseum* and *F. moniliformae*, inhibiting mycelial growth on plates by 78.2 and 84.3% respectively. Similarly, *Fusarium oxysporum* was checked

by different partially purified plant products components in the present studies. Rodriguez and Valendia (1998) while working with *Colletotrichum* species in vitro reported that extracts of clove (*Syzygium aromatium*) inhibited the growth and sporulation of the fungus. The growth of *Colletotrichum gloeosporioides* from yam was inhibited in the present experiment by the use of different plant products components. Sarma *et al.* (1999) also used extracts of common weed, *Solanum nigrum*, and reported that 90 per cent growth of *Rhizoctonia solani* was inhibited by the extracts of *Solanum nigrum* at 20 per cent concentration. In the present investigation, *Solanum indicum* tested was found effective in controlling the growth of *Alternaria cyamopsidis* and *Helminthosporium maydis*. Khan (1999) studied the effectiveness of seven partially purified plant product components and found that fruit components of bitter Temru and Datura leaves components were quite effective in inhibiting the mycelial growth of various fruit rot pathogens of mango and guava which confirms the present findings. Similarly, Roat (2000) screened seven partially purified plant product components against *Colletotrichum capsici* and *Alternaria alternata* from chilli and reported the effectiveness of bitter Temru fruits and Datura leaves components in inhibiting the mycelial growth of the fungi. Likewise, Sharma (2000) reported the effectiveness of bitter Temru fruits and Datura leaves components against fruit rot pathogens of tomato in inhibiting the mycelial growth of the pathogens. All of the aforementioned reports corroborate the findings of the present studies where similar plant components were undertaken.

Plant product components were also tested against spore germination of the four pathogens. viz., *Fusarium oxysporum*, *Alternaria cyamopsidis*, *Colletotrichum gloeosporioides* and *Helminthosporium maydis* and the spore germination was found minimum with partially purified component of bitter Temru fruits (50.00, 51.51, 54.76 and 25.83%, respectively) followed by Datura leaves (60.83, 57.54, 61.67 and 37.39%, respectively) at 2000 ppm concentration. The bitter Temru fruits component preparation was significantly effective than all other eight partially purified plant components used in the present investigation. Prasad and Simlot (1983) also recorded the similar results with bitter Temru fruits component against the spore germination of the pathogens viz., *Fusarium udum*, *F. lycopersici* and *Helminthosporium turcicum*.

Kumar et al. (1979) tested aqueous extract of different parts of onion, garlic, parthenium and Kalanchoe *in vitro* against *Fusarium oxysporum* and observed complete inhibition of spore germination. Kapoor et al. (1981) reported that extracts from *Convolvulus alsinoids* and *C. pluriculis* were almost completely effective against spore germination of *Fusarium oxysporum*. Similar results were recorded in present studies where spore germination of *Fusarium oxysporum* was inhibited by different plant products components. Ahmad and Prasad (1995) evaluated the aqueous foliar extracts of different plants against soft rot of *Luffa cylindrica* fruit caused by *Helminthosporium spiciferum* and *Fusarium scirpi* and observed that their conidial germinations were reduced to 75 per cent when their spores were treated with *Azadirachta indica*, *Catharanthus roseus*, *Datura fistulosa*, *Lantana camera* and *Ocimum sanctum*. Almost similar results

were recorded in present studies where different partially purified plant products components including *Datura stramonium* were effective in inhibiting the spore germination of *Fusarium oxysporum* and *Helminthosporium maydis*. Srivastava and Srivastava (1998) reported that maximum spore germination of *Alternaria alternata* was inhibited by leaf extracts of *Argemone mexicana*, which was also found effective in our present experiments. Khan (1999) reported the inhibition of spore germination of fruit rot pathogens of mango and guava with partially purified components of fruits of bitter temru and leaves of Datura. Similarly Roat (2000) reported the inhibition of spore germination of *Colletotrichum capsici* and *Alternaria alternata* from chilli with bitter Temru fruits and Datura leaves components. Likewise, Sharma (2000) showed the effectiveness of bitter Temru fruits and Datura leaves components in inhibiting the germination of spores of tomato fruit rot pathogens. The results obtained in present studies were similar to those observed by various above mentioned workers and thereby confirms the validity of the results obtained in present investigation..

*In vivo* studies effect of partially purified plant product components were carried out against *Helminthosporium maydis*, incitant of maydis leaf blight on maize, at two different locations. The *in vitro* studies revealed that bitter Temru fruits and Datura leaves components were most effective among all plant products components in controlling the plant pathogenic fungi so they were further used in these field trials. In addition to these two partially purified plant products components, two most commonly used fungicides viz., Indofil M-45 and Bavistin were also tried. The field

experiments were conducted in randomized black design using "Mahi Kanchan" variety during the kharif season of the year 2001. The treatments were tested as post-inoculation sprays i.e. fungicidal and plant product components sprays were given 48 hrs after inoculation with the fungi, *Helminthosporium maydis*.

In both the field experiments, Indofil M-45 and bitter Temru fruits components were significantly more effective in managing the disease incidence than other treatments viz., Bavistin and Datura leaves components. Although the best results were recorded with fungicide, Indofil M-45 but partially purified bitter Temru fruits components also showed encouraging results in the management of this disease, thus it can be recommended for field sprays in the future.

Practically no work has been done in managing the maydis leaf blight in the field with botanicals but there are reports of evaluation of different plant extracts against *Helminthosporium* species *in vitro*. Shekhawat and Prasad (1971) found that garlic and onion gave good inhibition of spore germination of *Helminthosporium sativum* due to pressure of allincin in garlic and protocatechuic acid and catechol in onion. Chaudhary *et al.* (1998) reported inhibition of *Helminthosporium maydis* from partially purified component extracted from Bitter Temru fruits which was similar to our present studies. Similarly, Ahmed and Prasad (1995) evaluated the aqueous foliar extracts of different plants against soft rot of *Luffa cylindrica* fruit caused by *Helminthosporium spiciferum* and observed that conidial germination of *Helminthosporium spiciferum* was reduced to 75 per cent when

the spores were treated with *Azadirachta indica*, *Catharanthus roseus*, *Datura* fistulosa, *Lantana camera* and *Ocimum sanctum*. The growth of *Helminthosporium maydis* was inhibited by different partially purified plant products components including *Datura stramonium* in the present studies also. Similarly, Qasem and Abu-blan (1996) while working with *Helminthosporium sativum*, reported that aqueous extracts of *Anagallis arnensis*, *Atriplex leucoclada*, *Crepis aspera*, *Runex cripus* and *Vicia norbenensis* were toxic to the fungus.

The results of the various above works prompted the present studies to be carried out in the field and the experiment showed fairly encouraging results although the effect of partially purified plant products components were not as much as to those compared with synthetic fungicides. The scope of use of purified plant products components is wide open and further research is needed as in the present investigation, due to lack of facilities, only the partially purified plant products components were used but if suitable facilities are available then the fully purified plant products components can be extracted out and tried out against various plant pathogens and they may prove to be effective as much as synthetic fungicides and all the deleterious effects due to the indiscriminate use of synthetic fungicides to the environment, soil, human and animals can dwindle in the long run by the introduction of these ecofriendly botanicals.

# *Chapter 6*

# *Summary*

# SUMMARY

Numerous plants have been tried out for the presence of antifungal activities. Hazards of fungicides and pesticides and induction of fungicidal resistant strains of plant pathogens beckons for advent of alternative strategies and it is now more or less imperative to gradually shift from synthetic fungicides to natural plant products for management of plant diseases.

Four plant pathogens namely, *Fusarium oxysporum* f. sp. *lycopersici, Alternaria cyamopsidis, Colletotrichum gloeosporioides* and *Helminthosporium maydis* were isolated from tomato, guar, yam and maize, respectively. Purification of the isolated fungi was done by single spore culture technique and their identity was confirmed from Indian type culture collection, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.

Nine partially purified plant products components namely, Amaltas seeds, Brhati fruits, Babool seeds, custard apple seeds, Datura leaves, Mehandi seeds, Sandal fruits, Satyanashi seeds and bitter Temru fruits were tried against aforementioned four plant pathogens.

Partially purified plant product components tried in the present investigation were found effective in controlling the different plant pathogens.

In poison food technique, all the partially purified plant product components significantly inhibited the growth of plant pathogens but the utmost inhibition of colony growth was recorded with bitter Temru fruits components followed by Datura leaves and Satyanashi seeds components. The other treatments were not so effective and minimum growth inhibition was recorded in *Fusarium oxysporum* f. sp. *lycopersici*, *Colletotrichum gloeosporioides* and *Alternaria cyamopsidis* with Brhati fruits and in *Helminthosporium maydis* with Sandal fruits at higher concentration (2000 ppm).

In case of spore germination technique, least spore germination was observed with bitter Temru fruits components followed by Datura leaves and Satyanashi seeds components. The other treatments were not much effective and maximum spore germination was recorded in *Fusarium oxysporum* f. sp. *lycopersici* with Babool seeds, in *Colletotrichum gloeosporioides* with Amaltas seeds, in *Alternaria cyamopsidis* with Sandal seeds and in *Helminthosporium maydis* with Brhati fruits components even at higher concentration (2000 ppm).

The most effective partially purified plant product components viz., bitter Temru fruits and Datura leaves alongwith two fungicides viz., Indofil M-45 and Bavistin (Carbendazim) were tested in the field against *Helminthosporium maydis*, the incitant of maydis leaf blight on maize. The results indicate that disease severity was much reduced with Indofil M-45 followed by bitter Temru fruits components.

It is concluded that the partially purified plant product components were effective in managing the plant pathogens and if due emphasis is given to them, they can gradually replace the synthetic or chemical fungicides easily. These plant product components may be environmentally non pollutive in nature and negate the possibility of development of fungicide resistant strains of plant pathogenic fungi.

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# LITERATURE CITED

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# "Efficacy of Partially Purified Plant Products Components *in vitro* and *in vivo* Against Plant Pathogens"

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## ABSTRACT

Plant protection through synthetic chemicals contributed significantly in reducing losses due to diseases and thereby increased agricultural production. However, chemical's share to environmental pollution through air, water, soil and by induction of resistance amongst pathogens, currently caused wide spread concern and hence the need to look for an alternative safer means for plant disease is felt greater now than ever before. Natural plant product components can replace synthetic chemicals effectively.

Four plant pathogenic fungi viz., *Fusarium oxysporum* f. sp. *lycopersici*, *Alternaria cyamopsidis*, *Colletotrichum gloeosporioides* and *Helminthosporium* [illegible] *maydis* were isolated from tomato, guar, yam and maize respectively.

Nine partially purified plant product components were screened for their efficacy in inhibiting the growth of the above pathogens under *in vitro* and the two most effective among them along with fungicides against *Helminthosporium maydis* under *in vivo* conditions.

In poison food technique, the utmost inhibition of growth of fungi was recorded with bitter Temru and Datura components and the same result was recorded in spore germination technique.

In the field trials, the disease severity of maydis leaf blight of maize was significantly reduced with Indofil M-45 followed by bitter Temru fruits component.

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# अनुक्षेपण

## अर्द्धशोषित पादप उत्पादित संघटकों का रोग जनकों पर पात्रे व जीवे क्षमता

**सुनिल जकारिया***
शोधकर्त्ता

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मुख्य सलाहकार

कृत्रिम रसायनों द्वारा पौध रक्षण से रोगों में कमी हुई है जिसके फलस्वरूप कृषि उत्पादन में बढ़ोतरी हुई है। किन्तु रसायनों के उपयोग के फलस्वरूप पर्यावरणीय प्रदूषण का बढ़ना एवं रोगजनकों में प्रतिरोध का विकसित होना आज विस्तृत चिंता का विषय हैं। इस कारण पौध व्याधि की रोकथाम हेतु वैकल्पिक सुरक्षित माध्यम की खोज की आवश्यकता नितांत अनिवार्य हो गया है। प्राकृतिक पौध उत्पाद निर्मित, कृत्रिम रसायनों पर स्वयं को प्रतिस्थापित कर सकती है।

निम्नलिखित चार पौध रोगजनक कवको, *फ्युसेरियम ओक्सीस्पोरम, आल्टरनेरिया सायमोपसिडिस, कोलेटोट्राइकम ग्लियोस्पोरोइडस, हेलमिन्थोस्पोरियम मेडिस* को क्रमशः टमाटर, ग्वार, रतालू एवं मक्का में से पृथक्क किया गया।

नौ अर्द्धशोषित पादप उत्पादित संघटकों को रोगजनकों के बढ़ोतरी पर अंकुश हेतु पात्रे परीक्षण किया गया तथा उनमें से श्रेष्ठ पाये गये दो अर्द्धशोषित पादप उत्पादित संघटकों कड़वा टिमरू एवं धतूरा को दो रसायनिक कवकनाशी इन्डोफिल एम-45 एवं बेविस्टीन के साथ खेत में मक्का पर *हेल्मिन्थोस्पोरियम मेडिस* के विरूद्ध परीक्षण किया गया।

विषाक्तविलोभक तकनीक एवं बीजाणु अंकुरण तकनीक में सबसे अधिक कवक विकास पर अंकुश कड़वा टिमरू एवं धतूरा संघटकों ने किया।

खेत पर किए गए परीक्षण में मक्का का मेडिस पत्ता झुलसा रोग का रोग उग्रता इन्डोफिल एम-45 एवं कड़वा टिमरू फल संघटक द्वारा कम पाया गया।

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# *Appendices*

# APPENDIX - I

**ANOVA for Table - 1**

| Source of variance | df | SS | MSS | F.CAL | F.TAB 5% | SEm± | CD 5% | CD 1% |
|---|---|---|---|---|---|---|---|---|
| Control/Rest | 1 | 767.8698 | 767.870 | 1867.528 | 4.013 | | | |
| Treatment (A) | 8 | 2119.5855 | 264.948* | 644.378 | 2.109 | 0.214 | 0.606 | 0.806 |
| Concentration (B) | 2 | 1270.9248 | 635.462* | 1545.502 | 3.162 | 0.123 | 0.350 | 0.465 |
| A x B | 16 | 1343.4203 | 83.964* | 204.207 | 1.828 | 0.370 | 1.049 | 1.396 |
| Error | 56 | 23.03 | 0.411 | | | | | |
| | | **CV%** | **3.94** | | | | | |

**ANOVA for Table - 2**

| Source of variance | df | SS | MSS | F.CAL | F.TAB 5% | SEm± | CD 5% | CD 1% |
|---|---|---|---|---|---|---|---|---|
| Control/Rest | 1 | 515.0002 | 515.000 | 2005.248 | 4.013 | | | |
| Treatment (A) | 8 | 512.9271 | 64.116* | 249.647 | 2.109 | 0.169 | 0.479 | 0.637 |
| Concentration (B) | 2 | 234.3254 | 117.163* | 456.195 | 3.162 | 0.098 | 0.276 | 0.368 |
| A x B | 16 | 334.7061 | 20.919* | 81.452 | 1.828 | 0.293 | 0.829 | 1.103 |
| Error | 56 | 14.38 | 0.257 | | | | | |
| | | **CV%** | **3.80** | | | | | |

* Significant at 5% level

# APPENDIX - II

**ANOVA for Table - 3**

| Source of variance | df | SS | MSS | F.CAL | F.TAB 5% | SEm± | CD 5% | CD 1% |
|---|---|---|---|---|---|---|---|---|
| Control/Rest | 1 | 8099.6329 | 8099.633 | 47567.619 | 4.013 | | | |
| Treatment (A) | 8 | 1788.7404 | 223.593* | 1313.117 | 2.109 | 0.138 | 0.390 | 0.519 |
| Concentration (B) | 2 | 3264.0766 | 1632.038* | 9584.654 | 3.162 | 0.079 | 0.225 | 0.299 |
| A x B | 16 | 1434.2250 | 89.639* | 526.433 | 1.828 | 0.238 | 0.675 | 0.898 |
| Error | 56 | 9.54 | 0.170 | | | | | |
| | | **CV%** | **0.78** | | | | | |

**ANOVA for Table - 4**

| Source of variance | df | SS | MSS | F.CAL | F.TAB 5% | SEm± | CD 5% | CD 1% |
|---|---|---|---|---|---|---|---|---|
| Control/Rest | 1 | 7302.4916 | 7302.492 | 8156.746 | 4.013 | | | |
| Treatment (A) | 8 | 6195.2069 | 774.401* | 864.991 | 2.109 | 0.315 | 0.894 | 1.189 |
| Concentration (B) | 2 | 2804.3716 | 1402.186* | 1566.215 | 3.162 | 0.182 | 0.516 | 0.687 |
| A x B | 16 | 952.2473 | 59.515* | 66.478 | 1.828 | 0.546 | 1.548 | 2.060 |
| Error | 56 | 50.14 | 0.895 | | | | | |
| | | **CV%** | **1.88** | | | | | |

* Significant at 5% level

# APPENDIX - III

ANOVA for Table - 5

| Source of variance | df | SS | MSS | F.CAL | F.TAB 5% | SEm± | CD 5% | CD 1% |
|---|---|---|---|---|---|---|---|---|
| Control/Rest | 1 | 1458.6808 | 1458.681 | 156.079 | 4.013 | | | |
| Treatment (A) | 8 | 2843.5104 | 355.439* | 38.032 | 2.109 | 1.019 | 2.887 | 3.843 |
| Concentration (B) | 2 | 3706.4300 | 1853.215* | 198.294 | 3.162 | 0.588 | 1.667 | 2.219 |
| A x B | 16 | 416.4807 | 26.030* | 2.785 | 1.828 | 1.765 | 5.000 | 6.656 |
| Error | 56 | 523.36 | 9.346 | | | | | |
| | | **CV%** | **4.09** | | | | | |

**ANOVA for Table - 6**

| Source of variance | df | SS | MSS | F.CAL | F.TAB 5% | SEm± | CD 5% | CD 1% |
|---|---|---|---|---|---|---|---|---|
| Control/Rest | 1 | 758.2875 | 758.288 | 80.305 | 4.013 | | | |
| Treatment (A) | 8 | 2142.3894 | 267.799* | 28.361 | 2.109 | 1.024 | 2.902 | 3.863 |
| Concentration (B) | 2 | 4673.5856 | 2336.793* | 247.475 | 3.162 | 0.591 | 1.675 | 2.230 |
| A x B | 16 | 497.0949 | 31.068* | 3.290 | 1.828 | 1.774 | 5.026 | 6.690 |
| Error | 56 | 528.78 | 9.443 | | | | | |
| | | **CV%** | **3.82** | | | | | |

* Significant at 5% level

# APPENDIX - IV

ANOVA for Table - 7

| Source of variance | df | SS | MSS | F.CAL | F.TAB 5% | SEm± | CD 5% | CD 1% |
|---|---|---|---|---|---|---|---|---|
| Control/Rest | 1 | 3563.5776 | 3563.578 | 610.870 | 4.013 | | | |
| Treatment (A) | 8 | 946.3121 | 118.289* | 20.277 | 2.109 | 0.805 | 2.281 | 3.036 |
| Concentration (B) | 2 | 1987.2486 | 993.624* | 170.327 | 3.162 | 0.465 | 1.317 | 1.753 |
| A x B | 16 | 997.8903 | 62.368* | 10.691 | 1.828 | 1.394 | 3.951 | 5.259 |
| Error | 56 | 326.68 | 5.834 | | | | | |
| | | **CV%** | **3.72** | | | | | |

**ANOVA for Table - 8**

| Source of variance | df | SS | MSS | F.CAL | F.TAB 5% | SEm± | CD 5% | CD 1% |
|---|---|---|---|---|---|---|---|---|
| Control/Rest | 1 | 6952.2615 | 6952.262 | 853.832 | 4.013 | | | |
| Treatment (A) | 8 | 4444.7385 | 555.592* | 68.234 | 2.109 | 0.951 | 2.695 | 3.587 |
| Concentration (B) | 2 | 1070.5999 | 535.300* | 65.742 | 3.162 | 0.549 | 1.556 | 2.071 |
| A x B | 16 | 648.8609 | 40.554* | 4.981 | 1.828 | 1.647 | 4.667 | 6.213 |
| Error | 56 | 455.98 | 8.142 | | | | | |
| | | **CV%** | **5.60** | | | | | |

* Significant at 5% level

# APPENDIX - V

**ANOVA for Infection Index at RCA in Table - 9**

| Source of variance | df | SS | MSS | F.CAL | F.TAB 5% | SEm± | CD 5% | CD 1% |
|---|---|---|---|---|---|---|---|---|
| Replication | 2 | 1.9328 | 0.9664 | 0.4729 | 3.555 | | | |
| Treatment (A) | 3 | 1915.1587 | 638.3862* | 312.4298 | 3.160 | 0.5835 | 1.7338 | 2.3755 |
| Concentration (B) | 1 | 310.3923 | 310.3923* | 151.9077 | 4.414 | 0.4126 | 1.2260 | 1.6797 |
| A x B | 3 | 58.3102 | 19.4367* | 9.5124 | 3.160 | 0.8252 | 2.4520 | 3.3595 |
| No spray/Rest | 1 | 2617.1303 | 2617.1302* | 1280.838 | 4.414 | | | |
| Water spray/Fact. T | 1 | 3413.0145 | 3413.0145* | 1670.348 | 4.414 | | | |
| Error | 18 | 36.7793 | 2.0432 | | | | | |
| | | **CV%** | **3.35** | | | | | |

* Significant at 5% level

# APPENDIX - VI

**ANOVA of P.E.D.C. in Table - 9**

| Source of variance | df | SS | MSS | F.CAL | F.TAB 5% | SEm± | CD 5% | CD 1% |
|---|---|---|---|---|---|---|---|---|
| Replication | 2 | 7.5948 | 3.7974 | 0.9372 | 3.555 | | | |
| Treatment (A) | 3 | 3718.6843 | 1239.5614* | 305.9255 | 3.160 | 0.8217 | 2.4416 | 3.3452 |
| Concentration (B) | 1 | 654.8075 | 654.8075* | 161.6074 | 4.414 | 0.5810 | 1.7264 | 2.3654 |
| A x B | 3 | 118.5972 | 39.5324* | 9.7566 | 3.160 | 1.1621 | 3.4529 | 4.7308 |
| No spray/Rest | 1 | 5290.5130 | 5290.5129* | 1305.7060 | 4.414 | | | |
| Water spray/Fact. T | 1 | 6613.1412 | 6613.1412* | 1632.1330 | 4.414 | | | |
| Error | 18 | 72.9331 | 4.0518 | | | | | |
| | | **CV%** | **5.05** | | | | | |

* Significant at 5% level

# APPENDIX - VII

**ANOVA for Infection Index at Farmer's Field in Table - 9**

| Source of variance | df | SS | MSS | F.CAL | F.TAB 5% | SEm± | CD 5% | CD 1% |
|---|---|---|---|---|---|---|---|---|
| Replication | 2 | 2.7738 | 1.3869 | 0.4885 | 3.555 | | | |
| Treatment (A) | 3 | 2138.9924 | 712.9974* | 251.1364 | 3.160 | 0.6878 | 2.0438 | 2.8001 |
| Concentration (B) | 1 | 357.35884 | 357.3588* | 125.8711 | 4.414 | 0.4864 | 1.4451 | 1.9800 |
| A x B | 3 | 319.1098 | 106.3699* | 37.4662 | 3.160 | 0.9728 | 2.8903 | 3.9600 |
| No spray/Rest | 1 | 1563.5151 | 1563.5150* | 550.7109 | 4.414 | | | |
| Water spray/Fact. T | 1 | 2064.1822 | 2064.1821* | 727.0590 | 4.414 | | | |
| Error | 18 | 51.1035 | 2.8390 | | | | | |
| | | **CV%** | **3.86** | | | | | |

* Significant at 5% level

# APPENDIX - VIII

ANOVA for P.E.D.C at Farmer's Field in Table - 9

| Source of variance | df | SS | MSS | F.CAL | F.TAB 5% | SEm± | CD 5% | CD 1% |
|---|---|---|---|---|---|---|---|---|
| Replication | 2 | 78.8365 | 39.4182 | 7.9019 | 3.555 | | | |
| Treatment (A) | 3 | 5012.6548 | 1670.8849* | 334.9518 | 3.160 | 0.9118 | 2.7091 | 3.7117 |
| Concentration (B) | 1 | 844.3134 | 844.3134* | 169.2542 | 4.414 | 0.6447 | 1.9156 | 2.6246 |
| A x B | 3 | 754.1995 | 251.3998* | 50.3965 | 3.160 | 1.2895 | 3.8313 | 5.2492 |
| No spray/Rest | 1 | 3674.3104 | 3674.3104* | 736.5659 | 4.414 | | | |
| Water spray/Fact. T | 1 | 4592.8880 | 4592.8880* | 920.7074 | 4.414 | | | |
| Error | 18 | 89.7918 | 4.9884 | | | | | |
| | | **CV%** | **6.73** | | | | | |

* Significant at 5% level

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