

**STUDIES ON LEAF BLIGHT OF
CANNA (*Canna indica* L.) INCITED BY
Alternaria alternata (Fr.) Keissler.**

By
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DAPOLI- 415 712, DIST. RATNAGIRI (M.S.)**

MAY, 2012

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A thesis submitted to the
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(Agricultural University)
Dist. Ratnagiri (Maharashtra State), India

in partial fulfilment of the requirements for the degree of
MASTER OF SCIENCE (AGRICULTURE)

in

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CERTIFICATE

This is to certify that, the thesis entitled **STUDIES ON LEAF BLIGHT OF CANNA (*Canna indica* L.) INCITED BY *Alternaria alternata* (Fr.) Keissler** submitted to the faculty of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (Maharashtra State), in the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY**, embodies the results of a piece of *bona-fide* research carried out by **Miss. PATIL ROOPA** under my guidance and supervision and that no part of this thesis has been submitted for any other degree or diploma or published in other form. All the assistance and help received during the course of investigation and the sources of literature has been duly acknowledged by her.

Place : Dapoli
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Dated:
Chairman,

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(P.A.

Advisory

**DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE, DAPOLI**

Title of thesis : Studies on blight disease of canna (*Canna indica* L.) incited by *Alternaria alternata* (Fr.) Keissler

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THESIS ABSTRACT

Canna (*Canna indica* L.), an important bulbous crop, the only flowering genus of family Cannaceae, is valued for its flowers as well as foliage. It has gained much importance and attraction of gardeners and floriculturists for use in garden decoration throughout the year.

The severe blight disease incidence was observed on Canna at the Hi- tech Farm, College of Agriculture, Dapoli during March in 2011.

The pathogenic fungus was isolated on PDA medium and the pathogenicity test was confirmed on the test plant. The pathogen was taxonomically identified as *Alternaria alternata* (Fr.) Keissler by the Chief Mycologist, Agharkar Research Institute, Pune.

The host range study indicated that the pathogen could infect Gerbera (*Gerbera jamesonii*), Marigold (*Tagetes erecta*), Heliconia (*Heliconia psittacorum*) and Mussaenda (*Mussaenda erythrophylla*).

Among different fungicides tested *in vitro*, Propiconazole (0.1 per cent), and Hexaconazole (0.1 percent) completely inhibited the growth of the test fungus.

In vitro screening of the plant extracts revealed that the nut extract of soapnut (*Sapindus laurifolius* L.) was most effective in inhibiting the growth of the test fungus followed by neem.

The data on antagonistic effect of the fungal bioagents against *Alternaria alternata* revealed that *Trichoderma harzianum* significantly inhibited the mycelial growth of the test fungus followed by *Trichoderma viride* and *Trichoderma koningii*.

CHAPTER-I

INTRODUCTION

Ornamental Horticulture is a branch of Horticulture which deals with commercial growing of flowers, ornamental plants and beautification of surroundings. This subject assumes great importance in improving total environment and checks visual pollution by way of beautification (Arora, 2010).

Gardening includes the entire field of Horticulture, which of course, includes ornamentals also. The importance of ornamental gardening as a hobby or for commercial purpose does not need any emphasis. However, the ornamentals do have a therapeutic value in such intensifications where they care for mentally diseased. In growing ornamental plants, the fungal diseases affecting different plant species play a damaging role in reducing the scenic and economic value of these precious crops.

The study of ornamental Horticulture comprises of two parts i.e. the knowledge of growing of plants like annuals, shrubs, climbers, trees, bulbs, succulents and cacti's, shade loving plants, lawns, etc. and their use in beautification programme. Beautification involves the principles of art, and gardening styles.

Horticulturally the bulbous plants include the plants with true bulbs, corms, rhizomes, tubers and tuberous roots. Bulbous plants are usually herbaceous perennials which play an important role in the beautification of our landscape. They add beauty and grace to the landscape. There are a large number of different types of bulbs, offering variation in form, fragrance, color and lasting display. These plants can be used in formal as

well as informal landscape in beds, borders, pots, etc (John *et al.*, 2007).

Canna, an important bulbous crop, the only flowering genus of family Cannaceae, is valued for its flowers as well as foliage. It has gained much importance and attraction of gardeners and floriculturists for use in garden decoration throughout the year (Bihari *et al.*, 2009).

Canna (*Canna indica* L.) commonly known as Indian shot belongs to family Cannaceae, is native of Central and South America and the West Indies. It is a tall growing shrub (1.5-2m) with flowers of different colour shades like yellow, red, etc. It is one of the best garden flowers suitable for growing in beds. The different coloured varieties are planted separately and sometimes different colours may be grouped together in the same bed, keeping in view their plant height, colour and bold effect, (Bose and Mukherjee, 2005).

The canna rhizome is rich in starch and it has many uses in agriculture. All aerial canna plant parts have commercial value, rhizomes for starch (consumption by human beings and livestock), stems and foliage for animal fodder and young shoots are used for vegetable purpose by many people. The seeds are used as beads in jewelry. In more remote regions of India, cannas are fermented to produce alcohol. The plant yields a fiber from the stem which is used as a jute substitute. A fiber obtained from the leaves is used for making paper. A purple dye is obtained from the seed. Smoke from the burning leaves is said to have insecticidal property. Cannas are used to extract many undesirable pollutants in a wetland environment as they have high tolerance to contaminants.

The successful maintenance of ornamental gardening needs plant protection measures, since ornamentals also succumb to many diseases like other plants. The leaf blight is supposed to be the major disease of canna as it affects the leaves of plant severely. However, the following major fungal diseases have been reported on *Canna indica* L.,

Sr. no	Disease	Causal organism
1.	Rust	<i>Puccinia cannae</i> Henn.
2.	Botrytis blight	<i>Botrytis cinerea</i> Pers.(Fr)
3.	Rhizome rot	<i>Sclerotium rolfsii</i> Sacc.
4.	Blight	<i>Alternaria alternata</i> (Fr.) Keissler

The severe incidence of leaf blight disease on *Canna indica* L. was noticed in the nursery of Department of Horticulture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. Symptoms of blight were observed as small spots, which gradually increased, ultimately giving a completely charred and burnt appearance to the plant. The disease gradually caused drying and withering of the blighted leaves.

The severity of *Alternaria* blight disease in the experimental plot of Canna was found to be quite high. So far, no research has been carried out on Canna leaf blight in Konkan. Looking towards the magnitude of the disease and severity and adverse effect caused on ornamental value of this plant, the present investigations were carried out on the leaf blight of *Canna indica* L. with following objectives:

- 1) To isolate the organism associated with blight disease.
- 2) To prove the pathogenicity on the host plant.
- 3) To study symptomatology on the host plant under artificial inoculations in the laboratory.

- 4) To study host range of the causal organism.
- 5) To study *in vitro* efficacy of different fungicides against the pathogen.
- 6) To study *in vitro* efficacy of different bioagents against the pathogen.
- 7) To study *in vitro* efficacy of phytoextracts against the pathogen.

CHAPTER II

REVIEW OF LITERATURE

2.1 Disease reports:

Wellman (1949) recorded leaf blight of *Petunia hybrida* caused by *Alternaria tenuis*.

Edward (1957) first recorded *Alternaria zinnia* on marigold from Allahabad (India) causing leaf and inflorescence blight.

Sharma (1959) demonstrated leaf blight of hollyhock caused by *Alternaria tenuis*.

Kanjanasoon and Mathur (1962) reported *Alternaria* blight of *Zinnia elegans* caused by *Alternaria tenuis*.

Rao (1962) recorded *Alternaria zinniae* on *zinnia elegans* Jacq. From Maharashtra state (India).

Rao (1965) noticed *Alternaria tenuis* Auct. (*Alternaria alternata* (Fr.) Keissler.) causing leaf blight and blossom blight of *Chrysanthemum indicum* L. from Maharashtra.

Srinath and Sarwar (1965) for the first time reported *Alternaria* blight of *Chrysanthemum cinerariifolium* (Trev.) var. *pyrethrum* caused by *Alternaria tenuissima* (Fries) Wiltshire from Bangalore.

Waterworth and Povish (1968) isolated *Alternaria tenuis* from lesions on newly introduced crop plants of *Chrysanthemum viscidihirtum* and *Hibiscus esculentus*.

Rangaswamy *et al.* (1970) recorded *Alternaria zinniae* on zinnia crop from Karnataka state.

Bedi and Singh (1972) reported leaf blight of rose in the Punjab caused by *Alternaria alternata*. The same disease was also reported by Sezgin *et al.* (1973) in Turkey.

Kulibaba (1972) described *Alternaria dauci* and *Alternaria tenuis* (*Alternaria alternata*) on Gerbera.

Sahni (1973) reported *Alternaria* leaf blight of rose incited by *Alternaria alternata* in rose gardens of Simla and Delhi.

Srivastava and Mathur (1979) recorded *Alternaria alternata* on *Jasminum sambac*.

VenkatRao and Reddy (1979) described *Bougainvillea spectabilis*, a new host of *Alternaria alternata* inciting leaf spot for the first time from Andhra Pradesh.

Chase (1982) reported *Alternaria alternata* causing the leaf blight disease which severe on *Calathea bella*.

Agarwal and Gupta (1983) recorded a new leaf spot disease of *Petunia* caused by *Alternaria alternata* from nurseries and gardens in Agra and Aligarh.

Shotri *et al.* (1983) isolated *Alternaria alternata* and *Alternaria zinnia* from the seeds of Gaillardia, *Alternaria alternata* was also reported to be foliar pathogen on *Gaillardia picta*.

Hegde (1988) reported the leaf blight of *Chrysanthemum morifolium* Ramat caused by *Alternaria tenuissima* from Dharwad in Karnataka.

Sarbajna (1989) recorded *Alternaria alternata* on *Alternanthera sessile*.

Shinde (1991) reported foliar blight of Marigold (*Tagetes erecta* L.) caused by *Alternaria alternata* from Konkan Krishi Vidyapeeth, Dapoli.

Cavallini *et al.* (1992) observed *Alternaria alternata* on commercial chrysanthemum in Italy.

Sunita and Gupta (1996) described a new leaf spot on *Gerbera jamesonii* caused by *Alternaria alternata* from Himachal Pradesh.

Kadam (1997) noticed leaf spot of gerbera caused by *Alternaria alternata*.

Mirkova and Konstantinova (2003) for the first time reported the *Alternaria* leaf spots of gerbera (*Gerbera jamesonii*) in Bulgaria.

Yadav *et al.* (2010) for the first time described *Alternaria alternata* on orchids in Uttarakhand.

2.2 Pathogenicity:

Wellman (1949) confirmed the pathogenicity of *Alternaria tenuis* by inoculating healthy leaves of Petunias (*Petunia hybrida*) with a spore suspension. The typical symptoms of leaf blight appeared within two days.

Edward (1957) proved the pathogenic behavior of *Alternaria zinniae*, causing leaf and inflorescence blights on marigold.

Kanjansoon and Mathur (1962) reported that *Alternaria alternata* causing blight of *Zinnia elegans* was infectious only in wound inoculations.

Rao (1962) confirmed *Alternaria zinniae* inciting leaf spot on zinnia under artificial inoculation.

Bedi and Singh (1972) proved the pathogenicity of *Alternaria alternata* on rose by inoculating a spore suspension of the pathogen from 10 days old culture. The typical symptoms appeared within 6 days of inoculation.

Srivasthava and Gupta (1983) confirmed the pathogenicity of *Alternaria alternata*, *Alternaria zinniae* on seeds of Zinnia causing seed rot and death of seedlings.

Crisan and Szenyei (1987) proved the pathogenicity of *Alternaria alternata* on *Dahlia variabilis* and *Canna indica*.

Shinde (1991) confirmed the pathogenicity of *Alternaria alternata* by inoculating leaves of Marigold.

Kadam (1997) conducted the pathogenicity test by inoculating gerbera leaves with spore suspension of *Alternaria alternata*.

Xu-GaoJuan and FaDi (2009) proved the pathogenicity of *Alternaria tenuissima* and *Alternaria alternata* on chrysanthemum.

Yadav *et al.* (2010) confirmed the pathogenicity of *Alternaria alternata* by inoculating leaves of *Cymbidium* spp.

Zhou *et al.* (2010) conducted the pathogenicity test by using an isolated leaf method to discuss the potentiality of *Alternaria alternata* for controlling *Alternanthera philoxeroides*.

2.3 Symptomatology:

Kanjanasoon and Mathur (1962) described the symptoms of blight of *Zinnia elegans* caused by *Alternaria tenuis* as large, brown, dry lesions with concentric rings on leaves.

Rao (1965) reported *Alternaria tenuis* Auct. (*Alternaria alternata* (Fr.) Keissler.) causing leaf blight and blossom blight of *Chrysanthemum indicum* L. from Maharashtra. He described that the spots were oval to irregular or angular, dark brown to black, inciting leaf blight, blossom blight and defoliation.

Melville and Chalk (1968) recorded symptoms produced by *Alternaria tenuis* on Anthurinum. The symptoms appeared as pale brown lesions on the leaves, mainly around the edges and on the growing points of seedlings.

Rangaswamy *et al.* (1970) reported that the symptoms appeared on zinnia crop as reddish brown spots with greyish white centres on the leaves, small in the beginning but rapidly increased in size and formed irregular brown patches. *Alternaria zinniae* was capable of infecting the stem resulting in the blighting and drying of plants.

Bedi and Singh (1972) described the symptoms produced by *Alternaria alternata* on rose. The leaf margin showed browning which started from the apex downwards to the base. The lesions extended towards the centre in humid weather. The tip and margin of an infected leaf became brittle and the colour changed from yellowish brown to dark-brown. Defoliation of the infected plant was quite common. In persisting humid weather, flower buds and flowers were also infected.

Srivastava and Mathur (1979) reported the symptoms produced by *Alternaria alternata* on *Jasminum sambac*. Spots were mostly marginal starting from the tip of leaves or petiolar end. The irregular, wood brown lesions on leaves turned tissue

into dark colour bands. The conidial masses were present on either side of the leaves.

Agawal and Gupta (1983) recorded the symptoms of *Alternaria alternata* on *Petunia hybrida* as small, isolated brown spots on the upper surface of the leaf which spread rapidly to form circular lesions. Characteristic concentric rings were also noticed in some cases. Some of the lesions coalesced to cover large areas and caused death of the infected portion. In some cases the leaves dried up from the tip. In severe infection blighting of entire leaf also took place.

Srivastava and Gupta (1983) observed the symptoms produced by *Alternaria alternata* on zinnia leaves. Initially, chlorotic areas were appeared which later on turned to yellowish brown. These zones further advanced to form irregular large lesions which covered the whole area of the leaves.

Shinde (1991) recorded the symptoms produced by *Alternaria alternata* causing foliar blight of marigold as necrotic spots on leaf surface which coalesced and produced blight symptoms. Necrotic spots were also observed on stem whereas, petals of the flowers were attacked severely, presenting a burnt appearance.

Mirkova and Konstantinova (2003) reported the symptoms on the leaves of gerbera produced by *Alternaria* spp. which were characterized by the development of brown, small, scattered dots, which gradually enlarged and coalesced to form large, oval, circular or irregular, brown to black lesions with concentric rings.

Yadav et al. (2010) described the symptoms produced by *Alternaria alternata* on *cymbidium* spp. Initially the symptoms started from the tip or margin of the leaves and progressed up to the basal part of the leaves, which later caused blight of the entire plant.

2.4 Host range study:

Bedi and Singh (1972) studied the relative reaction of 209 varieties of rose to the leaf blight caused by *Alternaria alternata*. Fifty four varieties were free from infection and remaining 155 varieties were infected to varying degree of fairly resistant to highly susceptible.

Nagi and Raghava (1984) from Indian Institute of Horticultural research, Bangalore screened 42 varieties of chrysanthemum for resistance to *Alternaria* and *Septoria* diseases and reported three varieties “Indira”, “Pinkcasket”, and “Sundhari kalikata” as tolerant. The chrysanthemum varieties “Naskar’spride”, “Phillips-11” and “Summer Gem” and the hybrid

32-27-78 were tolerant to both *Alternaria* and *septoria* diseases (Nagi and Raghava, 1985).

Crisan and Szenyei (1987) screened the varieties of *Dahlia variabilis* and *Canna indica* against *Alternaria alternata*. The cultivars viz; Justicia, opera, D.W. and Prof, Al. Borza of *Dahlia variabilis*, and Pictor Grigorescu, Agnes, Extra, America, Cardinal and Signal of *Canna indica* were resistant.

Karlatti and Hiremath (1989) collected marigold flowers heavily infected by *Alternaria zinniae* from a garden in Dharwad district of Karnataka. Seeds were separated, dried and plated on potato dextrose agar (PDA) medium. Some of the seeds were surface sterilized. Spore suspensions were prepared and inoculated onto seedlings of 10 plants belonging to the Asteraceae. *A. zinniae* was successfully isolated from apparently healthy and discoloured seeds and from those that had been surface sterilized. The isolated fungus infected Ageratum, Aster, Chrysanthemum, Cosmos and Sunflower seedlings. Safflower, Tridax, Niger and Parthenium were not infected.

Hilal and Kamel (1990) studied the reaction of carnation varieties against the blight disease caused by *Alternaria dianthi*. The cultivar Leza was resistant to disease while New Arthur was very susceptible. Amber Rose, Lena and Yellow Sim were resistant to *Alternaria dianthi*.

Mirkova and Konstantinova (2003) recorded the relative reaction on 10 gerbera cultivars against *Alternaria* spp. The cultivars screened were Diplomat, Queen, Ellyna, Berta, Maria, Raysa, Myrah, Milena, Lady and mycus all of them which were susceptible to *Alternaria* leaf spot.

2.5 Efficacy of the fungicides against the pathogen:

Crisan and Messeou (1970) reported that Captan (0.25 and 0.5 per cent) was effective in inhibiting the growth and sporulation of *Alternaria alternata* completely in *in vitro* studies.

Bedi and Singh (1972) demonstrated the inhibition of *Alternaria alternata* causing leaf blight of rose with Ziram and Thiram @ 0.2 per cent concentration.

Sahni (1973) reported that Dithane M-45 was not effective against *Alternaria alternata* in *in vitro* test.

Singh and Milne (1974) observed that Mancozeb and Captan were the most promising fungicides in inhibiting the growth and sporulation of *Alternaria alternata* in *in vitro* test.

Shorti *et al.* (1984) reported the complete control of seed borne fungus, *Alternaria alternata* with treatments comprising Ceresan, Dithane M-45 (Mancozeb) and Aureofungin.

Ungaro and Azevedo (1984) studied the efficacy of Zineb and Captan against *Alternaria alternata* in *in vitro* test and observed that Zineb was most effective in controlling the fungus.

Crisan and Szenyei (1987) tested different fungicides *in vitro* against *Alternaria alternata* causing leaf blight of canna and dahlia. Among those fungicides, Mycodifol, Euparen (dichlofluanid) and Vitavax (carboxin) were most effective.

Mallikarjun (1996) found that in *in vitro* evaluation of eight fungicides against *A. alternata* causing leaf blight of turmeric, Propiconazole (tilt) was superior in inhibiting the growth of the fungus while Ziram a non systemic fungitoxicant was found to be most effective in inhibiting the growth of the test fungus.

Kamble *et al.* (2000) tested six fungicides against *A. alternata* under *in vitro* conditions. They reported that Mancozeb was highly effective in inhibiting the mycelial growth followed by Copper Oxychloride and Iprodione at 1000, 2000 and 3000 ppm respectively.

Bagade (2006) reported complete inhibition of *Alternaria alternata* due to alone Propiconazole (0.05 percent) and was followed by Mancozeb + Copper oxychloride combination with 82.31 per cent inhibition over control.

Mane (2008) tested different fungicides *in vitro* against *Alternaria alternata* causing leaf blight of chilli. Among these Mancozeb (0.2 per cent), Propiconazole (0.05 per cent) and Copper oxychloride (0.2 per cent) were effective in inhibiting the growth of the fungus.

2.6 Efficacy of Bio-agents against the test fungus:

Pandey (1985) reported that culture filtrates of *Aspergillus* & *Trichoderma viride* retarded the growth of *Alternaria alternata*.

Kadam (1997) studied the effectiveness of *Trichoderma* spp. *in vitro* against *Alternaria alternata* causing leaf spot disease of gerbera and reported that *Trichoderma viride* and *Trichoderma harzianum* were quite effective in inhibiting the mycelial growth of *Alternaria alternata*.

Kota (2003) reported that *Trichoderma harzianum* and *Trichoderma virens* were highly effective in inhibiting the growth of *A. alternata* under *in vitro* condition.

Sanjeet kumar *et al.* (2005) observed that in dual culture, all the three antagonist, *viz.* *Trichoderma virens*, *Trichoderma harzianum* and *Trichoderma viride* overgrew the colony of *Alternaria alternata* but *T. viride* parasitized the test fungus well in advance.

2.7 Effectiveness of plant extracts *in vitro* against test fungus:

Shekhawat and Prasad (1971) reported that out of nine plant extracts *Allium cepa* L., *Allium sativum* L., *Ocimum sanctum* L., *Mentha piperita* L. and *Beta vulgaris* L. showed strong inhibitory effect against *A. tenuis*.

Saksena and Tripathi (1985) showed that the leaf extracts of *Lantana camara* L. was effective in inhibiting the spore germination of *Alternaria alternata*.

Meena and Mariappan (1993) reported that the leaf extracts of *Azadirachta indica* inhibited mycelial growth and spore germination of the seed borne mycoflora of sorghum including *Alternaria tenuis* (*Alternaria alternata*).

Senthilnathan and Narasimhan (1993) observed the effect of plant extracts on mycelial growth of *Alternaria tenuissima* inciting the blight of onion. The bulb extract of garlic and leaf extract of neem showed 33.57 and 44.9 per cent inhibition, respectively over control. The seed oil of neem and undi recorded 59.8 and 63.8 per cent inhibition of *Alternaria tenuissima*.

Kadam (1997) tested five different plants extracts against *A.alternata* causing leaf spot of gerbera at 10 per cent concentration. He observed that maximum inhibition of the fungus was recorded due to bulb extract of garlic (89.30 per cent) followed by vekhand, undi (82.30 per cent) and neem (75.61 per cent).

Karade and Sawant (1999) tested different plant extracts on solid and liquid media against *Alternaria alternata* and reported that the bulb extract of *Allium sativum* L. was most effective.

Rahman *et al.*, (1999) observed that the bishkatali (*Polygonum hydropiper* L.), garlic (*A. sativum* L.), ginger (*Zingiber officinale* Rosc) and neem (*Azadirachta indica* A. Juss) extracts were effective against *Alternaria alternata*.

Singh and Majumdar (2001) tested water and acetone leaf extracts of neem, datura, tulsi and rhizome or bulb extracts of ginger, turmeric, onion and garlic against *A. alternata* and found that datura, garlic, ginger, neem and turmeric were effective.

Jadhav (2003) studied antifungal effects of different plant extracts against *A.alternata* causing leaf spot of Gaillardia and reported that bulb extract of *Allium sativum*, leaf extract of *Calophyllum inophyllum* and *Ocimum basilicum* were most

effective in inhibiting the mycelial growth @ 10 per cent concentration.

Kota (2003) recorded maximum inhibition of mycelial growth of *A. alternata* in 10 per cent chromalaena leaf extract, while garlic bulb extract at 10 per cent recorded maximum inhibition of spore germination of the same fungus.

Mamatha and Yashoda (2006) screened eleven plant extracts against *Alternaria alternata* causing leaf blight of turmeric. Asfoetida at 10 per cent concentration was most effective in controlling the pathogen followed by Neem seed kernel extract at 5 per cent and garlic bulb extract at 10 per cent concentration.

CHAPTER-III

MATERIALS AND METHODS

The leaves showing typical symptoms of the blight disease were collected from diseased canna plants from the nursery of the Department of Horticulture, College of Agriculture, Dr. Balasaheb Sawant Konkan Vidyapeeth, Dapoli. The samples were brought to the laboratory for further studies.

3.1 Examination of diseased samples

3.1.1 Visual

Visual observations and measurements were recorded to find out manifestation of different symptoms.

3.1.2 Microscopic examination

Fresh samples of diseased leaves, showing typical leaf blight symptoms were brought to the laboratory. These samples were washed under running tap water to remove extraneous material. The diseased leaf tissues were cut in sterile Petri plate filled with little bit of water to observe whether there was oozing of bacterial sap. Before going for isolation, microscopic examination was done by making temporary mounts of diseased tissue. A drop of lacto phenol cotton blue was taken on clean micro-slide on which diseased tissue was mounted and covered with cover slip. The slides were observed under microscope to identify the pathogen.

3.1.3 Incubation of the diseased samples in humid chamber

Small pieces of infected leaves were placed on the surface sterilized micro-slide. Each micro slide was kept on a pair of sterilized glass rods in a sterilized Petri plate internally lined with sterilized moist blotting paper. These plates were incubated at

room temperature ($27 \pm 1^\circ\text{C}$) for 48 hours and later on examined under microscope to identify the pathogen.

3.2 Isolation and purification of the causal organism

3.2.1 Isolation

The fresh leaf samples of Canna showing typical symptoms of leaf blight disease were collected from Hi-Tech Farm, College of Agriculture, Dapoli and brought to the laboratory in clean polyethene bags. Tissue isolation was followed using Potato Dextrose Agar medium (PDA). The leaf samples were washed in clean water to remove extraneous material. Small bits of affected portion along with healthy portion were cut from margins of the spots. These bits were then surface sterilized with 0.1 per cent aqueous solution of Mercuric chloride (HgCl_2) for 1 min and then washed with three successive changes of sterilized water to remove the traces of Mercuric chloride. Each bit was blot dried and aseptically transferred equidistantly in Petri plates containing solidified Potato Dextrose Agar (PDA). These plates were incubated at room temperature ($27 \pm 1^\circ\text{C}$) for seven days. The pure fungal growth that appeared on Potato Dextrose Agar plates around the host tissue was transferred aseptically to Potato Dextrose Agar slants & maintained in pure for further studies.

3.2.2 Identification of the causal organism

The fungus isolated from diseased specimen and established in pure form was identified temporarily on the basis of colony and morphological characters and then it was sent to the Chief Mycologist, Agharkar Research Institute, Pune for identification and confirmation of the test fungus upto species level.

3.3 Pathogenicity

3.3.1 Inoculation

Canna seedlings were planted in earthen pots and plants were raised. The young leaves were selected for proving pathogenicity of the test fungus. The young leaves of the test plant were surface sterilized with 0.1 per cent Mercuric chloride solution with the help of cotton swab and were washed with sterile water to remove traces of mercuric chloride. Very light injuries were made on the surface of the leaves by gently pressing the sand paper (No. 40) so as to allow easy penetration by the test fungus. The spore suspension of 15 days old culture on PDA was prepared by pouring 10 ml of sterilized water on the colony surface in each Petri plate and gently scrapped with the help of sterilized needle. The suspension from ten Petri plates was strained through sterilized muslin cloth and collected in 250 ml sterilized Erlenmeyer conical flask for inoculating the test plant. The homogeneous spore suspension (>200 conidia per microscopic field at low power) prepared in sterile water was sprayed by sterilized atomizer on the surface of leaves. After inoculation, plants were covered with clean polyethene sheets of appropriate size for maintaining humid conditions. Control plants sprayed only with sterilized distilled water were also kept under similar conditions. The observations on the development of symptoms were recorded daily for a period of 15 days after inoculation

3.3.2 Reisolation

The causal organism was reisolated from the artificially inoculated leaves showing typical symptoms on leaf lamina in the same way as described under the sub-head, in isolation and purification of the causal organism. The fungal growth obtained on PDA medium by reisolation was compared with the original culture obtained from naturally infected leaves under field conditions.

3.4 Host range

This study was undertaken to determine the ability of the test fungus to infect different types of ornamental plants. The technique of inoculation was same as described earlier. Adequate control plants were simultaneously maintained in each host plant tested. The observations for disease development were recorded after a period of 10 days of inoculation. The following different types of ornamental plants were used.

1. Gerbera : *Gerbera jamesonii* Hook.
2. Marigold : *Tagetes erecta* L.
3. Hibiscus : *Hibiscus cannabinus* L.
4. Spider lily : *Hymenocallis littorals* Herb. Var. *longituba*
5. Heliconia : *Heliconia psittacorum* L.
6. Dracena : *Cordyline fruticosa* (L.) Kunth.
7. Mussaenda : *Mussaenda erythrophylla* Schumach & Thonn.
8. Croton : *Croton variegatum* L.
9. Acalypha : *Acalypha californica* Benth.
10. Aglonema : *Aglonema commutatum* Schott.

3.5 Efficacy of different fungicides against causal organism in vitro (Poisoned Food Technique)

Eight fungicides belonging to different groups were tested (Table 1) against the test fungus by using 'Poisoned Food Technique' as described by Nene and Thapliyal (1979). Potato Dextrose Agar medium (PDA) was used as basal medium and distributed in 100 ml aliquots in each 250 ml Erlenmeyer conical flasks, which were sterilized at 10.54 kg/cm² pressure for 20 minutes. The quantity of fungicide for each concentration was calculated for 100 ml medium separately. The weighed quantity of the fungicide was added in molten PDA at 45°C, mixed thoroughly and poured into sterilized plates and allowed to solidify. The mycelial discs of 5mm diameter were cut from 7 day old culture with the help of sterile cork borer. Each disc was transferred aseptically to the centre of the petriplates containing PDA. The PDA plates containing no fungicide but inoculated with fungal culture, served as control.

The plates were incubated at room temperature (27 ± 1°C). Three replications per treatment were maintained. The observations on colony diameter and sporulation were recorded when Petri plates in control treatment were fully covered with mycelial growth.

Per cent inhibition of growth of the test fungus was calculated by the following formula (Horsfall, 1956)

$$X = \frac{Y - Z}{Y} \times 100$$

Where,

X = Per cent inhibition

Y = Growth of fungus in control (cm)

Z = Growth of fungus in treatment (cm)

Table 1: List of fungicides tested against the pathogen

Sr. No.	Common name	Trade name and formulation	Fungicide conc. (%)
1.	Carbendazim	Bavistin (50WP)	0.1
2.	Mancozeb	CurrentM-45 (75 WP)	0.2
3.	Propiconazole	Tilt (25 EC)	0.1
4.	Thiophenate methyl	Roko(70 WP)	0.1
5.	Difenconazole	Score(25 EC)	0.1
6.	Copper oxychloride	Blitox (50 WP)	0.2
7.	Hexaconazole	Contaf (5 EC)	0.1
8.	Captan	Captan (50 EC)	0.2
9.	Control	-	-

3.6 Efficacy of different Bio agents against the test fungus

To study the effectiveness of fungal bioagents *viz.*, *Trichoderma harzianum*, *T. viride*, *T. koningii*, *Aspergillus niger* and yeast were tested against the pathogen in *in vitro*. *Trichoderma harzianum*, *T. viride* *T. koningii*, *Aspergillus niger* and *Saccharomyces cerevisiae* were separately grown on PDA in Petri plates. The fungal discs of 5 mm diameter were placed in such a way that both the fungi would get equal opportunity for their growth. The placement details are illustrated in Table 3.

Table 3: The details of bioagents tested against the pathogen

T ₁	Th Tf Th Th	T ₂	Tf Th Tf Tf
T ₃	Tv Tf Tv Tv	T ₄	Tf Tv Tf Tf
T ₅	Tk Tf Tk Tk	T ₆	Tf Tk Tf Tf
T ₇	An Tf An An	T ₈	Tf An Tf Tf
T ₉	Y Tf Y Y	T ₁₀	Tf Y Tf Tf
T ₁₁	Control		

Where,

Tf = Test fungus

Th = *Trichoderma harzianum*

Tv = *Trichoderma viride*

Tk = *Trichoderma koningii*

An = *Aspergillus niger*

Y = Yeast (*Saccharomyces cerevisiae*)

Each treatment was replicated three times. The plates were incubated at room temperature ($27 \pm 1^\circ\text{C}$) for seven days. The observations on colony diameter and sporulation of the test

fungus were recorded seven days after inoculation when Petri plate in control treatment was fully covered with mycelial growth of the fungus and the per cent inhibition of growth was calculated as discussed earlier in this chapter.

3.7 Effectiveness of plant extracts *in vitro* against the test fungus

3.7.1 Selection of the test plants

Test plants were selected (Table 2) on the basis of their antifungal properties against *Alternaria* sp. as reported in the chapter on review of literature.

Table 2: The list of plant extracts tested against the pathogen

Sr. No.	Common name	Botanical name	Plant part used	Concentration (%)
1.	Castor	<i>Ricinus communis</i> L.	Leaf	10
2.	Neem	<i>Azadirachta indica</i> A. Juss	Leaf	10
3.	Heena	<i>Lawsonia inermis</i> L.	Leaf	10
4.	Ghaneri	<i>Lantana camera</i> L.	Leaf	10
5.	Sarpagandha	<i>Rauwolfia serpentine</i> (L.) Benth.	Leaf	10
6.	Tulsi	<i>Ocimum sanctum</i> Linn	Leaf	10
7.	Garlic	<i>Allium sativum</i> Linn	Clove	10
8.	Soapnut	<i>Sapindus laurifolius</i> L.	nut	10
9.	Onion	<i>Allium cepa</i> L.	bulb	10

10	Control	-	-	-
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3.7.2 Processing of plant materials to test their antifungal properties

➤ Crude extraction

One hundred grams (100 g) of the fresh plant material was weighed and thoroughly washed with clean water to remove dirt. The plant material was then blended in a food processor by adding 100 ml of sterilized distilled water. The macerate was then filtered through double layered muslin cloth and was centrifuged at 4000 rpm for 5 min. After centrifuging, the supernatant was taken out whereas pellet was discarded. This supernatant was then filtered through Whatman's filter paper No. 1. The filtered extract was then passed through Sintered glass filter to avoid bacterial contamination. Thus, the standard plant extract solution (100 per cent) was obtained.

3.7.3 Effect of plant extract on mycelial growth and sporulation of the test fungus

All the glassware used in the study were sterilized before their use. The effect of plant extracts on mycelial growth was studied by 'Poisoned Food Technique' (Nene and Thapliyal, 1979).

All the plant extracts were tested at 10 per cent concentration against *Alternaria alternata* using potato dextrose agar medium (PDA) as a basal medium. To obtain 10 per cent plant extract medium, 90 ml PDA was poured in 100 ml sterilized conical flask and 10 ml of plant extract was poured in each flask with the help of sterilized pipette. Then 20 ml of such

medium was poured in each sterilized Petri plate and allowed to solidify. Mycelial discs of 5 mm diameter were cut from seven day old culture of the fungus with the help of sterilized cork borer and transferred aseptically to the centre of Petri plate already poured with poisoned medium. Medium devoid of plant extract served as control. Petri plates were incubated at room temperature ($27 \pm 1^{\circ}\text{C}$) for growth. Three replications per treatment were maintained.

The observations on colony diameter of the fungus and sporulation were recorded when Petri plate in control treatment was fully covered with mycelial growth.

The per cent inhibition of growth was calculated by using the following formula (Horsfall, 1956) as mentioned earlier in this chapter.

CHAPTER IV

EXPERIMENTAL RESULTS

4.1 Identification of disease cause

4.1.1 Examination of diseased samples

4.1.1.1 Visual

The leaf blight disease of Canna (*Canna indica* L.) caused by *Alternaria* sp. was noticed in severe form at the Hi- tech Farm, College of Agriculture, Dapoli during March 2011. The disease appeared initially as small brownish spots on the upper surface of leaf lamina later, dark brown coloured lesions were produced on upper surface of the leaves. These lesions gradually increased and formed larger patches. Leaf blight and complete drying of the affected plants were notable symptoms in advanced stage of the disease (Plate I).

4.1.1.2 Microscopic examination

Microscopic examination of temporary mounts prepared from the diseased samples in lactophenol cotton blue revealed constant association of *Alternaria* sp. with diseased tissue.

4.1.1.3 Incubation of diseased samples in humid chamber

The diseased samples incubated at room temperature (27 ± 1 °C) for 48 hrs. in humid chamber, produced light to dark grey colony with abundant conidia of *Alternaria* sp. Therefore, it was decided to isolate the pathogen.

4.2 Isolation of causal organism

The pathogen was repeatedly isolated on potato dextrose agar medium in the laboratory. The visible mycelial growth was developed around the surface of sterilized pieces after 2-3 days of incubation. Initially, the colony appeared as light gray, later

on it turned black in colour with abundant conidia of *Alternaria* sp.

4.2.1 Identification of the causal organism

On the basis of cultural and morphological characters, the test fungus was identified as *Alternaria* sp. The further identification at the species level was confirmed by the Chief Mycologist, Agharkar Research Institute, Pune. The causal organism was identified as *Alternaria alternata* (Fr.) Keissler.

4.3 Pathogenicity

4.3.1 Inoculation of fungal culture

Surface sterilized healthy leaves of canna when inoculated with bits of mycelium and spore suspension of fungal culture, developed typical symptoms within 8 to 10 days under laboratory conditions (Plate II).

The injured inoculated leaves of canna produced typical symptoms of the disease. The symptoms produced on the inoculated plants were identical to those observed in the field. Initially, leaf showed brownish spots on the upper surface of leaf lamina. Later, the dark brown coloured lesions were produced on upper surface of leaves. However, the plant kept as control and sprayed with only sterilized water remained healthy and did not produce any kind of symptoms.

4.3.2 Reisolation

The fungus was reisolated from the infected leaves and found to be identical with the original isolate, thereby confirming the test of pathogenicity.

4.4 HOST RANGE STUDY:

In the present investigation 10 ornamental plants were tested for their reaction to *Alternaria alternata* isolated from canna.

Table 4: Host range of *Alternaria alternata* (canna isolate)

Sr.No.	Common name	Botanical name	Reaction
1.	Gerbera	<i>Gerbera jamesonii</i>	+
2.	Marigold	<i>Tagetes erecta</i>	+
3.	Hibiscus	<i>Hibiscus canabinus</i>	-
4.	Spider lily	<i>Hymenocallis littorals</i>	-
5.	Heliconia	<i>Heliconia psittacorum</i>	+
6.	Dracena	<i>Cordyline fruticosa</i>	-
7.	Mussaenda	<i>Mussaenda erythrophylla</i>	+
8.	Croton	<i>Croton variegatum</i>	-
9.	Acalypha	<i>Acalypha californica</i>	-
10.	Aglonema	<i>Aglonema commutatum</i>	-

The data from table 4 indicated that among the different ornamental plants tested for their reaction to *Alternaria alternata*, Marigold (*Tagetes erecta*), Heliconia (*Heliconia psittacorum*), Mussaenda (*Mussaenda erythrophylla*) showed characteristic leaf blight symptoms within 9-12 days after inoculation, but in Gerbera, the symptoms developed after 7 days of inoculation. Other hosts viz., Spider lily (*Hymenocallis*

littorals), Hibiscus (*Hibiscus cannabinus*), Dracena (*Cordyline fruticosa*), Croton (*Croton variegatum*), Acalypha (*Acalypha californica*), Aglonema (*Aglonema commutatum*) failed to express any kind of symptom.

4.5 Efficacy of different fungicides against causal organism *in vitro* (Poisoned Food Technique)

Eight fungicides belonging to different groups were tested for their efficacy against *Alternaria alternata* by employing Poisoned Food Technique. The data obtained on the effect of different fungicides on the vegetative growth of *Alternaria alternata* are presented in Table 5, Plate III and depicted in Fig. 1.

Table5: Efficacy of different fungicides on growth and sporulation of *Alternaria alternata* (Fr.) Keissler

Sr. No.	Common name	Conc. (%)	Mean colony diameter (cm)*	Per cent inhibition
1.	Carbendazim	0.1	5.54	38.44
2.	Difenconazole	0.1	1.02	88.66
3.	Propiconazole	0.1	0.00	100
4.	Thiophenate methyl	0.1	4.35	51.66
5.	Copper oxychloride	0.2	3.12	65.33
6.	Mancozeb	0.2	3.70	58.88
7.	Captan	0.2	5.03	44.11
8.	Hexaconazole	0.1	0.00	100
9.	Control	-	9	-
S.E.m ± : 0.0274			C.D. at 1% : 0.1115	

* Mean of three replications.

In the present study all the fungicides tested were significantly effective in reducing the mycelial growth of *A. alternata* (Plate 4 and Fig. 1). The inhibition of the growth over the control of the *A. alternata* ranged from 38.44 per cent to 100.00 per cent, irrespective of the concentrations, Propiconazole and Hexaconazole completely inhibited mycelial growth of the test fungus. These two fungicides were superior over all other fungicides. The next treatment in order a merit was Difenconazole (88.66%) which was on par with Copper oxychloride (65.33%) whereas, Carbendazim was least effective in reducing the fungal growth (38.44%).

4.6 Efficacy of bioagents against the test fungus

4.6.1 Dual culture technique

The laboratory experiment was conducted by dual culture method with five bioagents *viz.*, *Trichoderma harzianum*, *T. viride*, *T. koningii*, *Aspergillus niger* and *Saccharomyces cerevisiae*. The data obtained on the effect of bioagents on growth of *Alternaria alternata* are presented in Table 6, Plate V and depicted in Fig. 3.

Table 6: Effect of different bioagents on growth and sporulation of *Alternaria alternata* (Fr.) Keissler

Sr. No.	Placement details	Mean colony diameter (cm)*	Per cent inhibition over control
1.	Th Tf Th	0.00	100
2.	Tf Th Tf	0.00	100
3.	Tv Tf Tv	2.10	76.66
4.	Tf Tv Tf	1.63	81.88
5.	Tk Tf Tk	1.65	81.66
6.	Tf Tk Tf	2.27	74.70
7.	An Tf An	1.77	80.33
8.	Tf An Tf	2.27	74.77
9.	Y Tf Y	6.19	31.22
10.	Tf Y Tf	5.08	43.55
11.	Control	9	-
S.E.m ±: 0.067		C.D.at 1% : 0.270	

* Mean of three replications.

Where,

Tf = *Alternaria alternata* Th = *Trichoderma harzianum*
Tv = *Trichoderma viride* Tk = *Trichoderma koningii*
An = *Aspergillus niger* Y = *Saccharomyces cerevisiae*

It was revealed from the data presented in table 6 that *Trichoderma harzianum*, *T. viride* and *T. koningii* significantly inhibited the mycelial growth of the test fungus over control. Maximum inhibition (100%) was recorded due to *Trichoderma harzianum*, when the test fungus was placed at the centre as well as at the periphery. *Trichoderma viride*, *Trichoderma koningii* and *Aspergillus niger* were also effective in inhibiting the growth of the test fungus (76.66-81.88, 81.66-74.70 and 80.33-74.77 per cent inhibition, respectively). *Saccharomyces cerevisiae* showed least inhibition of the test fungus, 31.22-43.55 per cent.

4.7 Effectiveness of plant extracts against the test fungus *in vitro*

The water extracts of nine plant species were tested against *Alternaria alternata* to exploit their antifungal properties. All the plant extracts were tested at 10 per cent concentration by Poisoned Food Technique. All of the plant extracts under study, showed antifungal activity against *Alternaria alternata*. The data obtained on the effect of plant extracts on growth and sporulation of the test fungus, are presented in Table 7, Plate IV and depicted in Fig. 2.

Table 7: Effectiveness of different plant extracts on growth and sporulation of *Alternaria alternata* (Fr.) Keissler

Sr. No.	Common name	Conc. (%)	Mean colony diameter (cm)*	Per cent inhibition
1.	Castor	10	8.35	7.22
2.	Neem	10	6.10	32.22
3.	Heena	10	8.03	10.77
4.	Ghaneri	10	7.70	14.44
5.	Sarpagandha	10	8.35	7.22
6.	Tulsi	10	7.2	20.00
7.	Garlic	10	7.23	19.66
8.	Soapnut	10	5.53	38.55
9.	Onion	10	7.87	12.55
10.	Control		9	-
S.E.m ±: 0.033		C.D.at 1% :0.133		

* Mean of three replications.

The data presented in Table 7 revealed that the nut extract of Soapnut (*Sapindus laurifolius* L.) recorded maximum inhibition (38.55%) of mycelial growth and was significantly superior to rest of the treatments. This was followed by Neem (*Azadirachta indica*) recording 32.22 per cent inhibition over control. The leaf extract of Tulsi (*Ocimum sanctum* Linn), Garlic (*Allium sativum* Linn) and Ghaneri (*Lantana camera* L.) recorded 20 per cent, 19.66 percent and 14.44 per cent inhibition of mycelial growth, respectively over control. Castor (*Ricinus communis* L.) and Sarpagandha (*Rauvolfia serpentine* (L.) Benth.) proved to be least effective in inhibiting the growth of the test fungus 7.22 per cent inhibition.

CHAPTER- V

DISCUSSION

5.1 Disease reports

Alternaria leaf blight is one of the most common diseases of many cultivated and wild plants. The incidence of the leaf blight of canna incited by *Alternaria alternata* (Fr.) Keissler, was recorded at the Hi-tech Farm, College of Agriculture, Dapoli. This disease incited by *Alternaria alternata* has not been reported on canna crop from Konkan region of Maharashtra, so far.

The leaf blight of canna caused by *Alternaria alternata* (Fr.) Keissler was reported in Romania by Crisan and Szenyei (1987). This constitutes the only reference of *Alternaria* inciting in canna. *Alternaria* species is a common pathogen of cultivated crops including floriculture and ornamental crops. *Alternaria alternata* was reported to infect Chrysanthemum (Rao, 1965), Rose (Bedi and Singh, 1972), Gerbera (Kadam, 1997), Dahlia (Smits and Palacios, 1999), Orchids (Yadav *et al.* 2010).

Since no comprehensive work on this disease was carried out in Konkan region, it was decided to undertake the systematic studies on some important aspects like pathogenicity, host range and control measures.

5.2 Isolation and identification

The pathogenic fungus was easily isolated from infected leaf tissue and cultured in laboratory on PDA.

Culture obtained by tissue isolation method was identified as *Alternaria* sp. on the basis of cultural and morphological characters. However, the identification upto species level was confirmed by the Chief Mycologist, Agharkar Research Institute (MACS), Pune. The causal fungus was identified as *Alternaria alternata* (Fr.) Keissler. The same pathogen was identified by

Crisan and Szenyei (1987) on blight infected canna and dahlia plants.

5.3 Pathogenicity

Pathogenicity of *Alternaria alternata* on canna was confirmed. The typical symptoms appeared on injured inoculated leaves after eight days of inoculation. These symptoms produced on inoculated plant were similar to those observed in natural conditions on canna. Reisolation from inoculated leaves always yielded the original pathogenic fungus.

Crisan and Szenyei (1987) confirmed the pathogenicity of *Alternaria alternata* affecting canna and dahlia with mycelia or typical conidia from *Alternaria alternata* culture. The pathogenicity of *Alternaria* sp. on different ornamental and floriculture plants was proved by various workers. Bedi and Singh (1972) confirmed the pathogenicity of *Alternaria alternata* affecting rose, Mirkova and Konstantinova (2003) confirmed its pathogenicity on Gerbera, Yadav *et al.* (2010) proved its pathogenicity on *cymbidium* spp.

5.4 Host range

Host range study indicated that the pathogen could infect Marigold (*Tagetes erecta*), Gerbera (*Gerbera jamsonii*), Heliconia (*Heliconia psittacorum*), Mussaenda (*Mussaenda erythrophylla*). These results indicated that the pathogen is not very host specific and infects other species of plants also signifying that these hosts play an important role in active survival of the pathogen in nursery. Crisan and Szenyei (1987) reported that the cultivars *viz.*, Justicia, opera, D.W. and Prof, AI. Borza of *Dahlia variabilis*, and Pictor Grigorescu, Agnes, Extra, America, Cardinal and Signal of *Canna indica* were resistant against *Alternaria* leaf blight. Karlatti and Hiremath (1989) recorded that

Ageratum, Aster, Chrysanthemum, Cosmos and Sunflower were the hosts of *Alternaria alternata*. Ingawale (1996) reported that the fungus *A.alternata* was not host specific and it infected 15 different host plants which included cereals, vegetables and ornamental crops.

5.5 Efficacy of different fungicides against causal organism (Poisoned Food Technique)

During present investigation, the fungicides varied in their effectiveness in inhibiting the growth of the test fungus. Among eight different fungicides tested, Propiconazole (0.1%) and Hexaconazole caused 100 per cent inhibition of *Alternaria alternate*. These results are in conformity with those of Mallikarjun (1996) who reported that Propiconazole completely inhibited the growth of *Alternaria alternata* causing leaf blight of turmeric. Mesta *et al.* (2003) and Gorawar (2004) also revealed that Propiconazole completely inhibited the growth of *Alternaria alternata* causing leaf blight of sunflower and turmeric. Bagade (2006) demonstrated that Propiconazole (0.05%) completely inhibited the spore germination of *Alternaria alternata* affecting watermelon. Arun Kumar, (2008) recorded that Hexaconazole at all the concentrations used (0.1%, 0.2%, and 0.3 %), was highly effective resulting in 100% inhibition of mycelial growth causing leaf blight of chrysanthemum.

Difenconazole (0.1%) caused moderate inhibition of *Alternaria alternata* followed by copper oxychloride. Kamble *et al.* (2000) reported that Copper Oxychloride at 2000 ppm was quite effective against *Alternaria alternata*. Mane (2008) also proved that Mancozeb (0.2 per cent), Propiconazole (0.05 per cent) and Copper oxychloride (0.2 per cent) were effective against *Alternaria alternata* causing leaf blight of chilli.

Carbendazim (0.1%) was found to be the least effective fungicide in present investigation and recorded only 38.44 per cent inhibition of *Alternaria alternata* over control. Ahmad (2009) also found that Carbendazim was less effective fungicide which recorded 33.1 per cent inhibition of *Alternaria mali* inciting apple blotch.

5.6 Efficacy of bioagents against the test fungus

The antagonistic effect of fungal bioagents was studied in the present investigation against *Alternaria alternata* under dual culture technique. *Trichoderma harzianum* was most effective against *Alternaria alternata* and recorded 100 per cent inhibition, followed by *Trichoderma viride*, and *Trichoderma koningii* causing 81.88 per cent and 74.70 per cent inhibition over control, respectively, when the bioagents were placed at the centre. These findings are almost similar to those of Mane (2008) who reported that the inhibition of *Alternaria alternata* by *Trichoderma harzianum*, *T. viride* and *T. koningii* was to the tune of 86.11 per cent, 81.33 per cent and 79.66 per cent, respectively. Similarly, *Trichoderma harzianum* was also reported to cause the inhibition of *Alternaria alternata* by Kadam (1997) and Jadhav (2003) under *in vitro* conditions. Effectiveness of *Aspergillus* and *Trichoderma viride* was demonstrated by Pandey (1985) against *Alternaria alternata*.

5.7 Effectiveness of plant extracts against the test fungus

In the present investigation, nine plant extracts were tested for their antifungal properties against *Alternaria alternata* by poisoned food technique. Among all the plant extracts tried, Soapnut (*Sapindus laurifolius*) was found to be most effective and

recorded 38.55 per cent inhibition over control followed by Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), Garlic (*Allium sativum*) and Ghaneri (*Lantana camera*) which recorded 20 per cent, 19.66 percent and 14.44 per cent inhibition of mycelial growth, respectively over control. These findings are almost similar to those of Karade and Sawant (1999) who screened different plant extracts on solid and liquid media against *Alternaria alternata* and reported that *Allium sativum* L. was found to be more effective. Jadhav (2003) also reported that bulb extract of *Allium sativum* was most effective against *Alternaria alternata*. Similarly, Effectiveness of tulsi (*Ocimum sanctum*) was demonstrated by Mamata and Yashoda (2006) against *Alternaria alternata* causing leaf blight of turmeric. Saksena and Tripathi (1985) observed that leaf extract of *Lantana camara* L. were effective in inhibiting the spore germination of *Alternaria alternata*.

Castor (*Ricinus communis*) and Sarpagandha (*Rauvolfia serpentine*) proved to be least effective in inhibiting the growth of the test fungus recording 7.22 per cent inhibition. The fungicidal toxicity of different plant extracts in the present study might be due to the antifungal metabolites present in different plant species.

However, all the effective fungicides, bioagents and plant extracts reported under present investigation, need to be tested at field level for further confirmation of present results and their utility to the farming community.

CHAPTER VI

SUMMARY AND CONCLUSION

The blight disease of canna (*Canna indica* L.) caused by *Alternaria* spp. was first time noticed in severe form at Hi-tech Farm, College of Agriculture, Dapoli during March 2011.

The pathogenic fungus was isolated on potato dextrose agar medium from infected leaves of canna. The pure culture of the fungus was obtained by tissue isolation and identified as *Alternaria alternata* (Fr.) Keissler. The pathogenicity of the fungus was confirmed by proving Koch's Postulates.

The disease appeared initially as small brownish spots on the upper surface of leaf lamina, which increased gradually in size. Several spots coalesced producing blight symptoms, resulting in drying of the plant.

The host range study indicated that the pathogen could infect Gerbera (*Gerbera jamesonii*), Marigold (*Tagetes erecta*), Heliconia (*Heliconia psittacorum*) and Mussaenda (*Mussaenda erythrophylla*).

In vitro evaluation of various fungicides indicated that Propiconazole (0.1%) and Hexaconazole (0.1%) caused 100 per cent inhibition of growth of *Alternaria alternata* followed by Difenconazole (0.1%) and Copper oxychloride (0.2%) which were also effective in minimizing the growth of the fungus.

The antagonistic effect of bioagents viz., *Trichoderma viride*, *T. harzianum* and *T. koningii* revealed that maximum inhibition of growth and sporulation was achieved due to *Trichoderma harzianum* followed by *T. viride*. All the fungal bioagents used, significantly inhibited the mycelial growth of the fungus.

Among the various plant extracts (10% concentration) tested against *Alternaria alternata* for their antifungal effect, Soapnut extract was found to be most effective in inhibiting the mycelial growth of *Alternaria alternata* followed by neem & Tulsi extracts.

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Place: Dapoli

Date:

(Patil Roopa)

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*Original not seen

TREATMENTS

(For Plate IV and Fig. 1)

Efficacy of different fungicides against *Alternaria alternata* (Fr.) Keissler

Tr. No.	Fungicides	Conc. (%)
T ₁	Carbendazim	0.1
T ₂	Mancozeb	0.2
T ₃	Propiconazole	0.1
T ₄	Thiophenate methyl	0.1
T ₅	Difenconazole	0.1
T ₆	Copper oxychloride	0.2
T ₇	Hexaconazole	0.1
T ₈	Captan	0.2
T ₉	Control	-

TREATMENTS

(For Plate V and Fig. 2)

Effect of different bioagents on growth of *Alternaria alternata* (Fr.) Keissler

I) Pathogen at centre

II) Pathogen at periphery

Tr. No.	Placement details	Tr. No.	Placement details
T ₁	Th Tf Th Th	T ₂	Tf Th Tf Tf
T ₃	Tv Tf Tv Tv	T ₄	Tf Tf Tf
T ₅	Tk Tf Tk Tk	T ₆	Tf Ad Tf Tf
T ₇	An Tf An An	T ₈	Tf An Tf Tf
T ₉	Y Tf Y	T ₁₀	Tf Y Tf Tf
T ₁₁	Control		

Where,

Tf = Test fungus

Th = *Trichoderma harzianum*

Tv = *Trichoderma viride*

Tk = *Trichoderma Koningii*

An = *Aspergillus niger*

Y = Yeast (*Saccharomyces cerevisiae*)

TREATMENTS

(For Plate VI and Fig. 3)

Effectiveness of different plant extracts on growth of *Alternaria alternata* (Fr.) Keissler

Tr. No.	Plant extract	Conc. (%)
T ₁	Castor	10
T ₂	Neem	10
T ₃	Heena	10
T ₄	Ghaneri	10
T ₅	Sarpagandha	10
T ₆	Tulsi	10
T ₇	Garlic	10
T ₈	Soapnut	10
T ₉	Onion	10
T ₁₀	Control	-

Plate-I Field symptoms of *Alternaria* blight on Canna under natural conditions



Fig 2: Effect of different bioagents on the growth of *Alternaria alternata*

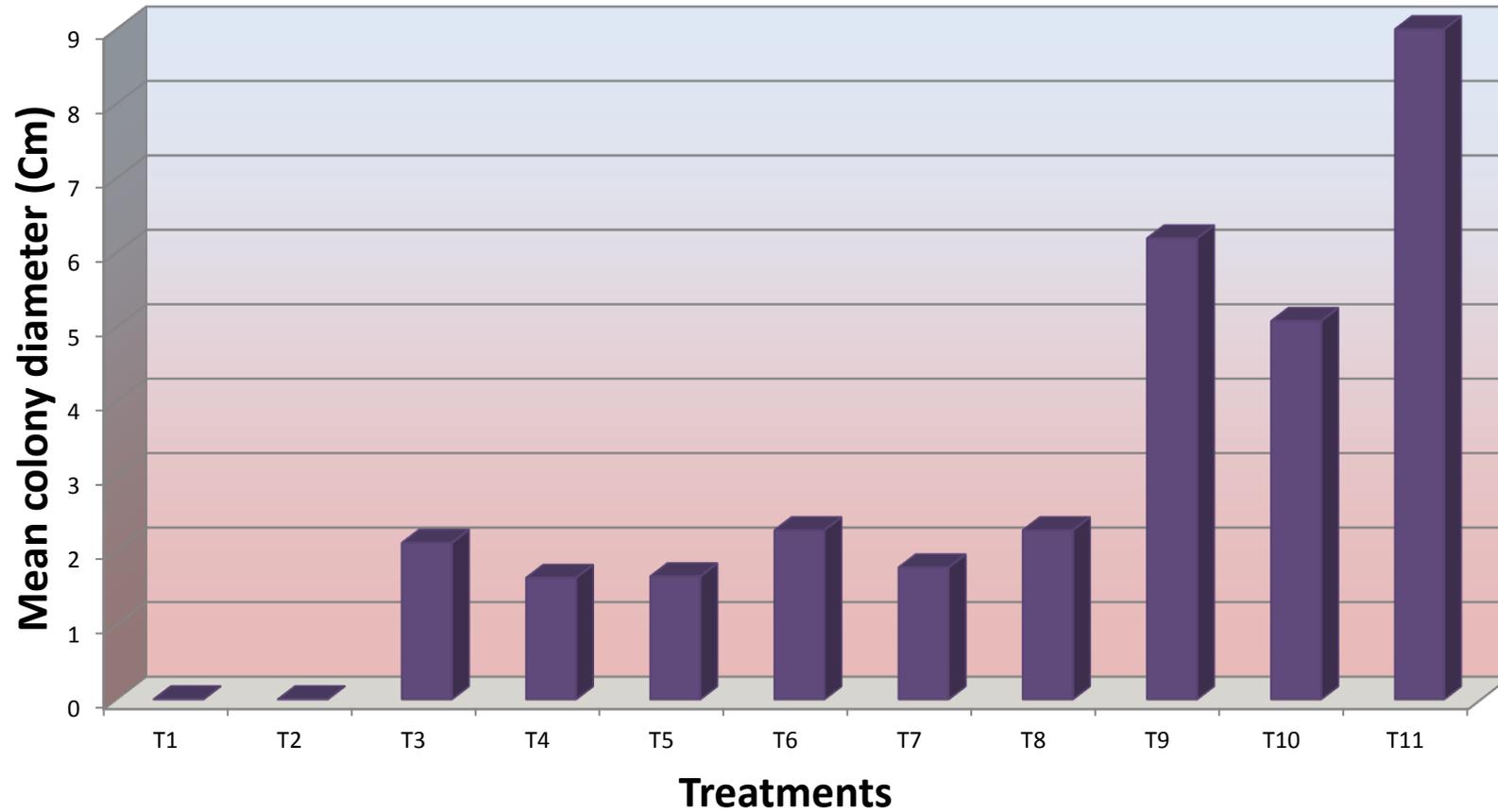


Fig 3: Effectiveness of different plant extracts on growth of *Alternaria alternata*

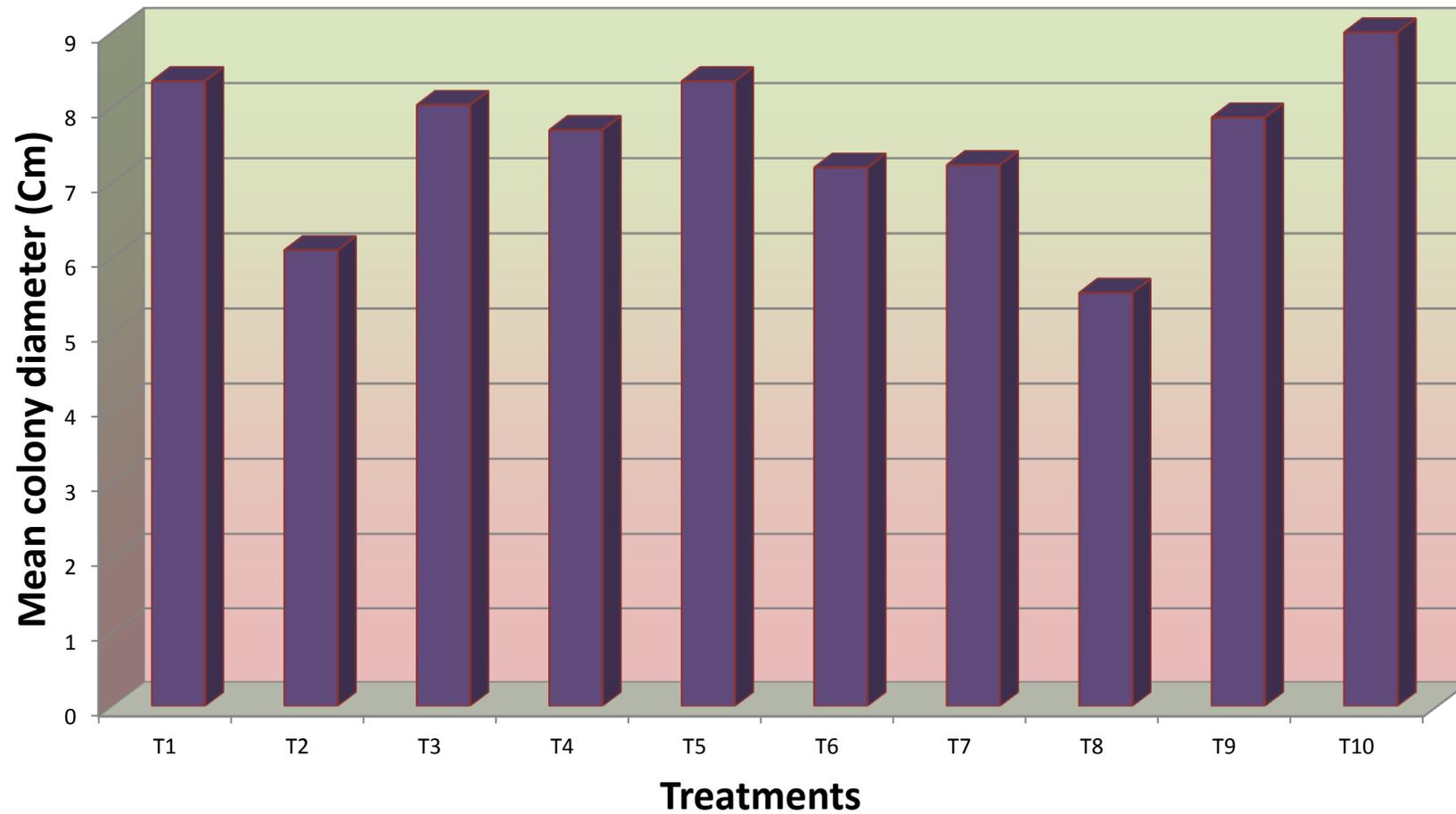


Fig 1: Efficacy of different fungicides on growth of *Alternaria alternata*

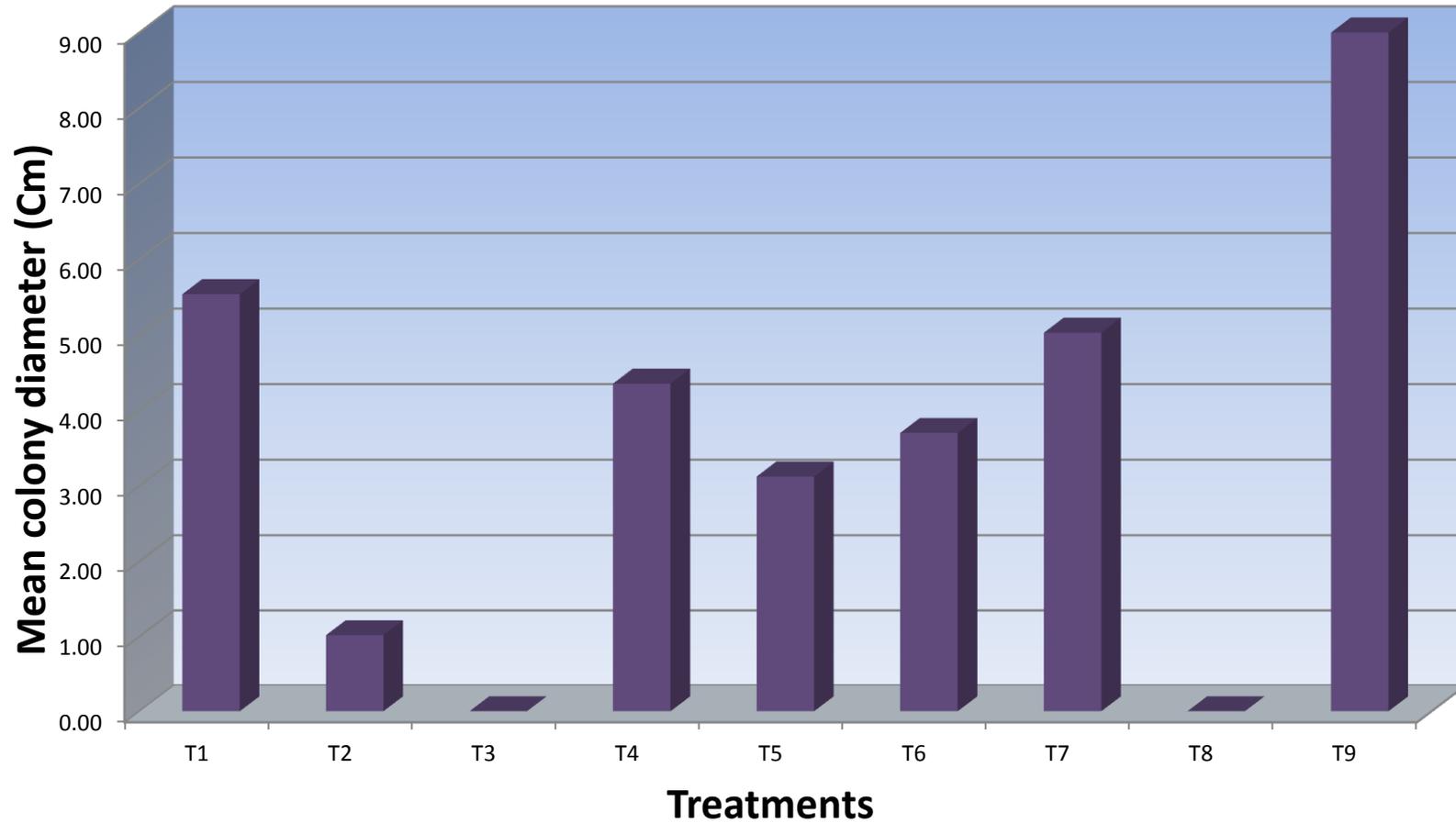


Plate III: Host range study
Symptoms produced by the pathogen on different hosts



Mussaenda



Heliconia



Marigold



Gerbera

**Plate II: Pathogenicity test for *Alternaria alternata* (Fr.)
Keissler on artificially inoculated plant of canna**



A

B

A: HEALTHY PLANT

B: INOCULATED PLANT

