"HISTOPATHOLOGY, EPIDEMIOLOGY, VARIETAL SCREENING AND MANAGEMENT OF LEAF SPOT OF GERBERA (Gerbera jamesonii H. Bolux ex J.D. Hook) CAUSED BY Alternaria alternata (Fr.) Keissler UNDER CONTROLLED CONDITIONS"

A

THESIS SUBMITTED TO THE NAVSARI AGRICULTURAL UNIVERSITY NAVSARI

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MASTER OF SCIENCE (HORTICULTURE) IN HORTICULTURAL PATHOLOGY

BY

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ABSTRACT
HISTOPATHOLOGY, EPIDEMIOLOGY, VARIETAL SCREENING AND MANAGEMENT OF LEAF SPOT OF GERBERA (Gerbera jamesonii H. Bolux ex J. D. HOOK) CAUSED BY Alternaria alternata (Fr.) Keissler UNDER CONTROLLED CONDITIONS

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ABSTRACT

Gerbera (Gerbera jamesonii H. Bolux ex J. D. Hook) is one of the important crop primarily grown for cut flower in India. During the survey, occurrence of leaf spot disease in gerbera was noticed in serious proportion affecting severely on plant growth and flower quality around Navsari areas. Considering the seriousness of the problem, the present investigation was carried out to pinpoint exact cause and to find out suitable control measure for the disease. The repeated isolations from infected leaves revealed the association of Alternaria sp., which was identified as Alternaria alternata (Fr.) Keissler (I. T. C. C. No. 8774-12). The Pathogenicity was proved by different artificial inoculation methods with positive results.
Abstract

The histopathology study of pathogen revealed that the hyphae penetrated between the walls of cell after 72 hours and developed fruting body like structure in leaves.

In the present investigation biochemical characterization of gerbera cultivar was carried at pre (45 DAP) and post-infection (57 DAP i.e. 7 days after infection). Results indicated that total soluble sugar was higher in resistant variety at pre-infection stage, whereas susceptible variety had higher total soluble sugar at post-infection stage. Total phenol content at pre-infection stage did not show inherent resistance or susceptibility. Total phenol was found to be higher in susceptible variety. Peroxidase (POX) activity were observed higher in susceptible variety at pre-infection stage as compared to resistant variety at pre-infection stage. POX activity were higher in both the resistant and susceptible variety in post infection stage as compared to the pre-infection stage. Catalase (CAT) activity were higher in resistant variety at both the stages of analysis but decreased at post-infection compared to pre-infection stage.

Thirteen varieties were screened under greenhouse conditions. Among these, minimum percent disease intensity was recorded in Kento and Jaffana, while the variety Venezia proved resistant and Binaka, Torbin were found moderately resistant. Further Ice queen, Lion, Diego, Fana and Stanza recorded moderately susceptible and C.F. orange, Cherany and C.F. gold were found susceptible.
The observations on leaf spot intensity were recorded at weekly interval from C. F. Gold variety in greenhouse during entire crop season. Initiation of leaf spot was started after 60 days of transplanting and since then it was found continuously progressing and reaching up to 30.48 per cent at harvest during September. There was main peak of leaf spot between 23rd to 30th July which considered as window period. Among the various weather parameters, average temperature was found to have key role affecting comparatively more on the disease development. Multiple correlation (R) value and co-efficient of determination value were found 0.97 per cent and 91.29 per cent, respectively. The multiple regression equation, $y = 389.55 + 828.8508 (x^1)$ where $X^1 =$ Average temperature was established on the basis of the observations. The observed and predicted per cent leaf spot intensity at any peak was found closely related.

Standard weak 44th was the peak period of highest disease index. The highest AUDPC value observed in variety Cherany (2044) followed by Diego (1772) and Stanza (1675).

Eight fungicides were screened in vitro against A. alternata where propiconazole (200, 300 & 500 ppm), tebuconazole (300 & 500 ppm) and difenoconazole at higher concentration proved very effective in inhibiting the mycelia growth of the pathogen. Among combination fungicides, tebuconazole + trifloxystrobin, captan + hexaconazole, femamidone + mancozeb, iprovalicarb + propineb, bayleton +
triadimefon also proved effective especially at higher concentration.

The fungicide concentration those found effective under laboratory screening were further screened under pot conditions against Alternaria leaf spot disease. Maximum percent disease control was recorded by the spraying of propiconazole (79.60 %), tebuconazole + trifloxystrobin (75.85 %) and tebuconazole (72.50 %).
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CERTIFICATE

This is to certify that the thesis entitled  
“HISTOPATHOLOGY, EPIDEMIOLOGY, VARIETAL SCREENING AND MANAGEMENT OF LEAF SPOT OF GERBERA (Gerbera jamesonii H. Bolux ex J.D. Hook) CAUSED BY Alternaria alternata (Fr.) Keissler UNDER CONTROLLED CONDITIONS.” submitted by Mr. PANSARE MAHESH KERUNATH in partial fulfillment of the requirements for the award of the degree of MASTER OF SCIENCE (HORTICULTURE) in HORTICULTURAL PATHOLOGY of the NAVSARI AGRICULTURAL UNIVERSITY is a record of bonafied research work carried out by him under my guidance and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

Place: Navsari  
Date: 8th July, 2013.  
(PUSHPENDRA SINGH)  
Major Advisor
DECLARATION

This is to declare that the whole of the research work reported in the thesis in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (HORTICULTURE)** in **HORTICULTURAL PATHOLOGY** by the undersigned is the result of investigations done by me under direct guidance and supervision of **Dr. PUSHPENDRA SINGH** Associate Professor, Gujarat Agricultural Biotechnology Institute, Navsari Agricultural University, Surat and no part of the work has been submitted for any other degree so far.

**Place:** Navsari  
**Date:** 8th July, 2013.

Countersigned by

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I. INTRODUCTION

Gerbera is commonly known as ‘Transval daisy’, Barberton daisy or African daisy. It belongs to the family Compositae. The genus *Gerbera* consists of about forty species of hardy and perennial flowering plants. Out of which only *Gerbera jamesonii* is under cultivation. It is important flower grown throughout the world under wide range of climatic conditions.

Gerbera is native to South Africa and Asiatic regions. In India, they are distributed in the temperate Himalayas from Kashmir to Nepal at the altitude of 1300 to 3200 meters. In Gujarat mostly South region is dominant for cultivation of gerbera. A well-drained, rich, light, neutral or slightly alkaline soil is most suitable for gerbera production. Now a days it is being cultivated in polyhouses also for better yield and quality. In Gujarat, the production of gerbera under polyhouse condition is increasing. Thus, gerbera is becoming one of the most important cut flower crops in both fields as well as in polyhouse conditions.

The genus *Gerbera* was named in the honor of German naturalist, Traugott Gerber. It is a beautiful flower remarkable for the extra ordinary geometrical regularity of its form, which often looks artificial. It is ideal for beds, borders, pots and rock gardens. The large daisy like bloom certainly does give the best impression. Plants are stem less and tender perennials herbs, leaves are radical, petioles, lanoeolate and deeply lobed. Flower head is solitary, many flowered and with conspicuous ray florets.
are in wide range of colours, such as yellow, orange, cream-white, pink, brick red, scarlet, salmon and various other intermediate shades. Based on flower head types or forms they are grouped into single, double and semidouble cultivars. The flower stalks are long thin and leafless. This characteristic made gerbera very popular and is of great demand in market for preparation of bouquets. Because of its graceful appearance, hardiness, ability to stand the vigour. It transpiration admirably and its long lasting flower quality.

Floriculture has considerable potential for export looking towards the commercial enterprise. In India area under floriculture is 1,91,000 ha with production 10,31,000 MT of loose flowers and 69,027 million cut flowers in 2010-11 (Anon. 2011). Gujarat, endowed with varied agro-climatic condition, has immense scope for cultivation of various flower crops and is advancing rapidly in the lucrative flora business. The state is producing around 49,500 MT of loose flowers, 5063 lakh cut flowers from nearly 12,500 ha area (Anon. 2011).

The crop is affected by a number of fungal diseases viz., root rot (Pythium irregularae and Rhizoctonia solani Kuhn.); foot rot (Fusarium oxysporium Snyder and Hansen and Phytophthora cryptogea); sclerotium rot (Schererlivrum rolfsii Sacc); blight (Botrytis cinerea); powedery mildew (Erysiphe cichoracearum DC. and Oidium crysiphoides f. sp. gerbera); leaf spot (Phyllosticta gerbarae, Alternaria sp.); downy mildew (Bremia lactucae), bacterial disease viz., bacterial blight
(Xanthomonas sp.) and viral diseases *viz.*., tobacco rattle virus and mosaic virus (gerbera mosaic virus) (Bose and Yadav, 1989). Among all fungal, bacterial, and viral diseases, fungal disease *Alternaria* leaf spot caused by *A. alternata* is a serious disease in south Gujarat, causing considerable losses in green house conditions.

Leaf spot of gerbera is caused by several species of *Alternaria* (Jacob and Folk, 1986; Saini *et al*., 1989; Sunita *et al*., 1996 and Mirkova, 1998); *A. alternata*, *A. dauci*, *A. porri*, *A. solani* (Pape, 1964; Kulibaba, 1972; Jacob & Folk 1986), *A. gerberae* (Wick and Disklow, 2000). *A. alternata* was noticed causing leaf spot on all the varieties of gerbera in greenhouse (Ghosh *et al*., 2002). It is severe and common disease in the entire gerbera growing areas in the country. Leaf spot caused by *Alternaria alternata* was observed in South Gujarat region causing considerable losses to the crop. Looking to the seriousness of this disease under south Gujarat, present investigation was carried out on the following objectives.

1. Isolation, purification, identification, pathogenicity and Symptomatology
2. To study Histopathology of the diseased plants/Leaves
3. To study biochemical parameters for disease resistance
4. To study varietal screening against Alternaria blight
5. Epidemiology of the disease
6. Screening of different fungicides against disease *in vitro* and in pot condition
REVIEW OF LITERATURE
Plant diseases are the major constrain in the crop production as they inflict heavy losses. Gerbera is affected by many fungal diseases viz., root rot (*Pythium irregularae* and *Rhizoctonia solani* Khunn.); foot rot (*fusarium oxysporium* Snyder and Hansen and *Phytophthora cryptogea*); sclerotium rot (*S. rolfsii* Sacc.); blight (*Botrytis cinerea*); powdery mildew (*Erysiphe cichoracearum* DC. and *Oidium crysophoides* f. sp. *gerbera*); leaf spot (*Phyllosticta gerberae, Alternaria sp.*); downy mildew (*Bremia lactucae*); bacterial disease viz., bacterial blight (*Xanthomonas* sp.) and viral disease viz., tobacco rattle virus and mosaic disease (gerbera mosaic virus) causing severe losses (Bose and yadav, 1989). Alternaria leaf spot caused by *Alternaria alternata* (fr.) Keissler is one of the important disease of gerbera (*Gerbera jamesonni* H. Bolux J. D. Hook) (Gosh *et al.* 2002).

During the survey, gerbera crop was found severely affected by Alternaria leaf spot in nurseries, gardens, greenhouses and fields of ASPEE College of Horticulture and Forestry, N.A.U, Navsari and surrounding areas throughout the crop season causing a serious threat to its cultivation. Research worked carried out by various workers on different aspects of the gerbera Alternaria leaf spot caused by *A. alternata* is reviewed here as under.

### 2.1 Occurrence

The leaf spot of gerbera caused by several *Alternaria* species like *A. alternata, A. dauci, A. porri* and *A. solani* are
commonly found on gerbera (*G. jamesonii* H. Bolux ex J.D. Hook) worldwide (Jacob and folk, 1986; Saini *et al.*, 1989; Sunita *et al.*, 1996; Mirkova, 1998). *Alternaria gerberae* was reported by Wick and Disklow (2000) from Netherland while Alternaria leaf spot caused by *A. alternata* on gerbera was reported from Bulgaria by Mirkova and Konstantinova (2003). Gosh *et al.*, (2002) also reported leaf spot of gerbera caused by *A. alternata* from Pune (India).

### 2.2 Causal organism

The genus *Alternaria* belongs to the family Dematiaceae of the order Moniliales under the class Hyphomycetes.

*Alternaria* sp. are among the most widely distributed and most common form of fungi Imperfecti. There are very few economically important crops throughout the world that escape from infection by one or more species of *Alternaria*. The genus *Alternaria* was first described by Nees in 1816.

Neergaard (1945) divided species of this genus into three groups on the basis of size of the conidial chain and the presence or absence of the chain. They are:

a) Longicatenatae, with 10 or more conidia in a chain *e.g.* *A. tenuis*

b) Bravicatenatae, with shorter chain of 3 to 5 conidial chain *e.g.* *A. citri.*
c) Noncatenatae, solitary conidia with long and filiform beak, *e.g.* *A. solani*.

Lucas (1971) suggested the name of *Alternaria longipes* as *A. tenuis* for which according to international rules of nomenclature, the valid name is *A. alternata*.

Several *Alternaria* species are commonly found on gerbera worldwide. *A. alternata*, *A. dauci*, *A. porri* and *A. solani* were also isolated from gerbera leaves with some of the cultivars being very susceptible (Pape, 1964; Kulibaba, 1972; Jacob & Folk 1986, Saini *et al.*, 1989; Sunita *et al.*, 1996 and Mirkova, 1998).

### 2.3 Isolation of the pathogen

Solanki (2004) isolated *Alternaria alternata* from leaf spot of gerbera. The fungus produced profuse mycelial growth on PDA, which gradually turned light grayish and brown to dark grayish in colour within 10 days. The microscopic observation revealed that the hyphae were septate and light to dark brown in colour. The mycelium was irregularly branched at acute angle, conidiophores were light brown, long conidia with 3-5 transverse and 0-3 longitudinal septa measuring 23.40 -32.76 X 5.27 -9.95 µm.

Pandey and Vishwakarma (1999) isolated *A. alternata* from Uttar Pradesh, causing leaf blight disease of brinjal.

Bhatt *et al.* (2000) isolated *A. alternata* from Kumaon hills of Uttar Pradesh, which was causing leaf blight disease in tomato and chilli.
2.4 Identification of pathogen

Sreekantiah et al. (1973) described the cultural characters of *A. alternata* (leaf spot and fruit spot pathogen of chilli) in details. Initially the fungus produced whitish aerial hyphae, soon turned greenish brown to brown revealing a clean concentric ring pattern covering entire area within 4-5 days on potato dextrose agar (PDA) medium.

Mirkova and Konstantinova (2003) observed that the conidia of *A. alternata* isolated from gerbera were catenated in long, sometimes branched chains of 5-12 spores, variable in size and shape, usually ovoid to ellipsoid or obclavate and with a usually pale long oval conidia, pale brown to brown, with 3-6 transverse and 0-2 longitudinal or oblique septa and measured 25-35x5-10 µm.

According to Nees (1816) clones of *A. alternata* were usually black or olivaceous, sometimes grey. Conidiophores arising singly or in small groups, small or branched, straight or flexous, sometimes geniculate, pale to mid olivaceous of golden brown, smooth, up to 50 µm long, 3 to 6 µm thick with one or several conidial scars. Conidia formed in long, often branched chains, obclavate, ovoid and ellipsoidal often with a short conical or cylindrical beak, one third length of the conidium, pale to mid golden brown, smooth or verruculose, with up to 8 transverse, overall length 20 to 63 (37) µm, 9 to 18 (13) µm thick in the broadest part; beak pale and 2 to 5 µm thick.
Bedi and Singh (1972) observed blackish green, septate and branched mycelium of *A. alternata* isolated from rose. The condiphores and conidia were light yellowish brown, ovoid and pyriform with a short cylindrical beak, the length varied from 10.5 to 47.5 µm and breadth from 3.5 to 17.5 µm with 3 to 6 transverse and one or two longitudinal septa.

Patil (2003) noted the morphological characters of *A. alternata* isolated from marigold. The mycelium was irregular at acute angle, the hyphae were smooth, septate, profusely branched, light to dark brown in colour, 3.7-5.5 µm in diameter, conidiphores were erect, grouped, unbranched, brown to dark brown, septate (1-6 septa), 37.2-124.0 µm x 6.2-9.3 µm in size, muriform conidia with 3-9 transverse and 1-4 longitudinal septa in younger and 8-14 transverse and 3-6 longitudinal in older ones, deep brown in colour, showed a wide central part-tapering at both the ends and measuring 186-217 µm x 8.6-31.0 µm in size.

Keissler (1912) had given the morphology of *A. alternata*. According to him the colonies were usually black or olivaceous black and sometimes grey. Conidiophores produced singly or in small groups, simple or branched, straight or flexuous, sometimes geniculate, pale to mid olivaceous or golden brown, smooth, up to 50 m long, 3-6 m thick, with one or several conidial scars. Conidia formed in long often branched chains, obclavate, pyriform, ovoid or ellipsoidal often with short conical or cylindrical beak some times up to but not more than one third the length of the conidium, pale to mid golden brown,
smooth or verruculose with up to eight transverse and usually several longitudinal or oblique septa. Overall length 20-63 μm, 9-18 μm thick in the broadest part, beak pale, 2-5 μm thick.

Pandey and Vishvakarma (1999) mentioned the growth characters of *A. alternata* (leaf spot of brinjal), the mycelial growth was circular, profuse cottony and wooly, olivaceous black, with white periphery on potato dextrose agar medium.

### 2.5 Symptomatology

The leaf spot of gerbera caused by *A. alternata* is characterized by the development of brown, small, scattered dots approximately 1 mm in diameter initially. They gradually enlarge and coalesce to form large patches (20-25 mm). Oval and circular to irregular, brown to black lesions with concentric rings are produced. Lesions are often numerous and enlarge quickly to blight the leaf tissues. The spots are also found on petioles, which get desiccated and sunken, the adjacent leaves shrivel, turn yellow and finally dry up. The disease attacks first the lower older leaves and progress upwards. Severely infected leaves drop off from the plant. Lesions on the stem are sunk and dark. The plants that survived showed lower vitality, suppressed development and smaller, distorted in shape and fewer in number flowers. The disease is usually observed on mature plants but young seedlings can also be girdles (Mirkova and Konstantinova, 2003).
Sahni (1973) reported *A. alternata* on rose, producing small, oval to irregular brown lesions at the margin as well as at the apex of the leaf which later extended all over the leaf surface. The spots were scattered and subsequently enlarged showing concentric rings or ridges. The infected leaves became weak and finally dropped.

Waraitch *et al.* (1974) noticed *A. alternata* on a new host, *Crataeva religiosa*, producing minute spots, circular in outline and light brown with raised margin and depressed centre, giving pimple appearance, scattered on the leaf let or in abundance, near the margin, mostly isolated. Sometimes coalesced forming large, irregular necrotic lesions, which become papery and latter on the dead tissues, there may fall off to give the shot hole effect.

Mc Kenzie and Dingley (1996) recorded *A. alternata* causing leaf spot of petunia. The spots were circular, brown later turned dark brown. Under high humidity and temperature these spots enlarged rapidly, coalesced with each other giving the plant-blighted appearance.

Verma and Sharma (1999) and Patil (2003) observed leaf spot of marigold first on lower older leaves and progressing upward. *A. alternata* caused circular, brown lesions that later enlarged, coalesced and turned dark brown to black. Severely infected plant became black, appeared scorched and eventually died. On blooming, the inflorescence axis and flower heads were attacked severely and turned dark brown to black.
Yu et al. (2001) reported that A. iridicola causing a leaf blight disease of blackberry-lily (Belamcanda chinensis) producing lesions on leaves were elliptical to irregularly oval in shape, yellow brown to dark brown, sometimes concentrically zonate with diffuse margins, frequently surrounded by light coloured haloes. The infection often started at leaf tips and progressed to the base of leaves as symptoms developed. In severe infection, lesions enlarged and coalesced, resulting in blighting of leaves.

2.6 Pathogenicity

Sreekantiah et al. (1973) proved the pathogenicity of A. alternata with wire brush inoculation method on fruits and leaves of chilli plants and observed the typical fruit rot and leaf spot symptoms within seven days of inoculation.

Hotchkiss and Baxter (1983) proved the pathogenicity of A. alternata on Tagetes sp. causing blight of marigold characterized by dark lesions on leaves, stem and petals.

Thippeswamy et al. (2006) isolated the A. solani from the infected leaf of brinjal and proved the pathogenicity by spraying of spore suspension on abaxial and adaxial surface of leaves.

Umamaheshawari et al. (2008) proved the pathogenicity of A. alternata and A. cucumerina on cucurbits, causing leaf blight symptoms. Many workers have proved the pathogenicity of
A. alternata (A. tenuis Nees) on various plant species successfully (Dutta et al., 1971; Chaturvedi, 1972 and Bedi and Singh, 1972). Mirkova and Konstantinova (2003) reported that, *Alternaria alternata* causing leaf spot of gerbera was pathogenic when artificially inoculated. They found positive results in all 10 gerbera varieties tested.

Waraitch et al. (1974) reported pathogenic ability of *A. alternata* on *Crataeva religiosa* Forst. Leaves under natural conditions, where lesions appeared within 10 days after inoculation with 7 days old culture of the fungus.

Mistry (1992) had successfully proved pathogenicity of *A. alternata* by spraying of spore suspension on papaya leaves. *A. alternata* infected all inoculated leaves and produced percent infection within fifteen days.

Patil (2003) have proved pathogenicity of *A. alternata* on marigold successfully through all the inoculation techniques, however, pin prick inoculation method appeared more effective followed by tooth brush injury and carborandum powder injury inoculation method while the pathogenicity of *A. alternata* isolated from green gram was proved by different artificial inoculation methods with positive results by Patel (2003).

Marcinkowska (1982) reported that under *in vitro* conditions, all the isolates of *A. alternata* caused slight seedling infection in tomato. In glass house tests, *A. solani* was highly pathogenic to leaves and stems of tomato, while *A. alternata* was
weakly pathogenic. *A. solani* penetrated through undamaged fruit surface while *A. alternata* attacked injured fruits only.

### 2.7 Histopathological study of the Disease.

Dita *et al.* (2007) carried out histopathological analyses of the infection process of *A. solani* in three potato cultivars with different levels of early blight resistance. In order to study the effect of leaf age, leaf samples were collected separately, in the lower, middle and upper third of the plants. These results suggest that HR may be one of the mechanisms associated with age-related and genetic resistance to early blight in potato.

Jose Cristino *et al.* (2004) observed that the histopathology of *A. alternata* on tomato crop after 6, 12, 24, 48, 72 hour of inoculation and they observed that pathogen produces, appresoria, superiolesion, penetration peg and ostiole for conidial formation. Further, they also reported that penetration by *Alternaria* sp. commonly occurs from appresoria and which proves essential characters of proving pathogenesis.

### 2.8 Biochemical parameters for disease resistance

#### 2.8.1 Total soluble sugar

Prasad *et al.* (1997) observed the gradual decrease in total starch, total sugar, reducing sugar and individual sugars in the leaflets of Khesari due to downy mildew disease. Non-reducing sugar tended to increase.
Kumar et al. (2002) reported higher content of total sugars and reducing sugars in the rust susceptible genotypes of pearl millet. After rust infection (caused by *Puccinia pennisetii*), the level of total sugars and reducing sugars increased in the resistant and moderately resistant genotypes, but decreased in the susceptible genotypes.

### 2.8.2 Total phenol content

Phenolics are well known anti-fungal, anti-bacterial and anti-viral compounds occurring in plants. According to Matern and Kneusal (1988), the first step of the defense mechanism in plants involves a rapid accumulation of phenols at the infection site. Phenols by their simple structure penetrate the microorganisms cause considerable damage to the cell metabolisms.

Yadav et al. (1998) reported the changes in phenol content due to downy mildew infection. Total phenols was higher in leaves of highly resistant genotype (P-310-17) compared with the moderately resistant (ICMP 451) and highly susceptible (70425 MS) genotypes. After infection phenol increased during the early stage of plant growth (30 DAS) but decreased with plant age and increased due to infection.

Gogoi et al. (2001) concluded from their investigation that the level of total phenol was highest in HD 29 (highly resistant) genotype of wheat at 2 days after infection (DAI) with karnal bunt pathogen and then declined significantly. In the
susceptible wheat WL 711, total phenol was the highest at 0 DAI and decline significantly to 6 DAI but total phenol content was higher than resistant genotype.

2.8.3 Peroxidase

Peroxidase, a group of heme-containing glycosylated protein was found to play significant roles in defense mechanisms and development of plants (Hiraga et al. 2001)

Activity of peroxidase was found to be maximum in resistant variety of maize 'Ganga-5' followed by 'VL-42' and 'Malan' infected with Helminthosporium maydis. However, in H. turcicum leaf blight the enzyme activity was found to be higher in susceptible variety 'Malan' followed by 'Ganga-5' and 'VL-42' (Sukhwal and Purohit, 2003).

2.8.4 Catalase activity

Catalase is thought to play an important role in removing H2O2 from plant tissues. Vanacker et al. (1998) showed that CAT activity in barley apoplast increased 20 fold in a susceptible cultivar after infection, whereas in a susceptible oat cultivar it decreased approximately 50%. After inoculation with Botrytis cinerea, CAT activity in the apoplast increased similarly in both less susceptible and more susceptible cultivars of tomato. In the control (uninoculated), the level of CAT activity was a little higher in more susceptible cultivar (Patykowski and Urbanek, 2003).
Guo et al. (2003) studied the changes in catalase activity in 4 maize cultivars in response to gray leaf spot (*Cereospora zeae-maydis*). The catalase activity was higher in resistant and susceptible cultivars at start of inoculation but then decreased. The range of the changes in resistant cultivars was larger than those in the susceptible cultivars.

2.9 Varietal screening against *Alternaria* sp.

The long term disease management strategy includes use of disease resistant varieties. It is considered as the best alternative, being most effective, cheaper and eco friendly also.

Bedi and Singh (1972) screened 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 degrees of fairly resistant to highly susceptible. For this, the plants of test varieties were grown in earthen pots. These earthen pots were kept on the glasshouse under polythene moist chamber for 48 hrs after inoculation for providing sufficient humidity.

Singh and Singh (1998) screened 29 Phaseouls vulgaris varieties against *A. alternata* in field and Kentucky wonder, EC-26392 and PBL-14-1 were found resistant. EC-57080, Sel-9, MB-Radish spotted, sum-1, p-37, sel-48-2-3 and sel48-1-2 gave moderately resistant reaction. MB light brown, Arka Komal, Mbwhite, Pusa Parvati Contender, D- 48, Pusa Hem Lata, Sel- 2, Sel- 4 and SB black were moderately susceptible.
Angadi et al. (2002) Screened 10 varieties of China aster against Alternaria leaf spot in field. The observations on intensity of disease were recorded by using 0 to 5 point disease rating scale and also percent disease index was worked out. Among the cultivars, eight cultivars were moderately resistant and two cultivars were moderately susceptible. There were no cultivars which were either resistant.

Shivanna and Shetty (1991) Screened eight cluster bean varieties and HG 182 found showing the lowest percent infection of *A. Cyamopsisidis* in both pot and field trials while Bharodia et al. (1993) found cluster bean variety GAU 434, the variety with high gum content as moderately resistant to bacterial blight, Alternaria leaf spot and Powdery mildew.

Kushwaha et al. (1993) reported that the seedlings of 12 faba bean cultivars, inoculated with *A. alternata* and maintained under JV-1 proved moderately resistant (6 to 10 Percent infection).

Maheshwari et al. (1997) Screened 44 varieties of Lablab purpureus for their reaction of *Alternaria alternata* and found that Arka Vijay, JDL- 77, Pusa early prolific and Rajani were Resistant while Arka jai, Culture- 6802, 7001, HA- 3, HD- 1, 81, JDL- 85 and Kalyanpur Tpe- 2 were susceptible to highly susceptible.

Pangavhane (2001) Screened cluster bean varieties against leaf spot and blight caused by *A. Cyamopsisidis*, variety
Sona-51 and Dipali were found as moderately susceptible while Neelam-51 as susceptible and Pusa Narbahar as highly Susceptible.

2.10 Epidemiology Study of leaf spot disease.

Environment plays a vital role in the manifestation of the disease. Datar and mayee(1981) recorded severe incidence of early blight of tomato caused by *A. solani* in crop sown during july. Gupta and pathak (1990) observed that disease severity of papaya fruits inoculated at mature and semi ripe stage was significantly higher than those inoculated at immature stage. The optimum temperature for *Alternaria* sp. was 25°C.

Ahamad and Narian (2000) noticed severe leaf spot (*A. cucumerina*) in bitter gourd during July while Sharma and Bhargava (1977) observed severe fruit rot of bitter gourd (*A. tenuissima*) during June to September.

Singh *et al.* (1995) reported maximum disease intensity of leaf spot of cluster bean (*A. cyamopsidis*) when the temperature was ranging from 25° C to 30° C and relative humidity was 80 per cent with high rain fall.

Fitt *et al.* (1991) noted that the incidence of *Alternaria* sp. was 25°C linseed cotyledons was greatest at 10°C and at least 19°C temperature, when germination was most rapid.

Sinha and mahmood (1993) indicated that *Alternaria* leaf blight of pigeon pea appeared in the second week of
November when the temperature, relative humidity and rainfall were 24.8°C, 72.50 percent and 0.0 mm, respectively.

Patel (2003) observed relative humidity related significantly and negatively while maximum temperature and sunshine hours related significantly and positively with the leaf spot intensity in green gram (*A. alternata*) during kharif season. He also noted that the leaf spot intensity was negatively correlated with minimum temperature and relative humidity in the crop grown in late kharif.

Suhag *et al.* (1982) recorded Pod and Leaf blight of radish infected by *Alternaria alternata* (Fr) Keissler considered as a minor problem till recently, has caused considerable losses in Haryana and elsewhere.

Narain *et al.* (1985) recorded severe leaf blight and fruit rot of water melon (*A. alternata*) during wet and humid weather. Furgo *et al.* (1993) observed that sowing of water melon on 1st and 5th Nov resulted in significantly low incidence of late blight. They also noted that high humidity accompanied by optimum temperature played an important role in reducing the development of the disease severity.

Ruchi and Dohroo (2002) recorded influence of environmental factors *viz.*, air, temperature, relative humidity and rainfall on the leaf spot of ginger. The disease development was found to an extent of 85.5 percent.
Rajivkumar and Singh (1996) studied the influence of weather factors on development of leaf spot of sunflower caused by *A. helianthi* under field conditions during kharif 1990 and 1991. The most important weather factors favoring disease development were the temperature and relative humidity ranging from 27°C – 29°C and 78 - 80 per cent respectively, whereas rainfall did not affect the disease development because it was erratic and abnormal distribution during both the years. The disease intensity was highest in last week of August in both the years. Thereafter there was a gradual decline in disease severity.

2.10.1 *Area under disease progression curve (AUDPC)*

Sundhar *et al.* (1993) studied Alternaria leaf blight disease caused by *Alternaria brassicae* in mustard (*Brassica Juncea* L.) Czern and Coss). In that they studied that Disease measured using AUDPC for both the leaves and pods was significantly higher in all cultivars in unsprayed plots than in sprayed plots.

Raghvendra (2005) observed that maximum disease index of powdery mildew with AUDPC was observed in (2004) Std weak of 40th to 1st weak of (2005). Further he also noted that all the five varieties had significant effect of powdery mildew their increase or decrease of disease on similar trends.
2.11 Chemical control

2.11.1 Bio-efficacy of fungicides against pathogen *in vitro*

Khanna and Chandra (1981) reported that Aureofungin was responsible for complete inhibition of *Alternaria alternata* at 500 ppm level.

Choulwar and Datar (1987) screened nine fungicides against early blight of tomato and found that Mancozeb (0.2%) was best followed by Captafol (0.2%) and Zineb (0.2%).

Akbari and Parakhia (2007) reported that among systemic group of fungicides, Propiconazole, Hexaconazole, Difenoconazole and Tridemorph, and in non systemic fungicides, Thiram and Mancozeb were inhibitory which gave percent inhibition of *A. alternata* at 50 and 500 ppm conc. respectively causing Alternaria blight of sesame in Kharif season.

Ghosh *et al.* (2002) reported that the fungicide, tridemorph (0.1%) was proved the best followed by ziram (0.25%), mancozeb (0.25%) and carbendazim + mancozeb (0.05 + 0.25%) in inhibiting the growth of *A. alternata* isolated from gerbera *in vitro*.

Lal *et al.* (2000) reported that Rovral at 2000 mg/ml completely inhibited the growth of *A. alternata* isolated from pigeonpea. Indofil M-45 was also significantly effective followed by Blitox- 50 while Kavach and carbendazim were less inhibitory.

Kalra and Sohi (1985) reported complete inhibition of mycelial growth of *A. alternata* isolated from tomato by Thiram
(0.05-0.2%), Dithane M-45 (mancozeb) (0.1-0.2%) and Difolatan (captafol 0.2%) while systemic fungicides were ineffective except Calixin (tridemorph).

Murthy and Shenoi (2001) screened twentyeight fungicides at 100, 500 and1000 ppm concentrations by poisoned food technique against *A. alternata*, the incident of brown spot disease of tobacco. Out of them, Score (difenconazole), Tilt (propicanazole) and Indofil M-45 (mancozeb) were potent in inhibiting the mycelial growth even at 100 ppm concentration. Barnwal *et al.* (2002) tested six fungicides *viz.*, Bavistin (carbendazim), Blitox-50 (copper oxychloride), Indofil M-45 (mancozeb), Kavach (chlorothalonil), Kitazin (iprobenfos) and Roko (thiophanate-methyl) at their recommended doses against *A. tenuissima* of marigold by poisoned food technique. Indofil M-45 was the best fungicide, inhibiting cent mycelial growth and sporulation of the pathogen. The second best fungicide was Roko followed by Kavach. Bavistin was least effective in terms of inhibition of the radial growth.

Patil (2003) reported that propiconazole (Tilt 25 EC at 250, 500, and 1000 ppm), difenconazole (Score 25 EC at 500 and 1000ppm) gave cent percent inhibition of the mycelia growth and spore formation of *A. alternata* isolated from marigold. Patel (2003) also recorded propiconazole totally inhibiting the mycelia growth of the *A. alternata* of greengram at 250, 500 and 1000 ppm concentrations. Propineb, ziram and thiophanate methyl were also observed effective especially at higher concentrations.
Mixed fungicides viz., metalaxyl + mancozeb and carbendazim + mancozeb proved moderately effective while mancozeb and copper oxychloride were less effective. Carbendazim and chlorothalonil were proved poorest in their effectiveness.

Pandey and Vishwakarma (1999) reported that the radial growth of *A. alternata* (brinjal isolate) was completely inhibited by Thiram and Copper oxychloride at 1000 ppm.

### 2.11.2 Bio-efficacy of fungicides against leaf spot of *gerbera* in pot condition

Karunanithi *et al.* (1996) suggested spray schedule comprising of two sprays of mancozeb (0.2 %) at fifteen days intervals, which was most effective and also gave highest cost benefit ratio (1:4:3) and was adequate for the management of Alternaria leaf blight of sesamum.

Gaikwad (2000) reported that combination *i.e* carbendazim and mancozeb were effective for controlling leaf and fruit spot disease of pomegranate caused by *A. alternata*. The fungicidal combinations also improved fruit size and weight thereby increasing fruit quality yield and economic returns.

Tripathi and Lal (1992) found Difolatan (0.2%) and Dithane M-45 (0.2%) most effective to minimize the leaf blight of carnation caused by *A. dianthi*.

Jitendra Singh and Majumdar (2002) found Tilt as the most effective fungicide in controlling fruit rot caused by
A. alternata on pomegranate giving cent control on the 8th day of inoculation by worm solution treatment.

Murthy and Shenoi (2001) reported that the fungicides viz., Tilt, Score, Foltal and Indofil M-45 were effective in decreasing order of potency. The performed well in terms of disease management as they significantly reduced PDI and percent leaves with severe damage besides improving the yield parameters of FCV tobacco.

Barnwal et al. (2002) reported that out of five fungicides screened for their efficacy in controlling blight disease of marigold caused by A. tenuissima, two sprays of Indofil M-45 recorded the minimum disease intensity (11.1%) with highest disease control (79.6%), followed by Roko which showed a 69.4 percent disease control. The rest of the fungicides in order of superiority were Blitox- 50, Kavach and Kitazin.

Patil (2003) found propiconazole while Patel (2003) found propiconazole and ziram as the most effective fungicides in controlling marigold and greengram leaf spot (A. alternata) diseases, respectively.

Ghosh et al. (2002) reported that the fungicide, tridemorph (0.1%) was significantly superior in controlling the leaf spot disease caused by A. alternata on gerbera which was followed by ziram (0.25 %) and mancozeb (0.25 %).

Khanna and Chandra (1981) reported benlate controlling leaf blight of wheat, flax, Citrus microcarpae and
*Pisidium friehrichstelianum* caused by *A. alternata* to a large extent, more than 60 per cent control was always obtained with this fungicide.

Singh and Sharma (1986) got best results with 2 sprays of Topsin-M (thiophanate methyl) and Dithane M-45 (mancozeb) to control the *Alternaria* fruit rot of tomato. Mathur and Shekhawat (1986) reported that Blitox-50 (copper oxychloride) was the most effective fungicide for controlling early blight of tomato caused by *A. solani*, followed by Difolatan (captafol) and Dithane M-45 (macozeb).

Chaudhari and Patel (1987) found that Dithane M-45 (mancozeb) applied at 20 days interval proved the most effective fungicide in controlling leaf blight of fennel. Singh *et al.* (1988) proved Topsin-M (thiophanate methyl) and Dithane M-45 (mancozeb) as highly effective fungicides against *A. alternata* causing storage rot of tomato.

Maheswari and Singh (1997) reported that spraying of Thiram and Bavistin (carbendazim) followed by Benlate (benomyl) and Topsin-M (thiophanate methyl) gave complete control of Alternaria leaf spot of dolichos bean while Maheswari and Singh (1998) reported effective control of Alternaria leaf spot dolichos bean by three applications of either thiophanate methyl (0.2 %) or carbendazim (0.1 %) at an interval of ten days.

Dubey *et al.* (2000) reported that three sprays of Topsin-M (0.1 %) gave the best control of *Alternaria* blight of
broad bean in field along with maximum yield and net cost benefit ratio (Rs. 6.7) followed by T
opsin-M (0.05%) + Indofil M-45 (0.1%). Lal et al. (2000) reported that Rovral showed minimum disease intensity of 22.83 percent, maximum disease control (64.60 per cent) and also gave highest yield (1444 kg/ha). Indofil M-45 showed 24.66 per cent disease intensity, 61.76 per cent control of disease and 1333 kg/ha yield ranked second in controlling leaf blight disease (A. tenuissima) of pigeonpea.

Amiresh et al. (2000) observed that chlorothalonil (0.2%), mancozeb (0.2 %) and cyproconazole (0.1 %) gave effective control of the Alternaria leaf blight of sunflower. Maximum grain yield (9.21 q/ha) was recorded in the chlorothalonil treatment, which was similar to the yields of the cyproconazole (9.13 q/ha) and mancozeb (9.07 q/ha) treatments.
MATERIALS AND METHODS
The details of the material methods adopted in the present investigation are described here as under.

3.1 Pathological investigation

3.1.1 Collection of samples

The infected leaves of gerbera plant showing typical, well developed, dark brown spots with concentric rings were collected from the polyhouse of floriculture, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari. The infected leaves were brought into the laboratory, placed in blotting papers under herbarium press and preserved for further studies. The symptoms and signs observed in nature were critically observed.

3.1.2 Isolation of the pathogen

Isolation from freshly infected leaves of gerbera showing typical well developed dark brown spots with concentric rings was carried out and the association of the pathogen with the gerbera plant was studied at Departmental Laboratory. The infected area was subjected to isolation. The infected portion of the plant was cut into small pieces in such a way that each bit consisted of infected as well as healthy tissues.
MATERIALS AND METHOD

The bits were surface sterilized with 0.1 per cent mercuric chloride (HgCl$_2$) solution for 30 seconds followed by three washing with sterilized distilled water. These bits were aseptically transferred using laminar airflow system on sterilized petriplates containing 20 ml Potato Dextrose Agar (PDA) medium (peeled potato 200g, dextrose 20g and agar-agar 20g in 1000 ml distilled water) and these petriplates were incubated at (27 ± 2°C) temperature. The fungal hyphae developing from the infected tissues were sub-cultured aseptically on PDA slants and pure culture thus obtained was maintained. This culture was microscopically examined for identification and was further purified by using single spore isolation technique. The single spore culture obtained and later maintained on PDA slants for further investigation.

3.2 IDENTIFICATION OF PATHOGEN

Identification of the pathogen causing leaf spot of gerbera was carried on the basis of cultural and morphological characters and pathogenicity studies. The cultural characters were recorded right from the initiation of growth till the period of 10 days. The morphological characters of spore viz., length and breadth were measured under low power magnification (10 X) from 10 days old culture of Alternaria sp. using stage and ocular micrometer and were compared with those given in literature. The photomicrograph of the spores was also taken. The pure culture was sent to Indian Type Culture Collection (I.T.C.C.), Division of
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plant pathology, I.A.R.I., New Delhi-110012 for further confirmation.

3.3 Pathogenicity test

Pathogenicity was proved on susceptible variety by detached leaf technique Patel (1983).

For testing the Pathogenicity of the isolated fungus healthy young leaves were cut from susceptible variety C. F. Gold of gerbera from healthy plants along with petiole and brought into the laboratory, washed thoroughly with tap water. Later leaves were surface sterilized with 0.1 percent mercuric chloride solution and the same was removed by washing three times with sterilized water. Flask of 500ml capacity was taken and filled with 2% sucrose solution (1000ml Sterilized water + 20g sucrose). Surface sterilized leaves were placed in each of these flask to conduct inoculation test.

Ten days old culture of A. alternata was used to prepare inoculum. Inoculum were prepared by washing culture plates of A. alternata with sterilized distilled water and spore strength 1x10^6 was adjusted using haemacytometer. In both the methods detached leaves were inoculated with spore suspension of A. alternata. Suitable controls with only distilled sterilized water spray were maintained. All the leaves were then kept under moist condition covered with polythene bag. The observations on infection, and symptom development were recorded. Periodically fungus was reisolated from the inoculated diseased leaves and
morphological and cultural characteristics were studied and compared.

a) **Tooth Brush injury**

Sterilized tooth brush was dipped in the spore suspension (1x10^6 spores/ml) and then inoculated with gentle rubbing on entire leaves.

b) **Carborundum powder**

Leaves were injured with carborandum powder (600 mesh) and uniform quantity of inoculum was (1x10^6 spores/ml) sprayed at the injured area, by using automizer.

3.4 **Histopathological study of the disease**

To study the histopathology of disease, different sequences of events from pathogen attachment to disease development were studied under compound light microscope. The plants of gerbera which were raised in earthen pots having diameter 18 (cm) and pots were kept in green house. These gerbera plants were inoculated with the spore suspension (1x10^6 conidia/ml) of test culture with the help of automizer and kept under polythene moist chamber for 12, 24, 48 and 72 hrs. These sequences of disease development were recorded as photomicrographs.

Histopathological analysis of the infection processs of *Alternaria alternata* was accomplished on gerbera leaves. The cross section was cut from infected leaves and they were softened in distilled water for 2 h and then fixed in 70% ethanol for 48 h.
They were dehydrated through tertiary butyl alcohol (TBA) series. Serial microtome sections 12-15 µm thick were stained with cotton blue and light green combination, cleared in clove oil and mounted in lactophenol. Conidial germination, appresoria formation, penetration were qualitatively assessed at, 12, 24, 48 and 72 hr after inoculation at room temperature.

3.4.1 **Following sequences of disease development were studied under compound microscope**

a. Fungal structures inside the host.

b. Formation of appresoria and infection peg in host surface.

c. Invasion inside the host.

d. Spore structure and spore germination.

3.5 **Biochemical parameters**

Fresh leaf samples for biochemical analysis at both stages (Diseased and healthy) will be collected from second upper leaf and washed twice with tap water and then with millipore water. Infected and uninfected leaves without mid rib will analyze in triplicate for following biochemical estimation.

a. Total sugar

b. Total phenols

c. Catalase

d. Peroxidase
(a) **Total Soluble Sugar content**

Leaves (100 mg) were extracted with 5 ml of 80% ethanol and centrifuged at 3000 rpm for 10 minutes. Extraction was repeated 4 times with 80% ethanol and supernatants were collected into 25 ml volumetric flasks. Final volume of the extract was made to 25 ml with 80% ethanol. The extract (0.3 ml) was pipetted from treatments into separate test tubes and the tubes were placed in a boiling water bath for 3 minutes to evaporate the ethanol. One ml of millipore water and 4 ml of 0.2% anthrone reagent (200 mg in 100 ml H$_2$SO$_4$) were added in each test tube and placed in ice cold water. Reagent blank was prepared by adding 1 ml of distilled water and 4 ml of anthrone reagent. The intensity of colour was read at 600 nm on spectrophotometer. A standard curve was prepared using 10 mg glucose per 100 ml distilled water (Franscistt *et al.*, 1971).

\[
\text{Total soluble sugar (mg/g)} = \frac{\text{Sample O.D.} \times \text{Standard O.D.} \times \text{Dilution factor}}{}
\]

(b) **Total Phenol**

One ml of supernatant was taken from ethanol extract prepared for total soluble sugar analysis and evaporated to dryness in water bath. One ml of millipore water in each test tube and 0.5 ml of Folin & Ciocalteu reagent (1:1 with water) was added and kept for 3 min. After this 2 ml of 20% Na$_2$CO$_3$ was added and mixed thoroughly. The tubes were placed in boiling water for exactly one minute and cooled in ice water. The absorbances were read at 650 nm against a reagent blank
Materials and Methods

A standard graph was prepared using pyrocatachol ranging between 0-25 µg concentrations.

The amount of phenols present in the sample will be calculated as:

Phenol (mg/g) = Sample O.D. x Standard O.D. x Dilution factor

(c) Catalase

Total catalase (EC 1.11.1.6) activity was determined in the homogenates by measuring the decrease in absorption at 240 nm as \( \text{H}_2\text{O}_2 \) (\( \varepsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1} \)) was consumed according the method of Aebi (1984) and enzyme activity expressed as µmol \( \text{H}_2\text{O}_2 \) oxidized min\(^{-1}\) g\(^{-1}\) protein. The 3 ml mixture containing 50 mM sodium phosphate buffer (pH 7.0), 10mM \( \text{H}_2\text{O}_2 \) and 50 µl enzyme extract.

(d) Peroxidase

POX (EC 1.11.1.7) activity was determined in the homogenates by measuring the increase in absorption at 470 nm due to the formation of tetraguaiacol (\( \varepsilon = 26.6 \text{ mM}^{-1} \text{ cm}^{-1} \)) in a reaction mixture containing 50 mM sodium phosphate buffer pH 7.0, 0.1 mM EDTA, 0.05 ml enzyme extract, 10 mM guaiacol and 10 mM \( \text{H}_2\text{O}_2 \) (Costa et al., 2002).

3.6 Varietal screening against leaf spot of gerbera

The main object of this study was to find out the relative resistance or susceptibility of the selected varieties of gerbera against \textit{Alternaria alternata}. The experiment was laid out
in complete randomized design with three repetitions. Plants of gerbera varieties were raised in earthen pots having diameter 18 (cm). These earthen pots were kept in green house. These varieties of gerbera were inoculated with the spore suspension (1x10^6 conidia/ml) of test culture with the help of automizer and kept under polythene moist chamber for 48hrs after inoculation for providing sufficient humidity. The different varieties screened against A. alternata are shown in (Table-3.1). The observation on disease incidence will be recorded. These varieties will be grouped under different degrees of resistant on the basis of 0-5 scale (Mathur et al 1972).

**Rating scale**

<table>
<thead>
<tr>
<th>% Area infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
</tbody>
</table>

**Grading of varieties**

<table>
<thead>
<tr>
<th>PDI</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 to 10.1</td>
<td>Resistant ( R )</td>
</tr>
<tr>
<td>10.1 to 20.0</td>
<td>Moderately Resistant ( MR )</td>
</tr>
<tr>
<td>20.1 to 40.0</td>
<td>Moderately Susceptible ( MS )</td>
</tr>
<tr>
<td>40.1 to 60.0</td>
<td>Susceptible ( S )</td>
</tr>
<tr>
<td>Above 60.0</td>
<td>Highly Susceptible</td>
</tr>
</tbody>
</table>
Table-3.1: Different varieties of Gerbera evaluated for resistance to *Alternaria alternata*

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Varieties</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Stanza</td>
<td>(Red)</td>
</tr>
<tr>
<td>2.</td>
<td>Fana</td>
<td>(Red)</td>
</tr>
<tr>
<td>3.</td>
<td>C.F. Gold</td>
<td>(Yellow)</td>
</tr>
<tr>
<td>4.</td>
<td>Diego</td>
<td>(Orange)</td>
</tr>
<tr>
<td>5.</td>
<td>Cherany</td>
<td>(Pink)</td>
</tr>
<tr>
<td>6.</td>
<td>C.F. Orange</td>
<td>(Orange)</td>
</tr>
<tr>
<td>7.</td>
<td>Lion</td>
<td>(Yellow)</td>
</tr>
<tr>
<td>8.</td>
<td>Venezia</td>
<td>(Violet)</td>
</tr>
<tr>
<td>9.</td>
<td>Torbin</td>
<td>(White)</td>
</tr>
<tr>
<td>10.</td>
<td>Binaka</td>
<td>(White)</td>
</tr>
<tr>
<td>11.</td>
<td>Kento</td>
<td>(Yellow)</td>
</tr>
<tr>
<td>12.</td>
<td>Jaffana</td>
<td>(Orange)</td>
</tr>
<tr>
<td>13.</td>
<td>Ice Queen</td>
<td>(White)</td>
</tr>
</tbody>
</table>
3.7 Epidemiology

Epidemiological encompasses the study of all the factors associated with disease development. The four major components of the disease *i.e.* susceptible host, virulent pathogen, human and favourable weather in time and space causes epidemic. Studies on epidemiology were carried out in green house under natural disease development condition.

3.7.1 Progress of leaf spot during entire crop season under green house- conditions.

The observation on leaf spot infection was recorded starting from the transplanting of the crop in pot by selecting three leaves from each pot. Such five pots was tagged. The PDI were weekly recorded from C.F. gold variety of gerbera.

3.7.2 Correlation of leaf spot intensity with weather parameters

\[
\text{PDI} = \frac{\sum \text{ratings of infected leaves observed}}{\text{No of leaves observed} \times \text{maximum disease score}} \times 100
\]

The observation was recorded during the entire crop season starting from transplanting on weather parameters *viz*, temperature, humidity, leaf spot infection were also recorded at weekly interval in the pot on C.F. gold variety using 0-5 scale. The percent disease intensity was calculated by using the formula devised by Mathur *et al.* (1972).
Leaf spot intensity observed during the crop season at weekly interval was correlated with different weather parameters and multiple regression equations was also worked out.

3.7.3 **Area under disease progress curve (AUDPC)**

Using following formula given by Shaner and Finney (1977),

$$
AUDPC = \sum_{i=1}^{k} \frac{X_i + X_{i-1}}{2} \times d
$$

xi = Disease Severity at the end of the ith week

k = Number of successive evaluation of Alternaria leaf spot.

D = Day’s interval between two observation.

3.8 **Chemical control**

3.8.1 **Bio-efficacy of fungicides against Alternaria alternata in vitro**

Eight fungicides with their different concentrations as listed in Table-3.2 were tested for in vitro efficacy against A. alternata by adopting poisoned food technique (Nene and Thapliyal, 1993).

The desired quantity of test fungicide was diluted with autoclaved lukewarm PDA medium in conical flask. The flask containing fungicidal medium was shaken well to facilitate uniform mixture and 20 ml was distributed to each sterilized petriplate. The inoculum disc of 7 mm diameter was cut with the help of sterilized cork borer from 10 days old pure culture and placed at the centre on petriplate containing solidified fungicidal medium. Three repetitions of each treatment were kept. The
Table-3.2: Fungicides tested against *Alternaria alternata* in *vitro*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Technical name</th>
<th>Trade name</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Tebuconazole50% + Trifloxystrobin25% WG.</td>
<td>Nativo</td>
<td>200 300 500</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Propiconazole</td>
<td>Tilt 25% EC</td>
<td>200 300 500</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Iprovalicarb5.5% + Propineb61.25%WP</td>
<td>Melody DUO</td>
<td>200 300 500</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Femamidone10% + Mancozeb50%WG.</td>
<td>Sectin</td>
<td>200 300 500</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt;</td>
<td>Tebuconazole 25.9% w/w (25 w/w)</td>
<td>Folicur 25Ec</td>
<td>200 300 500</td>
</tr>
<tr>
<td>T&lt;sub&gt;6&lt;/sub&gt;</td>
<td>Captan + Hexaconazole</td>
<td>Taquat</td>
<td>200 300 500</td>
</tr>
<tr>
<td>T&lt;sub&gt;7&lt;/sub&gt;</td>
<td>Bayleton25%WP+Triadimefon25%WP</td>
<td>Bayleton</td>
<td>200 300 500</td>
</tr>
<tr>
<td>T&lt;sub&gt;8&lt;/sub&gt;</td>
<td>Difenoconazole25%EC</td>
<td>Score(25EC)</td>
<td>200 300 500</td>
</tr>
<tr>
<td>T&lt;sub&gt;9&lt;/sub&gt;</td>
<td>Control (without fungicides)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
medium without fungicide served as control. The inoculated plates were incubated at \((27 \pm 2^\circ C)\) temperature. The colony diameter of the fungus was recorded from three repetitions after 10 days of incubation. From the eight fungicide tested the best concentration of each fungicide was taken for pot studies. The percent growth inhibition over control was calculated by using following formula suggested by Vincent (1972).

\[
\text{PGI} = \frac{100 \times (\text{DC}-\text{DT})}{\text{DC}}
\]

\(\text{PGI}\) = Percent growth inhibition

\(\text{DC}\) = Average diameter of mycelial colony of control set (mm)

\(\text{DT}\) = Average diameter of mycelial colony of treated set (mm)

3.8.2 Bio-efficacy of fungicides against leaf spot of gerbera in pot condition

Considering the importance of the disease and variation in the recommendations of different fungicides by various workers for the control of leaf spot disease. An experiment under greenhouse was carried out to evaluate different fungicides concentration on gerbera plants in pots. Only those fungicides concentrations which were found effective in poisoned food technique were taken for pot condition experiment.

The experiment was laid out in complete randomized design with three repetitions and eight treatments. The fungicides used were listed in Table-3.3. The efficacy of each fungicide was compared with control pots, which was sprayed with water
<table>
<thead>
<tr>
<th>Treatments</th>
<th>Technical name</th>
<th>Effective concentration (ppm)</th>
<th>Trade name</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Tebuconazole50% + Trifloxystrobin25% WG.</td>
<td>500</td>
<td>Nativo</td>
</tr>
<tr>
<td>T2</td>
<td>Propiconazole</td>
<td>200</td>
<td>Tilt 25% EC</td>
</tr>
<tr>
<td>T3</td>
<td>Iprovalicarb5.5% + Propineb61.25% WP</td>
<td>500</td>
<td>Melody DUO</td>
</tr>
<tr>
<td>T4</td>
<td>Femamidone10% + Mancozeb50% WG.</td>
<td>500</td>
<td>Sectin</td>
</tr>
<tr>
<td>T5</td>
<td>Tebuconazole 25.9% w/w (25 w/w)</td>
<td>300</td>
<td>Folicur 25Ec</td>
</tr>
<tr>
<td>T6</td>
<td>Captan + Hexaconazole</td>
<td>500</td>
<td>Taquat</td>
</tr>
<tr>
<td>T7</td>
<td>Bayleton25% WP+Triadimefon25% WP</td>
<td>500</td>
<td>Bayleton</td>
</tr>
<tr>
<td>T8</td>
<td>Difenoconazole25%EC</td>
<td>500</td>
<td>Score(25EC)</td>
</tr>
<tr>
<td>T9</td>
<td>Control (without fungicides)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
control only. Two sprays of the fungicides, first at the time of initiation of the disease, second at fifteen days after first spray was carried out. Ten days after second spraying, leaves were selected from each pot for recording observations. From one plant five leaves were observed.

Disease rating was done by using 0-5 scale and PDI was calculated as mentioned earlier.

**Experimental details**

(1) **Location** : Green house of floriculture.

N.A.U., Navsari-396450

(2) **Crop** : Gerbera

(3) **Variety** : C.F.gold

(4) **Replication** : Three

(5) **Design** : C.R.D

(6) **Number of plants/treatment** : 3

(7) **Date of planting** : 1st week of September
RESULTS
AND
DISCUSSION
Gerbera is one of the important crops in India grown for flower purpose. It is considered to be native of Natal and Transvaal and grown widely throughout the world under wide range of climatic conditions. Diseases are major constraint in economic crop production as they inflict heavy losses. Among the various diseases affecting gerbera, leaf spot caused by *Alternaria alternata* (Fr.) Keissler has become a severe threat to successful and profitable cultivation of gerbera in South Gujarat. Taking this fact into consideration, the present investigation was carried out on various aspects to generate scientific information on this important pathological problem and suitable management strategies to prevent crop losses. The leaf spot of gerbera caused by *A. alternata* reported here for the first time from Gujarat and studies on various aspects of the pathogen and the diseases are new information.

4.1 Pathological investigation

4.1.1 Collection of samples and isolation of pathogen

The diseased leaves of gerbera plant showing typical dark brown, small scattered dots with concentric rings were collected from the polyhouse of floriculture, of ASPEE College of Horticulture and Forestry, Navsari Agricultural University. The leaf spot disease was observed in single floret cultivar in polyhouse conditions. The symptoms and signs were observed visually and the presence of pathogen was confirmed by
Results and Discussion

microscopic examination. The diseased leaves collected from infected field were subjected to repeated tissue isolation on PDA medium after confirming the presence of pathogen by microscopic examination. Isolation from disease leaves yielded *Alternaria* sp., which was maintained on PDA slants and used throughout the investigations.

4.1.2 Symptomatology

The symptoms of leaf spot of gerbera were recorded from the nursery of ASPEE College of Horticulture and Forestry, Navsari Agriculture University, Navsari. The symptoms and signs produced in natural conditions were recorded which are as under.

In the initial stage of infection small brown, scattered spots appeared on leaves which gradually enlarged and coalesced to form large oval and circular to irregular black to brown lesions with concentric rings (Plate-I). In severe infection, several lesions merged together and enlarged quickly to give blighted appearance of the leaves. The disease symptoms first appeared on lower, older leaves and progressed upwards. Several infected leaves withered and dropped from the plants. The disease symptoms were usually observed on mature plants but young seedlings can be also found infected (Plate-II). Affected plant showed lower vitality, suppressed development and fewer, smaller, distorted in shape of flowers. The disease symptom initiated generally during July *i.e.* after 45 days of transplanting and progressed throughout the year. The systemic studies on *Alternaria* leaf spot symptoms of gerbera under greenhouse
Plate I: Infected leaf showing brown spots
condition revealed that it was by and large similar to the description provided by the earlier workers (Kulibaba, 1972; Saini et al. 1989; Wick and Dicklow, 2000 and Mirkova and Monstantinova, 2003) on gerbera. Similarly, effect was also noticed by Patil (2003) of marigold due to leaf spot (*A. alternata*).

4.2 **Taxonomy and identification of the pathogen**

The morphological and cultural characters of the fungus grown on PDA were studied for the purpose of identification and taxonomy.

4.2.1 **Cultural characters**

The colony of *A. alternata* grew fast on PDA attained diameter of 9 cm within 10 days at (27 ± 2°C) temperature with profuse mycelial growth. The mycelium was whitish in colour when young but gradually turned light greyish and brown to dark greyish in colour and produced clear cut zonations. The whitish crystal formation was also observed in mycelial mat (Plate-III).

4.2.2 **Morphological characters**

The fungus produced profuse mycelial growth on PDA, which gradually turned light greyish and brown to dark greyish in colour within 10 days. The microscopic observations revealed that the hypha was septate and light to dark brown in colour. The mycelium was irregularly branched at acute angle (Plate-IVA) conidiophores were light brown, simple and septate (Plate-IVB) bearing pale brown to brown, long conidia with 3-5 transverse
A : Mycelium and conidia

Plate IV: Photomicrograph of *Alternaria alternata*
Results and Discussion

and 0-3 longitudinal septa measuring 23.40 - 32.76 x 5.27 - 9.95µm. Conidia borne singly or in chains of 5 to 8, variable in size and shape usually ovoid to ellipsoid, with cylindrical beak and dark brown in colour.

The studies on morphological and cultural characters of isolated Alternaria sp. showed its close identity with Alternaria alternata (Fr.) Keissler as described by Mirkova and Konstantinova (2003) isolated from gerbera. Similarly, it showed close identity with the description of Neergaard (1945), Bedi and Singh (1972), Mistry (1992) and Patil (2003). The identity was also confirmed with Indian Type Culture Collection (I.T.C.C.), Division of Plant Pathology, I.A.R.I., New Delhi-110 112 as Alternaria alternata (Fr.) Keissler and assigned the I.T.C.C., No. 8774-12.

Hence, the cause of leaf spot of gerbera in south Gujarat region was confirmed as Alternaria alternata (Fr.) Keissler.

4.3 Pathogenicity test

The pathogenicity test of isolated Alternaria sp. was carried out by both tooth brush and carborandum powder injury techniques on detached leaves of gerbera. Leaves were inoculated with A. alternata spore suspension (1x10^6 conidia/ml).

It is evident from the results presented in (Table 4.1) and symptoms produced (Plate-V) that the fungus is pathogenic to gerbera. The leaf spot pathogen A. alternata was able to infect
Plate 5: Pathogenicity test of *A. alternata* under different inoculated methods.
Table-4.1: Pathogenicity test of *Alternaria alternata* on gerbera under laboratory condition

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Inoculation technique</th>
<th>No. of Leaves</th>
<th></th>
<th></th>
<th>Per cents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inoculated</td>
<td>Infected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Carborandum powder</td>
<td>3</td>
<td>2</td>
<td></td>
<td>80.00</td>
</tr>
<tr>
<td>2</td>
<td>Injury by tooth brush</td>
<td>3</td>
<td>1</td>
<td></td>
<td>40.00</td>
</tr>
<tr>
<td>3</td>
<td>Control (spraying sterilized distilled water on uninjured surface)</td>
<td>3</td>
<td>0</td>
<td></td>
<td>0.00</td>
</tr>
</tbody>
</table>
Results and Discussion

and develop the symptoms on gerbera leaves irrespective of inoculation techniques. Spraying with sterilized water (control) produced no disease symptoms. When leaf inoculation was done, typical symptoms were developed after sixth days of inoculation as small, brown bright spots on the leaves. Later on, these spots were enlarge and coalesced to form large oval, and circular to irregular, black to brown lesion with concentric rings. In severity, several lesions merged together and enlarged quickly to give blighted appearance of the leaves. Reisolation from artificial inoculated diseased leaves yielded *Alternaria alternata* which was identical with the original one.

Typical symptoms of the disease developed, which were similar to those described by Mirkova and Konstantinova (2003) on gerbera. Various workers have proved the pathogenicity of *A. alternata* on various plant species (Dutta, *et al.*, 1971; Chuturvedi, 1972; Bedi and Singh, 1972 and Patil, 2003) earlier. The present finding is also on the same line and confirmed the pathogenicity of the organism isolated.

4.4 Histopathology of Disease

The study on histopathology of leaf spot disease of Gerbera, after critical examination of pathogen in compound light microscope using cotton blue stain. Formation of mycelium was observed after 6 hours as well as 12 hrs (Plate-VI). Formation of appressoria was observed laterally on mycelium after 24 hours. The morphologically appresoria were rounded or calviform with septum not always apparent. After the 48 hours formation of
A. Histopathology of gerbera leaves indicating presence of mycelium

B. Microphotograph showing penetration of mycelium in the leaves

C. Formation of fruiting body like structure in leaves

Plate VI: Histopathology of leaf spot of gerbera showing mycelium
A) Conidia seen in chain

B) Conidia of *Alternaria alternata* showed vertical and horizontal septa

Plate-VII: Histopathology of leaf spot of gerbera
Results and Discussion

mycelium penetration observed in epidermal cells that generally positioned over the junction between two cells (Plate VI B). However, critical examination of pathogen showed that hyphae penetrated between the walls of cells after 72 hours and developed fruting body like structure in leaves (Plate VI C). Conidiophores were light brown, simple and septate bearing pale brown to brown long conidia with 3-5 transverse and 0-3 longitudinal septa measuring 23.40 – 32.76 x 5.27-9.95 µm (Plate-VII A). Conidia borne singly or in chains of 5 to 8, (Plate-VII A) variable in size and shape usually ovoid to ellipsoid, with cylindrical beak and dark brown in colour. Similar study on histopathology of Alternaria alternata was also carried out by Jose et al. (2004) of tomato. They observed that pathogen produces, appresoria, penetration peg for conidial formation. Further, they also reported that penetration by Alternaria sp. commonly occurs from appresoria and which proves essential characters of proving pathogenesis. It was also supported by Dehpour et al. (2007) reported Conidia borne singly or in chains of 5 to 8, variable in size and shape usually ovoid to ellipsoid, with cylindrical beak and dark brown in colour of Alternaria alternata on minneola Tangelo.

4.5 Biochemical activity

4.5.1 Total Soluble Sugar content

Sugars also play a major role in disease resistance since sugars are the precursors for the synthesis of phenolics and
phytoalexins which suppress the pectolytic and cellulolytic enzymes that are essential for pathogenesis.

The perusal of data presented in (Table 4.2) showed that the total soluble sugar varied in resistant and susceptible genotypes at pre-infection stage. The total soluble sugar was found 2 fold higher in resistant varieties like, Venezia (11.060) and Kento (12.940) than susceptible Fanna (5.930) and Diego (6.690) variety. The total soluble sugar in resistant as well as susceptible variety was found more at pre-infection than post-infection stage.

Total soluble sugar decreased in all the varieties at 7 days after infection (d.a.i.) except in Cherang in which it increased 4.6 per cent. The reduction was more pronounced and significant in resistant variety. Reduction in total soluble sugar with the increase in age of crop observed in the present study is also in confirmity with Kumar and Singh (1996) and Saharan and Saharan (2004).

The total soluble sugar was 2-3 fold more in susceptible variety C.F Gold (8.473) and Cherang (9.055) compared to resistant variety at 7 days after infection (57 DAS) (Fig. 4.1.) The higher soluble sugar indicated that susceptible variety may have more efficient sugar synthesizing capacity and facilitates a higher rate of transport across the host pathogen interface to support the mycelium growth and sporulation. Moreover, availability of lower sugar content in the intercellular fluid of the resistant varieties may be responsible for the
Figure 4.1 - Total Sugar (mg g\textsuperscript{-1} FW) at Pre and post-infection in Gerbera

Resistant

- Binaka
- Venezia
- Kento
- Torbin
- C.F. Gold

Susceptible

- Fana
- Diego
- Cherang

Sugar (mg g\textsuperscript{-1} FW)

Pre- infection

Post- infection
inhibition of the growth and multiplication of the pathogen (Borkar and Verma, 1989).

4.5.2 Total Phenol

Phenolic compounds are the most important group implicated in both constitutive and induced resistance and a distinct correlation between the degree of plant resistance and phenolics present in plant tissue has been demonstrated.

The total phenol content (mg g\(^{-1}\) FW) varied in all variety at pre-infection stage (Table 4.2) but susceptible variety Cherang (1.247) had the highest phenol.

Total phenols were increased in susceptible varieties and decreased in resistant varieties after infection with Leaf spot pathogen. The magnitude of induction was the highest in susceptible Variety C.F. Gold (2.030) which was about 2 fold higher than constitutive level. Furthermore, susceptible variety has 2-2.5 fold higher induced phenol compared to resistant variety (Fig. 4.2.) Similar observations were recorded at pre-infection stage by Gogoi et al. (2001) in Karnal bunt susceptible wheat WL-711 and in grey mildew susceptible cotton lines by Chakrabarty et al. (2002) Although, in the present study the level of constitutive phenols did not indicate distinct pattern in susceptible and resistant variety.

Jalali et al. (1976) did not find any significant difference in the constitutive total phenol contents of the bacterial blight resistant and susceptible cotton plants. The
Figure 4.2 - Total Phenol at (mg g⁻¹ FW) Pre and post-infection in Gerbera

- **Resistant**
  - Pre-infection
  - Post-infection

- **Susceptible**
  - Pre-infection
  - Post-infection
Results and Discussion

apparent decrease in the total phenol content in resistant genotypes reflects that phenols are channeled for biosynthesis of lignin. Brammachari and Kolte (1983) reported a decrease in total phenols in groundnut cultivars resistant towards *Cercospora*. Similar results have also been reported in muskmelon varieties resistant to powdery mildew (Jindal *et al.* 1979).

**4.5.3 Catalase**

Catalase activity was also found significantly higher in resistant varieties than susceptible varieties at both stages (Fig. 4.3). In resistant varieties at (μ mol H₂O₂ min⁻¹ g⁻¹ protein) Binaka (43.47), Venezia (90.30), Kento (58.80), Torbin (50.60) at pre-infection and at post infection (34.57), (61.77), (43.37), (37.29) respectively. In susceptible varieties at pre-infection C.F. gold (21.65), Fanna (16.13), Diego (17.13), Cherang (26.50) and at post infection (16.50), (12.15), (13.92), (20.16) respectively. Although the activity was decreased after infection in all varieties (Table 4.2). However, resistant varieties had about 2-3 fold higher CAT activity than susceptible varieties at both the stages. The range of decrease in resistant varieties (20.9-31.6%) was higher than those in the susceptible varieties (22.9-27.6%). These results are consistent with previous results of maize cultivars in response to grey leaf spot (Guo *et al.* 2003).

These observations suggest that the disease induced stress might somehow cause direct structural or functional effect on CAT protein, preventing induction of activity after pathogen infection. Thus, ongoing protein synthesis is required to maintain
Figure: 4.3- CAT activity at (µ mol H₂O₂ min⁻¹ g⁻¹ protein) Pre and post-infection in Gerbera

- Binaka
- Venezia
- Kento
- Torbin
- C.F.Gold
- Fana
- Diego
- Cherang

- Resistant
- Susceptible

Pre-infection
Post-infection
catalase activity under conditions in which degradation exceeds resynthesis, otherwise CAT activity will decrease.

On the other hand, inhibition of catalase activity is a phenomenon that occurs in many plant species exposed to oxidative stress and is related to the accumulation of salicylic acid (Shim et al. 2003).

4.5.4 Peroxidase

Leaf guaiacol peroxidase (POX) activity was found (Table 4.2) lower in resistant varieties (132.0 - 154.2 µmol guaiacol min\(^{-1}\) g\(^{-1}\) protein) than the susceptible varieties (180.2 - 215.6 µmol guaiacol min\(^{-1}\) g\(^{-1}\) protein) at pre-infection stage. The POX activity was enhanced after inoculation with A.alternata in all varieties (Fig. 4.4.). The degree of increase in susceptible varieties C.F.Gold, Fanna, Diego and Cherang was 2.72, 3.20, 2.96 and 2.62 fold higher, respectively than the resistant variety (2.20 - 2.52 fold).

In the green and red pepper fruit inoculated with Colletotrichum gloeosporiodes, the peroxidase genes were strongly activated in the green fruit by anthracnose disease (Lee et al. 2000) but not in red fruit resistant to anthracnose disease. These results suggest that the peroxidase genes may be inducible during the pathogenesis of the anthrachose disease rather than in the disease resistance response. Data presented in (Table 4.2) suggested that the guaiacol-POX activity, total phenol content activity in gerbera variety showed some degree of
Figure 4.4: Pox activity at (µ mol guaiacolmin⁻¹ g⁻¹ protein) Pre and post-infection in Gerbera

Pre-infection  | Post-infection
---|---
Binaka  |  
Venezia  |  
Kento  |  
Torbin  |  
C.F.Gold  |  
Fana  |  
Diego  |  
Cherang  |  
Resistant  |  
Susceptible  |  
(µ mol guaiacolmin⁻¹ g⁻¹ protein) peroxidase
Table 4.2: Pre- and post-infectional changes of metabolites constituents in Alternaria Leaf spot resistant and susceptible Variety of Gerbera

<table>
<thead>
<tr>
<th>No. and name of genotypes</th>
<th>Total soluble sugar (mg g\textsuperscript{-1} FW)</th>
<th>Total phenols (mg g\textsuperscript{-1} FW)</th>
<th>POX activity (µ mol guaiacol min\textsuperscript{-1} g\textsuperscript{-1} protein)</th>
<th>CAT activity (µ mol H\textsubscript{2}O\textsubscript{2} min\textsuperscript{-1} g\textsuperscript{-1} protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-infection</td>
<td>Post-Infection</td>
<td>Pre-Infection</td>
<td>Post-Infection</td>
</tr>
<tr>
<td><strong>Resistant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Binaka</td>
<td>8.750</td>
<td>3.040</td>
<td>0.959</td>
<td>0.854</td>
</tr>
<tr>
<td>2. Venezia</td>
<td>11.060</td>
<td>3.337</td>
<td>1.026</td>
<td>0.992</td>
</tr>
<tr>
<td>3. Kento</td>
<td>12.940</td>
<td>4.480</td>
<td>0.946</td>
<td>0.618</td>
</tr>
<tr>
<td>4. Torbin</td>
<td>11.207</td>
<td>4.650</td>
<td>1.175</td>
<td>0.855</td>
</tr>
<tr>
<td><strong>Susceptible</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Fana</td>
<td>5.930</td>
<td>5.573</td>
<td>1.132</td>
<td>1.830</td>
</tr>
<tr>
<td>7. Diego</td>
<td>6.690</td>
<td>5.490</td>
<td>1.060</td>
<td>1.690</td>
</tr>
<tr>
<td>S.Em. C.D. at 5%</td>
<td>0.071</td>
<td>0.055</td>
<td>0.008</td>
<td>0.012</td>
</tr>
<tr>
<td>C.V. %</td>
<td>1.33</td>
<td>1.73</td>
<td>1.22</td>
<td>1.69</td>
</tr>
</tbody>
</table>

Pre-infection at 45 DAP, Post-infection at 57 DAS (7 days after infection), FW-Fresh weight
positive correlation. Interestingly, POX activity, total phenol content activities were higher in susceptible varieties than the resistant Varieties. Apart from toxic H$_2$O$_2$ scavanging via POX-activity in plants, it is also involved in the biosynthesis of cell wall components and lignification. High POX activity under salt-stress conditions has been correlated with reduction of plant growth (Lin and Kao, 2002) and this reduction has been attributed to POX catalysis of ferulolylation of hemicellulose and insolubilization of hydroxy proline-rich glycoproteins causing cell wall stiffening (Dionisio-Sese and Tobita, 1998).

### 4.6 Varietal Screening

Thirteen varieties were selected for screening against leaf spot disease under artificial inoculation in pot condition. The result are presented in (Table 4.3). The observation on leaf spot incidence was recorded on the basis of 0-5 disease scale (Plate-VIII); they were grouped under different degrees of resistance.

Out of thirteen varieties screened under greenhouse conditions minimum percent disease intensity was recorded in Kento (7.50), Jaffana (8.00), Venezia (7.67) proved resistant, while Binaka (15.50), Torbin (19.50), were found moderately resistant. Ice queen (34.50), Lion (32.33), Diego (27.17), Fana (35.83), Stanza (23.67) found moderately susceptible and C.F. orange (44.00), Cherany (47.67), C.F. gold (55.17) were found susceptible.
Table 4.3: Evaluation of different varieties of Gerbera against *Alternaria alternata*

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Variety</th>
<th>PDI</th>
<th>Grading Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stanza</td>
<td>23.67</td>
<td>MS</td>
</tr>
<tr>
<td>2</td>
<td>Fana</td>
<td>35.83</td>
<td>MS</td>
</tr>
<tr>
<td>3</td>
<td>C.F. gold</td>
<td>55.17</td>
<td>S</td>
</tr>
<tr>
<td>4</td>
<td>Diego</td>
<td>27.17</td>
<td>MS</td>
</tr>
<tr>
<td>5</td>
<td>Cherany</td>
<td>47.67</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>C.F. orange</td>
<td>44.00</td>
<td>S</td>
</tr>
<tr>
<td>7</td>
<td>Lion</td>
<td>32.33</td>
<td>MS</td>
</tr>
<tr>
<td>8</td>
<td>Venezia</td>
<td>7.67</td>
<td>R</td>
</tr>
<tr>
<td>9</td>
<td>Torbin</td>
<td>19.50</td>
<td>MR</td>
</tr>
<tr>
<td>10</td>
<td>Binaka</td>
<td>15.50</td>
<td>MR</td>
</tr>
<tr>
<td>11</td>
<td>Kento</td>
<td>7.50</td>
<td>R</td>
</tr>
<tr>
<td>12</td>
<td>Jaffana</td>
<td>8.00</td>
<td>R</td>
</tr>
<tr>
<td>13</td>
<td>Ice queen</td>
<td>34.50</td>
<td>MS</td>
</tr>
</tbody>
</table>

**Grading of varieties**

<table>
<thead>
<tr>
<th>PDI</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 to 10.1</td>
<td>Resistant (R)</td>
</tr>
<tr>
<td>10.1 to 20.0</td>
<td>Moderately Resistant (MR)</td>
</tr>
<tr>
<td>20.1 to 40.0</td>
<td>Moderately Susceptible (MS)</td>
</tr>
<tr>
<td>40.1 to 60.0</td>
<td>Susceptible (S)</td>
</tr>
<tr>
<td>Above 60.0</td>
<td>Highly Susceptible</td>
</tr>
</tbody>
</table>
Plate-VIII: 0-5 grading of the leaf spot

0 = No visible symptoms

1 = 01 to 10 per cent leaf area affected

2 = 11 to 20 per cent leaf area affected

3 = 21 to 30 per cent leaf area affected

4 = 31 to 40 per cent leaf area affected

5 = Above 40 per cent leaf area affected
Results and Discussion

Bedi and Singh (1972), Crisan and Szenyei (1987), and Hilal and Kamel (1990) have screened the varieties of ornamental and flowering plant other than gerbera against *Alternaria sp.* They have indicated the presence of resistance or susceptibility in the various genotypes.

Based on the result of varietal screening, it is suggested to grow resistant to moderately resistant varieties in this area to maximize the production.

4.7 Epidemiological study

4.7.1 Progress of leaf spot during entire kharif crop season in variety C. F. Gold under greenhouse conditions.

The observations on leaf spot intensity were recorded at weekly interval from C.F. Gold variety grown at greenhouse of Floriculture of ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari. Starting from the planting to harvesting of the crop in entire crop season, 2011. The results are presented in Table: 4.4 (Fig.4.5).

The first leaf spot intensity was recorded after 60 days of planting on 16th July (29th std. week). Since, then there was linear progress of the disease during the entire crop season. It was initiated on 16th July (2.11 %) and reached at its highest on 10th September (30.48 %). There was maximum increase in leaf spot intensity during 23rd July (6.93 %) to 30th July (13.15 %). This can be considered as window period for leaf spot of gerbera (Table- 4.4).
### Table-4.4: Progress of leaf spot during entire crop season in CV. C. F. Gold under green house conditions.

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Date observation</th>
<th>Std week</th>
<th>Percent leaf spot intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9 July-012</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>16 July-012</td>
<td>29</td>
<td>2.11</td>
</tr>
<tr>
<td>3</td>
<td>23 July-012</td>
<td>30</td>
<td>6.93</td>
</tr>
<tr>
<td>4</td>
<td>30 July-012</td>
<td>31</td>
<td>13.15</td>
</tr>
<tr>
<td>5</td>
<td>6 Aug-012</td>
<td>32</td>
<td>17.24</td>
</tr>
<tr>
<td>6</td>
<td>13 Aug-012</td>
<td>33</td>
<td>20.97</td>
</tr>
<tr>
<td>7</td>
<td>20 Aug-012</td>
<td>34</td>
<td>23.11</td>
</tr>
<tr>
<td>8</td>
<td>27 Aug-012</td>
<td>35</td>
<td>25.42</td>
</tr>
<tr>
<td>9</td>
<td>3 Sep-012</td>
<td>36</td>
<td>28.88</td>
</tr>
<tr>
<td>10</td>
<td>10 Sep-012</td>
<td>37</td>
<td>30.48</td>
</tr>
</tbody>
</table>
Figure: 4.5- Progress of leaf spot during entire crop season in CV. C.F.Gold under green house conditions

Percent disease intensity vs. Standard Week

- Percent leaf spot intensity
Table-4.5: Correlation of leaf spot intensity in CV. C. F. Gold with weather parameters.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Date of observation</th>
<th>Std. week</th>
<th>Atmospheric temp (°C)</th>
<th>Relative humidity (%)</th>
<th>Rainfall (mm)</th>
<th>SSH (km/hr)</th>
<th>Rainfall days</th>
<th>Observed leaf spot intensity (%) X</th>
<th>Predicted leaf spot intensity (%) (Y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9 July-012</td>
<td>28</td>
<td>31</td>
<td>25.75</td>
<td>28.37</td>
<td>5.24</td>
<td>93</td>
<td>82</td>
<td>88</td>
</tr>
<tr>
<td>2</td>
<td>16 July-12</td>
<td>29</td>
<td>30.84</td>
<td>26.78</td>
<td>28.81</td>
<td>4.05</td>
<td>91</td>
<td>82</td>
<td>86</td>
</tr>
<tr>
<td>3</td>
<td>23 July-12</td>
<td>30</td>
<td>30.07</td>
<td>26.97</td>
<td>28.52</td>
<td>3.1</td>
<td>90</td>
<td>85</td>
<td>87</td>
</tr>
<tr>
<td>4</td>
<td>30 July-12</td>
<td>31</td>
<td>29.74</td>
<td>25.78</td>
<td>27.76</td>
<td>3.9</td>
<td>91</td>
<td>85</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>6 Aug-012</td>
<td>32</td>
<td>30</td>
<td>25.82</td>
<td>27.91</td>
<td>4.17</td>
<td>93</td>
<td>87</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>13 Aug-12</td>
<td>33</td>
<td>29.64</td>
<td>25.67</td>
<td>27.65</td>
<td>3.97</td>
<td>89</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>7</td>
<td>20 Aug-12</td>
<td>34</td>
<td>30.1</td>
<td>24.97</td>
<td>27.53</td>
<td>5.12</td>
<td>95</td>
<td>79</td>
<td>87</td>
</tr>
<tr>
<td>8</td>
<td>27 Aug-12</td>
<td>35</td>
<td>30.72</td>
<td>25.57</td>
<td>28.15</td>
<td>5.15</td>
<td>91</td>
<td>78</td>
<td>85</td>
</tr>
<tr>
<td>9</td>
<td>3 Sep-012</td>
<td>36</td>
<td>29.1</td>
<td>24.94</td>
<td>27.02</td>
<td>4.15</td>
<td>96</td>
<td>90</td>
<td>93</td>
</tr>
<tr>
<td>10</td>
<td>10 Sep-012</td>
<td>37</td>
<td>29.07</td>
<td>24.77</td>
<td>26.92</td>
<td>4.3</td>
<td>92</td>
<td>83</td>
<td>88</td>
</tr>
</tbody>
</table>

SSH: Sunshine hours  
WS: Wind speed  
Std: Standard week
Figure: 4.6- Average temperature and observed leaf spot intensity

- **Avg. Temp.**
- **Observed Leaf Spot Intensity**
Figure 4.7 - Observed and Predicted leaf spot intensity

Observed leaf spot intensity

Predicted leaf spot intensity
4.7.2 Correlation of leaf spot intensity with weather parameters

The epidemiological studies are generally divided into two groups (1) those established for studying the effect of as many variables as possible (Holistic approach) of host, pathogen and environment on disease development; and (2) those in which only key variables are studied to explain most of the effect governing the epidemic (Meristemic approach). The decision whether to employ holistic or meristemic approach depends not only to the purpose of study and/or to the research facilities, but also on financial, instrumental and staff situation etc. Because of some limitations, meristemic approach was adopted in pot experiment suitable for studies within a disease triangle.

The disease started appearing in the younger stage of the crop and epidemiological studies were performed right from this to harvesting. The leaf spot infection in susceptible variety C. F. Gold and corresponding weather parameters at weekly interval were recorded which are presented in (Table 4.5).

The data presented (Table 4.5) revealed that the disease was initiated at younger stage (16\textsuperscript{th} July) and gradually increased and progressed up to harvesting stage (10\textsuperscript{th} September) continuously.

The leaf spot was more progressive during second fortnight of July (23\textsuperscript{rd} to 30\textsuperscript{th} July) when maximum, minimum and range temperatures were around 30\textdegree C, 26\textdegree C, and 3\textdegree C,
respectively; morning, evening and average humidity were around 90, 85, and 87 per cent, respectively; sunshine hours, wind speed, rainfall and rainy days were 0.41, 8.7 km/hr, 19 mm and 5 days, respectively.

Correlation matrix worked out (Table: 4.6) showed that maximum temperature (-0.71038), average temperature (-0.85800), minimum temperature (-0.79767) and wind speed (-0.63050) were significantly and negatively correlated with the leaf spot intensity. All these factors jointly played an important role in the disease development.

However, among all the weather parameters, average temperature was found to have key role on leaf spot disease development (Table- 4.6 and Fig-4.6).

**Multiple linear regression**

The regression co-efficient based on multiple linear regression analysis for percent leaf spot intensity of gerbera with respect to weather parameters have been worked out.

The regression co-efficient for average temperature was found to be negative and significant (Table- 4.7). It has been also observed that multiple correlation R value was high (0.97) indicating a strong association between per cent disease intensity and average temperature. The co-efficient of determination value was found to be high *i.e.* 91.29 per cent (Table- 4.7).

This clearly indicates that at least 80 per cent of variation in leaf spot intensity can be explained by the function of
Table-4.6: Significance of leaf spot intensity and weather parameters

<table>
<thead>
<tr>
<th>Atmospheric temp (°C)</th>
<th>Max</th>
<th>Min</th>
<th>Average (X₁)</th>
<th>Wind speed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.71038*</td>
<td>-0.79767*</td>
<td>-0.85800*</td>
<td>-0.63050*</td>
</tr>
</tbody>
</table>

Note: +\- 0.6297 Critical value (0.05%)

*Significant

Table-4.7: Multiple linear regression for gerbera leaf spot on different weather parameter

<table>
<thead>
<tr>
<th>Multiple linear regression equation</th>
<th>Multiple R</th>
<th>Co-efficient of determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y=a+b₁x₁ + b₂x₂ +...+bₙxₙ</td>
<td>0.97</td>
<td>91.29</td>
</tr>
<tr>
<td>Y= 389.55+828.8508(x₁)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Where,

Y= Predicted disease incidence

X₁= Average temperature
Results and Discussion

weather parameters as evident from multiple linear regression equation \[ Y=389.55 + 828.8508 \times (x_1) \] where \( x_1 = \text{Average temperature} \).

The observed and predicted leaf spot intensity plotted in (Fig-4.7) clearly indicated that both the lines were closer justifying the validity of the regression equation formulated.

Thus, observed leaf spot intensity and predicted leaf spot intensity found closely related and regression equation established as \( Y =389.55 + 828.8508 \times (x_1) \) which may be most reliable and useful for forecasting of the leaf spot incidence. The loss caused by the leaf spot can be saved giving fore warming to the farmers and there by controlling the same at proper time.

Ahamad and Narain (2000) observed the leaf spot \((A. cucumerina)\) of bitter gourd when temperature was ranging from 25 to 28°C and relative humidity was more than 80 per cent. Rain promoted disease development and susceptibility gradually declined with increasing plant age. Fugro (1993) observed more incidence of late blight \((A. cucumerina)\) in watermelon when higher humidity accompanied by optimum temperature. Singh et al. (1995) observed maximum leaf spot intensity in cluster bean \((A. Xymopsidis)\) when temperature was ranging from 25 to 30°C; relative humidity was 80 per cent with high rain fall.

Patel (2003) observed relative humidity significant and negative while maximum temperature and sunshine hours significant and positive co-relation with the leaf spot intensity in
green gram (A. alternata) during Kharif season while, minimum temperature and relative humidity was negatively correlated in the crop grown in the late Kharif.

4.7.3 Area under disease progression curve (AUDPC)

To know the disease progress and apparent rate of infection in six varieties of gerbera an experiment was carried out during 2012-2013 at greenhouse of Floriculture, N.A.U., Navsari. Disease severity was recorded at weekly interval from the onset of disease till 46th meteorological week (Mw) of 2012. Further, PDI on foliage apparent rate of infection and AUDPC were calculated and presented in (Table 4.8).

The disease onset was simultaneously seen in all the six varieties on 40th, reached peak on 44th and gradually declined up to 46th std week of 2012. The result on percent disease intensity (PDI) in all the varieties showed in the range of 2.45 to 67.21, 2.25 to 40.54, 1.75 to 45.25, 4.5 to 75.21, 3.25 to 62.35 and 2.5 to 54.21 percent such as Stanza, Venezia, Torbin, Cherany, Diego and Binaka respectively.

The maximum PDI was recorded in Cherany (75.21) followed by Stanza (67.21) and least in Diego (62.35). Later sudden increase in the disease progress was observed between 41st to 43rd std weak and from 44th std weak onwards all the 6 varieties showed gradual decrease in disease (Table 4.8).

All the Six varieties recorded more than 1000 AUDPC values. However, maximum AUPDC value was recorded in
Table 4.8: Area under disease progression curve (AUDPC) worked out of six varieties

<table>
<thead>
<tr>
<th>Std. week</th>
<th>Weekly intervals</th>
<th>Per cent disease index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stanza</td>
</tr>
<tr>
<td>40</td>
<td>October 7th</td>
<td>2.45</td>
</tr>
<tr>
<td>41</td>
<td>October 14th</td>
<td>5.7</td>
</tr>
<tr>
<td>42</td>
<td>October 21st</td>
<td>24.75</td>
</tr>
<tr>
<td>43</td>
<td>October 28th</td>
<td>45.25</td>
</tr>
<tr>
<td>44</td>
<td>November 4th</td>
<td>67.21</td>
</tr>
<tr>
<td>45</td>
<td>November 11th</td>
<td>59.65</td>
</tr>
<tr>
<td>46</td>
<td>November 18th</td>
<td>55.22</td>
</tr>
<tr>
<td></td>
<td>AUDPC</td>
<td>1675</td>
</tr>
</tbody>
</table>
Cherany (2044) followed by Diego (1772) and the least AUPDC value was observed in Stanza (1675).

This result of present investigation is also line with those obtained by Raghvendra (2005), he observed that maximum disease index of powdery mildew with AUDPC was observed in (2004) at Std weak of 40th and (2005) in 1st weak. Further, he also noted that all the five varieties had significant effect of powdery mildew their increase or decrease of disease on similar trends of present investigation.

4.8 Chemical control

4.8.1 Bio-efficacy of fungicides against *Alternaria alternata in vitro*

Different Eight fungicides with three concentrations were evaluated *in vitro* by poisoned food technique for their efficacy against *A. alternata*.

It is evident from the results presented in (Table-4.9), (Fig.-4.8) and (Plate-IX) that all the fungicides evaluated at different concentrations significantly reduced the growth of *A. alternata* as compared to the control. Out of these, propiconazole (Tilt), at all the three concentrations and tebuconazole (Folicur) at 300 and 500 ppm and difenoconazole (Score) at 500 ppm cent per cent growth inhibition of the pathogen was recorded. Significantly lower mycelial growth was recorded in tebuconazole + trifloxystrobin (Nativo) at 500 ppm (6.50 mm) and at 300 ppm (6.83 mm) as compared to the rest.
Results and Discussion

Next best in order of merit was captan + hexaconazole (Taquat) at 500 ppm (7.67 mm) and tebuconazole + trifloxystrobin at 200 ppm (7.83 mm) followed by tebuconazole at 200 ppm (8.50 mm) and captan + hexaconazole 300 ppm (8.67 mm). Rest of the fungicides, there also significantly lower mycelial growth was observed. This includes, captan + hexaconazole at 200 ppm (9.83 mm), difenoconazole at 300 and 200 ppm (14.50, 15.83 mm), femamidone + mancozeb (Sectin) at 500 ppm (24.83 mm), iprovalicarb + propineb (Melody DUO) at 500 ppm (26.50 mm) and bayleton + triadimefon (Bayleton) at 500 ppm (29.50 mm).

Propiconazole at all the three concentrations and tebuconazole at 300 and 500 ppm, difenoconazole at 500 ppm showed cent per cent growth inhibition and appeared as the most effective over all the fungicides tested followed by tebuconazole + trifloxystrobin at 500 ppm (92.74%) and at 300 ppm (92.36%). Next best in order of merit was captan + hexaconazole at 500 ppm (91.43%) and tebuconazole + trifloxystrobin at 200 ppm (91.25%) followed by captan + hexaconazole at 200 ppm (89.01%), difenoconazole at 300 and 200 ppm (83.80%, 82.31%), femamidone + mancozeb at 500 ppm (72.25%), iprovalicarb + propineb at 500 ppm (70.39%) and bayleton+ triadimefon at 500 ppm (67.04%).

Considering the effect of fungicides on growth of *A. alternata*, propiconazole, tebuconazole, difenoconazole proved the most effective followed by tebuconazole + trifloxystrobin. This suggested further testing of the chemicals by spraying in pot
Table-4.9: Evaluation of different fungicide against *Alternaria alternata* in vitro

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Technical and trade names of fungicides</th>
<th>Concentration (ppm)</th>
<th>Average colony diameter of pathogen (mm)</th>
<th>Per cent inhibition over control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tebuconazole50% + Trifloxystrobin25% WG. (Nativo)</td>
<td>200</td>
<td>2.88*(7.83)**</td>
<td>91.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>2.70 (6.83)</td>
<td>92.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>2.64 (6.50)</td>
<td>92.74</td>
</tr>
<tr>
<td>2</td>
<td>Propiconazole (Tilt 25% EC)</td>
<td>200</td>
<td>0.71 (0.00)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>0.71 (0.00)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>0.71 (0.00)</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>Iprovalica5.5% + Propineb61.25%WP (Melody DUO)</td>
<td>200</td>
<td>5.87 (34.00)</td>
<td>62.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>5.65 (31.50)</td>
<td>64.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>5.19 (26.50)</td>
<td>70.39</td>
</tr>
<tr>
<td>4</td>
<td>Femamidone10% + Mancozeb50%WG (Sectin)</td>
<td>200</td>
<td>5.58 (30.67)</td>
<td>65.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>5.35 (28.17)</td>
<td>68.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>5.03 (24.83)</td>
<td>72.25</td>
</tr>
<tr>
<td>5</td>
<td>Tebuconazole 25.9% w/w (25 w/w) (Folicur 25Ec)</td>
<td>200</td>
<td>2.99 (8.50)</td>
<td>90.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>0.71 (0.00)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>0.71 (0.00)</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>Captan + Hexaconazole (Taquat)</td>
<td>200</td>
<td>3.21 (9.83)</td>
<td>89.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>3.02 (8.67)</td>
<td>90.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>2.85 (7.67)</td>
<td>91.43</td>
</tr>
<tr>
<td>7</td>
<td>Bayleton25%WP+Triadimefon25%WP (Bayleton)</td>
<td>200</td>
<td>6.01 (35.67)</td>
<td>60.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>5.65 (31.50)</td>
<td>64.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>5.47 (29.50)</td>
<td>67.04</td>
</tr>
<tr>
<td>8</td>
<td>Difenoconazole25%EC (Score25EC)</td>
<td>200</td>
<td>4.04 (15.83)</td>
<td>82.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>3.87 (14.50)</td>
<td>83.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>0.71 (0.00)</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>Control (without fungicides)</td>
<td></td>
<td>9.48 (89.5)</td>
<td></td>
</tr>
</tbody>
</table>

* Figures indicate SQR+ 0.5 transformed values  
** Figures in the parentheses indicate original values
## Figure: 4.8 - Evaluation of different Fungicides against *Alternazta alternata* in vitro

<table>
<thead>
<tr>
<th>Treatment</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Tebuconazole+ Trifloxy Strobin</td>
<td>200</td>
<td>300</td>
<td>500</td>
</tr>
<tr>
<td>T2 Propiconazole</td>
<td>200</td>
<td>300</td>
<td>500</td>
</tr>
<tr>
<td>T3 Iprovalicarb+ Propineb</td>
<td>200</td>
<td>300</td>
<td>500</td>
</tr>
<tr>
<td>T4 Femamidone+ Mancozeb</td>
<td>200</td>
<td>300</td>
<td>500</td>
</tr>
<tr>
<td>T5 Tebuconazole</td>
<td>200</td>
<td>300</td>
<td>500</td>
</tr>
<tr>
<td>T6 Captan + Hexaconazole</td>
<td>200</td>
<td>300</td>
<td>500</td>
</tr>
<tr>
<td>T7 Bayleton+Triadimefon</td>
<td>200</td>
<td>300</td>
<td>500</td>
</tr>
<tr>
<td>T8 Difenoconazole</td>
<td>200</td>
<td>300</td>
<td>500</td>
</tr>
<tr>
<td>T9 Control</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Plate-IX: Effect of fungicides on the growth of *A. alternata*

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Fungicides</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Tebuconazole50% + Trifl oxy Strobin25%</td>
<td>200 300 500</td>
</tr>
<tr>
<td>T2</td>
<td>Propiconazole</td>
<td>200 300 500</td>
</tr>
<tr>
<td>T3</td>
<td>Iprovalicarb5.5% + Propineb61.25%WP</td>
<td>200 300 500</td>
</tr>
<tr>
<td>T4</td>
<td>Femamidone10% + Mancozeb50%WG</td>
<td>200 300 500</td>
</tr>
<tr>
<td>T5</td>
<td>Tebuconazole 25.9% w/w (25 w/w)</td>
<td>200 300 500</td>
</tr>
<tr>
<td>T6</td>
<td>Captan + Hexaconazole</td>
<td>200 300 500</td>
</tr>
<tr>
<td>T7</td>
<td>Bayleton25%WP+Triadimefon25%WP</td>
<td>200 300 500</td>
</tr>
<tr>
<td>T8</td>
<td>Difenonconazole25%EC</td>
<td>200 300 500</td>
</tr>
<tr>
<td>C</td>
<td>Control</td>
<td>200 300 500</td>
</tr>
</tbody>
</table>
for more confirmation of their efficacy and feasibility. Murthy and Shenoi (2001) reported that difenconazole and propiconazole was most effective fungicide for the control of A. tenuissima of marigold. Dubey et al. (2000) observed that hexaconazole (contaf) inhibited cent per cent growth of A. alternata. Patil (2003) and Patel (2003) reported propiconazole (Tilt) as the most effective fungicide against A. alternata of marigold and greengram, respectively. The results of earlier workers are also in agreement with the results obtained in the present investigation.

4.8.2 Bio-efficacy of fungicides against leaf spot of gerbera in pot condition

The chemicals concentration those was found effective under laboratory screening were further screened under pot conditions. The performance of each of these fungicides was compared with control where no fungicides were sprayed.

The observations were taken on the basis of 0-5 scale (Plate- VIII). The percent disease intensity (PDI) and percent disease control (PDC) was worked out and the results are presented in (Table 4.10) and (Fig.4.9).

The data presented in (Table 4.10) revealed that all the fungicidal treatments significantly reduced the disease intensity as compared to the control (Plate- X). Among them, propiconazole (Tilt) was found significantly superior over the rest as resulted minimum disease intensity (8.16%) but was at par with tebuconazole + trifloxystrobin (Nativo) (9.66%). The next
### Table-4.10: Evaluations of different fungicides for the management of Leaf spot of Gerbera in Pot condition.

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Fungicides</th>
<th>Concentration (ppm)</th>
<th>Per cent disease intensity</th>
<th>Per cent disease control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tebuconazole50% + Trifloxystrobin25% WG.(Nativo)</td>
<td>500</td>
<td>18.07 * (9.66)**</td>
<td>75.85</td>
</tr>
<tr>
<td>2</td>
<td>Propiconazole(Tilt 25% EC)</td>
<td>200</td>
<td>16.59 (8.16)</td>
<td>79.60</td>
</tr>
<tr>
<td>3</td>
<td>Iprovalicarb5.5% + Propineb61.25%WP (Melody DUO)</td>
<td>500</td>
<td>32.26 (28.50)</td>
<td>28.75</td>
</tr>
<tr>
<td>4</td>
<td>Femamidone10% + Mancozeb50%WG (Sectin)</td>
<td>500</td>
<td>27.37 (21.16)</td>
<td>47.10</td>
</tr>
<tr>
<td>5</td>
<td>Tebuconazole 25.9% w/w (25 w/w) (Folicur 25Ec)</td>
<td>300</td>
<td>19.35 (11.00)</td>
<td>72.50</td>
</tr>
<tr>
<td>6</td>
<td>Captan +Hexaconazole (Taquat)</td>
<td>500</td>
<td>20.25 (12.00)</td>
<td>70.00</td>
</tr>
<tr>
<td>7</td>
<td>Bayleton25%WP+Triadimefon25%WP (Bayleton)</td>
<td>500</td>
<td>31.40 (27.16)</td>
<td>32.10</td>
</tr>
<tr>
<td>8</td>
<td>Difenoconazole25%EC (Score25EC)</td>
<td>500</td>
<td>19.50 (11.16)</td>
<td>72.10</td>
</tr>
<tr>
<td>9</td>
<td>Control (without fungicides)</td>
<td>-</td>
<td>39.23 (40.00)</td>
<td>-</td>
</tr>
</tbody>
</table>

- S.Em. +
- C.D. @ 5%
- C.V. %

* Figures indicate arcsine transformed values
** Figures in the parentheses indicate original values
Figure: 4.9- Evaluation of Different fungicide for the control of Alternaria leaf spot of gerbera in Pot condition.
Results and Discussion

best treatment in order of merit was tebuconazole (Folicur) (11.00%), difenoconazole (Score) (11.16%), captan + hexaconazole (Tاقت) (12%), femamidone + mancozeb (Sectin) (21.16%), bayleton + triadimefon (Bayleton) (27.16%) and iprovalicarb + propineb (Melody) (28.50%) were also found effective.

The maximum disease control was recorded in the pots where propiconazole (Tilt) was sprayed (79.60%). This was followed by tebuconazole + trifloxystrobin (Nativo) (75.85%), tebuconazole (Folicur) (72.50%), difenoconazole (Score) (72.10%), captan + hexaconazole (Tاقت) (70%), femamidone + mancozeb (Sectin) (47.10%), bayleton + triadimefon (32.10%) and iprovalicarb + propineb (Melody) (28.75%) were also proved considerably effective. These confirm the finding of Murthy and Shenoi (2001) reported propiconazole (Tilt) as most effective fungicide for the control of leaf spot of tobacco (*A. alternata*).

Patil (2003) proved propiconazole (Tilt) while Patel (2003) proved propiconazole (Tilt) and ziram most effective fungicides in controlling marigold and green gram leaf spot disease (*A. alternata*), respectively. These findings are in agreement with the results obtained in the present investigation.
SUMMARY

AND

CONCLUSION
V. SUMMARY AND CONCLUSIONS

Leaf spot of gerbera has become a severe problem in recent years with a threat to successful and profitable cultivation in south Gujarat. Considering this fact, the present investigations were carried out on various aspects to generate scientific information on this important pathological problem and suitable management strategies to prevent crop losses.

Microscopic examination and tissue isolation from leaf of infected gerbera plant yielded culture of *Alternaria sp.* The typical leaf spot symptoms observed in the greenhouse were light brown and small lesions appeared on leaves, which later turned black to brown in colour and irregular in shape with concentric rings. With the age of infection, the lesions enlarged. In severe infection, several spots merged together and covered major portion of the leaf.

The morphological and cultural characters of *Alternaria sp.* isolated was studied, which were found closely identical with *Alternaria alternata* (Fr.) Keissler and this was also confirmed through identification by Indian Type Culture Collection, Division of Plant pathology, I.A.R.I., New Delhi (I.T.C.C. No. 8774-12). The pathogenicity test carried out by Carborandum powder and injury by tooth brush on leaves. Both these methods successfully produced typical leaf spot symptoms similar to those observed under natural condition and described in the literature and confirming pathogenic nature of the fungus.
Thus, the causal agent of gerbera leaf spot identified and confirmed as *Alternaria alternata* (Fr.) Keissler.

The critical examination of pathogen showed that hyphae penetrated between the walls of cell after 72 hours and developed fruting body like structure in leaves. Conidiophores were light brown, simple and septate bearing pale brown to brown long conidia with 3-5 transverse and 0-3 longitudinal septa measuring 23.40 – 32.76 x 5.27-9.95 µm. Conidia borne singly or in chains of 5 to 8, variable in size and shape usually ovoid to ellipsoid, with cylindrical beak and dark brown in colour.

At the pre-infection stage, total soluble sugar was found higher in resistant varities than susceptible varities and reverse trend was found at post-infection. The higher soluble sugar in susceptible varities at post-infection may support the fungal growth and sporulation. Total phenol, contents at pre-infection stage did not showed specific trend or correlation with inherent resistance or susceptibility to Leaf spot pathogen. Although the susceptible varities had higher phenol as compared to resistance varities at post-infection stage. Peroxidase activity was higher in susceptible varities at both constitutive and induced stages. POX activity was higher in both the resistant and susceptible varities in post infection stage as compared to the pre-infection stage. In the present study high phenol content, POX activity in susceptible varities showed some degree of positive correlation. Catalase activity was decreased in all varities after
infection. However, resistant varities had about 2-3 fold higher CAT activity than susceptible varities at both stages.

Out of thirteen varieties screened under greenhouse conditions minimum percent disease index was recorded in Kento, Jaffana, Venezia proved resistant, while Binaka, Torbin, were found moderately resistant. Ice queen, Lion, Diego, Fana, Stanza found moderately susceptible and C. F. orange, Cherany, C. F. gold were found susceptible.

The observations on leaf spot intensity were recorded weekly interval from variety C. F. Gold in the greenhouse during crop season to know the time\stage of infection and window period. The leaf spot was found initiated 60 days after planting and since then it was found continuously progressing and reaching up to 30.48 per cent at harvest during September. There was main peak of leaf spot between 23\textsuperscript{rd} to 30\textsuperscript{th} July which considered as window period for leaf spot of Gerbera.

The weekly disease intensity and the corresponding weekly weather parameters recorded during entire crop cycle were also analysed for correlation maximum temperature, average temperature, minimum temperature and wind speed were significantly and negatively correlated with the leaf spot intensity. Among them, Average temperature was found to have key role affecting comparatively more on the disease development.
The regression co-efficient for average temperature was highest. Multiple correlation (R) value received was 0.97 per cent indicating strong association of the disease with average temperature. The co-efficient of determination value was found 91.29 per cent. The multiple regression equation, \[ Y = 389.55 + 828.8508(x_1) \] where \( x_1 \) = Average temperature establish on the basis of the observations. The observed and predicted per cent leaf spot intensity at any peak was found closely related.

The results regarding to the AUDPC, seen that there is increase in disease in the standard weak wise, the highest AUDPC value observed in Cherany (2044) followed by Diego (1772) and Stanza (1675). Standard weak 44\(^{th}\) was the peak period of highest disease intensity.

Eight fungicides at three concentrations were screened \textit{in vitro} by the poisoned food technique against \textit{A. alternata}. It was found that propiconazole (Tilt), at all the three concentrations and tebuconazole (Folicur) at 300 and 500 ppm and difenoconazole (Score) at 500 ppm, were fungitoxic with a powerful prolonged persistent fungitoxic effect up to eight days giving cent per cent inhibition. Next best in order of merit was tebuconazole + trifloxy strobin (Nativo) at 500 ppm and at 300 ppm followed by captan + hexaconazole (Taquat) at 500 ppm, tebuconazole + trifloxy strobin at 200 ppm, tebuconazole at 200 ppm, captan + hexaconazole at 200 ppm, difenoconazole at 300 and 200 ppm, femamidone + mancozeb (Sectin) at 500 ppm,
iprovalicarb + propineb (Melody DUO) at 500 ppm and bayleton + triadimefon (Bayleton) at 500 ppm.

The fungicide concentration those found effective under laboratory screening were further screened under greenhouse in pot conditions on leaf spot of gerbera. It was found that minimum percent disease intensity and maximum percent disease control was recorded in the pots which were sprayed with propiconazole (79.60%) followed by tebuconazole + trifloxy strobin (75.85%), tebuconazole (72.50%), difenoconazole (72.10%), captan + hexaconazole (70%), femamidone + mancozeb (47.10%), bayleton + triadimefon (32.10%) and iprovalicarb + propineb (28.75%).


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* Original not seen
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Place: Navsari.  (Pansare M. K.)

Date: