PHYLOGENETIC VARIATION AND FUNGICIDAL MANAGEMENT OF CERCOSPORA ABELMOSCHI ELL. AND EV. INFECTING OKRA

By

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B. Sc. (Ag.)

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DECLARATION

I, **G. Amulya,** hereby declare that the thesis entitled **"PHYLOGENETIC VARIATION AND FUNGICIDAL MANAGEMENT OF CERCOSPORA ABELMOSCHI Ell. and Ev. INFECTING OKRA"** submitted to the **Acharya N. G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

Place: Bapatla

(G. AMULYA)
ID. No. BAM-15-48

Date:

CERTIFICATE

Ms. G. AMULYA has satisfactorily prosecuted the course of research and that thesis entitled **"PHYLOGENETIC VARIATION AND FUNGICIDAL MANAGEMENT OF CERCOSPORA ABELMOSCHI ELL. AND EV. INFECTING OKRA**" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by her for a degree of any University.

Date:

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CERTIFICATE

This is to certify that the thesis entitled **"PHYLOGENETIC VARIATION AND FUNGICIDAL MANAGEMENT OF CERCOSPORA ABELMOSCHI ELL. AND EV. INFECTING OKRA"** submitted in partial fulfilment of the requirements for the degree of '**Master of Science in Agriculture'** of the Acharya N. G. Ranga Agricultural University, Guntur is a record of the bonafide original research work carried out by **Ms. G. AMULYA** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

Thesis approved by the Student's Advisory Committee

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Date of final viva-voce:

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Place: Bapatla

Date: **(G. AMULYA)**

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LIST OF SYMBOLS /ABBREVIATIONS

%	:	Per cent
^{0}C	:	Degree Celsius
CD (P=0.05%)	:	Critical difference at 5 per cent level
cm	:	Centimeter
CV	:	Coefficient of variation
et al.	:	and other co-workers
Fig.	:	Figure
g	:	Gram (s)
h	:	Hour
i.e.	:	that is
kg	:	Kilogram
ac	:	Acre
kg ha^{-1}	:	Kilogram per hectare
q ha^{-1}	:	Quintal per hectare
m	:	Metre
m^2	:	Metre square
mg	:	Milligram
min	:	Minute
ml	:	Millilitre
M	:	Molarity
mM	:	Milli molar
nm	:	Nanometre
No.	:	Number
ppm	:	parts per million

RBD	:	Randomized Block Design
rpm	:	Revolutions per minute
s	:	Second
SEm	:	Standard Error of mean
sp., spp.	:	Species (singular or plural)
M t	:	Million tonnes
µg	:	Microgram
viz.	:	Namely
µl	:	Microlitre
l	:	Litre
bp	:	Basepair
ITS	:	Internal Transcribed Spacer regions
PCR	:	Polymerase Chain Reaction

ABSTRACT

Name of the author	:	**G. AMULYA**
I.D. No.	:	**BAM-15-48**
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The present study on phylogenetic variation and fungicidal management of *Cercospora abelmoschi* Ell. and Ev. infecting okra was taken up in the Agricultural College, Bapatla during 2016-2017.

Cercospora leaf spot was mainly observed on the lower surface of leaves in the form of sooty to dark olivaceous colour, indistinct or none, fruiting effuse, forming angular vein limited areas in dense patches of conidiophores and conidia.

Cercospora infected leaves were collected during *kharif* 2016 from eight different okra growing villages in Guntur district, Andhra Pradesh which were used for *in planta* isolation of fungal DNA using universal primers ITS 1 and ITS 4.

The 550 bp amplicon thus obtained was restricted with hexa cutters, EcoRI, BamHI and tetra cutter Taq1 to find variability among *Cercospora* isolates. EcoRI found two restriction sites in all isolates except the Yazali isolate while BamHI found single restriction site in all the isolates with length polymorphism in Yazali isolate. Taq1 restriction indicated a high degree of genetic diversity among the isolates and was represented by three different banding patterns while in three isolates there was no restriction sites.

Dendrogram constructed from similarity coefficients showed that Yazali isolate seperated into a group upon digestion with EcoRI and BamHI. Taq1 digestion of Yazali isolate clustered with Thimmareddipalem isolate. The results revealed that,

polymorphism existed among the *Cercospora* isolates collected from Guntur district. The changes in single nucleotide resulted in variation in restriction sites for the different restriction enzymes used.

Among all the tested fungicides, trifloxystrobin + tebuconazole @ 0.1% showed significant reduction of the disease on both lower leaves (35.61%) and upper leaves (12.98%), leading to the significant increase in yield (101.75 q ha^{-1}). However, highest benefit cost ratio was recorded with propiconazole @ 0.1% (3.62).

Phenol content in lower leaves(1.58 mg g^{-1}) and upper leaves (1.52 mg g^{-1}), total protein content in lower (9.32 mg g^{-1}) and upper leaves (9.23 mg g^{-1}) were significantly higher in trifloxystrobin + tebuconazole @ 0.1% applied plants. Total sugar content in lower (12.33 mg g^{-1}) and upper leaves (10.08 mg g^{-1}) were low in trifloxystrobin + tebuconazole @ 0.1% treatment.

The reduction in Chlorophyll a and chlorophyll b was observed to be lowest in lower leaf (1.15 and 1.06 mg g^{-1} respectively) and upper leaf (1.28 and 1.02 mg g^{-1} respectively) in trifloxystrobin + tebuconazole @ 0.1% sprayed plants. The reduction in the total chlorophyll content was relatively less in trifloxystrobin + tebuconazole @ 0.1% as compared to other treatments due to reduced disease intensity and thus maintaining greenness of the leaves.

Significant negative correlation existed between disease severity and total phenols (-0.903), total proteins (-0.903), chlorophyll a (-0.901), chlorophyll b (-0.788), total chlorophyll (-0.856) while significant positive correlation existed between total sugars and disease severity (0.932).

Significantly high positive correlation existed between proteins and phenols (0.862), total chlorophyll (0.889), chlorophyll a (0.844), chlorophyll b (0.868). Similarly, significant high positive correlation existed between phenols and total chlorophyll (0.854), chlorophyll a (0.803), chlorophyll b (0.839). Significantly high negative correlation between total sugars and phenols (-0.924), proteins (-0.836), chlorophyll a (-0.919), chlorophyll b (-0.838), total chlorophyll (-0.897) existed.

Chapter – I

INTRODUCTION

Okra, *Abelmoschus esculentus (L.)* Moench, is an important warm season vegetable crop grown mainly in the tropical or sub-tropical regions during summer and rainy seasons (Thomson and Kelly, 1957). It is called as "lady's finger" in England, "Gumbo" in United states and "Bhindi" in India (Chauhan, 1972). Okra was previously referred to as *Hibiscus* but was later, designated as *Abelmoschus*, which can be distinguished from the earlier by spatulate calyx, five short tooth, connate corolla and flower caduceus (Kundu and Biswas, 1973; Terrell and Winters 1974). Okra is the object of an intensive production system in urban and rural agriculture according to the United States Agency for International Development (USAID, 2006). Its nutritive values are above that of tomato (Sawadogo *et al.*, 2006).

Okra is known to be originated from West Africa (Joshi *et al.,* 1974). The major okra producing countries in the world include India (3.5 M t), Nigeria (0.73M t), Pakistan (0.12 M t), Ghana (0.10 M t) and Egypt (0.08 M t) (Badaru, 2011). Major Okra growing states in India are Uttar Pradesh, Bihar, Orissa, West Bengal, Andhra Pradesh and Karnataka. In India, it is grown in 503.7 thousand ha with a production of 5708 thousand M t and 11.3 M t/ha productivity. In Andhra Pradesh, it occupies an area of 18.6 thousand ha with a production of 211.2 thousand M t and productivity is 11.4 M t/ha (Ministry of Agriculture and Farmers Welfare, Govt. of India, 2014- 2015).

Okra is cultivated for a variety of uses but mainly for its edible leaves and immature green seed pods or fruits. Okra dry seeds contain 18-20% oil and 20-23% crude protein. Roasted and ground seed find their use as a coffee substitute. It is good for patients suffering from renal colic, leucorrhoea, chronic dysentery and general weakness (Siemonsma and Kouame, 2004).

Fresh okra leaves are used as vegetables while the roots and stems are used for clearing the cane juice from which 'gur' or brown sugar is prepared. Potassium, sodium, magnesium and calcium are the principal elements in pods, which contain about 17% seeds. Presence of iron, zinc, manganese and nickel also has been reported (Moyin-Jesu, 2007). Fresh pods are low in calories (20/100 g), practically no fat, rich in fiber, and with several valuable nutrients. Okra seed is mainly composed of oligomeric

catechins (2.5 mg g^{-1} of seeds) and flavonol derivatives (3.4 mg g^{-1} of seeds), while the mesocarp is mainly composed of hydroxycinnamic (0.2 mg g^{-1}) and quercetin derivatives (0.3 mg g^{-1}). Pods are rich in phenolic compounds with important biological properties like quartering derivatives, catechin oligomers and hydroxycinnamic derivatives (Arapitsas, 2008).

Diseases play a vital role in yield losses of the crop. Among them, fungi are one of the most important and prevalent pathogens which attack the crops from seedling to harvesting stage. Some of the fungal diseases that attack are Cercospora leaf spot (*Cercospora abelmoschi*), damping-off (*Pythium* sp. and *Rhizoctonia* sp.), powdery mildew (*Oidium* sp.), southern blight (*Sclerotium rolfsii*), verticillium wilt (*Verticillium albo-atrum*) and alternaria leaf spot (Raid and Palmateer, 2006).

Cercospora species (Family: *Mycosphaerellaceae,* Order: *Capnodiales)* are commonly associated with leaf and fruit spots on a wide range of cultivated and wild plants worldwide (Bakhshi *et al.,* 2015). The presence of this fungus is especially abundant and diverse in tropical and subtropical areas (Braun *et al.,* 2014). In India, two species of *Cercospora viz., C. malayensis* Stev. and Solh. and *C. abelmoschi* Ell. and Ev. were found to cause leaf spots in okra. These species differ in symptom production. Due to the paucity of useful morphological and physiological characteristics for identification, the taxonomy of the genus *Cercospora* remains confusing and depends heavily on the other hosts it affects. Of late, Cercospora leaf spot of okra has become endemic in fields of Bapatla and has been causing a major threat to okra cultivation. Hence, the present study is proposed with the following objectives:

1. To evaluate the diversity of *Cercospora abelmoschi* infecting okra in Guntur district using ITS primers.

2. To evaluate fungicides for the management of Cercospora leaf spot in okra.

Chapter-II

REVIEW OF LITERATURE

The review of literature on "Phylogenetic variation and fungicidal management of *Cercospora abelmoschi* Ell. and Ev. infecting okra" and other relevant literature to the present investigation is presented in this chapter.

2.1 THE DISEASE

Okra crop suffers from number of biotic and abiotic stresses. Among biotic factors, fungal diseases are reported to pose serious problem in okra cultivation. In fungal diseases, leaf spots were reported to be a threat to the crop (Jha and Dubey, 2000; Kochhar, 2005; Jiskani, 2006). Anam *et al.* (2002) reported seed-borne fungal diseases like cercospora leaf spot, anthracnose, stem rot, corynespora leaf spot and ascochyta blight on okra in India. Cercospora leaf spot and anthracnose were reported to be the important diseases causing major yield losses in okra (Singh, 1999). All India Co-ordinated Vegetable Improvement Project identified Bhubaneswar as hotspot for Cercospora blight because of its greater impact on economic loss in Orissa (Beura *et al.*, 2007) as productivity was found to be low in Orissa (Ministry of Agriculture and Farmers Welfare, Govt. of India, 2014-2015).

Fakir (2000) reported fungal diseases due to *Aspergillus, Cercospora, Fusarium, Colletotrichum, Macrophomina* and *Penicillium* on okra grown in Bangladesh. *Cercospora* leaf spot of okra is called as black mould in Bangladesh and was reported as a common disease when okra is grown as a late winter or as an early summer crop causing tremendous yield losses (Pabitra, 2009 and Jiskani, 2011).

Species belonging to the genus *Cercospora* sp. Fresen are distributed worldwide and cause leaf spots on major plant families (Crous and Braun, 2003). Considerable damage was reported on kenaf (Prasad *et al.*, 1960), avocado (Darvas, 1977), okra (Chauhan *et al.*, 1980), cowpea (Amadi, 1994), safflower (Lartey *et al.*, 2005), sugar beet (Hashem and Farrag, 2005), sesame (Enikuomehin, 2005), maize (Crous *et al.*, 2006), groundnut (Ambang *et al.*, 2011) and oyster plant (Rooney-Latham *et al.*, 2011).

2.2 THE FUNGUS

The occurrence of *Cercospora abelmoschi* on *Abelmoschus esculentus* was first recorded by Ellis and Everhart (1893) in Jamaica. Hennings (1904) and Reitsma and Sloof (1950) noted infection of *C. hibisci- manihotis* on *Hibiscus manihot* L. in Tokyo. Fresenius introduced the genus *Cercospora* one of the largest genera of hyphomycetes, has been linked to *Mycosphaerella* teleomorphs (Crous *et al.*, 2000). The genus *Mycosphaerella* Johanson, contains more than 3000 names (Aptroot, 2006), and has been linked to more than 30 well-known anamorphic genera (Crous and Groenewald, 2006a and 2006b). It has a worldwide distribution from tropical and subtropical to warm and cool regions (Crous, 1998; Crous *et al.*, 2000 and 2001). *Mycosphaerella*, however, has been associated with at least 27 different coelomycete or hyphomycete anamorph genera (Kendrick and DiCosmo, 1979), 23 of which were accepted by Crous *et al*. (2000). More than 3000 names have been published in Cercospora (Pollack, 1987).

Cercospora was first monographed by Chupp (1954), who accepted 1419 species. Crous and Braun (2003) reported that Deighton, Sutton and Braun had divided *Cercospora* in to 50 different genera that were morphologically similar and distinct with each other. "Cercosporoid fungi" a collective term was used to a group of fungi belonging, to the genus *Cercospora* and its allied genera, namely *Pseudocercospora, Passalora, Asperisporium, Corynespora* and *Cladosporium*. Differences among them are based mainly on a combination of characters that include the structure of conidiogenous loci (scars) and hila, presence or absence of pigmentation and ornamentation in conidiophores and conidia, geniculate or non-geniculate conidiophore and rare presence of additional or unique features such as knotty appearance of conidiophores.

Sawada (1922), Chandrasekaran and Rangaswami (1960) reported that *Hibiscus syriacus* L. was infected by *C. hibisci – cannabini*. Chupp (1954) and Rao (1962) considered that *C. hibisci, C. hibisci- manihotis* and *C. hibisci – cannabini* as synonyms of *C. abelmoschi.*

In the MycoBank fungal database, approximately 2,721 *Cercospora* species have been described and verified. However, the revised data on *Cercospora* by Crous and Braun (2003) recognized only 659 names in the genus *Cercospora*.

2.3 SYMPTOMATOLOGY

Cercospora leaf spot on *A. esculentus* was reported to manifest itself as sooty to dark olivaceous colour, indistinct or none, fruiting effuse, that form mere specks in an area or to a large part of lower leaf surface (Ellis and Everhart, 1893).

Deighton (1976) described Cercospora leaf spot in okra as distinctly angular vein limited areas in dense patches of dark brown conidiophores and conidia occurring on the lower surface of the leaves but without any distinct leaf lesion that coalesced up to 0.6 cm wide. Leaf tissues were chlorotic at later stages where leaf rolling, wilting occurs. Defoliation was reported to occur when the leaves were completely covered with conidiophores and conidia.

According to Dhancholia and Singh (1992) initial disease symptoms occur on the lower surface of leaves as indistinct spots in the form of dark olivaceous specks that later turn into light brown to grey due to the growth of the fungus. As disease advances from lower leaves, necrotic spots were reported on the upper surface and ultimately infected leaves became sooty- black, dried and defoliated with similar symptoms on fruits and stem (Hasan *et al.,* 2009).

However, leaf spot caused by *Cercospora malayensis* in okra was reported as long fairly broad leaf spots that later changed to brown streaks between major veins of leaf frequently extending from the edge of the leaf to midrib. The conidiophores form dark brown, sooty patches resulting in discoloured areas with indefinite boarders (Solheim and Stevens, 1931).

2.4 DIVERSITY OF THE PATHOGEN

Chupp (1954) monograph on Cercosporoid hyphomycetes was based on generic concept in broad sense equating many cercosporoid genera as synonyms to the genus *Cercospora*. Contrary to his approach, Deighton (1967, 1973, 1976, 1979) and Ellis (1971, 1976) narrowed the generic concept of *Cercospora* sp. and divided it into smaller morphological units. Deighton (1987 and 1990) reclassified numerous species into several allied genera based on two distinct taxonomic categories *i.e.,* thickened conidial scar as *Cercospora* and its allied genera such as, *Passalora* and *Stenella*, while unthickened scars as characteristic to the genera *Pseudocercospora* and *Stigmina*.

2.5 STUDIES ON MOLECULAR VARIABILITY

Crous *et al.* (2000) and Crous and Braun (2003) reviewed the genera of Cercosporoid fungi that were classified earlier based on morphological characters and rearranged them into four phylogenetic genera based on DNA sequences for the first time *viz. Cercospora, Passalora, Pseudocercospora* and *Stenella.* As the work progressed towards establishing a stable phylogeny for the Mycosphaerellaceae members (Arzanlou *et al.,* 2007, Crous *et al.,* 2007, 2009a, 2009b, Braun *et al.,* 2013, Crous *et al.,* 2013a, Groenewald *et al.,* 2013), most of the assumptions made by Crous and Braun (2003) regarding generic circumscriptions have been confirmed.

Intra specific polymorphism was reported in ectomycorrhizal fungi due to length mutations, ranging from 5 to 15 bp in four of the seven polymorphic species (Karen *et al.,* 1997) and there are reports on host- specific specialization (*formae speciales*) of *C. canescens* from *Vigna mungo* (Kaushal *et al.,* 1993) and *V. radiata* (Chand *et al.,* 2000). Genetic heterogeneity was reported in fungi like *Ascochyta rabiei* (Morjane *et al.,* 1994) and *Rhynchosporium secalis* (Dermott *et al.,* 1989). Samples from a single chickpea field infected with *A. rabiei* were reported to have a large amount of subtle genetic variation, with more than one *A. rabiei* haplotype being present on single host plant even with in single lesion.

Almeida *et al.* (2005) studied pathogenecity, molecular characterization and cercosporin content of 72 Brazilian isolates of *Cercospora kikuchii* and RAPD analysis clustered all isolates into seven groups. No relationship was observed between RAPD groups and geographic origin or cercosporin content but the sequence of the internal spacer regions from 13 isolates chosen according to the previous RAPD and clustering analysis showed high similarity (97%-100%) to the GeneBank sequences of *C. kikuchii* from which it is clear that Brazilian isolates of *C. kikuchii* from different geographic regions, are variable in relation to virulence, RAPD profiles and cercosporin content.

Joshi *et al.* (2006) studied genetic diversity in eleven different isolates of *C. canescens* (Ellis & Martin), the causative agent of leaf spot in legumes. Isolates from different geographical locations, with different morphological and pigment production characters were reported to be polymorphic when studied with RAPD marker and variation in the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA). RAPD profiling clustered the isolates into three clusters. They reported considerable

genetic diversity in the isolates from the same geographical location. rDNA analysis was reported to show length variation in ITS regions of two isolates from mungbean, with one 600 bp band common to both. Restriction analysis was reported to differentiate between the common 600 bp bands of the two isolates.

Crous *et al.* (2013) has partially sequenced the 28S nuclear ribosomal RNA gene of a selected set of isolates to resolve phylogenetic generic limits within the *Pseudocercospora* complex and 14 clades were recognised, six of which were reported to clustered in Mycosphaerellaceae. Host specificity was reported for 146 species of *Pseudocercospora* occurring on 115 host genera from 33 countries. Based on the partial nucleotide sequence data for ITS, EF-1α, and ACT loci, they suggested that the majority of these species as host specific. They further reported that species identified on the basis of host, symptomatology and general morphology, within the same geographic region, frequently differed phylogenetically.

Yuliarni *et al.* (2013) determined the relationship between host plants and multilocus sequence variations (ITS rDNA including 5.8S rDNA, elongation factor 1-α, and calmodulin) in *Cercospora* spp. to investigate the host specificity and phylogenetic variability.

Bakhshi *et al.* (2015) conducted phylogenetic studies based on partial gene sequences of the LSU, ITS, ACT, TEF1-α and HIS loci and elucidated a *cercospora*-like taxon from Bishop's flower (*Ammi majus* L.) to cluster in a clade apart from *Cercospora s. str*. They emphasized that their collection represented the concrete evidence to the fact that the morphological characters previously attributed to *Cercospora s. str*. has evolved more than once in the Mycosphaerellaceae. Thus, they introduced a genus *Neocercospora* to accommodate the Iranian taxon occurring on *A. majus*.

Nguanhom *et al.* (2015) studied morphological and molecular phylogenetic analysis of sixty *Cercospora* spp. isolated from 29 host species grown in northern Thailand (representing 16 plant families). Partial nucleotide sequence data for two gene loci (ITS and cmdA) were generated for all the isolates.

2.6. MANAGEMENT OF CERCOSPORA LEAF SPOT

Use of chemicals for the control of CLS has been practiced for a long time having varying degree of success (Backman *et al.,* 1977). The leaf spot disease was reported to be controlled commonly by different fungicides *viz.* Benlate-T, Dithane M-45, Topsin-M, Rovral, Mancozeb, Iprodion, Tridemorph, Ziram, Bavistin, Pencozeb, Derosol, Signum 334 WG, Amistar 250 AC, Boscalid, Pyraclostrobin (Mondal *et al*., 1986; Orozco and Mexico, 1991; Masirevic, 1993; Ghosh *et al.,* 2002; Khalequzzaman *et al.*, 2003; Ayoub and Qureshi, 2004; Surviliene *et al.,* 2006; Rizzolli and Acler, 2006) applied as foliar spray (Beura *et al.*, 2007).

Meriggi *et al.* (2000) reported that traditional protective fungicide such as maneb or systemic fungicide chlorothalonil being used worldwide for the control of Cercospora leaf spot. Sukand *et al.* (2005) reported that chlorothalonil was more efficient over propiconazole and mancozeb for managing Cercospora leaf spot and rust of groundnut. Beura *et al.* (2007) studied the efficacy of seven different fungicides *viz.* carbendazim 0.15% , copper oxychloride 0.3%, mancozeb 0.3%, propineb 0.25%, copper hydroxide 0.3%, thiophanate methyl 0.1% and ziram 0.25% against Cercospora leaf spot of okra and found carbendazim to be effective. Hasan *et al.* (2009) studied the effect of combined management practices for Cercospora leaf spot of okra. Soil amendment with mustard oil cake (50 g pot^{-1}) + carbendazim (0.15%) spray at 65 DAS followed by two sprays at 15 days interval were reported to be most effective in controlling the disease with maximum seed yield (22.03 g $plant^{-1}$). Farrag (2011) reported that foliar application of Topsin M-70 WP @ 0.15 % concentration showed 100% inhibition of the pathogen. Palakshappa *et al.* (2012) evaluated different fungicides against Cercospora leaf spot of sesame and found Carbendazim as effective fungicides in controlling the disease.

Khunti *et al.* (2002) found that hexaconazole to be effective while penconazole was statistically on par. Sulphur, propiconazole and carbendazim were reported as moderately effective in reducing Cercospora leaf spot of greengram. Hundekar *et al.* (2005) reported that spraying hexaconazole (0.1%) or propiconazole (0.1%) or carbendazim (0.05%) to be better in managing the frog-eye leaf spot of bidi tobacco in Karnataka. Bhattiprolu (2010) reported that taqat (hexaconazole 5% + captan 70 % WP) was on par with propiconazole in controlling Cercospora leaf spot of cotton and

both increased the yield. Kibria and Mian (2012) evaluated the efficacy of five fungicides *viz.,* Aimcozim 200 EW (carbendazim) @ 0.10%, Emivit 50 WP (copper oxychloride) @ 0.35%, Indofil M-45 (mancozeb) @ 0.20%, Tilt 250 EC (propiconazole) @ 0.05% and Folicur 250 EC (tebuconazole) @ 0.10% against black mould of okra seed crops. The maximum reduction in disease severity and increase in plant growth, yield and yield attributes was achieved with Emivit followed by Aimcozim, Folicur and Tilt. Beura *et al.* (2013) studied eight fungicides *viz.,* carbendazim + mancozeb @ 0.2%, thiophanate methyl @ 0.15%, metalaxyl + mancozeb @ 0.2%, difenconazole @ 0.05%, hexaconazole @ 0.05%, copper hydroxide @ 0.3% and copper oxychloride @ 0.3% against Cercospora leaf spot of okra and reported that difenconazole was found to be the most effective fungicide followed by carbendazim + mancozeb. Kumar *et al.* (2015) tested 13 fungicides *in-vitro* against Cercospora leaf spot of okra, of which tebuconazole, propiconazole and carbendazim were reported to be most effective in inhibiting the fungal growth. Under field conditions, minimum disease incidence (0.17%) and maximum fruit yield of 145.16 q ha^{-1} was reported when tebuconazole was sprayed thrice.

Strobilurin fungicides act as strong inhibitors of early stages in the infection cycle (Godwin *et al.,* 1994). Time of strobilurin application plays a crucial role for successful disease management at the early stages of the disease epidemic (Bartlett *et al*., 2002; Koller *et al*., 2004). Karadimos and Karaoglanidis (2006) reported that among four strobilurins, trifloxystrobin as the most efficient against Cercospora leaf spot of sugarbeet followed by pyraclostrobin. Bdliya and Kura (2007) reported that Nativo (Trifloxystrobin+ Tebuconazole) was the most effective to control Cercospora leaf spot of groundnut.

2.7 STUDIES ON BIOCHEMICAL VARIABILITY

2.7.1 Total Phenols

Phenol accumulation was reported to be more rapid in incompatible host pathogen complex than in the compatible ones (Kiraly and Farkas, 1962). Borkar and Verma (1991) reported that the leaves of bacterial blight resistant cotton cv. 101-102B contained 69% total phenol more than the leaves of susceptible cotton cv. Acala-44. Sorghum genotypes resistant to foliar diseases were reported to possess higher content of phenols compared to susceptible ones (Kalappanavar and Hiremath, 2000).

Rani and Reddy (1998) reported that susceptible cultivars of groundnut have lower amounts of phenols compared to resistant cultivars against Cercospora leaf spot disease. Sindhan *et al.* (1999) and Garain *et al.* (2003) reported that high phenol in less susceptible greengram genotypes.

Ghosh *et al.* (2003) studied the changes in biochemical parameters in leaf spot of mulberry leaves and reported increased phenol content in infected samples over healthy samples. Jyosthna *et al.* (2004) reported increase in total phenols in thirteen groundnut cultivars resistance to *Phaeoisariopsis personata.* Total phenols were higher in resistant cultivar upon infection. Chatterjee and Ghosh (2008) reported decrease in phenols due to yellow vein mosaic disease in mesta.

Jabeen *et al.* (2009) studied two resistant and six susceptible chilli (*Capsicum annum* L.) genotypes and twelve F1 hybrids with variable degree of resistance to Fusarium wilt for phenols and phenolic enzymes, under both uninoculated and inoculated conditions at different growth stages. They found that total phenols, ortho-dihydroxy phenols and the enzyme activity to be invariably high in resistant parents and hybrids irrespective of growth stages, while, in case of susceptible parents the phenols content and enzyme activities were comparatively less. Significantly high total phenols was reported in the Okra Yellow Vein Mosaic Virus resistant wild okra and their inter-specific hybrids compared to susceptible cultivated okra cultivar (Prabu and Warade, 2009).

Kulkarni and Benagi (2013) reported increased phenols in greengram at a higher rate in resistance over susceptible genotypes due to *C. truncatum* infection. Decrease in phenols was reported in *Cymbopogon martinii* and *C. citratus* plants infected by the rust pathogen *Puccinia nakanishikii* (Tamuli *et al.,* 2013). Chavan and Suryvanshi (2014) evaluated susceptible and resistant varieties of soybean and found that total phenols on infection with *Colletotrichum truncatum* (Schw.) to be high (0.9 mg g^{-1}) in healthy leaves of resistant genotypes than susceptible (0.5 mg g^{-1}).

2.7.2 Total Proteins

Arjunan *et al.* (1976) reported changes in protein content in sorghum leaves infected by *Helminthosporium turcicum* pass. Its content in healthy and infected leaves was 0.31 and 0.39 per cent, respectively in ten day old plants and 0.24 and 0.02 per cent, respectively in sixty day old plants.

Patel and Vaishnav (1987) reported increase in protein content in groundnut leaves infected with rust as compared to healthy leaves. Kalappanavar and Hiremath (2000) reported that the multiple foliar disease resistant sorghum genotypes possessed higher content of protein compared to those of susceptible genotypes. Malhotra (2009) reported that there was marked significant increase of protein in resistant tomato plants, while it decreased significantly in susceptible cultivars in relation to Fusarium wilt.

Nandagopal (1995) reported that the wheat genotypes resistant to leaf spot caused by *Exherohilum hawaiiensis* contains lowest crude protein content than that of the susceptible genotypes. Decrease in total soluble proteins was reported in mesta plants infected with yellow vein mosaic disease (Chatterjee and Ghosh, 2008), *C. martinii* and *C. citratus* infected by leaf rust pathogen *P. nakanishikii* (Tamuli *et al.*, 2013).

Mary and Subramanian (2014) reported biochemical and enzymatic response of *Cajanus cajan* plant towards biotic stress induced by *Fusarium oxysporum udum.* Due to infection total protein was found to be comparatively higher in resistant cultivar than susceptible cultivar.

2.7.3 Total Sugars

Ramdayal and Joshi (1968) studied the post infection changes in *H. sativum* infected barley leaves and reported decrease in reducing, non reducing and total sugars than the healthy leaves and similar reports were given by Sindhan and Jaglan (1987) and Sindhan *et al.* (1987) in *Cercospora* resistant groundnut cultivars as compared to the susceptible one.

Subramanyam *et al.* (1990) studied the influence of inoculation of *Dreschslera hawaiiensies* (Bugnicourt) on biochemical parameters of wheat leaves. They found higher total and reducing sugar in resistant cultivars than in susceptible ones and further they observed that both the sugar content decreased after the disease development in susceptible and resistant cultivars. Total sugar content of resistant cultivar of maize to turcicum blight was higher than the susceptible variety but it was reverse in case of reducing sugar. Due to infection there was sudden decrease in total sugar as well as reducing sugar in susceptible variety (Sharma *et al.*, 1992).

Suryawanshi *et al.* (1993), also reported higher amounts of sugars (reducing and total) in *P. personata* susceptible groundnut. Tanmai (1997) found increased levels of sugars with increased severity of late leaf spot of groundnut. Ghosh *et al*. (2003) reported decrease in total sugar, reducing sugar, non-reducing sugar, in leaf spot infected resistant mulberry samples, Prakasha (2009) in *BYVMV* resistant Arka Anamika genotype, Sunil *et al.* (2009) in Cercospora leaf spot resistant and moderately resistant greengram genotypes, Waghmare *et al.* (2012) in *Alternaria alternata* leaf spot of rose genotypes, Kulkarni and Benagi (2013) in *C. truncatum* infected resistant greengram than susceptible or healthy samples. Tamuli *et al.* (2013) reported decrease in sugars in *C. martinii* and *C. citratus* samples due to leaf rust caused by *P. nakanishikii*. Chavan and Suryvanshi (2014) reported low amount of carbohydrates in resistant soybean genotypes that imparts resistance against *C. truncatum.*

Younes and Elyousr (2014) screened some okra genotypes to powdery mildew resistance and yield under Agro- climatic conditions of Assiut, Egypt. They reported that disease severity was positively correlated with yield, total sugars and peroxidase activity, but negatively correlated with phenol contents.

2.7.4 Total Chlorophyll

Jyosthna *et al.* (2004) studied chlorophyll content in thirteen groundnut cultivars resistance to *P. personata.* They reported that total chlorophyll was high in resistant cultivar while it reduced upon infection in all cultivars. Chatterjee and Ghosh (2008) observed decrease in chlorophyll content in mesta plants infected with yellow vein mosaic disease.

Ghose *et al.* (2010) studied the changes of photosynthetic pigments in mulberry leaf infected with leaf blight pathogen. They observed drastic reduction of total chlorophyll, chlorophyll a, chlorophyll b in blight infected leaves. The pigment content decreased with the increasing pathogenesis by *Phyllostica* sp.

Kulkarni and Benagi (2013) studied changes in chlorophyll, in resistant and susceptible genotypes of greengram. The chlorophyll was found to decrease due to the infection of *C. truncatum* and the rate of decrease was more in susceptible genotypes than resistant genotypes.

Mali and Rakesh (2013) reported increase in chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll contents when sprayed with different chemicals and botanicals constituting dimethoate 30% EC (1 ml/l), imidacloprid 17.8% SL (1 ml/3 L), azadirachtin 1500 ppm (5 ml/l) and karanj oil (2%). When sprayed alone or in combination to control *Yellow Vein Mosaic Virus* (*YVMV*) in infected okra.

Tamuli *et al.* (2013) reported a decrease in chlorophyll of *C. martinii* and *C. citratus* when infected by the fungal rust pathogen *P. nakanishikii.*

Chavan and Suryvanshi (2014) chlorophyll a, b and total chlorophyll content was reported to reduce drastically over healthy cultivars, but at higher rate in susceptible than in resistant variety of soybean when infected with *C. truncatum.*

Kumar *et al.* (2017) studied morphological and biochemical parameters in leaves of *YVMV* resistant and susceptible lines of okra and observed that phenol and total chlorophyll contents were positively correlated to each other while total soluble sugar content was negatively correlated with phenol and total chlorophyll contents.

Chapter III

MATERIAL AND METHODS

The present investigations on "Phylogenetic variation and fungicidal management of *Cercospora abelmoschi* Ell. and Ev. infecting okra" was carried out during *kharif* 2016-2017 in the Agriculture College Farm, Bapatla and Advanced PG Center, Lam, Guntur. The material used and methods followed are described in this chapter.

3.1 MATERIALS

3.1.1 Glassware

The glassware used in the present investigation was of Borosil and Corning make. Conical flasks, test tubes, pipettes, measuring cylinders, beakers were used in the present study. Axygen make micropipettes and microtips were used.

3.1.2 Cleaning of Glassware

Glassware was washed first with detergent powder and then washed under tap water. Later they were kept overnight in cleaning solution (potassium dichromate 75 g, concentrated sulphuric acid 500 ml and water 1000 ml) and rinsed with tap water followed by distilled water.

3.1.3 Sterilization

All the glassware used in the present investigation was sterilized in a hot air oven at 160^0C for two h. All the buffers for DNA isolation were sterilized in an autoclave at 15 psi for 15 min at 121^0C.

3.1.4 Chemicals and Water

Analytical reagent grade chemicals of M/s Himedia and distilled water were used in all the laboratory experiments in the present study.

3.2 PHYLOGENETIC VARIATION OF CERCOSPORA ABELMOSCHII

3.2.1 Collection of Diseased Samples

Selection of suitable diseased samples is a prerequisite for successful study on phylogenetic variation. Diseased leaf samples were collected from the okra growing villages of Guntur district in Andhra Pradesh during *kharif* 2016. Samples were designated based on the place of collection. Two samples were collected from two different fields in Bapatla, one from Nandirajuthotha, three samples from different fields of Dhundivaripalem and one each from Yazali and Thimmareddipalem.

The collected leaf samples were screened for the diseased portion, required amount of leaf was weighed, properly labeled and packed in polythene bags and were stored at -40^{0}C temperature for *in-Planta* isolation of fungal DNA.

3.2.2 *In- Planta* Fungal DNA Isolation

The DNA from *Cercospora* infected okra leaf samples were isolated by CTAB method (cetyltrimethyl ammonium bromide) as described by Murray and Thompson (1980) with modifications. Frozen plant material (1 g) kept at - 40^{0}C was ground in a pre-sterilized chilled pestle and mortar until a fine powder was obtained and the homogenate was transferred to sterile 30 ml polypropylene tube. To this, 10 ml of pre-heated (65^0 C) extraction buffer (1 M Tris (pH 8.0), 5 M NaCl, 0.5 M EDTA (pH 8.0), 2% CTAB, 1% PVP, 0.1 % mercaptoethanol) and 5 µl of proteinase- k, 4% (W/V) PEG were added and incubated for an hour at 65^{0}C. To this equal volume of chloroform and isoamyl alcohol (24:1) was added and tubes were centrifuged at 10,000 rpm for 10 min at room temperature (24^{0}C). The upper aqueous phase was carefully transferred to a sterile polypropylene tube and the DNA was precipitated with 0.6 volume of ice cold isopropanol and 0.1 volume of 3 M sodium acetate (pH 4.8) mixed gently to precipitate the nucleic acid and kept at -20^{0}C for an hour. The DNA was precipitated by centrifugation at 10,000 rpm for 15 min at 4^{0}C. Supernatant was discarded and DNA pellet was washed with 70% ethanol and centrifuged at 10,000 rpm for 10 min at 4^{0}C. Pellet was air dried for 15 min at room temperature and was dissolved in 200 µl of molecular grade water. The DNA was stored at -20^{0}C for further use. the DNA was checked on a 1.2% agarose gel in 1X TAE [40 mM Tris-acetate, 1 mM EDTA (pH 8.0)] buffer.

3.2.3 DNA Quantification

Quantification of DNA was done by visualizing 5 µl DNA on 1.2 % Agarose gel in 1X TAE buffer containing ethidium bromide (10 mg ml^{-1}). The quantity and concentration of the extracted DNA was checked by measuring the absorbance on Nano-Drop ND-1000 spectrophotometer (Nanodrop Technologist) at 260 and 280 nm. Quantification was analyzed by absorbance ratios *i.e.,* 260/280 nm.

3.2.4 PCR Amplification

In-planta isolation of *C. abelmoschi* DNA collected from different villages of Guntur district was based on the amplification of Internal Transcribed Spacer (ITS) region using universal primers previously described by White *et al.* (1990). Forward and reverse primers, *viz.,* ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS 4 (5' TCCTCCGCTTATTGATATGC 3') respectively, synthesized based on conserved 18S and 28S coding regions of the nuclear rDNA were used. Amplification was carried out with 25 µl reaction mixture containing 2.5 µl of 10X PCR buffer, 0.5 µl 10 mM dNTPs, 1 µl of each primer, 1.5 µl of 25 mM $MgCl_2$, 0.5 units of Taq polymerase**,** 15 µl of water and 3µl of template DNA. Amplification was performed in 0.2 ml thin walled PCR tubes using a thermocycler (Biorad) programmed for initial denaturation at 94^0C for 5 min, followed by 35 cycles of denaturation at 94^0C for 30 sec, annealing at 56.9^0C for 1 min, primer extension at 72^0C for 1.5 min and a final extension at 72^0C for 7 min and hold at 4^0C.

3.2.5 Analysis of PCR Products by Agarose Gel Electrophoresis

Amplified product was analysed by agarose gel electrophoresis as described by Sambrook and Russell (2001). Agarose gel of 1% (w/v) was prepared by dissolving 0.5 g of agarose in 50 ml of 1 X TAE buffer. At lukewarm temperature, 2.5µl of ethidium bromide (10 mg ml^{-1}) was added and poured into gel casting tray of mini horizontal electrophoresis unit (Genaxy, India). The DNA samples were loaded after mixing with loading dye and the electrophoresis was carried in 1 X TAE buffer at 60 V till the dye front reached the lower part of the agarose gel. The migration pattern of the DNA fragments in the gel was recorded using gel documentation system (Biorad, USA) in an auto exposure mode.

3.2.6 Restriction Enzyme Analysis of ITS regions

Polymorphism was determined by digesting the amplicon obtained using ITS primers with three different restriction endonucleases, *i.e.*, hexa basepair cutters - *Eco*RI, *Bam*HI and tetra basepair cutter - *Taq*I. The restriction fragments were size separated by electrophoresis on 2.0% agarose gel and were viewed under UV light and phylogenetic analysis was done using the Dendro-UPGMA (Unweighted Pair Group Method with Arithmetic mean) (Garcia-Vallve *et al.*, 1999) software.

Restriction bands were analysed, wherein each band with a different electrophoretic mobility was assigned a position number and based on the presence or absence of the band it was named as binary digits 1 or 0. Only reproducible bands were considered for analysis. Bands common to all isolates were incorporated into the analysis. Based on the similarity coefficients, a dendrogram was constructed by the unweighted pair group method with arithmetic mean (UPGMA) hierarchical clustering algorithm.

3.3 MANAGEMENT OF CERCOSPORA LEAF SPOT OF OKRA

Field experiment was conducted during *kharif* 2016-17 at the Agriculture College Farm, Bapatla, Guntur district, Andhra Pradesh situated at an altitude of 5 m above mean sea level and seven km away from the coast of Bay of Bengal. Geographically it is located at 80^0 30´E longitude and 15^0 54´N latitude.

3.3.1 Design and Layout

The experiment was laid out in Randomized Block Design (RBD) with seven treatments and four replications (Plate 3.1). The plan of layout of the experiment is as shown in (Fig. 3.1).

3.3.2 Date of Sowing

Kharif : 01-08-2016

3.3.3 Spacing and Plot Size

Plot size: 5.4 x 4.2 m	No. of rows: 9	No. of plants/ row: 14
Spacing: 60 x 30 cm	Net plot: 4.2 x 3.0 m	No. of plants/ row: 10

3.3.4 Variety

A susceptible F_1 hybrid, Sahiba was used in this experiment.

3.3.5 Land preparation

The land was prepared by thorough ploughing and harrowing and soil was brought to a fine tilth.

3.3.6 Fertilizer application

48:24:24 N: P_2O_5: K_2O kg ac^{-1} were applied to the crop. Entire P_2O_5 and K_2O were applied as basal and 48 kg N was applied in three splits at 30, 60 and 90 days after sowing.

3.3.7 Weed management

Pendimethalin @ 2 l $acre^{-1}$ was sprayed as pre-emergence application.

3.3.8 Intercultivation

Weeding was carried out regularly during cropping season.

3.3.9 Harvesting

Medium sized (7-10 cm long) tender pods, which can be easily snapped from the plant were harvested and pickings were carried out once in 3-4 days. Net plot yield was taken to avoid boarder effect.

3.3.10 Treatments

Commonly used fungicides and new fungicides available in the market were tested for the management of Cercospora leaf spot disease of okra. The treatments are as mentioned in Table 3.1.

Table 3.1. Fungicides used for managing Cercospora leaf spot of okra

Treatments	Common Name	Trade Name	Formulation	Recommended Conc. (%)
T1	Mancozeb	Dithane M-45	75% WP	0.25%
T2	Thiophanate methyl	Control	70% WP	0.1%
T3	Trifloxystrobin (25%) + Tebuconazole (50%)	Nativo	75 WG	0.1%
T4	Pyraclostrobin	Headline	20%WG	0.1%
T5	Hexaconazole	Contaf	5% SC	0.2%
T6	Propiconazole	Tilt	25% EC	0.1%
T7	Control (Water spray)			

Two foliar sprays were scheduled, where initial spray was at immediate initiation of the disease and second at fifteen days after first spray. Data on yield and per cent disease severity were recorded.

3.3.11 Collection of Experimental Data from Experimental Field

Cercospora leaf spot disease severity was recorded at weekly intervals from each micro plot and disease was scored from initial appearance of symptoms till two weeks after second spray schedule, adopting modified scale of Mayee and Datar (1986) as mentioned in Table 3.2 and Plate 3.2.

Table 3.2. Modified disease rating scale for Cercospora leaf spot of okra (Mayee and Datar,1986)

Scale	Leaf area covered with the spots
0	No symptoms on leaf.
1	Small, round to irregular fungal spots covering up to 1% or less of leaf area.
3	Fungal spots covering >1% - 10 % of the leaf area.
5	Fungal spots covering 11-25% of the leaf area.
7	Fungal spots covering 26-50% of the leaf area. Moderate defoliation occurs.
9	Fungal spots expanding and covering 51% or more of leaf area. Heavy shedding of leaves.

The per cent disease index (PDI) was computed from above (0-9) scale by using the following formula (Wheeler, 1969).

$$PDI = \frac{\text{Sum of all the numerical ratings}}{\text{Number of observations} \times \text{Maximum disease grade}} \times 100$$

3.4 BIOCHEMICAL ANALYSIS

Healthy and diseased leaf samples were collected from different plots two weeks after complete execution of treatments. The collected leaf samples were properly labeled and packed in polyethylene bags for biochemical analysis .

3.4.1 Estimation of Total Phenols

3.4.1.1 Materials:

1. 80% Ethanol
2. Folin- Ciocalteau Reagent
3. 20% Na_2CO_3
4. Standard (100 mg Catechol in 100 ml water)

Diluted 10 times for a working standard.

3.4.1.2 Procedure: One gram of okra leaf sample was ground with a pestle and mortar in 10 ml of 80% ethanol and the homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was saved and re-extracted. The residue was re-extracted five times the volume of 80% ethanol, centrifuged and the supernatants were pooled and evaporated to dryness. The residue was dissolved in a known volume of distilled water (5 ml).

Different aliquots (0.2 to 2 ml) were pipetted into tubes and the volume in each tube was made up to 3 ml with water. 0.5 ml of Folin- Ciocalteau reagent was added to each tube and after 3 min, 2 ml of 20% Na_2CO_3 was added. The contents were mixed thoroughly, the tubes were placed in boiling water for exactly one min, cooled and absorbance at 650 nm was measured against a reagent blank. A standard curve was prepared using different concentrations of catechol.

3.4.1.3 Calculation: Using the standard curve the concentration of phenols in the test sample were expressed as mg phenols per g plant sample on fresh weight basis (Mallick and Singh, 1980).

3.4.2 Estimation of Total Proteins

Total Protein estimation was carried out according to the procedure described by the Lowry *et al.* (1951).

3.4.2 .1 Reagents:

1. 2.0% sodium carbonate in 0.1 N NaOH..... (A)
2. 0.5% copper sulphate in 1% potassium sodium tartrate.....(B)
3. Alkaline copper sulphate solution: 50 ml of (A) and 1 ml of (B) were mixed prior to use ..... (C)
4. Folin- Ciocalteau reagent..... (D)
5. Protein solution (stock standard): Exactly 50 mg of bovine serum albumin was weighed accurately and dissolved in distilled water to make up the volume to 50 ml in standard flask. 10 ml of the stock solution was diluted to 50 ml with distilled water in a standard flask so that one ml of this solution contains 200 µg of protein.
6. Extraction was carried out with 0.1 M phosphate buffer.

3.4.2.2 Extraction procedure: 500 mg of okra leaf tissue of healthy and diseased from lower and upper leaves was taken and ground well using pestle and mortar in 5-10 ml of buffer, centrifuged and the supernatant was used for protein estimation.

3.4.2.3 Procedure for estimation: 0.2, 0.4, 0.6, 0.8, and 1 ml of the working standards were pipetted into a series of test tubes and 0.2 ml of the sample extract was pipetted in to other test tube. Then the volume was made up to 1 ml in all the test tubes and a tube with 1 ml of distilled water served as blank. Five ml of reagent (C) was added to each test tube including the blank, mixed well and was allowed to stand for 10 min. Then, 0.5 ml of reagent (D) was added. After thorough mixing, reaction mixture was incubated at room temperature in dark for 30 min. After the development of blue colour the readings were taken at 660 nm. Standard graph was drawn and the amount of protein in the sample was calculated from it and expressed as mg per g of sample.

3.4.3 Estimation of Total Sugars

Total sugars were estimated following anthrone method (Gerhardt *et al.*, 1994).

3.4.3.1 Reagents: Anthrone reagent : 2 g of anthrone dissolved in 1 l of concentrated H_2SO_4.

3.4.3.2 Extraction procedure: 100 mg of the sample of healthy and diseased from both lower and upper leaves was weighed and extracted for sugars with hot 80% ethanol and the extraction was done twice (5 ml each time). The supernatant was collected and evaporated in a water bath at 80^0C. 10 ml of water was added to dissolve the sugars.

3.4.3.3 Methodology: Aliquots of 1 ml of the extract was pipetted into test tubes. To each tube, 4 ml of anthrone reagent was added and the reagent was allowed to run down through the wall of the test tube. Glass marble was placed on top of each tube to prevent the loss of water by evaporation. Tubes were placed in a boiling water bath for 10 min. The tubes were removed and cooled to room temperature in a water bath. A reagent blank was treated similarly. The absorbance of the blue-green solution was measured at 630 nm. The amount of sugars present in the extract was calculated using a standard curve prepared from glucose.

3.4.4 Chlorophyll Estimation

Total chlorophyll was estimated following the method given by Arnon, 1949.

3.4.4 .1 Materials: Analytical grade acetone was diluted to 80% acetone.

3.4.4.2 Procedure: The leaves of healthy and diseased samples from upper and lower leaves were collected, washed with distilled water and blot dried. One gram of leaf sample was weighed exactly into clean mortor and was ground to a fine pulp by adding 20 ml of 80% acetone. After centrifugation at 5000 rpm for 5 min the supernatant transferred to 100 ml volumetric flask. The residue was further ground with 20 ml of 80% acetone, centrifuged and supernatant was transferred to the same volumetric flask. The procedure was repeated until the residue was colourless. Finally the mortor and pestle were washed thoroughly with 80% acetone and clear washings

were collected into the volumetric flask. Volume was made up to 100 ml with 80% acetone and absorbance at 645, 663and 652 nm against the solvent (80% acetone) blank were measured.

3.4.4.3 Calculation: The amount of chlorophyll a, chlorophyll b and total chlorophyll was estimated using the following formulae.

Chlorophyll a (mg g^{-1} tissue) = [12.7 (A663) - 2.69 (A645)] × V/ 1000 × W

Chlorophyll b (mg g^{-1} tissue) = [22.9 (A645) - 4.68 (A663)] × V/ 1000 × W

Total Chlorophyll (mg g^{-1} tissue) = [20.2 (A645) +8.02 (A663)]x V/1000 x W

Where:

A = Optical density at respective wave length (nm)

V = Final volume of chlorophyll extract in 80% acetone

W = Fresh weight of the tissue extracted

3.5 STATISTICAL ANALYSIS

The data obtained from all the experiments were statistically analyzed following the standard procedures (Gomez and Gomez, 1984).

Chapter IV

RESULTS AND DISCUSSION

Results obtained in the study on phylogenetic variation, biochemical variability and its management are presented and discussed in this chapter.

4.1. DISEASE SYMPTOMATOLOGY

The typical okra leaf spot symptoms were observed on lower leaves. Symptoms were characterized by the presence of sooty to dark olivaceous colour, indistinct or none, fruiting effuse, forming angular vein limited areas in dense patches of conidiophores and conidia on the lower surface of leaves. Initially spot size ranged from 0.1 to 0.2 cm (Plate 4.1) which later coaleased to form spots of 0.6 to 1.0 cm. Under severe infection, symptoms were observed on upper surface of the leaf. Similar symptom description was provided by Ellis and Everhart (1893) and Deighton (1976).

4.2. VARIABILITY STUDIES

4.2.1 Collection of Isolates

Cercospora infected leaves were collected during *kharif* 2016 from eight different okra growing villages in Guntur district and were designated as described in the Table 4.1. The samples were screened for the diseased portion, required amount of leaf was weighed, properly labeled, packed in polythene bags and stored at -40^0C temperature for further investigations.

As the pathogen survives in infected leaves as stromata, its conidia and conidiophores were easily identified using light microscope. The leaf samples were selected for DNA isolation. DNA was isolated by modified CTAB method (Murray and Thompson, 1980). The concentration of DNA was determined using the Nano-Drop ND-1000 spectrophotometer (Nano Drop Technologist). *In-planta* expression of pathogenic DNA was tested as the technique avoids the usage of liquid nitrogen, its simplicity, low cost, fast and safe protocol.

Table 4.1. Okra samples collected from different locations of Guntur district

S. No.	Place of collection	Sample designation
1	Bapatla	Bpt1
2	Bapatla	Bpt2
3	Nandirajuthotha	Nt
4	Dhundivaripalem	Dp1
5	Dhundivaripalem	Dp2
6	Dhundivaripalem	Dp3
7	Yazali	Yz
8	Thimmareddipalem	Tp

4.2.2. PCR Amplification

Molecular identification of *Cercospora abelmoschi* was performed using ITS 1 and ITS 4 primers, previously designed by White *et al*. (1990) to amplify the internal transcribed spacer (ITS) regions located between the 18 S rRNA and 28 S rRNA genes (Joshi *et al.,* 2006).

The DNA extracted (Plate 4.2) from *Cercospora* infected leaf samples were amplified and amplicon of 550 bp in all the samples (Plate 4.3) confirmed that the quality of DNA extracted using the protocol was suitable for the purpose. No size variation was found among the amplified ITS regions.

The two hexa cutter restriction endonucleases EcoRI and BamHI tested, showed restriction sites in the ITS region and revealed polymorphism in only one isolate collected from Yazali.

Seven isolates tested with EcoRI enzyme produced three digested products at 550, 450 and 200 bp products (Plate 4.4). BamHI also gave similar variation in the restriction site with Yazali isolate where only one digested product at 450 bp was observed as against two in other isolates (Plate 4.5).

Based on the dendrogram construction utility software DendroUPGMA, the similarity coefficients were transformed into distances and clustering was done using the Unweighted Pair Group Method with Arthmetic mean (Garcia-Vallve *et al.,* 1999). The dendrogram constructed indicated that the Yazali isolate differed in restriction digestion. PCR with primers produced a DNA fragment of approximately 550 bp for the eight isolates tested. No size variation was found among the amplified ITS regions. With the two hexa base pair restriction enzymes used, seventh isolate collected from Yazali has clustered separately due to difference in restriction sites. EcoRI restriction resulted in three fragments in all seven isolates except the isolate collected from Yazali (Fig. 4.1). Restriction with BamHI exhibited single restriction site in all seven isolates except in isolate from Yazali which differed in length polymorphism (Fig. 4.2).

Restriction with tetra base pair cutter Taq1 indicated a high degree of genetic diversity among the isolates of okra collected from different geographical locations in the study which was represented by the differences in banding pattern. Based on similarity coefficients, two major clusters were formed which further divided into four groups (Fig. 4.3). Bapatla 1 and Dhundivaripalem 3 isolates had similar banding patterns with six restriction sites that were clustered into a group. The isolates Nandirajuthota, Dhundivaripalem 1 and Dhundivaripalem 2 did not have any restriction sites for tetra cutter Taq1, thus were grouped together. Isolate Bapatla 2 had only one restriction site and was separately grouped where as the isolates Yazali, Thimmareddipalem had three restriction sites and were in a group (Plate 4.6).

The present results agree with the similar study conducted on ectomycorrhizal fungi in Fennoscandia that showed intra specific polymorphism in seven species. The polymorphism was reported due to length mutations, ranging from 5 to 15 bp in four of the seven polymorphic species (Karen *et al.,* 1997).There are reports on host- specific specialization (*formae speciales*) of *C. canescens* from *V. mungo* (Kaushal *et al.,*1993) and *V. radiata* (Chand *et al.,* 2000). Genetic heterogeneity previously has been observed for other fungi like *Ascochyta rabiei* (Morjane *et al.,* 1994) and *Rhynchosporium secalis* (Dermott *et al.,* 1989). In case of *A. rabiei,* population sampled from a single chickpea field contained a large amount of subtle genetic variation, with more than one *A. rabiei* haplotype being present on single host plant even with in single lesion.

In the present study, it is confirmed that variability existed in *Cercospora* isolates collected from Guntur district. The different banding patterns with hexa and tetra cutters revealed that polymorphism existed within the isolates which may be due to variations in single nucleotide resulting in variation in restriction sites.

4.3. MANAGEMENT OF CERCOSPORA LEAF SPOT OF OKRA WITH FUNGICIDES

Management of Cercospora leaf spot disease of okra was studied under field conditions during *kharif* 2016.

4.3.1. Field Studies

Cercospora leaf spot in okra was chemically managed by spraying chemicals twice first at first appearence of disease symptoms and later fifteen days after first spray.

4.3.2. Effect of Fungicides on Cercospora Leaf spot Disease Severity

Significant difference was observed in cercospora leaf spot symptom development with respect to leaf position. Lower leaves were found with severe infection (45.27%) over upper leaves (7.36%). Prior to spray schedule, there was no significant difference in disease severity either on lower or upper leaves scored indicating equal threshold of inoculum (Table 4.2).

A week after first spray, significant difference in Cercospora leaf spot disease severity was observed in lower and upper leaves where lower leaves had 75.55% PDI as against 19.15% in upper leaves. A combination fungicide (trifloxystrobin + tebuconazole) @ 0.1% was found with significantly low disease severity (23.75%) compared to other fungicides. All fungicides tested were significantly effective in managing the disease over control (water spray) (68.69%). Among the interaction between position of leaves and fungicide treatments, trifloxystrobin+ tebuconazole @ 0.1% was found significant in controlling disease in upper leaves (10.83%) as against control (90.00%) and other fungicides tested. Thiophanate methyl @ 0.1% was found to be least effective with 83.89% PDI on lower leaves which was on a par with mancozeb @ 0.25% (81.95%), pyraclostrobin @0.1% (81.67%) and hexaconazole @

0.2% (79.17%). Greater control was obtained on upper leaves by spraying fungicides in comparison to unsprayed control (47.38% PDI). Among all the fungicides, trifloxystrobin + tebuconazole @ 0.1% treatment resulted in lowest PDI (10.83%) on upper leaves followed by thiophanate methyl (14.72%), pyraclostrobin (14.72%) and propiconazole (15.28%) which were on a par (Table 4.3).

Even after two weeks of first spray there was significant difference in disease severity between lower (76.75%) and upper leaves (50.89%). Significantly low mean disease severity (24.58%) was observed in trifloxystrobin+ tebuconazole @ 0.1% sprayed plants compared to other fungicides tested. All other test fungicides were equally effective over control (83.67%) and on a par with each other. Interaction effect between leaf position and fungicide treatments indicated that the combination product trifloxystrobin + tebuconazole @ 0.1% was significantly superior with only 35.56% PDI on lower leaves and 13.61% PDI on upper leaves compared to all other fungicides and the unsprayed control (93.33% PDI on lower leaves and 74.00% PDI on upper leaves) (Plate 4.7, Table 4.4).

Significant difference in disease severity between lower (72.74%) and upper leaves (57.32%) was observed one week after second spray. All fungicides tested were significantly effective in controlling the disease over control (88.40%). Among fungicide treatments, trifloxystrobin+ tebuconazole @ 0.1% continued to be highly significant in reducing the disease (24.05%) over other fungicides (59.17 to 73.87%). Interaction effect revealed continued superiority of trifloxystrobin + tebuconazole @ 0.1% containing the disease significantly low compared to other fungicides and unsprayed control irrespective of the leaf position. With trifloxystrobin + tebuconazole @ 0.1%, PDI on lower leaves was 31.39% while on upper leaves 16.70%. In unsprayed control, the PDI on lower leaves was 94.45% and on upper leaves 82.35% (Table 4.5).

The protection due to trifloxystrobin+ tebuconazole @ 0.1% continued even after two weeks of second spray (Table 4.6). Mean PDI in trifloxystrobin + tebuconazole @ 0.1% sprayed plants was only 24.03% followed by propiconazole @ 0.1% (50.66%) which differed significantly between them and with unsprayed control (92.09%).

Over all the fungicides tested, mean PDI was higher in lower leaves (71.95%) compared to upper leaves (55.02%) (Fig. 4.4; Fig. 4.5). Combination fungicide trifloxystrobin + tebuconazole @ 0.1% was superior to all other fungicides and only water spray because on lower leaves PDI was only 34.11% and on upper leaves PDI was 16.38% which differed significantly with other test fungicides and water control.

Beura *et al.* (2007) studied the efficacy of seven different fungicides *viz.*, carbendazim 0.15%, copper oxychloride 0.3%, mancozeb 0.3%, propineb 0.25%, copper hydroxide 0.3%, thiophanate methyl 0.1% and ziram 0.25% against Cercospora leaf spot of okra and found carbendazim with the lowest disease incidence. Beura *et al.* (2013) studied eight fungicides *viz.*, carbendazim + mancozeb @ 0.2%, thiophanate methyl @ 0.15%, metalaxyl + mancozeb @ 0.2%, difenconazole @ 0.05%, hexaconazole @ 0.05%, copper hydroxide @0.3% and copper oxychloride @0.3% against Cercospora leaf spot of okra and reported that difenconazole was found to be the most effective fungicide followed by carbendazim + mancozeb. Kumar *et al.* (2015) tested 13 fungicides *in-vitro* against Cercospora leaf spot of okra, of which tebuconazole, propiconazole and carbendazim were reported to be most effective in inhibiting the fungal growth. Under field conditions maximum seed germination (98.33%), minimum disease incidence (0.17%) and maximum fruit yield of 145.16 q ha^{-1} was reported when tebuconazole was sprayed thrice.

Khunti *et al.* (2002) found that hexaconazole to be more effective while penconazole was statistically on par with hexaconazole and sulphur while propiconazole and carbendazim were moderately effective in reducing Cercospora leaf spot of greengram. Hundekar *et al.* (2005) reported that hexaconazole (0.1%) or propiconazole (0.1%) or carbendazim (0.05%) spray was found to be better in managing the frog-eye leaf spot of bidi tobacco in Karnataka. Karadimos and Karaoglanidis (2006) reported that among four strobilurins, trifloxystrobin to be the most efficient against Cercospora leaf spot of sugarbeet followed by pyraclostrobin. Bdliya and Kura (2007) reported that Nativo (trifloxystrobin + tebuconazole) was the most effective to control Cercospora leaf spot of groundnut. Bhattiprolu (2010) reported that Taqat (hexaconazole 5% + captan 70% WP) was on par with propiconazole in controlling Cercospora leaf spot of cotton that increased the yield.

The present results corroborate with earlier results when tested with the strobilurin, triazole and other groups of fungicide molecules. Fungicides were applied immediately after the first appearance of the symptoms. It was suggested that time of strobilurin application plays a crucial role in successful disease management as they provide the maximum protection when applied prior to infection or at the early stages of the disease epidemic (Bartlett *et al.*, 2002; Koller *et al.*, 2003). It was well established that strobilurin fungicides act as strong inhibitors in early stages of the infection cycle including spore germination and penetration (Godwin *et al.*, 1994).

Traditional protective fungicides such as maneb or chlorothalonil were reported against Cercospora leaf spot worldwide that adequately controls the disease, early in the season when inoculum pressure is low. However, they fail later in the season when inoculum pressure increases (Meriggi *et al.*, 2000). The results in the present studies indicated similar response of the pathogen to mancozeb and thiophanate methyl.

Fungicide Resistance Action Committee (FRAC) guidelines suggests that strobilurin fungicides should not constitute more than 30 to 50%of the total number of fungicide spray applications made to the crop during one season as they result in resistance development (Bartlett *et al.*, 2002). Therefore, reduced number of strobilurin fungicide treatments is a pre requisite for delaying the development of resistance to this fungicide class, Consequently, the strobilurin fungicides provide the maximum of their activity prior to infection or at the early stages of the disease epidemic. It was reported that post-infection applications of strobilurin fungicides increase the risk for resistance development because they may allow the growth of isolates with sensitivity lower than the average and, consequently, accelerate their speed of selection (Koller *et al*., 2003). The present results depicted that when leaf position and fungicide treatments were compared, the combination fungicide trifloxystrobin and tebuconazole was found to be superior over other fungicides. Therefore, use of combination of trifloxystrobin and tebuconazole also satisfies the guidelines suggested by FRAC where in the amount of strobilurin is present in reduced but at effective concentration.

4.3.3. Effect of Fungicides on Fruit Yield (q ha^{-1}) and B: C Ratio

Fruit yield (q ha^{-1}) in all fungicide treated plants was significantly higher (89.92 to 101.75 q ha^{-1}) over control (87.86 q ha^{-1}). Significantly the highest yield was recorded in trifloxystrobin + tebuconazole @ 0.1% treated plots (101.75 q ha^{-1}) and was followed by propiconazole @ 0.1% (97.86q ha^{-1}) which was on a par with hexaconazole @ 0.2% (97.22 q ha^{-1}) and pyraclostrobin (96.75 q ha^{-1}) (Table 4.7).

Though trifloxystrobin + tebuconazole @ 0.1% was found to be efficient in controlling Cercospora leaf spot pathogen but B: C ratio was found low (3.45) when compared to propiconazole @ 0.1% treatment(3.62). Lower B: C ratio with trifloxystrobin + tebuconazole @ 0.1% compared to propiconazole @ 0.1% is attributed to its high cost. Incremental B: C ratio was found to be high in trifloxystrobin + tebuconazole @ 0.1% (0.607) treated plots. If the fungicide cost is decreased, this fungicide would be a very useful fungicide in managing Cercospora leaf spot of okra.

Currently, control of Cercospora leaf spot worldwide was reported to be based on fungicides that are applied preventively. Incorporation of some of the strobilurin combination fungicides especially as a preventive spray or during early stages of infection would reduce the cost of cultivation. Spray schedules with trifloxystrobin + tebuconazole would be a valuable tool in order to achieve both satisfactory control of the disease and can delay the evolution of resistance. The results presented in this study provide important information related to the efficacy of interaction of mixture of strobilurin fungicides with triazole, other fungicides or as a protectant fungicide alone.

4.4. STUDIES ON BIOCHEMICAL CHANGES IN CERCOSPORA INFECTED OKRA LEAVES

4.4.1. Total Phenols Content (mg g^{-1})

Variation in phenol content was observed to certain extent in infected leaves with respect to their position. In lower leaves phenol content ranged from 0.98 to 1.58 mg g^{-1}while in upper leaves, it varied between1.25 and 1.52 mg g^{-1} (Table 4.8, Fig. 4.6).

Phenol content in lower leaves was found significantly high (1.58 mg g^{-1}) over other treatments when infected leaves were sprayed with a combination fungicide trifloxystrobin + tebuconazole @ 0.1%. Combination fungicide treated upper leaves (1.52 mg g^{-1}) and propiconazole sprayed upper leaves were on a par (1.48 mg g^{-1}) with phenol content. However, in upper leaves the amount of phenols were low compared to lower leaves probably due to the low inoculum pressure in trifloxystrobin + tebuconazole sprayed plants.

Nearly, three fold high per cent increase over control in phenols was observed in lower leaves (61.23%) over upper leaves(21.60%) in trifloxystrobin + tebuconazole fungicide treated plants. In the same treatment a maximum of 97.50 % increase in phenols was observed over healthy in lower leaves and 87.65% in upper leaves.

Newton and Anderson (1929) observed that rust resistance in wheat plants was due to the release of phenolics that inhibited the growth of the pathogen in the host cells. Tomiyama (1963) reported that the accumulation of phenolics in diseased plants was a common phenomenon observed in many host pathogen interactions. The increase in phenolics concentration might arise from the release of phenol from their glucosides by the enzyme glucosidase of their host or pathogen (Pridham, 1965; Sharma

*et al.,*1983). It was stated that the higher amount of phenolic compounds in diseased leaves may be due to either enhancement of synthesis or translocation of phenolics to the site of infection which helped in arresting the spread of the pathogen (Sharma *et al.,*1983). Therefore the early accumulation of phenolic compounds at the infection site will trigger defense responses thereby slowing down the pathogen development through rapid cell death (Fernandez and Heath, 1989). The accumulation was reported to be more common in resistant varieties while in susceptible varieties, as the symptoms develop, phenols decrease (Thimmaiah, 1999). The present study corroborate with the earlier findings and it was found that in addition to phenol accumulation there was an additive impact of fungicides that reduced the amount of inoculum thus reducing the disease severity.

A significant negative correlation (-0.903) existed between total phenols and disease severity (Table 4.12). Similar report was given by Younes and Elyousr (2014) where disease severity was negatively correlated with phenol contents in okra powdery mildew resistant genotypes.

4.4.2. Total Protein Content (mg g^{-1})

There was no significant difference in total protein content in okra lower and upper leaves on infection with Cercospora leaf spot and it ranged from 5.98 (control with water spray) to 9.32 mg g^{-1} (trifloxystrobin + tebuconazole) and 6.27 (control with water spray) to 9.23 mg g^{-1} (trifloxystrobin + tebuconazole), respectively. However, significantly low protein content was observed in control which was ranged from 5.98 (lower leaves) to 6.27 mg g^{-1} (upper leaves) (Table 4.9, Fig. 4.7).

A significant negative correlation (-0.903) existed between total proteins and disease severity (Table 4.12). Significantly high total protein (9.32 and 9.23 mg g^{-1} in lower and upper leaves) was present in fungicide treated plants that expressed low per cent disease severity than other fungicide treated plants with high PDI. Per cent decrease in protein content over healthy was maximum in control (42.77% - lower and 38.04% - upper leaves) while it was minimum in trifloxystrobin + tebuconazole treated plants (9.25% - lower and 8.79% - upper leaves).

Plant pathogens such as viruses, bacteria, fungi and nematodes elicit the synthesis of host proteins which help in restricting the multiplication and spread of pathogens in the healthy tissue (Datta *et al.,* 1999). Present results clearly confirms the earlier statement where the healthy tissue was found with high amount of protein content either in lower (10.27 mg g^{-1}) or upper leaves (10.12 mg g^{-1}). Due to the

amount of inoculum that has established there was considerable reduction in the total protein content in diseased leaf samples. Difference in the treated diseased samples might be due to variations in the efficacy of the chemical fungicides. More effective the chemical lesser was the degradation in proteins which could be substantiated by Datta *et al.* (1999) that decrease in protein content in susceptible cultivars might be due to degradation of the host proteins by the proteolytic enzymes secreted by the virulent pathogens or may be due to the changes in the metabolic activity because of plant, pathogen (Mary and Subramanian, 2014) and fungicide interactions.

4.4.3. Total Sugar Content (mg g^{-1})

Lower and upper leaves of okra on infection with Cercospora leaf spot resulted in varied amounts of total sugar content that ranged from 12.33 (trifloxystrobin + tebuconazole) to 18.25 mg g^{-1} (control with water spray) in lower leaves and from 10.08 (trifloxystrobin + tebuconazole) to 15.94 mg g^{-1} (control with water spray) in upper leaves (Fig. 4.8). Total sugar content was significantly low in fungicide treated samples than in control or healthy samples. Similarly, decrease in the quantity of total sugars was observed compared to the healthy samples (Table 4.10). Infection by the pathogen in plant induces changes in biochemical constituents like total sugars, amino acids etc (Siddaramaiah and Hegde, 1990) resulting in poor nutritive value thus indicating reduction in sugars (Madhavarao *et al.,* 1981) due to increased disease severity (Naik *et al.*, 1988; Ali, 1995 and Ghosh, 1996). Sunil *et al.* (2009) reported total sugars, reducing sugars and non reducing sugars to be higher in healthy leaves of susceptible genotypes than resistant genotypes of greengram against Cercospora leaf spot. Waghmare *et al.* (2012) reported that the rose plant infected with leaf spot caused by *A. alternata* showed a decrease in the quantity of total sugars compared to the healthy plant.

A significant positive correlation (0.932) existed between total sugars and disease severity (Table 4.12). The results are in agreement with Younes and Elyousr (2014) where disease severity was positively correlated with total sugars in okra genotypes screened for powdery mildew resistance. Among the infected samples maximum PDI and total sugar was recorded in control ((83.98- lower and 60.04 % upper leaves) (18.25 lower and 15.94 mg g^{-1} upper leaves) respectively). The amount of decrease in total sugar content over both control and healthy samples was found maximum in trifloxystrobin + tebuconazole treated samples. Tamuli *et al.* (2013)

reported decreased in sugars in infected plants where percent loss in reducing sugars was 45.312 and 45.45 in *C. martinii* and *C. citratus* respectively due to leaf rust infection.

Reduction in the amount of total sugars in the fungicide treatment with least PDI values could be due to the effect of chemical on the pathogen in managing it. In less effective fungicides infection was high and there will be a greater demand for sugars by the pathogen compared to the treatments with less PDI. Thus, in an successful host pathogen interaction, there will be requirement of sugars for increased respiration or utilization of sugars by the fungi which in turn depends on the capability of fungi to secrete carbohydrate degrading enzyme (Prasad *et al*., 1960).

4.4.4. Chlorophyll a Content (mg g^{-1})

Significant reduction in Chlorophyll a was noticed in infected leaves (0.73 lower and 0.79 mg g^{-1} upper leaves) compared with healthy (1.21 lower and 1.32 mg g^{-1} upper leaves). In upper leaves, relatively high concentration of chlorophyll a (0.79 to 1.28 mg g^{-1}) was observed indicating lesser damage due to reduced infection than lower leaves (0.73 to 1.15 mg g^{-1}) (Fig. 4.9 and Fig. 4.10). Among all the treatments the lowest reduction in Chlorophyll a was recorded in trifloxystrobin + tebuconazole sprayed treatment and was only 4.96% decrease over healthy in lower leaves and 3.03% in upper leaves (Table 4.11).

A significant negative correlation (-0.901)existed between chlorophyll a and disease severity (Table 4.12).

4.4.5 Chlorophyll b Content (mg g^{-1})

The amount of chlorophyll b content was relatively low over chlorophyll a and Chlorophyll b reduction was comparatively high than the chlorophyll a in both lower (Fig. 4.9) and upper leaves (Fig. 4.10). In lower leaves, chlorophyll b content ranged from 0.41 (Control) to 1.06 mg g^{-1} (trifloxystrobin + tebuconazole) and from 0.44(Control) to 1.02 mg g^{-1} (trifloxystrobin + tebuconazole) in upper leaves. In lower leaves, reduction in chlorophyll b in trifloxystrobin + tebuconazole was found on a par with hexaconazole and propiconazole treated samples while in upper leaves in addition to the earlier it was found on par with pyraclostrobin. Per cent decrease over healthy in different treatments varied from 7.83 to 61.74% in lower leaves while it varied from 7.27 to 48.18% in upper leaves (Table 4.11).

A significant negative correlation (-0.788) existed between chlorophyll b and disease severity (Table 4.12).

The results were in agreement with Muqit *et al.* (2007) who reported the high Chl a/b ratio which indicates more degradation or lower synthesis of chlorophyll 'b' than 'a' due to *okra Yellow Vein Mosaic Virus* infection.

4.4.6 Total Chlorophyll Content (mg g^{-1})

Significant variation in total chlorophyll content was recorded among different treatments in lower (Fig. 4.9) and upper leaves (Fig. 4.10) trifloxystrobin + tebuconazole treated samples were found to be least effected due to infection, with significantly high amount of total chlorophyll (2.22 mg g^{-1} in lower and 2.30 mg g^{-1} in upper leaves) among the treatments. Within the treatments maximum reduction in total chlorophyll was observed in mancozeb treated samples (44.92% in lower and 36.36% in upper leaves) while it was least in trifloxystrobin + tebuconazole treated samples (5.93% in lower and 4.96% in upper leaves) (Table 4.11).

A significant negative correlation (-0.856) existed between total chlorophyll and disease severity (Table 4.12).

Ghose *et al.* (2010) studied the changes in aminoacids and photosynthetic pigments when mulberry leaf was infected with leaf blight pathogen. They observed drastic reduction of total chlorophyll, chlorophyll a, chlorophyll b and β-carotene in blight infected leaves. The pigment contents were reported to decrease with increasing pathogenesis due to *Phyllostica* sp. leaf blight pathogen on mulberry leaves.

Rajesh and Jayakumar (2013) studied variation in growth, biochemical and yield parameters of okra under different concentrations of panchagavya ranging from nil to 10% concentration. The photosynthetic pigment contents such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoid of *A. esculentus* (L.) Moench were reported to increase with 3% panchagavya spray and decreased in control and other concentration.

Chavan and Suryvanshi (2014) reported that the chlorophyll content reduced drastically over healthy cultivars, but at higher rate in susceptible than in resistant variety of soybean when infected with *C. truncatum*.

Kulkarni and Benagi (2013) studied changes in chlorophyll, sugar and phenolic compounds in resistant and susceptible genotypes of greengram. The chlorophyll and sugar content were found to decrease due to the infection of *C. truncatum* and the rate of decrease was more in susceptible genotypes than resistant genotypes.

Bawden (1999) reported that abnormalities in the form and destruction of chloroplasts are common features of disease tissue in plants infected with pathogens, which usually exhibited reduced photosynthetic rate, phosphorylation, hill reaction and carbon dioxide assimilation. Palanisamy *et al.* (2009) reported the effect of okra *Yellow Vein Mosaic Virus* infection on PS II efficiency and thylakoid membrane integrity. The present studies corroborate with the earlier findings. The disease under study was found to develop in the form of sooty effuse and has strong impact on photosynthetic area and the pathogen during the process of establishment may bring about rapid changes in the cells that may result in accumulation of reactive oxygen species that in turn may affect the integrity of thylakoid membrane. Thylakoids being the centres for chlorophyll synthesis get affected due to the disruption of thylakoid integrity.

Table 4.12. Correlation coefficients between biochemical parameters and Per cent disease index (PDI) due to Cercospora infection on okra leaves

Biochemical parameters	PDI (%)
Total Phenols	-0.903
Total Proteins	-0.903
Total Sugars	0.932
Total chlorophyll	-0.856
Chlorophyll a	-0.901
Chlorophyll b	-0.788

4.5 CORRELATION BETWEEN BIOCHEMICAL PARAMETERS

Significantly high positive correlation existed between proteins and phenols, total chlorophyll, chlorophyll a and chlorophyll b. Conversion of majority of the proteins in to phenols to overcome the stress at infection site will slow down the pathogen development.

Similarly, significantly high positive correlation existed between phenols and total chlorophyll, chlorophyll a and b. As phenols play an important role in resisting the pathogen entry and establishment more the concentration of phenols lesser will be the degradation of chlorophylls.

Between total sugars and phenols, proteins, chlorophyll a, chlorophyll b and total chlorophyll significantly high negative correlation was observed (Table 4.13). As more sugars are demanded by the invading pathogen for its biomass production and thus the production of protein or phenols gets reduced. As the pathogen establishes chlorophyll pigments will be degraded.

Kumar *et al.* (2017) observed that phenol content and total chlorophyll content was positively correlated to each other while total soluble sugar content was negatively correlated with other both the traits in leaves of *YVMV* resistant and susceptible lines of okra.

Table 4.13. Correlation between biochemical parameters due to Cercospora leaf spot on okra leaves

	Phenols	**Proteins**	**Sugars**	**Total chlorophyll**	**Chlorophyll a**	**Chlorophyll b**
Phenols	1					
Proteins	0.862*	1				
Sugars	-0.924*	-0.836*	1			
Total Chlorophyll	0.854*	0.889*	-0.897*	1		
Chlorophyll a	0.803*	0.844*	-0.919*	0.942*	1	
Chlorophyll b	0.839*	0.868*	-0.838*	0.982*	0.861*	1

* Significant at 5 %

r = 0.556 No. of observations = 14

Chapter - V

SUMMARY AND CONCLUSIONS

Cercospora leaf spot infected leaves with typical symptoms on lower leaf surface were collected from eight different okra growing villages in Guntur district. *In-planta* isolation of pathogen DNA was done and amplified with universal primers ITS 1 and ITS 4. No size variation was found among the amplified ITS regions. Hexa cutter restriction endonuleases *i.e.*, EcoRI and BamHI were used to restrict the ITS region PCR products of the isolates and polymorphism was revealed in Yazali isolate. EcoRI was found with two restriction sites in the isolates except Yazali isolate, on restriction with BamHI one restriction site was observed in all isolates. However, Yazali isolate differed in length polymorphism. Restriction with tetra base pair cutter Taq1 indicated a high degree of genetic diversity among the isolates and was represented by the differences in banding pattern. Bapatla 1 and Dhundivaripalem 3 isolates were found with six restriction sites, Yazali and Thimmareddipalem isolates with three restriction sites and Bapatla 2 isolate with one restriction site. Nandirajuthota, Dhundivaripalem 1 and Dhundivaripalem 2 isolates were not found with any restriction site for Taq1enzyme.

The dendrogram constructed based on similarity coefficients indicated that the Yazali isolate differed in restriction digestion with hexa cutters while with tetra cutter, it got clustered with Thimmareddipalem isolate. The results revealed that the presence of notable genetic variation in population sampled within the geographic region of Guntur district may be due to to variations in single nucleotide polymorphism.

Significant difference was observed in Cercospora leaf spot symptom development with respect to leaf position. Before spraying, there was no significant difference in disease severity either within lower or upper leaves scored, indicating equal threshold of inoculum. After a week of first spray, significant difference in Cercospora leaf spot disease severity was observed in lower and upper leaves where lower leaves had 75.55% PDI as against 19.15% in upper leaves. All fungicides tested were found to be significantly effective in managing the disease over control (water spray) (68.69%). Even after two weeks of first spray there was significant difference in disease severity between lower (76.75%) and upper leaves (50.89%). Significantly low

disease severity was observed in trifloxystrobin + tebuconazole @ 0.1% sprayed plants (24.58%) compared to other fungicides tested (65.00 to 71.67%), while all the fungicides were equally effective over control (83.67%). It was found that interaction between leaf position and fungicide treatments, trifloxystrobin + tebuconazole @ 0.1% effect on upper leaves was found significant with low PDI (13.61%) over other treatments (35.56 to 87.79%) and was followed by same chemical spray on lower leaves (35.56%).

One week after second spray, significant difference in disease severity was observed between lower (72.74%) and upper leaves (57.32%). Among fungicide treatments, trifloxystrobin + tebuconazole @ 0.1% spray continued to be significant in reducing the PDI (24.05%) over other fungicides (59.17 to 73.87%). It was followed by propiconazole @ 0.1% (59.17%) and hexaconazole @ 0.2 % (66.95 % PDI) that were on a par with each other. Among the interactions, trifloxystrobin + tebuconazole @ 0.1% on upper leaves was found with significantly low PDI (16.70 %) compared to other interactions (31.39 to 94.45%).

Marked difference was observed in PDI in upper and lower leaves irrespective of the time and number of spray when noticed two weeks after second spray. Among all the fungicides tested for their efficacy against *C. abelmoschi*, trifloxystrobin + tebuconazole @ 0.1% was found significantly superior (PDI 24.03%) over all fungicides (PDI ranged from 50.66 to 72.21%). In interactions, the amount of disease was much reduced in both upper (16.38%) and lower leaves (31.68%) in trifloxystrobin + tebuconazole @ 0.1% treated plants as against control (88.62 (upper leaves) to 95.55% (lower leaves)). Incorporating trifloxystrobin + tebuconazole in the spray schedule as a preventive spray or during early days of infection would reduce the evolution of resistance strains, disease and cost of cultivation.

Phenol content (1.58 mg g^{-1} in lower leaves) and total protein content (9.32 in lower and 9.23 mg g^{-1} upper leaves) was found significantly high over other treatments when infected leaves (PDI 35.61 (lower) and 12.98% (upper leaves)) were sprayed with fungicide trifloxystrobin + tebuconazole @ 0.1%. Total sugar content was significantly low in fungicide treated samples than in control or healthy samples. In lower and upper leaves the total sugar content ranged from 12.33 (trifloxystrobin + tebuconazole) to 18.25 mg g^{-1} (control) and from 10.08 (trifloxystrobin + tebuconazole) to 15.94 mg g^{-1}

(control) respectively. Reduction in the amount of total sugars in the fungicide treatment with least PDI values could be due to the effect of chemical on the pathogen in managing it. Among all the treatments the lowest reduction in Chlorophyll a was recorded in trifloxystrobin + tebuconazole sprayed treatment and was only 4.96 % decrease over healthy in lower leaves and 3.03 % in upper leaves. In both lower and upper leaves chlorophyll b (1.06 and 1.02 mg g^{-1} respectively) and total chlorophyll (2.22 and 2.30 mg g^{-1} respectively) was significantly high in trifloxystrobin + tebuconazole treated plants.

Significant negative correlation existed between total phenols (-0.903), total proteins (-0.903), chlorophyll a (-0.901), chlorophyll b (-0.788), total chlorophyll (-0.856) and disease severity while significant positive correlation (0.932) existed between total sugars and disease severity.

Significantly high positive correlation existed between proteins and phenols (0.862), total chlorophyll (0.889), chlorophyll a (0.844) and chlorophyll b (0.868). Similarly, significant high positive correlation existed between phenols and total chlorophyll (0.854), chlorophyll a (0.803) and chlorophyll b (0.839). Conversion of majority of the proteins in to phenols is required to overcome the stress at infection site thus slows down the pathogen development. Increased phenols will resist the pathogen entry and establishment thus the chlorophyll pigments are kept intact without degradation.

Significantly high negative correlation between total sugars and phenols (-0.924), proteins (-0.836), chlorophyll a (-0.919), chlorophyll b (-0.838), total chlorophyll (-0.897) existed because sugars are required by the invading pathogen for its biomass production that lead to reduction in protein or phenols. Once the pathogen establishes chlorophyll pigments get degraded.

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*** Original not seen**

Note: The pattern of literature cited presented above is in accordance with the guidelines for Thesis presentation for Acharya N G Ranga Agricultural University, Guntur.

Fig. 4.1 Dendrogram showing clustering pattern of *Cercospora* isolates using EcoRI by UPGMA method

Fig. 4.2 Dendrogram showing clustering pattern of *Cercospora* isolates using BamHI by UPGMA method

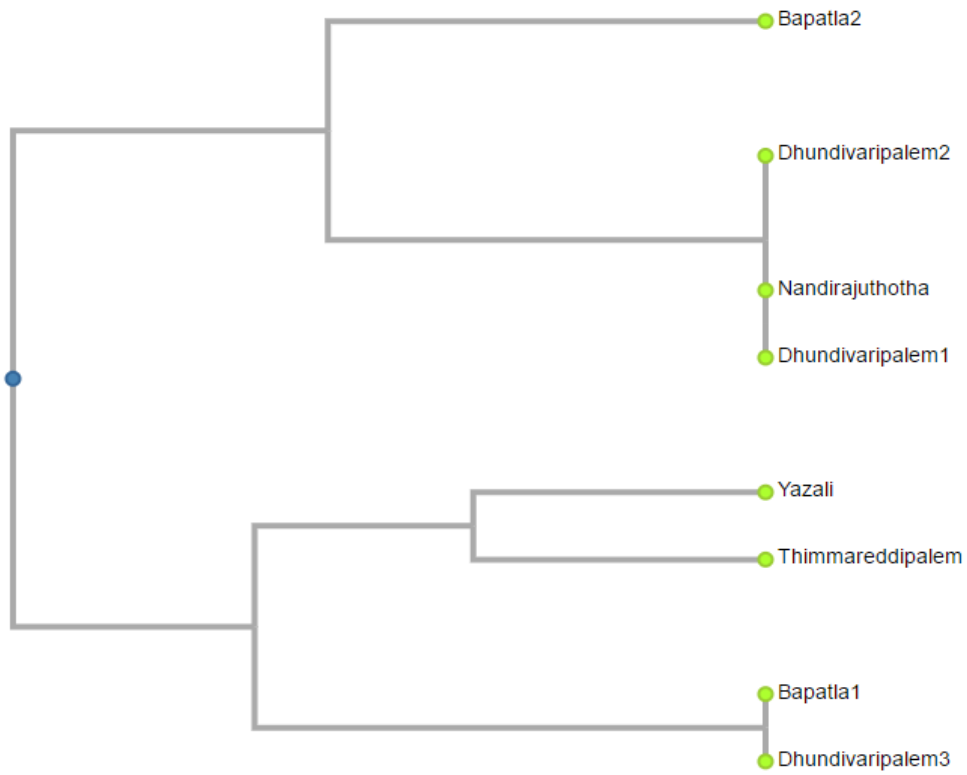


Fig. 4.3 Dendrogram showing clustering pattern of *Cercospora* isolates using Taq1 by UPGMA method

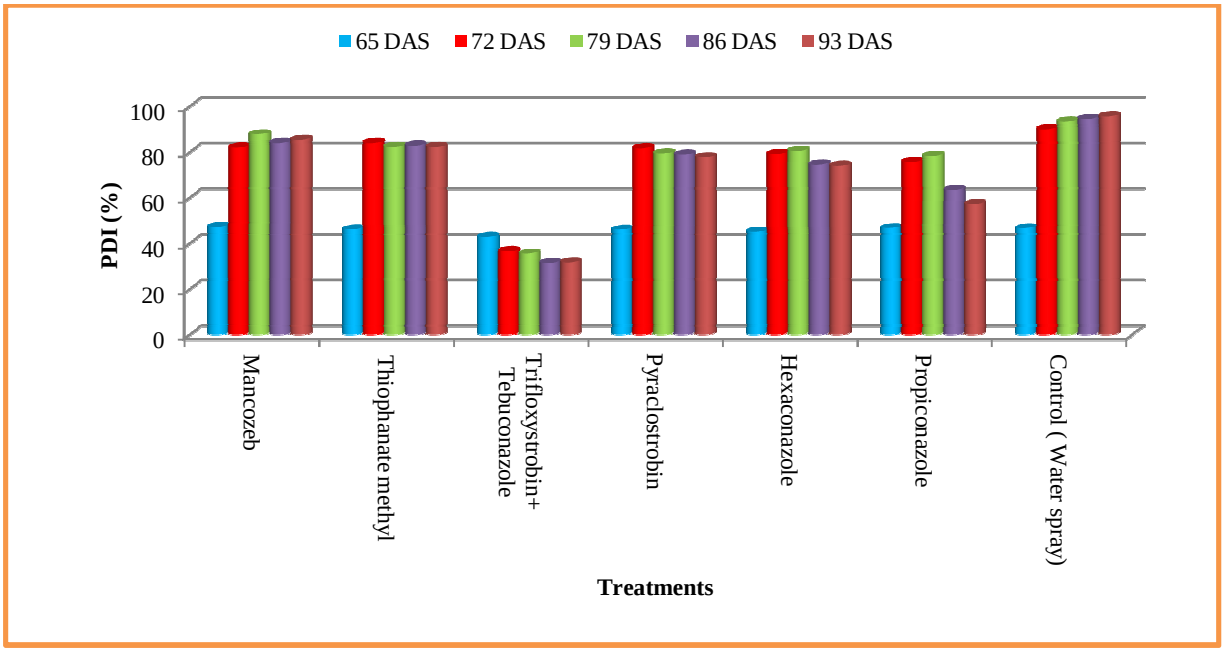


Fig. 4.4. Per cent disease index of Cercospora leaf spot on okra in different treatments at different crop stages in lower leaves

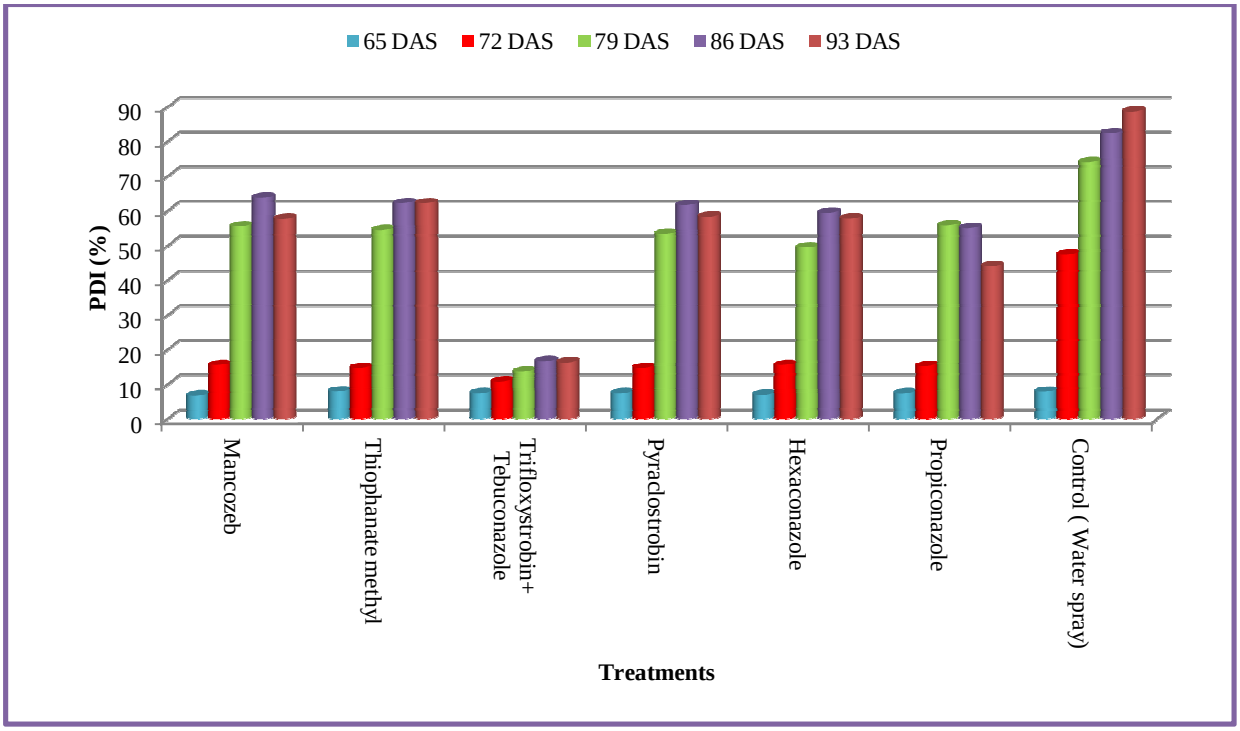


Fig. 4.5. Per cent disease index of Cercospora leaf spot on okra in different treatments at different crop stages in upper leaves.

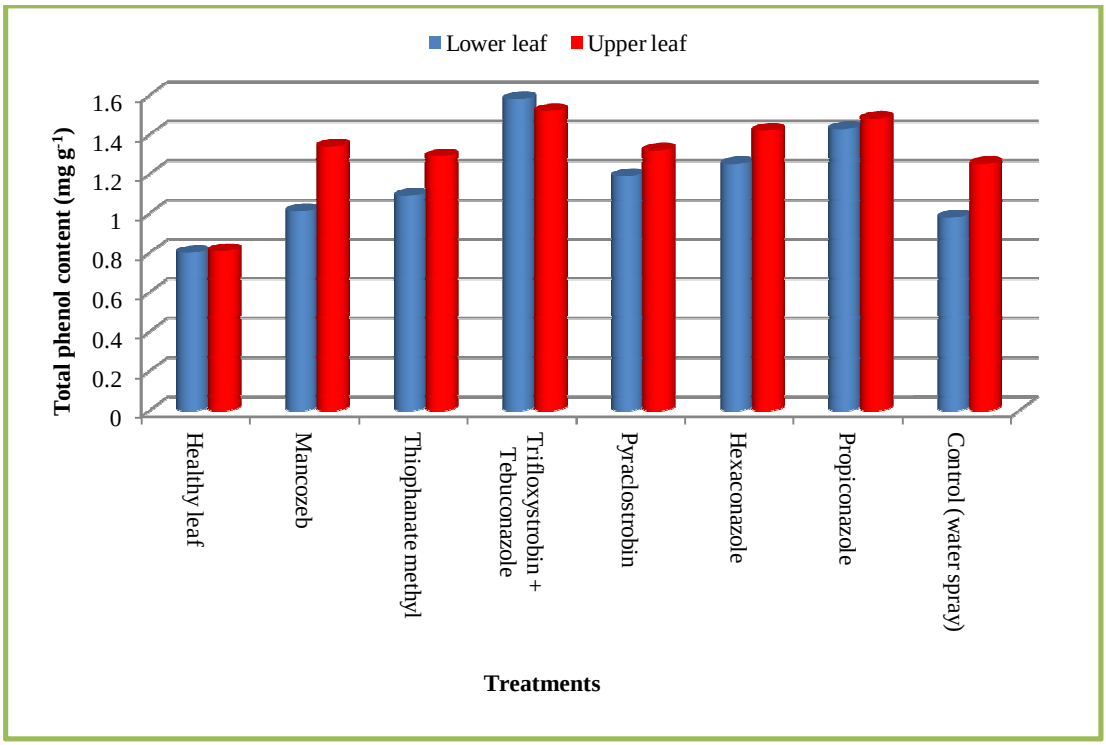


Fig. 4.6. Effect of fungicides on total phenol content (mg g^{-1}) due to Cercospora leaf spot infection in lower and upper leaves of okra during *kharif* 2016-17

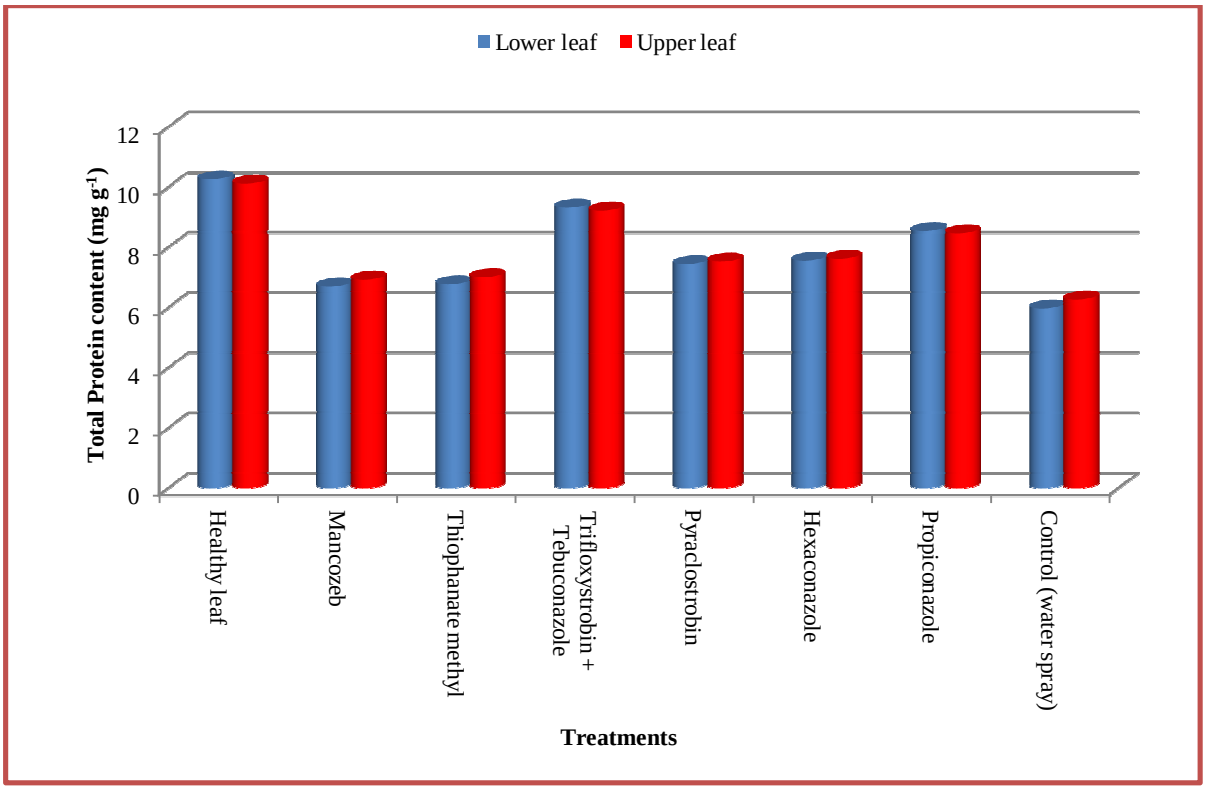


Fig.4.7. Effect of fungicides on total protein content (mg g^{-1}) due to Cercospora leaf spot infection in lower and upper leaves of okra during *kharif* 2016-17

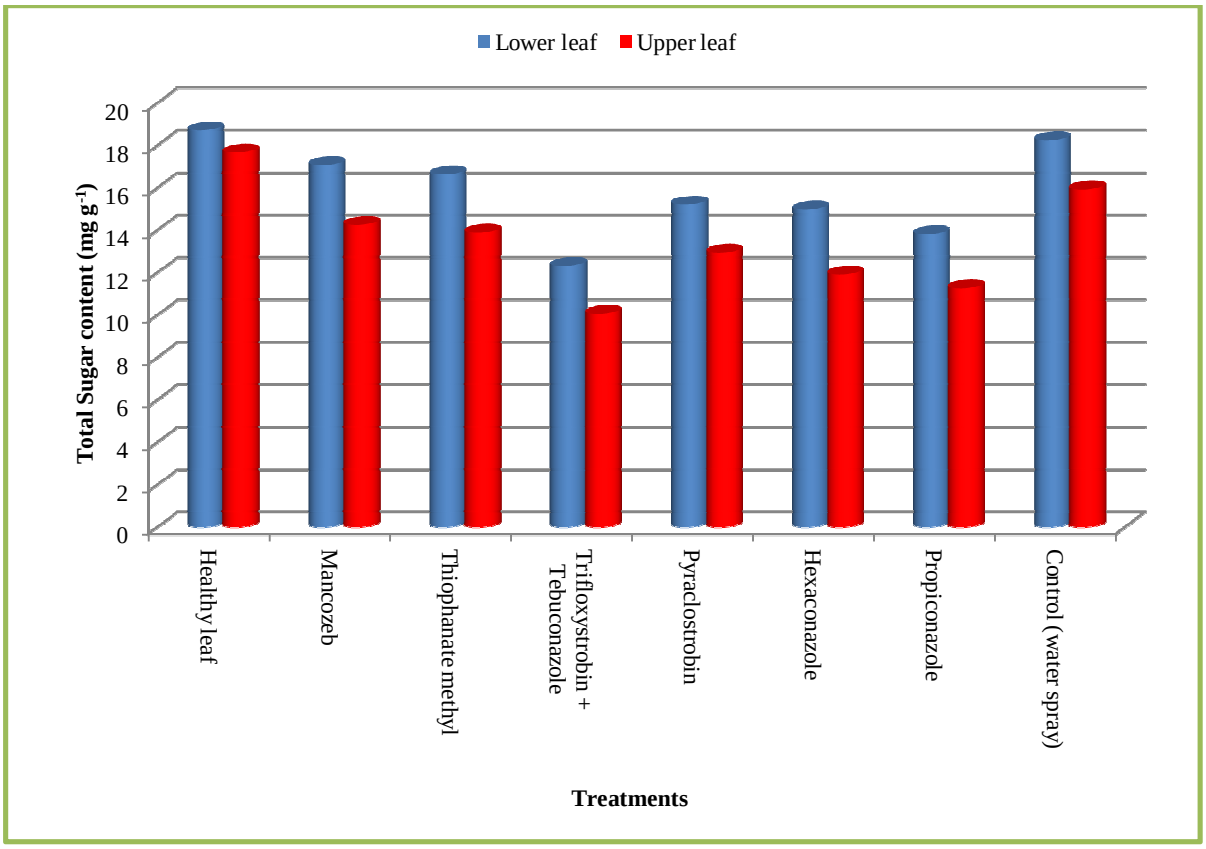


Fig.4.8. Effect of fungicides on total sugar content (mg g^{-1}) due to Cercospora leaf spot infection in lower and upper leaves of okra during *kharif* 2016-17

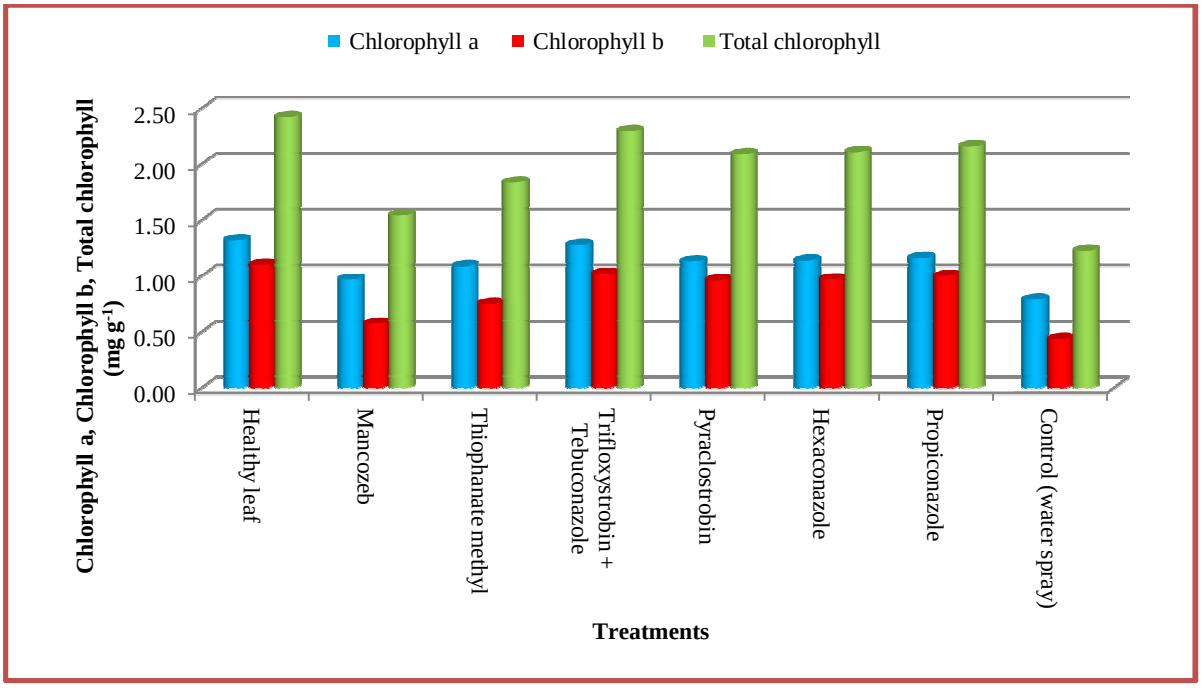


Fig. 4.9. Effect of fungicides on chlorophyll a, chlorophyll b and total chlorophyll content (mg g^{-1}) in Cercospora infected lower leaves of okra during *kharif* 2016-17

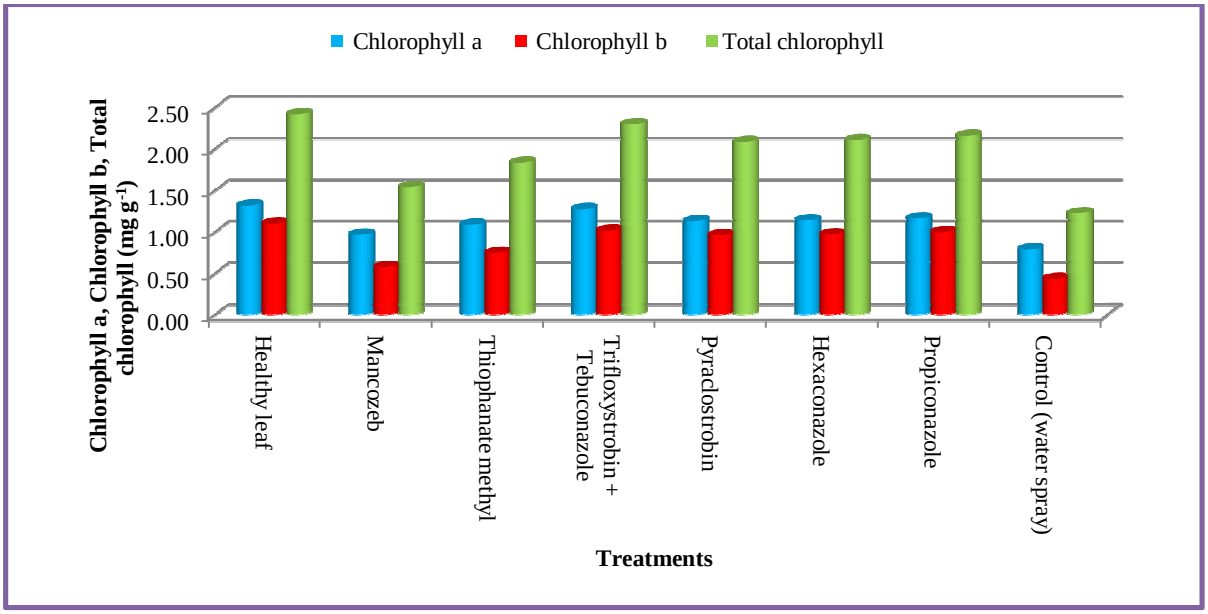


Fig. 4.10. Effect of fungicides on chlorophyll a, chlorophyll b and total chlorophyll content (mg g^{-1}) in Cercospora infected upper leaves of okra during *kharif* 2016-17

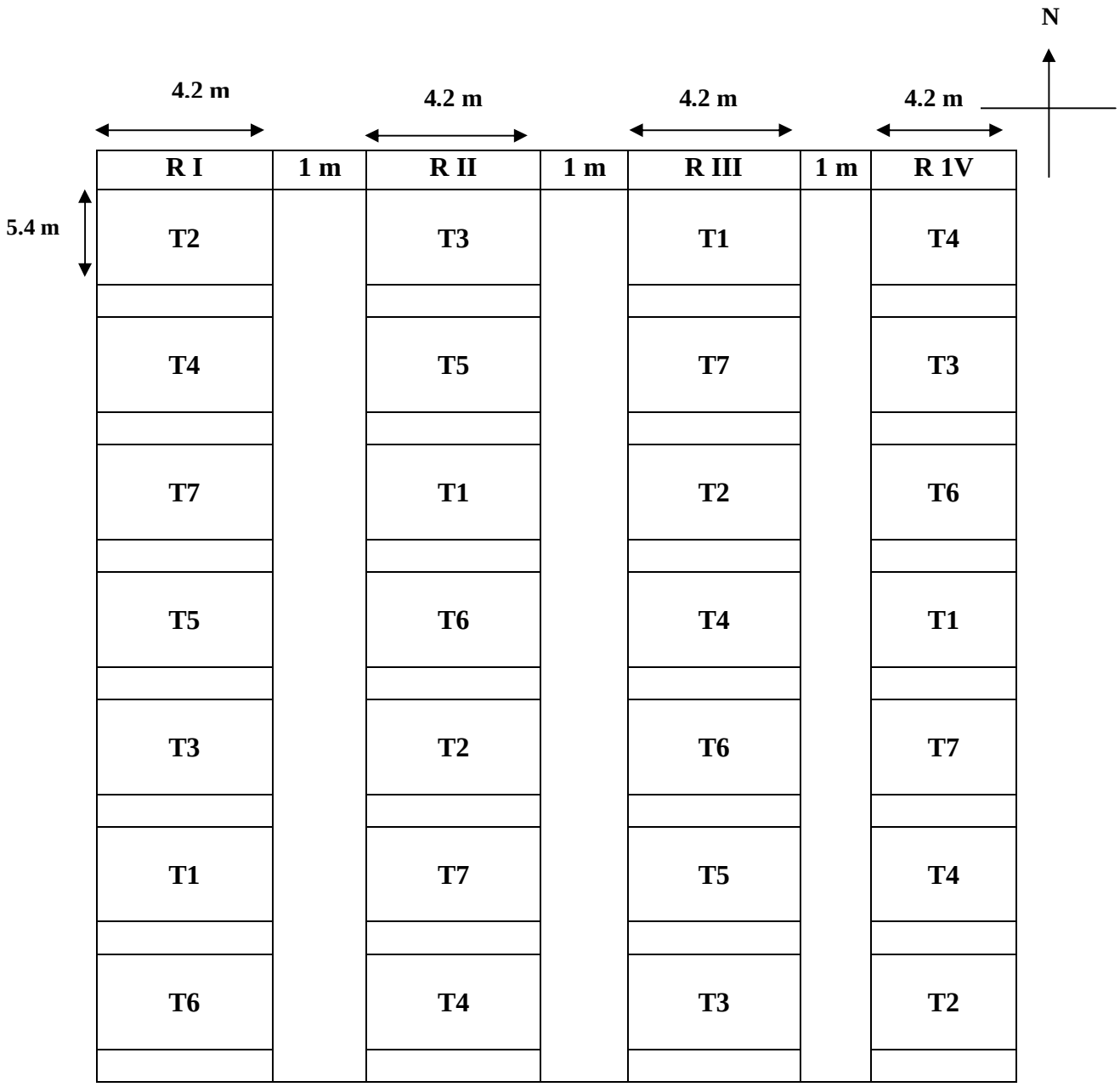


Fig.3.1. Layout for field experiment on management of Cercospora leaf spot of okra with the application of fungicides

T1- Mancozeb @ 0.25%

T2- Thiophanate methyl @ 0.1%

T3- Tebuconazole + Trifloxystrobin @ 0.1%

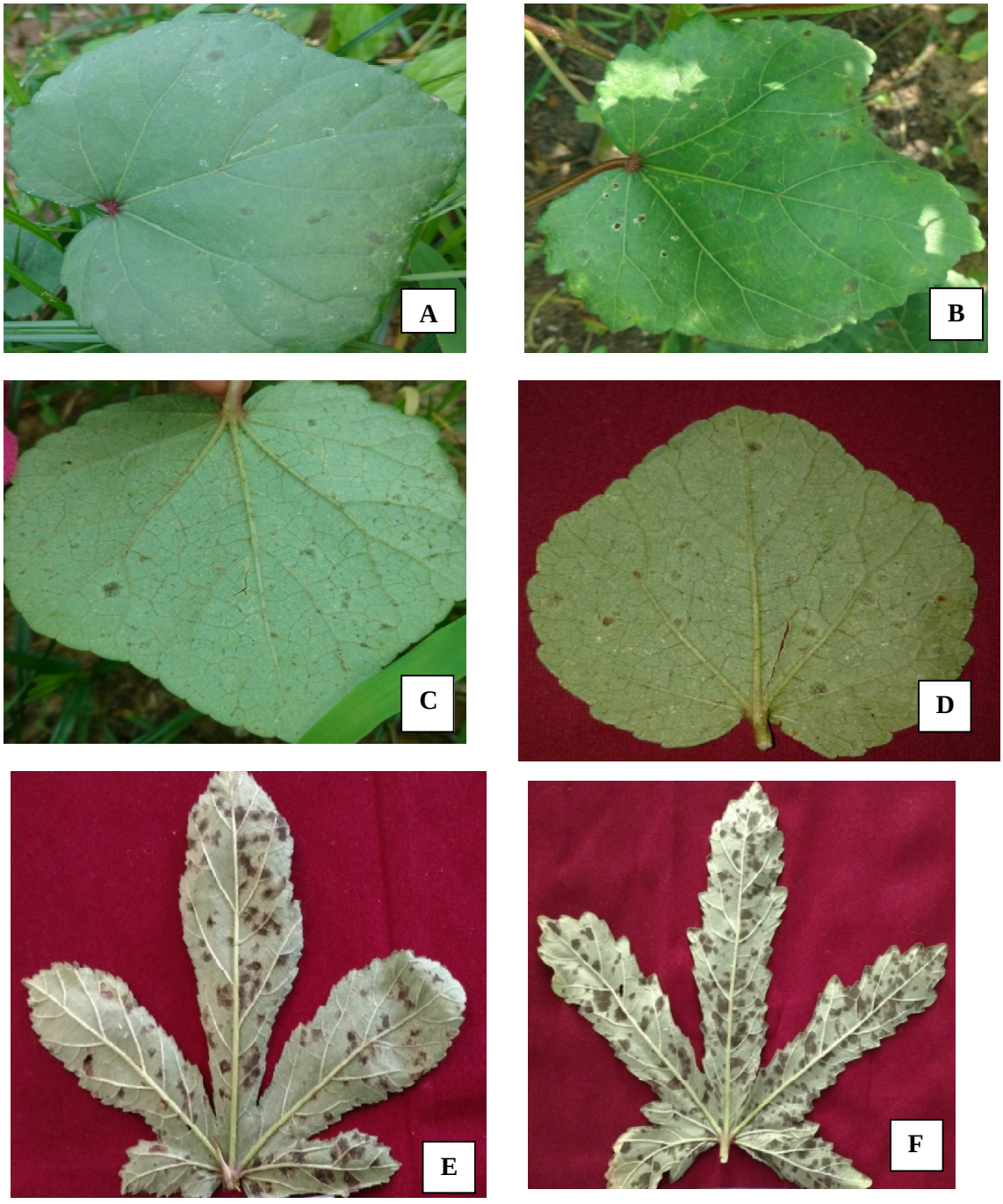
T4- Pyraclostrobin @ 0.1%

T5- Hexaconazole @ 0.2%

T6- Propiconazole @ 0.1%

T7- Control

Plate 3.1 View of field experiment on management of Cercospora leaf spot of okra with fungicides during *kharif* 2016-17.



A, B - Initial symptoms on adaxial surface of leaves; C, D - Initial symptoms on abaxial surface of leaves;

E, F - Severe symptoms on abaxial surface of leaves.

Plate 4.1 Cercospora leaf spot disease symptoms on adaxial and abaxial leaf surfaces of okra

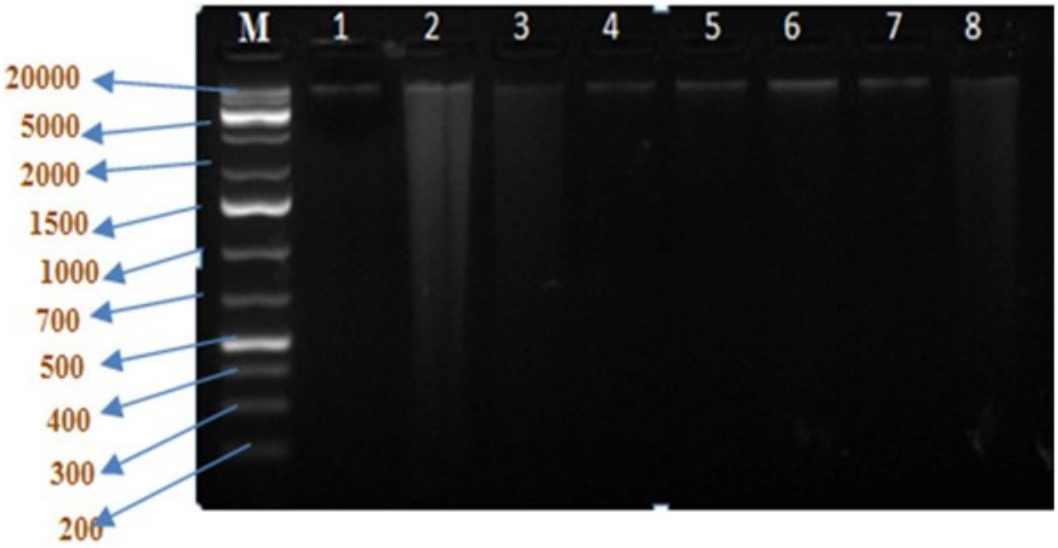


Plate 4.2 Agarose gel electrophoresis showing DNA of *Cercospora abelmoschi* isolated from infected leaves. Lanes 1-8 represent isolates Bpt1, Bpt2, Nt, Dp1, Dp2, Dp3,Yz, Tp. Lane M indicates the molecular weight marker 1kb plus ladder.

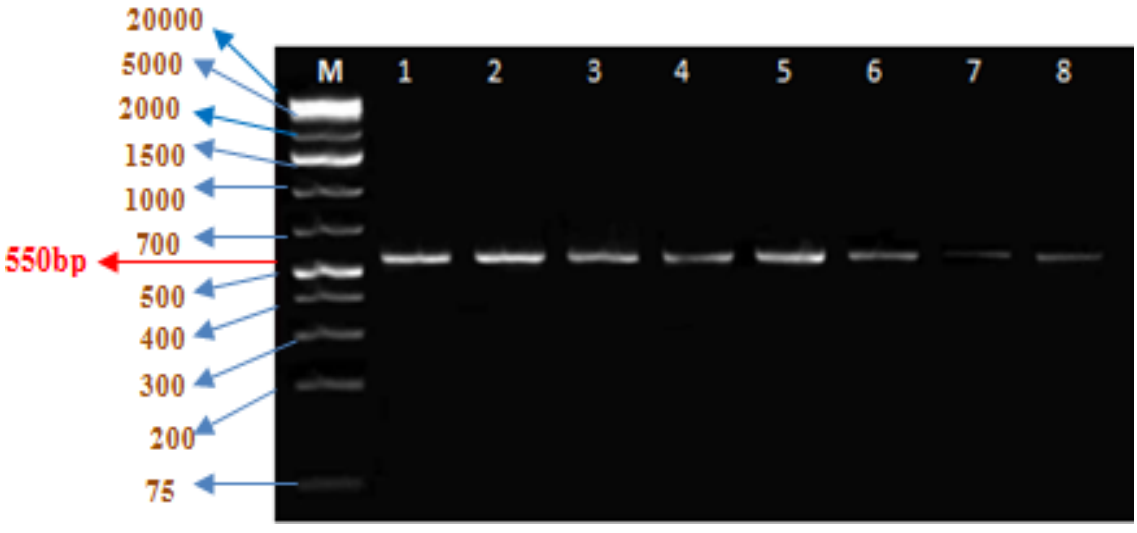


Plate 4.3 Agarose gel electrophoresis showing amplicon amplified by universal Internal Transcribed Spacer (ITS) primers in eight isolates. Lanes 1-8 represent isolates Bpt1, Bpt2, Nt, Dp1, Dp2, Dp3,Yz, Tp. Lane M indicates the molecular weight marker 1kb plus ladder

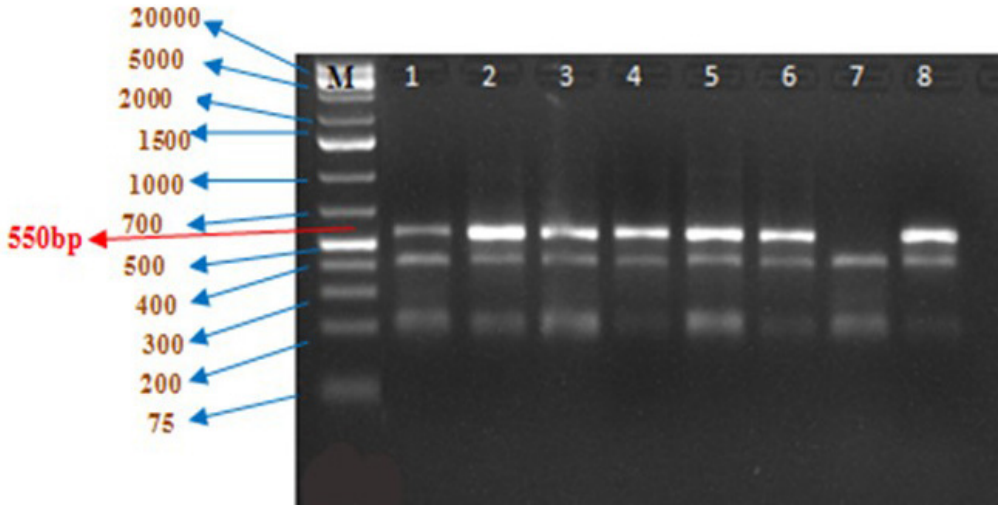


Plate 4.4 Restriction analysis of amplicons obtained from ITS primers with EcoRI. Lanes 1-8: Digested products of ITS region from isolates Bpt1, Bpt2, Nt, Dp1, Dp2, Dp3,Yz, Tp. Lane M indicates the molecular weight marker 1kb plus ladder

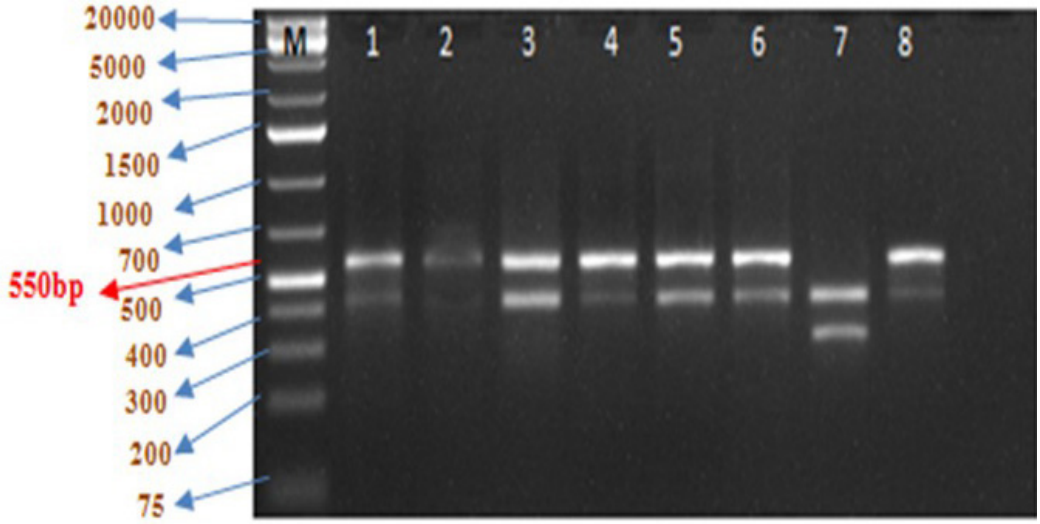


Plate 4.5 Restriction analysis of amplicons obtained from ITS primers with BamHI. Lanes 1-8: Digested products of ITS region from isolates Bpt1, Bpt2, Nt, Dp1, Dp2, Dp3,Yz, Tp. Lane M indicates the molecular weight marker 1kb plus ladder

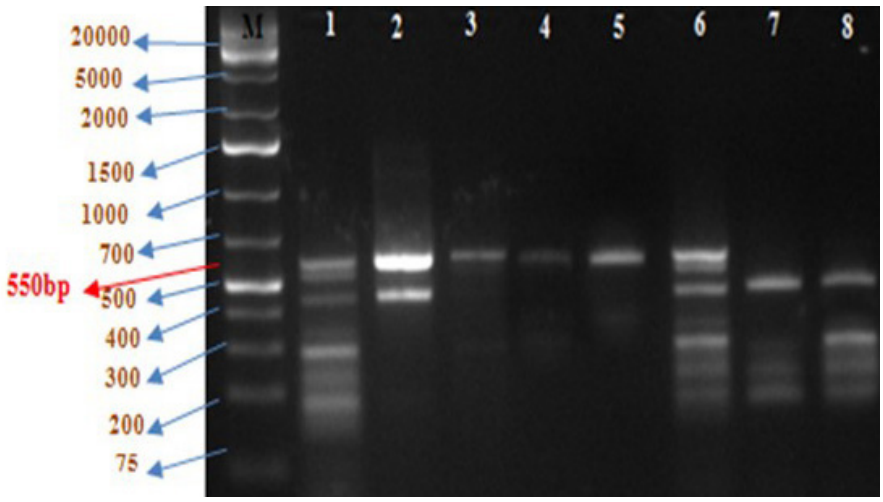


Plate 4.6 Restriction analysis of amplicons obtained from ITS primers with Taq1. Lanes 1-8: Digested products of ITS region from isolates Bpt1, Bpt2, Nt, Dp1, Dp2, Dp3,Yz, Tp. Lane M indicates the molecular weight marker 1kb plus ladder

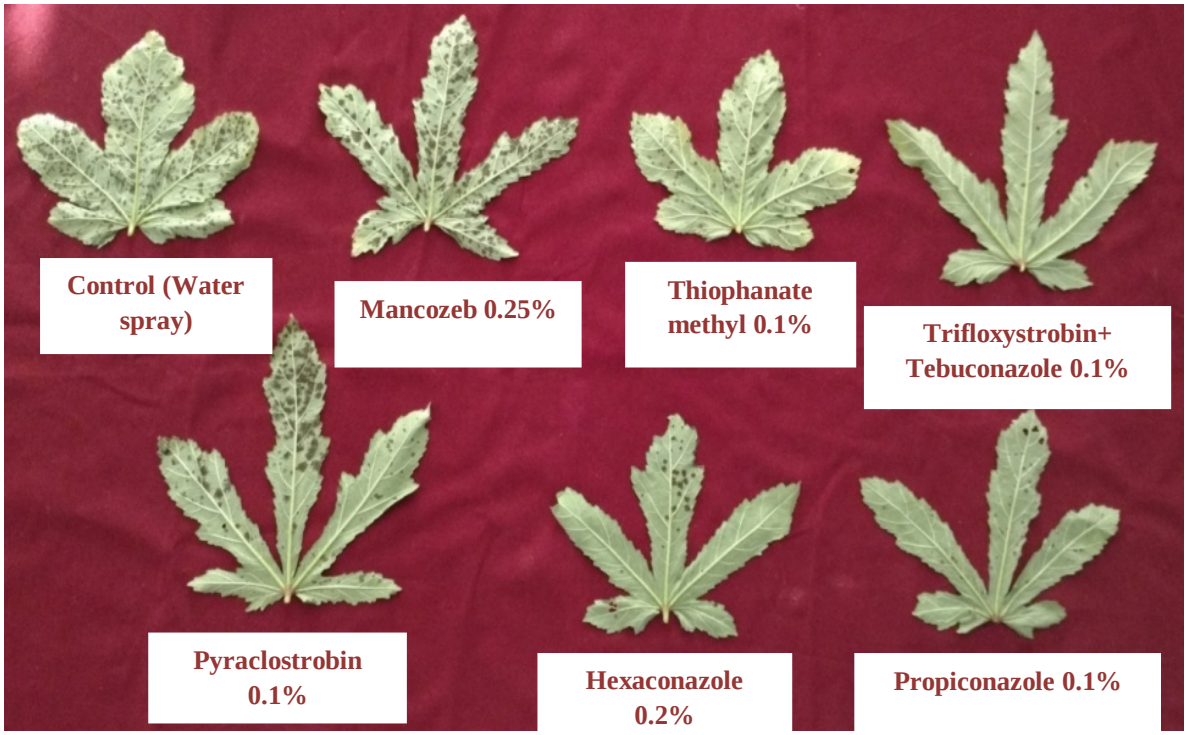


Plate 4.7 Disease severity of Cercospora leaf spot in different fungicide treatments after first spray of fungicides

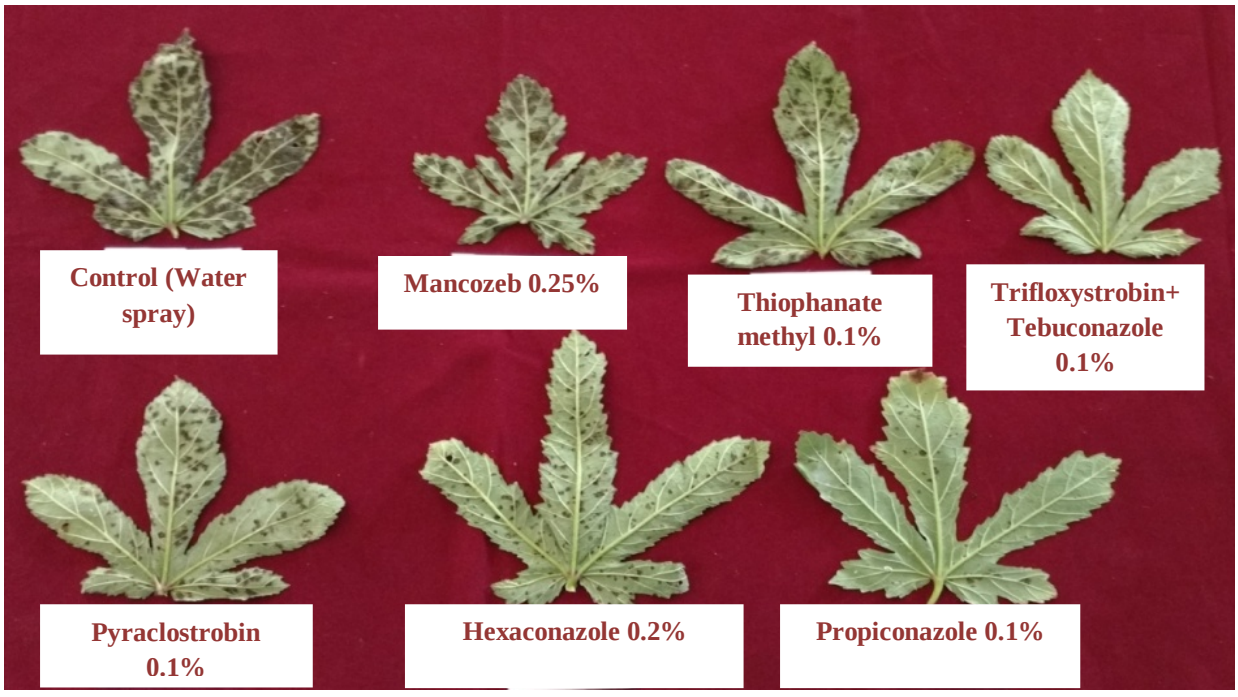


Plate 4.8 Disease severity of Cercospora leaf spot in different fungicide treatments two weeks after second spray of fungicides

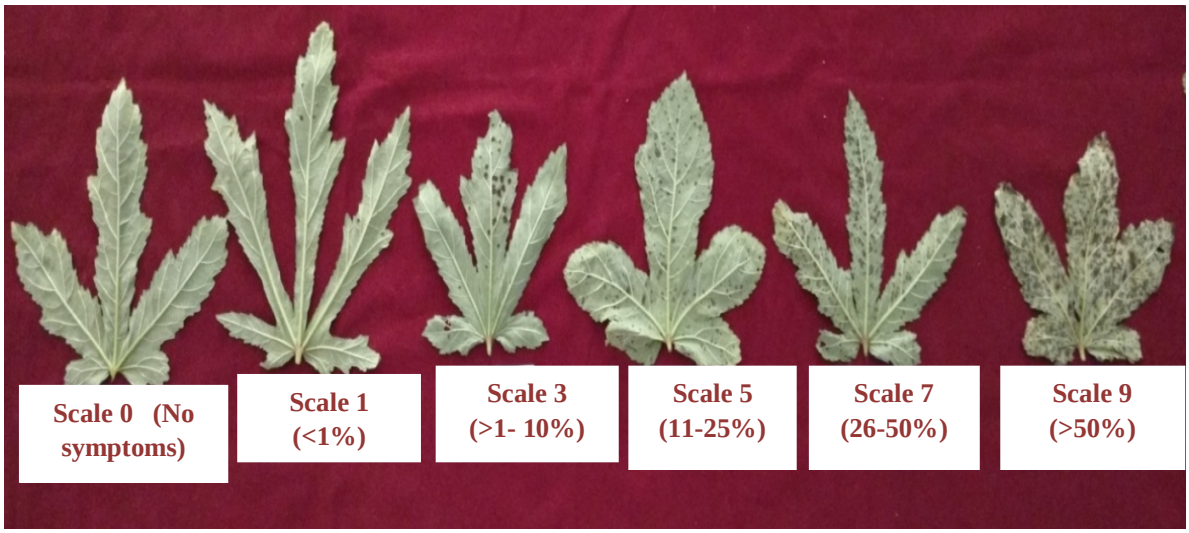


Plate 3.2 Disease rating scale of Cercospora leaf spot of okra

Table 4.2. Per cent disease index (PDI) of Cercospora leaf spot of okra before first spray of fungicides.

PDI (%) - before first spray								
Treatments / Position of leaf	**Mancozeb**	**Thiophanate methyl**	**Trifloxystrobin+ Tebuconazole**	**Pyraclostrobin**	**Hexaconazole**	**Propiconazole**	**Control (Water spray)**	**Mean**
Lower leaf	47.23 (43.36)*	46.11 (42.73)	45.27 (42.27)	46.00 (42.68)	45.00 (42.11)	46.67 (43.07)	46.58 (43.02)	45.26 (42.54)
Upper leaf	6.75 (14.89)	7.92 (15.93)	7.40 (15.76)	7.36 (15.58)	6.94 (15.22)	7.33 (15.59)	7.85 (16.13)	7.36 (15.59)
Mean	26.66 (29.13)	27.01 (29.33)	26.34 (28.29)	26.68 (29.13)	25.97 (28.66)	27.00 (29.33)	27.21 (29.58)	
	LEAVES			**TREATMENTS**			**L X T**	
SEm ±	0.51			0.96			1.35	
C.D. (0.05)	1.46			2.74			3.88	
CV%	9.32							

* Figures in parenthesis are arcsine transformed values

Mean of four replications

Table 4.3. Effect of fungicides on Per cent disease index (PDI) of Cercospora leaf spot of okra a week after first spray

PDI (%) - one week after first spray								
Treatments / Position of leaf	**Mancozeb**	**Thiophanate methyl**	**Trifloxystrobin+ Tebuconazole**	**Pyraclostrobin**	**Hexaconazole**	**Propiconazole**	**Control (Water spray)**	**Mean**
Lower leaf	81.95 (64.95)*	83.89 (66.43)	36.67 (37.22)	81.67 (64.70)	79.17 (62.84)	75.54 (60.53)	90.00 (71.84)	75.55 (61.22)
Upper leaf	15.56 (23.19)	14.72 (22.51)	10.83 (19.20)	14.72 (22.53)	15.56 (23.21)	15.28 (22.95)	47.38 (43.48)	19.15 (25.30)
Mean	48.75 (44.07)	49.31 (44.47)	23.75 (28.21)	48.20 (43.61)	47.36 (43.03)	45.41 (41.74)	68.69 (57.66)	
	LEAVES			**TREATMENTS**			**L X T**	
SEm ±	0.51			0.95			1.34	
C.D. (0.05)	1.45			2.71			3.83	
CV%	6.19							

* Figures in parenthesis are arcsine transformed values

Mean of four replications

Table 4.4. Effect of fungicides on Per cent disease index (PDI) of Cercospora leaf spot of okra after two weeks of first spray.

PDI (%) - two weeks after first spray								
Treatments / Position of leaf	Mancozeb	Thiophanate methyl	Trifloxystrobin+ Tebuconazole	Pyraclostrobin	Hexaconazole	Propiconazole	Control (Water spray)	Mean
Lower leaf	87.79 (69.53)*	82.22 (65.06)	35.56 (36.58)	79.44 (63.28)	80.56 (64.35)	78.34 (62.43)	93.33 (75.11)	76.75 (62.33)
Upper leaf	55.56 (48.19)	54.45 (47.54)	13.61 (21.56)	53.34 (46.90)	49.44 (44.66)	55.84 (48.52)	74.00 (59.61)	50.89 (45.28)
Mean	71.67 (58.86)	68.33 (56.30)	24.58 (29.07)	66.39 (55.09)	65.00 (54.51)	67.09 (55.48)	83.67 (67.36)	
	LEAVES			TREATMENTS			L X T	
SEm ±	0.81			1.52			2.15	
C.D. (0.05)	2.32			4.34			6.14	
CV%	7.98							

* Figures in parenthesis are arcsine transformed values

\# Mean of four replications

Table 4.5. Effect of fungicides on Per cent disease index (PDI) of Cercospora leaf spot of okra after a week from second spray

PDI (%) - one week after second spray								
Treatments / Position of leaf	**Mancozeb**	**Thiophanate methyl**	**Trifloxystrobin+ Tebuconazole**	**Pyraclostrobin**	**Hexaconazole**	**Propiconazole**	**Control (Water spray)**	**Mean**
Lower leaf	83.89 (66.36)*	82.78 (65.51)	31.39 (34.01)	78.89 (62.68)	74.45 (59.80)	63.34 (52.83)	94.45 (76.71)	72.74 (59.70)
Upper leaf	63.86 (53.03)	62.22 (52.06)	16.70 (24.02)	61.67 (51.78)	59.45 (50.53)	55.00 (49.87)	82.35 (65.15)	57.32 (49.21)
Mean	73.87 (59.70)	72.50 (58.78)	24.05 (29.02)	70.28 (57.23)	66.95 (55.17)	59.17 (50.35)	88.40 (70.93)	
	LEAVES			**TREATMENTS**			**L X T**	
SEm ±	0.74			1.38			1.96	
C.D. (0.05)	2.11			3.95			5.59	
CV%	7.18							

* Figures in parenthesis are arcsine transformed values

Mean of four replications

Table 4.6. Effect of fungicides on Per cent disease index (PDI) of Cercospora leaf spot of okra after two weeks from second spray

PDI (%) - two weeks after second spray								
Treatments / Position of leaf	**Mancozeb**	**Thiophanate methyl**	**Trifloxystrobin+ Tebuconazole**	**Pyraclostrobin**	**Hexaconazole**	**Propiconazole**	**Control (Water spray)**	**Mean**
Lower leaf	85.28 (67.54)*	82.23 (65.26)	31.68 (34.11)	77.78 (61.89)	73.90 (59.54)	57.25 (49.16)	95.55 (79.75)	71.95 (59.61)
Upper leaf	57.72 (49.43)	62.20 (52.05)	16.38 (23.64)	58.35 (49.79)	57.80 (49.47)	44.07 (41.55)	88.62 (70.32)	55.02 (48.04)
Mean	71.50 (58.49)	72.21 (58.66)	24.03 (28.88)	68.06 (55.84)	65.85 (54.50)	50.66 (45.35)	92.09 (75.04)	
	LEAVES			**TREATMENTS**			**L X T**	
SEm ±	0.78			1.46			2.06	
C.D. (0.05)	2.23			4.18			5.91	
CV%	7.67							

* Figures in parenthesis are arcsine transformed values

Mean of four replications

Table 4.7. Effect of fungicides on yield (q ha^{-1}) due to Cercospora leaf spot infection in okra and B: C ratio

S. No.	Treatment	PDI (%)		Yield in kg plot^{-1}	Yield in q ha^{-1}	Gross income	Expenditure	Net income	B: C ratio	Incremental B: C ratio
		Lower leaf	Upper leaf							
1	Mancozeb @ 0.25%	85.28 (67.54)	57.72 (49.43)	11.65	92.46	184921	43215	141706	3.28	0.21
2	Thiophante methyl @ 0.1%	82.23 (65.26)	62.20 (52.05)	11.33	89.92	179841	42075	137766	3.27	0.09
3	Trifloxystrobin + Tebuconazole @ 0.1%	31.68 (34.11)	16.38 (23.64)	12.82	101.75	203492	45715	157777	3.45	0.61
4	Pyraclostrobin @ 0.1%	77.78 (61.89)	58.35 (49.79)	12.19	96.75	193492	43740	149752	3.42	0.41
5	Hexaconazole @ 0.2%	73.90 (59.54)	57.80 (49.47)	12.25	97.22	194444	42235	152209	3.60	0.44
6	Propiconazole @ 0.1%	57.25 (49.16)	44.07 (41.53)	12.33	97.86	195714	42325	153389	3.62	0.47
7	Control (Water spray)	95.55 (79.75)	88.62 (70.32)	11.07	87.86	175714	41715	133999	3.21	
	SEm ±			0.046	0.37					
	C.D. (0.05)			0.137	1.09					
	CV%			1.57	1.57					

* Figures in parenthesis are arcsine transformed values

*The conversion factor for yield per hectare is 793.65.

Table 4.8. Total phenol content (mg g^{-1}) due to Cercospora leaf spot in okra leaves

S. No.	Treatments	PDI (%)		Total Phenol content (mg g^{-1})		Per cent increase over control		Per cent increase over healthy	
		Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf
1	Mancozeb	85.28 (67.54)	57.72 (49.43)	1.01	1.34	3.06	7.2	26.25	65.43
2	Thiophante methyl	82.23 (65.26)	62.20 (52.05)	1.09	1.29	11.22	3.2	36.25	59.26
3	Trifloxystrobin + Tebuconazole	31.68 (34.11)	16.38 (23.64)	1.58	1.52	61.23	21.6	97.50	87.65
4	Pyraclostrobin	77.78 (61.89)	58.35 (49.79)	1.19	1.32	21.4	5.6	48.75	62.96
5	Hexaconazole	73.90 (59.54)	57.80 (49.47)	1.25	1.42	25.55	13.6	56.25	75.3
6	Propiconazole	57.25 (49.16)	44.07 (41.53)	1.43	1.48	45.91	18.4	78.75	82.72
7	Control (water spray)	95.55 (79.75)	88.62 (70.32)	0.98	1.25				
8	Healthy	0.00	0.00	0.8	0.81				
	SEm±			0.015	0.013				
	CD (P≤0.05)			0.045	0.039				
	CV %			5.40	4.17				
	T cal value			10.37* (df 26), P ≤ 0.05					

* Figures in parenthesis are arcsine transformed values

Table 4.9. Total protein content (mg g^{-1}) due to Cercospora leaf spot in okra leaves

S. No.	Treatments	PDI (%)		Total Protein content (mg g^{-1})		Per cent increase over control		Per cent decrease over healthy	
		Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf
1	Mancozeb	85.28 (67.54)	57.72 (49.43)	6.71	6.93	12.2	10.5	34.66	31.52
2	Thiophante methyl	82.23 (65.26)	62.20 (52.05)	6.78	7.01	13.4	11.8	33.98	30.73
3	Trifloxystrobin +Tebuconazole	31.68 (34.11)	16.38 (23.64)	9.32	9.23	55.9	47.2	9.25	8.79
4	Pyraclostrobin	77.78 (61.89)	58.35 (49.79)	7.45	7.54	24.6	20.3	27.51	25.49
5	Propiconazole	73.90 (59.54)	57.80 (49.47)	7.57	7.63	26.6	21.7	26.29	24.6
6	Hexaconazole	57.25 (49.16)	44.07 (41.53)	8.54	8.47	42.8	35.1	16.84	16.3
7	Control (water spray)	95.55 (79.75)	88.62 (70.32)	5.98	6.27			41.77	38.04
8	Healthy	0.00	0.00	10.27	10.12				
	SEm±			0.09	0.16				
	CD (P≤0.05)			0.27	0.47				
	CV %			4.73	8.24				
	T cal value			9.32* (df 26)), P ≤ 0.05					

* Figures in parenthesis are arcsine transformed values

Table 4.10. Total sugar content (mg g^{-1}) due to Cercospora leaf spot in okra leaves

S. No.	Treatments	PDI (%)		Total Sugar content (mg g^{-1})		Per cent decrease over control		Per cent decrease over healthy	
		Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf
1	Mancozeb	85.28 (67.54)	57.72 (49.43)	17.08	14.26	6.41	10.53	9.58	19.3
2	Thiophante methyl	82.23 (65.26)	62.20 (52.05)	16.64	13.89	8.82	12.86	11.91	21.4
3	Trifloxystrobin + Tebuconazole	31.68 (34.11)	16.38 (23.64)	12.33	10.08	32.44	36.76	34.73	42.95
4	Pyraclostrobin	77.78 (61.89)	58.35 (49.79)	15.22	12.94	16.6	18.82	19.43	26.77
5	Hexaconazole	73.90 (59.54)	57.80 (49.47)	14.98	11.92	17.91	25.22	20.6	32.54
6	Propiconazole	57.25 (49.16)	44.07 (41.53)	13.82	11.28	24.27	29.27	26.84	36.16
7	Control (water spray)	95.55 (79.75)	88.62 (70.32)	18.25	15.94			3.39	9.79
8	Healthy	0.00	0.00	18.89	17.67				
	SEm±			0.041	0.04				
	CD (P≤0.05)			0.12	0.13				
	CV %			1.04	1.29				
	T cal value			8.18* (df 26), P ≤ 0.05					

* Figures in parenthesis are arcsine transformed values

Table 4.11. Total chlorophyll, chlorophyll a, chlorophyll b content (mg g^{-1}) due to Cercospora leaf spot in okra leaves

S. No.	Treatments	Chlorophyll a (mg g^{-1})		Per cent decrease over healthy		Chlorophyll b (mg g^{-1})		Per cent decrease over healthy		Total chlorophyll (mg g^{-1})		per cent decrease over healthy	
		Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf	Lower leaf	Upper leaf
1	Mancozeb	0.88	0.97	27.27	26.52	0.44	0.57	61.74	48.18	1.30	1.54	44.92	36.36
2	Thiophante methyl	0.96	1.09	20.66	17.42	0.47	0.75	59.13	31.81	1.43	1.84	39.41	40.90
3	Trifloxystrobin+ Tebuconazole	1.15	1.28	4.96	3.03	1.06	1.02	7.83	7.27	2.22	2.30	5.93	4.96
4	Pyraclostrobin	0.99	1.13	18.18	14.39	0.60	0.96	47.83	12.72	1.60	2.09	32.20	13.63
5	Hexaconazole	1.04	1.14	14.05	13.63	1.00	0.97	13.04	11.82	2.04	2.11	13.56	12.81
6	Propiconazole	1.06	1.16	12.39	12.12	1.05	1.00	8.69	9.09	2.10	2.16	11.02	10.74
7	Control (Water spray)	0.73	0.79	39.67	40.15	0.41	0.44	35.65	6.00	1.14	1.23	51.69	49.17
8	Healthy	1.21	1.32			1.15	1.10			2.36	2.42		
	SEm±	0.01	0.01			0.02	0.03			0.04	0.03		
	CD (P≤0.05)	0.04	0.02			0.06	0.08			0.11	0.09		
	CV %	5.27	3.00			12.20	12.91			9.12	6.06		
	T cal value	10.42* (df 26), P ≤ 0.05				10.46* (df 26), P ≤ 0.05				10.29* (df 26), P ≤ 0.05			

* Figures in parenthesis are arcsine transformed values