CHARACTERIZATION OF *Mycosphaerella* spp. CAUSING SIGATOKA LEAF SPOT DISEASE COMPLEX OF BANANA IN KERALA AND ITS MANAGEMENT

by

Milsha George
(2015-21-009)

THESIS

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DEPARTMENT OF PLANT PATHOLOGY
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DECLARATION

I, hereby declare that the thesis entitled **"Characterization of *Mycosphaerella* spp. causing Sigatoka leaf spot disease complex of banana in Kerala and its management"** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Place : Vellanikkara
Date : 30/03/2019

Milsha George
(2015-21-009)

CERTIFICATE

Certified that thesis entitled **"Characterization of *Mycosphaerella* spp. causing Sigatoka leaf spot disease complex of banana in Kerala and its management"** is a record of research work done independently by Ms. Milsha George under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Place : Vellanikkara
Date : 30 /03/2019

Dr. Anita Cherian K.
Professor and Head and Major Advisor
Department of Plant Pathology
College of Horticulture, Vellanikkara

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Milsha George, a candidate for the degree of **Doctor of Philosophy in Agriculture**, with major in **Plant Pathology**, agree that the thesis entitled **"Characterization of *Mycosphaerella* spp. causing Sigatoka leaf spot disease complex of banana in Kerala and its management"** may be submitted by Ms. Milsha George in partial fulfillment of the requirement for the degree.

7/3/19

Dr. Anita Cherian K.
(Chairman, Advisory Committee)
Professor and Head
Department of Plant Pathology
College of Horticulture, Vellanikkara

7/3/19

Dr. Pushpalatha P.B
(Member, Advisory Committee)
Professor and Head
Banana Research Station, Kannara

7/3/19

Dr. Vimi Louis
(Member, Advisory Committee)
Professor
Banana Research Station, Kannara

7/3/19

Dr. Reshmy Vijayaraghavan
(Member, Advisory Committee)
Assistant Professor
Department of Plant Pathology
College of Horticulture, Vellanikkara

7/3/19

Dr. Deepu Mathew
(Member, Advisory Committee)
Assistant Professor
CPBMB, Vellanikkara

2.3.19

EXTERNAL EXAMINER

Dr. R. Suseela Bhai
Principal Scientist (Pathology)
Indian Institute of Spices Research (ICAR)
Calicut - 673 012, Kerala

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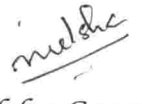

Milsha George

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Introduction

INTRODUCTION

Banana is an important fruit crop grown in many tropical and subtropical countries as a source of livelihood security and an export commodity. The multifaceted uses of banana as a source of food, fiber, fuel and for therapeutic purposes has made it a crop of wide popularity. Banana is a rich source of vitamins like vitamin A, B and C and minerals like calcium, magnesium with the trace quantities of iron and zinc. These aspects clearly place banana as one among the healthiest of fruits. In India, cultivation of banana covers an area of 860 ha with an average productivity of 30.47 million tonnes (FAOSTAT, 2017). Although the crop is grown widely under diverse agro climatic conditions, the existence of the crop is often threatened by the incidence of various pests and diseases and this limits the cultivation and production of some popular cultivars like banana var. Nendran (AAB). The diseases that pose threat to *Musa* biodiversity include leaf spot diseases, wilt, bacterial and viral diseases. Among the fungal diseases, Sigatoka leaf spot disease complex caused by *Mycosphaerella* spp. is a serious constraint to banana cultivation as it destroys the photosynthetic green leaf tissue through necrotic leaf lesions and results in an yield loss of 11% to 80% (Shanthiyaa *et al.*, 2013).

Sigatoka leaf spot disease is widely distributed throughout the banana growing tracts of the world. The occurrence of yellow Sigatoka was first reported from Java in 1902 (Zimmermann, 1902) and later in Sigatoka valley on the island of Viti Levu, Fiji (Philpott and Knowles, 1913), hence named as Sigatoka. The presence of the disease was later reported from other parts of the world such as Australia, Sri Lanka, Africa, Asia and America (Graham, 1969). In India, the occurrence of Sigatoka leaf spot disease is more prevalent in states of Kerala, Tamil Nadu, Karnataka, Maharashtra, West Bengal and Tripura where the maximum disease severity of upto 90-100% was reported on different cultivars. Among the different cultivars, banana var. Nendran (AAB), which is grown extensively in southern most states of India especially in Kerala state is more prone to the infection

of Sigatoka leaf spot pathogen (Selvarajan *et al.,* 2001).

Globally three species of related ascomycetous fungi *viz., Mycosphaerella musicola* R. Leach. Ex. J.L. Mulder (anamorph *Pseudocercospora musae* (Zimm.) Deighton), *Mycosphaerella fijiensis* M. Morelet (anamorph *Pseudocercospora fijiensis* (M. Morelet) Deighton) and *Mycosphaerella eumusae* Crous & Mourichon (anamorph *Pseudocercospora eumusae* Crous & Mourichon) have been reported as the causal agents of different Sigatoka leaf spot diseases *viz.,* yellow Sigatoka, black Sigatoka and Eumusae leaf spot respectively (Arzanlou *et al.,* 2007). Among these, yellow and black Sigatoka leaf spot diseases are considered as the major leaf spot diseases of banana driving the popular cultivars out of cultivation and thereby making banana production a less profitable enterprise. Carlier *et al.* (2000) reported the presence of Eumusae leaf spot in the South India causing an yield loss of 20 - 40 % in banana.

The symptoms produced by all the three species of the pathogens were found to be similar and confusing. The initial symptom of the disease is the appearance of pale yellow or dark brown indistinct linear streaks lying parallel to the veins on the lower leaves. In the advanced stages of the disease, these streaks enlarge to form necrotic lesions with yellow halo and light grey centres. These necrotic lesions later coalesced leading to complete drying of the leaves followed by severe defoliation which then caused delayed flowering, reduction in number of hands and fingers, premature ripening of the fingers and peel splitting of the fruits (Surridge *et al.,* 2003). The similarity of symptoms expressed by the three types of Sigatoka leaf spot diseases along with the difficulty in artificial culturing of these pathogens limits the identification of the pathogen specifically associated with each disease. Also the reports on rapid replacement of yellow Sigatoka leaf spot by black Sigatoka due to the aggressive nature of *M. fijiensis* and the presence of Eumusae leaf spot caused by *M. eumusae* in South India (Carlier *et al.,* 2000) necessitate the need to identify the pathogen specifically associated with Sigatoka leaf spot disease affecting banana in Kerala. The three species of *Mycosphaerella* infecting banana could be identified based on the differences in their base pairs using molecular

techniques (Surridge *et al.*, 2003).

Sigatoka leaf spot disease can be effectively managed by more intensive spraying schedules involving chemical fungicides and bioagents. In the wake of the recent ban of many currently recommended plant protection chemicals, there is an urgent need to identify alternate new molecules of fungicides for the management of the disease. Recently, agricultural scenario of our state is giving more thrust on organic farming and hence it is necessary to develop an effective management package integrating organic preparations, bioagents and new molecules of fungicides against leaf spot disease of banana. Apart from organic/inorganic and chemical control, breeding for resistance has been credited as the most appropriate and effective method to achieve sustainable disease management in banana.

Under these circumstances, the present study was taken up to characterize different species of the pathogen associated with Sigatoka leaf spot disease complex occurring in commercial varieties grown in Kerala, to study the basis of host plant resistance and to develop an effective package of recommendations utilizing genetic resistance, fungicides, biocontrol agents, organic and inorganic preparations for the management of Sigatoka leaf spot disease of banana. The study was undertaken in the following lines:

- Survey and symptomatology of the disease
- Isolation and characterization of *Mycosphaerella* spp. associated with the disease
- Host plant resistance and evaluation of the basis of host plant resistance in selected genotypes of banana
- Validation of markers to identify the disease resistant gene
- Management of Sigatoka leaf spot of banana using chemical fungicides and organic / inorganic preparations

Review of Literature

REVIEW OF LITERATURE

Sigatoka leaf spot disease complex caused by the three related ascomycetous fungi such as *Mycosphaerella musicola, M. fijiensis, M. eumusae* and is considered as a devastating disease which trims down the green life of banana, causing considerable reduction in yield of the crop. Yellow Sigatoka leaf spot caused by *M. musicola* was reported as the first leaf spot disease having a global impact on banana cultivation. The incidence of this disease was first reported in Java during 1902 (Zimmermann, 1902). Later during 1913 the disease had a severe outbreak at Fiji in the Sigatoka valley, hence known by the name "Sigatoka" (Massee, 1914). In India, the first report about the incidence of the disease was from Assam (Meredith, 1970).

The incidence of black Sigatoka leaf spot disease was first reported in 1964 and the symptoms of the disease were detailed by Meredith and Lawrence (1969). Jones (2000) reported the rapid replacement of yellow Sigatoka leaf spot by black Sigatoka leaf spot disease caused by *M. fijiensis* in tropical coastal regions. The presence of *M. fijiensis* inciting black Sigatoka was reported in southern states of India (Selvarajan *et al.*, 2001).

Apart from the above described yellow and black Sigatoka leaf spot diseases, the presence of another type of Sigatoka leaf spot namely, Eumusae leaf spot caused by *M. eumusae* was reported from South India (Carlier *et al.*, 2000). The incidence of Sigatoka leaf spot disease, expressing different types of symptoms are spreading at a faster rate in banana growing tracts in the state of Kerala, posing threat to banana cultivation in the state. Therefore, it is essential to characterize the pathogen associated with Sigatoka leaf spot disease complex affecting commercial cultivation of banana in Kerala, to identify the sources of resistance and to develop an integrated management package. The literature related to symptomatology, characterization of the pathogen, basis of anatomical, biochemical and molecular mechanism of defense during host pathogen interaction as well as the management of the disease has been reviewed and detailed below.

2.1. SYMPTOMATOLOGY

2.1.1. Symptomatology of yellow Sigatoka leaf spot disease

Yellow Sigatoka leaf spot disease is considered as one of the most destructive disease of banana causing huge economic loss in banana cultivation worldwide. The symptoms of the disease were described in detail by many authors.

The individual leaf spots were larger and almost spherical on leaves of young banana plants. The size of the mature spots measured 4 to 12 mm in length. Leach (1946) categorized the symptom development from specks to spots into six stages *viz.,* initial streak stage, second streak stage, third streak stage, first spot stage, second spot stage and third spot stage, whereas Klein (1960) divided these symptoms into five stages, viz., very early yellow streak, early to advanced yellow streak, early brown to advanced brown streaks and early spots and advanced spots.

The symptoms first appeared as yellow streaks on the lower surface of the leaf which later converted into brown streaks and finally into dark brown spots having greyish centres. The shape of the spot varied from oval or linear with pointed ends (Stover, 1972). The streaks first appeared along the margins of the banana leaves 14 to 21 days after inoculation (Stover and Simmonds, 1987).

The earliest symptoms were usually first noticed on the third or fourth leaf counting down from the first fully opened leaf in an active state of growth, but under favourable conditions of disease development the symptom appeared on the second leaf. Mature spot symptoms were observed on older leaves. Spotting was more common along the margins and at the apex of the leaf then spreads to the midrib (Jeger *et al.,* 1995).

Leaf tissues surrounding spots turned yellow in colour, which were initially more pronounced on the leaf margin. When the infection index was high, large areas of leaf tissue surrounding the spots turned yellow and eventually become necrotic with dark brown borders (Jones, 2000).

The effect of Sigatoka disease on the fruit is indirect and depended upon the stage of symptom development, the severity of the infection and the duration of the epidemic state of infection. The fingers of the bunches which approached harvesting stage, remained undersized and more or less angular. During severe conditions, the pulp developed a pale ochraceous colour (Wardlaw, 1939).

2.1.2. Symptomatology of black Sigatoka leaf spot disease

Black Sigatoka disease is caused by *M. fijiensis* which spreads at a faster rate replacing *M. musicola* and are considered as the dominant and most serious leaf spot pathogen of banana. Though the incidence of black Sigatoka leaf spot disease was first reported in 1964, the symptoms of the disease were detailed by Meredith and Lawrence (1969). The symptom expression on Cavendish group of banana were categorized into six stages such as initial speck stage, first streak stage, second streak stage, first spot stage, second spot stage, third or mature spot stage (Meredith and Lawrence, 1969).

Foure (1985) redefined the symptoms described by Meredith and Lawrence. Accordingly diploid (AA) and triploid (AAA) *accuminata* groups infected with the disease initially produced whitish or yellowish mark on the lower surface of the leaf which could be visible when held against the sunlight. These marks later turned to reddish brown speck. In the second stage of disease development, the reddish brown streaks appeared on both surface of the leaves. During the next stage, the streaks became wider and changed the colour from red to dark brown colour. In stage 4, the spot appeared as dark brown on the under surface while black on the upper surface. These black spots were surrounded by yellow halo and became slightly depressed in the fifth stage. During the last stage, the centre of the lesions became whitish to grey surrounded by dark brown to black border.

Fullerton (1994) reported that initial symptom of the disease appeared 14 - 20 days after infection of the pathogen. The time period between each developmental stage varied in length according to the cultivar and the severity of

infection. The disease was found to be more severe in susceptible varieties when compared to resistant cultivars where the symptom development was too slow. Marin *et al.* (2003) observed that under favorable conditions the incubation periods of *M. fijiensis* was short as 13-14 days in susceptible host and during unfavourable conditions it extended upto 30 days.

2.1.3. Symptomatology of Eumusae leaf spot disease

Carlier *et al.* (2000) reported the occurrence of a previously undescribed leaf spot disease on banana from southern India, Sri Lanka, Thailand, Malaysia, Vietnam, Mauritius, and Nigeria. The causal agent of disease was identified as *M. eumusae* with *Septoria eumusae* (changed to *Pseudocercopsora* eumusae) as its anamorph.

The symptom initially appeared as faint brown streaks on both surface of the leaves. During the next stage of symptom development, the streaks turned oval or elliptical spots with greyish centre surrounded by well defined dark brown margin. These spots under high infection density, coalesced forming large necrotic areas leading to complete drying of the leaves (Crous and Mourichon, 2002).

Selvarajan *et al.* (2001) reported the variation of symptom expression on four commercially grown cultivars in India. On banana var. Nendran the disease initiated as brown thin streaks of size 6 x 3 mm to 30 x 5 mm appeared on the under surface of the leaf. Later these streaks converted to chain of oval spots spreading from the tip towards the base of the leaf. The mature spots were with greyish centre and light black margin surrounded by prominent bright yellow halo. On banana var. Rasthali, the symptoms developed as light brown streaks on the lower surface of the leaves which then turned to black colour on both surfaces of the leaf. In case of mature spots, grey centre was light in colour and had no distinct margin. In advanced stages of disease development, the spots were coalesced giving a blighted appearance to the crop. While on banana var. Robusta, light brick reddish brown streaks appeared on the lower surface of the leaf corresponding to which translucent mild yellow streaks were observed on the upper leaf surface. The mature spots

turned light grey to bright grey with definite black margin surrounded by yellow halo. On banana var. Poovan, two types of mature lesions were observed. The mature lesions either appeared as bright grey centre surrounded by black margin without prominent yellow halo or with light grey centre having light brown to black margin and clear yellow halo.

Amani and Avangyan (2014) observed that the symptoms initiated as faint brown streaks on the lower surface of the leaves which later developed into oval shaped lesions with greyish centers with definite dark brown margins. When the infection density was higher these spots coalesced causing complete drying of the leaves.

2.2. CHARACTERIZATION OF THE PATHOGENS

Mulder and Stover (1976) reported that the three related ascomycetous fungi inciting Sigatoka leaf spot disease complex, were distinguished based on the morphological differences of conidia and condiophores, while the morphological characters were found to be similar among the three species of *Mycosphaerella* spp.

Stover (1980) reported that *M. musicola* could be distinguished based on the presence of stroma at the origin of conidiophores, which was found to be absent in *M. fijiensis* as well as in *M. eumusae.* He also reported that morphological characters of *M. fijiensis* and *M. eumusae* were found to be similar except the presence of occasional septations on the conidiophores of the *M. eumusae.* Also, the size of sporodochia, conidiophores and conidia of *M. eumusae* were found to be smaller in size as compared with other two species.

2.2.1. Morphological characterization of *M. musicola*

Perham (1935) reported the imperfect stage of the pathogen inciting yellow Sigatoka leaf spot disease on banana as *Cercospora musae* Zimm. and observed the occurrence of irregular rounded bodies of varying size, while artificially culturing which might be the immature perithecia of the perfect stage of the fungus. Leach (1941) demonstrated the presence of the ascospore stage of *C. musae* in

Jamaica, hence the fungus was renamed as *M. musicola* Leach.

The mature spots were characterized by the presence of perithecia on the upper surface of the leaf. The perithecia were dark brown or black having short protruding ostiole. The size of the perithecia varied from 36.8 µm to 72.0 µm. Inside the perithecia, asci were present which were septate, hyaline and obtuse ellipsoidal with the upper cell slightly broader than the lower measuring 14.4-18.0 µm x 3-4 µm in size (Leach, 1941).

Stover (1962) reported from Honduras that perithecia and sporodochia of *M. musicola* were produced when the temperature dropped below 21°C. Fulton (1962) reported that the discharge of ascopsores occurred from necrotic banana leaf tissue which had been moist for 10 h.

Under favourable conditions the emergence of conidiophores were observed as first brown spot stage of the disease. The studies carried out by Meredith (1970) revealed that the development of the sporodochia occurred in the substomatal air chamber and the conidiophores originating from the sporodochia grew through the stomatal pore in a tuft like fashion. When more number of conidiophores emerged, the sporodochia became erumpent causing disruption of the guard cells.

The presence of spermagonia was observed on leaves just before the spots reached maturity. Spermagonia appeared as small, black, immersed flask shaped structures measuring 46.77 µm x 37.63 µm. The reproductive cells *i.e.,* spermatia were very minute, hyaline cells formed as chains inside the spermagonium. The spermatia were ejected from the spermagonium to outside through the ostiole present at its apex (Meredith and Lawrence, 1969).

Conidiophores were pale to olivaceous brown coloured either straight or slightly curved. The condiophores were rarely branched without any septations or conidial scars. Conidia are usually 2 to 5 septate or more and usually measured 10 - 80 µm x 2 - 6 µm (Mulder and Holliday, 1974). Flask shaped spermagonia and spermatia were produced by many isolates, but the sexual fruiting body

perithecia were not observed in culture which might be due to the heterothallic nature of these fungi (Mourichon *et al.*, 1990).

2.2.2. Morphological characterization of *M. fijiensis*

Like *M. musicola,* the conidiophores of the pathogen emerged from substomatal cavity bearing multiple conidia from single conidiophores. Conidiophores were pale to light brown coloured with 0 - 3 septate, occasionally branched and produced prominent spore scars. Conidia were obclavate or cylindroclavate with one to six septae having distinct basal hilum or scar. Both the conidiophores and conidia were produced on the lower surface of the leaves at first streak stage (Meredith and Lawrence, 1969).

The anamorphic and telomorphic stages of *M. fijiensis* were found to be concurrently present on the infected leaves. The germination of both the conidia and ascospore were observed when the temperature was low and relative humidity was high (Jacome *et al.*, 1991).

Earlier, the anamorphic stage of the pathogen was described as *Paracercospora musae* (Zimmerm.) Deighton but Crous *et al.* (2002) sequenced the rDNA of the internal transcribed spacer (ITS) region of the fungus and revealed that *Paracercospora* is synonymous with *Pseudocercospora*, hence *Pseudocercospora fijiensis* (Morelet) Deighton is considered as the anamorphic stage of *M. fijiensis.*

Zapater *et al.* (2008) concluded that the telemorphic stages of the three species of *Mycosphaerella* were found to be very similar and the separations of the taxa at the species level were based on the difference on their conidial morphology. The fruiting body of the fungi was identified as perithecia. The perithecia of *M. fijiensis* closely resembled to that of *M. musicola* except the ascospores were singly septate and germinated from both polar ends, with each germ tube parallel to the long axis of the spore.

The spermatia or the male gametes of *M fijiensis* were observed during the

second streak stage on the under surface of the leaves. The male gametes were produced inside the spermagonia and were hyaline, rod shaped and single celled. Under favourable conditions these spermatia were released from the spermagonia to outside through the ostiole of the spermagonium (Liberato *et al.*, 2009).

2.2.3. Morphological characterization of *M. eumusae*

Jones (2000) reported that the pathogen produced two distinct types of fruiting bodies, which were observed more on the upper surface of the lesions. The first structure was identified as flasked shaped pycnidia which was ostiolated when young while acervuli like when mature. The second structure was identified as perithecia which were globose in shape with short protruding ostiole containing oblong asci bearing two celled ascospores.

Carlier *et al.* (2000) described the conidial morphology of *M. eumusae* and observed two types of fruiting structures *i.e.,* pycnidia and perithecia which were closely associated with the lesions caused by *M. eumusae*. These fruiting bodies were more prevalent in lesions on upper leaf surface than on the lower surface. Pycnidia were pear-shaped measured about 31 to 42 µm wide, embedded inside the leaf tissue, more or less erumpent and ostiolated when young but acervuli like when matured. Fusiform, hyaline conidia which were three to five septate and measured between 21.2 and 41.6 µm long were observed in the pycnidia. Based on the characteristics of this imperfect stage the fungus was identified as *Septoria*. The sexual fruiting body, perithecia were dark brown measured 42 to 51 µm in diameter, amphigenous, scattered and globose, with a short protruding ostiole. These were with well-defined dark wall and contained oblong asci. Ascospores were two-celled and measured about 12.0 to 16.5 × 3.0 to 4.5 µm based on the following characters the perfect stage of the fungus were described as *Mycosphaerella.*

Udugama (2002) reported that the anamorphic stage of *M. eumusae* were characterized by the non septate conidiophores and found to be shorter when compared with *C. musicola* and *C. fijiensis*. The conidia were produced from the apex of the conidiophores. The conidia were hyaline with 0-5 septae and boarder

than the other two species of *Cercospora.* The telomorphic stages produced two distinct types of fruiting bodies.

Crous and Mourichon (2002) reported that the telomorphic stage produced pseudothecia. The ascospores of *M. eumusae* possessed mucoid sheath but failed to display the germ tube distortions but had three to four parallel or lateral germ tubes which was a distinguishing characteristic of *M. eumusae* (Arzanlou *et al.*, 2008).

Amani and Avagyan (2014) reported that the asexual fruiting body pycnidia were found to be closely associated with the lesions of the infected leaf samples. The young pycnidia were ostiolated which measured about 30 – 40 µm wide. The conidia were hyaline with 3 – 5 septations and 21 – 40 µm long.

2.2.4. Cultural Characterization of the Pathogens

Meredith and Butler (1939) was the first to observe sporulation of *C. musicola* in banana leaf agar medium. Later, Leach (1941) based on his observations on banana leaf infected with *C. musae* concluded that the perfect stage of the pathogen as *M. musicola.*

Calpouzos (1954) tested the growth of *C. musicola* in six different media, *viz.*, mycophil agar, potato dextrose agar, corn meal agar, enriched synthetic medium, mineral salt and glucose with no growth factors added and plain agar with pH approximately 6.2 and observed that the pathogen showed good growth on mycophil agar, potato dextrose agar (PDA), fair amount of growth on corn meal and on enriched synthetic agars and no growth on non-enriched synthetic medium and plain agar.

Meredith and Lawrence (1970) observed the growth of *C. musicola* on potato dextrose agar and V-8 juice agar. They observed that on PDA *C. musicola* produced small, pale to medium grey colonies after two to three days of incubation at 26°C which later turned dark green to black within 10-12 days of incubation, while the colonies remained pale to medium grey on V-8 juice medium.

On PDA, *C. musicola* produced aerial mycelium in clumps from twisted hyphae with numerous short side branches. Stromata was absent in culture while dense fascicles of conidiophores consisting of conidia were observed after 2 to 3 days of incubation. As colonies grew older, these became raised, lobed and irregular. The centre of the colony were hallow with outer shell made of thick black rind of dumb bell shaped hyphae. Small colonies with 2.0 cm diameter were noticed after 60 days of growth of the pathogen on PDA (Meredith and Lawrence, 1970).

According to Jones (2000), *C. musicola* produced colonies within 4 to 6 days after inoculation with ascospores or conidia on the agar medium. He also reported that the growth of the pathogen was slow and required an optimum temperature of 25°C for the growth. The centre of the colony was raised with unevenly folded margins. The colours of the colonies were white, light to dark grey, pink and dark green.

Many researchers reported about the colony character of *M. fijiensis* on different media. Stover (1980) reported that *M. fijiensis* produced two types of colonies in culture with dark grey or grey brown colony with crenate edge and pale grey or pink colony. The former produced more number of conidia and became unstable with time while the latter produced few conidia and was stable in culture.

Leiva - Mora *et al.* (2010) studied the growth of *M. fijiensis* on nine culture media *viz.,* carboxymethyl cellulose medium (CMC) at 1 % pH 6.8, filter paper wetted with sterile distilled water, filter paper wetted with 2 ml of $CaCO_3$ solution at 3 %, potato dextrose agar (pH 5.6), agar-mycophil 3.6 % (pH 5.6), water agar 3 % (pH 5.6), agar poor synthetic nutrient medium (pH 5.6), potato carrot agar (PCA) (pH 5.6) and V-8 (pH 6.8) and concluded that enchanced conidial production were obtained on potato carrot agar and V-8 media while in filter paper, filter paper with $CaCO_3$ and agar poor synthetic nutrient medium had lower conidial production.

M. fijiensis produced greenish grey or pink colour colonies in culture. The colonies were raised to hemispherical, velvety in appearance with dark stromatic

mycelia which were compact and slow growing (Mourichon and Fullerton, 1990). Rivas (2001) reported that nutritional supplement components such as inorganic and organic charcoal, nitrogen source, microelements like Ca, Mn, Zn, Cu, Mo, Fe and quantities of vitamins stimulated sporulation of *M. fijiensis*. Crous and Mourichon (2002) reported that colonies of *M. eumusae* on PDA appeared pale olivaceous grey to rosy vinaceous on the upper surface and brown vinaceous on the lower surface. The colony produced aerial mycelium with uneven margins and obtained a diameter of 10mm after 2 months of incubation at 25^0C.

Carlier (2002) observed that the cultures of *M. eumusae* were initiated from single *Mycosphaerella* ascopores ejected from perithecia. The pathogen produced black stroma-like structures in culture and appeared similar to cultures of *M. fijiensis*. He also observed that the pathogen produced numerous conidia and pycnidial structures in modified V-8 juice medium. The conidia produced hyaline, straight or slightly flexuous, three to five celled conidia.

2.2.5. Molecular characterization of pathogens

The ribosomal DNA genes are highly conserved and possessed characteristics that were suitable for the detection of pathogens at the species level. This region contains geneic and non geneic or spacer regions and each repeat unit consisted of a copy of 18S, 5.8S and 28S like rDNA and two spacers, the internal transcribed spacer (ITS) and intergenic spacer (IGS). Variation within the rDNA repeat units can be used to measure intraspecific divergence (O'Donnell, 1992). The rDNA genes had been employed to analyze major evolutionary events as it is highly conserved, whereas, the rDNA internal transcribed spacer (ITS 1 and ITS 2) is more variable so that it had been used for the investigation of the species level relationship (Bruns *et al.,* 2001) and used in classifying fungal species due to its systemic and taxonomic usefulness.

Johanson and Jeger (1993) reported that polymerase chain reaction (PCR) of DNA isolated from the infected leaf tissue is the most reliable technique used for the characterization of pathogen associated with yellow and black Sigatoka disease

of banana and plantain. Two 21- base oligonucleotide primers *viz.*, MF137 and MM137 were synthesized from the variable region of the internally transcribed spacer (ITS) 1 region of ribosomal DNA (rDNA) of *M. fijiensis* and *M. musicola*. MF137 in association with R635 (from a conserved sequence of the rDNA) primer yielded an amplification product of 1000bp in *M. fijiensis* and MFF137 along with R635 produced an amplified product of approximately 1000bp with DNA from *M. musicola*. Though primers amplified similar sized fragments from the DNA isolated from banana leaf tissue infected with *M. fijiensis* and *M. musicola*, the fungal origin of these fragments were confirmed by Southern hybridization analysis.

Johanson (1994) reported that random amplification of polymorphic DNA (RAPD) by polymerase chain reaction (PCR) can be used as efficient tool to differentiate and identify isolates of *Mycosphaerella* species which caused Sigatoka disease of banana in Southeast Asia. The pathogen *M. musicola* and *M. fijiensis* produced distinct RAPD banding patterns when amplified with specific primers. *M. fijiensis* produced an amplicon of 1250bp when amplified with RC07 primer while *M. musicola* produced RAPD fragment of 1180bp when amplified with PM06 primer.

Larena *et al.* (1999) reported that the primers ITS 1F and ITS 4R could efficiently amplify the internal transcribed spacers (ITS) of the ribosomal DNA (rDNA) of ascomycetous fungi at an annealing temperature of 62°C and 64°C thereby detecting the pathogen at species level. Their studies also revealed that the primer provided information about affinities of many mitosporic fungi with their perfect stage.

Carlier *et al.* (2000) based on ITS sequences had developed a molecular diagnostic method for identification of banana leaf spot pathogens using digestion of PCR amplified ITS regions with restriction enzymes. ITS sequence analysis of *M. eumusae, M. fijiensis*, *M. musicola* and one isolate each of *M. musae* and *Phaeoseptoria musae* produced PCR product between 530 and 550bp using ITS 1

and ITS 4 primers. Phylogenic analysis of the ITS sequences of *M. eumusae, M. musicola, M. fijiensis, M. musae* and *Phaeoseptoria musae* confirmed that all these pathogens were different at species level and showed >98% similarity to that previously published for other species.

The phylogenetic analysis of ITS 1, 5.8S, ITS 2 were used for the separation of three species of *Mycosphaerella* spp. *M. musicola, M. fijiensis* and *M. eumusae* inciting Sigatoka leaf spot disease complex in banana and plantain. ITS sequence data obtained was sufficient to distinguish pathogens which belonged to morphologically similar taxa (Crous *et al.,* 2002).

Arzanlou *et al.* (2007) developed an optimized qualitative and quantitative molecular detection technique for the three dominant *Mycosphaerella* spp. causing Sigatoka leaf spot complex on banana. They designed species specific primers based on sequence data of β-tubulin gene could differentiate *M. fijiensis, M. musicola* and *M. eumusae* from each other and also from other fungal species commonly infected on banana leaves without any undesired cross-reaction. The selective primers yielded an amplicon of ≤142 and were monitored by the level of fluorescence.

2.3. SOURCE OF RESISTANCE TO SIGATOKA LEAF SPOT PATHOGENS

Screening trials undertaken with the wild species in the *Eumusa* sections of *Musa* revealed that *M. schizocarpa, M.balbisiana, M. accuminata* ssp. *malaccensis, microcarpa, siamea* and *truncate* were highly resistant to Sigatoka leaf spot disease (Ceesman and Wardlaw, 1937).

The banana accessions belonging to the genotype AA group varied in their response to the diseases. Among the 180 accessions screened against Sigatoka leaf spot disease 45 were highly resistant, 25 were partially resistant and 110 were susceptible to the disease (Vakili, 1968).

Stover and Simmonds (1987) based on their study concluded that the banana genotypes belonging to ABB group were highly resistant to Sigatoka leaf spot disease which was due to the high 'B' genomic content. The general rule is that higher the B genomic content, greater is the resistance to Sigatoka leaf spot disease.

KAU (1978) reported that varieties like Chenkadali, Sanna chenkadali, Pisang Lilin, Paka, Tongat, Adakkakunnan and Thiruvanathapuram were found to be tolerant to Sigatoka leaf spot disease.

All wild *Musa acuminata* except few, were found to be either highly resistant or resistant to both *M. musicola* and *M. fijiensis.* Rowe (1984) reported that several sub species of wild *M. acuminata* showed high levels of resistance against black Sigatoka disease and used to develop resistant bred diploids.

Jones and Monteel (1988) observed that eight accessions of banana collected from Papua New Guinea were resistant to *M. fijiensis,* whereas *Musa balbisiana* were highly resistant to both *M. musicola* and *M. fijiensis* at all conditions.

Montel (1989) reported that diploids (AA) and triploids (AAA and AAB) were considered to be highly susceptible to *M. musicola* though their parents *Musa acuminata* and *M. balbisiana* were highly resistant to yellow Sigatoka disease.

Estelitta *et al.* (1991) screened various accessions in the banana germplasm against Sigatoka leaf spot disease and concluded that varieties like Pisang Lilin, Sanna Chenkadali, Manoranjitham and Thiruvanathapuram were resistant to Sigatoka leaf spot disease.

Fullerton and Olsen (1994) tested the disease reactions of selected *Musa* genotypes against *M. fijiensis.* Their studies revealed that sixty three strains of *M. fijiensis* obtained from Papua New Guinea and the Pacific were susceptible to genotypes such as SF215, 11-249 ('Saimea'), Grande Naine while resistant to T8

and Calcutta 4. Some strains of the pathogen exhibited susceptible reaction on juvenile plants of genotypes Paka and Pisang Lilin.

Mourichon *et al.* (1997) reported that *M. musicola* (yellow Sigatoka) attacks only bananas and not plantains while *M. fijiensis* (black Sigatoka) attacks both bananas as well as plantains. Under favourable conditions of disease development cultivars such as Yangambi (AAA) and Sweet Plantain (AA) had short incubation period when compared to other cultivars.

Cherian *et al.* (2002) studied the disease reaction of various accessions maintained in the field gene bank of Banana Research Station (BRS), Kannara and observed the disease reactions of various cultivars against Sigatoka leaf spot disease. Among the various accessions screened, varieties like Calcultta 4, Pisang Lilin, Chenkadali, Sanna Chenkadali, Paka, Tongat, BRS 1 and BRS 2 *etc.*, were found to be tolerant to Sigatoka leaf spot diseases. Most of the cultivars belonging to AAB group were either resistant or moderately resistant to the disease.

Compatible interactions of *M. fijiensis* with host (banana) were characterized by rapid development of disease from stage 1 to necrosis followed by sporulation of the pathogen whereas in incompatible interactions, development of symptoms was blocked without any sporulation. The banana variety Grand Naine (AAA) were found to be highly susceptible to the disease while varieties like Calcultta 4, Pisang Lilin were resistant to infection of the pathogen. Some banana varieties such as Pisang Berlin, Pisang Mas, Pisang Ceylan (AAB, subgroup Mysore) and Fougamou (ABB, sub-group Pisang Awak) showed partial resistance by slowing down the disease development time with low sporulation (Mourichon and Fullerton, 1990).

The studies on disease reaction of twelve morphotypes of French plantain cultivar (*Musa* AAB) against Sigatoka leaf spot revealed that Manjeri Nendran II was the least susceptible morphotype with maximum yield while Changalikodan was the most susceptible morphotype (Cherian *et al.*, 2007). Churchill (2010) reported that *M. fijiensis,* the pathogen inciting black Sigatoka leaf spot disease in

banana caused a major yield loss in Cavendish or AAA subgroup.

2.3.1. Anatomical basis of resistance

The ability of the host plant to defend against the attack by a pathogen is termed as plant disease resistance. The plant protects itself from pathogen either by pre formed structures and chemicals or by infection induced responses of the immune system. The pre formed structures or the anatomical structures of the plant provide the initial basis of resistance against the pathogen.

2.3.1.1. Role of cell wall in disease resistance

Kosack and Jones (1996) reported that fortification of the cell wall in gerbera could increase the resistance of the plants against the attack of the pathogens. Their studies concluded that sealing of the cell wall could prevent the growth of extracellular biotrophs such as *Pseudomonas syringae* or *Cladosporium fulvum* by blocking the leakage of cytoplasmic contents, thereby reducing nutrient availability for the pathogens. Similarly the toxins and enzymes released by the necrotrophic pathogen *Botrytis cinerea* could not reach the sensitive plant cells thereby reducing the lesion formation upto 80%.

Vorwerk *et al.* (2004) reported that the high degree of structural complexity of plant cell wall polysaccharides in plants play a major role in host defense of host during host pathogen interaction. They also observed that the cell wall components elicit plant defense responses through signals that are released during the pathogen infection.

2.3.1.2. Role of epidermis and stomata in disease resistance

The waxes, cutin acids, ethanol soluble and water soluble fractions present on variously aged cotton bolls exhibited fungistatic action against nine species of fungi associated with boll rots. Wang and Pinckard (1973) observed that the thickness of the cuticle, wax quantity and the amount of cutin acids were found to be the highest in cotton bolls of intermediate age which accounts for high level of

resistance of bolls of this age to fungal penetration.

Jorgensen and Mortensen (1977) reported that the germination percentage of conidia on epidermal layers of barley by the pathogen *Erysiphe graminis f. sp. hordei* were found to be very less in resistant varieties when compared to susceptible varieties.

Rijkenberg *et al.* (1980) based on the studies on tomato fruits concluded that the varieties with thicker cuticle prevented infection process of *Botrytis cinerea*, the pathogen inciting gray mold disease in tomato.

Knogge (1996) observed that the enzyme cutinase was inhibited either by the chemical inhibitors or by cutinase specific antibodies which prevented the infection of *Nectria haematococca* (*Fusarium solani f. sp. pisi*) on pea stem.

Trujillo *et al.* (1997) conducted anatomical studies on leaf blades of four cultivars of *Musa* sp. showing varying levels of resistance to *M. musicola* causing yellow Sigatoka leaf spot of banana and reported that the resistant cultivars contained a single abaxial epidermal layer with rectangular thin walled cells, the hypodermis with two or three layers of cells having thick walls with low stomatal density and several layers of spongy and mesophyll tissue.

Mahajan and Gill (1998) reported a positive correlation between downy mildew of cauliflower and the number of stomata. They observed that sporulation and stomatal count were more on the dorsal surface of the leaves. The plants with more number of stomata were highly susceptible to *Perenospora parasitica*, the causal agent of downy mildew in cauliflower.

Anatomical barriers of twenty two muskmelons genotypes were screened against downy mildew pathogen (*Pseudoperenospora cubensis*) by Inder *et al.* (1999) and noticed that a significant correlation existed between disease resistance with stomatal and trichome size. They observed that epidermis and cuticle thickness were greater in resistant varieties than susceptible varieties.

Dagade (1999) reported that the resistant genotype *Piper colubrinum* had compact arrangement of cells with small epidermal, mesophyll and spongy parenchyma cells. They also possessed thick epiderm with large and less number of stomata while susceptible genotype Panniyur 1 possessed thin palisade and collenchymas tissues, small vascular bundles, thin epidermis with small cells and numerous numbers of stomata.

The mechanism of structural and biochemical basis resistance of groundnut genotypes resistant GPBD- 4 (resistant), DH-22 (resistant), K-134 (moderately resistant), R-8808 (moderately resistant), KRG-1 (susceptible) and TMV-2 (susceptible) to *Puccinia arachidis* were studied in detail by Sunkad and Kulkarni (2006). They concluded that the resistant and moderately resistant genotypes were characterized by higher epidermal cum cuticular thickness and high wax content with less number of epidermal cells and stomata. Bessire *et al.* (2007) reported that the plant cuticle composed of cutin, a lipid-derived polyester and cuticular waxes arrested the growth of both *Botrytis cinerea* and *Sclerotinia from inducing infection in Arabidopsis plant.*

Homa *et al.* (2016) evaluated twenty *Ocimum* cultivars including sweet cinnamon (*O. basilicum*), clove (*O. basilicum*), citrus (*O. africanum* syn. *O. citriodorum*), spice (*O. americanum* syn. *O. canum*) and holy basils (*O. tenuiflorum* syn. *O. sanctum*) for susceptibility to downy mildew and concluded that sweet basils were determined to be the most susceptible, cinnamon, clove and Thai types were moderately susceptible while citrus, spice and holy types were least susceptible to downy mildew. They also observed that basil species possessed higher stomatal densities, shortest stomatal lengths and greatest downward leaf curvature were highly susceptible to disease incidence.

2.3.1.3. Role of mesophyll tissues in disease resistance

Lazorovits and Higins (1976) observed that the mode of penetration of *Cladosporium fulvum* race 1 was same in susceptible, resistant and immune varieties of tomato but the development of mycelia was slower in resistant varieties.

They also reported that fungal development was restricted to few cells of mesophyll tissue in both resistant and immune variety due to extensive deposition of callus like material around the mesophyll tissue during the attack of the pathogen.

Harder *et al.* (1979) reported that the primary leaves of wheat containing resistant gene *Sr5* and *Sr6* showed incompatible reaction to avirulent races of stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn.).

The interaction of barley (*Hordeum vulgare*) with powdery mildew pathogen *Blumeria graminis* caused H_2O_2 accumulation in mesophyll cells which in turn induce the production of glutathione leading to hypersensitive response in barely plants thereby arresting the growth of the pathogen (Vanacker *et al.,* 2000).

2.3.1.4. Role of vascular tissue in disease resistance

The studies conducted by Olson and Varner (1993) revealed that the production of hydrogen peroxide in the cells during host pathogen interaction caused the lignifications of tracheary elements, phloem fibres and some epidermal cells thereby preventing the entry of the pathogen into the host cell.

The stems of susceptible and resistant cassava plants were cytologically investigated for their defence reaction against *Xanthomonas campestris* pv. *manihotis.* Lignification and suberization with callose deposition of xylem and phloem acted as the barrier mechanism that limited the bacterial growth within the infected plants (Kpemoua *et al.,* 1996).

Wasternack *et al.* (2006) reported that the synthesis of jasmonic acid in the vascular bundles of tomato plants which in turn produced induced systemic resistance in tomato plants.

Lopez *et al.* (2007) noticed the accumulation of Mir1-CP (a novel defense cysteine protease) in the vascular tissues of resistant maize genotype Mp708 after 24 h of infection by the pathogens which in turn confers resistance against the entry

of the pathogen.

Cohen and Moshelion (2012) reported that the bundle sheath tissues surrounding the vascular system function as pipe that acted as a solute transport regulating barrier which prevented excessive quantities of toxins of pathogen from entering the leaves by pushing the toxins towards the hydathodes.

2.3.2. Biochemical basis of resistance

The resistance offered by the plant to the pathogen is not only associated with anatomical difference but also to biochemical, physiological and genetic basis (Walker and Stahmann, 1955). The host plant exhibited an array of biochemical conditions and reactions to prevent the spread of disease when the host is attacked by the pathogen (Bell, 1981). He also noticed that the biochemical mechanism were present in plants even before the attack of the pathogen, but became activated only upon infection by a pathogen.

During host pathogen interaction, certain antimicrobial compounds were produced at the site of penetration of the pathogen which resistant the entry of the pathogen to the host. The magnitude of accumulation of the antimicrobial components inside the plants varied with the cultivar – parasite combinations (Bailey and Mansfield, 1982). Therefore, it is necessary to understand the physiological and biochemical basis of resistance offered by the plant to the invasion by the pathogen.

2.3.2.1. Role of phenols in disease resistance

Musa balbisiana variety Kattuvazhi, which is resistant to *Cercopsora musae* contained slightly more total phenol content than the susceptible *Musa paradisiaca* var. *monthaniana* (Jayapal and Mahadevan, 1968). They also noticed an increase in phenolic compounds in the resistant variety in response to the attack of the fungus.

Howell *et al.* (1976) reported that an aromatic ring bearing a hydroxyl

substituent *i.e.,* phenolic substances play a major role in plants defense by acting as protective compounds against disease agents such as fungi, bacteria and viruses. They also noticed that the resistant varieties had more phenolics than that of the susceptible varieties to defend itself against the pathogen.

Chattopadhyay and Bera (1980) reported that the phenols and polyphenol oxidase activity were increased in rice leaves infected with *Helminosporium oryzae.* In resistant variety CH13, the polyphenol oxidase converted phenols to quinones which were responsible for the resistance in CH13 than the susceptible variety Benibhog.

Matern and Kneusel (1988) concluded that the first line of defense in plant against the pathogens were by the rapid synthesis of phenolics and their polymerization in the plant cell wall. Friend (1979) reported that the phenolic compounds produced in the potato tuber during the attack of *Phytophthora infestans* possessed antimicrobial activity thereby acted as a protective component against the pathogen.

Nicholson and Hammerschmidt (1992) reported that phenols occurred constitutively in plants and were associated with non host resistance. The phenols formed in the host plants during the entry of the pathogen possessed a role in the active expression of resistance in the host against the specific pathogen.

Paul (1998) noticed that during the interaction of *Ralstonia solanacearum* with chilli and brinjal, there was an increase in levels of phenolic content in resistant varieties than in susceptible ones.

Beckman (2000) proposed a relationship between the location of phenols storing cells within plant tissue and the host defense. The phenols were synthesized by specialized cells in plants and stored them in their vacuoles (distributed within most of the tissues) during the normal processes of differentiation. At the site of penetration of the wilt pathogen, the cells storing phenols undergo decompartmentation causing the rapid oxidation of their phenolic content thereby

causing lignification and suberization of cells and cell death which seal off infections or injuries at the site. He also observed that if the first mechanism of defense failed and the stress still persisted, these processes promoted the prolonged build up of IAA and ethylene thereby providing peridermal defense.

Banu (2001) reported that the total phenolic content were high in resistant banana variety Manoranjitham when compared with the highly susceptible variety Grand Naine before the attack of *C. musicola* but a gradual decrease in phenolic content was noticed in the resistant variety following the attack of the pathogen, whereas an increased level of phenolic content was observed in Grand Naine variety.

Dagade (2003) conducted a study to estimate the total phenol content present in different genotypes of *Piper* spp. and concluded that higher content of total phenolics were present in *P. columbrinum* which is highly resistant to foot rot disease and low content of phenolics was noticed in susceptible genotype Panniyur 1 whereas in tolerant genotype Kalluvally the total phenolic content were expressed in medium values.

Bera *et al.* (2004) reported that the total phenol content was higher in groundnut varieties that were resistant to the attack of *Cercospora* pathogen. Similarly Singh *et al.* (2009) noticed higher amount of phenols in resistant varieties of *Brassica* spp. when attacked by downy mildew and white rust pathogen.

Takahama (2004) reported that the phenolics undergo oxidation or reduction forming quinones which was involved in resistance in plants as they are highly toxic to microorganism. They also noticed that the phenolic compounds present on the brown scales of the onion bulbs produced hydrogen peroxide by autoxidation thereby exhibiting hypersensitive response to the pathogen.

2.3.2.2. Role of sugars in disease resistance

Horsfall and Diamond (1957) reported that the plant tissues having low sugar content were more susceptible to diseases than plants having high sugar

content. Niesh (1964) concluded that a reduction in sugar content of the plants infected with the pathogen, which might be due to the synthesis of poly phenols from sugars.

Easwaran (1967) noticed a high content of reducing sugar in both susceptible and moderately susceptible variety of sorghum against bacterial wilt pathogen. The inoculation of the pathogen led to reduction of the sugars in both the varieties.

The study conducted by Jayapal and Mahadevan (1968) revealed that the resistant banana leaves of Kattuvazhi contained higher amounts of reducing sugars than the susceptible Monthan varieties. Inoculation of the pathogen *C. musae* on both varieties led to the decrease in amount of reducing sugars while the non reducing sugars was more in resistant variety than susceptible one.

Abraham (1986) reported that the betel vine cultivars resistant to bacterial leaf spot pathogen showed higher levels of reducing, non-reducing and total sugars when compared to the susceptible cultivars.

Veermohan *et al.* (1994) noticed that the capsicum leaves infected with *Alternaria solani* showed decreased amount of reducing, non reducing and total sugars with decrease in photosynthetic efficiency and content of the chlorophylls.

The chilli and tomato varieties resistant to bacterial wilt pathogen possessed higher levels of soluble sugars (Paul, 1998). Mahajan (1999) observed a positive correlation between reducing sugar and downy mildew disease incidence in cauliflower.

Bera *et al.* (1999) reported the presence of higher level of soluble sugars in *Cercospora* resistant genotype of groundnut which was absent in susceptible cultivars. Singh *et al.* (1999) concluded that the reducing sugars present in rape and mustard contributed significantly to disease resistance to Alternaria blight.

Banu (2001) reported that before the incidence of *C. musicola* on resistant variety Manoranjitham higher quantities of non reducing and total sugars were

present when compared with the highly susceptible variety Grand Naine though reducing sugar was higher in Grand Naine. After the inoculation of the pathogen, their occurred a reduction in non reducing and total sugars in resistant variety.

Qin *et al.* (2013) observed that in higher plants during infection, the pathogen uses sugars present in the plants for their own needs which in turn forced the plants to modify their sugar content thereby offering resistance against the pathogen.

Morkunas and Ratajczak (2014) reported that the presence of high levels of sugars in plants during plant fungal interaction enhanced plant resistance against the pathogen. Sugars present enhanced oxidative burst at early stages of infection, increasing lignification of cell walls, stimulate the synthesis of flavonoids and induce certain PR proteins. Some sugars also acted as priming agents inducing higher plant resistance to pathogens.

2.3.2.3. Role of defense related enzymes in disease resistance

2.3.2.3.1. Peroxidase

Bell and Mace (1981) revealed a positive correlation between peroxidase activity and plant disease resistance. They observed ten fold increase in peroxidase activity in resistant cultivars of wheat when infected with vascular pathogen.

The release of peroxidase into the intercellular spaces as a mode of defense reponse was reported in resistant cultivar of capsicum infected with *P. capsici* (Alcazar *et al.*,1995). They also noticed that the level of peroxidase was very low in susceptible and moderately resistant cultivars.

Higher activity of peroxidase in conjugation with polyphenol oxidase and OD phenol was noticed in resistant cultivars of chilli against bacterial wilt pathogen (Markose, 1996) and brinjal (Paul, 1998).

Alternaria blight resistant cultivars of cluster beans exhibited high polyphenol oxidase and peroxidase activity in comparison with susceptible

cultivars at 65 days after sowing. The amount of peroxidase activity was several times higher compared to polyphenol oxidase activity (Saharan *et al.,* 1999).

Banu (2001) reported that the peroxidase activity was higher in resistant cultivar Manoranjitham than the susceptible variety Grand Naine before the attack of *C. musicola.* She also noticed a decrease in peroxidase activity in resistant cultivars during the attack of the pathogen.

Kortekamp and Zyprian (2003) observed higher basal activity of peroxidase enzymes in resistant grapevine cultivar. They also noticed a sudden increase in peroxidase activity in grapevine immediately after the inoculation with downy mildew pathogen.

Kawano (2003) reported that the peroxidases secreted by the plants during the attack of the pathogen have the ability to catalyze the generation of reactive oxygen species (ROS) coupled with oxidation of plant hormone indole-3-acetic acid (IAA) and defense-related compounds salicylic acid (SA), aromatic monoamines (AMAs) and chitooligosaccharides (COSs) which in turn stimulate the intracellular Ca^{2+} signaling required for induction of defense responses in plants.

The peroxidases produced in cell line cultures of Norway spruce (*Picea abies*) and *Zinnia elegans* caused the polymerization of cell wall lignin thus help in defense response (Fagerstedt *et al.,* 2010).

The levels of peroxidase, phenols and catalase activity showed a negative significant association with disease resistance in Glory Lily when infected with leaf blight pathogen (Rajamani *et al.,* 2013).

Francoz *et al.* (2015) reported that peroxidases in addition to the production of reactive oxygen species also played a role in cell wall loosening and stiffening thereby providing multiple defense response in plants against the invaded pathogen.

2.3.2.3.2. Role of polyphenol oxidase in disease resistance

Polyphenol oxidase causes the oxidation of phenols to quinones in plants and assumed to be involved in plant defense against pests and diseases. Bobes *et al.* (1987) observed variation in activities of peroxidase and polyphenol oxidase in resistant and susceptible potato plants infected with *P.infestans* and *Erwinia carotovora* var. *atroseptica.* The activity of the enzymes were found to be more in resistant varieties thereby conferring resistance.

Srivastava (1987) conducted studies on the activity of peroxidase and polyphenol oxidase in resistant and susceptible cultivars of *Brassica juncea* at definite intervals after inoculation with *Macrophomina phaseolina* and recorded that, fungus infected tissues possessed relatively higher activities of these oxidative enzymes in comparison to healthy plants. At the early stages of the infection *i.e.,* 6^{th} day, resistant plants showed higher activities of peroxidase and polyphenol oxidase when compared to susceptible varities. Very low activity of these enzymes was recorded in healthy plants.

Trandafirescu *et al.* (1999) observed a positive correlation between the increase in activity of polyphenol oxidase and resistant cultivars of *Stereum purpureum.* Khirbat and Jalai (1999) noticed an increase in activity of polyphenol oxidase in resistant genotypes of chickpea, after 6 to 10 days of inoculation with the pathogen *Ascochyta rabiei* when compared with the susceptible variety.

Banu (2001) observed an intermediate value for polyphenol oxidase activity in both resistant and susceptible variety of banana before infection with *C. musicola*, but after inoculation with the pathogen the polyphenol oxidase was found to be more in resistant cultivars than susceptible ones.

Li and Steffens (2002) reported a thirty fold increase in polyphenol oxidase in transgenic tomato plants with cauliflower mosaic virus 35S promoter which in turn resulted in enhanced disease resistance against *Pseudomonas syringae.*

Mohammadi and Kazemi (2002) measured polyphenol activity in resistant and susceptible cultivars of wheat at flowering, milk, dough and ripening stages after inoculation with the pathogen *Fusarium graminearum*. The results of the study concluded that polyphenol activity reached the maximum level in wheat heads during milky stage and declined subsequently. The activity was found to be three times more in inoculated resistant cultivar than the susceptible cultivar.

Gandia-Herrero *et al.* (2005) reported that polyphenol oxidase hydroxylase monophenols to o-diphenols, which then oxidised to quinones, which were often more toxic to the microorganisms thereby offering disease resistance against the infection of pathogens.

Raj *et al.* (2006) analyzed polyphenol activity in seedlings of resistant and susceptible cultivars of pearl millet with and without inoculation of *Sclerospora graminicola*, the pathogen inciting downy mildew in pearl millet. The results of the study revealed that among the cultivars, the resistant cultivars showed more polyphenol oxidase activity than the susceptible ones and also the seedlings inoculated with the pathogen had more polyphenol oxidase activity than uninoculated seedlings.

The studies conducted by Poiatti *et al.* (2009) concluded that the basal leaves of potato plants inoculated with *Xanthomonas axonopodis* and *Ralstonia solanacearum* showed higher activities of peroxidase and polyphenol oxidase activity when compared with the apical leaves. They also noticed that leaves with single inoculation sites had more polyphenol oxidase activity than multiple wounded leaves.

Rajamani *et al.* (2013) observed a highly positive correlation with polyphenol oxidase activity with disease resistance in GS 05 and GS 07 genotype of Glory Lily against leaf blight disease.

2.3.2.3.3. Role of phenylalanine ammonia lyase in disease resistance

Henderson and Friend (1978) observed a rapid increase in phenylalanine ammonia lyase (PAL) activity in resistant potato tubers infected by *P. infestans* than the susceptible tubers. They also concluded that the activity PAL contributed to disease resistance.

Edwards *et al.* (1985) reported that when pathogen interacted with *Phaseolus vulgaris,* it elicited rapid stimulation of PAL mRNA synthesis as early defense response leading to accumulation of phenyl propanoid-derived phytoalexin. Cahill and McComb (1992) observed a rapid increase in PAL activity in roots of resistant varieties of eucalyptus infected with *P. cinnamomi* when compared with susceptible variety leading to disease resistance.

Nagarathna *et al.* (1993) analysed the activity of PAL in different genotypes of pearl millet inoculated with *Sclerospora graminicola.* The result revealed higher activity of PAL was observed in resistant cultivars while a reduced activity in susceptible genotypes.

Mani and Slusarenko (1996) reported that in downy mildew resistant cultivar of *Arabidopsis,* PAL act as the precursors for the production of salicylic acid thereby inducing resistance against the disease.

When the expression of PAL was suppressed in tobacco plants, the plants failed to develop systemic acquired resistance against infection caused by *Tobacco mosaic virus* (Pallas *et al.,* 1996).

Way *et al.* (2002) observed an eight fold increase in PAL activity in transgenic tobacco plants with phenylalanine ammonia lyase gene from *Stylosanthes humilis,* which enchanced disease resistance but slowed plant growth.

Shadle *et al.* (2003) reported that transgenic plants over expressing PAL produced high levels of chlorogenic acid which induced resistance against infection caused by fungal pathogen *Cercospora nicotianae.*

Umesha (2006) reported that, the resistant cultivar of tomato inoculated with *Clavibacter michiganensis* subsp. *michiganensis,* pathogen inciting bacterial canker, showed an increased activity of PAL while the activity was low in susceptible cultivar. The activity of PAL was positively correlated with disease resistance in plants.

The studies carried by Chandra *et al.* (2007) revealed that the application of salicylic acid on cowpea caused an increase in PAL activity in cowpea, which in turn enhanced resistance against *Rhizoctonia solani.*

Twenty different cultivars of tomato were analyzed for the activity of PAL against bacterial wilt pathogen *Ralstonia solanacearum* following the inoculation of the pathogen. Among the different cultivars analyzed, an increase in enzyme activity was noticed in resistant cultivar (Vanitha *et al.*, 2009).

Ngadze *et al.* (2012) reported that polyphenol oxidase, peroxidase, PAL, chlorogenic acid and total soluble phenols were formed during the attack of soft rot pathogens which imparted disease resistance in potato plants.

2.4. VALIDATION OF MARKERS TO IDENTIFY THE DISEASE RESISTANT GENE

The RAPD markers were used to characterize the banana diploids (AA) with varying levels of black Sigatoka and yellow Sigatoka resistance (Ferreira *et al.*, 2004).

Nwauzoma *et al.* (2011) identified a SCAR *(*sequence characterized amplified region) marker through RAPDs that linked tightly to Sigatoka leaf spot disease in banana, but the marker failed to discriminate between the resistant and the susceptible samples.

The SCAR marker SuscPD-F/SuscPD-R generated were validated on twenty eight genotypes of banana which were resistant or susceptible to Panama wilt disease, indicated high degree of specificity of markers and were able to discriminate between the resistant and susceptible genotypes (Cunha *et al.,* 2015).

Das *et al.* (2016) reported that the SCAR markers developed through RAPDs when validated among twenty six diverse genotypes of banana across India, showed a positive correlation with Sigatoka leaf spot resistant lines. The marker amplified an amplicon of size 640bp in resistant cultivars and were absent in susceptible ones.

Among the 276 accessions of banana screened using SCAR primer ScaU1001 that were tightly linked to Fusarium tropical race 4 resistance revealed that, ScaU1001 was efficient at discriminating accessions with possible resistance in 36.6% of the evaluated accessions (Silva *et al.*, 2016).

2.5. MANAGEMENT OF SIGATOKA LEAF SPOT DISESAE OF BANANA

The integrated management approach used to control *Mycosphaerella* spp. involved the use of new molecular chemicals, organic / inorganic preparations and biocontrol agents.

2.5.1. Management using chemical fungicides

Evans *et al.* (1961) observed that aerial spraying of copper based fungicides was effective in controlling Sigatoka leaf spot disease by inhibiting the sporulation of the pathogen. Prahl (1967) reported that oil based application of maneb controlled conidial infection of Sigatoka leaf spot disease and found to be less phytotoxic to banana plants.

Stover (1969) observed the effect of different fungicides on germination of ascopsores of *M. musicola.* Among the different fungicides tested, benomyl were found to be very effective in inhibiting the germination of spores of *M. musicola.*

Stover and Simmonds (1987) reported that systemic fungicides such as benomyl, thiabendazole, thiophanate methyl, imazalil, propiconazole, bitertanol, flusilazol and tridemorph were found effective in controlling Sigatoka leaf spot disease. Foure (1988) reported that triadimenol and pyrazophos were very effective in management of *M. fijiensis* on banana in Cameroon.

Anaso and Olatunde (1989) observed that banana plants sprayed with mancozeb, benomyl, thiophanate methyl, thiophanate methyl + hydroxyl quinoline copper (as Topsin MD075) or mancozeb + benomyl had significantly less yellow Sigatoka disease and higher bunch yield than those plants treated with captafol, captan or zineb. The application of systemic fungicides was found to be more effective than protectants fungicides as they penetrated into the plant tissue and arrested the growth of *M. fijiensis* (Hewitt, 1998).

Saxeena *et al.* (1994) recommended foliar application of Dithane M-45 + Bavistin in 2 : 1 and 1 : 2 ratio alternatively at 15 days interval for controlling Sigatoka leaf spot disease of banana.

Cherian *et al.* (2002) reported the effectiveness of tebuconazole and propiconazole for the management of Sigatoka leaf spot disease.

Marin *et al.* (2003) reported that Sigatoka leaf spot disease can be controlled by application of protectant chemical such as mancozeb and chlorothalonil and systemic fungicides belonging to the benzimidazole, triazole, morpholine and strobilurin (QoI) groups, applied either in oil or in oil - water emulsion.

Vawdrey and Grice (2005) tested the effect of selected strobilurin fungicides (trifloxystrobin, pyraclostrobin and azoxystrobin), triazole fungicide (propiconazole) and mancozeb against Sigatoka leaf spot disease of banana and concluded that among the fungicides tested trifloxystrobin, pyraclostrobin and azoxystrobin proved to be more effective in controlling the disease than the other two fungicides.

Hermanto *et al.* (2010) evaluated the efficacy of new generation molecules against Sigatoka disease and reported the effectiveness of azoxystrobin against *Mycosphaerella* leaf spot diseases.

Foliar spraying of carbendazim (0.05%) or alternate spraying tridemorph (0.08%), mancozeb (0.2%), carbendazim (0.05%) were recommended for the control of Sigatoka leaf spot disease (KAU, 2011).

Shinde (2013) reported that azoxystrobin (0.15%) and tebuconazole (0.1%) were effective in reducing Sigatoka leaf spot disease with concomitant increase in yield. Nayana (2015) reported that the foliar application of Mg (2 g/ 1) + Zn (3 g/ 1) + B (2 g / 1) plus tebuconazole (0.1%) gave maximum disease suppression of Sigatoka leaf spot disease under Kerala conditions.

Among the five chemicals *viz.,* difenoconazole (0.1%), propiconazole (0.1%), hexaconazole (0.1%), carbendazim (0.05%), azoxystrobin (0.05%) were tested for the management of Cercospora disease of cowpea, difenoconazole recorded the minimum disease incidence of 31.88% and disease severity (13.97%) compared to other treatments (Ravi, 2017).

2.5.2. Management using biocontrol agents and organic/ inorganic preparations

Philpott and Knowles (1913) in Fiji was the first to advocate the use of Bordeaux mixture for the control of Sigatoka leaf spot disease. Riveros *et al.* (2002) reported that biocontrol agents *Serratia marcescens*, *S. entomophyla* and *Bacillus* spp. inhibited the mycelial growth of *M. fijiensis* by 40%. Davis (2003) reported that garlic oil (10%) and *Trichoderma viride* (0.4%) were effective in controlling Cercospora leaf spot of ivy gourd.

Kim *et al.* (2003) reported that curcumin when applied at 500mg/l showed fungicidal activity against *Botrytis cineria, Erysiphe graminis, P. infestans, Puccinis recondita* and *Rhizoctonia solani.*

Vawdrey *et al.* (2004) evaluated five mineral oils, four plant oils and a plant derived non ionic sticker adjuvant against yellow Sigatoka leaf spot disease of banana in the field. The result of the study revealed that among the various treatments, the five petroleum based mineral oils were found to be effective for the control of yellow Sigtaoka leaf spot disease.

Alvindia (2012) observed the effect of biocontrol agents (*T. harzianum*

DGA01 and *B. amyloliquefaciens* DG14), plant oils (Piper betel oil (2.5% EC), garlic oil (15% EC), capsicum oil (20% EC) and inorganic salt (Potassium bicarbonate (14% EC)) on mycelial growth of *M. fijiensis* under *in vitro* conditions. Among the various treatments, the biocontrol agents completely inhibited the mycelial growth of *M. fijiensis* while piper betel oil (2.5% EC), garlic oil (15% EC), capsicum oil (20% EC) delayed the development of *M. fijiensis* by 37, 9 and 43% while potassium bicarbonate (14% EC) restricted the growth of *M. fijiensis* by 43%.

Elisee *et al.* (2014) reported that salicylic acid showed an inhibitory effect on mycelial growth and spore germination of *M. fijinesis.* Chemical elicitors such as salicylic acid (SA), β-amino butyric acid (BABA), chitosan (CHT) and 2,6-dicholoroisonicotinic acid (INA) when applied to plants induced disease resistance in plants by increasing the activity of defense related enzymes.

Hedge and Mesta (2014) reported that *Pseudomonas fluorescens* and *B. subtilis* were effective in reducing the disease pressure caused by *M. musicola* thereby increasing the yield of the plant.

Castro *et al.* (2015) observed that foliar application of *Trichoderma* based bioproduct on banana var. Williams, increased plant growth along with providing some degree of disease control.

Application of 1% Bordeaux mixture, 1% mineral oil and bioagents like *P.fluorescens* (20 g/l) or *B. subtilis* (5 g/l) were effective in management of Sigatoka leaf spot disease (Nayana, 2015).

Ravi (2017) reported the effect of organic formulations such as mineral oil (0.1%), neem oil (0.5%) and *P. fluorescens* (2%) against Cercospora leaf spot of vegetable cowpea. Among the different preparations, mineral oil (0.1%) gave maximum (14.32%) disease suppression and lowest disease incidence of 40.57%.

Materials and Methods

3. MATERIALS AND METHODS

The present study on "Characterization of *Mycosphaerella* spp. causing Sigatoka leaf spot disease complex of banana in Kerala and its management" was conducted in the Department of Plant Pathology, College of Horticulture, Vellanikkara and Banana Research Station, Kannara during 2015 - 2018. The details of the materials used and the techniques adopted for the investigation are described below.

3.1. SURVEY AND SYMPTOMATOLOGY

3.1.1. Survey and Collection of samples

Purposive sampling surveys were conducted in different agroclimatic zones *viz.,* Malappuram (Northern zone), Palakkad (Northern zone), Ernakulam (Central zone), Wayanad (High range zone) and Thiruvananthapuram (Southern zone) during April – May, 2017 (pre monsoon). In Thrissur district, intensive surveys were conducted in eighteen selected panchayaths (Fig 3.1) twice *i.e.,* during April – May, 2017 (pre monsoon) and July – August, 2018 (post monsoon). These surveys were conducted with the objective to document each type of symptoms occurring in the field under field conditions, to assess disease severity, to record the youngest leaf spotted (YLS) and to identify the pathogen associated with Sigatoka leaf spot disease complex of banana in Kerala. The weather parameters *viz.,* rainfall and temperature were also collected from each locations.

Twenty plants were selected randomly from each plot to assess the per cent disease severity and the number of the youngest leaf spotted. The variations in symptoms observed on different varieties grown in each location were also recorded. The samples of the leaves exhibiting differences in symptoms were collected and brought to the laboratory for characterization and identification of the pathogen associated with the disease.

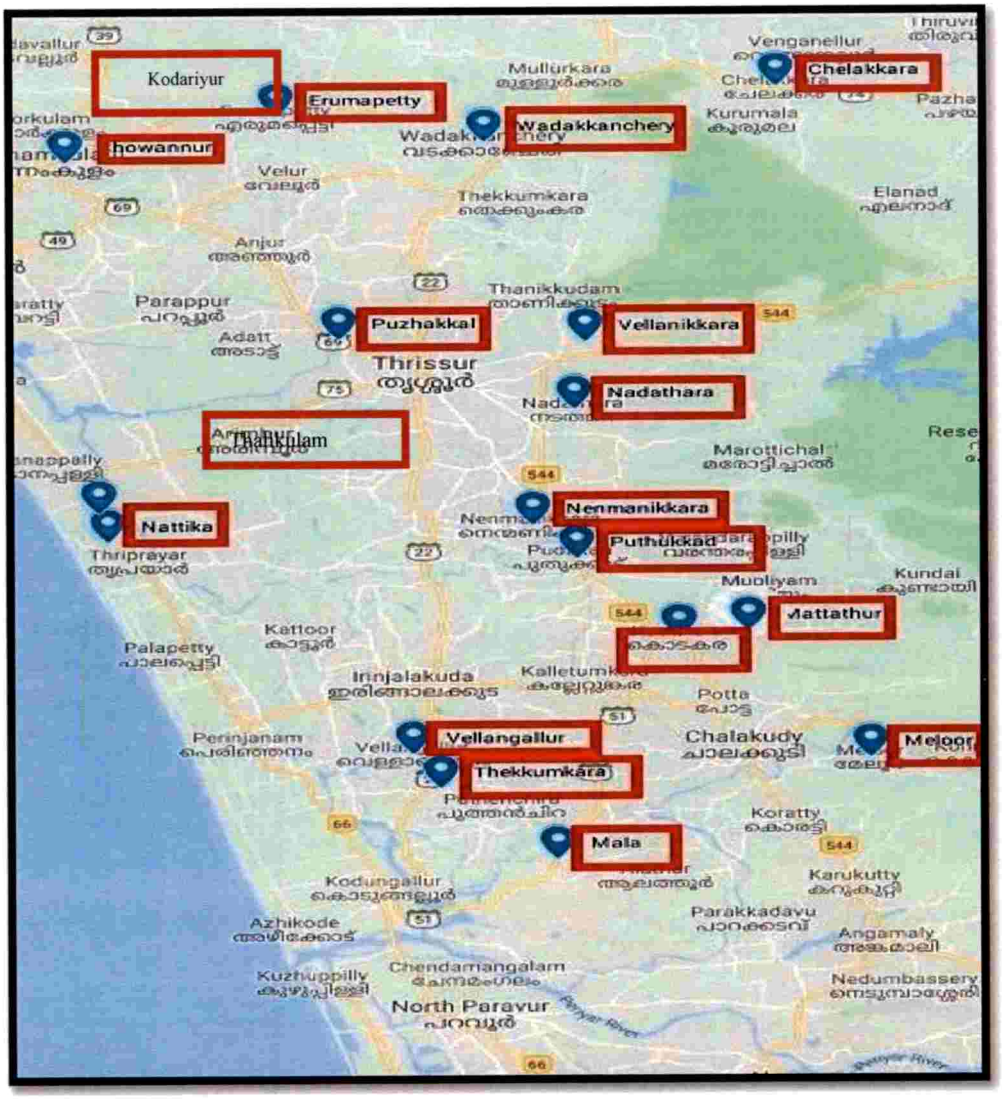

Fig 3.1: Locations of survey

The youngest leaf spotted was calculated by counting the position of the youngest leaf showing at least ten leaf spots (Fig 3.2).

The disease severity was assessed using a standard score chart (Meredith and Lawrence, 1969) (Fig 3.3) using the 0 - 6 scale as mentioned below:

Score	**Symptoms**
0	no symptom
1	< 1% of lamina with symptoms
2	1 to 5% of lamina with symptoms
3	6 to 15% of lamina with symptoms
4	16 to 33% of lamina with symptoms
5	34 to 50% of lamina with symptoms
6	51 to 100% of lamina with symptoms

The per cent disease severity was calculated using the formula:

$$\text{Per cent disease severity (PDS)} = \frac{\sum nb}{(N-1)T} \times 100$$ where,

n= number of leaves in each grade,

b= grade

N=Number of grade used in the scale

T= total number of leaves

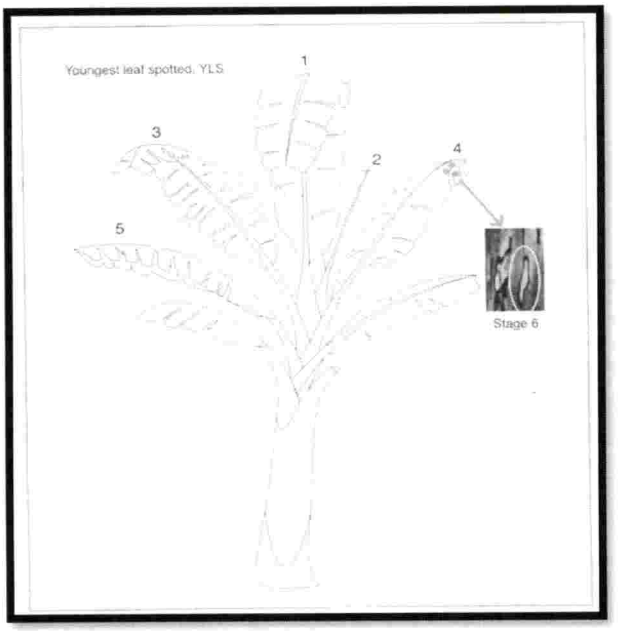

Fig 3.2: Youngest leaf spotted

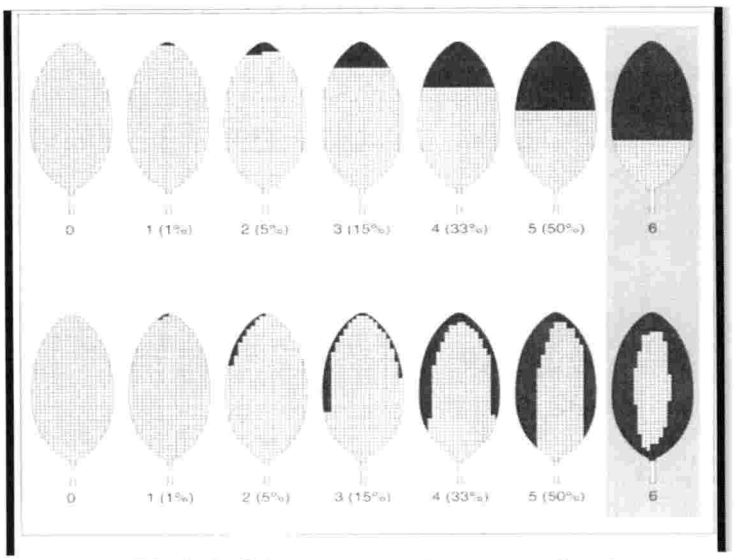

Fig 3.3: Disease severity score chart

3.1.2. Correlation analysis of disease severity with weather parameters

The data on per cent disease severity and the weather data collected during the survey were subjected to correlation analysis to assess the correlation of disease severity with weather parameters.

3.1.3. Symptomatology

The infected leaves were closely observed for symptoms from initial spots to mature lesions on different varieties to document the variation in symptoms exhibited by different varieties grown in different geographical locations.

3.1.4. Development of symptoms

The developmental stages of the symptom were studied during summer and rainy season on four commercially grown banana varieties *viz*., Nendran (AAB), Grand Naine (AAB) and Njalipoovan (AAA). The flag leaves of these varieties were tagged and observed to document the development of symptoms of Sigatoka leaf spot disease and also to study the developmental stages of the diseases. Thirty five lesions at the initial stage symptoms were marked on the leaves of each variety and developmental stages of symptom expression were observed and recorded. The symptoms, lesion size and the time taken for symptom development from one stage to another were observed and documented. The stages of symptom development were categorized as reported by Leach (1941).

3.2. ISOLATION OF THE PATHOGEN

The pathogen associated with Sigatoka leaf spot disease of banana was isolated by leaf bit method. The leaf samples exhibiting various stages of the disease were collected from the field in polythene bags and brought to the laboratory for isolation of the pathogen.

3.2.1. Leaf bit isolation method

The infected leaves were washed thoroughly under running water, wiped with blotting paper, air dried and were cut into small bits containing diseased portions along with the healthy portion. These bits were then surface sterilized using 1% sodium hypochlorite for 30 sec. followed by washing in three changes of sterile water. The bits were then transferred into a sterile filter paper for the absorption of excess water and were cultured on potato dextrose agar (PDA) medium poured into sterile Petri plates under aseptic condition. The Petri plates were then incubated in BOD incubator at 24^0C and observed daily for fungal growth. The fungal growth produced on the medium was subcultured and purified by hyphal tip method. The purified culture of the fungus were maintained on PDA and stored at 24°C in BOD incubator.

3.2.2. Pathogenicity studies

The pathogenicity studies of the isolated fungus were carried out on healthy banana leaves collected from the field. The leaves were washed under running water followed by surface sterilization using 70% ethanol. Artificial inoculation of the pathogen was carried out after wounding the leaves by definite number of pin pricks using a sterile stainless steel needle. Then mycelial discs from three week old culture of the fungus were cut and placed on the leaves where pin pricks were given. The site of inoculation was covered with moist sterile cotton. After inoculation, the leaves were kept in polythene covers in order to maintain humidity and incubated at room temperature. The inoculated leaves were observed daily for the appearance of symptoms. The pin pricked leaves covered with moist cotton swab alone served as the control. The fungus was re-isolated from the infected leaves and was compared with the original culture. Twelve replicates of inoculated leaves along with control were kept.

3.3. CHARACTERIZATION OF THE PATHOGEN

The characterization of the pathogen was carried out by studying the

cultural, morphological and molecular characters of the pathogen.

3.3.1. Cultural characterization of the pathogen

The cultural characters of the pathogen was studied by growing the fungus on different solid media such as potato dextrose agar, carrot agar, V8- juice agar and banana leaf extract agar medium. The compositions of the media are given in Appendix 1. Seven mm sized culture disc from three week old culture was placed at the centre of Petri plates poured with different media and incubated in BOD incubator maintained at 24°C. The cultural characters such as colour, shape, texture and growth rate of the fungus were recorded on each media at 24 h interval. Three replicates were kept for each media.

3.3.2. Morphological characterization of the pathogen

The morphological characterization of the pathogen was done by two methods *viz.,* Cellophane tape method and Potassium hydroxide method (Udugama, 2002).

3.3.2.1. Cellophane tape method

The leaves with lesions at stage 1, 2 and 3 were selected for studying the asexual characters *viz.,* conidiophores, size and nature of the conidia. The lesions were cleaned using a sterile tissue paper. A stripe of clear cellophane tape was placed on the under surface of the lesions and left for one day. The tape was pressed in order to adhere properly to the lesions. After 24 h, the cellophane tape was removed carefully and transferred on to a slide containing lactophenol cotton blue and observed under the microscope.

3.3.2.2. Potassium hydroxide (KOH) method

The leaves exhibiting stage 5 and 6 lesions of the disease were cut into small bits of $2cm^2$ size. These bits were then dipped in 10% KOH (which decolorizes

plant tissue) overnight followed by washing the bits in sterile water. The bits were blot dried and the lesions were scrapped using a sterile needle. The scrapings were then transferred to a slide containing a drop of lactophenol cotton blue stain and were observed under microscope for the presence of fungal sexual structures such as perthecia, asci and ascospores.

3.3.3. Molecular characterization of the pathogen

3.3.3.1. Collection of samples

During the purposive sampling surveys as described in 3.1, the leaf samples were collected for molecular characterization of the pathogens associated with the disease. The samples were collected and placed immediately in ice box and brought to the laboratory for further studies.

3.3.3.2. Isolation of DNA

The DNA from 50 isolates collected during the survey was isolated using *DNeasy* Plant Mini Kit (Qiagen) by following the protocol supplied by the manufacturer. About 100 mg of the leaf tissue containing mature lesions (stage 5 & 6) was homogenized using liquid nitrogen in pestle and mortar and the powdered tissue was transferred to a microcentrifuge tube. 400 µl of AP1 buffer was added into the tube and inverted for one min. Four µl of RNAase A solution was added to the tube and vortexed vigorously for 30 sec. The homogenate was then incubated at 65°C in a water bath for 10 min. During incubation the homogenate was mixed 2 or 3 times by slowly inverting the tube. AP2 buffer (130µl) was then added to the lysate, mixed well and incubated on ice for 5 min. The lysate was pipetted into the QIAshredder Minispin column placed in a 2 ml collection tube and centrifuged at 14,000 rpm for 2 min. The flow through liquid was collected into a new tube without disturbing the cell debris pellet. 1.5 volumes of AP3 buffer was added to the cleared lysate and was mixed by pipetting. 650 µl of the mixture was transferred into *DNeasy* Mini Spin column placed in a 2ml collection tube. The mixture was then centrifuged for one min. at 8,000 rpm and the flow through liquid was

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discarded. The column was then placed into 2ml collection tube and 500 µl of AW buffer was added which was then centrifuged at 8,000 rpm for one min. and the flow though liquid was discarded. Again 500 µl of AW buffer was added to the column and centrifuged at 14,000 rpm for 2 min. to dry the membrane. The column was transferred to a new 1.5 ml tube and 100 µl of AE buffer was added directly into the *DNeasy* membrane and incubated at room temperature (15-25°C) for 5 min. The column was then centrifuged at 8000 rpm for one min. to elute the DNA. The eluted DNA was stored at 4°C.

3.3.3.3. Assessment of purity

The purity of the isolated DNA was checked using agarose gel electrophoresis. Agarose gel (0.8%) was prepared in 1X TAE (Tris acetate EDTA) (Appendix II) containing 2 µl ethidium bromide. One µl of 6X gel-loading dye was added to 5µl of DNA and was mixed well by pipetting and loaded on 0.8% agarose gel. Electrophoresis was performed with 1X TBE (Tris borate EDTA) as electrophoresis buffer at a constant voltage of 75 V for 2-3 h. The gels were visualized in UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.3.3.4. Polymerase Chain Reaction (PCR)

Polymerase chain reaction for the molecular diagnosis and subsequent characterization was developed by standardizing the PCR conditions especially the annealing temperature for the primers specific to ITS (Internal Transcribed Spacer) – rDNA region.

3.3.3.4.1. Standardization of annealing temperature

The annealing temperature was standardized by gradient PCR (Eppendorf). The range of temperature used for the study is given below:

Sl No	Temperature ^{0}C
1.	60.1
2.	60.4
3.	61.2
4.	62.6
5.	64.3
6.	66.2
7.	68.2
8.	70.2
9.	72.0

The PCR amplifications were performed in 25 µl reaction volume. The reaction mixture include:

Sl. no.	Contents	Quantity
1.	PCR master mix	12.5µl
2.	Primer	0.5 µl (each primer)
3.	Nuclease free water	9.5 µl
4.	DNA	50 ng
	Total volume	**25 µl**

Takara EmeraldAmp® GT PCR Master Mix was used for PCR mix.

Nucleotide sequence of the primers used is given below:

Target	Primer Name	Direction	Sequence (5' → 3')
ITS	ITS-1F	Forward	TCCGTAGGTGAACCTTGCGG
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC

The thermal profile followed for PCR amplification is given below:

PCR thermal profile

Process	Temperature (°C)	Time
Initial denaturation	94	3 min.
Denaturation	94	45 sec.
Annealing	*	45 sec.
Extension	72	1 min.
No: of cycles: 35		
Final extension	72	10 min.

* Annealing temperature standardized under session 3.3.3.4.1 using gradient PCR was set.

3.3.3.4.2. PCR Amplification

The ITS region of fungal pathogens of all the 50 isolates collected during the survey were amplified using ITS 1 and ITS 4 primer sets using the standardized annealing temperature with the same reaction mixture given above and PCR amplification thermal profile as described in 3.3.3.4.1.

3.3.3.4.3. Analysis of the PCR products

Ten µl PCR products were loaded along with 100bp ladder on 1.2% agarose gel prepared in 1X TAE containing 2 µl ethidium bromide to detect the presence and size of the amplicons. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad). The amplicons were then eluted using DNA elution kit (Qiagen).

3.3.3.5. Sequencing of the amplicons

The eluted amplicons obtained from all the 50 isolates were sequenced using automated sequencing facility available at AgriGenome Pvt Ltd., Kakkanad, Kochi.

3.3.3.5.1. *In silico* analysis of the sequences

The sequence data set corresponding to the ITS- rDNA region of the fungus and other reference sequences were retrieved from NCBI Genbank database (USA) using BLASTn algorithm and were compared.

3.3.3.5.2. Phylogenetic Analysis

Evolutionary analysis of the isolates were done using MEGA X software and the evolutionary history was inferred using the UPGMA method (Sokal and Michener, 1958). The bootstrap consensus tree inferred from 500 replicates was taken to represent the evolutionary history of the taxa of the isolate analyzed and were evaluated by construction of phylogenetic tree.

3.4. EVALUATION OF HOST PLANT RESISTANCE

Banana accessions maintained in the field gene bank of Banana Research Station (BRS), Kannara, Kerala Agricultural University were screened to assess the level of disease resistance. This was done by assessing the per cent disease severity (PDS) calculated using the formula given in 3.1 and the youngest leaf spotted of each accessions at six months after planting and at shooting stage. The accessions

were then categorized based on the disease reaction as reported by Estelitta *et al.* (1991).

Score	Per cent disease severity	Disease reaction categories
0	0	Immune
1	<10	Resistant
2	10 – 25	Moderately resistant
3	26 – 40	Susceptible
4	41 – 75	Moderately susceptible
5	>75	Highly susceptible

3.4.1. Anatomical and biochemical basis of host plant resistance

Five resistant and susceptible varieties maintained in the field genebank of BRS Kannara were selected based on their disease reaction assessed under 3.4. and evaluated for anatomical, biochemical and molecular basis of host plant resistance.

3.4.1.1. Collection of samples

The healthy and diseased leaf samples at early stage of infection were collected from seven month old plants of selected accessions maintained in the field genebank of BRS, Kannara.

3.4.1.2. Anatomical studies

The anatomical parameters such as the thickness of cuticle, epidermis,

spongy and palisade tissue were studied as per the protocol described by Vasquez *et al.* (1989). From each selected varieties, three replicates each were taken from four portions of the leaf lamina *viz.*, tip, centre, base and middle (Fig 3.4). Thin transverse hand sections of 2- 3cm of the leaf samples were taken using a sharp razor and the bits were transferred to a watch glass containing water. The thinnest sections were taken with the help of a camel brush and were transferred to a clean watch glass with water. Few drops of safranin stain was added to the watch glass with water and left for 3-5 min. The excess stain was drained off and the sections were placed in the centre of a clean glass slide. A drop of glycerine was placed over the section, covered with coverslip and observed under microscope at 100X and 400X .

The observations on stomata *viz.,* number of stomata / microscopic field and pore width were studied by the protocol of Rajeevan (1985). From the selected varieties, three replicates were taken from each of the four portion of the leaf lamina (Fig 3.4). The adaxial and abaxial surfaces of the healthy and diseased leaves were smeared with an adhesive, 'Quick fix' which facilitated easy removal of the peel. These peels were then stained with safranin and were observed under 400X magnification of the microscope.

3.4.2. Biochemical studies

The biochemical basis of resistance was studied by quantifying the biochemical parameters such as phenols, reducing and non-reducing sugars and defense related enzymes (peroxidase, polyphenol oxidase and phenylalanine ammonia lyase) following standard procedures. Two replicates from each of the four portions of the leaf lamina were taken for the analysis.

3.4.2.1. Phenols

Phenols in the leaf samples were estimated based on the protocol described by Sadasivam and Manickam (1996). 500mg - 1 g of the leaf samples were taken and ground using a pestle and mortar in ten volume of 80% ethanol (1:10 w/v). The

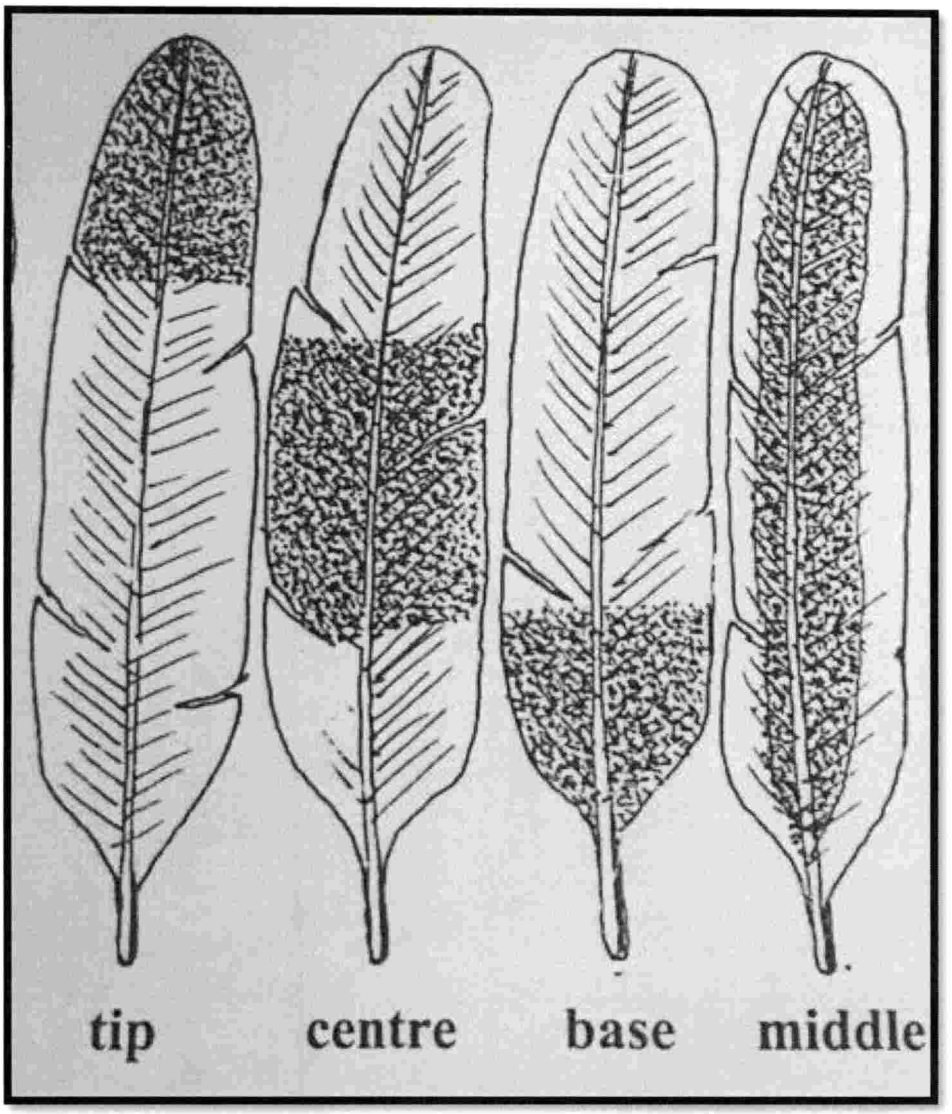

Fig 3.4: Different portions of leaf lamina used for anatomical and biochemical analysis

homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was taken in a boiling tube and the residue was re-extracted with 80% ethanol (1:5 w/v). The homogenate was then centrifuged and the supernatant was pooled. The boiling tube containing the supernatant was transferred to a water bath and the contents were evaporated to dryness. This was then dissolved in 5 ml of distilled water. From this, different aliquots (0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1 ml and 1.2 ml) were pipetted out into different test tubes and the volume was made upto 5 ml using distilled water. 0.5ml of Folin- Ciocalteau reagent was then added into each test tubes and mixed well. After 3 min., 2ml of 20% Na_2CO_3 was added to each tube and mixed thoroughly. The tubes were then placed in boiling water for exactly one min. and cooled. The absorbance was measured at 650nm against a reagent blank. The quantity of the phenols were estimated from standard curve prepared using different concentrations of catechol.

3.4.2.2. Reducing sugars

The reducing sugar content of the leaf samples were estimated using Dinitrosalicylic acid (DNS) method as described by Miller (1959). 200 mg of the leaf samples was ground well in 2 ml of methanol and transferred to a test tube. The extracts were evaporated in a water bath for 5 min. 3 ml of DNS reagent were added into the aliquot of extract. The extract was then heated for 10 min. in a boiling water bath, after adding one ml of 40% Rochelle salt and allowed to cool. The intensity of the red colour of the extract was read at 575 nm in a spectrometer. Glucose was used as the standard for the preparation of the standard curve to estimate the quantity of reducing sugars.

3.4.2.3. Non reducing sugars

The non reducing sugar content of the leaf samples were estimated by Dinitrosalicylic Acid (DNS) method as per the protocol of Miller (1959). One ml of alcohol extract of the leaf sample was taken in a test tube and evaporated the content to dryness in a water bath. After adding one ml of distilled water and one

ml of 1N H_2SO_4 into the test tubes, the content was hydrolysed for 30 min. at 49^0C in a water bath. The tubes were then taken out of the water and allowed to cool followed by adding of two drops of methyl red indicator to it. The content was neutralized by adding 1N NaOH, and the volume was made up to 10 ml using distilled water. From this aliquot, 5 ml was taken along with 3 ml of DNS reagent and 1 ml of 40% Rochelle salt. The extract was heated in boiling water for 10 min followed by cooling of the extract. The non reducing sugar present in the hydrolysate was estimated by reading the intensity of the red colour at 575 nm in a spectrometer. Glucose was used for the preparation of the standard curve to quantify the non reducing sugar content.

3.4.2.4. Peroxidase

The peroxidase activity of the leaf samples were assessed following the protocol described by Sadasivam and Manickam (1996). The enzyme extract was prepared by grinding one g of fresh leaf tissue in 3 ml of 0.1 M phosphate buffer of pH 7 in a pre cooled mortar and pestle. The homogenate was centrifuged at 18,000 rpm at 5 ^{0}C for 15 min. The supernatant was used as enzyme source for the assay. The spectrophotometer was set at 436 nm with 3.1 ml of phosphate buffer. Three ml of buffer solution, 0.05 ml of guaiacol (240 mg in 100 ml of water), 0.02 ml of enzyme extract and 0.03 ml of hydrogen peroxidase (0.14 ml of 30 per cent hydrogen peroxide in 100 ml water) were taken in a cuvette and mixed well. The cuvette was then placed in a spectrophotometer until the absorbance increased by 0.05. The stop watch was started and noted the time required in minutes (Δ t) to increase the absorbance by 0.1. The peroxidase activity of the extract was calculated using the formula:

$$\text{Enzyme activity} = \frac{3.18 \times 0.1}{6.39 \times \Delta t \times 0.1}$$

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3.4.2.5. Polyphenol oxidase

Polyphenol oxidase activity was assayed by the method suggested by Malik and Singh (1980).

The buffer solutions used for the extraction and assay were 0.1 M monobasic and dibasic sodium phosphate of pH 6.0. 0.01 M catechol dissolved in 100 ml of phosphate buffer (pH 6.0) was used as the substrate for the assay.

The enzyme extract was prepared by grinding one g of fresh leaf tissue in 3 ml of phosphate buffer in a pre cooled pestle and mortar. The resultant supernatant was used as the source of enzyme for the assay.

Three ml of buffered catechol and 0.5 ml phosphate buffer (pH 6.0) were taken in a cuvette and the absorbance was adjusted to zero in spectrometer at 495 nm. The reaction mixture containing three ml of phosphate buffer and 0.5 ml of enzyme extract were mixed well in a cuvette. The reaction was initiated by adding three ml of buffered catechol. The change in absorbance due to the oxidation of catechol by catechol oxidase was recorded at 495nm at 30 sec. interval upto 5 min.

3.4.2.6. Phenylalanine ammonia lyase

The enzyme activity was assessed using the protocol described by Sadasivam and Manickam (1996). The enzyme extract was prepared by homogenizing 500 mg of the leaf tissue in 5 ml of cold 25mM borate- HCl buffer of pH 8.8 containing 5mM mercaptoethanol (0.4 ml/l). The homogenate was centrifuged at 12,000 rpm for 20 min. The resultant supernatant was used for the assay.

0.5 ml of 0.2M borate buffer, 0.2 ml prepared enzyme extract and 1.3 ml of water was taken in a test tube and mixed well. The reaction was initiated by adding 1 ml of 0.1 M L - Phenylalanine solution (165 mg of L - Phenylalanine in 10 ml of

water with a pH of 8.7). The homogenate was incubated for 20 - 60 min. at 32^0C. The reaction was stopped by adding 0.5 ml of 1M trichloroacetic acid (16.3 g in 100 ml of water). Control was also run in which phenylalanine was added after addition of trichloroacetic acid but without any enzyme extract. The absorbance was measured at 290 nm. The standard graph was prepared using *trans* – cinnamic acid as the standard.

3.4.2.7. Statistical analysis

The statistical analysis was done following Factorial CRD (Completely Randomized Design) using OPSTAT software .

3.5. VALIDATION OF MARKERS TO IDENTIFY THE DISEASE RESISTANT GENE

3.5.1. Isolation of DNA

The genomic DNA was isolated from selected resistant and susceptible varieties using *DNeasy* Plant Mini Kit following the procedure supplied by the manufacturers given in 3.3.3.2.

3.5.2. Quality of DNA

The quality or the purity of the isolated DNA was checked by agarose gel electrophoresis as described in 3.3.3.3.

3.5.3. Quantity of DNA

The quantity of DNA isolated from the samples was assessed by reading the absorbance value of the DNA samples using Nano drop 2000C spectrophotometer (Thermo scientific). Absorbance was recorded at both wavelength and purity was indicated by the ratio A 260 /A 280.

3.5.4. PCR amplification in identification of disease resistant gene

PCR amplification was carried out by modifying the protocol of Das *et al.* (2016) using SCAR (Sequence characterized amplified region) primers to identify the gene linked to disease resistance in the resistant varieties.

3.5.4.1. Standardization of annealing temperature

The annealing temperature for PCR amplification was standardized by gradient PCR (Eppendorf). The range of the temperature used for the gradient PCR is given below:

Sl No	Temperature ^{0}C
1.	48.1
2.	49.4
3.	51.2
4.	52.6
5.	54.2
6.	56.2
7.	58.2
8.	60.2
9.	62.4

Amplification was carried out with 20µl of reaction mixture. The components of the reaction mixture is given below:

Sl. no	Components	Quantity
1	Buffer B	2 µl
2	dNTPs	1 µl
3	Primers	1 µl
4	Taq polymerase	0.3 µl
5	$MgCl_2$	0.7 µl
6	Nuclease free water	13 µl
7	DNA	50 ng
	Total volume	**20 µl**

The nucleotide sequence of SCAR primers used are given below:

Forward primer	5′-GCACCCACCAACAGTTTGA- 3′
Reverse primer	5′-CAGCACCCACTT CCAATT-3′

The thermal profile followed for PCR includes:

Process	Temperature (°C)	Time
Initial denaturation	94	5 min.
Denaturation	92	45 sec.
Annealing	*	45 sec.
Extension	72	1 min.
No: of cycles: 35		
Final extension	72	7 min.

* Annealing temperature standardized under 3.5.4.1. by gradient PCR was set

3.5.4.2. PCR amplification

PCR amplification was carried out using SCAR primers with PCR reaction mixture and thermal profile standardized as given in 3.5.3.1.

3.5.4.3. Analysis of PCR products

The analysis of the PCR amplicons was done using agarose gel electrophoresis as given in 3.3.3.4.3.

3.5.5. Sequencing of the amplicons

The eluted amplicons obtained from all the 50 isolates were sequenced using automated sequencing facility at AgriGenome Pvt Ltd., Kakkanad, Kochi.

3.5.5. 1. *In silico* analysis

The homology search was carried out by blasting the nucleotide sequences at NCBI Genebank database (USA) using BLASTn algorithm and then comparing the homology with the sequence available in the database. The gene conferring disease resistance was identified by BLASTp algorithm and then comparing the sequence obtained with the whole genome sequence of *Musa* spp.

3.6. Management of Sigatoka leaf spot disease of banana

Two separate field experiments entitled "Management of Sigatoka leaf spot using chemicals" and "Management of Sigatoka leaf spot using biocontrol agents, organic/inorganic preparations" were conducted in BRS, Kannara during May, 2016. The statistical design followed was Randomized Block Design (RBD). The variety used was Nendran (AAB) for both the trials. Pits of size 50 cm x 50 cm x 50 cm were taken and applied with lime. Healthy suckers were planted with a spacing of 2 x 2 m. The organic manures and the fertilizer application were done as per Package of Practices (POP) recommendations of KAU (KAU, 2011). The first spray of the treatments was given when ten leaf spot stage was visible on the lowest leaves of majority of plants and subsequently three sprays were given at fortnightly

intervals. Severity of the disease was taken at weekly intervals and the per cent disease severity (PDS) was calculated using Gauhl's modification of Stover's severity scoring system as given in 3.1

3.6.1. Experiment No: 1: Management of Sigatoka leaf spot disease using chemicals fungicides

The field experiment for the management of Sigatoka leaf spot disease using chemical fungicides was laid out in RBD design with seven treatments. Three replications of each treatment were maintained with nine plants in each replication. The details of the treatments and layout of the experimental field are given in Table 3.2 and Plate 3.1 respectively.

3.6.2. Experiment No: 2: Management of Sigatoka leaf spot disease using biocontrol agents and organic/inorganic preparations

The field experiment on management of Sigatoka leaf spot disease of banana using organic/ inorganic preparations was laid out in RBD design with nine treatments replicated thrice with nine plants in each replication. The details of the treatments and layout of the field are given in Table 3.3 and Plate 3.1 respectively.

3.6.3. Observations recorded

The observations taken during the experiment are given below:

1.Per cent disease severity (PDS)

The PDS was calculated using Gauhl's modification of Stover's severity scoring system and was calculated using the formula given in 3.1.

2. Youngest leaf spotted (YLS)

The youngest leaf spotted was calculated by counting the position of the youngest leaf showing at least ten leaf spots.

3. Disease development time (DDT)

DDT is the parameter which indicates the time or number of days taken by the leaves to reach ten leaf spots.

4. Per cent reduction of disease over control

Per cent reduction of disease over control was calculated by:

$$\text{PDS} = \frac{\text{PDS in control plants} - \text{PDS in treatments}}{\text{PDS in control plants}}$$

5. Vegetative characters

Vegetative characters such as plant height, girth and number of green leaves at the time of flowering was noticed.

6. Yield parameters

The yield characters such as bunch weight, number of hands, number of fingers, length and circumference of fingers, fresh and ripe weight of fingers, peel to pulp ratio and total soluble solids (TSS) were recorded.

3.6.4. Economic analysis

The economic analysis was carried out by calculating B: C ratio.

3.6.5. Statistical analysis

The statistical analysis was done following experimental design RBD (Randomized Block Design) using WASP software.

3.7. Residue analysis

During harvest one kg of the fruit samples were collected from T1

(pyraclostrobin), T4 (hexaconazole + captan) and T5 (trifloxystrobin + tebuconazole) for residue analysis. The analysis was done at Pesticide Residue Research and Analytical Lab, College of Agriculture, Vellayani.

Table 3.1: List of chemical treatments

Treatment no:	Treatments
T1	Foliar spraying of pyraclostrobin (1g/l)
T2	Foliar spraying of copper hydroxide (2g/l)
T3	Foliar spraying of propiconazole (1ml/l)
T4	Foliar spraying with hexaconazole (5%) + captan (70%) (2g/l)
T5	Foliar spraying with trifloxystrobin 25% + tebuconazole (50%) (0.4g/l)
T6	Foliar spraying of Bordeaux mixture (1%)
T7	Control

Table 3.2: List of organic/ inorganic preparations

Treatment no:	Treatments
T1	Foliar spraying with cowdung extract + *Pseudomonas fluorescens* (1%)
T2	Foliar spraying PGPR mix II (consortium of *P. fluorescens* and *Bacillus subtilis*) (20g/l)
T3	Foliar spraying of *Pseudomonas fluorescens* (20g/l)
T4	Foliar spraying of turmeric powder + baking soda mixture (5:1 per litre of water)
T5	Foliar spraying of salicylic acid (25mg/l)
T6	Foliar spraying of KAU micronutrient multimix (Sampoorna) (10g/l)
T7	Foliar spraying of petroleum based mineral oil (1ml/l) (POP)
T8	Foliar spraying of Bordeaux mixture (1%) (Chemical check)
T9	Untreated control

Field experiments
Location: Banana Reseach Station, Kannara

Imposing of treatments in the field

Tagging of cigar leaves

Plate 3.1: An overview of field

Results

4. RESULTS

The present study on "Characterization of *Mycosphaerella* spp. causing Sigatoka leaf spot disease complex of banana in Kerala and its management" was carried out at College of Horticulture, Vellanikkara and Banana Research Station, Kannara during 2015 - 2018. The experiments were carried under *in vivo* and *in vitro* conditions in order to study and document in detail the symptomatology, variations in symptoms and characterization of the pathogen inciting Sigatoka leaf spot disease in Kerala, to understand the basis of host plant resistance and to evolve different chemical and bioagents/organic/inorganic strategies for the management of the disease. The results obtained during the study are summarized below:

4.1. SURVEY AND SYMPTOMATOLOGY

Purposive sampling surveys were conducted during two seasons *ie.,* April – May, 2017 (pre monsoon) and July – August, 2018 (post monsoon) in the farmer's field of selected panchayaths of Thrissur district as well as in Malapuram (Northern zone), Palakkad (Northern zone), Ernakulam (Central zone), Wayanad (High range zone), Thiruvanathapuram (Southern zone) districts and the variation of symptoms between different cultivars as well as within the cultivars during both seasons were noted and are documented below. The latitude, longitude and weather parameters such as temperature and rainfall prevailed at the time of survey in different locations were also recorded from the data obtained from Marksim site (Table 4.1a&b).

4.1.1. Incidence and severity of the disease

The incidence of the disease was 100% in all the fields surveyed. The variety, age of plant, per cent disease severity (PDS) and the youngest leaf spotted (YLS) were documented from fields surveyed at different locations and is presented in Table 4.2a&b. The results of the survey revealed that the severity of the disease varied with

Table 4.1a: Details of weather parameters in different locations of Thrissur district

Locations of survey	Latitude ^{0}N	Longitude ^{0}E	April – May, 2017 (pre monsoon)			July – August, 2018 (post monsoon)		
			Maximum temperature (^{0}C)	Minimum temperature (^{0}C)	Rainfall (mm)	Maximum temperature (^{0}C)	Minimum temperature (^{0}C)	Rainfall (mm)
Wadakkanchery	10.66	76.24	31.55	24.85	182.50	28.50	23.00	691.00
Chelakkara	10.53	76.21	33.45	25.55	188.50	26.87	20.50	698.50
Erumapetty	10.68	76.16	34.15	25.10	194.00	28.40	23.20	570.50
Vellangallur	10.30	76.21	40.60	25.25	59.00	25.85	20.40	739.00
Meloor	10.30	76.38	34.20	25.20	60.00	27.56	22.45	706.50
Pudukkad	10.42	76.27	31.85	21.55	230.50	25.18	20.55	725.00
Kodakkara	10.37	76.31	31.15	22.05	196.50	26.95	20.65	410.50
Mattathur	10.37	76.33	33.25	25.40	136.00	26.95	20.65	410.50
Nenmanikkara	10.44	76.25	33.30	25.5	183.50	28.95	23.35	727.00
Vellanikkara	10.55	76.27	33.35	25.30	178.00	28.50	23.20	587.50

Table 4.1a (contd....): Details of weather parameters in different locations of Thrissur district

Locations of survey	Latitude ^{0}N	Longitude ^{0}E	April – May, 2017 (pre monsoon)			July – August,2018 (post monsoon)		
			Maximum temperature (^{0}C)	Minimum temperature (^{0}C)	Rainfall (mm)	Maximum temperature (^{0}C)	Minimum temperature (^{0}C)	Rainfall (mm)
Mala	10.66	76.24	37.75	25.45	57.00	28.70	23.30	578.00
Chowannur	10.53	76.21	33.05	25.45	67.50	26.00	21.89	712.50
Kodariyur	10.68	76.16	41.35	25.30	56.00	26.32	21.34	722.00
Nattika	10.30	76.21	33.65	25.30	61.50	28.40	23.30	592.50
Thalikulam	10.30	76.38	33.65	25.30	61.50	28.50	23.35	592.50
Puzhakkal	10.42	76.27	33.35	25.55	75.00	26.81	21.88	722.50
Thekkumkkara	10.37	76.31	33.60	25.25	67.00	26.91	20.59	738.50
Nadathara	10.37	76.33	34.20	25.05	68.00	24.67	19.78	717.00

Table 4.1b: Details of weather parameters in different locations of other districts

Sl. no.	District	Locations of survey	Temperature ^{0} C		Rainfall (mm)
			Minimum	Maximum	
1.	Malappuram	Vattumkulam	25.10	33.52	158.21
2.		Perinthalmanna	25.30	33.27	141.23
3.		Vengara	24.76	32.50	177.12
4.		A.R. Nagar	24.76	32.50	177.12
5.	Palakkad	Malampuzha	24.95	34.70	115.00
6.		Srikrishnapuram	25.30	34.14	165.13
7.		Vattapara	25.45	34.32	121.50
8.		Pattambi	25.60	33.50	180.19
9.		Thrithala	25.33	33.78	174.21
10.	Ernakulam	Kalady	22.50	33.80	183.21
11.		Kothamagalam	24.56	34.56	216.58
12.		Pothanicad	22.75	33.21	222.89
13.	Wayanad	Lakkadi	20.50	28.95	165.50
14.		Ambalavayal	19.60	26.88	182.18
15.		Sultan Bethery	20.10	27.36	177.12
16.	Thiruvanathapuram	Nedumangad	25.10	31.24	243.46
17.		Neyyattinkara	25.09	31.40	233.19
18.		Peringammala	24.80	32.00	255.37

the type of cultivars, the age of the plant, location and the season. In general, the highest PDS of 43.90% was recorded on nine months old banana var. Palayankodan at Pudukkad panchayath of Thrissur district during post monsoon survey and the lowest PDS of 3.33% was recorded during pre monsoon survey on three months old banana var. Njalipoovan at Thalikulam panchayath of Thrissur district.

During pre monsoon survey in Thrissur district the highest PDS of 19.41% was observed in Pudukkad panchayath on eight months old Nendran variety with YLS of 7.70. This was followed by PDS of 15.87% on six months old Nendran variety at Kodakkara panchayath with YLS of 7.00. The least PDS of 3.33% was recorded on three months old Njalipoovan variety in Thalikulam panchayath with YLS of 10.00.

The results of the post monsoon survey in Thrissur district revealed that the highest PDS of 43.90% was recorded on nine months old Palayankodan variety in Pudukkad panchayath with YLS of 3.78. This was followed by PDS of 43.88% on eight months old Nendran variety having YLS of 4.00 in Vellangallur panchayath. The lowest PDS 23.02% was recorded on seven months old Kadali variety with YLS of 6.40 in Erumapetty panchayath.

In Malappuram district (Northern zone) the highest PDS of 16.78% was recorded on nine months old Nendran variety with YLS of 6.12 at Vengara panchayath while the lowest PDS of 4.57% was recorded on five months old Nendran variety with YLS of 8.78 at Perinthalmanna.

In Palakkad district (Northern zone), the highest PDS was recorded on eight months old Nendran variety with YLS of 6.89 at Pattambi and the lowest PDS of 4.53% was noticed on five months old Nendran variety having YLS of 7.00 in Vattapara panchayath.

Among the three panchayaths surveyed in Ernakulam district (Central zone)

the highest PDS of 18.76% was recorded on seven months old Nendran variety with YLS of 6.00 in Pothanicad panchayath whereas the lowest PDS was recorded on eight months old Nendran variety with YLS of 5.70 at Kalady panchayath.

In case of Wayanad district (High range zone), the highest PDS of 23.41% was noticed in Ambalavayal panchayath on eight months old Nendran variety with YLS of 4.98 while lowest PDS of 14.01% was recorded on seven months old Nendran at Lakkadi panchayath with YLS of 6.01.

The disease scenario at Thiruvanathapuram (Southern zone) district the highest PDS of 29.40% was noticed on nine months old Nendran variety at Peringammala panchayath with YLS of 5.34. The lowest PDS (21.34%) was noticed on seven months old Nendran variety at Nedumangad panchayath with YLS of 6.00.

4.1.2. Correlation analysis of disease severity with weather parameters

The correlation analysis of disease severity with major weather parameters and age of the plants was performed using SPSS v 16.0 and the result is presented in Table 4.3.

It was observed that there was a significant positive correlation between per cent disease severity (PDS), rainfall and the age of the plants while it was negatively correlated with temperature (Fig 4.1, 4.2 & 4.3). Hence, the correlation analysis revealed that disease severity increased with increase in rainfall and age of the plants while PDS decreased with increase in temperature.

4.1.3. Variation in symptoms on different cultivars in different locations

The results of the survey revealed that there were variations in symptom expression according to the cultivars and the locations (Plate 4.1).

Table 4.2a: Severity of Sigatoka leaf spot disease at different locations surveyed in Thrissur district

Sl.no.	Location of survey	April – May, 2017 (pre monsoon)				July – August, 2018 (post monsoon)			
		Variety	Age of plants (MAP)*	PDS**	YLS***	Variety	Age of plants (MAP)*	PDS**	YLS***
1.	Wadakkanchery	Nendran	5.00	12.72	6.70	Palayankodan	7.00	30.08	6.90
2.	Chelakkara	Nendran	5.00	12.33	9.00	Nedunendran	7.00	43.23	3.80
3.	Erumapetty	Nendran	3.00	7.33	9.20	Nendran	8.00	36.92	4.40
		Kadali	3.00	5.42	7.52	Kadali	7.00	23.02	6.40
		Poovan	5.00	13.49	8.00	Nendran	7.00	34.85	6.30
4.	Vellangallur	Nendran	4.00	6.12	7.40	Nendran	8.00	43.88	4.00
5.	Meloor	Nendran	3.00	7.48	10.30	Nendran	5.00	38.23	6.10
6.	Pudukkad	Nendran	8.00	19.41	7.70	Palayankodan	8.00	42.07	5.30
7.	Kodakkara	Nendran	6.00	15.87	7.00	Njalipoovan	4.00	26.98	7.10
						Nendran	5.00	32.79	6.00
8.	Mattathur	Kadali	4.00	10.85	7.00	Kadali	5.00	35.61	5.40
9.	Nenmanikkara	Nendran	5.00	13.85	10.10	Nendran	8.00	39.92	6.60

*Months after planting, ** Per cent disease severity, ***Youngest leaf spotted

Table 4.2a (contd...) : Severity of Sigatoka leaf spot disease at different locations surveyed in Thrissur

Sl.no.	Location of survey	April – May, 2017 (pre monsoon)				July – August, 2018 (post monsoon)			
		Variety	Age of plants (MAP)*	PDS**	YLS***	Variety	Age of plants (MAP)*	PDS**	YLS***
10.	Vellanikkara	Nendran	4.00	11.09	10.80	Nendran	6.00	26.08	6.80
11.	Mala	Nendran	3.00	6.40	10.40	Nendran	7.00	34.53	5.50
12.	Chowannur	Nendran	4.00	10.13	11.50	Nendran	9.00	40.12	5.80
13.	Kodariyur	Nendran	2.00	5.57	10.12	Robusta	7.00	38.28	6.90
						Nendran	7.00	41.87	5.40
14.	Nattika	Nendran	3.00	7.98	7.80	Njalipoovan	5.00	31.44	6.30
15.	Thalikulam	Nendran	5.00	7.86	11.7	Njalipoovan	6.00	23.58	7.20
		Njalipoovan	3.00	3.33	10.00				
16.	Puzhakkal	Nendran	7.00	10.34	11.60	Nendran	8.00	40.49	4.90
17.	Thekkumkkara	Palayankodan	5.00	8.35	8.12	Palayankodan	9.00	42.57	4.10
18.	Nadathara	Nendran	7.00	8.36	11.62	Palayankodan	9.00	43.90	3.78

*Months after planting, ** Per cent disease severity, ***Youngest leaf spotted

Table 4.2b : Severity of Sigatoka leaf sot disease at different locations surveyed in other districts

Sl. no.	District	Locations of survey	Variety	MAP*	PDS**	YLS***
1.	Malappuram	Vattumkulam	Nendran	5	6.50	7.33
2.		Perinthalmanna	Nendran	5	4.57	8.78
3.		Vengara	Nendran	10	16.78	6.12
4.		A.R. Nagar	Nendran	9	10.23	6.81
5.	Palakkad	Malampuzha	Nendran	3	3.62	8.01
6.		Srikrishnapuram	Nendran	7	11.03	6.25
7.		Vattapara	Nendran	5	4.53	7.00
8.		Pattambi	Nendran	8	12.50	6.89
9.		Thrithala	Nendran	7	10.13	6.31
10.	Ernakulam	Kalady	Nendran	7	14.34	5.70
11.		Kothamagalam	Nendran	8	12.96	6.69
12.		Pothanicad	Nendran	7	18.76	6.00
13.	Wayanad	Lakkadi	Nendran	7	14.01	6.01
14.		Ambalavayal	Nendran	8	23.41	4.98
15.		Sultan Bethery	Nendran	9	19.08	5.67
16.	Thiruvanathapuram	Nedumangad	Nendran	7	21.34	6.00
17.		Neyyattinkara	Nendran	6	21.79	6.10
18.		Peringammala	Nendran	9	29.40	5.34

*Months after planting, ** Per cent disease severity, *** Youngest leaf spotted

Table 4.3: Correlation analysis of disease severity with weather parameters

Sl. no.	Weather parameters	Correlation coefficient
		PDS **
1	Minimum temperature	-0.886*
2	Maximum temperature	-0.906*
3	Rainfall	0.965*
4	Age of the plants	0.704*

*Correlation is significant at 0.05 level

** Per cent disease severity

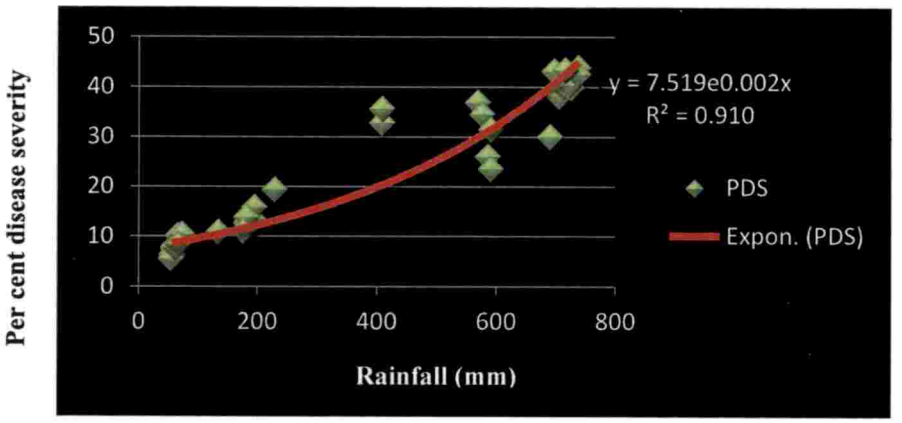


Fig 4.1: Influence of rainfall on disease severity

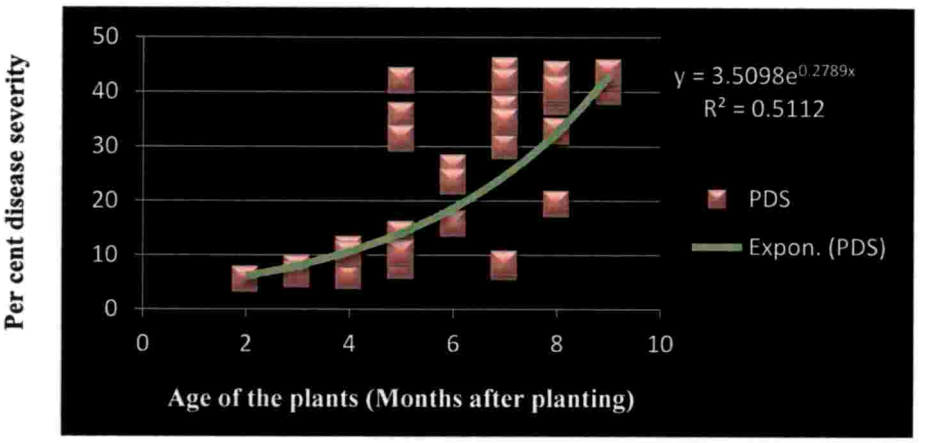


Fig 4.2: Influence of age of plants on disease severity

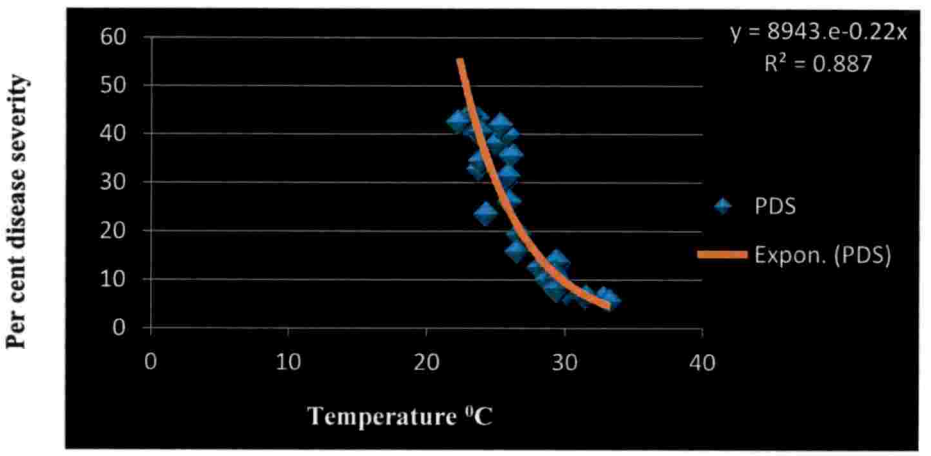


Fig 4.3: Influence of temperature on disease severity

4.1.3.1. Banana var. Nendran

Predominantly six different types of symptoms were noticed on Nendran cultivars during both the seasons *viz.*, pre monsoon and post monsoon.

4.1.3.1.1. Type 1 symptom

The symptom initiated as small light green or yellow spots on the lower surface of the leaves. The streaks then changed into faint brown visible streaks. Later, these streaks expanded in length and breadth and became linear in shape with pointed tips and appeared adjacent to the veins of the leaves (Plate 4.1a). The mature spots observed on the lower leaves had greyish centre with brown margin surrounded by prominent yellow halo. On the lower most leaves, these mature spots coalesced resulting in complete necrosis of the leaves.

4.1.3.1.2. Type II symptom

The initial symptoms appeared as small light yellow coloured round spots on the lower surface of the leaf. During the next stage of infection, the spots enlarged and turned to dark brown streaks on the lower side of the leaves. These streaks later appeared as linear dark brown spots on the interveinal areas of the upper surface of the leaves. In the advanced stages of infection, the streaks changed into oval spots with greyish centre and prominent brown margin (Plate 4.1b). These spots coalesced together causing the complete drying up of the leaf. These symptoms were similar to those described for Eumusae leaf spot by Carlier *et al.* (2000).

4.1.3.1.3. Type III symptom

Small yellow coloured round spots were observed on the under surface of the leaves. Moving down the lower leaves small faint brown streaks appeared on the lower surface of the leaves, which during next stage started appearing on the upper surface as dark brown streaks. These streaks expanded both in length and breadth and

became oval in shape. Chains of these oval spots were noticed along the veins of the leaves (Plate 4.1c). The oval spots were initially appeared on the edge of the leaves which later spreads towards the midrib. The mature oval spots contained greyish centre with definite light brown margin surrounded by yellow halo. In the final stage of infection, the mature spots coalesced, causing complete necrosis of the leaves. Type III symptoms is the commonly observed symptoms in banana var. Nendran.

4.1.3.1.4. Type IV symptom

The initial visible symptom appeared as faint brown streaks on the adaxial surface of the leaves. During the next stage, these streaks turned rusty brown on the adaxial surface of the leaves, which then developed into oval or elliptical brown spots surrounded by yellow halo. The mature spots had greyish centre surrounded by irregular dark brown border having black pin head like fruiting bodies embedded in it (Plate 4.1d). The spots appeared more along the veins than on the inetrveinal spaces of the leaves. Upon heavy infection, the spots coalesced leading to complete necrosis of the leaves thereby destroying the functional green tissues of the leaves.

4.1.3.1.5. Type V symptom

The initial symptoms appeared as light faint brown rusty streaks on the lower surface of the leaves. Later, these streaks started to appear on the upper surface of the leaves. These streaks then increased in length rather than breadth and appeared as brownish black elongated streaks instead of oval elliptical spots without prominent yellow halo. In the advanced stages of infection the streaks coalesced causing necrosis of the leaf.

4.1.3.1.6. Type VI symptom

These types of the symptoms were noticed during post monsoon surveys. The initial visible symptoms appeared as faint rusty streaks on the under surface of the leaf. Upon the next stage of infection, these streaks started appearing as black oval

shaped spots along the vein on the upper surface of the leaves. The chain of spots when mature had greyish centre with well defined black margin which was surrounded by yellow halo, which get coalesced from the edge to the midrib causing necrosis and drying up of the leaves. No other variation in symptom expression was noticed in Nendran variety surveyed during post monsoon surveys at different locations.

4.1.3.2. Variation in symptoms on other varieties

There were two types of symptoms observed on Palayankodan (AAB) and Njalipoovan (AB) varieties, while only one type of symptom was recorded on Robusta (AAA) and Kadali (AA) varieties (Plate 4.2).

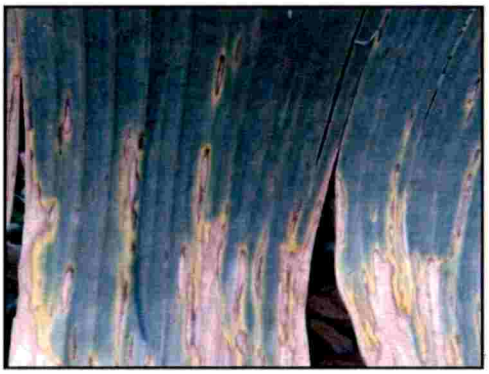
4.1.3.2.1. Banana var. Palaynkodan (AAB)

4.1.3.2.1.1. Type I symptom

The initial symptom appeared as light brown streaks on the lower surface of the leaves. Corresponding to the streaks on the lower surface, mild yellow colour appeared on the upper surface. During the next stage, the streaks changed into brown colour and appeared on the upper surface of the leaf. These streaks then became irregularly oval in shape with greyish centre without definite margins, which later coalesced causing complete necrosis of the leaves (Plate 4.2a). The lesions were more prominent along the interveinal space rather than on the veins. The symptoms were noticed during pre monsoon survey.

4.1.3.2.1.2. Type II symptom

The symptom initiated as small rusty brown streaks on the lower surface of the leaf which later appeared as black thin streaks on the upper surface. These black streaks increases in length and get coalesced along the line of the vein even before maturation giving an unusual black appearance to the foliage. Water soaked lesions

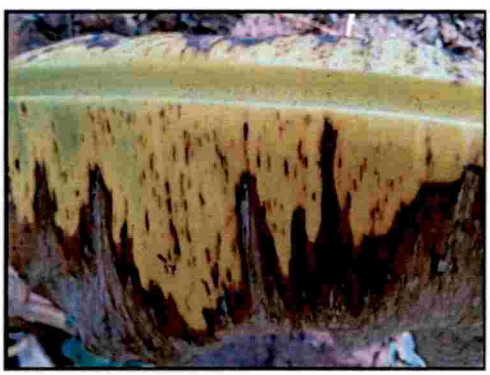


4.1a: Type 1 symptom

4.1b: Type II symptom

4.1c: Type III symptom

4.1d: Type IV symptom



4.1e: Type V symptom

4.1f: Type VI symptom

Plate 4.1: Variation in symptoms on banana var. Nendran (AAB)

were found on the under surface of the leaves corresponding to the black streaks on the upper surface. This type of symptom were more common during post monsoon survey (Plate 4.2b).

4.1.3.2.2. Banana var. Njalipoovan (AB)

4.1.3.2.2.1. Type I symptom

These symptoms were noticed during the pre monsoon survey. Yellowish orange streaks were observed on the lower side of the leaves. The streaks were then converted into rusty brown in appearance. Dark brown colour lesions were observed along the veins. During the next stage of infection, the brown coloured lesions changed to elongated linear spots with blended tips with prominent yellow halo. The mature lesions observed on 6^{th} and 7^{th} leaves were elongated having greyish centre with broad brown margin. The mature lesions coalesced along the veins and finally causes drying up of the whole leaf (Plate 4.2c).

4.1.3.2.2.2. Type II symptom

The initial symptoms appeared as yellowish orange elongated streaks on the underside of the leaves. During the next stage of infection, the yellowish orange streaks changed to brown streaks, which later started appearing on the upper surface of the leaves and were slightly depressed. The streaks then expand in size to become elliptical or oval in shape, corresponding to which water soaked lesions were observed on the under surface of the leaves. The lesions coalesced before maturation causing complete drying of the leaves (4.2d). On the dried leaves, greyish areas were noticed with black pin headed fruiting bodies embedded in the lesion discoloured area. These types of symptoms were more prevalent during post monsoon surveys.

4.1.3.2.3. Banana var. Kadali (AA): Type I symptom

The symptom expression on Kadali cultivar were similar during both the

seasons. Light brownish black coloured streaks appeared on the lower surface of the leaves. Corresponding to these streaks, black coloured streaks appeared on the upper surface of the leaves along the veins. These streaks increased in length rather than in breadth and became slightly depressed on the leaves. Greyish centre with black margin surrounded by yellow halo was observed on mature spots which later coalesced, causing necrosis of the leaves (Plate 4.2e).

4.1.3.2.4. Banana var. Robusta (AAA): Type I symptom

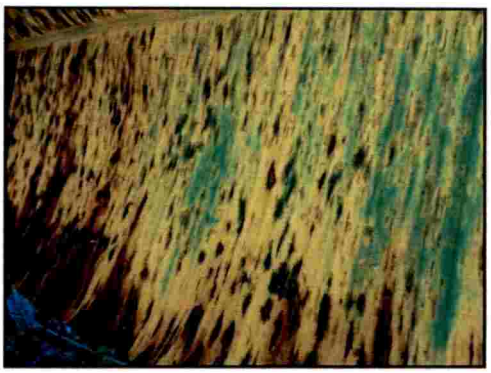
Similar symptoms were noticed in Robusta cultivars during the pre monsoon and post monsoon seasons of survey. Light brown coloured streaks appeared initially on the tip of the leaves which later spreads uniformly on the leaf lamina. These initial streaks then changed into linear black streaks on the upper surface of the leaf. The streaks were present both along the margin as well as in the interveinal areas and were slightly depressed, giving the leaf an unusual black appearance. The mature lesions had greyish centre with thick black margin surrounded by irregular yellow halo. The spots were found to be coalesced before maturation (Plate 4.2f). These symptoms expressed were very much similar to the symptom described for black Sigatoka disease by Meredith *et al.* (1969).

4.1.4. Developmental stages of symptom expression

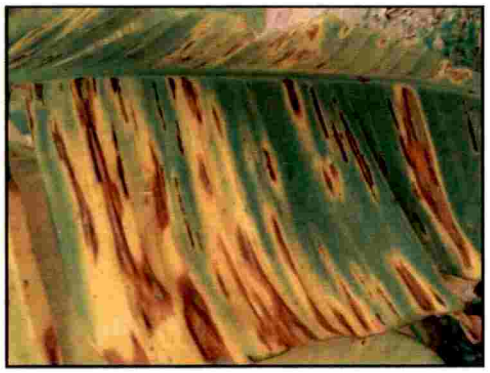
The developmental stages of symptoms were categorized into six stages during summer as well as rainy season (Plate 4.3 & 4.4).

During summer, the first external symptoms appeared as small yellow pigmented spots on the undersurface of the leaves and are designated as stage 1 (Plate 4.3a). This became more visible only when the leaves were held against sunlight. In the second stage of the disease development, the colour of the spots changed progressively to brown faint rusty stripes on the lower surface of the leaves (Plate 4.3b). During the next stage of disease development *i.e.,* stage 3, the brown

4.2a: Type 1 symptom (Palayankodan)



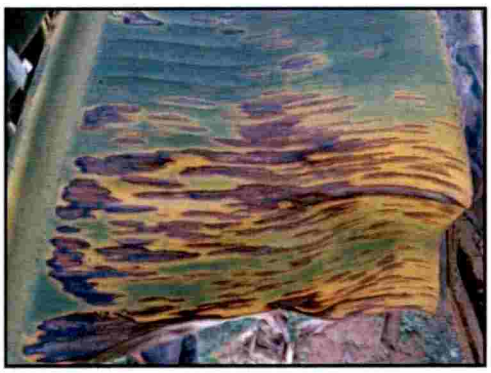
4.2b: Type 11 symptom (Palayankodan)



4.2c: Type 1 symptom (Njalipoovan)

4.2d: Type 11 symptom (Njalipoovan)

4.2e: Type 1 symptom (Kadali)



4.2f: Type 1 symptom (Robusta)

Plate 4.2: Variations in symptom on other cultivars

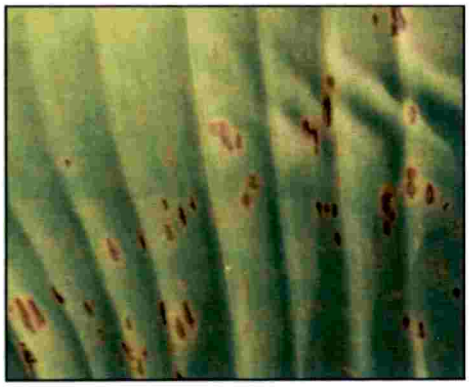
streaks/ stripes became elongated in size and the streaks appeared on both surfaces of the leaf (Plate 4.3c). These streaks then developed into elliptical spot surrounded by prominent yellow halo which were represented as stage 4 (Plate 4.3d). At stage 5, the elliptical spots enlarges into lesions, the centre of the spots dried out and turned greyish in colour surrounded by definite dark brown borders containing black pin headed like fruiting body embedded on it (Plate 4.3e). When the infection density was high, these lesions coalesced causing complete necrosis of the leaf (stage 6), thereby reducing the photosynthetic area of the leaves which in turn reduced the yield of the plant (Plate 4.3f).

During rainy season a slight variation in symptoms were noticed (Plate 4.4). Though all the six stages were noticed in all cultivars during rainy season, the lesions appeared more darker in colour. Initially the symptoms appeared as small yellow spots on the lower surface of the leaves (Plate 4.4a) which later changed to light faint brownish black streaks (Plate 4.4b). Later these streaks changed to black in colour which were slightly depressed and appeared on the upper surface of the leaves (Plate 4.4c). The streaks increased in size and formed oval spots with irregular margins (Plate 4.4d). During the next stage of infection, light grey to bright grey centers were noticed on the mature lesions with thick dark black margins surrounded by yellow halo giving the foliage the appearance of black Sigatoka disease (Plate 4.4e). At the final stage of infection the spots coalesced causing necrosis of the leaf (Plate 4.4f).

The lesion size and the symptom development time *i.e.,* days taken to shift from one stage to other on three cultivars *viz.,* Grand Naine, Nendran and Njalipoovan were recorded during summer and rainy season and presented in Table 4.4 and 4.5. The lesion size and the time taken to shift from one stage to the next differed with stage of symptom development and seasons. The studies on the lesion size and the time taken to shift from stage 1 to stage 6 of the three cultivars during summer and rainy season revealed that the size of the lesions were smaller and the days required for symptom development was more during the summer season than in

4.3a : Stage 1

4.3b : Stage 2



4.3c : Stage 3

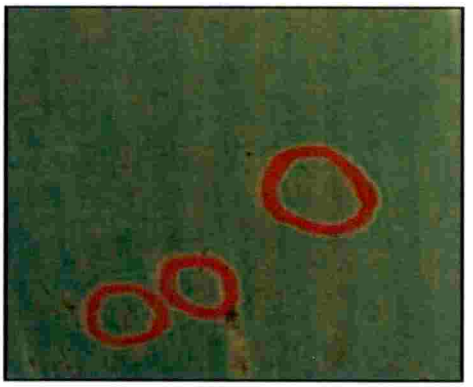
4.3d : Stage 4

4.3e : Stage 5

4.3f : Stage 6

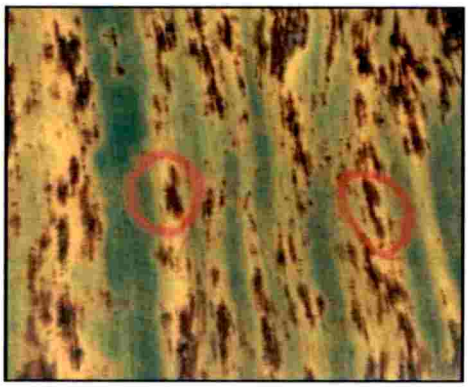
Plate 4.3: Developmental stages of Sigatoka leaf spot disease (summer season)

4.4a : Stage 1



4.4b : Stage 2

4.4c : Stage 3



4.4d : Stage 4

4.4e : Stage 5

4.4f : Stage 6

Plate 4.4: Developmental stages of Sigatoka leaf spot disease (rainy season)

the rainy season.

During summer, on banana var. Nendran, the initial stage lesion had a size of 0.10 cm x 0.01 cm whereas the mature lesions had a size 1.65 cm x 0.1 cm and took 79.02 days for the development of symptoms from stage 1 to stage 6 while during rainy season the size of the lesions ranged from 0.32 cm x 0.04 cm to 2.49 cm x 1.01 cm. The time taken to shift from stage 1 to stage 6 was observed to be 31.74 days.

While on banana var. Njalipoovan, an average of 75.97 days were taken for the development of the symptoms from stage 1 to stage 6 with an initial lesion size of 0.10 cm x 0.06 cm and the mature lesions attained a size of 1.59 cm x 0.42 cm during summer season. During rainy season, the size of the lesions ranged from 0.18 cm x 0.06 cm to 2.32 cm x 0.98 cm and took 35.78 days for development of symptom from stage 1 to stage 6.

The initial lesions developed during the summer season in Grand Naine cultivar had a size of 0.32 cm x 0.05 cm while the mature lesions size was observed to be 1.92 cm x 0.48 cm. The time taken to shift from the initial stage to last stage was 74.27 days. Whereas during rainy season, the size of the lesions ranged from 0.4 cm x 0.20 cm to 2.52 cm x 1.02 cm and took 31.48 days for symptom development from stage 1 to 6.

4.2. ISOLATION OF THE PATHOGEN

4.2.1. Isolation of the pathogen on PDA

The isolation of the pathogen was carried using leaf bit isolation technique on potato dextrose agar (PDA) medium. The growth of the fungus was very slow and appeared 10 days after isolation when incubated at 24°C in BOD incubator. On PDA the fungal growth appeared pale greyish in colour with raised centre. The colonies had irregular margins with velvety appearance. While the fungal colony appeared

Table 4.4: Development of symptoms on different cultivars (Summer season)

Variety	Days taken to shift to next stage						Lesion size (cm x cm)					
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
Nendran	32.3	10.50	12.10	10.69	8.32	5.11	0.10 x 0.01	0.26x 0.03	0.42 x 0.1	1.04 x 0.58	1.65 x 0.1	-
Njalipoovan	31.03	9.61	8.34	10.05	9.71	7.23	0.10 x 0.06	0.29x 0.08	0.46 x 0.11	0.92 x 0.24	1.59 x 0.42	-
Grand naine	30.11	12.70	9.56	7.23	7.66	7.01	0.32x 0.05	0.45 x 0.12	0.51 x 0.21	0.77 x 0.35	1.92 x 0.48	-

Table 4.5: Development of symptoms on different cultivars (Rainy season)

Variety	Days taken to shift to next stage						Lesion size (cm x cm)					
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
Nendran	10.35	5.34	5.95	4.18	3.37	2.56	0.32 x 0.04	0.70 x 0.1	0.84 x 0.25	1.78 x 0.44	2.49 x 1.01	-
Njalipoovan	11.25	4.44	6.62	5.97	4.45	3.05	0.18 x 0.06	0.40 x 0.1	0.64 x 0.18	1.56 x 0.42	2.32 x 0.98	-
Grand naine	8.33	5.01	6.38	4.61	3.85	3.30	0.4 x 0.20	0.61 x 0.28	0.78 x 0.37	1.42 x 0.52	2.52 x 1.02	-

black coloured on the reverse side of the Petri plates (Plate 4.5a & b). No sporulation was observed. The fungal growth produced on the medium was subcultured and purified by hyphal tip method. The purified culture of the fungus were maintained in PDA slants and stored at 24°C in BOD incubator.

4.2.2. Pathogenicity studies

The pathogenicity of the isolate was confirmed by proving Koch's postulates. Artificial inoculation was carried out on healthy banana leaves collected from the field with mycelial discs cut from three week old fungal culture grown on PDA medium after giving definite number of pinpricks with a sterile stainless steel needle. Inoculated leaves produced yellow coloured lesions within 7 days after inoculation, which later changed to brown colour, while no symptoms were noticed in the control (Plate 4.6). Re-isolation of the pathogen from the artificially inoculated leaves yielded fungal culture identical to the original culture.

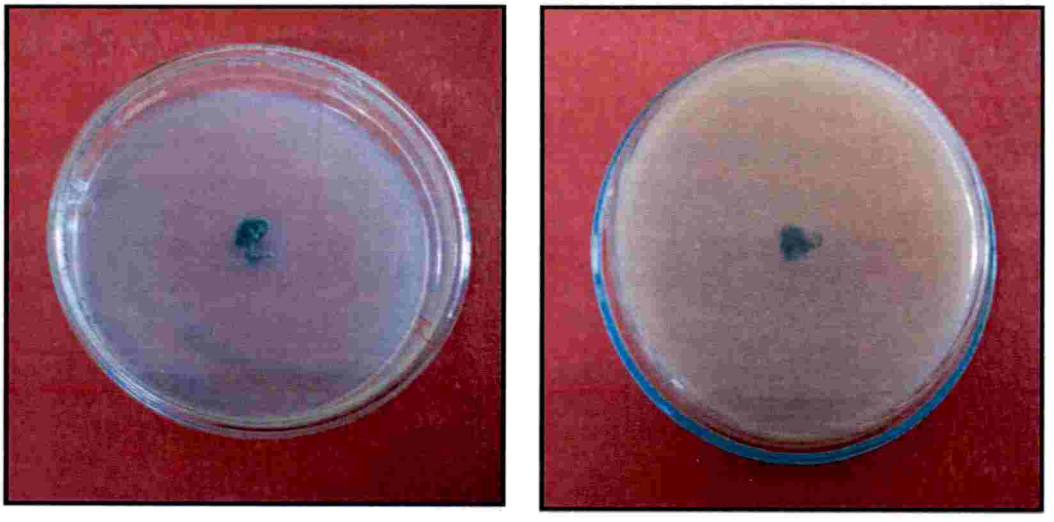
4.3. CHARACTERIZATION OF THE PATHOGEN

The characterization of the pathogen was carried out by studying the cultural, morphological and molecular characters of the pathogen.

4.3.1. Cultural characters

The mycelial growth of the pathogen on different solid media such as Potato dextrose agar (PDA), Carrot agar, V8 – juice agar and Banana leaf extract agar were studied and the best medium for the growth of the fungus was determined (Plate 4.7). The fungal colony produced maximum radial growth of 2 cm on PDA while the minimum radial growth of 0.31 cm on carrot agar 21 days after incubation. The results observed on growth pattern and growth rate of the pathogen is presented in Table 4.6.

On PDA, the three week old fungal culture produced small greyish colony



4.5a: Upper surface 4.5b: Lower surface

Plate 4.5: Mycelial growth of fungus on PDA

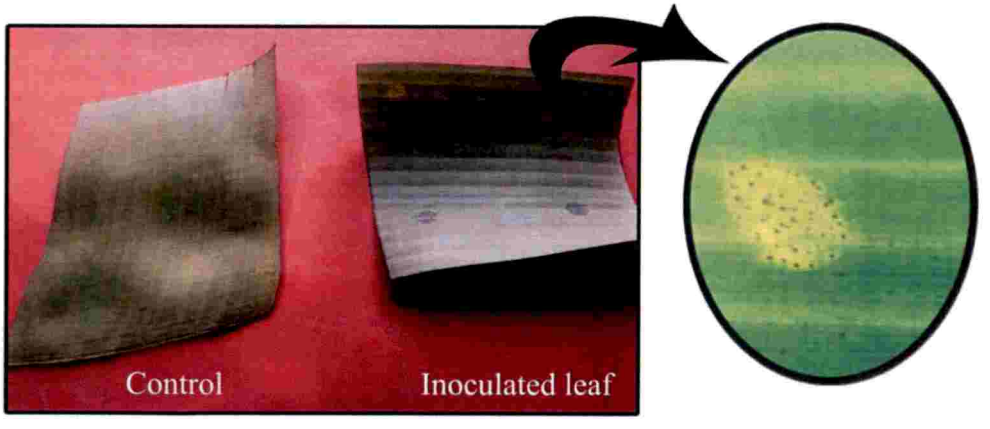


Plate 4.6: Pathogenicity of isolate on banana leaves

with raised centre with a radial growth of 2 cm (Plate 4.7a).

On carrot agar, the fungus produced greyish colony with slightly raised centre. The growth rate was very slow compared to PDA and the three week old culture produced radial growth of 0.31 cm only (Plate 4.7b).

On V8 juice agar, the fungal colony appeared small with an appressed growth. The culture initially appeared greyish in colour which later changed to black. The three week old culture produced a radial growth of 1.06 cm (Plate 4.8c).

On banana leaf extract agar, the fungus produced small, raised greyish colony with a radial growth of 1.93 cm (Plate 4.7d).

4.3.2. Morphological characters

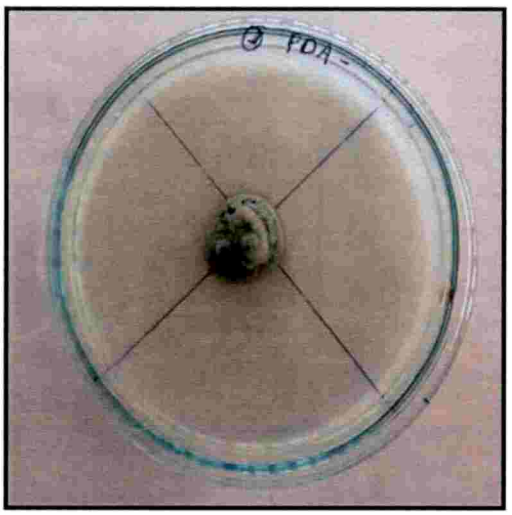
The mycelial characters of the pathogen were studied using fungal culture. The characters of the reproductive structures were studied directly from the leaf lesions using cellophane tape method and potassium hydroxide method.

4.3.2.1. Mycelial characters of the pathogen

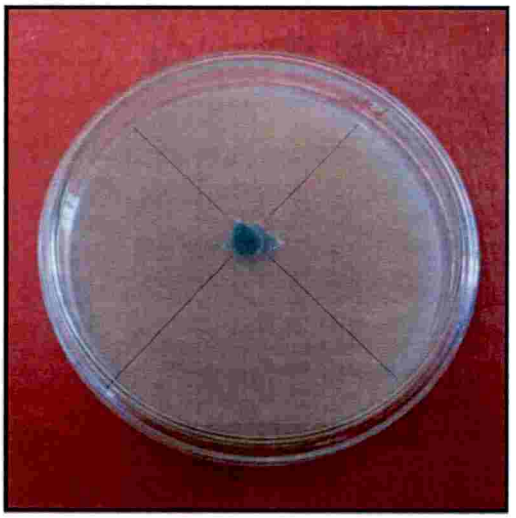
The microscopic observations of the mycelium revealed that the hyphae were septate and brown coloured. Hyphal anastomosis or fusion of the hyphae was observed in the culture which is a key identifying character of fungus belonging to genus *Pseudocercospora* (Plate 4.8) (Meredith *et al.*,1969).

4.3.2.2. Reproductive structures of the pathogen

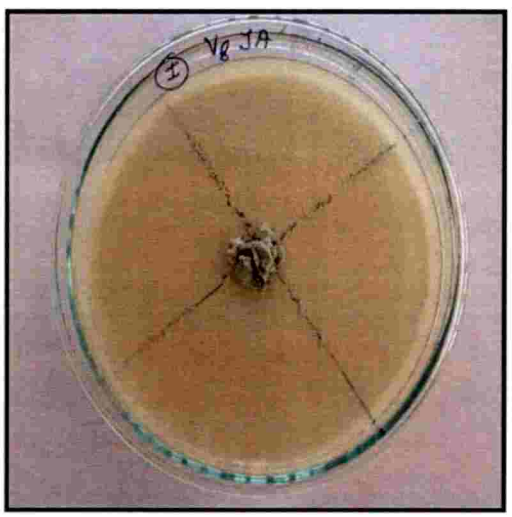
The asexual and sexual fungal structures were found to be closely associated with different stages of the leaf lesions. The conidial structures were found to be more prevalent in stage 1, 2 and 3 lesions as described in 4.1.4 whereas the perithecial structures were observed in the mature lesions of Stage 5 and 6 (Plate 4.9 & 4.10).



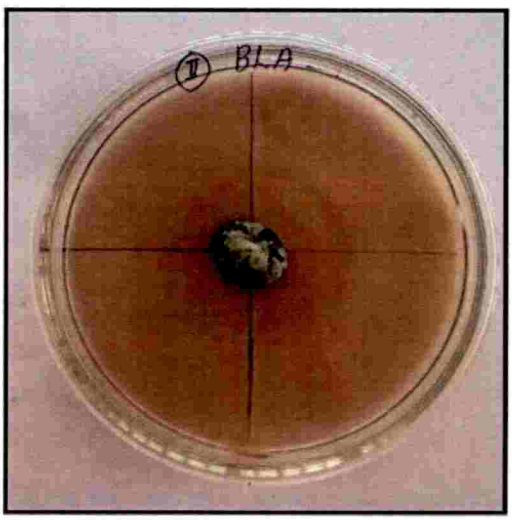
4.7a: Potato dextrose agar



4.7b: Carrot agar



4.7c: V8- juice agar



4.7d: Banana leaf extract agar

Plate 4.7: Cultural characters of the fungus on different solid media

Table 4.6: Growth pattern and growth rate of the fungus on different media

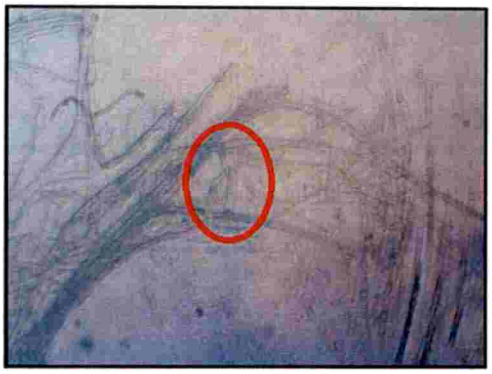
Sl.no.	Media	Growth pattern of the fungus	Radial growth of the fungus (cm)
1.	Potato dextrose agar	Small greyish colonies with raised centre	2.40 [a]
2.	Carrot agar	Small greyish colonies with slightly raised centre	0.31[d]
3.	V8- juice agar	Small greyish colonies with appressed, which later change to black colour	1.06 [c]
4.	Banana leaf extract agar	Small greyish colonies with slightly raised centre	1.93 [b]
	CD (0.05)		0.35

The conidiophores and the conidia were found to be emerged from the subepidermal region of the leaves. The conidiophores were seen as dense fascicles on the dark stroma (Plate 4.9a). These were pale brown in colour, straight with 2 – 5 septae arising from conidiogenous cells terminating in truncate ends (Plate 4.9b) which were absent in *P. fijiensis*. Conidia were thin, hyaline to olivaceous brown, straight to curved with a round base having septations without any visible scars. The size of the conidia ranged from 24.29 – 71.89 µm x 0.91 – 2.40 µm with 3- 6 septations (Plate 4.9c).

The presence of anastomosis, septate conidiphore emerging from conidiogenous cell and hyaline, septate conidia without any scar helps in identifying *Pseudocercospora eumusae* from the other two species *i.e., P.musicola* and *P.fijiensis* (Carlier *et al.*, 2000). Hence, based on the characters of the mycelium and asexual structures the pathogen is identified as *Pseudocercospora eumusae*.

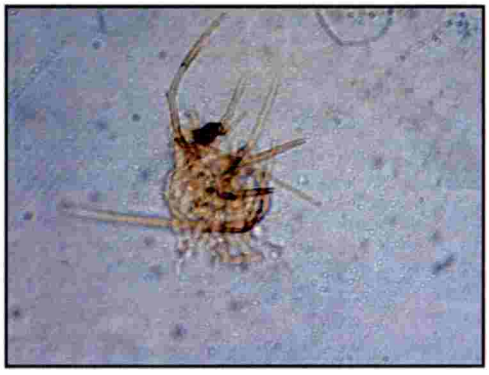
The microscopic observations of the leaf samples treated with 10% KOH revealed the presence of flask shaped perithecia and such sexual structures were closely associated with the mature lesions. The immature perithecia appeared to be round in shape (Plate 4.10a) which were embedded in the plant tissue whereas mature perithecia were flask shaped, 45.6-50.7 µm long x 30- 51.12 µm wide, ostiolated and were dark brown in colour (Plate 4.10b). The bursting of mature perithecia (Plate 4.10c) resulted in the release of asci bearing eight ascospores inside it (Plate 4.10d). The ascospores were 2-3 septate measured 12.0 to 14.5 µm × 2.23 to 4.8 µm and were twined around each other (Plate 4.10e). Similar observations were made by Jones (2000) while studying the character of the sexual fruiting bodies associated with *Mycosphaerella* sp. inciting Sigatoka leaf spot disease on banana.

Based on these characters of teleomorphic stage *i.e.,* the perfect stage of the fungus was identified as *Mycosphaerella* sp. The identification was further confirmed by molecular characterization of the pathogen.

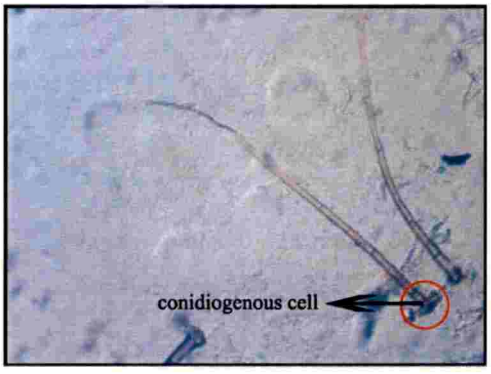


Hyphal anastomosis (400X)

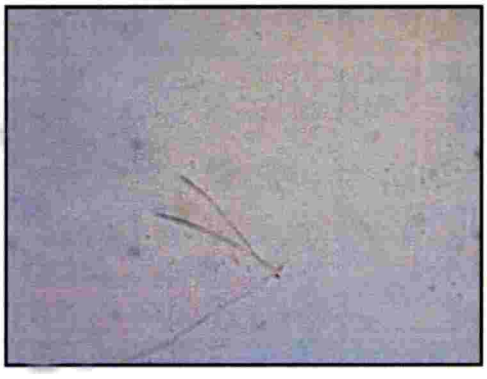
Plate 4.8: Mycelial characters of the fungus

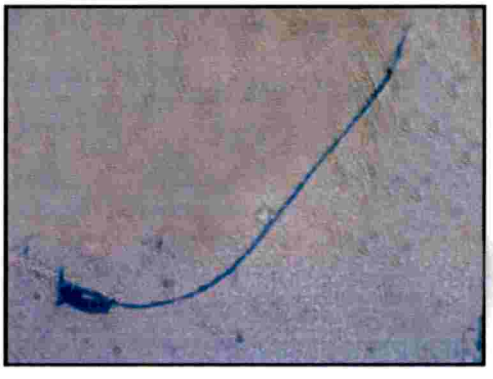


4.9a: Conidiophores formed on dark stroma (400X)



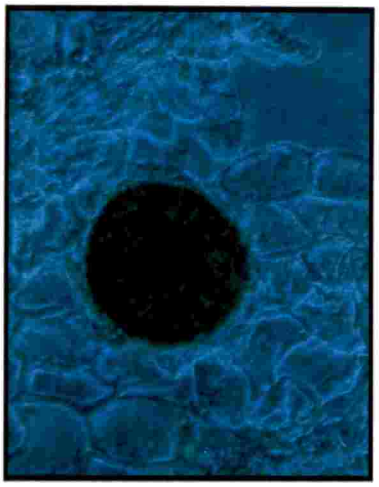
4.9b: Conidiophores arising from conidiogenous cell (400 X)



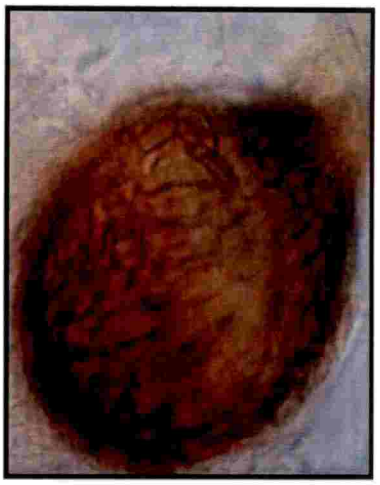


4.9c: Conidia (400X)

Plate 4.9: Asexual characters of the fungus

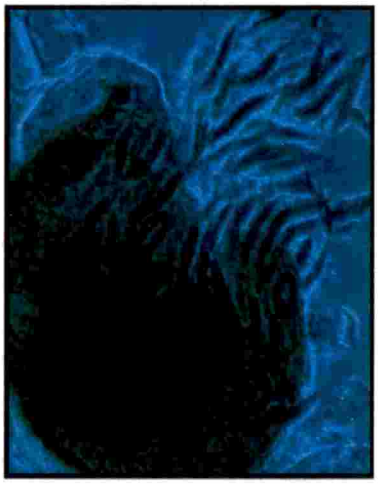


4.10a: Immature perithecia (400X)

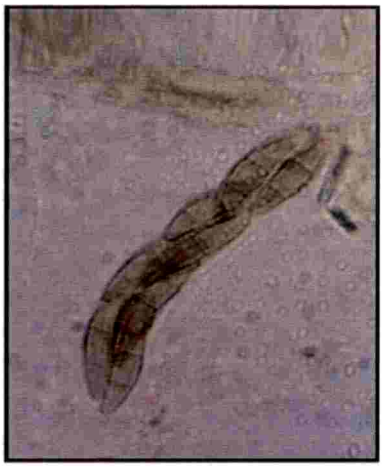


4.10b: Mature flask shaped perithecia (400X)

4.10c: Bursting of mature perithecia (400 X)



4.10d: Release of asci from perithecia (400 X)



4.10e: Ascus containing ascospores (400 X)

Plate 4.10: Sexual structures of the fungus

4.3.3 Molecular characterization of the pathogen

4.3.3.1. Isolation of DNA

The genomic DNA was isolated from infected leaf samples / isolates collected from different locations.

4.3.3.2. Assessment of purity of DNA

The agarose gel electrophoresis of the genomic DNA produced high quality single undegraded bands and were obtained for all the 50 isolates (Plate 4.11).

4.3.3.3. Polymerase Chain Reaction (PCR)

4.3.3.3.1. Standardization of annealing temperature

The standardization of the annealing temperature using gradient PCR revealed that among the range of temperatures used, good band of amplicons were produced at 60.4°C. Therefore the best annealing temperature was chosen as 60.4 °C for further PCR amplification of other isolates (Plate 4.12).

4.3.3.3.2. PCR Amplification

The amplification of the genomic DNA isolated from the 50 infected leaf samples/isolates was carried out using ITS primers (ITS 1 and ITS 2). The PCR thermal profile is given below:

Process	Temperature (°C)	Time
Initial denaturation	94	3 min.
Denaturation	94	45 sec.
Annealing	60.4	45 sec.
Extension	72	1 min.
No: of cycles: 35		
Final extension	72	10 min.

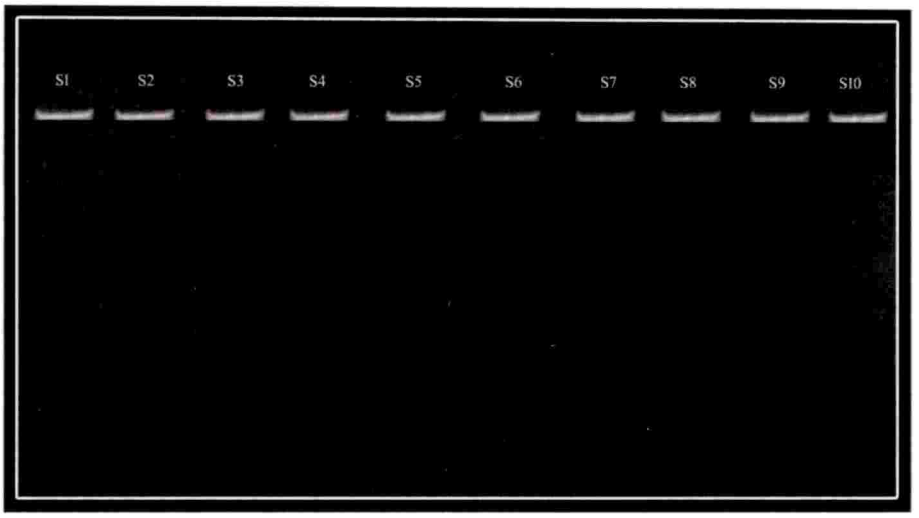


Plate 4.11: DNA isolated from infected samples

1. 60.1°C, 2. 60.4°C, 3. 61.2°C, 4. 62.6°C, 5. 64.3°C, 6. 66.2°C, 7. 68.2°C, 8. 70.2°C, 9. 72.0°C

Plate 4.12: Standardization of annealing temperature

4.3.3.3.3. Analysis of amplicons

The amplicons produced bands with expected molecular size at 540-580bp when visualized on 1% agarose gel. While in control no bands were produced confirming the validity and accuracy of PCR reactions (Plate 4.13a - c).

4.3.3.4. Sequencing of the amplicons

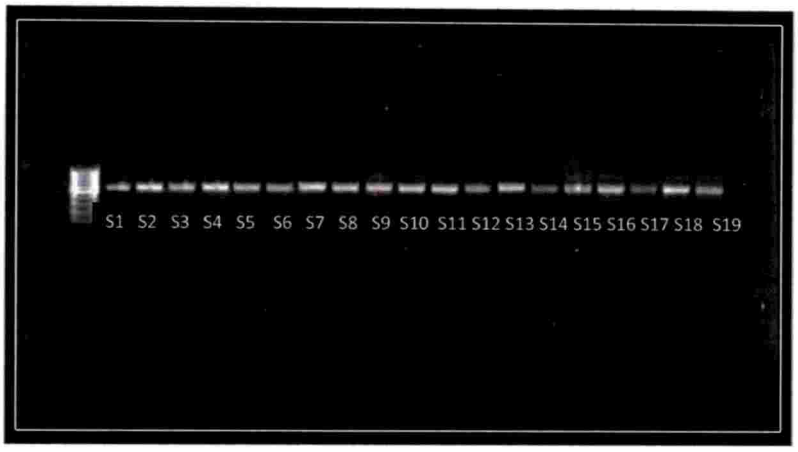
The sequencing of the amplicons was done using automated sequencing facility at AgriGenome Pvt Ltd., Kakkanad, Kochi. The details of nucleotide sequences obtained from 50 isolates are given in Appendix III.

4.3.3.4.1. *In silico* analysis of the sequences

The nucleotide sequences of the isolates were compared with the sequences of *Mycosphaerella* spp. available in the GeneBank of NCBI database using BLASTn program. The analysis revealed that the local isolates showed 99 - 100% sequence homology to *Mycosphaerella eumusae* (Fig 4.4) (Table 4.7). The amplified sequence included partial sequences of 18S rDNA and 28S rDNA and full sequence of ITS 1 region, 5.8S rDNA and ITS 2 region. The phylogenetic analysis was also performed using bioinformatics tool.

4.3.3.5. Phylogenetic analysis

The phylogenetic analysis of the 50 local isolates and the three *Mycosphaerella* spp. *viz., M. musicola, M.fijiensis* and *M.eumusae* revealed that all the fungus formed a monophyletic group which indicated that the Sigatoka leaf spot pathogens have been originated from a common ancestor. However, the local isolates showed greater similarity to the isolates of *M. eumusae* from CIRAD than to *M.musicola* and *M.fijiensis* (Fig 4.5). Though all the local isolates were pylogenetically similar to *M. eumusae,* the phylogenetic tree revealed that the local isolates belonged to different subclusters of the main cluster which indicated the



4.13a: Isolates from Thrissur district

4.13b: S20- S25: Isolates from Trivandrum,
S26- S31: Isolates from Kollam, S32- S34: Isolates fromErnakulam

4.13c: S35- S39: Isolates from Wayanad,
S40- S46: Isoaltes from Malappuram, S47-S50: Isolates from Pattambi

Plate 4.13: Gel profile of amplicons (540 - 580 bp)

Table 4.7: Nucleotide BLASTn analysis of *Mycosphaerella* isolates

Isolate	Query coverage	E value	Identity %	Most similar isolate
S1	100	0.0	100	**KC966891.1 (*Mycosphaerella eumusae*)**
S2	100	0.0	100	
S3	100	0.0	100	
S4	100	0.0	100	
S5	100	0.0	100	
S6	100	0.0	100	
S7	100	0.0	100	
S8	100	0.0	100	
S9	99	0.0	100	
S10	100	0.0	100	
S11	100	0.0	100	
S12	100	0.0	100	
S13	100	0.0	100	
S14	100	0.0	100	
S15	99	0.0	100	
S16	100	0.0	100	
S17	99	0.0	100	
S18	100	0.0	100	
S19	100	0.0	100	
S20	100	0.0	100	
S21	100	0.0	100	
S22	100	0.0	100	
S23	100	0.0	100	
S24	100	0.0	100	
S25	100	0.0	100	

Table 4.7 (contd....): Nucleotide BLASTn analysis of *Mycosphaerella* isolates

Isolate	Query coverage	E value	Identity %	Most similar isolate
S26	100	0.0	100	**KC966891.1 (*Mycosphaerella eumusae*)**
S27	100	0.0	100	
S28	100	0.0	100	
S29	100	0.0	100	
S30	100	0.0	100	
S31	100	0.0	100	
S32	100	0.0	100	
S33	100	0.0	100	
S34	100	0.0	100	
S35	100	0.0	100	
S36	99	0.0	100	
S37	100	0.0	100	
S38	100	0.0	100	
S39	100	0.0	100	
S40	100	0.0	100	
S41	100	0.0	100	
S42	99	0.0	100	
S43	100	0.0	100	
S44	90	0.0	100	
S45	100	0.0	100	
S46	90	0.0	100	
S47	100	0.0	100	
S48	100	0.0	100	
S49	100	0.0	100	
S50	100	0.0	100	

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Mycosphaerella eumusae isolate 74ME 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer	896	896	100%	0.0	100%	KC966893.1
Mycosphaerella eumusae isolate 70ME 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer	896	896	100%	0.0	100%	KC966889.1
Mycosphaerella eumusae isolate 26ME internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and	896	896	100%	0.0	100%	KC966846.1
Mycosphaerella eumusae from banana cv. Myndoli (Nendran-Plantain-AAB) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA g	896	896	100%	0.0	100%	GU168026.1
Mycosphaerella eumusae isolate UQ 5E2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spac	896	896	100%	0.0	100%	AY923757.1
Mycosphaerella eumusae isolate 52ME internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and	893	893	99%	0.0	100%	KC966871.1
Mycosphaerella eumusae isolate 66ME 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer	891	891	100%	0.0	99%	KC966885.1
Mycosphaerella eumusae isolate 35ME internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and	891	891	100%	0.0	99%	KC966855.1
Mycosphaerella eumusae from banana cv. Kachkel (ABB) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal t	891	891	99%	0.0	100%	GU168024.1
Mycosphaerella eumusae from banana cv. Grand naine (AAA) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inter	889	889	99%	0.0	100%	GU168036.1
Mycosphaerella eumusae from banana cv. Ladan (Pome-AAB) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inte	889	889	99%	0.0	100%	GU168031.1
Mycosphaerella eumusae isolate 50ME 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer	887	887	98%	0.0	100%	KC966869.1
Mycosphaerella eumusae isolate 46ME 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer	887	887	98%	0.0	100%	KC966865.1
Mycosphaerella eumusae isolate 28MEF 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed space	887	887	99%	0.0	99%	KC966848.1
Mycosphaerella eumusae isolate 42me 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer	885	885	100%	0.0	99%	KC966861.1
Mycosphaerella eumusae isolate 25ME internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and	885	885	98%	0.0	100%	KC966845.1
Mycosphaerella eumusae isolate 20ME 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer	885	885	98%	0.0	100%	KC966840.1
Mycosphaerella eumusae voucher 487THA internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence;	883	883	98%	0.		
Mycosphaerella eumusae isolate 24ME 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer	883	883	99%	0.		

Fig 4.4: Results of sequences blasted at NCBI obtained through amplification of ITS region

presence of genetic diversity among *M. eumusae* isolates collected from different agroclimatic zones of Kerala (Fig 4.6).

The clade containing isolates of *M. eumusae* and local isolates differentiated from *M. fijiensis* clade by 10 base pairs while differed from *M. musicola* clade by 4 base pairs. Based on the first identical base in all alignment which had a motif of GGC (position 50) there observed a gap of 1bp in 75th position and 9bp from 81- 90 positions in *M.fijiensis* (Fig 4.7) while a gap of 4bp were observed in *M. musicola* at 460 - 463th positions (Fig 4.8), but were present in all the 50 local isolates and *M. eumusae*.

Therefore the molecular characterization of the ITS region of the local isolates revealed that the pathogen inciting Sigatoka leaf spot in Kerala was confirmed and identified as *Mycosphaerella eumusae* Crous & Mourichon (anamorph *Pseudocercospora eumusae* Crous & Mourichon). The pathogen belongs to the Kingdom: fungi, Phylum: Ascomycota, Class: Dothedeomycetes, Order: Capnodiales, Family: Mycosphaerellaceae

4.4. A NEW SPOT ON BANANA var. NENDRAN FROM KERALA

During the surveys, a leaf spot similar to Sigatoka leaf spot was observed on banana var. Nendran in Thalikulam and Erumapetty panchayaths of Thrissur district. The leaf samples were brought to the laboratory for characterization and identification of the pathogen (Plate 4.14).

4.4.1. Symptoms

The symptoms appeared mostly on the upper surface of the lower leaves as angular dark brown circular necrotic spots. As the necrotic lesions increased in size, it resulted in the formation of concentric rings on the spots. The size of the necrotic lesion ranged from 1.2 – 2.5 × 1.2 - 2cm. The concentric circles appeared dark brown

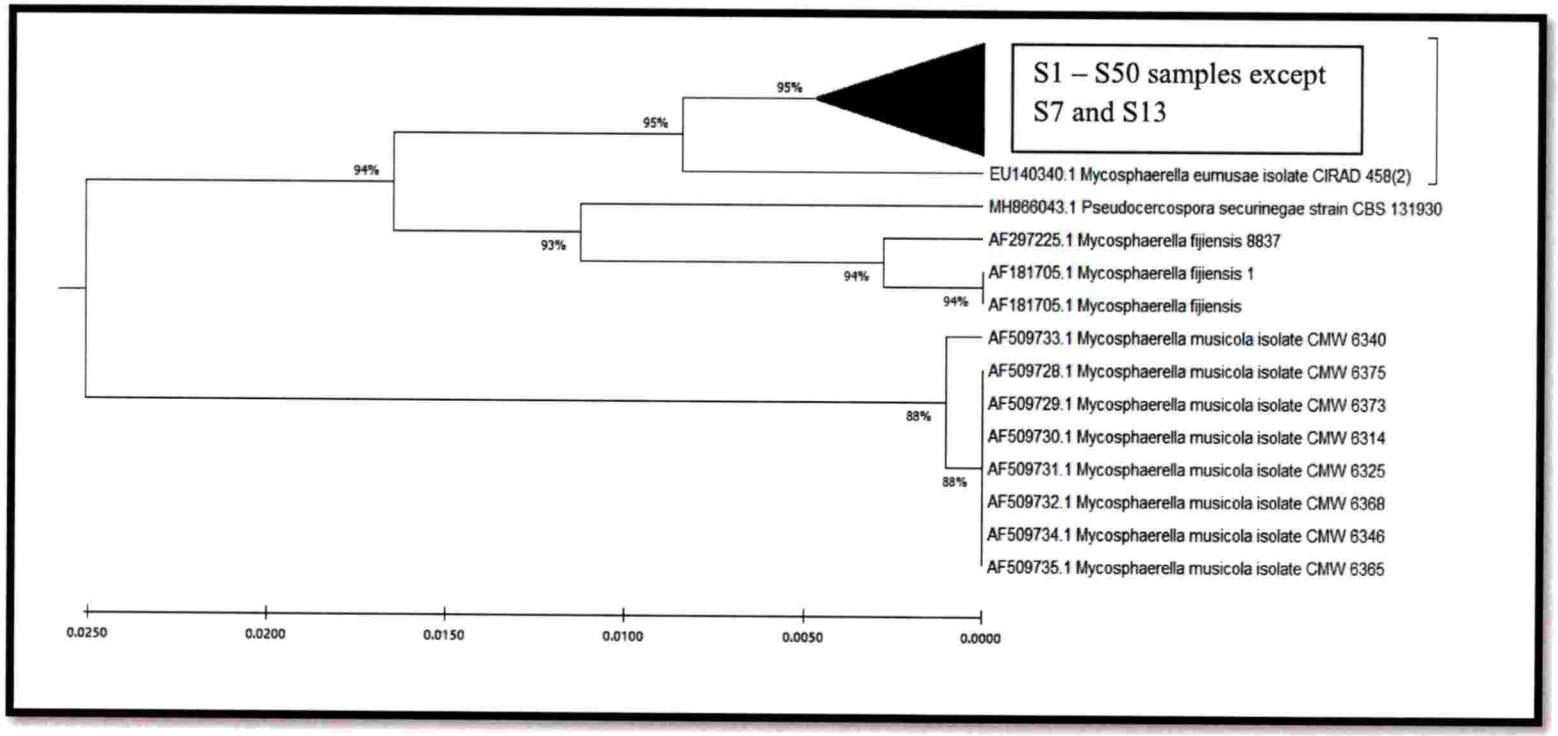


Fig 4.5: Phylogenetic tree generated from ITS – rDNA sequences of *Mycosphaerella* spp.

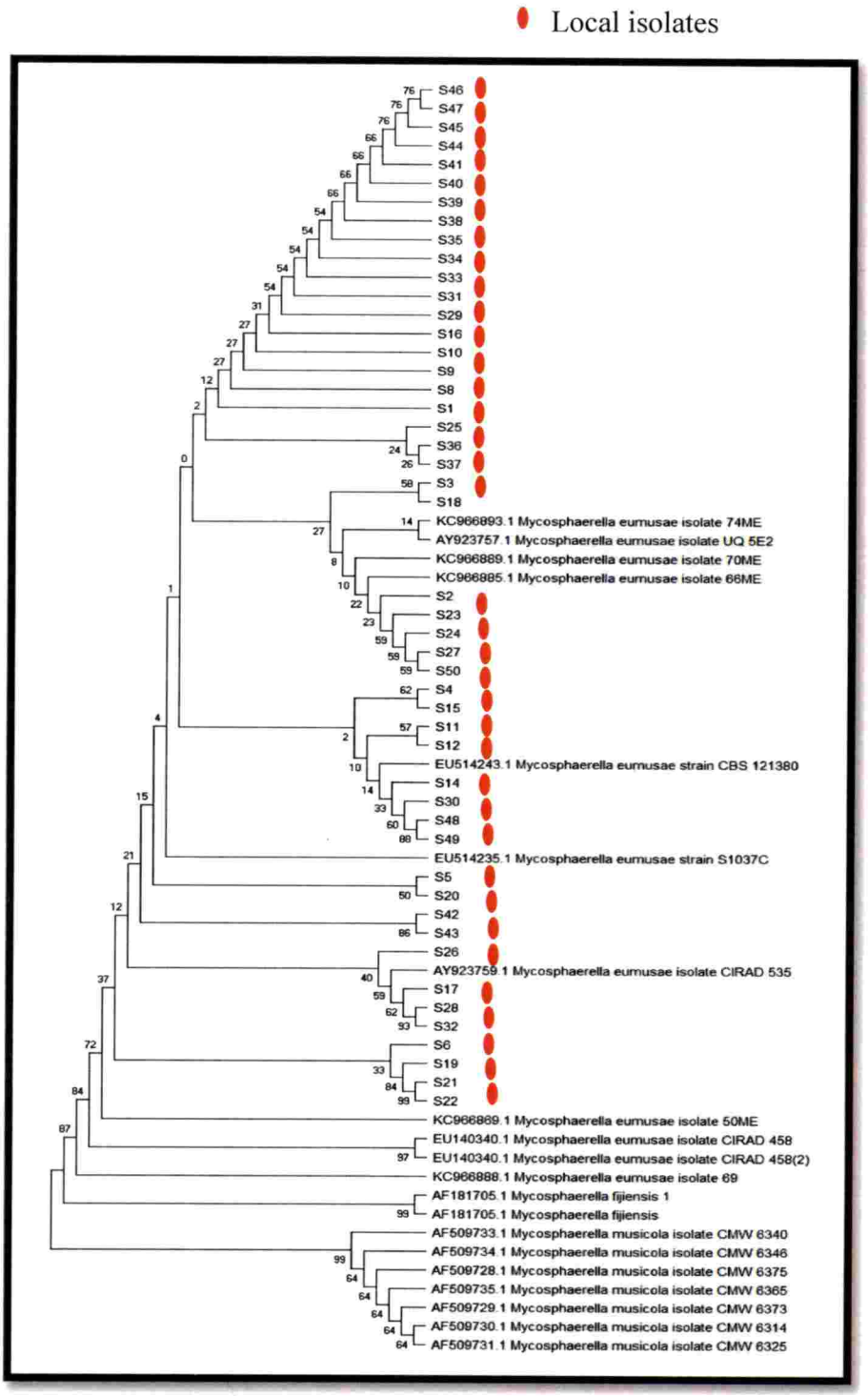


Fig 4.6: Phylogenetic tree generated from ITS – rDNA sequences of *Mycosphaerella* sp.

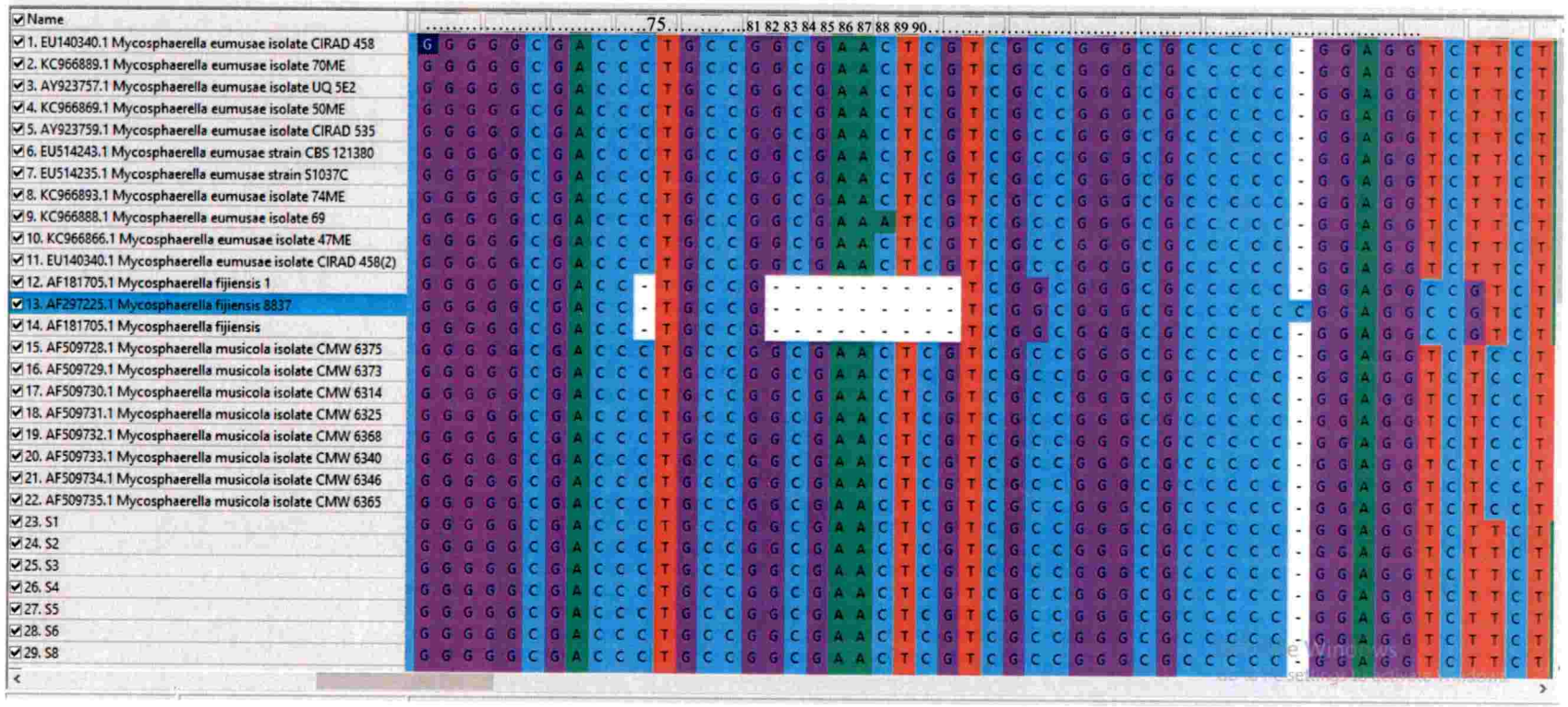


Fig 4.7: Variation in sequences of *Mycosphaerella eumusae* compared to *M. fijiensis*

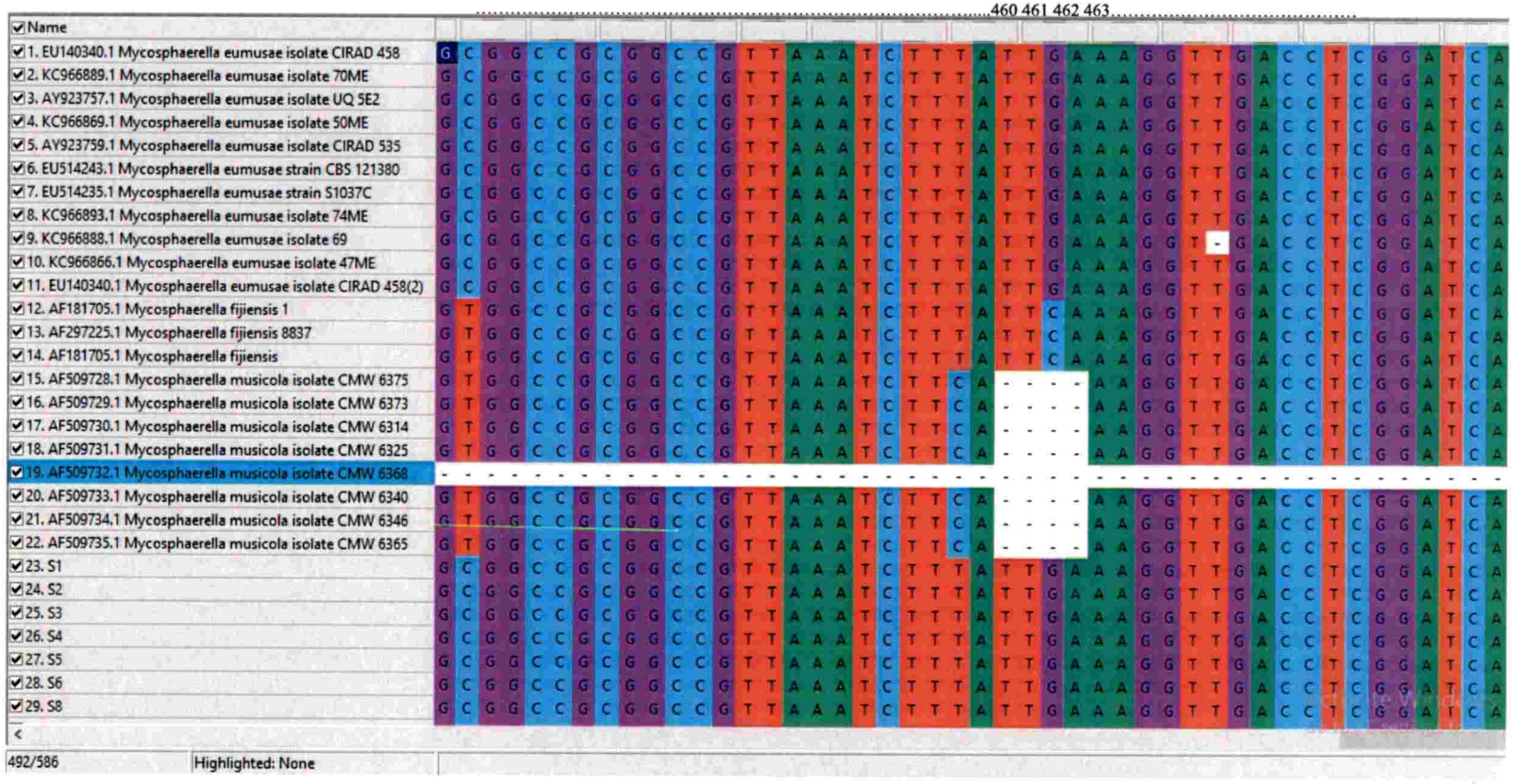


Fig 4.8: Variation in sequences of *M. eumusae* compared to *M. musicola*

towards the centre and became light coloured towards the peripheral region (Plate 4.14a).

4.4.2. Isolation of the pathogen

The growth of the pathogen appeared 2-3 days after isolation when incubated at room temperature. The pathogen initially produced white coloured mycelial growth on PDA which later changed to greyish colour after 4 days (Plate 4.14b). When culture discs of 7 mm was cut out from three day old culture and placed at the centre of 9 cm Petri plates containing PDA medium and incubated at room temperature, the isolate covered the whole Petri plates within 5-7 days. The fungal cultures were purified by hyphal tip method and maintained on PDA slants by periodic sub culturing.

4.4.3. Pathogenicity

The pathogenicity of the isolate was confirmed by proving Koch's postulates. Artificial inoculation was carried out on healthy banana leaves with mycelial discs cut from one week old fungal culture grown on PDA medium after giving definite number of pinpricks with a sterile stainless steel needle. Inoculated leaves produced brown coloured lesions within 3 days after inoculation, while no symptoms were observed in the control (Plate 4.14c). Re- isolation of the pathogen from the artificially inoculated leaves yielded fungal culture identical to the original culture.

4.4.3. Characterization and identification of the pathogen

4.4.3.1. Morphological characterization of the pathogen

The pathogen produced light brown coloured septate hyphae. The conidia arise in chains from a short condidiophore. The conidia were dark brown in colour, obclavate with a distinct long beak. The conidia were attached to the conidiophores with the boarder end. The conidia were divided by transverse and longitudinal septa.

The size of the conidia ranged from 5.18 -16.3 µm long and 2.6 – 10.67 µm wide with 3-8 transverse and 0-3 longitudinal septa (Plate 4.14d). Based on the cultural and morphological characters the pathogen was identified as *Alternaria* sp.

4.4.4. Molecular characterization of the pathogen

4.4.4.1. Isolation of DNA

The genomic DNA was isolated from the infected leaf samples/ isolates collected during the survey.

4.4.4.2. Assessment of purity

The agarose gel electrophoresis of the DNA produced single undegraded bands and were seen for the two isolates (Plate 4.15a).

4.4.4.3.Polymerase Chain Reaction Amplification

The amplification of the genomic DNA isolated from two infected leaf samples/ isolates was carried out using ITS primers (ITS 1 and ITS 4). The PCR thermal profile which gave good amplification was the same as given in 4.3.3.3.2.

4.4.4.3.1. Analysis of PCR products

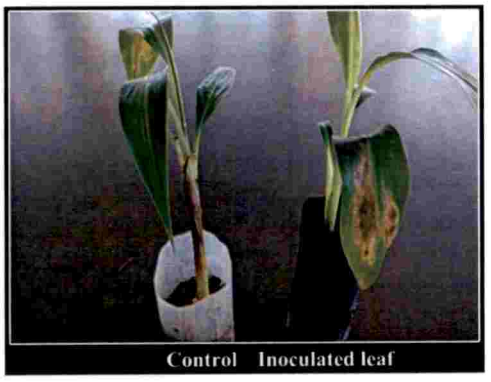
The amplicons produced bands with expected molecular size at 530bp when visualized on 1% agarose gel. While in control, no bands were produced confirming the validity and accuracy of PCR reactions (Plate 4.15b).

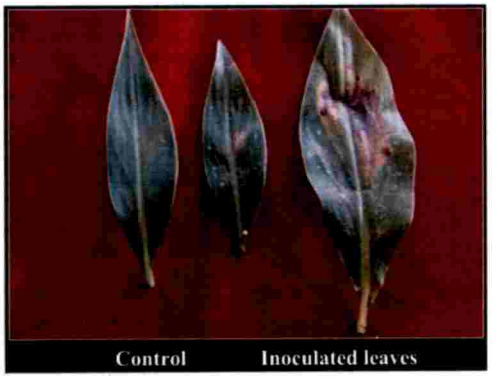
4.4.4.4. Sequencing of the amplicons

The sequencing of the amplicons was done using automated sequencing facility at AgriGenome Pvt. Ltd., Kakkanad, Kochi. The details of nucleotide sequences of the isolates are given in Appendix IV.

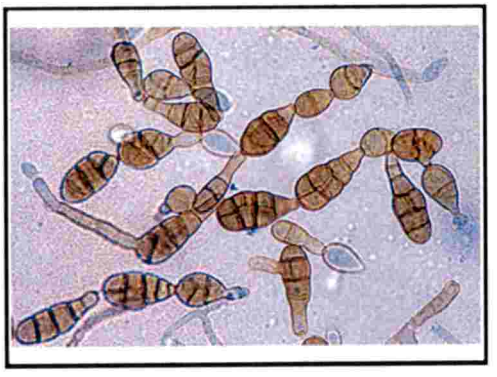
4.14a: Symptom

4.14b: Mycelial growth of fungus on PDA





4.14c: Pathogenicity of isolates



4.14d: Conidia

Plate 4.14: New leaf spot on banana var. Nendran

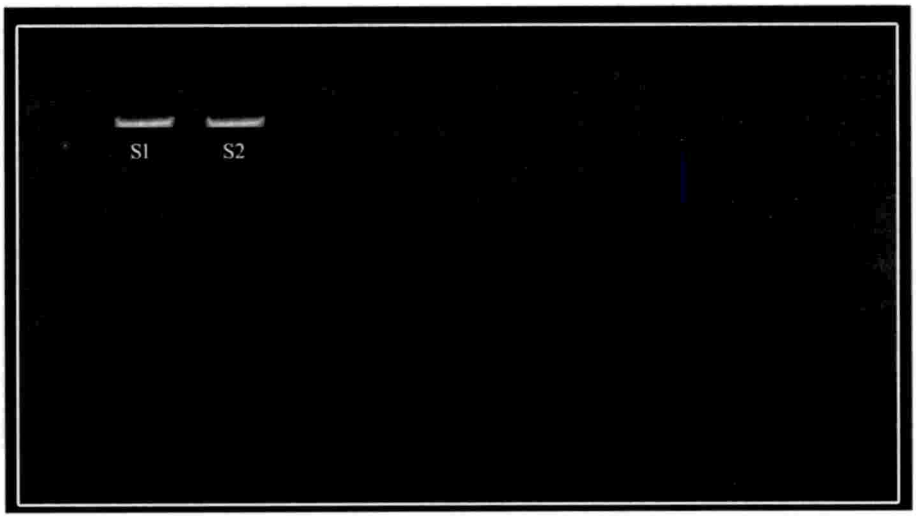


Plate 4.15a: DNA isolated from infected samples

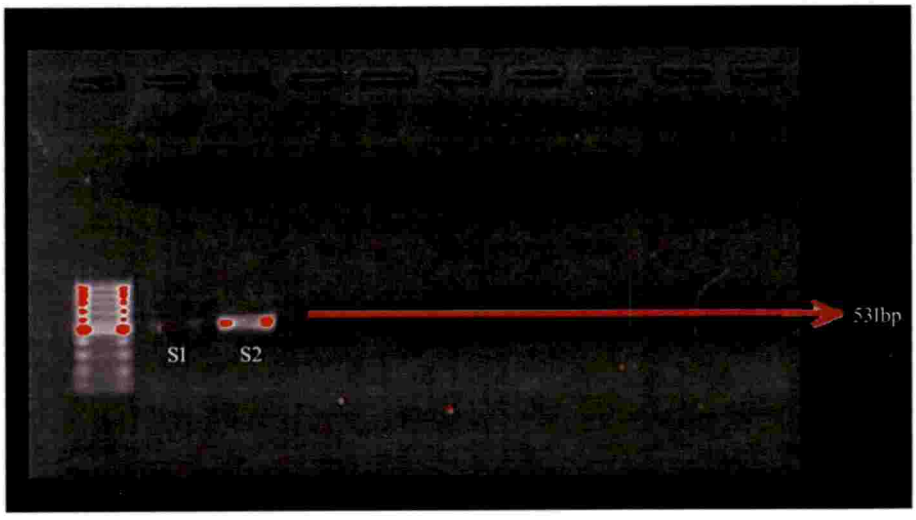


Plate 4.15b: Gel profile of amplicons

Plate 4.15: Molecular characterization of pathogen associated with new leaf of spot disease

4.4.4.4.1. *In silico* analysis of the sequences

The nucleotide sequences of the isolates were compared with the sequences available in the GenBank of NCBI database using BLASTn program. The analysis revealed that the local isolates showed 100% sequence homology to *Alternaria alternata*. The amplified sequence included partial sequences of 18S rDNA and 28S rDNA and full sequence of ITS 1 region, 5.8S rDNA and ITS 2 region. Phylogenetic analysis were also performed using bioinformatics tool. The nucleotide BLASTn analysis of *Alternaria* isolates are given below:

Isolate	Query coverage	E value	Identity %	Most similar isolate
S1	100%	0.0	100	**MG203920 (*Alternaria alternata*)**
S2	100%	0.0	100	

4.4.4.4.2. Phylogenetic analysis

The results of the phylogenetic tree revealed that the isolates collected during the survey were closely related to *Alternaria alternata.* The local isolates formed into the subcluster indicating there was no genetic diversity among the local isolates. Hence, the pathogen associated with the new leaf spot disease of banana var. Nendran is identified as *Alternaria alternata* (Fig. 4.9).

4.5. Evaluation of host plant resistance

The banana accessions maintained in the field genebank of Banana Research Station, Kannara were screened based on per cent disease severity (PDS) and the disease reaction of the accessions were assessed (Table 4.8). Among the 100 accessions screened, the most resistant cultivar was recorded as Calcutta 4 (AA) having PDS of 2.37% while the most susceptible was observed to be Grand

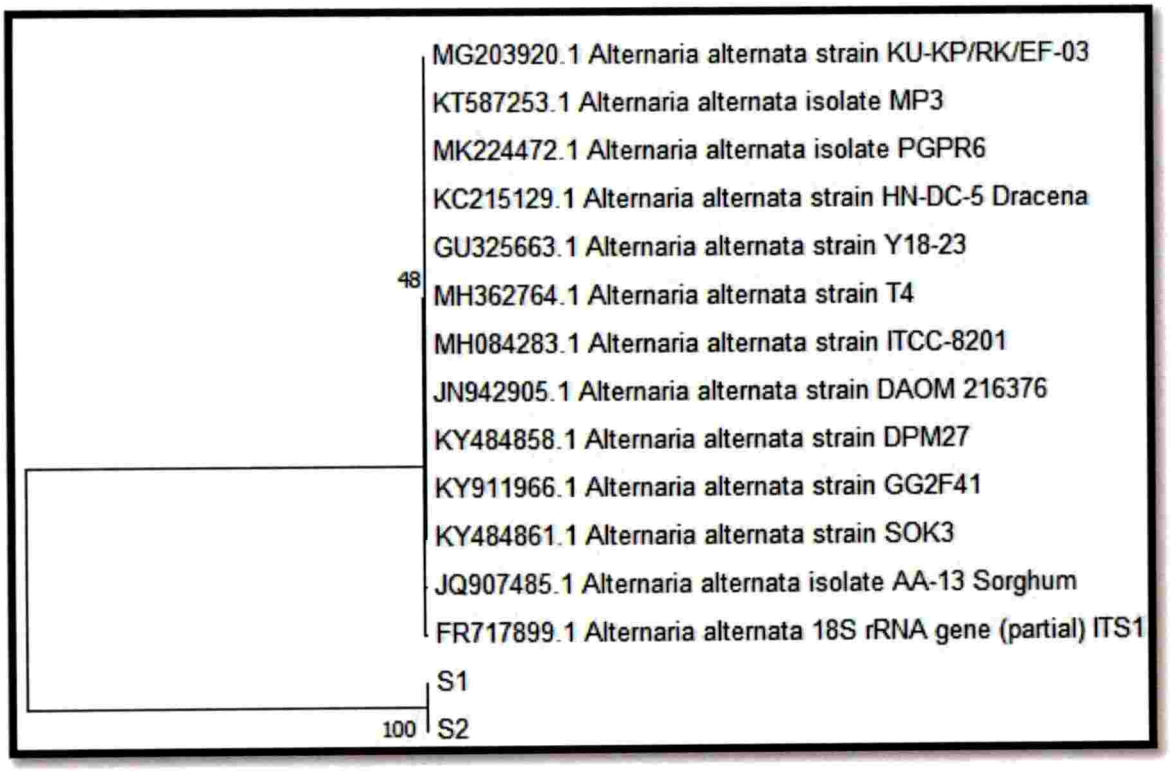


Fig 4.9:Phylogenetic tree generated from ITS – rDNA sequences of *Alternaria* sp.

Table 4.8: Classification of banana genotype based on disease reaction

Genotype	Disease reaction	Variety	PDS*	YLS**
AA	Resistant	Calcutta 4	2.37	8.00
		Musa ornate	4.87	7.70
		Pisang lilin	4.50	8.00
		Sikuzani	6.52	7.54
	Moderately resistant	Sanna Chenkadali	16.56	6.23
		Tongat	12.37	7.10
		Pisang seribu	18.75	6.25
	Moderately susceptible	Namari	37.21	5.10
		Matti	25.37	6.28
		Kadali	37.68	5.72
		Anaikomban	27.71	6.23
		Erachivazha	29.26	6.89
AAA	Resistant	Rajvazha	8.12	8.01
		Manoranjitham	7.88	7.91
	Moderately resistant	Chenkadali	18.75	6.12
		Pachakkappa	21.25	5.75
	Susceptible	Amamalu	33.72	5.67
		Sapumal	30.05	6.54
		Highgate	35.21	6.12
		Basari	31.33	6.33
		Wather	27.12	7.00
		Lactan	29.31	6.34
		Gros Michel	30.45	5.91

*Per cent disease severity, ** Youngest leaf spotted

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Table 4.8 (contd...) : Classification of banana genotype based on disease reaction

Genotype	Disease reaction	Variety	PDS*	YLS**
AAA	Susceptible	Robusta	46.11	4.67
		Moris	47.25	5.12
		Amrit sagar	40.36	6.43
		Grand naine	47.85	4.98
AB	Moderately resistant	Adukkan	17.36	7.10
		Valiakunnan	21.26	6.89
	Moderately susceptible	Padathi	33.12	7.33
		Njalipoovan	35.36	6.65
		Krishnavazha	25.66	7.24
		Sirumalai	39.41	6.11
		Agniswar	34.12	6.58
	Susceptible	Virupakshi	40.85	5.91
AAB	Resistant	Namkanika	7.22	8.23
		Dudhsagar	8.12	7.68
		BRS 2	9.76	7.45
		Thiruvanathapuram	6.31	7.13
		Poomkalli	7.33	7.00
		Adakkakunnan	8.35	6.92
		BRS 1	7.33	7.66
	Moderately resistant	Nakitemb	15.76	7.01
		Annan	21.53	6.44
		Thekkanthulladan	18.92	6.81
		Valiyapoovan	19.33	6.99
		Perumpadali	20.67	7.03

*Per cent disease severity, ** Youngest leaf spotted

Table 4.8 (contd...) : Classification of banana genotype based on disease reaction

Genotype	Disease reaction	Variety	PDS*	YLS**
AAB	Moderately resistant	Mysore ethan	21.33	6.75
		Sugandhi	20.91	6.94
		Mootapoovan	18.12	7.56
		Malakali	16.33	7.34
		Nattupoovan	24.25	6.98
		Malbhog	19.76	7.02
	Susceptible	Palayankodan	31.45	6.25
		Lady's finger	35.17	5.10
		Redia sire	27.33	6.28
		Pachanadan	26.26	5.72
		Mannan	30.12	6.23
		Kodappanilla kunnan	32.22	6.89
		Dakshin sagar	26.71	7.32
		Rasthali	26.23	6.12
		Marthaman	34.12	5.96
		Nendranpadathy	34.58	5.49
		Zanzibar	36.11	5.83
	Moderately susceptible	Nendran	40.16	5.67
		Charapadathy	42.23	6.21
		CO-1	39.69	6.37

*Per cent disease severity, ** Youngest leaf spotted

Table 4.8 (contd...) : Classification of banana genotype based on disease reaction

Genotype	Disease reaction	Variety	PDS*	YLS**
ABB	Resistant	Kalibow	8.26	7.89
		Boothibale	7.67	6.98
		Burkel	4.56	7.44
		Malaimonthan	5.35	6.86
	Moderately resistant	Monthan	22.56	
		Chetti	18.78	6.77
		TMP X 2829	22.18	6.48
		KNR 2/75	16.33	6.91
		Karpooravally	23.98	5.95
	Susceptible	Pidimonthan	27.26	5.18
		Kallar	31.99	6.33
		Pisang mas	34.22	6.12
		Karimbontha	29.26	5.99
		Velipadathi	37.12	4.88
	Moderately susceptible	Panchabontha	40.23	4.57
		Ennabenian	41.76	5.98
	Susceptible	Padathi	38.66	6.35
AAAA	Resistant	FHIA 23	8.18	7.82
	Moderately resistant	Bodles altafort	20.25	6.77
AAAB	Resistant	FHIA 1	9.23	7.00

*Per cent disease severity, ** Youngest leaf spotted

Naine (AAA) (47.85%).

Among the 12 varieties belonging to AA genotype, four were resistant, three were moderately resistant and five were moderately susceptible. The highest PDS of 37.68% was recorded in Kadali followed by Erachivazha accession which recorded PDS of 29.26% while the lowest PDS (2.37%) was observed in Calcutta 4.

Of the 17 varieties belonging to AAA genotype, two were resistant, two were moderately resistant, eight were susceptible and five were moderately susceptible. The highest PDS of 47.85% was noticed in Grand Naine cultivar followed by Moris accession (47.25%). While the lowest PDS of 7.88% was recorded in Manoranjitham accession.

Among the nine varieties belonging to AB genotype, two were moderately resistant and six were moderately susceptible and one was susceptible. The highest PDS of 40.85% was recorded in Virupakshi accession followed by Sirumalai accession with PDS of 39.31%. The lowest PDS of 17.36% was noticed in Adukkan accession.

Of the 37 varieties belonging to AAB genotype, nine varieties were resistant, 13 were moderately resistant, 12 were susceptible and 3 were moderately susceptible. The highest PDS (42.23%) was observed in Nendran cultivar followed by Charapadathy accession (40.16%) while the lowest PDS of 7.33% was recorded in Namkanika accession.

Among the 21 varieties belonging to ABB genotype, four were resistant, six were moderately resistant, nine were susceptible and two were moderately susceptible. The highest PDS of 41.76% was recorded in Pachabontha accession followed by Enna benian accession (40.11%) while the lowest PDS was recorded in Burkel accession (4.56%).

Among the two cultivar belonging to AAAA group, FHIA 23 hybrid were

found to be resistant with PDS of 9.23% while Bodles Altafort accession was moderately susceptible with PDS of 20.25%. The FHIA 1 hybrid belonging to AAAB genotype was found to be resistant with PDS of 9.23%.

In general the accessions belonging to AAA group were observed to be susceptible to Sigatoka leaf spot disease while the accessions belonging to AB, AAB, ABB and AAAB groups were observed to be resistant or moderately resistant to Sigatoka leaf spot disease.

From the different accessions screened, five resistant accessions *viz.,* Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23 and five susceptible accessions *viz.,* Nendran, Robusta, Grand Naine, Moris and Kadali were selected for evaluating anatomical, biochemical and molecular basis of resistance(Plate 4.16a & b) .

4.4.1. Anatomical studies

The anatomical studies on ten varieties *i.e.,* five resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) and susceptible accessions (Nendran, Robusta, Grand Naine, Moris and Kadali) were carried out to determine the mechanism of resistance. Transverse sections from four portions of both healthy and infected leaves of these ten selected accessions were compared to study the anatomical difference between the accessions (Plate 4.17a & b).

4.4.1.1. Upper cuticle

In resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) the infected leaves exhibited higher cuticle thickness than the healthy leaves. While no variations in cuticle thickness was observed for the susceptible accessions (Nendran, Robusta, Grand Naine, Moris and Kadali) both in healthy and infected leaves (Table 4.9).

Statistically significant differences was observed in case of thickness of cuticle

Pisang lilin (AA)

FHIA 01 (AAAB)

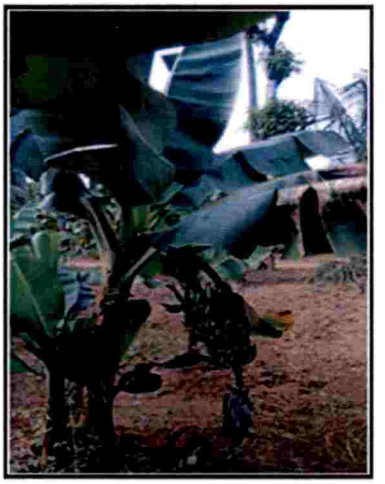
FHIA 23 (AAAA)

BRS 1 (AAB)

BRS 2 (AAB)

Plate 4.16a: Resistant accessions selected for evaluation of host plant resistance

Robusta (AAA)



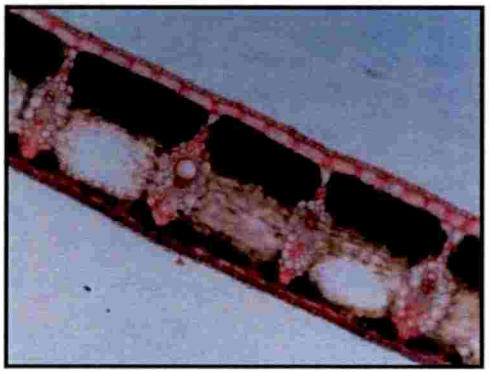
Moris (AAA)

Grand naine (AAA)

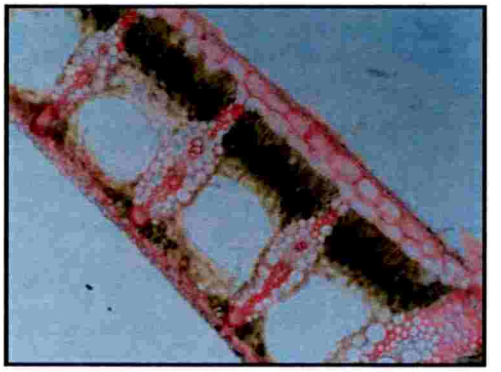
Nendran (AAB)

Kadali (AA)

Plate 4.16b: Susceptible accessions selected for evaluation of host plant resistance

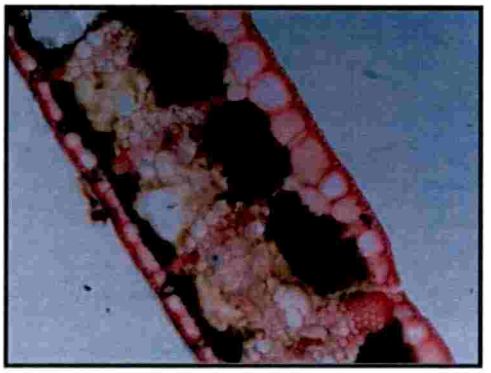


Healthy section

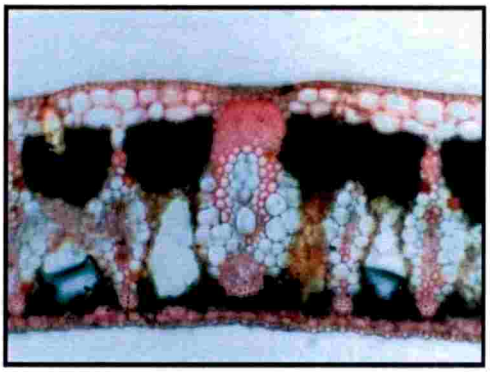


Infected section

Pisang lilin

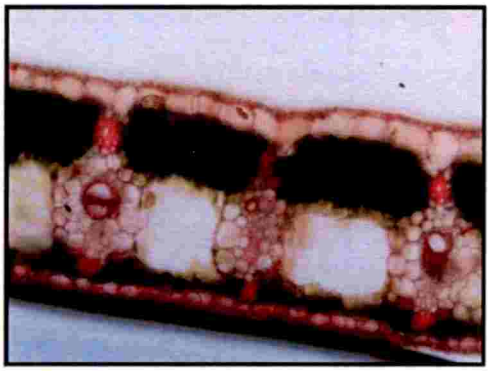


Healthy section

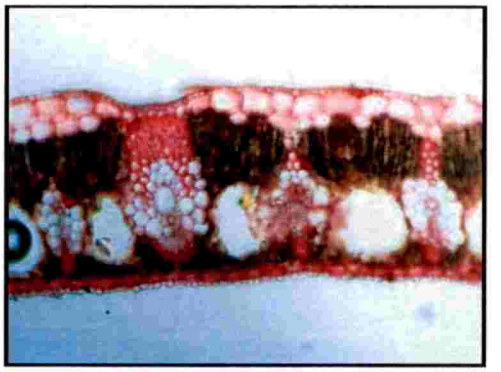


Infected section

FHIA 01



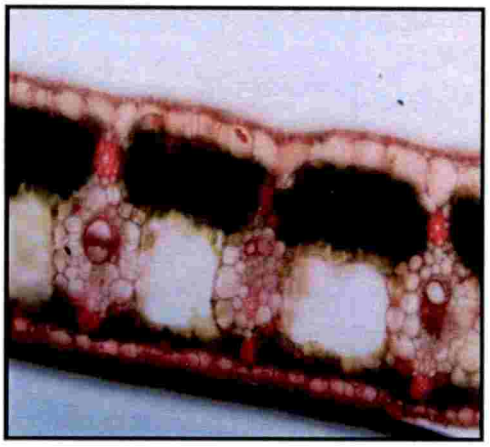
Healthy section



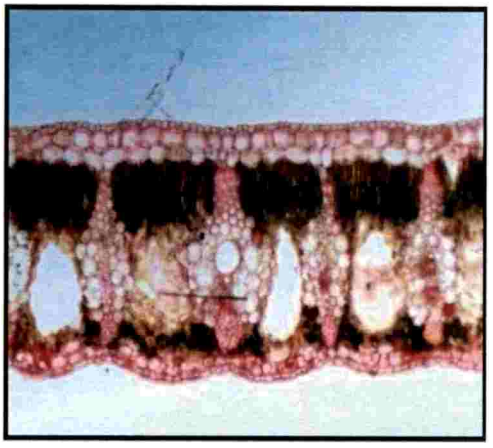
Infected section

BRS 2

Plate 4.17a: Transverse sections of healthy and infected leaves of resistant accessions (400X)

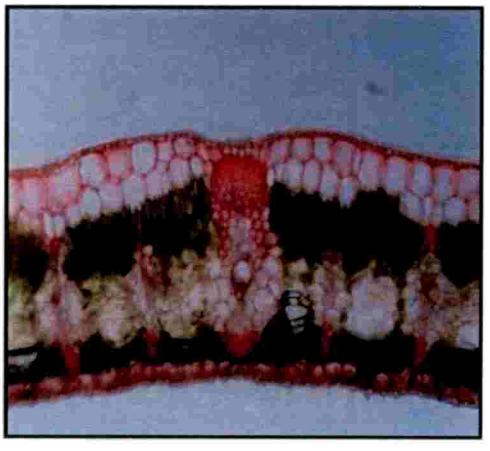


Healthy section

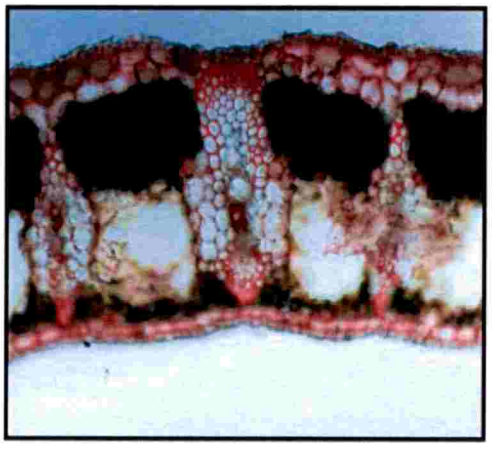


Infected section

FHIA 23



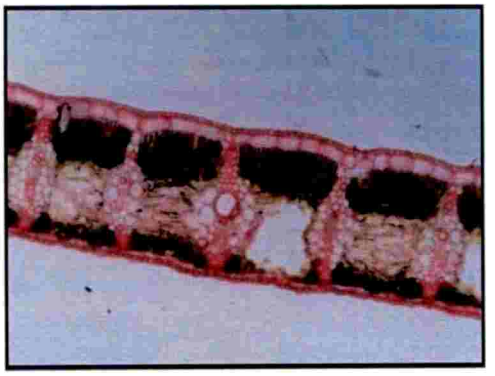
Healthy section



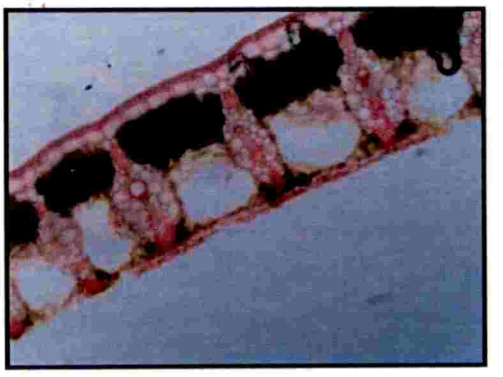
Infected section

BRS 1

Plate 4.17a (contd...): Transverse sections of healthy and infected leaves of resistant accessions (400X)

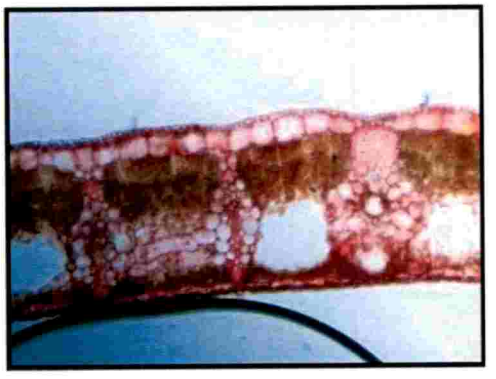


Healthy section

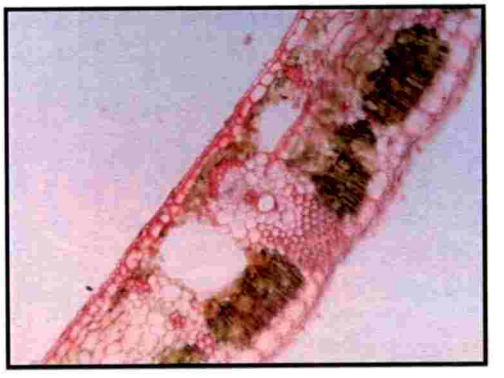


Infected section

Kadali

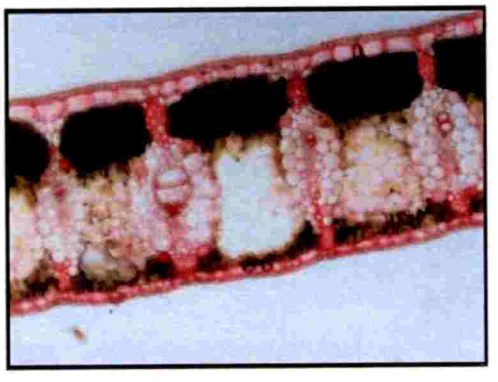


Healthy section

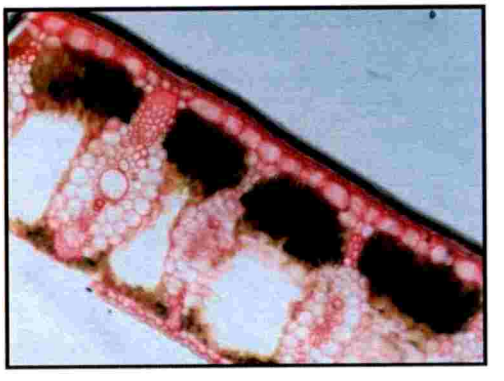


Infected section

Robusta



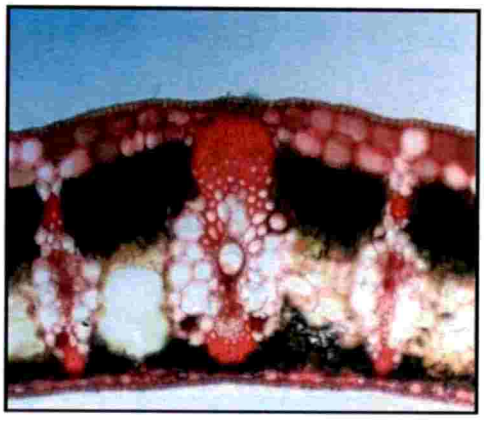
Healthy section



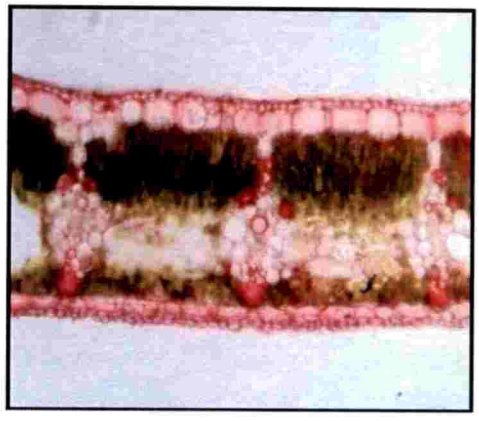
Infected section

Grand naine

Plate 4.17b: Transverse sections of healthy and infected leaves of susceptible accessions (400X)

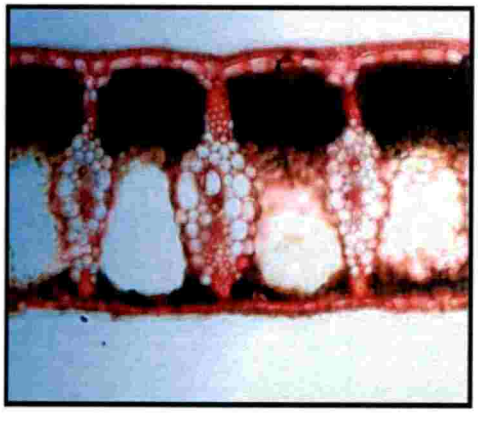


Healthy section

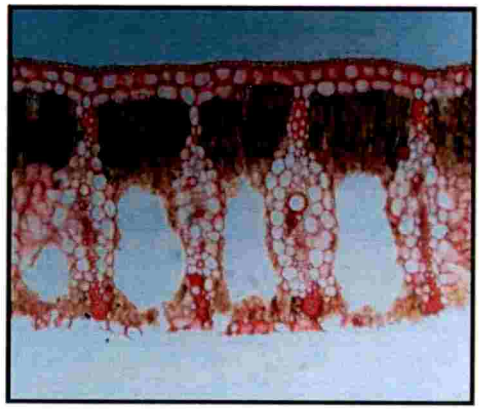


Infected section

Nendran



Healthy section



Infected section

Moris

Plate 4.17b (contd...): Transverse sections of healthy and infected leaves of susceptible accessions (400X

in healthy and infected leaves of resistant and susceptible accessions. In healthy leaves, the highest cuticle thickness was observed in resistant accession FHIA 23 (9.19 µm) followed by FHIA 01 (7.86 µm), Pisang Lilin (7.25 µm), BRS 1 (7.24 µm), BRS 2 (7.04 µm) and BRS 2 (6.88 µm). The lowest cuticle thickness was observed in the susceptible cultivar Grand Naine (5.09 µm). While in infected leaves, the highest cuticle thickness was observed in FHIA 23 (9.26 µm) followed by BRS 2 (8.94 µm) and the lowest cuticle thickness was observed in Grand Naine cultivar (5.09 µm).

Among the different portions of the leaves compared, significantly higher cuticle thickness was recorded in the central portions of the healthy leaves (8.75 µm) followed by the base portion (6.67 µm) while the lowest thickness was observed in the tip portion (5.65 µm) of the leaves (Fig 4.10). Whereas in infected leaves the highest cuticle thickness was observed in the central portion (8.82 µm) followed by middle portion (7.29 µm) while the lowest thickness was observed in the tip portion (5.74 µm) (Fig 4.11).

4.4.1.2. Upper epidermis

In resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) the infected leaves exhibited higher epidermal thickness than the healthy leaves. While no variations in thickness of upper epidermis was noticed in healthy and infected leaves of susceptible accessions (Nendran, Robusta, Grand Naine, Moris and Kadali) (Table 4.10).

Statistically significant differences were observed in case of upper epidermal thickness in healthy and infected leaves of resistant and susceptible accessions. In healthy leaves, highest epidermal thickness was recorded in BRS 2 (49.59 µm) followed FHIA 01 (47.15 µm). The lowest epidermal thickness of 20.85 µm was noticed in the susceptible cultivar Kadali. While in infected leaves, the highest epidermal thickness was recorded in BRS 2 (63.52 µm) followed by

Table 4.9: Histopathological changes in upper cuticle of different portions of leaf lamina in resistant and susceptible varieties

	Healthy leaves					Infected leaves				
Name of the varieties	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**
Pisang Lilin*	7.76	5.56	6.92	8.75	**7.25**	8.34	8.00	6.95	8.94	**8.05**
BRS 1*	6.76	7.53	5.80	8.88	**7.24**	6.99	8.60	5.93	8.71	**7.56**
BRS 2*	6.95	6.83	6.00	8.40	**7.04**	8.61	12.06	6.99	8.10	**8.94**
FHIA 01*	8.27	6.36	5.66	11.18	**7.86**	8.89	6.84	6.34	12.67	**8.68**
FHIA 23 *	9.07	8.72	7.73	11.26	**9.19**	8.00	8.90	6.82	13.31	**9.26**
Nendran **	5.78	7.10	3.87	9.05	**6.45**	5.78	7.10	3.87	9.05	**6.45**
Robusta**	6.21	5.98	5.15	6.94	**6.07**	6.21	5.98	5.15	6.94	**6.07**
Grand Naine**	4.8	4.79	4.75	6.01	**5.09**	4.82	4.79	4.75	6.01	**5.09**
Moris**	6.02	5.37	5.34	6.79	**5.88**	6.02	5.37	5.34	6.79	**5.88**
Kadali**	5.07	5.32	5.33	6.24	**5.49**	5.07	5.32	5.33	6.24	**5.49**
Mean	**6.67**	**6.35**	**5.65**	**8.75**		**6.87**	**7.29**	**5.74**	**8.82**	

Interaction of leaves x portions: CD =0.50, Interaction of leaves x cultivars: CD=0.79

*Resistant varieties, ** Susceptible varieties

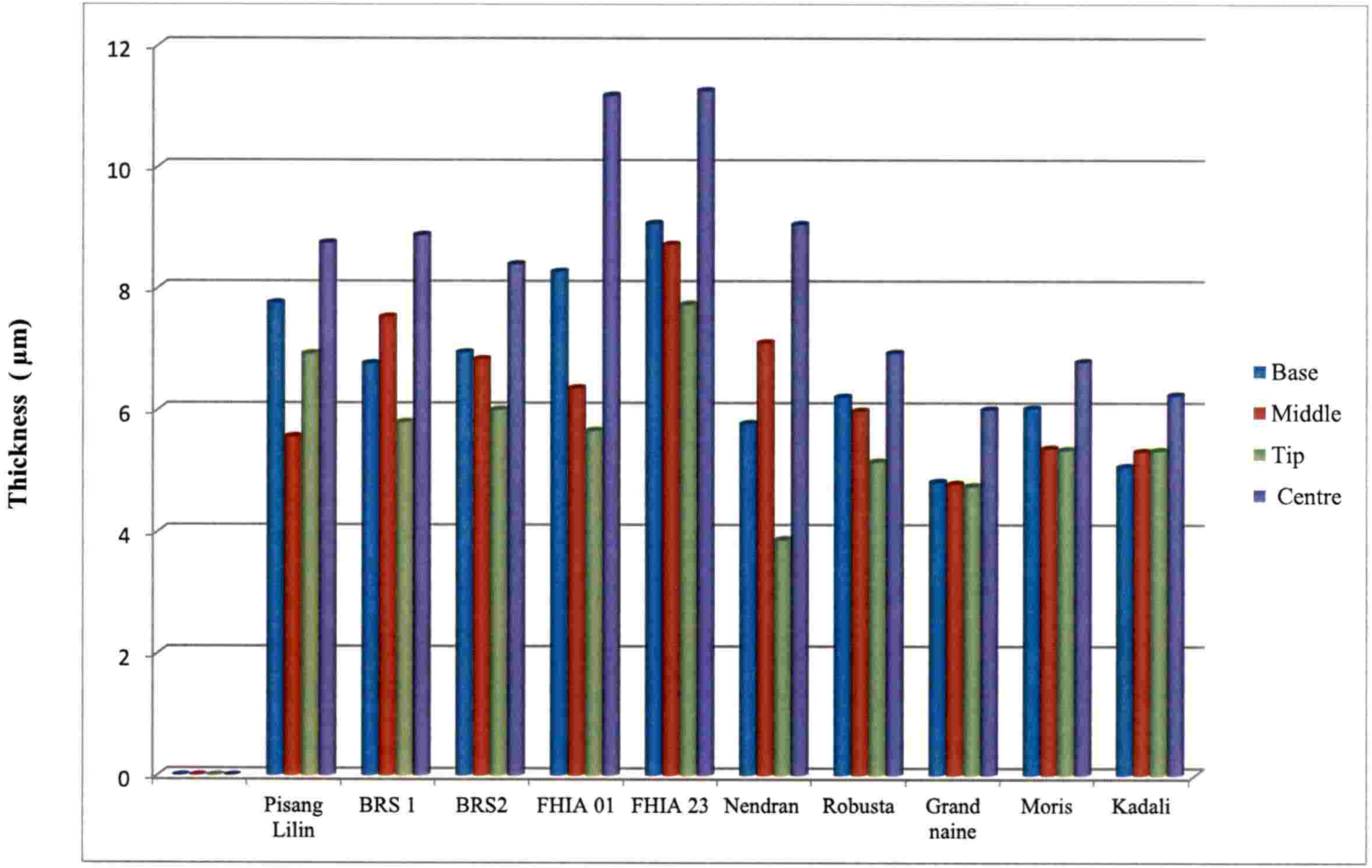


Fig 4.10: Histopathological changes in upper cuticle of different portions of healthy leaf lamina in resistant and susceptible varieties

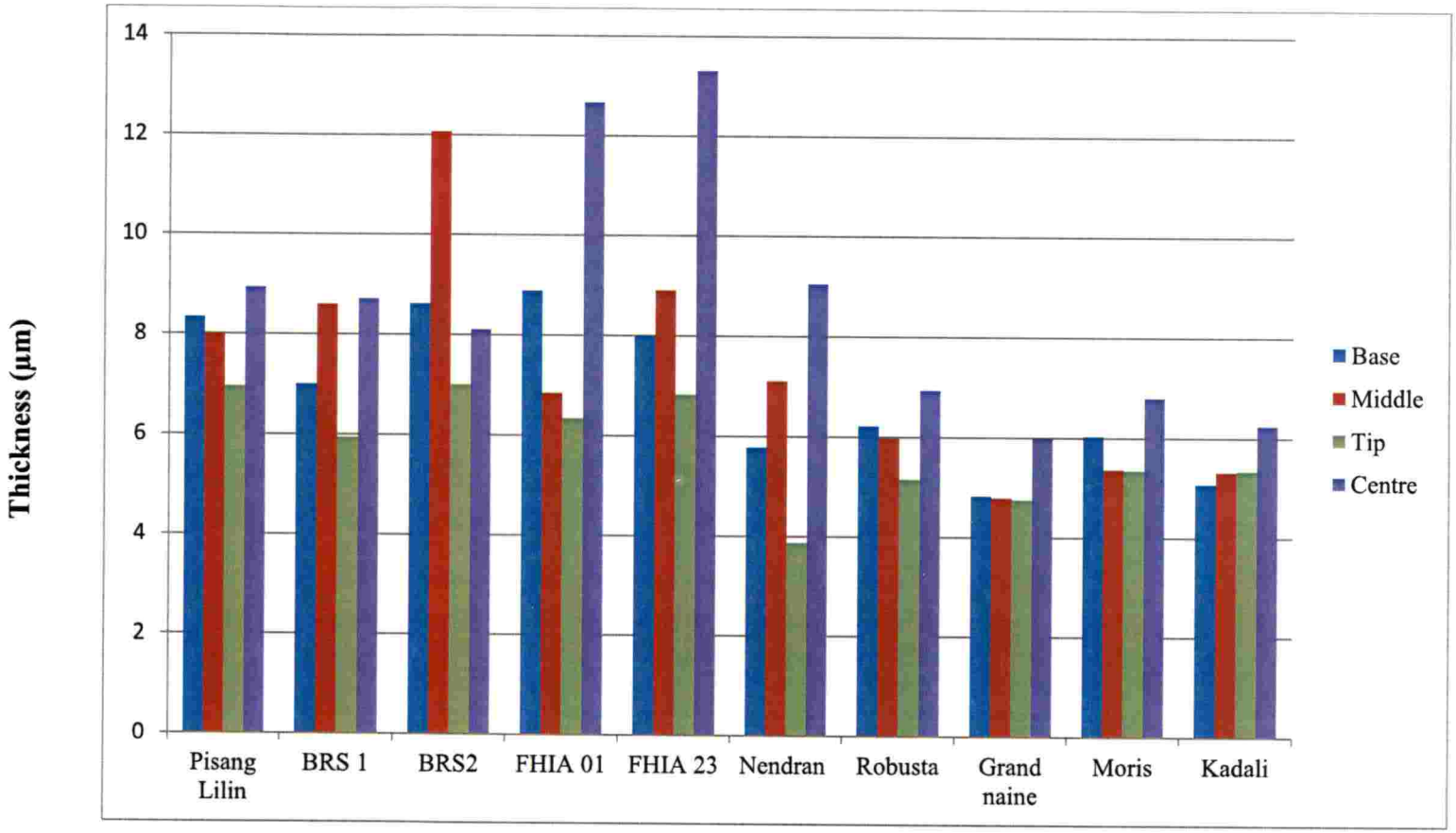


Fig 4.11: Histopathological changes in upper cuticle of different portions of healthy leaf lamina in resistant and susceptible varieties

FHIA 01 (57.84 µm), whereas the lowest epidermal thickness was observed in Kadali cultivar (20.85µm).

No significant difference was observed in case of upper epidermal thickness of healthy and infected leaves. In healthy leaves, the highest epidermal thickness was noticed in central portion (34.56 µm) followed by the base portion (33.46 µm), tip portion (29.87 µm) and middle portion (29.37 µm). In infected leaves, similar trend as in healthy leaves was noticed where the highest epidermal thickness of 37.26 µm was exhibited by central followed by base, tip and middle having an epidermal thickness of 37.24 µm, 35.08 µm and 31.73 µm respectively.

4.4.1.3. Palisade tissue

In healthy leaves, the thickness of the palisade tissue was found to be more in resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) than that of susceptible accessions (Nendran, Robusta, Grand Naine, Moris and Kadali). Whereas in infected leaves, an increase in thickness of palisade tissue was recorded in resistant accessions while decrease in thickness of palisade tissue was noticed in susceptible accessions except in the susceptible cultivar Kadali (Table 4.11).

Statistically significant difference was recorded in case of thickness of palisade tissue in healthy and infected leaves of resistant and susceptible accessions. In healthy leaves, highest palisade tissue thickness of 141.19 µm was noticed in FHIA 23 followed by FHIA 01 (125.62 µm) while the lowest thickness was noticed in the susceptible cultivar Kadali (58.74 µm). While in case of infected leaves, the highest thickness of the palisade tissue was recorded in BRS 2 (291.54 µm) followed by BRS 1 (188.52 µm) and the lowest was recorded in the susceptible cultivar Grand Naine (62.39 µm).

No significant differences was observed in thickness of palisade tissue among different portions of healthy and infected leaves. In healthy leaves, the highest

Table 4.10: Histopathological changes in upper epidermis of different portions of leaf lamina in resistant and susceptible varieties

	Healthy leaves					Infected leaves				
Name of the varieties	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**
Pisang Lilin*	37.18	39.81	39.88	43.33	**40.05**	51.54	52.18	53.98	58.17	**53.72**
BRS 1*	37.96	34.04	27.16	34.97	**33.53**	41.06	36.36	48.42	38.70	**46.14**
BRS 2 *	45.42	37.90	55.53	59.52	**49.59**	66.91	53.45	65.56	68.18	**63.52**
FHIA 01*	48.19	39.78	41.11	59.52	**47.15**	59.22	50.88	61.45	59.82	**57.84**
FHIA 23*	41.88	36.15	36.89	26.44	**35.33**	23.12	27.37	26.34	39.86	**46.17**
Nendran **	29.60	20.43	26.26	32.21	**27.12**	29.60	20.43	26.26	32.21	**27.12**
Robusta**	16.67	32.12	18.87	38.82	**26.62**	16.67	32.12	18.87	38.82	**26.62**
Grand Naine**	27.00	24.70	26.76	20.71	**24.79**	27.00	24.70	26.76	20.71	**24.79**
Moris**	28.41	21.04	19.58	24.41	**23.36**	28.41	21.04	19.58	24.41	**23.36**
Kadali**	26.29	19.76	17.65	19.71	**20.85**	26.29	19.76	17.65	19.71	**20.85**
Mean	**33.46**	**29.37**	**29.87**	**34.56**		**37.24**	**31.73**	**35.08**	**37.26**	

Interaction of leaves x portions: CD = NS, Interaction of leaves x cultivars: CD= 6.76

*Resistant varieties, ** Susceptible varieties

thickness of palisade tissue was noticed in the central portion (114.07 µm) followed base, tip and middle portion with thickness of 99.73 µm, 98.20 µm and 96.19 µm. In infected leaves, the middle portion of the leaves exhibited highest thickness of the palisade tissue (129.83 µm) followed by base portion (121.69 µm) while the lowest thickness was observed in tip portion (103.63 µm).

4.4.1.4. Spongy tissue

In healthy leaves, the thickness of the spongy tissue was observed to be more in resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) than that of susceptible accessions (Nendran, Robusta, Grand Naine, Moris and Kadali). Whereas in infected leaves, an increase in thickness of spongy tissue was recorded in resistant accessions while a decrease in thickness of spongy tissue was noticed in susceptible accessions (Table 4.12).

Statistically significant difference was recorded in case of thickness of spongy tissue in healthy and infected leaves of resistant and susceptible accessions. In healthy leaves, the highest thickness of spongy tissue was recorded in Pisang Lilin having thickness of 83.71 µm followed by BRS 1 (80.00 µm) while the lowest thickness was noticed in the susceptible cultivar Grand Naine (16.20 µm). Whereas in infected leaves, the maximum thickness of 98.88 µm was recorded in the resistant accession Pisang Lilin which was followed by BRS 1(97.10 µm) while the lowest thickness of 12.86 µm was recorded in Grand Naine cultivar.

Significant difference was recorded in thickness of the spongy tissue among different portions of healthy and infected leaves. In healthy leaves, the highest thickness of spongy tissue (62.38 µm) was recorded in the middle portion followed by base, central and tip portions of the leaves with thickness of 58.84 µm, 56.23 µm and 49.85 µm respectively (Fig 4.12). In infected leaves, the highest thickness of 83.36 µm was recorded in the middle portion followed by base, central and tip portions having thickness of 73.52, 67.29 and 63.46 µm (Fig 4.13).

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Table 4.11: Histopathological changes in palisade tissue of different portions of leaf lamina in resistant and susceptible varieties

	Healthy leaves					Infected leaves				
Name of the varieties	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**
Pisang Lilin*	102.56	135.26	78.21	129.74	**111.44**	127.25	156.15	94.65	147.48	**131.38**
BRS 1*	114.79	112.15	112.02	121.46	**115.10**	198.39	194.97	181.99	178.73	**188.52**
BRS 2*	70.11	81.78	129.24	139.23	**105.09**	270.89	195.57	140.62	159.09	**291.54**
FHIA 01*	73.52	160.745	115.92	152.30	**125.62**	92.45	192.43	143.10	173.53	**150.37**
FHIA 23*	139.98	138.97	169.21	116.59	**141.19**	177.45	203.37	171.80	156.26	**150.37**
Nendran **	75.83	83.28	69.92	115.51	**86.13**	63.98	77.41	53.75	81.08	**69.05**
Robusta **	159.38	124.43	98.10	128.80	**100.18**	88.15	67.93	79.95	77.20	**78.31**
Grand Naine**	67.54	77.25	77.42	78.34	**75.14**	58.55	60.00	72.28	58.75	**62.39**
Moris**	133.86	98.89	69.10	105.67	**101.88**	81.26	87.98	39.03	70.03	**69.57**
Kadali**	59.77	59.15	62.93	53.09	**58.74**	58.55	62.51	59.16	74.84	**63.76**
Mean	**99.73**	**96.19**	**98.20**	**114.07**		**121.69**	**129.83**	**103.63**	**117.60**	

Interaction of leaves x portions: CD = NS, Interaction of leaves x cultivars: CD= 78.51
*Resistant varieties,** Susceptible varieties

Table 4.12: Histopathological changes in spongy tissue of different portions of leaf lamina in resistant and susceptible varieties

	Healthy leaves					Infected leaves				
Name of the varieties	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**
Pisang Lilin*	89.26	81.89	89.69	74.01	**83. 71**	97.13	92.14	106.75	99.50	**98.88**
BRS 1*	82.46	84.90	73.66	79.97	**80.00**	105.72	99.03	92.05	91.60	**97.14**
BRS 2*	77.22	71.69	66.95	62.41	**69.57**	89.52	88.14	78.62	75.34	**82.90**
FHIA 01*	69.99	89.15	66.28	62.86	**72.07**	87.56	126.38	76.84	77.90	**92.17**
FHIA 23*	71.75	83.68	81.39	69.46	**76.82**	79.32	91.30	88.57	78.52	**84.43**
Nendran **	47.99	38.45	29.60	43.83	**39.97**	39.57	21.76	19.60	38.70	**29.91**
Robusta**	23.34	18.06	29.62	35.46	**26.62**	29.06	21.00	18.89	33.35	**25.57**
Grand Naine**	19.00	20.80	14.01	10.98	**16.20**	15.21	17.24	10.07	8.94	**12.86**
Moris**	32.48	27.94	31.88	26.35	**29.66**	33.24	23.49	20.92	13.67	**22.83**
Kadali**	34.92	28.29	27.49	17.00	**26.92**	18.90	20.15	20.31	17.39	**19.19**
Mean	**58.84**	**62.38**	**49.85**	**56.23**		**73.52**	**83.36**	**63.46**	**67.29**	

Interaction of leaves x portions: CD = 2.25, Interaction of leaves x cultivars: CD= 3.56

*Resistant varieties, ** Susceptible varieties

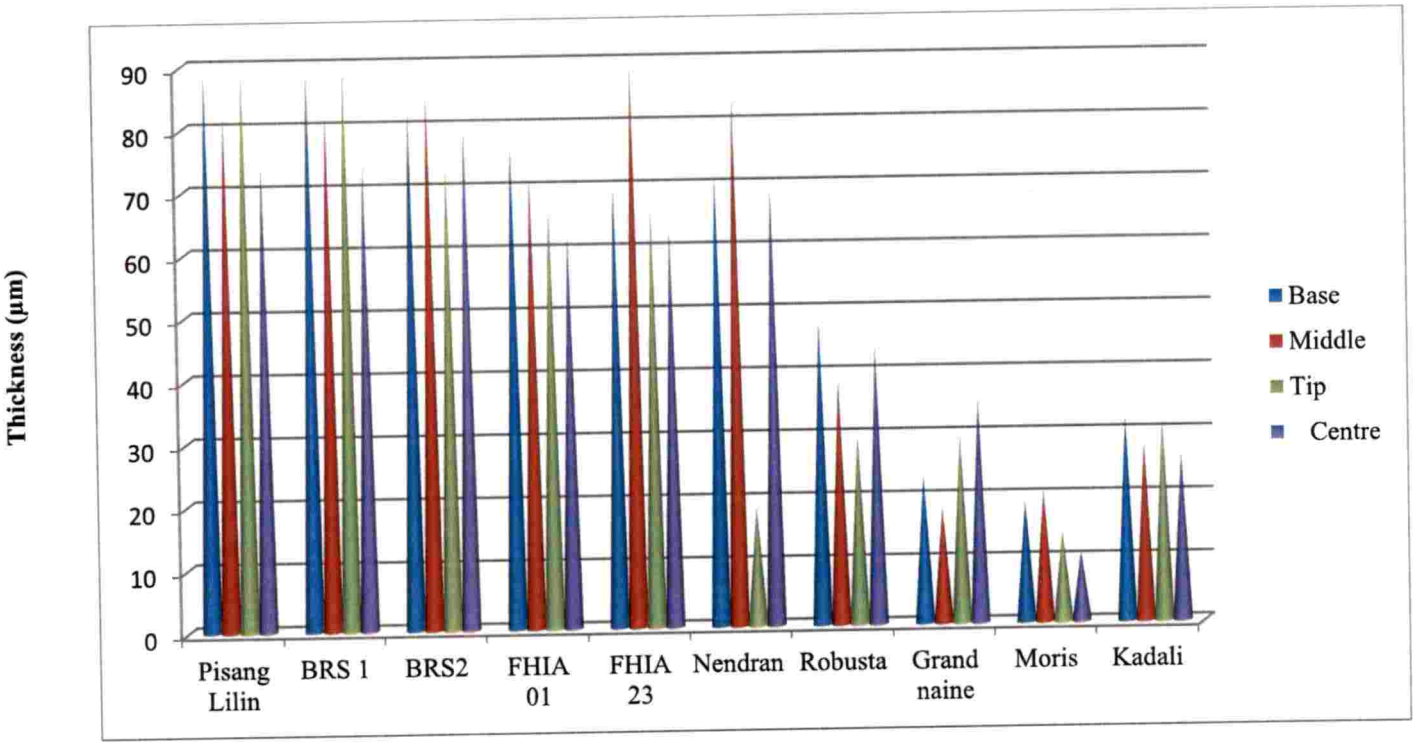

Fig 4.12: Histopathological changes in spongy tissue of different portions of healthy leaf lamina in resistant and susceptible varieties

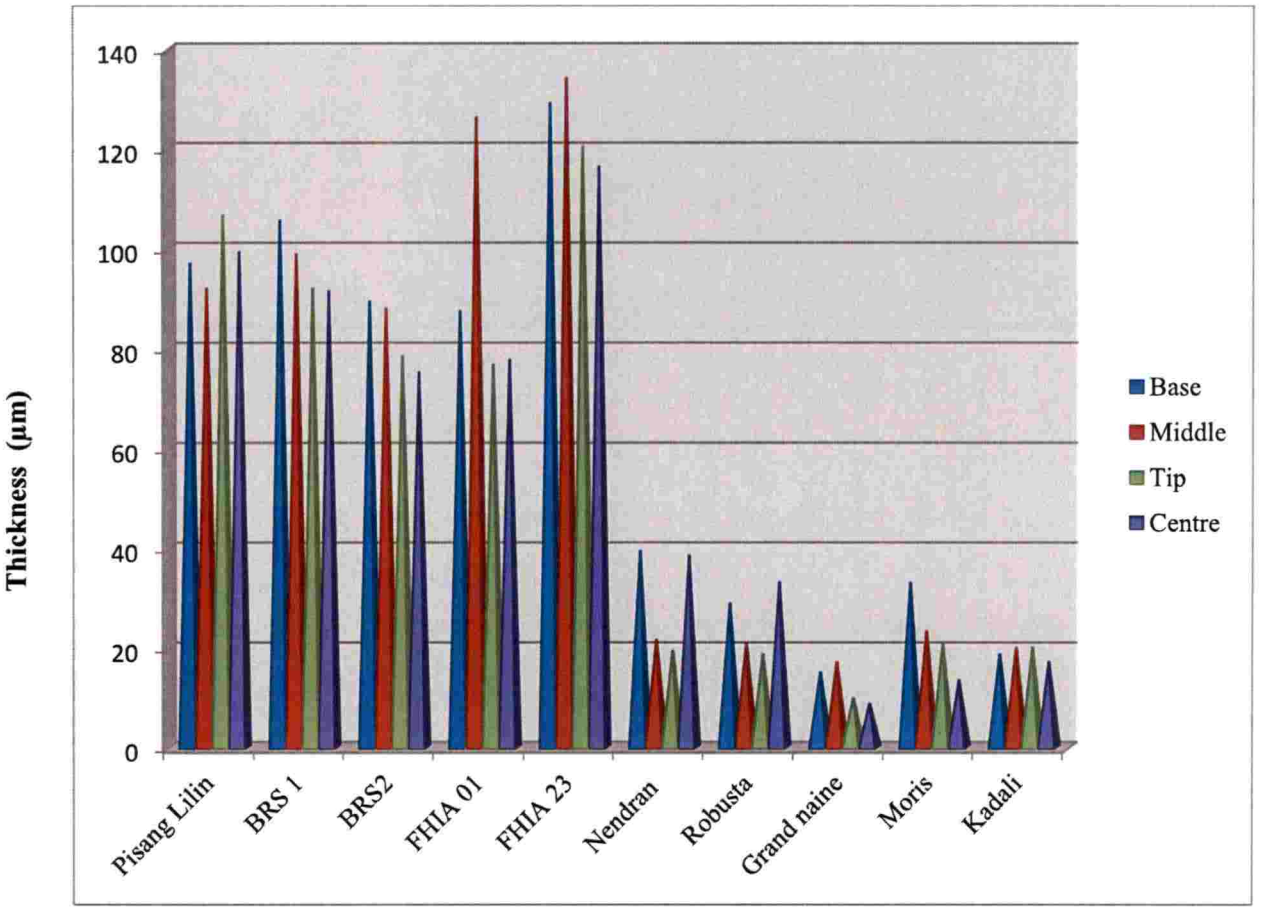

Fig 4.13: Histopathological changes in spongy tissue of different portions of infected leaf lamina in resistant and susceptible varieties

4.4.1.5. Lower epidermis

In healthy leaves, the thickness of the lower epidermis was observed to be more in resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) than that of susceptible accessions (Nendran, Robusta, Grand Naine, Moris and Kadali). Whereas in infected leaves, an increase in thickness of lower epidermis was recorded in resistant accessions while a decrease in thickness was noticed in susceptible accessions (Table 4.13).

Statistically significant difference was observed in the thickness of lower epidermis in healthy and infected leaves of resistant and susceptible accessions. In healthy leaves, the highest lower epidermal thickness (14.53 µm) was recorded in FHIA 23 followed by Pisang Lilin (14.40 µm) while the lowest thickness of the epidermis was observed in the susceptible variety Kadali (5.61 µm). In infected leaves, the lower epidermal thickness was highest in FHIA 23 (23.73 µm) which was followed by Pisang Lilin having thickness of 18.60 µm while the lowest thickness (5.04 µm) was observed in Kadali cultivar.

Significant differences were observed in thickness of the lower epidermis among different portions of healthy and infected leaves. In healthy leaves, highest epidermal thickness was recorded in the central region (12.38 µm) followed by base region (11.76 µm) while the middle and tip portions recorded thickness of 10.17 µm and 9.78 µm respectively (Fig 4.14). In infected leaves, the highest thickness of lower epidermis was observed in the central portion having thickness of 16.27 µm which was followed middle (13.67 µm), base (12.48 µm) and tip (10.67 µm) portions (Fig 4.15).

4.4.1.6. Lower cuticle

In healthy leaves, the thickness of the lower cuticle was found to be more in resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) than that of

Table 4.13: Histopathological changes in lower epidermis of different portions of leaf lamina in resistant and susceptible varieties

	Healthy leaves					Infected leaves				
Name of the varieties	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**
Pisang Lilin*	8.82	17.12	14.88	16.78	**14.40**	11.90	19.38	19.21	23.93	**18.60**
BRS 1 *	10.85	11.84	12.21	11.13	**11.51**	11.86	11.83	14.96	13.88	**13.13**
BRS 2*	15.50	9.9	13.29	12.77	**12.76**	18.13	14.43	9.35	27.82	**17.43**
FHIA 01*	16.99	10.00	15.04	12.51	**13.66**	18.79	19.82	12.25	15.09	**16.49**
FHIA 23*	19.56	10.31	8.04	20.22	**14.53**	18.25	28.37	16.63	31.68	**23.73**
Nendran **	9.86	8.66	8.78	11.68	**9.74**	8.99	7.65	8.00	10.67	**8.83**
Robusta**	7.15	8.56	5.74	10.09	**7.88**	6.61	7.92	5.41	9.62	**7.39**
Grand Naine**	10.54	9.09	7.17	11.66	**9.61**	9.88	7.90	6.94	11.12	**8.96**
Moris**	13.09	10.62	7.66	8.63	**10.00**	12.50	9.71	6.90	8.00	**9.28**
Kadali**	5.24	5.92	4.99	6.30	**5.61**	4.76	5.13	4.51	5.78	**5.04**
Mean	**11.76**	**10.17**	**9.78**	**12.38**		**12.17**	**13.21**	**10.41**	**15.76**	

Interaction of leaves x portions: CD = 0.90, Interaction of leaves x cultivars: CD=1.42

*Resistant varieties, **Susceptible varieties

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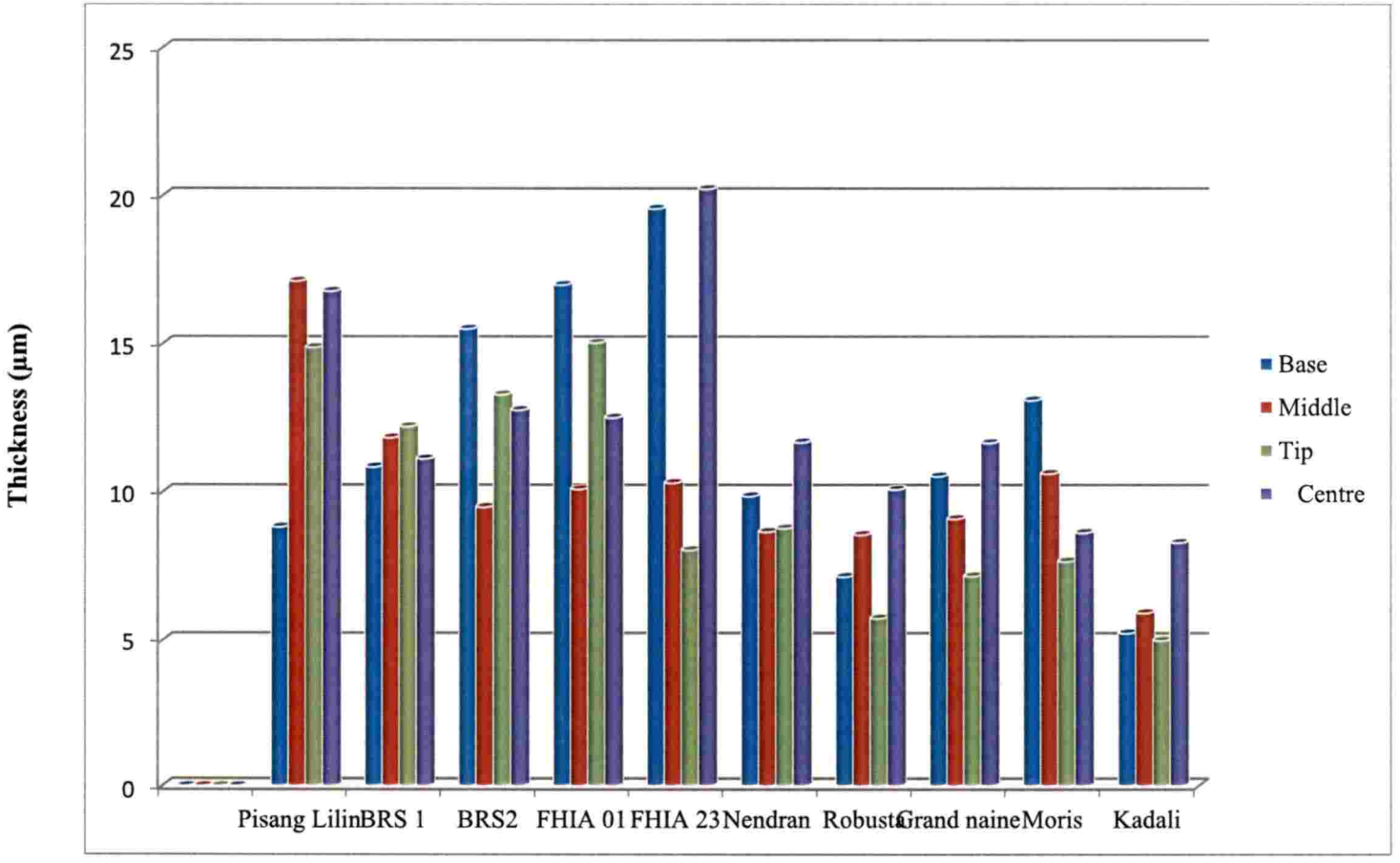


Fig 4.14: Histopathological changes in lower epidermis of different portions of healthy leaf lamina in resistant and susceptible varieties

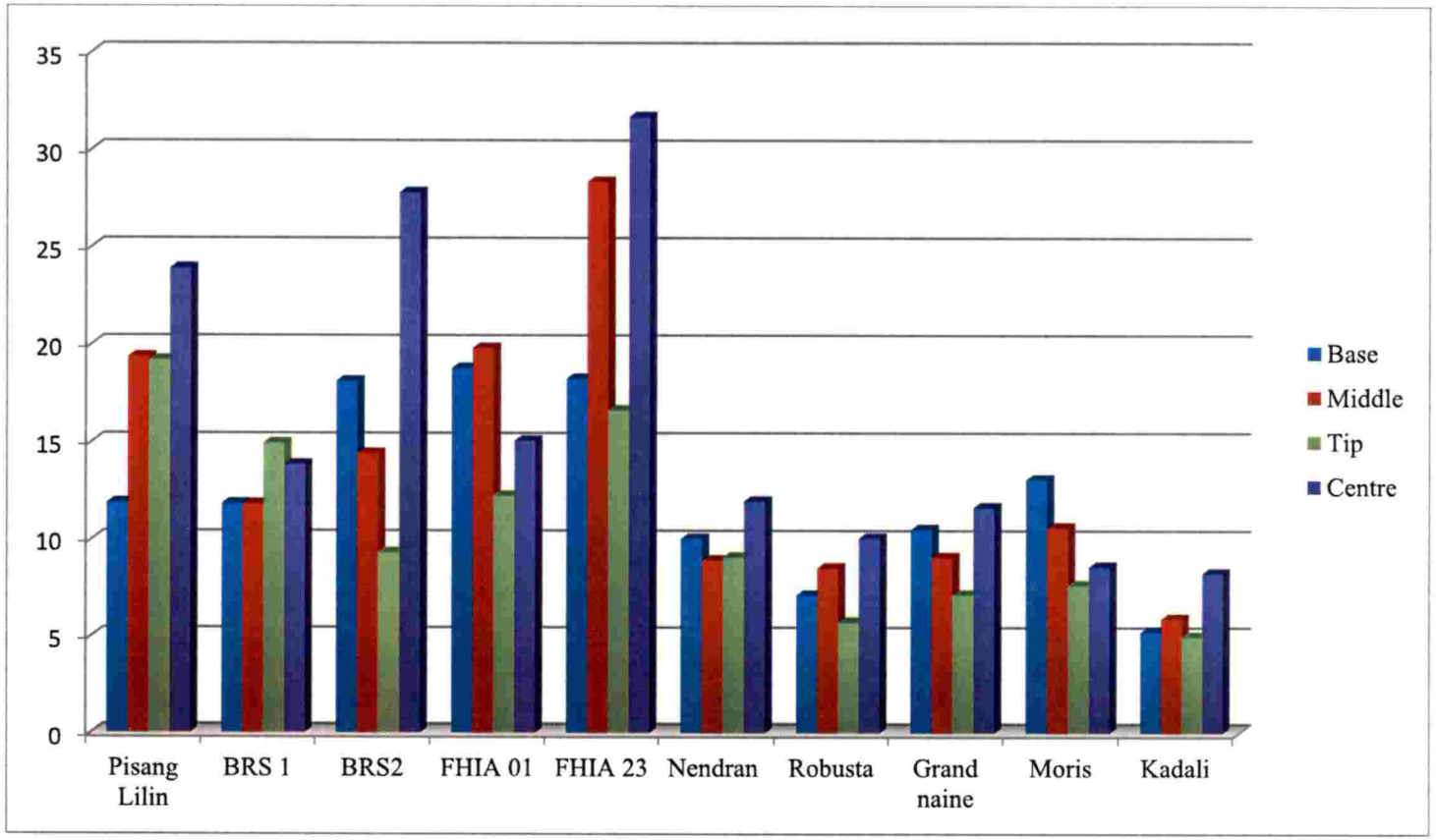


Fig 4.15: Histopathological changes in lower epidermis of different portions of infected leaf lamina in resistant and susceptible varieties

susceptible accessions (Nendran, Robusta, Grand naine, Moris and Kadali). Whereas in infected leaves, an increase in thickness of lower cuticle was noticed in resistant accessions while a decrease in thickness was noticed in susceptible accessions except in Nendran cultivar (Table 4.14).

No significant differences were recorded in thickness of lower cuticle in healthy and infected leaves of resistant and susceptible varieties. In healthy leaves, the highest cuticle thickness was recorded in the resistant accession FHIA 23 (6.91 µm) followed by BRS 2 (6.40 µm) and the lowest thickness was observed in susceptible accession Kadali (4.56 µm). In infected leaves, the highest cuticle thickness was noticed in FHIA 23 (7.42 µm) followed by FHIA 01 (7.35 µm) while the lowest cuticle thickness was recorded in Moris accession (4.35 µm).

Significantly no difference in thickness of lower cuticle was observed among different portions of healthy and infected leaves. In both the healthy and infected leaves, the highest thickness of the lower cuticle was observed in the middle portion which was followed by central, base and tip portions. The healthy leaves recorded a thickness of 6.35, 5.72, 5.32 and 5.31 µm in the middle, base, central and tip portions while in infected leaves the middle, central , base and tip region recorded thickness of 6.91, 5.95, 5.87 and 5.17 µm respectively.

4.4.1.7. Number of stomata

The number of stomata was found to be less in the upper surface of both healthy and infected leaves of resistant and susceptible varieties when compared with the lower surface (Plate 4.18a & b).

4.4.1.7.1. Number of stomata on upper surface

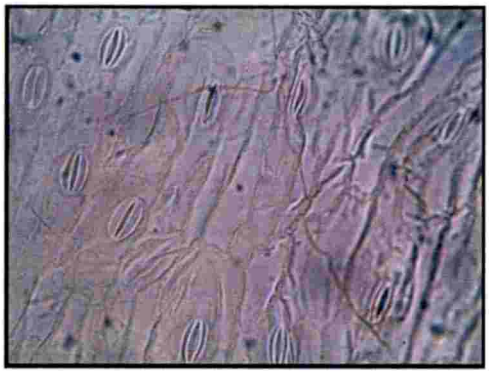
The number of stomata was observed to be more in the susceptible accessions (Nendran, Robusta, Grand Naine, Moris and Kadali) than that of resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) in healthy and infected leaves.

Table 4.14: Histopathological changes in lower cuticle of different portions of leaf lamina in resistant and susceptible varieties

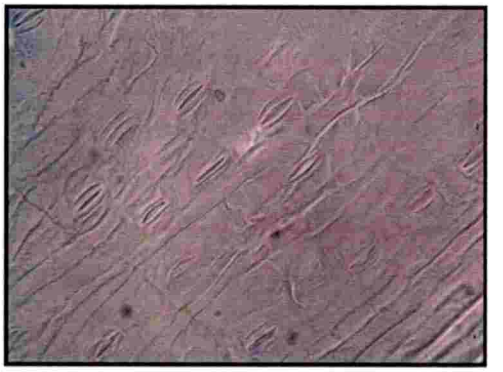
	Healthy leaves					Infected leaves				
Name of the varieties	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**
Pisang Lilin*	7.60	6.21	5.09	5.98	**6.22**	8.00	6.98	5.13	6.63	**6.68**
BRS 1*	6.43	6.21	5.50	6.77	**6.23**	6.58	7.53	4.88	7.82	**6.70**
BRS 2*	6.88	7.56	5.10	6.06	**6.40**	7.87	8.24	4.82	6.90	**6.96**
FHIA 01*	6.19	7.54	5.01	6.18	**6.23**	6.16	9.13	6.83	7.30	**7.35**
FHIA 23*	6.76	7.83	6.51	6.54	**6.91**	7.48	9.27	5.84	7.11	**7.42**
Nendran**	5.01	5.15	5.67	6.65	**5.62**	5.91	6.82	6.02	6.78	**6.38**
Robusta**	4.99	8.52	4.00	5.10	**5.65**	4.01	7.90	3.35	4.30	**4.89**
Grand Naine**	5.70	4.23	7.24	3.70	**5.22**	4.66	3.80	6.92	3.29	**4.67**
Moris**	4.53	4.89	4.93	5.48	**4.95**	4.07	4.44	4.00	4.91	**4.35**
Kadali**	4.15	5.36	4.04	4.72	**4.56**	4.00	4.97	3.94	4.51	**4.36**
Mean	**5.32**	**6.35**	**5.31**	**5.72**		**5.87**	**6.91**	**5.17**	**5.95**	

Interaction of leaves x portions: CD = NS, Interaction of leaves x cultivars: CD =NS

*Resistant varieties, ** Susceptible varieties

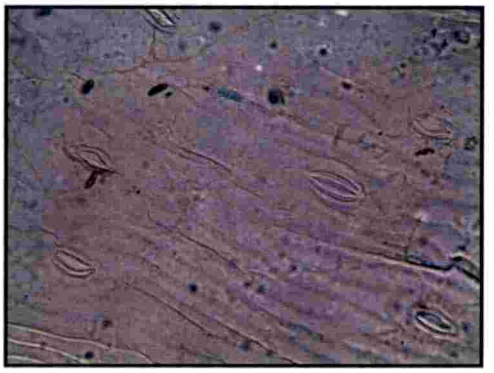


Upper surface

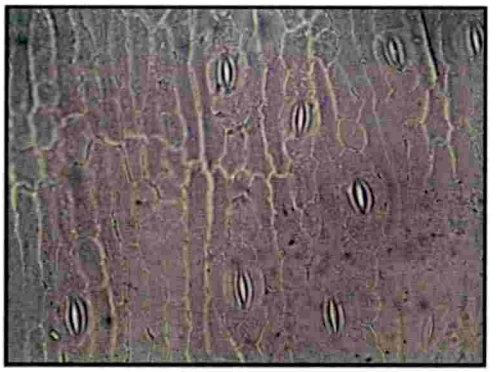


Lower surface

Pisang lilin

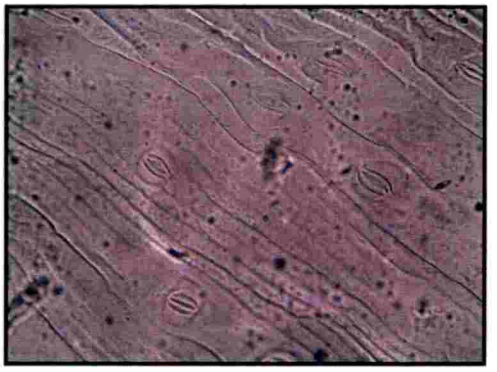


Upper surface

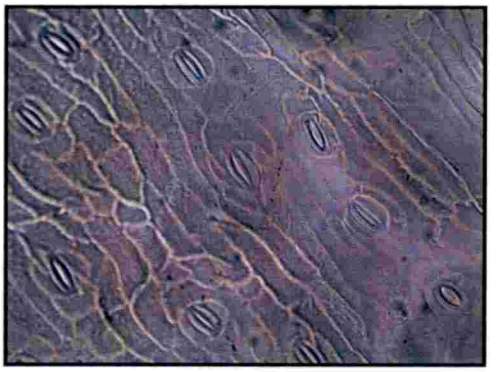


Lower surface

BRS 1



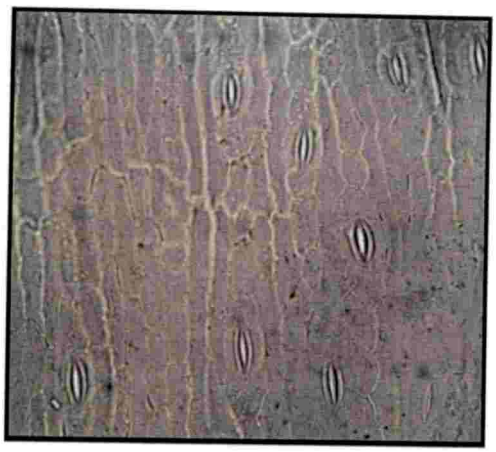
Upper surface



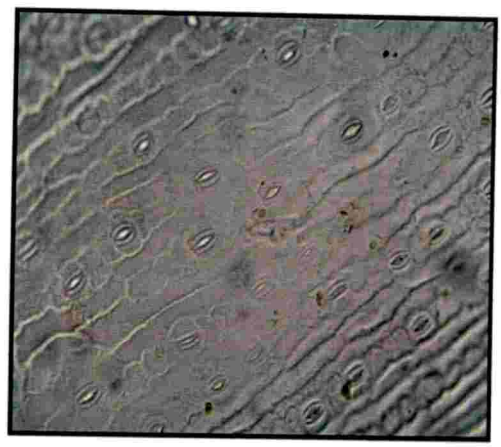
Lower surface

BRS 2

Plate 4.18a: Distribution of stomata on upper and lower surface of leaves of resistant accessions (400X)

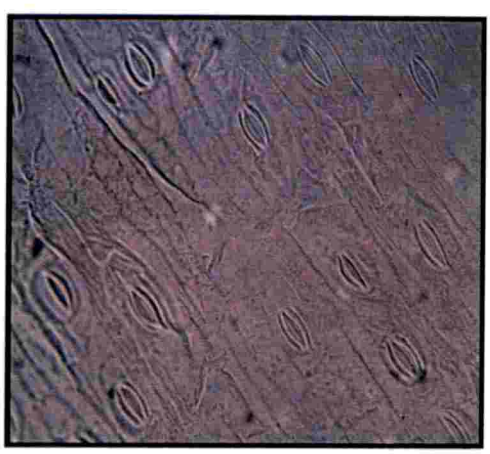


Upper surface

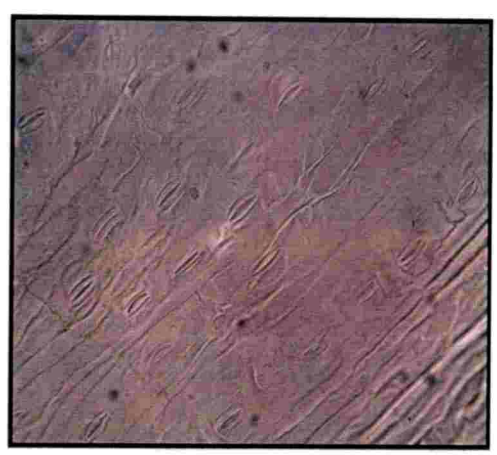


Lower surface

FHIA 01



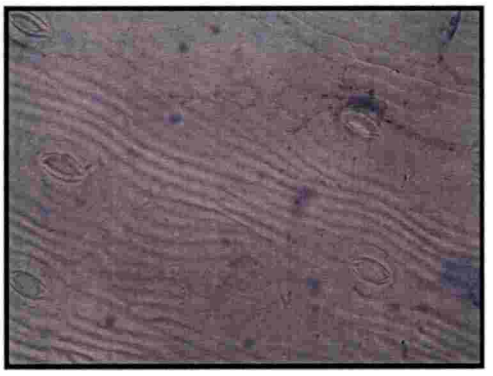
Upper surface



Lower surface

FHIA 23

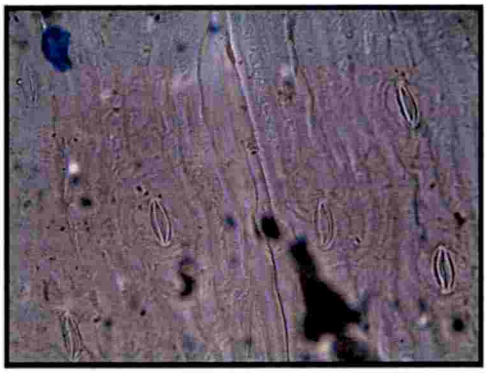
Plate 4.18a (contd...): Distribution of stomata on upper and lower surface of leaves of resistant accessions (400X)



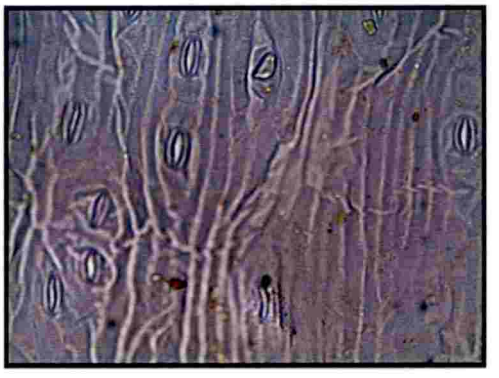
Upper surface

Lower surface

Robusta



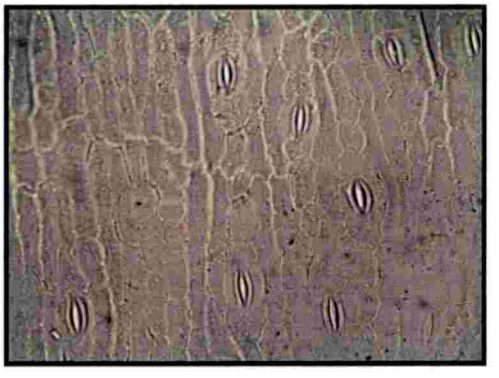
Upper surface



Lower surface

Grand naine

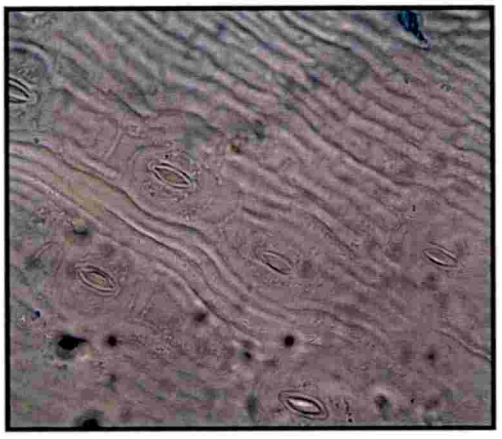
Upper surface



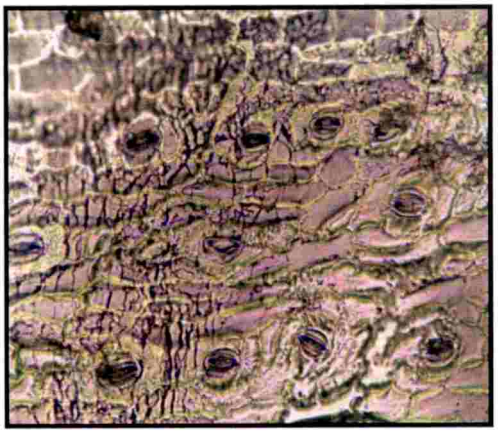
Lower surface

Moris

Plate 4.18b : Distribution of stomata on upper and lower surface of leaves of susceptible accessions (400X)

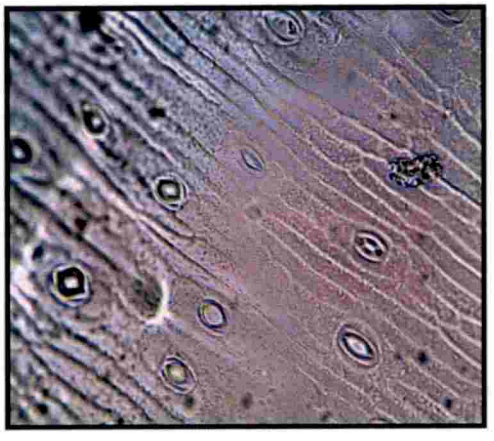


Upper surface

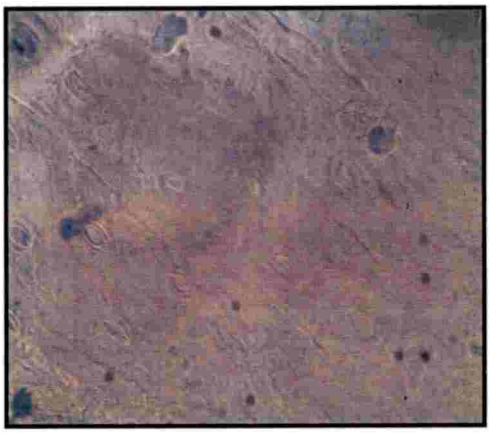


Lower surface

Kadali



Upper surface



Lower surface

Nendran

Plate 4.18b (contd...) : Distribution of stomata on upper and lower surface of leaves of susceptible accessions (400X)

Not much variations in number of stomata on the upper surface was noticed between the healthy and infected leaves of resistant and susceptible accessions (Table 4.15).

Statistically significant differences were recorded in number of stomata/microscopic field on the upper surface of healthy and infected leaves of resistant and susceptible accessions. In healthy leaves, the more number of stomata/microscopic field (19.75) was recorded in susceptible cultivar Nendran followed by Grand Naine (15.87) while the lowest number of stomata was observed in the resistant accession BRS 2 (7.12). In infected leaves, more number of stomata/microscopic field was noticed in susceptible Nendran accession (20.37) followed by Robusta (15.00) while the lowest number of stomata was noticed in the resistant accession BRS 1 (6.37).

No significant difference in number of stomata /microscopic field was noticed among different portions of healthy and infected leaves. In both healthy and infected leaves, the number of stomata was observed to be more in the centre followed by middle, base and tip portions. The mean number of stomata in the central, middle, base and tip portions were 14.45, 13.60, 11.30 and 10.90 stomata respectively in healthy and 14.20, 12.85, 11.20 and 10.80 in infected leaves.

4.4.1.7.2. Number of stomata on lower surface

The number of stomata was observed to be more in the susceptible accessions (Nendran, Robusta, Grand Naine, Moris and Kadali) than that of resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) in healthy and infected leaves. Not much variations in number of stomata on the lower surface was recorded between the healthy and infected leaves of resistant and susceptible accessions (Table 4.16).

No significant differences was noticed in the number of stomata/microscopic field on the lower surface of healthy and infected leaves of resistant and susceptible

Table 4.15: Distribution of stomatal density/ per microscopic field on upper surface of different portions of leaf lamina in resistant and susceptible cultivars

	Healthy leaves					Infected leaves				
Name of the varieties	**Base**	**Middle**	**Tip**	**Centre**	**Mean**	**Base**	**Middle**	**Tip**	**Centre**	**Mean**
Pisang lilin*	12.00	14.00	11.50	14.50	**13.00**	11.50	10.00	9.00	15.50	**11.50**
BRS 1*	5.00	8.00	6.00	11.00	**7.50**	5.00	5.00	7.00	8.50	**6.37**
BRS 2*	9.00	6.50	6.00	7.00	**7.12**	8.50	5.00	6.00	7.00	**6.62**
FHIA 01*	12.50	8.00	8.00	15.50	**11.00**	12.50	8.50	7.00	12.50	**10.12**
FHIA 23*	8.50	10.50	7.50	12.00	**9.65**	8.00	10.50	7.00	12.00	**9.37**
Nendran**	15.00	24.50	18.50	21.00	**19.75**	14.50	27.50	15.50	24.00	**20.37**
Robusta**	14.50	14.50	14.00	18.00	**15.25**	14.00	15.00	13.50	17.50	**15.00**
Grand naine**	16.00	17.50	13.50	16.50	**15.87**	16.50	14.00	15.00	13.50	**14.75**
Moris**	11.50	16.00	13.50	18.00	**14.75**	10.00	17.50	13.00	17.00	**14.37**
Kadali**	9.00	16.50	10.50	11.00	**11.75**	12.56	15.50	15.00	14.50	**14.12**
Mean	**11.30**	**13.60**	**10.90**	**14.45**		**11.20**	**12.85**	**10.80**	**14.20**	

Interaction of leaves x portions: CD = NS, Interaction of leaves x cultivars: CD = 0.92

*Resistant varieties, ** Susceptible varieties

Table 4.16: Distribution of stomata/ per microscopic field on lower surface of different portions of leaf lamina in resistant and susceptible varieties

	Healthy leaves					Infected leaves				
Name of the varieties	**Base**	**Middle**	**Tip**	**Centre**	**Mean**	**Base**	**Middle**	**Tip**	**Centre**	**Mean**
Pisang lilin*	15.50	19.50	13.00	21.50	**17.37**	15.00	19.50	13.50	18.50	**16.62**
BRS 1*	25.00	20.50	19.00	23.00	**21.87**	26.00	21.50	19.50	20.50	**21.87**
BRS 2*	18.50	17.50	17.50	22.00	**18.87**	19.00	17.50	18.50	22.00	**19.25**
FHIA 01*	19.00	26.00	21.50	23.00	**22.37**	15.00	26.00	19.00	23.50	**20.87**
FHIA 23*	20.50	23.50	19.00	21.00	**21.00**	24.50	24.50	18.50	22.50	**22.50**
Nendran **	24.50	27.50	25.00	28.50	**26.37**	24.00	29.00	25.50	29.00	**26.87**
Robusta**	27.50	29.00	24.50	26.50	**26.87**	26.50	30.50	24.50	29.50	**27.75**
Grand naine**	25.00	30.50	28.00	28.50	**28.00**	24.50	27.50	27.50	30.00	**27.37**
Moris**	29.00	27.50	27.50	26.50	**27.62**	27.00	30.50	25.50	27.00	**27.50**
Kadali**	24.50	31.00	28.50	30.50	**28.62**	27.50	28.50	25.00	30.50	**27.87**
Mean	**22.90**	**25.25**	**22.35**	**25.10**		**22.90**	**25.50**	**21.70**	**25.30**	

Interaction of leaves x portions: CD = NS, Interaction of leaves x cultivars: CD = NS

*Resistant varieties , **Susceptible varieties

accessions. In healthy leaves, the more number of stomata was recorded on the lower surface of susceptible cultivar Kadali (28.62) followed by Grand Naine cultivar (28.00) while the lowest number of stomata was found on Pisang Lilin (17.37). While in infected leaves, the more number of stomata (27.87) was observed in Kadali followed by Robusta (27.75) whereas the lowest number of stomata was observed in Pisang Lilin (16.62).

No significant differences was recorded in number of stomata /microscopic field among different portions of healthy and infected leaves. In both healthy and infected leaves, the number of the stomata was found to be more in the middle portion (25.25) followed by central (25.10), base (22.90) and tip (22.35) portions in healthy and 25.50, 25.30, 22.90 and 21.70 respectively in infected leaves.

4.4.1.8.Stomatal pore width

4.4.1.8.1. Stomatal pore width on upper surface

The stomatal pore width was observed to be more on upper leaf surface in the susceptible accessions (Nendran, Robusta, Grand Naine, Moris and Kadali) than that of resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) in healthy and infected leaves. (Table 4.17).

Statistically significant differences were recorded in stomatal pore width on the upper surface of healthy and infected leaves of resistant and susceptible accessions. In healthy leaves, the highest pore width was recorded in the susceptible accession Nendran (5.51 µm) which was followed by Robusta cultivar (5.51 µm) while the lowest pore width (1.87 µm) was recorded in Pisang Lilin. In infected leaves, the susceptible accession Moris exhibited the highest pore width of 5.67 µm followed by Robusta (5.55 µm) while the lowest pore width was recorded in the resistant accession Pisang Lilin (2.43 µm).

No significant differences were recorded in stomatal pore width among

Table 4.17: Histopathological changes in stomatal pore width on upper surface of different portions of leaf lamina in resistant and susceptible cultivars

	Healthy leaves					Infected leaves				
Name of the varieties	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**
Pisang lilin*	2.36	2.24	1.29	1.61	**1.87**	2.89	2.56	1.92	2.36	**2.43**
BRS 1*	2.78	2.87	3.10	3.38	**3.03**	2.44	3.05	3.47	3.40	**3.09**
BRS 2*	3.88	3.24	3.21	4.05	**3.59**	4.05	4.12	3.72	4.30	**4.04**
FHIA 01*	2.99	3.90	4.06	3.95	**3.73**	5.36	3.89	4.31	4.98	**4.63**
FHIA 23*	2.23	2.58	2.50	3.04	**2.59**	2.52	3.41	3.49	3.16	**3.14**
Nendran **	4.78	4.21	7.16	5.89	**5.51**	4.71	5.06	4.08	3.89	**4.43**
Robusta**	4.99	5.49	5.73	5.82	**5.51**	4.86	5.39	6.00	5.95	**5.55**
Grand naine**	4.09	5.85	2.33	5.83	**4.52**	3.68	4.09	4.19	4.40	**4.09**
Moris**	5.10	4.98	4.71	4.88	**4.92**	5.60	5.35	5.13	6.62	**5.67**
Kadali**	4.35	4.16	5.81	4.83	**4.79**	4.50	4.29	6.00	5.00	**4.95**
Mean	**3.83**	**4.07**	**3.96**	**4.46**		**4.06**	**4.12**	**4.23**	**4.40**	

Interaction of leaves x portions: CD = NS, Interaction of leaves x cultivars: CD = 0.49

*Resistant varieties, **Susceptible varieties

different portions of healthy and infected leaves. In healthy leaves, the highest stomatal pore width (4.46 µm) was recorded in the central region followed by middle, tip and base portions with stomatal pore width of 4.07, 3.96 and 3.83 µm. In infected leaves, the highest stomatal pore width (4.40 µm) was recorded in central region followed by tip, middle and base portions with stomatal pore width of 4.23, 4.12 and 4.06 µm respectively.

4.4.1.8.2. Stomatal pore width on lower surface

The stomatal pore width was recorded to be more on the lower surface in the susceptible accessions (Nendran, Robusta, Grand Naine, Moris and Kadali) than that of resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) in healthy and infected leaves. (Table 4.18).

No significant differences was recorded in stomatal pore width on the lower surface of healthy and infected leaves of resistant and susceptible accessions. In healthy leaves, the highest pore width was noticed in the susceptible accession Nendran (6.72 µm) followed by Robusta (5.75 µm) while the lowest pore width was noticed in the resistant accession Pisang Lilin (1.95 µm). Whereas in infected leaves, the highest pore width was noticed in Nendran cultivar (7.17 µm) followed by Robusta (6.10 µm) and the lowest pore size width of was recorded in Pisang Lilin (2.45 µm).

No significant differences was noticed in stomatal pore width among different portions of healthy and infected leaves. In healthy leaves, the highest pore width (4.37 µm) was observed in the middle portion followed by central (4.29 µm), tip (4.26 µm) and base (4.15 µm) portions. While in infected leaves, the maximum (4.738 µm) pore width was observed on the base region followed by central, middle and the tip portions having stomatal pore width of 4.69, 4.66 and 4.63 µm respectively.

Table 4.18: Histopathological changes in stomatal pore width on lower surface of different portions of leaf lamina in resistant and susceptible cultivars

	Healthy leaves					Infected leaves				
Name of the varieties	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**	**Base (µm)**	**Middle (µm)**	**Tip (µm)**	**Centre (µm)**	**Mean (µm)**
Pisang lilin*	2.15	1.89	1.99	1.77	**1.95**	2.26	2.60	2.61	2.34	**2.45**
BRS 1*	3.21	3.40	3.13	2.77	**3.14**	3.58	3.95	3.60	3.43	**3.64**
BRS 2*	3.20	3.00	4.27	3.58	**3.51**	4.95	3.55	4.66	3.88	**4.26**
FHIA 01*	4.01	4.14	3.27	3.99	**3.73**	3.70	4.53	4.11	4.48	**4.20**
FHIA 23*	2.08	2.45	3.11	2.77	**2.61**	2.50	1.92	3.50	2.97	**2.72**
Nendran**	7.03	7.60	5.45	6.79	**6.72**	7.14	7.98	6.02	7.56	**7.17**
Robusta**	5.36	5.67	6.10	5.88	**5.75**	5.98	5.97	6.39	6.06	**6.10**
Grand naine**	4.75	4.80	4.56	5.10	**4.80**	5.84	5.16	4.95	5.39	**5.33**
Moris**	4.90	5.94	5.22	6.17	**4.92**	5.41	4.82	5.01	5.14	**5.09**
Kadali**	5.65	5.99	5.21	5.46	**5.58**	5.99	6.14	5.46	5.69	**5.82**
Mean	**4.15**	**4.37**	**4.26**	**4.29**		**4.73**	**4.66**	**4.63**	**4.69**	

Interaction of leaves x portions: CD = N/A, Interaction of leaves x cultivars: CD = N/A

*Resistant varieties, ** Susceptible varieties

4.5.2. Biochemical studies

The biochemical studies were carried out by quantifying biochemical parameters such as phenols, reducing sugars, non reducing sugars and defense related enzymes *viz.,* peroxidase, polyphenol oxidase and phenylalanine ammonia lyase.

4.5.2.1. Phenols

In healthy leaves, the phenolic content was observed to be more in resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) than that of susceptible accessions (Nendran, Robusta, Grand Naine, Moris and Kadali). Whereas in infected leaves, decrease in phenolic content was observed in resistant as well as in susceptible varieties (Table 4.19).

Statistically significant differences were recorded in phenolic content among healthy and infected leaves of resistant and susceptible accessions. In healthy leaves, the highest phenolic content of 23.84 mg/g was recorded in the resistant accession Pisang Lilin followed by BRS 1 (20.57 mg/g) while the lowest phenolic content of (15.36 mg/g) was recorded in susceptible Grand Naine cultivar. In infected leaves, the highest phenolic content (16.69 mg/g) was recorded in the susceptible accession Kadali followed by Robusta (16.00 mg/g) while the lowest phenolic content (9.60 mg/g) was recorded in the resistant cultivar BRS 2.

Significant differences were recorded in phenolic content between different portions of healthy and infected leaves. In healthy leaves, the highest phenolic content (20.02 mg/g) was recorded in the middle portion followed by central, tip and base portions with phenolic content of 17.96 mg/g, 17.57 mg/g and 17.04 mg/g (Fig 4.16). In infected leaves, the highest phenolic content (14.946 mg/g) was recorded in the middle portion, followed by central, base and tip portions with phenolic content of 13.63 mg/g, 13.05 mg/g and 11.97 mg/g (Fig 4.17).

Table 4.19: Phenolic content in different portions of leaf lamina in healthy and infected leaves of selected varieties

	Healthy leaves					Infected leaves				
Name of the varieties	**Base (mg/g)**	**Middle (mg/g)**	**Tip (mg/g)**	**Centre (mg/g)**	**Mean (mg/g)**	**Base (mg/g)**	**Middle (mg/g)**	**Tip (mg/g)**	**Centre (mg/g)**	**Mean (mg/g)**
Pisang lilin*	21.99	24.81	25.80	22.80	**23.84**	10.68	14.73	9.88	16.49	**12.94**
BRS 1*	18.03	22.35	20.37	21.55	**20.57**	14.19	15.13	10.40	6.77	**11.62**
BRS 2*	17.86	18.88	17.07	18.25	**18.01**	10.00	10.35	9.28	8.79	**9.60**
FHIA 01*	17.96	22.61	17.57	18.93	**19.26**	13.70	15.70	13.25	15.26	**14.47**
FHIA 23*	16.06	19.41	17.85	18.81	**18.03**	9.69	13.68	8.59	13.11	**11.27**
Nendran **	15.34	21.00	15.09	14.44	**16.46**	14.29	14.79	10.65	13.52	**13.31**
Robusta**	16.97	17.66	16.75	17.13	**17.13**	15.79	16.36	15.70	16.13	**16.00**
Grand naine**	14.97	17.73	14.32	14.43	**15.36**	12.52	14.50	12.04	13.86	**13.23**
Moris**	15.63	16.57	15.38	16.25	**16.00**	15.20	16.02	14.86	15.38	**15.40**
Kadali**	15.63	19.23	15.55	17.13	**16.88**	14.47	18.20	15.09	17.02	**16.69**
Mean	**17.04**	**20.02**	**17.57**	**17.96**		**13.05**	**14.94**	**11.97**	**13.63**	

Interaction of leaves x portions: CD = 0.57, Interaction of leaves x cultivars: CD = 0.90

*Resistant varieties, ** Susceptible varieties

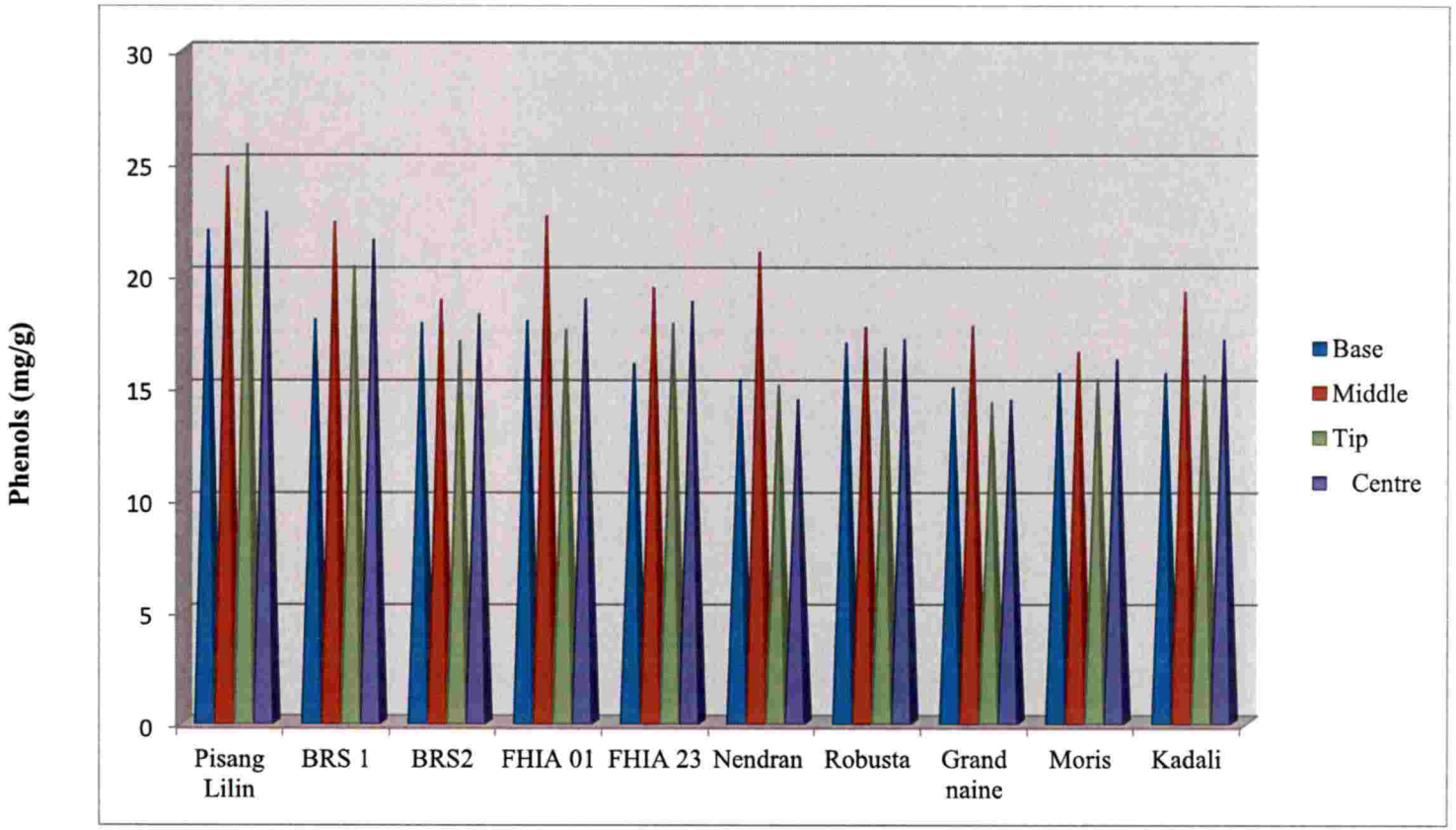


Fig 4.16: Phenolic content in different portions of leaf lamina in healthy leaves of resistant and susceptible varieties

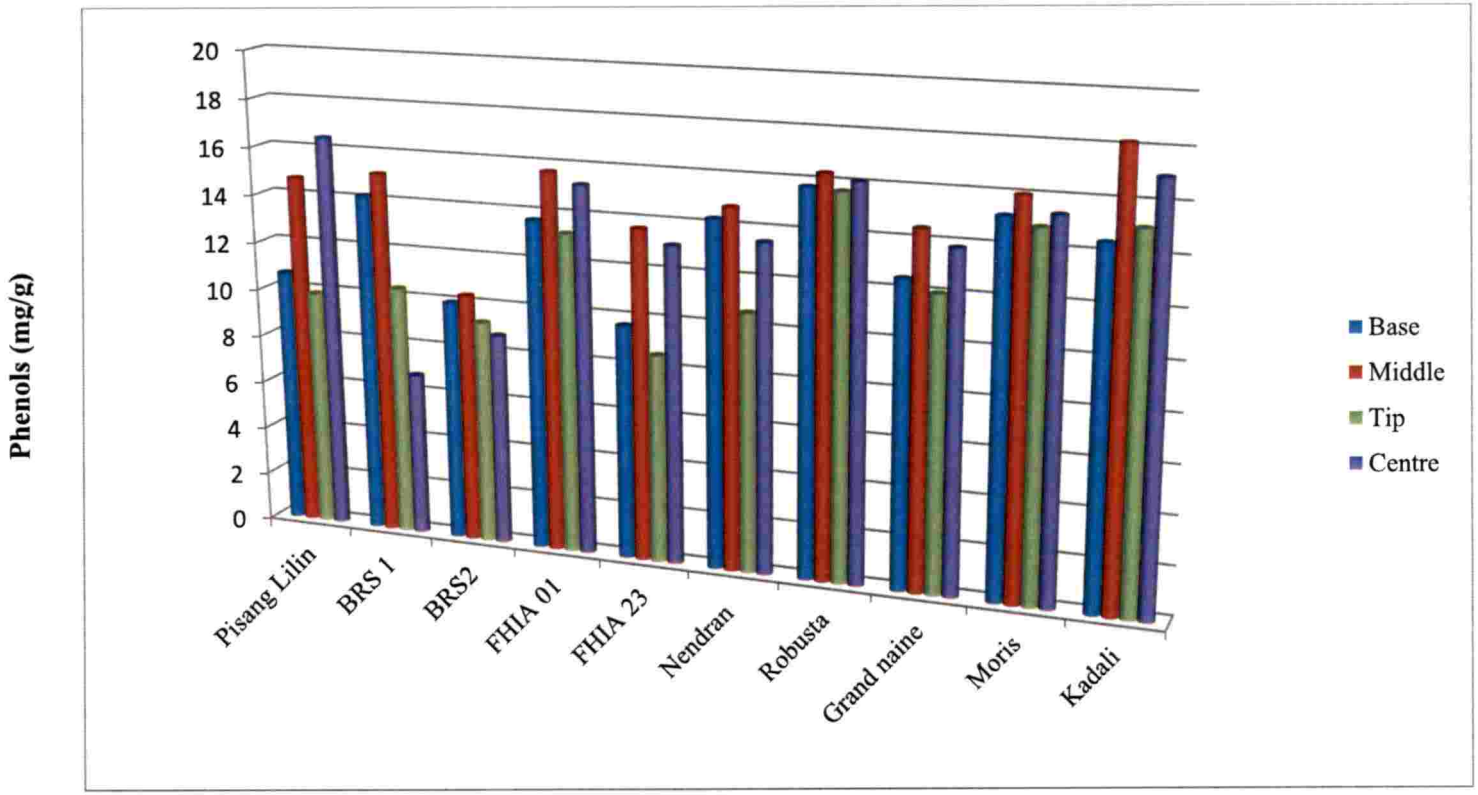


Fig 4.17: Phenolic content in different portions of leaf lamina in infected leaves of resistant and susceptible varieties

4.5.2.2. Reducing sugars

The quantity of reducing sugar in healthy leaves was noticed to be more in the resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) compared susceptible accessions (Nendran, Robusta, Grand Naine, Moris and Kadali). While in infected leaves, reduction in quantity of reducing sugar was observed both in resistant and susceptible accessions (Table 4.20)

No significant differences were recorded in quantity of reducing sugar in healthy and infected leaves of resistant and susceptible accessions. The quantity of reducing sugars was recorded highest (15.98 mg/g) in the resistant accession BRS 2 followed by BRS 1 (13.23 mg/g) while the lowest quantity of reducing sugar (8.563 mg/g) was recorded in susceptible cultivar Grand Naine. While in infected leaves, the quantity of reducing sugars was recorded to be highest (13.17 mg/g) in the resistant accession BRS 2 followed by BRS 1 (11.23 mg/g) and the lowest (7.80 mg/g) quantity of reducing sugar was recorded in the susceptible accession Grand Naine.

While comparing the reducing sugar content of different portions of healthy and infected leaves there was no significant differences noticed. The central portion of both healthy and infected leaves exhibited highest (12.78 mg/g and 11.04 mg/g) quantity of reducing sugar followed by middle (11.93 mg/g & 10.12 mg/g) and base (10.72 mg/g and 9.15 mg/g) portions while the lowest quantity of reducing sugar was observed in the tip portion (9.64 mg/g and 8.44 mg/g).

4.4.2.3. Non reducing sugars

In healthy leaves the quantity of non reducing sugar was recorded to be more in the susceptible accessions such as Nendran, Grand Naine and Kadali followed by the resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23). While in infected leaves the quantity of non reducing sugar increased in resistant accessions

Table 4.20: Reducing sugar content in different portions of leaf lamina in healthy and infected leaves of selected varieties

	Healthy leaves					Infected leaves				
Name of the varieties	**Base (mg/g)**	**Middle (mg/g)**	**Tip (mg/g)**	**Centre (mg/g)**	**Mean (mg/g)**	**Base (mg/g)**	**Middle (mg/g)**	**Tip (mg/g)**	**Centre (mg/g)**	**Mean (mg/g)**
Pisang Lilin*	10.49	11.10	11.18	13.00	**11.44**	9.01	9.08	8.84	10.22	**9.28**
BRS 1*	12.66	13.88	11.35	15.03	**13.23**	10.89	11.88	9.14	13.04	**11.23**
BRS 2*	15.99	16.71	13.84	17.38	**15.98**	13.02	13.16	11.07	15.44	**13.17**
FHIA 01*	11.88	13.17	10.93	14.22	**12.55**	9.15	10.48	9.09	11.50	**10.05**
FHIA 23*	12.94	13.87	11.21	14.83	**13.21**	10.21	11.15	9.77	12.85	**10.99**
Nendran **	8.04	9.66	7.04	10.58	**8.83**	7.06	8.44	6.74	9.27	**7.88**
Robusta**	8.54	9.84	7.75	10.06	**9.04**	7.55	8.89	6.73	8.10	**7.82**
Grand Naine**	7.95	9.30	7.11	9.88	**8.56**	7.27	8.55	6.44	8.96	**7.80**
Moris**	8.88	10.15	8.11	10.90	**9.51**	8.06	9.56	9.43	10.45	**9.37**
Kadali**	9.86	11.63	7.88	11.92	**10.32**	9.33	9.99	7.27	10.62	**9.30**
Mean	**10.72**	**11.93**	**9.64**	**12.78**		**9.15**	**10.12**	**8.44**	**11.04**	

Interaction of leaves x portions: CD = NS, Interaction of leaves x cultivars: CD = NS

*Resistant varieties, **Susceptible varieties

while a decrease in the levels of non reducing sugar content was recorded in the susceptible acccessions except Robusta and Kadali (Table 4.21).

Statistically significant differences were noticed in quantity of non reducing sugar on healthy and infected leaves of resistant and susceptible accessions. In healthy leaves, the quantity of non reducing sugar was recorded to be highest in susceptible accession Nendran (3.18 mg/g) followed by Kadali (2.99 mg/g) while the lowest (2.16 mg/g) quantity of non reducing sugar was observed in Robusta. While in infected leaves, the quantity of non reducing sugar content was highest in FHIA 23 (4.03 mg/g) followed by FHIA 01 (3.54 mg/g) and the lowest (1.95 mg/g) quantity was observed in the susceptible accession Moris.

Significant differences were recorded in quantity of non reducing sugar content among different portions of healthy and infected leaves. In healthy leaves, the quantity of non reducing sugar was observed to be highest in middle portion (2.76 mg/g) followed by central (2.75 mg/g), base (2.57 mg/g) and tip (2.56 mg/g) portions. In infected leaves, the middle portion exhibited highest quantity of non reducing sugar (3.04 mg/g) followed by central (2.99 mg/g) and base (2.96 mg/g) portions, while the tip portion recorded lowest quantity of 2.82 mg/g.

4.4.2.4. Peroxidase

In healthy leaves, the activity of peroxidase enzyme was observed to be highest in the resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) than susceptible accessions (Nendran, Robusta, Grand Naine, Moris and Kadali). Whereas in infected leaves, an increase in peroxidase activity was recorded in resistant accessions while a decrease in activity of enzymes was observed in the susceptible varieties (Table 4.22).

Statistically significant differences were recorded in activity of peroxidase enzyme on healthy and infected leaves of resistant and susceptible accessions. In

Table 4.21:Non reducing sugar content in different portions of leaf lamina in healthy and infected leaves of selected varieties

	Healthy leaves					Infected leaves				
Name of the varieties	**Base (mg/g)**	**Middle (mg/g)**	**Tip (mg/g)**	**Centre (mg/g)**	**Mean (mg/g)**	**Base (mg/g)**	**Middle (mg/g)**	**Tip (mg/g)**	**Centre (mg/g)**	**Mean (mg/g)**
Pisang Lilin*	2.39	2.56	2.45	2.43	**2.45**	3.38	3.56	3.43	3.52	**3.47**
BRS 1*	2.24	2.38	2.15	2.32	**2.27**	2.97	3.23	3.08	3.14	**3.16**
BRS 2*	2.53	2.64	2.44	2.59	**2.55**	3.10	3.12	2.88	3.04	**3.03**
FHIA 01*	2.93	3.04	2.82	2.97	**2.94**	3.53	3.57	3.43	3.62	**3.54**
FHIA 23*	2.77	2.88	2.61	2.84	**2.77**	4.04	4.13	3.88	4.09	**4.03**
Nendran **	3.18	3.26	3.10	3.17	**3.18**	3.09	2.64	2.44	2.61	**2.69**
Robusta**	2.01	2.16	2.39	2.08	**2.16**	2.46	2.52	2.62	2.28	**2.47**
Grand Naine**	2.61	2.78	2.13	3.68	**2.80**	2.20	2.15	1.52	2.83	**2.17**
Moris**	2.52	2.64	2.47	2.57	**2.55**	2.12	2.10	1.77	1.81	**1.95**
Kadali**	2.58	3.33	3.08	2.98	**2.99**	2.70	3.40	3.21	3.01	**3.08**
Mean	**2.57**	**2.76**	**2.56**	**2.76**		**2.96**	**3.04**	**2.82**	**2.99**	

Interaction of leaves x portions: CD = 0.07, Interaction of leaves x cultivars: CD =0.12

*Resistant varieties, ** Susceptible varieties

Table 4.22: Peroxidase activity in different portions of leaf lamina in healthy and infected leaves of selected varieties

	Healthy leaves					Infected leaves				
Name of the varieties	**Base (EU/g)**	**Middle (EU/g)**	**Tip (EU/g)**	**Centre (EU/g)**	**Mean (EU/g)**	**Base (EU/g)**	**Middle (EU/g)**	**Tip (EU/g)**	**Centre (EU/g)**	**Mean (EU/g)**
Pisang Lilin*	0.26	0.28	0.28	0.27	**0.27**	0.34	0.35	0.36	0.35	**0.35**
BRS 1*	0.23	0.23	0.23	0.27	**0.23**	0.33	0.34	0.35	0.34	**0.34**
BRS 2*	0.24	0.25	0.25	0.44	**0.24**	0.31	0.32	0.33	0.32	**0.32**
FHIA 01*	0.24	0.25	0.25	0.24	**0.24**	0.29	0.30	0.31	0.29	**0.30**
FHIA 23*	0.25	0.27	0.27	0.26	**0.26**	0.36	0.37	0.39	0.36	**0.37**
Nendran **	0.21	0.24	0.24	0.21	**0.23**	0.13	0.16	0.17	0.17	**0.16**
Robusta**	0.21	0.21	0.22	0.21	**0.21**	0.17	0.18	0.13	0.15	**0.16**
Grand Naine**	0.14	0.16	0.17	0.16	**0.16**	0.12	0.13	0.1	0.14	**0.13**
Moris**	0.19	0.21	0.19	0.23	**0.20**	0.13	0.13	0.15	0.20	**0.15**
Kadali**	0.21	0.21	0.22	0.21	**0.21**	0.23	0.23	0.24	0.23	**0.13**
Mean	**0.22**	**0.23**	**0.23**	**0.22**		**0.24**	**0.2**	**0.26**	**0.25**	

Interaction of leaves x portions: CD = NS, Interaction of leaves x cultivars: CD =0.01

*Resistant varieties, ** Susceptible varieties

healthy leaves, the highest peroxidase activity was recorded in the resistant accession Pisang Lilin (0.27 EU/g) followed by FHIA 23 (0.26 EU/g) and the lowest activity (0.16 EU/g) was recorded in susceptible accession Grand Naine. While in the infected leaves, the highest activity was recorded in resistant accession FHIA 23 (0.37 EU/g) followed by Pisang Lilin (0.35 EU/g) while the enzyme activity was recorded to be lowest (0.134 EU/g) in susceptible accession Kadali.

No significant differences were noticed in the activity of peroxidase enzyme among the different portions of healthy and infected leaves. The highest peroxidase activity was recorded in the tip portion of both healthy (0.23 EU/g) and infected leaves (0.26 EU/g) followed by middle and tip portions while the lowest activity was observed in the base portion (0.22 EU/g and 0.24 EU/g).

4.4.2.5. Polyphenol oxidase

In the healthy leaves, the polyphenol oxidase enzyme activity was observed to be highest in the resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) than susceptible accessions (Nendran, Robusta, Grand Naine, Moris and Kadali). While in infected leaves an increase in activity of the enzyme was recorded in both resistant and susceptible accessions (Table 4.23).

Statistically significant differences were recorded in activity of polyphenol oxidase enzyme in the healthy and infected leaves of resistant and susceptible accessions. In healthy leaves, the highest activity of polphenol oxidase enzyme was recorded in the resistant accession Pisang Lilin (2.53 EU/g) followed by FHIA 01 (2.41 EU/g) while the lowest activity was recorded in susceptible cultivar Nendran (0.50 EU/g). In infected leaves, the highest polyphenol oxidase was recorded in resistant accession Pisang Lilin (3.34 EU/g) followed by FHIA 01 (3.23 EU/g) while lowest activity was recorded in susceptible Moris accession (1.02 EU/g).

No significant differences were recorded in the activity of polyphenol oxidase

Table 4.23: Polyphenol oxidase activity in different portions of leaf lamina in healthy and infected leaves of selected varieties

	Healthy leaves					Infected leaves				
Name of the varieties	**Base (EU/g)**	**Middle (EU/g)**	**Tip (EU/g)**	**Centre (EU/g)**	**Mean (EU/g)**	**Base (EU/g)**	**Middle (EU/g)**	**Tip (EU/g)**	**Centre (EU/g)**	**Mean (EU/g)**
Pisang Lilin*	2.28	2.69	2.39	2.78	**2.53**	3.20	3.49	3.13	3.55	**3.34**
BRS 1*	1.96	2.18	2.23	2.38	**2.19**	2.84	3.68	3.07	2.98	**3.14**
BRS 2*	1.38	1.42	1.33	1.90	**1.50**	2.52	2.94	3.11	3.28	**2.96**
FHIA 01*	2.24	2.41	2.41	2.59	**2.41**	3.02	3.38	3.07	3.44	**3.23**
FHIA 23*	1.90	2.35	2.13	2.39	**2.19**	2.64	3.06	2.70	2.86	**2.81**
Nendran **	0.28	0.42	0.46	0.83	**0.50**	1.43	0.82	0.99	1.17	**1.10**
Robusta**	1.16	1.70	1.53	1.85	**1.56**	1.90	2.41	2.07	2.33	**2.18**
Grand Naine**	0.35	0.74	0.91	0.85	**0.71**	0.81	1.64	1.49	1.84	**1.44**
Moris**	0.62	0.77	0.73	0.88	**0.75**	0.87	1.08	0.88	1.26	**1.02**
Kadali**	0.70	0.94	0.88	1.06	**0.89**	1.13	1.70	1.58	1.82	**1.56**
Mean	**1.29**	**1.56**	**1.50**	**1.75**		**2.03**	**2.42**	**2.21**	**2.45**	

Interaction of leaves x portions: CD = NS, Interaction of leaves x cultivars: CD =0.180

*Resistant varieties, ** Susceptible varieties

among different portions of healthy and diseased accessions. The highest polyphenol oxidase activity was recorded in the centre portion of healthy (1.75 EU/g) and infected leaves (2.45 EU/g) followed by middle (1.56 EU/g and 2.42 EU/g), tip (1.50 EU/g and 2.21 EU/g) and base (1.29 EU/g and 2.03 EU/g) portions.

4.4. 2.6.Phenylalanine ammonia lyase (PAL)

In healthy leaves, the PAL enzyme activity was observed to be highest in the resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) than susceptible accessions ((Nendran, Robusta, Grand Naine, Moris and Kadali) . While in infected leaves, an increase in PAL enzyme activity was recorded both in the resistant and susceptible varieties (Table 4.24).

Statistically significant differences were noticed in activity of PAL enzyme on healthy and infected leaves of resistant and susceptible accessions. In healthy leaves, the highest PAL activity was recorded in the resistant accession FHIA 01 (2.65 µmol/g) followed by BRS 2 (2.44 µmol/g) while the lowest activity was recorded in the susceptible accession Moris (1.10 µmol/g). In infected leaves, the highest PAL activity was observed in resistant accession FHIA 01 (3.25 µmol/g) followed by Pisang Lilin (3.07 µmol/g) while the lowest activity of was recorded in susceptible Nendran (1.85 µmol/g) cultivar.

No significant differences were noticed in activity of PAL enzyme among different portions of healthy and infected leaves. The highest PAL enzyme activity was observed in the central portion of both healthy (2.22 µmol/g) and infected leaves (2.83 µmol/g) followed by middle (1.91 µmol/g and 2.56 µmol/g), tip (1.82 µmol/g and 2.52 µmol/g) and base (1.58 µmol/g and 2.22 µmol/g) portions.

4.5. VALIDATION OF MARKERS TO IDENTIFY THE DISEASE RESISTANT GENE

The Sequence characterized amplified region (SCAR) markers that were

Table 4.24: Phenylalanine ammonia lyase in different portions of leaf lamina in healthy and infected leaves of selected varieties

	Healthy leaves					Infected leaves				
Name of the varieties	**Base (µmol/g)**	**Middle (µmol/g)**	**Tip (µmol/g)**	**Centre (µmol/g)**	**Mean (µmol/g)**	**Base (µmol/g)**	**Middle (µmol/g)**	**Tip (µmol/g)**	**Centre (µmol/g)**	**Mean (µmol/g)**
Pisang Lilin*	2.05	2.47	2.37	2.69	**2.40**	2.75	3.01	3.14	3.39	**3.07**
BRS 1*	1.69	1.90	2.12	2.40	**2.02**	2.29	2.81	2.99	3.25	**2.83**
BRS 2*	2.16	2.52	2.36	2.74	**2.44**	2.59	2.99	2.86	3.18	**2.90**
FHIA 01*	2.29	2.83	2.56	2.93	**2.65**	2.91	3.37	3.22	3.52	**3.25**
FHIA 23*	1.70	1.99	2.09	2.24	**2.00**	2.87	2.90	2.89	3.03	**2.92**
Nendran **	0.97	1.56	1.12	1.72	**1.34**	1.57	1.84	1.80	2.18	**1.85**
Robusta**	1.22	1.59	1.59	2.06	**1.61**	1.89	2.16	2.14	2.48	**2.17**
Grand Naine**	1.32	1.49	0.98	1.61	**1.35**	1.69	2.04	1.59	2.10	**1.85**
Moris**	0.85	0.79	1.29	1.48	**1.10**	1.49	1.82	2.05	2.19	**1.89**
Kadali**	1.60	2.01	1.76	2.34	**1.92**	2.18	2.72	2.59	3.00	**2.62**
Mean	**1.58**	**1.91**	**1.82**	**2.22**		**2.22**	**2.56**	**2.52**	**2.83**	

Interaction of leaves x portions: CD = N/A, Interaction of leaves x cultivars: CD =0.149

*Resistant varieties, ** Susceptible varieties

tightly linked to the Sigatoka leaf spot disease resistance were used to identify the disease resistant gene in the selected accessions used in the study.

4.5.1. Isolation of genomic DNA

The genomic DNA was isolated from five selected resistant accessions *viz.*, Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23 and five selected susceptible accessions *viz.*, Nendran, Robusta, Grand Naine, Moris and Kadali using *DNeasy* Plant Minikit.

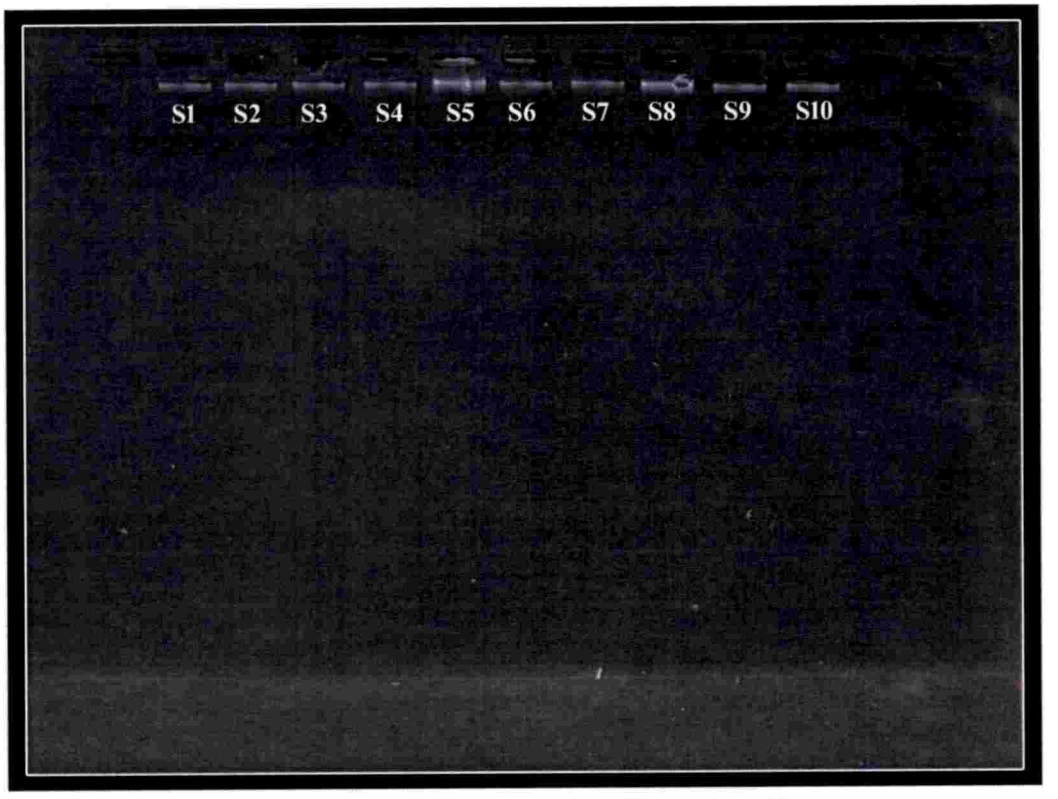
4.5.2. Quality of DNA

The quality of the DNA was examined by 0.8% agarose gel electrophoresis. The agarose gel electrophoresis of the genomic DNA revealed the presence of high quality single undegraded band in all the 10 samples (Plate 4.19a).

4.5.3. Quantity of DNA

The nanodrop spectrophotometer was used to quantify the DNA isolated from the 10 samples. The absorbance values ($A_{260/280}$) of the extracted DNA ranged from 1.6 – 2.10 which indicated purity of the DNA. The quantity of DNA present per µl of sample and Â 260/280 value of the ten samples are given below:

SL. No.	Isolate	Quantity (ng/µl)	Purity (Â 260/280)
1.	Pisang lilin	159.00	2.10
2.	BRS 1	101.90	1.88
3.	BRS 2	110.30	1.97
4.	FHIA 01	68.70	1.67
5.	FHIA 23	166.30	1.96
6.	Nendran	200.90	2.10
7.	Grand naine	89.60	1.82
8.	Robusta	86.40	1.80
9.	Kadali	112.30	1.94
10.	Moris	114.70	1.98



S1: BRS1 S2 : BRS2 S3 : FHIA 1 S4 : FHIA 23 S5 : Pisang lilin
S6 : Robusta S7: Nedunedran S8: Grand naine S9: Moris S10: Kadali

Plate 4.19a: DNA isolated from resistant and susceptible cultivars

Plate 4.19: Validation of SCAR marker to identify disease resistant gene

4.5.4. Standardization of the annealing temperature

The standardization of the annealing temperature using gradient PCR revealed that among the range of temperatures used good band of amplified DNA was produced at 54.2°C (Plate 4.19b). Therefore this temperature was further used for the PCR amplification.

4.5.5. PCR amplification

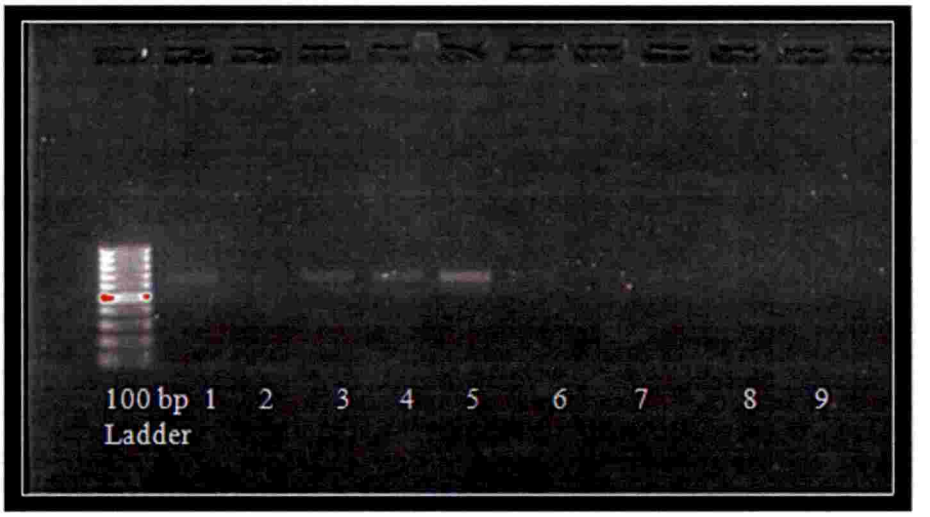
The amplification of the genomic DNA isolated from the five resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) and five selected susceptible accessions (Nendran, Robusta, Grand Naine, Moris and Kadali) was carried out using SCAR primers (SCAR F and SCAR R). The PCR thermal profile is given below:

The PCR thermal profile includes:

Process	Temperature (°C)	Time
Initial denaturation	94	5 min.
Denaturation	92	45 sec.
Annealing	54.2	45 sec.
Extension	72	1 min.
No: of cycles: 35		
Final extension	72	7 min.

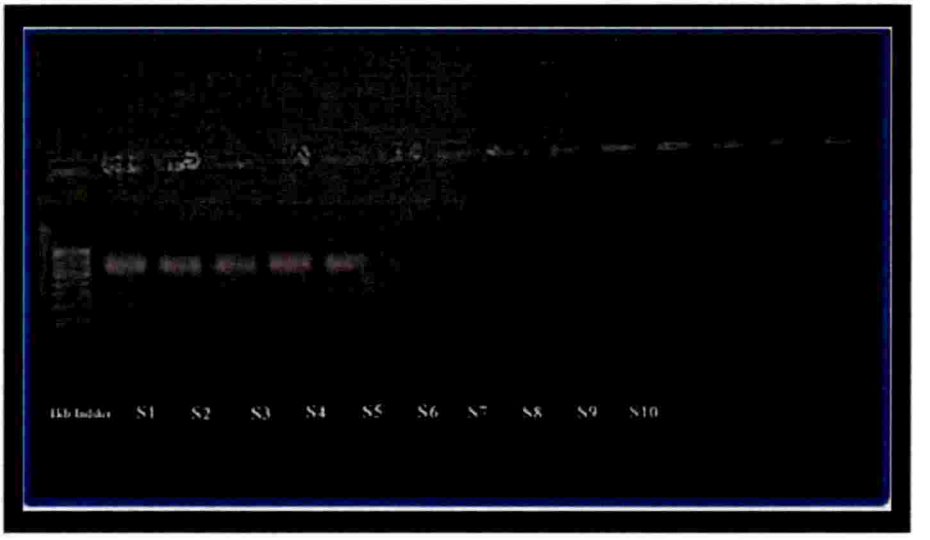
4.5.6. Analysis of the amplicons

The amplicons produced bands with expected molecular size at 644bp in resistant accessions (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) when visualized by agarose gel electrophoresis. These bands were absent in susceptible



1. 48.1°C, 2. 49.4°C, 3. 51.2°C, 4. 52.6°C, 5. 54.2°C, 6. 56.2°C, 7. 58.2°C, 8. 60.2°C, 9. 62.0°C

Plate 4.19b: Standardization of annealing temperature



S1: BRS1 S2 : BRS2 S3 : FHIA 1 S4 : FHIA 23 S5 : Pisang lilin S6 : Robusta S7: Nedunedran S8: Grand naine S9: Moris S10: Kadali

Plate 4.19c: Gel profile of amplicons (644 bp)

Plate 4.19: Validation of SCAR marker to identify disease resistant gene

accessions such as Nendran, Robusta, Grand Naine, Moris and Kadali.

4.5.6. Sequencing of the amplicons

The sequencing of the amplicons was done using automated sequencing facility at AgriGenome Pvt. Ltd., Kakkanad, Kochi. The details of nucleotide sequences of the isolates are given in Fig 4.18.

4.5.6.1. *In silico* analysis of the sequence

The nucleotide sequences were compared for similar sequence in NCBI database using BLASTn analysis and the data were presented in the Fig 4.19. Alignment of the sequences obtained from the amplicon with other known sequences of NCBI site showed 90% sequence homology to the SCAR markers that were tightly linked with Sigatoka leaf spot resistant gene of Kanthali clone1 (KJ801649.1). The BLASTp analysis of the sequences followed by aligning with the whole genome sequence of *Musa* spp. showed 99.08% identity to mito3 region of the *Musa* spp. This concluded that, the resistant gene present in the resistant accessions is a mitochondrial gene.

4.6. MANAGEMENT OF SIGATOKA LEAF SPOT DISEASE

4.6.1. EXPERIMENT NO: 1: MANAGEMENT OF SIGATOKA LEAF SPOT DISEASE USING CHEMICALS FUNGICIDES

The field experiment on management of Sigatoka leaf spot disease using chemical fungicides was laid in the field at Banana Research Station, Kannara. The inference of the experiment was drawn by statistical analysis of the data on per cent disease severity (PDS), youngest leaf spotted (YLS), disease development time (DDT), vegetative and yield characters.

ACCAACAGTTTGATTCTTGCTTTTGAGCGGTTAATTGAAGGGAACAGGTGGTTTATTGCAGGAAATGGGCAA
GGCCCTATAGCGTCGGGCCAGATTCTTTCTAATGAGTCTCCTTGTCTTTGTAACCAGCATGAATTCCGGAAGT
CAGCAACGAAAGATAGGTCTTAATTGTCAGCTCTCGAGTTAGCTAATGGCAGGAAGAAGCGGATAAGATCC
CGGAGGGACTACTCGCCTTCTAACAAGACTATATCTCTATTACCTGTCGTAGCTTACGTGAGAAACAGCAGC
TAACCTCTGGAACTATTTCATAACCAGCGGTGATAGGCTCTAGGAAGCAAGGTGGATATGTTCTTAGTGGCT
ATAAGTAGATCTTCCTCTGGCTCTTCTCTTCTGCATGCCAGTCGGATTATTCCCCTCCGAAATTCCTTTTTGAC
CATAGATGTTATTCTTCCAAATCCCTAGAGGCAGGCATGGGTCAATATAGAAGAAACAACTCAAGCTAGAA
ACCGACAGTGATTCAATAACCTACCAGTGATTCAATAACCTACCAGGCATGAGTAAAATAGAAGAAACAAC
TCAAGCTAGAAGCCGACAGTATTCAATAACCTACCGGCTATCATCAGATTTCTACTAATTGGAAGTGGGTGC
TGA

Fig 4.18: Nucleotide sequences of the amplicons amplified using SCAR markers

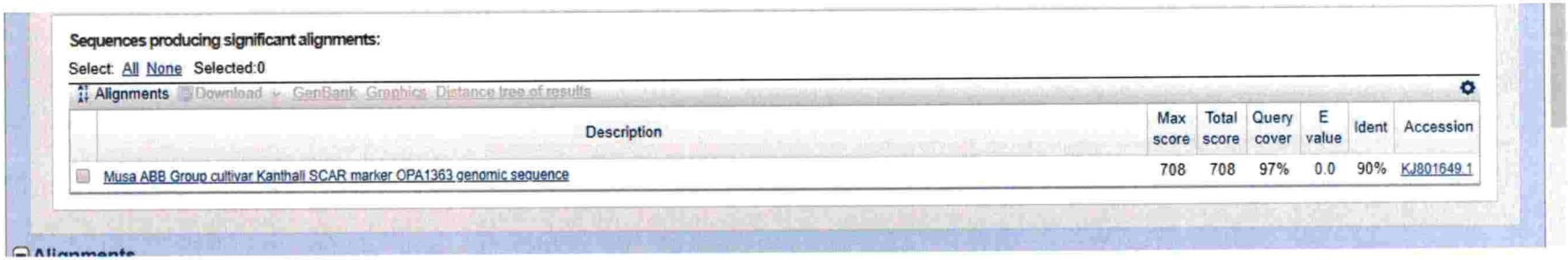


Fig 4.19: Blast result of sequence obtained by PCR amplification using SCAR markers

4.6.1. Per cent disease severity (PDS)

The statistical analysis of data on PDS at vegetative (six months after planting) and shooting stage revealed that all the treatments were significantly superior compared to the untreated control.

At vegetative phase, the PDS was recorded lowest (13.90%) in plants sprayed with T5 (trifloxystrobin (25%) + tebuconazole (50%), 0.4g/l) followed by foliar application of T3 (propiconazole, 1ml/l), T4 (hexaconazole (5%) + captan (75%), 2g/l) and T2 (copper hydroxide, 2g/l) with PDS of 15.55%, 16.04% and 16.12% respectively. While the PDS was highest in unsprayed control (31.33%) plants.

During shooting stage also, the least PDS (16.96%) was recorded in plants sprayed with T5 (trifloxystrobin (25%) + tebuconazole (50%), 0.4g/l). This was followed by foliar application of T2 (copper hydroxide, 2g/l), T6 (Bordeaux mixture, 1%) and T4 (hexaconazole (5%) + captan (70%), 2g/l) with PDS of 17.18%, 18.02% and 18.50%. While the highest PDS of 34.03% was recorded in the unsprayed control plants.

Comparing the mean values of PDS at vegetative and at shooting stage revealed that, the treatment T5 (trifloxystrobin (25%) + tebuconazole (50%), 0.4g/l) recorded the lowest PDS (15.43%) followed by T2 (copper hydroxide, 2g/l), T4 (hexaconazole (5%) + captan (70%), 2g/l), T6 (Bordeaux mixture,1%) with PDS of 16.65 %, 17.27% and 17.92% respectively (Table 4.25). The PDS was highest in unsprayed control (32.70%) plants (Fig 4.20).

When per cent disease reduction of disease over control was calculated the maximum per cent reduction of disease was recorded in T5 (trifloxystrobin (25%) + tebuconazole (50%),0.4g/l) (52.81%) followed by T2 (copper hydroxide, 2g/l), T4 (hexaconazole (5%) + captan (70%), 2g/l), T6 (Bordeaux mixture, 1%), T3 (propiconazole) and T1 (pyraclostrobin, 1g/l) with per cent disease reduction of

Table 4.25: Effect of chemical fungicides for the management of Sigatoka leaf spot disease (PDS)

Treatment details	*PDS at 6MAP**	*PDS at shooting	Mean PDS	Per cent disease reduction over control
T1: Pyraclostrobin	17.49 [b]	20.69 [bcd]	19.09	42.00
T2: Copper hydroxide	16.12[bc]	17.18 [e]	16.65	49.08
T3: Propiconazole	15.55[bc]	21.93 [b]	18.74	42.69
T4 : Hexaconazole + Captan	16.04[bc]	18.50 [cde]	17.27	47.18
T5 : Trifloxystrobin + Tebuconazole	13.90[c]	16.96 [e]	15.43	52.81
T6 : Bordeaux mixture	17.81[b]	18.02 [de]	17.92	45.19
T7 : Control	31.33[a]	34.03 [a]	32.70	-
CD (0.05)	**3.41**	**3.06**	**-**	**-**

*Per cent disease severity, ** Months after planting

49.08%, 47.18%, 45.19%, 42.69% and 42.00% respectively (Fig 4.21).

4.6.2.Disease development time (DDT)

Disease development time (DDT) is the parameter which indicates the time or number of days taken by the leaves to produce visible symptoms. Statistically significant differences were recorded in DDT among the treatments (Table 4.26). The maximum DDT (50.66 days) was recorded in plants sprayed with T5 (trifloxystrobin (25%) + tebuconazole (50%), 0.4g/l) followed by T2 (copper hydroxide, 2g/l) and T4 (hexaconazole (5%) + captan (70%), 2g/l) having DDT of 49.33 days for both. The lowest DDT (32.66 days) was recorded in unsprayed control plants (Fig 4.22).

4.6.3. Youngest leaf spotted (YLS)

Statistically significant difference in YLS was noticed among treatments both at vegetative (six months after planting) and shooting stage.

At vegetative stage, the highest YLS (8.91) was recorded in plants sprayed with trifloxystrobin (25%) + tebuconazole (50%) (T5) and was statistically on par with T3 (propiconazole) with YLS of 7.83. While the other treatments, T1 (pyraclostrobin), T2 (copper hydroxide), T4 (hexaconazole (5%) + captan (70%)), T6 (Bordeaux mixture) were found to be statistically on par with each other having YLS of 7.50, 7.50, 7.50 and 7.33 respectively. The lowest YLS (6.43) was recorded in T7 (unsprayed control).

At shooting stage also, the YLS was found to be highest (7.66) in plants sprayed with T5 (trifloxystrobin (25%) + tebuconazole (50%)) followed by T2 (copper hydroxide), T3 (propiconazole), T4 (hexaconazole (5%) + captan (70%)) and T6 (Bordeaux mixture) having YLS of 7.08, 7.08, 6.83 and 6.83 respectively. While lowest YLS (5.25) was recorded in unsprayed control plants (T7).

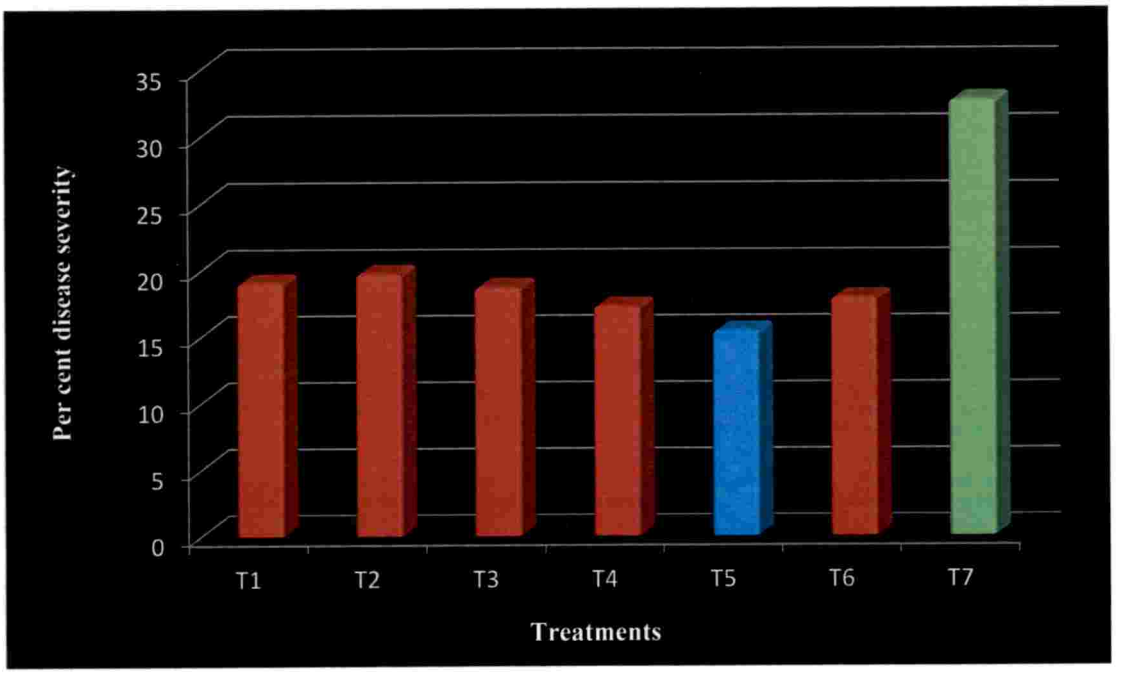


Fig 4.20: Effect of chemical fungicides on per cent disease severity (PDS)

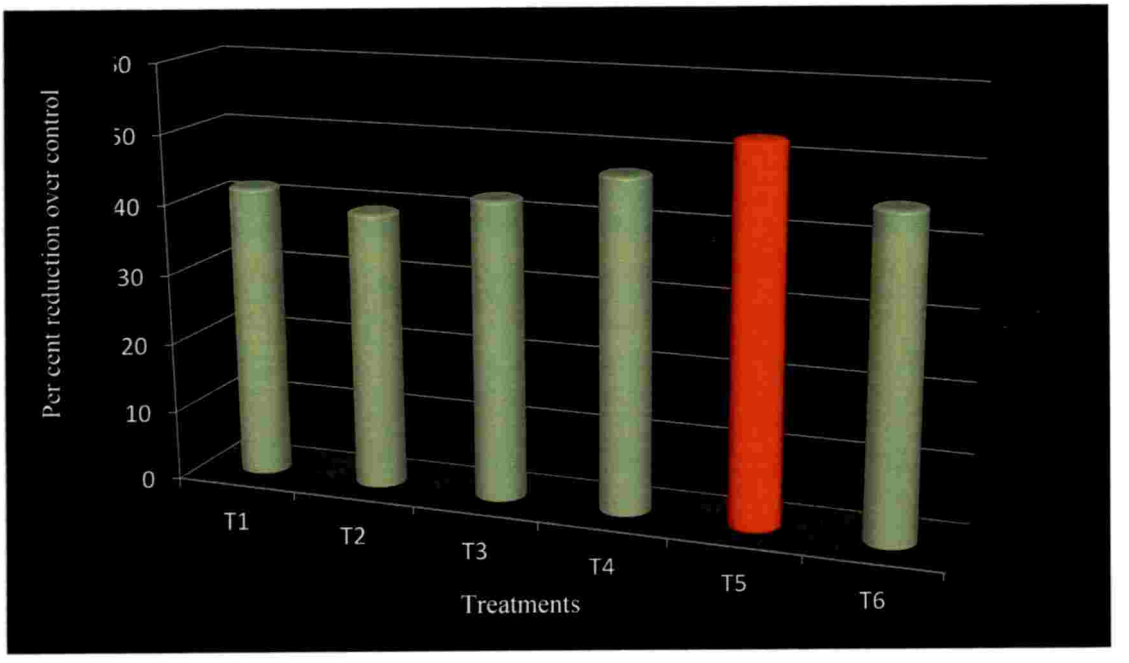


Fig 4.21: Per cent reduction of disease over control using chemical treatment

Comparing the mean values of YLS at vegetative and shooting stage revealed that, the highest YLS (8.00) was recorded in T5 (trifloxystrobin (25%) + tebuconazole (50%)) followed T3 (propiconazole), T2 (copper hydroxide) and T4 (hexaconazole (5%) + captan (70%)) with YLS of 7.45, 7.29 and 7.16 respectively. While the lowest YLS (5.18) was recorded in T7 (unsprayed control) (Table 4.26) (Fig 4.23).

4.6.4 Effect of chemicals fungicides on vegetative characters

The effect of chemical fungicides on vegetative characters plant height, girth and number of functional green leaves at the time of flowering was recorded and the results are presented in Table 4.27. Statistically no significant difference in plant height, girth and number of functional leaves was noticed among the treatments.

4.6.4.1 Plant height

The highest plant height (223.33cm) was recorded in plants sprayed with T3 (propiconazole) followed by T2 (copper hydroxide) and T6 (Bordeaux mixture) having plant height of 220.00 and 218.83 respectively. While the lowest plant height (199.16 cm) was recorded in plants sprayed with T5 (trifloxystrobin (25%) + tebuconazole (50%)).

4.6.4.2. Plant girth

The highest plant girth (57.66 cm) was recorded in plants sprayed with T3 (propiconazole) followed by T2 (copper hydroxide) having plant girth of 56.70 cm. While the lowest plant girth (52.41 cm) was recorded in T5 (trifloxystrobin (25%) + tebuconazole (50%)).

4.6.4.3.Number of green leaves

The maximum number of green leaves (7.00) was recorded in plants sprayed with T5 (trifloxystrobin (25%) + tebuconazole (5%)) followed by T3 (propiconazole),

Table 4.26: Effect of chemical fungicides on Youngest leaf spotted (YLS) and Disease development time (DDT)

Treatment details	*YLS at 6MAP**	YLS at shooting	Mean YLS	DDT***
T1: Pyraclostrobin	7.50 [b]	6.75 [b]	7.12	48.00 [b]
T2: Copper hydroxide	7.50 [b]	7.08 [ab]	7.29	49.33 [ab]
T3: Propiconazole	7.83 [a]	7.08 [ab]	7.45	47.43 [b]
T4 : Hexaconazole + Captan	7.50 [b]	6.83 [ab]	7.16	49.33 [ab]
T5 : Trifloxystrobin + Tebuconazole	8.91 [a]	7.66 [a]	8.00	50.66 [a]
T6 : Bordeaux mixture	7.33 [b]	6.83 [ab]	7.03	47.33 [b]
T7 : Control	6.43 [c]	5.25 [c]	5.18	32.66 [c]
CD(0.05)	**0.91**	**0.84**	-	**2.25**

*Youngest leaf spotted, ** Months after planting, ***Disease development time

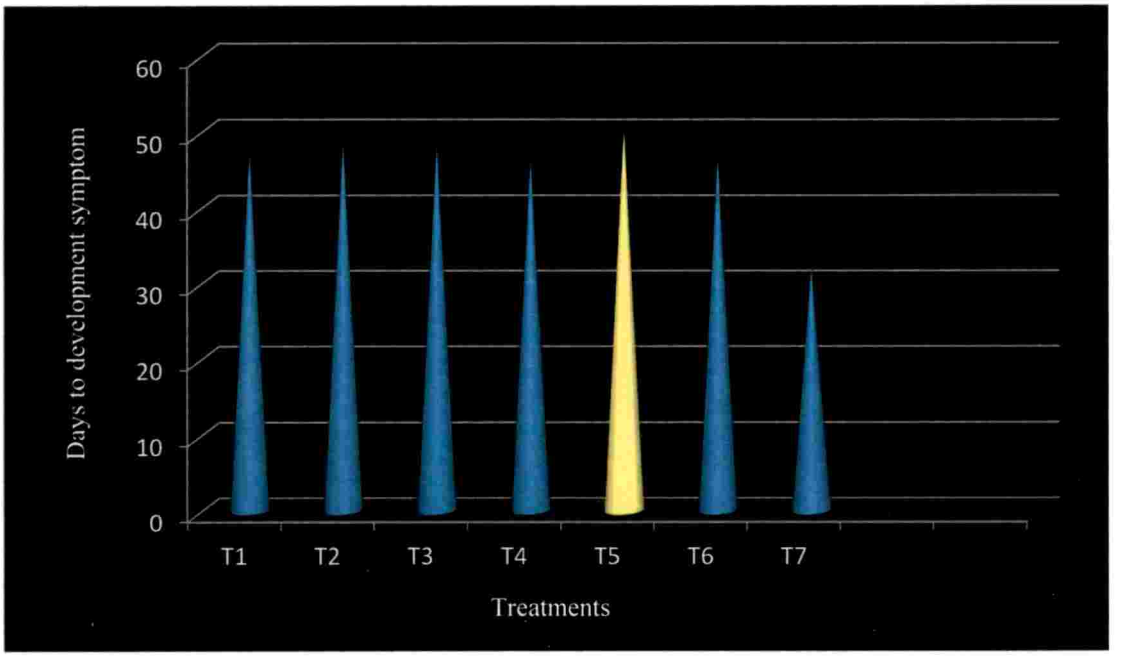


Fig 4.22: Effect of chemicals fungicides on disease development time (DDT)

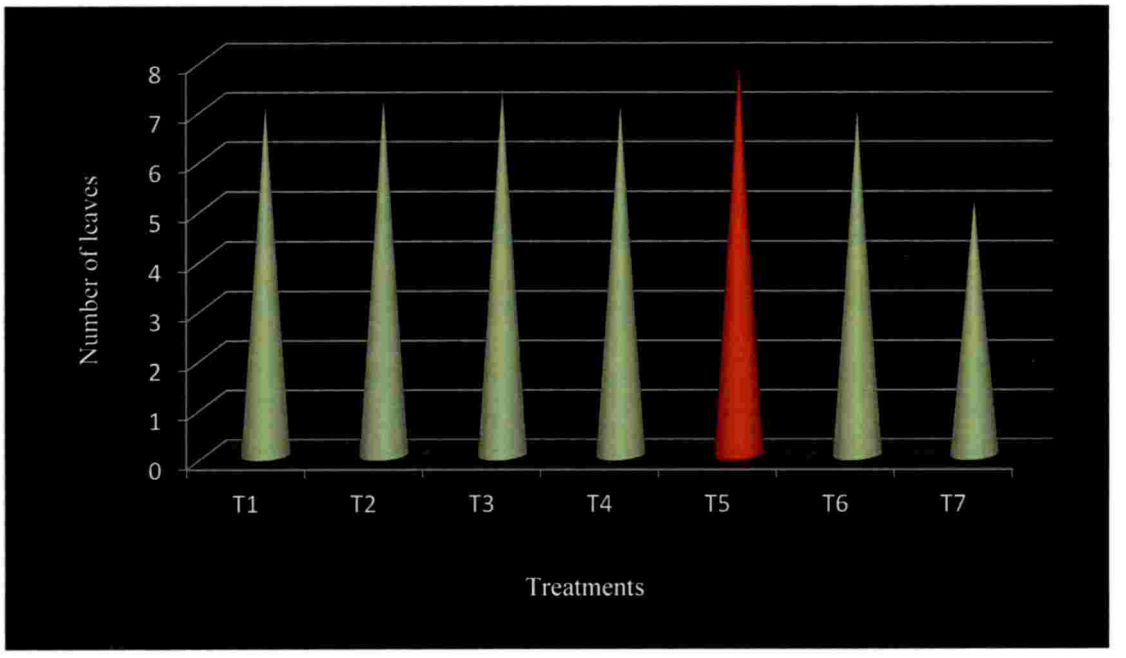


Fig 4.23: Effect of chemicals fungicides on youngest leaf spotted (YLS)

T2 (copper hydroxide), T1 (pyraclostrobin), T4(hexaconazole (5%) + captan (70%)), T6 (Bordeaux mixture) with 6.33, 6.00, 5.91, 5.83 and 5.83 number of green leaves respectively. While the minimum number of green leaves (5.41) was recorded in T7 (unsprayed control).

4.6.5. Effect of chemical fungicides on yield characters

The data on effect of chemical fungicides on yield characters is given in Table 4.28.

4.6.5.1. Bunch weight

The statistical analysis of data on bunch weight revealed that all the treatments were significantly superior to the untreated control. The yield in terms of the bunch weight was recorded highest in plants sprayed with copper hydroxide (T2) and Bordeaux mixture (T6) with bunch weight of 9.52kg. These treatments were found to be statistically on par with T4 (hexaconazole (5%) + captan (70%)) and T5 (trifloxystrobin (25%) + tebuconazole (50%)) which recorded bunch weight of 9.46kg and 9.49kg respectively. The lowest bunch weight (6.57) was recorded in control plants without any chemical treatments.

The per cent increase in yield over control was calculated. The results revealed that the maximum increase in yield (44.90%) was recorded in T2 (copper hydroxide) and T6 (Bordeaux mixture) which was followed by T5 (trifloxystrobin (25%) + tebuconazole (50%)), T4 (hexaconazole (5%) + captan (70%)), T3 (propiconaozle) and T1 (pyraclostrobin) with an yield increase of 44.44%, 43.98%, 26.09% and 16.89 % respectively.

4.6.5.2. Number of hands

Significantly no differences were recorded in number of hands among the treatments. The highest number of hands (6.58) was observed in plants sprayed with

Table 4.27: Effect of chemical fungicides on vegetative characters of banana var. Nendran

Treatment details	Plant height (cm)	Plant girth (cm)	No: of green leaves
T1: Pyraclostrobin	217.50	55.08	5.91
T2: Copper hydroxide	220.00	56.70	6.00
T3: Propiconazole	223.33	57.66	6.33
T4 : Hexaconazole + Captan	210.00	53.50	5.83
T5 : Trifloxystrobin + Tebuconazole	199.16	52.41	7.00
T6 : Bordeaux mixture	218.83	53.50	5.83
T7 : Control	216.00	55.21	5.41
CD (0.05)	**NS**	**NS**	**NS**

T4 (hexaconazole (5%) + captan (70%)) followed by T2 (copper hydroxide) with 6.50 hands. The lowest number of hands (5.75) was noticed in T7 (unsprayed control).

4.6.5.3. Number of fingers

No significant differences were recorded in number of fingers among the treatments. The total number of fingers was found to be the highest (66.08 no:s) in plants sprayed with T4 (hexaconazole (5%) + catan (70%)) followed by T2 (copper hydroxide) with 65.75 fingers. While the minimum number of fingers (63.04 no:s) was recorded in T7 (unsprayed control).

4.6.5.4. Length of fingers

No significant differences were recorded in length of fingers among the treatments. The maximum finger length (24.11cm) was recorded in plants sprayed with T3 (propiconazole) followed by T4 (hexaconazole (5%) + captan (70%)) with 24.01 cm. The lowest finger length (20.27cm) was recorded in T7 (unsprayed control).

4.6.5.5. Circumference of fingers

Significant differences were recorded in circumference of fingers among the treatments. The highest circumference of fingers (13.15 cm) was recorded in plants sprayed with T4 (hexaconazole (5%) + captan (70%)) followed by T2 (copper hydroxide) having circumference of 12.92 cm. The lowest circumference of fingers was noticed in T7 (unsprayed control) having circumference of 12.26 cm.

4.6.5.6. Fresh weight of fingers

Statistically significant difference in fresh weight of fingers was noticed among the treatments. The highest fresh weight of fingers were recorded T2 (copper hydroxide) having fresh weight of 152.23 g followed by Bordeaux mixture (148.07 g)

and T5 (trifloxystrobin (25%) + tebuconazole (50%)) with fresh weight of 147.32 g. The lowest fresh weight of fingers (120.11g) was observed in T7 (control plants).

4.6.5.7. Ripe weight of fingers

No significant differences in ripe weight of fingers was recorded among the treatments. The highest ripe weight of the fingers (135.73g) was observed in plants sprayed with T2 (copper hydroxide) followed by T3 (propiconazole) having ripe weight of 124.43g. While the lowest ripe weight of fingers (102.71g) was observed in T7 (control).

4.6.5.8. Peel to pulp ratio

Statistically significant differences were recorded in peel to pulp ratio among the treatments. The highest peel to pulp ratio (0.19) was noticed in control plants without any treatments followed by the plants sprayed with T1 (pyraclostrobin) (0.18). However, the lowest value (0.12) was observed in T2 (copper hydroxide) .

4.6.5.9. Total soluble solids (TSS)

The highest value for total soluble solids (TSS) was recorded in fingers taken from plants sprayed with copper hydroxide having 29.66^0brix. While the lowest value (26.66^0 brix) was recorded in plants without spraying. No significant differences were observed between the treatments.

4.6.5.10. Economic analysis

The benefit to cost ratio of different chemical treatments was calculated and presented in (Table 4.28). At the market price of Rs 40/kg, the highest B: C ratio was observed in T6 (Bordeaux mixture) with ratio of 1.75 which was followed by T5 (trifloxystrobin + tebuconazole) (1.71) while the lowest B: C ratio (1.35) was observed in T7 (control).

Table 4.28: Effect of chemical fungicides on yield characters of banana var. Nendran

Treatment details	Weight of bunch (kg)	No: of hands	No: of fingers	Per cent increase in yield over control	B: C ratio
T1: Pyraclostrobin	7.68 [bc]	5.91	59.91	16.89	1.32
T2: Copper hydroxide	9.52 [a]	6.50	65.75	44.90	1.50
T3: Propiconazole	8.89 [ab]	6.33	63.50	26.09	1.50
T4 : Hexaconazole + Captan	9.46 [a]	6.58	66.08	43.98	1.50
T5 : Trifloxystrobin + Tebuconazole	9.49 [a]	6.30	65.08	44.44	1.71
T6 : Bordeaux mixture	9.52 [a]	6.16	63.08	44.90	1.75
T7 : Control	6.57 [c]	5.75	55.25	-	
CD(0.05)	**1.49**	-	-	-	

Table 4.28 (contd....): Effect of chemical fungicides on yield characters of banana var. Nendran

Treatment details	Length of fingers (cm)	Circumference of fingers (cm)	Fresh weight of fingers (g)	Ripe weight of fingers (g)	Peel to pulp ratio	TSS (^{0} brix)
T1: Pyraclostrobin	23.02	12.27 ^{c}	130.55 ^{c}	117.48	0.18	28.66
T2: Copper hydroxide	23.46	12.92 ^{ab}	152.23 ^{a}	135.73	0.12	29.66
T3: Propiconazole	24.11	12.45 ^{bc}	140.12 ^{abc}	124.43	0.13	28.66
T4 : Hexaconazole + Captan	24.01	13.15 ^{a}	140.92 ^{abc}	124.01	0.17	27.66
T5 : Trifloxystrobin + Tebuconazole	23.05	12.52 ^{bc}	147.32 ^{ab}	114.13	0.16	28.33
T6 : Bordeaux mixture	23.01	12.61 ^{bc}	148.07 ^{ab}	123.13	0.15	27.66
T7 : Control	20.27	12.26 ^{c}	120.07 ^{d}	105.71	0.19	26.66
CD(0.05)	-	**0.515**	**15.29**	-	**22.73**	-

4.7.2. EXPERIMENT NO:2: MANAGEMENT OF SIGATOKA LEAF SPOT DISEASE USING BIOAGENTS / ORGANIC /INORGANIC PREPARATIONS

The field experiment on management of Sigatoka leaf spot disease using bioagents / organic / inorganic preparations was laid in the field at Banana Research Station, Kannara. The inference of the experiment was drawn by statistical analysis of the data on per cent disease severity (PDS), youngest leaf spotted (YLS), disease development time (DDT), vegetative and yield parameters

4.7.1. Per cent Disease Severity (PDS)

The statistical analysis of data on PDS revealed that all the treatments were significantly superior to the untreated control both at vegetative (six months after planting) and shooting stage (Table 4.29).

During vegetative phase, the lowest PDS (18.01%) was recorded in plants sprayed with T8 (Bordeaux mixture, 1%) which served as a chemical check. This treatment was found to be on par with T3 (*Pseudomonas fluorescens,* 2%), T5 (salicyclic acid, 25 ppm), T2 (PGPR mix II, 2%) and T7 (petroleum based mineral oil, 0.1%) having PDS of 18.59%, 19.17%, 19.82% and 20.04%. While the highest PDS (31.33%) was noticed in T9 (untreated control).

At shooting stage, the lowest PDS (18.75%) was observed in plants sprayed with T8 (Bordeaux mixture, 1%) which served as a chemical check. This treatment was on par with T2 (PGPR mix II, 2%), T1 (cowdung + *P.fluorescens,* 1%), T3 (*P.fluorescens*, 2%) and T4 (turmeric powder + baking soda, 5:1) with PDS of 22.21%, 23.19%, 24.86% and 25.34% respectively. While the highest PDS (34.03%) was recorded in T9 (untreated control). Though salicyclic acid (25 ppm) was effective in management of the disease at vegetative stage no effect was observed during the shooting stage.

Comparing the mean PDS at vegetative and shooting stage revealed that the

lowest PDS (20.84 %) was recorded in plants sprayed with T8 (Bordeaux mixture, 1%) which served as a chemical check. This was followed by T2 (PGPR mix II, 2%), T3 (*P. fluorescens,* 2%), T1 (cowdung extract + *P. fluorescens* (1%), T5 (salicylic acid, 25 ppm), T7 (petroleum based mineral oil, 0.1%), T4 (turmeric powder + baking soda mixture , 5:1), T6 (KAU micronutrient mix, 1%) with PDS of 20.93%, 21.72%, 22.74%, 22.76%, 23.44%, 23.79% and 24.53 % respectively (Table 4.29). The highest PDS (32.70%) was recorded in plants without any treatment (T9) (Fig 4.24).

The highest per cent disease reduction over control (36.26%) was noticed in plants sprayed with T8 (Bordeaux mixture, chemical check) followed by T2 (PGPR mix II), T3 (*P.fluorescens*), T1 (cowdung extract + *P. fluorescens*), T5 (salicyclic acid), T7 (petroleum based mineral oil) and T4 (turmeric powder + baking soda) having per cent disease reduction of 35.99%, 33.57%, 30.45%, 30.39%, 28.31% and 27.24 % respectively. While the lowest per cent reduction over control (24.98%) was noticed in plants sprayed with KAU micronutrient mix (1%) (Fig 4.25).

4.7.2. Disease development time (DDT)

Statistically significant differences were recorded in DDT among different treatments. The highest DDT (44.00 days) was recorded in plants sprayed with Bordeaux mixture (chemical check). This was followed by T4 (turmeric powder + baking soda mixture), T2 (PGPR mix II), T6 (KAU micronutrient mix), T3 (*P. fluorescens*), T5 (salicylic acid), T1(cowdung extract + *P. fluorescens*) and T7 (petroleum based mineral oil) having DDT of 43, 40, 39.66, 38.66, 38, 37.33, 35.33 days (Fig 4.26). The lowest DDT (32.66 days) was noticed in plants without any treatment (T9) (Table 4.30).

4.7.3. Youngest leaf spotted (YLS)

Statistically significant differences were recorded in YLS among treatments

Table 4.29: Effect of bioagents / organic/ inorganic treatments for management of Sigatoka leaf spot disease (PDS)

Treatment details	*PDS at 6 MAP**	PDS at shooting	Mean PDS	Per cent disease reduction over control
T1:Cowdung extract + *Pseudomonas fluorescens* (1%)	22.29 ^{b}	23.19 ^{de}	22.74	30.45
T2:PGPR mix II (2%)	19.82 ^{bcde}	22.21 ^{e}	20.93	35.99
T3:*P. fluorescens* (2%)	18.59 ^{be}	24.86 ^{bcde}	21.72	33.57
T4:Turmeric powder + baking soda mixture (5:1)	22.25 ^{bc}	25.34 ^{bcde}	23.79	27.24
T5: Salicylic acid (25 ppm)	19.17 ^{cde}	26.35 ^{bcd}	22.76	30.39
T6:KAU micronutrient multimix (Sampoorna) (1 %)	21.24 ^{bcd}	27.82 ^{b}	24.53	24.98
T7:Petroleum based mineral oil (0.1%) (POP)	20.04 ^{bcde}	26.83 ^{bc}	23.44	28.31
T8:Bordeaux mixture (1%) (Chemical check)	18.01 ^{e}	18.75 ^{cde}	**20.84**	**36.26**
T9:Untreated control	31.33 ^{a}	34.03 ^{a}	32.70	-
CD(0.05)	**3.10**	**3.53**	-	-

*Per cent disease severity, ** Months after planting

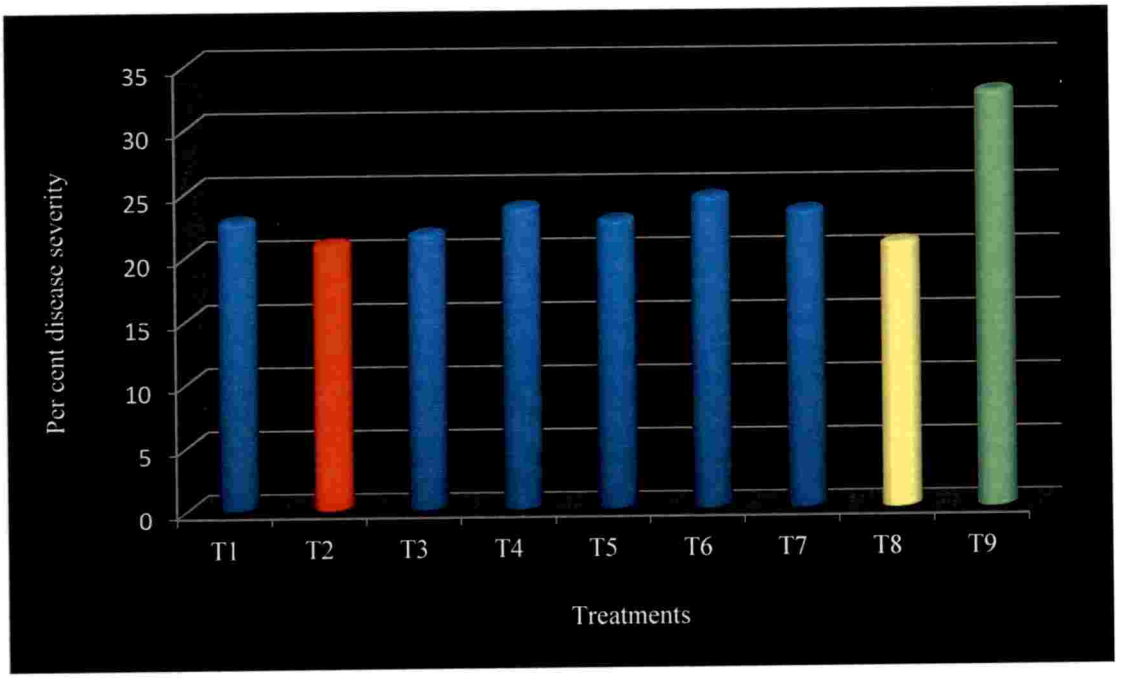


Fig 4.24: Effect of bioagents / organic / inorganic on per cent disease severity

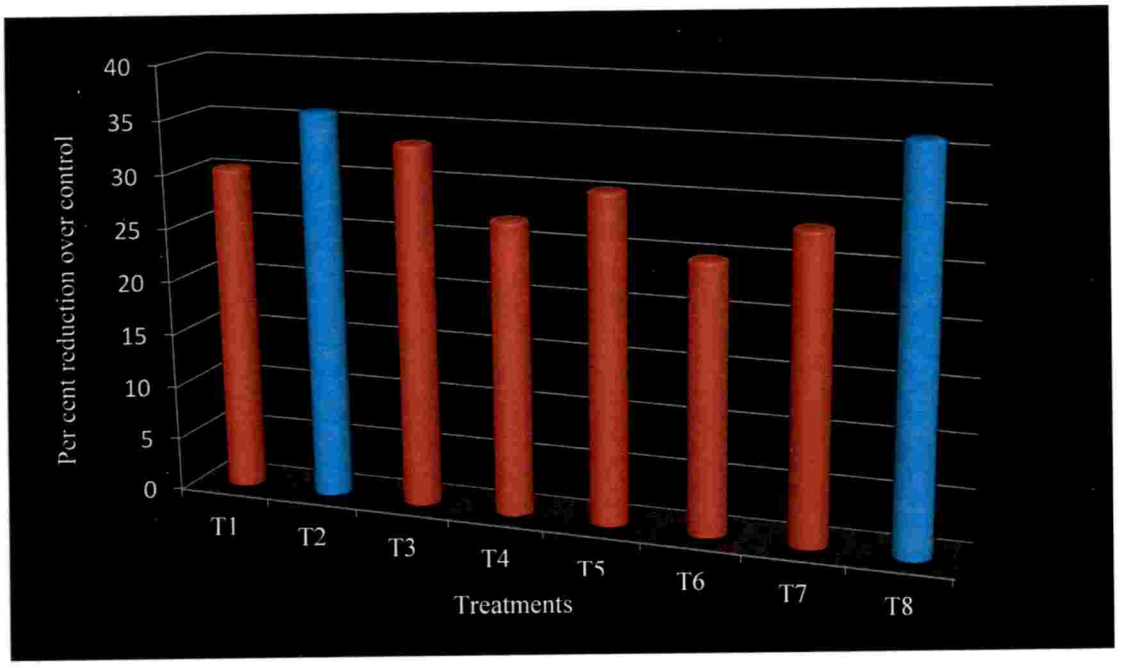


Fig 4.25 : Per cent reduction of disease over control using bioagents /organic treatments

both at vegetative (six months after planting) and shooting stage.

At vegetative stage, the highest YLS was recorded in plants sprayed with T3 (*P. fluorescens*) and T8 (Bordeaux mixture, chemical check) with YLS of 7.33. These treatments were followed by T7 (petroleum based mineral oil), T2 (PGPR mix II) and T5 (salicylic acid) with YLS of 7.16, 6.83 and 6.83 respectively. While the lowest YLS (6.38) was recorded in T9 (untreated control).

At shooting stage, the highest YLS was noticed in plants sprayed with T2 (PGPR mix II) followed by T8 (Bordeaux mixture, chemical check) and T3 (*P.fluorescens*) with YLS of 6.41 for both the treatments. While the lowest YLS (5.12) was observed in T9 (untreated control).

Comparing the mean value of YLS at vegetative and shooting stage revealed that the highest YLS (6.87) was recorded in T3 (*P. fluorescens,* 2%) as well as in T8 (Bordeaux mixture, chemical check). These treatments were followed by T2 (PGPR mix II, 2%), T7(petroleum based mineral oil, 0.1%), , T5 (salicylic acid, 25 ppm), T4 (turmeric powder + baking soda mixture, 5:1), T6 (KAU micronutrient mix, 1%) and T1 (cowdung extract + *P. fluorescens*, 1%) with YLS of 6.69, 6.67, 6.41, 6.37, 6.15 and 6.12 respectively. While the lowest YLS (5.16) was recorded in T9 (untreated control) (Fig 4.27) and (Table 4.30).

4.7. 4. Effect of bioagents/ organic/inorganic treatments on vegetative characters

The effect of bioagents/ organic / inorganic treatments on vegetative characters is given in Table 4.31.

4.7.4.1. Plant height

No significant differences were recorded in plant height among different treatments. The highest plant height (225.83 cm) was recorded in plants sprayed with T6 (KAU micronutrient mix) followed by T7 (petroleum based mineral oil)

Table 4.30: Effect of bioagents /organic / inorganic treatments on Youngest leaf spotted (YLS) and Disease development time (DDT)

Treatment details	*YLS at 6 MAP**	YLS at flowering	Mean YLS	DDT***
T1:Cowdung extract + *Pseudomonas fluorescens* (1%)	6.58 ^{b}	5.66 ^{cd}	6.12	37.33 ^{cd}
T2:PGPR mix II (2%)	6.83 ^{ab}	6.50 ^{a}	6.69	40.00 ^{abc}
T3:*P. fluorescens* (2%)	7.33 ^{a}	6.41 ^{ab}	6.87	38.66 ^{bcd}
T4:Turmeric powder + baking soda mixture (5:1)	6.41 ^{b}	6.33 ^{abc}	6.37	43.00 ^{ab}
T5: Salicylic acid (25 ppm)	6.83 ^{ab}	6.00 ^{abc}	6.41	38.00 ^{cd}
T6:KAU micronutrient multimix (Sampoorna) (1 %)	6.50 ^{b}	5.81 ^{bcd}	6.15	39.66 ^{abcd}
T7:Petroleum based mineral oil (0.1%) (POP)	7.16 ^{a}	6.25 ^{abc}	6.67	35.33 ^{de}
T8:Bordeaux mixture (1%) (Chemical check)	7.33 ^{a}	6.41 ^{ab}	6.87	44.00 ^{a}
T9:Untreated control	6.38 ^{c}	5.12 ^{d}	5.16	32.66 ^{e}
CD(0.05)	**0.57**	**0.67**	**-**	**4.52**

*Youngest leaf spotted, ** Months after planting, ***Disease development time

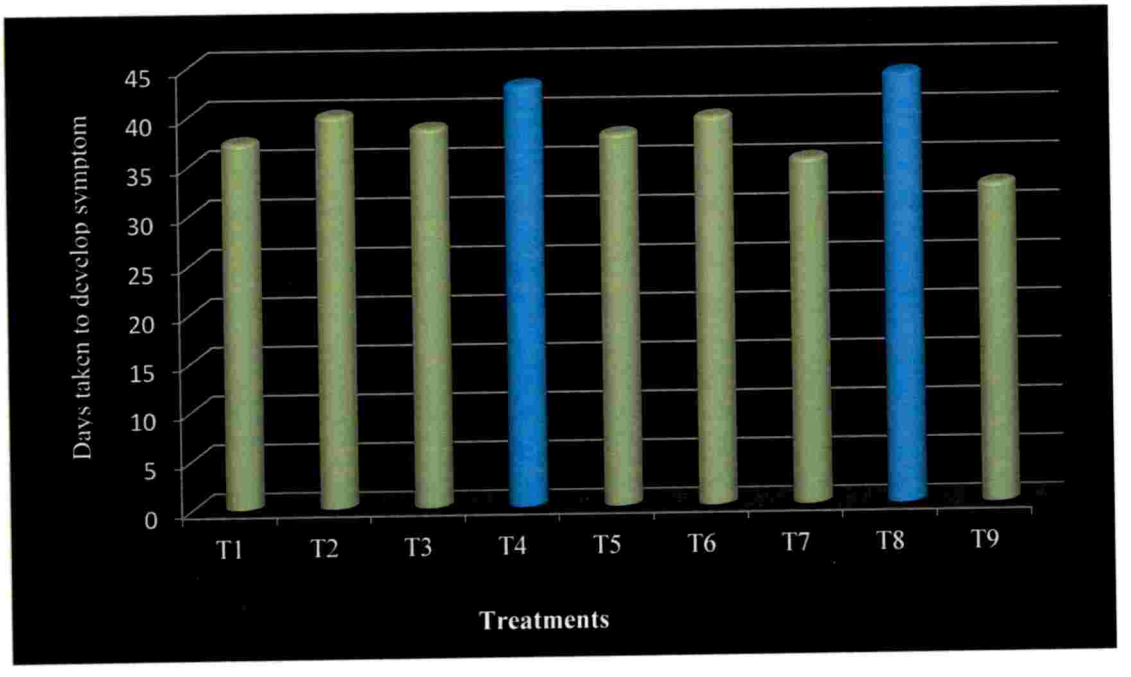


Fig 4.26: Effect of bioagents /organic preparations on disease development time

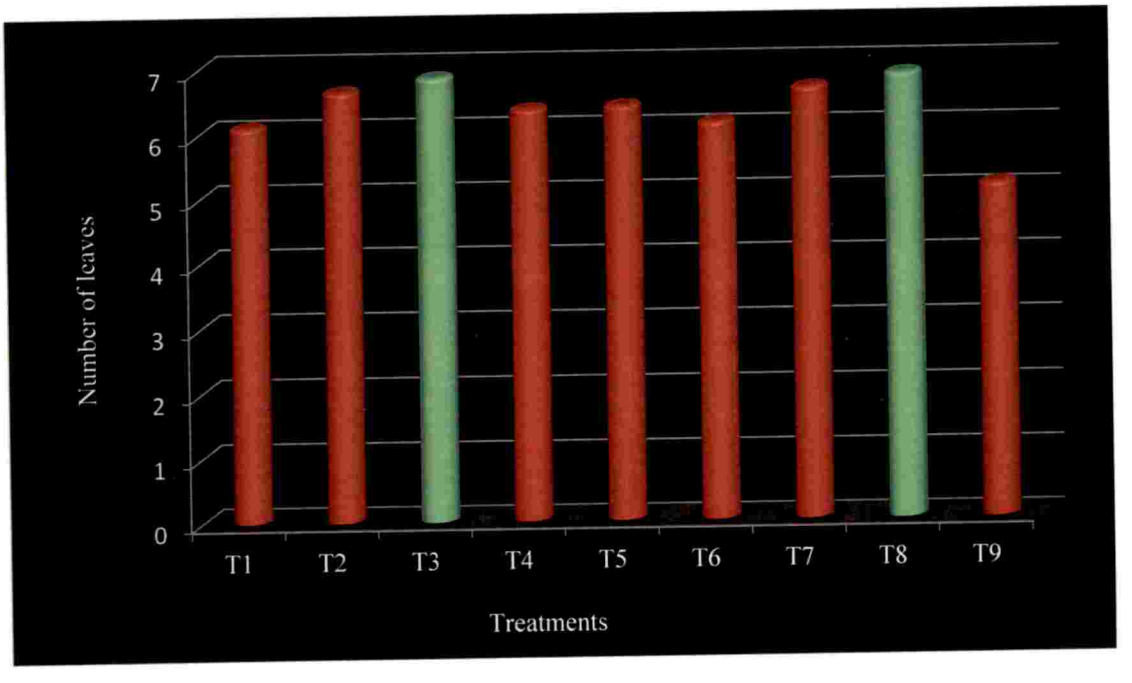


Fig 4.27: Effect of bioagents /organic preparations on youngest leaf spotted

(225.08 cm). While the lowest plant height was registered in T2 (PGPR mix II) with plant height of 158.16 cm.

4.7.4.2. Plant girth

Statistically significant differences were recorded in plant girth among different treatments. The highest plant girth (59.16 cm) was recorded in plants sprayed with T2 (PGPR mix II) followed by T7 (petroleum based mineral oil) and T1 (cowdung extract +*P. fluorescens*) and T6 (KAU micronutrient mix) having plant girth of 58.68 cm, 58.63 cm and 58.58 cm respectively. While the lowest plant girth (48.66cm) was recorded in plants sprayed with T4 (turmeric powder + baking soda mixture).

4.7.4.3. Number of green leaves

Statistically no significant differences were recorded in number of green leaves among different treatments. The highest number of green leaves (6.16) was recorded in plants sprayed with T5 (salicylic acid) followed by T3 (*P. fluorescens*) and T8 (Bordeaux mixture, chemical check) with 5.66 leaves in each treatment. The lowest number of green leaves (4.42) was noticed in T9 (untreated control).

4.7.5. Effect of bioagents / organic/inorganic preparations on yield characters

The effect of bioagents / organic/ inorganic treatments on yield characters are presented in Table 4.32. Statistically no significant difference was noticed in yield parameters except in circumference of fingers among the treatments.

4.7.5.1. Bunch weight

The highest bunch weight (8.82 kg) was recorded in plants sprayed with T8 (Bordeaux mixture, chemical check) followed by T3 (*P.fluorescens*) and T2 (PGPR mix II) having bunch weight of 8.47 kg and 7.93 kg respectively. The lowest bunch weight of 6.57 kg was noticed in T9 (untreated control).

Table 4.31: Effect of bioagents / organic / inorganic treatment on vegetative characters of banana var. Nendran

Treatment details	Plant height (cm)	Plant girth (cm)	No: of green leaves
T1:Cowdung extract + *Pseudomonas fluorescens* (1%)	213.33	58.63 [a]	4.91
T2:PGPR mix II (2%)	158.16	59.16 [a]	5.58
T3:*P. fluorescens* (2%)	195.00	50.58 [bc]	5.66
T4:Turmeric powder + baking soda mixture (5:1)	212.50	48.66 [c]	5.42
T5: Salicylic acid (25 ppm)	204.16	52.87 [bc]	6.16
T6:KAU micronutrient multimix (Sampoorna) (1 %)	225.83	58.58 [a]	5.50
T7:Petroleum based mineral oil (0.1%) (POP)	225.08	58.68 [a]	5.42
T8:Bordeaux mixture (1%) (Chemical check)	221.66	57.08 [ab]	5.66
T9:Untreated control	216.00	55.21 [ab]	4.42
CD (0.05)	**NS**	**1.32**	**NS**

The per cent increase yield over control was recorded highest (34.24%) was recorded in T8 (Bordeaux mixture, chemical check) followed by T3 (*P. fluorescens*) and T2 (PGPR mix II) having per cent yield increase of 28.91% and 20.70 % respectively.

4.7. 5.2. Number of hands

The highest number of hands (6.50) was recorded in plants sprayed with T3 (*P. fluorescens* ,2%), T2 (PGPR mix II, 2%) and T8 (Bordeaux mixture, chemical check) followed by T6 (KAU micronutrient mix (sampoorna). While the lowest number of hands (5.75) was recorded in T9 (untreated control).

4.7.5.3. Number of fingers

The highest number of fingers (65.83) was recorded in plants sprayed with T4 (turmeric powder + baking soda) and T8 (Bordeaux mixture) followed by T3 (*P. fluorescens*) and T6 (KAU micronutrient mix) with 63.66 and 63.58 number of fingers respectively. The lowest number of fingers (55.25) was recorded in T9 (untreated control).

4.7.5.4. Length of fingers

The highest length of fingers (24.88 cm) was noticed in plants sprayed with T5 (salicylic acid) which was followed by T3 (*P. fluorescens*) (24.32). While the lowest length of fingers (20.27 cm) was recorded in T9 (untreated control).

4.7.5.5.Circumference of fingers

Significantly highest circumference of fingers (13.11 cm) were recorded in plants sprayed with T3 (*P. fluorescens*) which was followed by T2 (PGPR mix II) 12.93 cm and T8 (Bordeaux mixture) having a circumference of 12.55. While the lowest width of fingers (11.92) was noticed in T6 (KAU micronutrient mix).

4.7.5.6. Fresh and ripe weight of fingers

The highest fresh (153.06 g) and ripe weight of fingers (136.03 g) was noticed in plants sparyed with T3 (*P. fluorescens*). While the lowest fresh weight (133.33 g) and ripe weight (115.17 g) of fingers were recorded in T9 (untreated plants).

4.7.5.7. Peel to pulp ratio

The highest peel to pulp ratio (0.19) was recorded in plants without any treatments (T9) which was followed by T5 (salicyclic acid) and T6 (KAU micronutrient mix) with a ratio of 0.18. The lowest (0.13) peel to pulp ratio was recorded in T3 (*P.fluorescens*.)

4.7.5.8. Total soluble solids (TSS)

The TSS of fruits obtained from all the treatments had TSS ranged from 26 - 29 ^{0} brix which was found to be in the normal range of TSS of banana var. Nendran. The highest TSS (29.33^0 brix) was recorded in T6 (KAU micronutrient mix (1%). While the lowest value (26^0 brix) was noticed in fingers of plants sprayed with PGPR mix II.

4.7.5.9. Economic analysis

The benefit to cost ratio of different bioagents/ organic / inorganic preparations were calculated and presented in (Table 4.33). At the market price of Rs 40/kg, the highest B: C ratio was observed in T8 (Bordeaux mixture) with a ratio of 1.60 while the lowest B: C ratio (1.14) was observed in T9 (control).

4.8. Residue analysis

The studies on persistence and degradation of the fungicides residues on banana fruit sprayed with trifloxystrobin (25%) + tebuconazole (50%), hexaconazole (5%) + captan (70%) and pyraclostrobin,revealed that no residues of the fungicides or

its metabolites were left in the banana fruits (Table 4.33). Therefore considering the efficiency and the safety of the chemicals on the crop indicated that the fungicide trifloxystrobin (25%) + tebuconazole (50%) could be effectively used for the management of the disease.

Table 4.32: Effect of bioagents / organic / inorganic treatments on yield characters of banana var. Nendran

Treatment details	Weight of bunch (kg)	No: of hands	No: of fingers	Per cent increase in yield over control	B: C ratio
T1:Cowdung extract + *Pseudomonas fluorescens* (1%)	7.37	5.91	60.33	12.17	1.21
T2:PGPR mix II (2%)	7.93	6.50	61.91	20.70	1.37
T3:*P. fluorescens* (2%)	8.47	6.50	63.66	28.91	1.37
T4:Turmeric powder + baking soda mixture (5:1)	7.62	5.75	65.83	15.98	1.35
T5: Salicylic acid (25 ppm)	7.86	6.08	57.58	19.63	1.32
T6:KAU micronutrient multimix (Sampoorna) (1 %)	7.67	6.16	63.58	16.74	1.37
T7:Petroleum based mineral oil (0.1%) (POP)	7.78	5.91	62.66	18.41	1.35
T8:Bordeaux mixture (1%) (Chemical check)	8.82	6.50	65.83	34.24	1.60
T9:Untreated control	6.57	5.75	55.25	-	1.14
CD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**

Table 4.32 (contd…) : Effect of bioagents / organic / inorganic treatments on yield characters of banana var. Nendran

Treatment details	Length of fingers (cm)	Circumference of fingers (cm)	Fresh weight of fingers (g)	Dry weight of fingers (g)	Peel to pulp ratio	TSS (^{0}brix)
T1:Cowdung extract + *Pseudomonas fluorescens* (1%)	22.96	12.33 ^{ab}	127.33	109.04	0.14	27.33
T2:PGPR mix II (2%)	23.51	12.93 ^{a}	133.46	110.17	0.17	26.00
T3:*P. fluorescens* (2%)	24.32	13.11 ^{a}	153.06	136.03	0.13	27.66
T4:Turmeric powder + baking soda mixture (5:1)	22.56	12.44 ^{ab}	125.01	89.67	0.14	27.00
T5: Salicylic acid (25 ppm)	24.88	12.44 ^{ab}	136.46	102.25	0.18	28.67
T6:KAU micronutrient multimix (Sampoorna) (1 %)	21.18	11.92 ^{c}	124.33	90.78	0.18	29.33
T7:Petroleum based mineral oil (0.1%) (POP)	23.48	12.43 ^{ab}	123.01	102.86	0.16	27.60
T8:Bordeaux mixture (1%) (Chemical check)	23.05	12.55 ^{ab}	140.75	129.10	0.12	28.00
T9:Untreated control	20.27	12.26 ^{c}	120.11	102.71	0.19	26.66
CD(0.05)	**NS**	**4.302**	**NS**	**NS**	**NS**	**NS**

Table 4.33: Report on the analysis of residues in the harvested fruits

Sl. No:	Sample	Identification code	Unique code	Residues (mg/kg)	LOQ* (mg/kg)
1.	Banana	Pyraclostrobin	RF 0091/617	Nil	0.05
2.	Banana	Hexaconazole	RF 0092/6/17	Nil	0.05
3.	Banana	Trifloxystrobin + Tebuconazole	RF 0094/6/17	Nil	0.05
4.	Banana	Hexaconazole + Captan	RF 0096/6/17	Nil	0.05

*Limit of Quantification

Discussion

DISCUSSION

Banana (*Musa* spp.) is one of the most popular fruit crop which is being commercially grown in tropical and subtropical countries. The state of Kerala is endowed with warm humid tropical climate and is the hub of biodiversity of edible banana. However, the commercial cultivation of banana in the state is challenged by the incidence of pests and diseases. Among the various fungal pathogens, associated with foliage disease of banana, Sigatoka leaf spot disease is considered to be one of the most important disease complex limiting banana and plantain production thereby causing a serious socio - economic impact to banana cultivation at global level.

Zimmermann (1902) reported the incidence of yellow Sigatoka leaf spot which is commonly called as Sigatoka leaf spot caused by *M. musicola* as the first foliage disease on banana having a global impact. Later, Leach (1941) reported the incidence of more virulent form of Sigatoka *i.e.,* black Sigatoka caused by *M. fijiensis,* which spreads at a rapid rate to new banana growing areas, thus replacing *M. musicola.* A survey was conducted during 1992 and 1995 to determine the world wide distribution of yellow Sigatoka and black Sigatoka in South and Southeast Asia, revealed the presence of a new leaf spot which were similar to the other two Sigatoka leaf spots (Carlier *et al.,* 1996). The pathogen associated with this new leaf spot is described as *M. eumusae* with *Septoria* as its anamorph (Carlier *et al.,* 2000). Now globally three related ascomycetous fungi *viz., M. fijiensis, M. musicola* and *M. eumusae* were reported to be associated with the Sigatoka leaf spot disease complex such as black Sigatoka, yellow Sigatoka and Eumusae leaf spot (Arzanlou *et al.,* 2007) in banana. Although wide spread incidence of yellow and very scattered incidence of black Sigatoka leaf spot disease has been previously reported from the state of Kerala, not much work has been done on the characterization of different species of the pathogen actually associated with Sigatoka leaf spot disease complex. This might be due to the difficulty in culturing the pathogen in artificial media. Considering the rapid rate at which black Sigatoka is spreading (Jones, 2000) and possibility of incidence of Eumusae leaf spot in

South India, a reappraisal of the etiology of Sigatoka disease complex occurring on banana in Kerala is necessary. With this view, the study entitled "Characterization of *Mycosphaerella* spp. causing Sigatoka leaf spot disease complex of banana in Kerala and its management" was undertaken in the Department of Plant Pathology at College of Horticulture, Vellanikkara during 2015 -2018.

Purposive sampling surveys were conducted in different agroclimatic zones *viz.,* Wayanad, Malappuram, Palakkad, Thrissur, Ernakulam and Thiruvanathapuram during two seasons *i.e.,* pre monsoon and post monsoon to assess disease severity, the youngest leaf spotted and the variations in symptom expression between different varieties in different locations. The results of the survey revealed that the incidence, severity, youngest leaf spotted and the variation in symptoms of the disease varied with seasons and cultivars.

The incidence of the disease was observed in all field throughout the year which might be due to the presence of conidia and ascopsores in the leaf lesions, which served as the source of inoculums of the pathogen. Similar findings were reported by Jacome and Schuh (1992). The severity of the disease was recorded maximum and the youngest leaf spotted was minimum during the post monsoon survey compared to the pre monsoon survey. This might be because the presence of moisture on leaf surface during the rainy season favored the germination and release of spores thereby increasing the severity of the disease. These findings were in tune with the reports of Jacome and Schuh (1992). Therefore, the impact of spores as a source of inoculum was observed during the rainy season than in the summer season which in turn increased disease severity while spores discharge and infection were less during the summer season due to the absence of moisture.

The correlation analysis of disease severity with weather parameters revealed that disease severity is positively correlated with rainfall whereas with temperature disease severity was negatively correlated. Therefore, low temperature and high rainfall are favorable for the germination of spore and fast spread of lesions thereby causing maximum severity of the disease during rainy

season than in the summer. These observations were in line with the reports of Mourichon *et al.* (1990) and Torres *et al.* (2000) who concluded that high temperature and low relative humidity were found to be unfavourable for the spore germination and development.

The studies on symptomatology revealed that there existed variations in symptom expression among different varieties grown in different locations. There were six types of symptoms noticed on banana var. Nendran (AAB), two types each on Palayankodan (AAB) and Njalipoovan (AB) cultivar, while only one type of symptom was recorded on Robusta (AAA) and Kadali (AA) cultivar.

On banana var. Nendran, type I symptom appeared as elongated oval spots showing pointed tips and appeared adjacent to the veins. In type II symptom the spots were elliptical with definite dark brown margin with greyish centre, this type of symptoms were similar to the symptoms reported by Carlier *et al.* (2000) for Eumusae leaf spot disease. In type III symptoms, chain of spots appeared along the veins of the leaves from the margin towards the midrib which later coalesced, causing complete drying of the leaves. The above observations were in tune with the reports of Selvarajan *et al.* (2001) on banana var. Nendran. Type IV symptoms were much similar to type II symptom but the mature spots had greyish centre surrounded by irregular dark brown border. Similar symptoms were reported by Selvarajan *et al.* (2001) on banana var. Rasthali. The mature lesions appeared streak like rather than spots in type V symptoms. Type VI symptoms were noticed during the rainy season and appeared similar to type III symptoms but were more darker in colour than type III symptoms which might be due to excessive sporulation of the pathogen during rainy season.

On banana var. Palayankodan type I symptom was noticed during pre monsoon survey where the mature spots were irregularly oval in shape without any definite margins whereas the type II symptom were observed during post monsoon survey. In banana var. Njalipoovan the type I symptom were observed during pre monsoon survey which appeared as elongated linear spots with

blend tips with prominent yellow halo whereas the type II symptom were observed during post monsoon survey and appeared as yellowish orange streaks which later changed into water soaked elliptical spots which coalesced before maturity causing complete necrosis of the leaves. In banana var. Robusta the mature spots were black in colour with slightly depressed on the leaves with greyish centre. In banana var. Kadali the lesions were appeared as black streaks rather than spots having a greyish centre. Therefore from the present study, it was observed that the symptoms of Sigatoka leaf spot disease differed with cultivar, locations and the season of survey. These findings were in line with the reports of Selvarajan *et al.* (2001).

The developmental stages of Sigatoka leaf spot disease were categorized into six stages. The symptoms initially appeared as small light green to yellow spots on the lower surface of the leaf which was visible only when the leaves were held against sunlight. This later changed into faint brown visible streaks. During the next stage, these streaks turned rusty brown on the adaxial surface of the leaves, which later developed into oval or elliptical brown spots with greyish centre surrounded by definite dark brown border with black pin head like fruiting bodies embedded in it. Upon heavy infection, the spots coalesced leading to complete necrosis of the leaves thereby destroying the functional green tissues of the leaves. These observations were in accordance with the reports of Leach (1946) who categorized the developmental stages of Sigatoka leaf spot disease into six stages. Crous and Mourichon (2002), Selvarajan *et al.* (2001) and Amani and Avangyan (2014) also reported six stages during the symptom development of the disease.

The present investigation clearly indicated that the size of the lesions were small and the days required for symptom development (stage 1 – 6) was more during the summer season than rainy season in the three commercial varieties *viz.,* Nendran, Njalipoovan and Grand Naine. During summer, the average days required for the development of disease ranged from 74. 27 days to 79.02 days while it took only 31.48 – 35.78 days for symptom development in all the three cultivars during rainy season which clearly indicated the influence of weather parameters on incidence and severity of the disease. These observations were in accordance with

the reports of Thammaiah (2003) who reported that the number of days taken for disease development varied with seasons and disease development time ranged from 14 - 50 days depending on the season of observations.

The pathogen was isolated on PDA using leaf bit isolation technique. The fungal growth appeared after 10 days of incubation at 24°C. Similar observations were reported by Meredith and Lawrence (1970) while isolating the pathogen associated with Sigatoka leaf spot disease. The fungal colonies appeared were small, raised and had greyish velvety in appearance. These observations were in tune with the reports of Meredith and Lawrence (1970), Jones (2000) and Crous *et al.* (2003).

The studies on the morphological characters of the pathogen revealed that both anamorphic and telomorphic structures were found to be closely associated with different stages of the lesions (Jones, 2000). The hyphae of the pathogen were brown coloured with septations. The phenomenon of anastomosis or fusion of the hyphae were noticed which might be due to the heterothallic nature of the fungi. The presence of anastomosis or fusion of hyphae is considered as one of the key identifying character observed in pathogen belonging to *Pseudocercospora*. Similar findings were reported by Meredith and Lawrence (1969). The sexual fruiting body *i.e.,* perithecia were dark brown coloured flask shaped structures which is 30 - 51.12 µm wide bearing asci containing eight ascospores. The ascospores were 2-3 septated measured 12.0 to 14.5 × 2.23 to 4.8 µm in dimension and were twined around each other. The conidiophores were formed from the dense stroma. The conidiophores were pale brown in colour, straight having 2 – 5 septation arising from a conidigenous cells terminating in truncate ends.

The conidia were hyaline to olivaceous brown in colour, straight to curved with a round base having septations without any visible scars at the ends having dimension of 24.29 - 71.89 µm x 0.91 - 2.40 µm with 3 - 12 sepations. The presence of anastomosis, septate conidiphore emerging from conidiogenous cell and hyaline, septate conidia without any scar helps in identifying *Pseudocercospora eumusae*

from the other two species *i.e., P. musicola* and *P. fijiensis.* These findings were in tune with the reports of Carlier *et al.* (2000). The size and the shape of the conidia and the perithecia were in tune with the descriptions given by Carlier *et al.* (2000) and Crous *et al.* (2002) for the morphological identification of the *Mycosphaerella eumusae.* Hence, based on the cultural and morphological characters the anamorphic stage of the pathogen is identified as *Mycosphaerella eumusae* with *Pseudocercospora eumusae* as its anamorph.

The molecular characterization of the isolates were carried out by the PCR amplification of ITS - rDNA region of the fungal DNA isolated from infected leaf samples using ITS 1 and ITS 4 primers. The PCR amplification yielded PCR product of size ranging between 540-580bp. The ITS region of the 50 isolates collected during the survey was sequenced and analyzed using BLASTn algorithm in the NCBI GeneBank. The results of the BLASTn analysis revealed that all the 50 isolates showed 99-100% sequence homology to *M. eumusae.* Hence, the present study clearly indicated that the pathogen associated with Sigatoka leaf spot disease in Kerala is *M. eumusae.* This findings were in line with the reports of Carlier *et al.* (2000) and Devi and Thangavelu (2014) who reported that the amplification of ITS region helped to distinguish between the three species of *Mycosphaerella i.e., M .musicola, M. fijiensis and M. eumusae.* The comparison of the sequences of ITS region of the local isolates collected from different district of Kerala with *M. fijiensis, M. musicola* and *M. eumusae* was carried out by aligning the sequence in Clustal omega. The analysis revealed that the isolates of *M. eumusae* and the local isolates differentiated from *M. fijiensis* by 10 base pairs (a gap of 1 bp in 75th position and 9bp from 81- 90 positions) while *M. eumusae* and local isolates differed from *M. musicola* by 4 base pairs in 460 - 463th positions. These results were similar to the reports of Surridge *et al.* (2003). The phylogenetic analysis of the local isolates with three species of *Mycosphaerella* indicated that the local isolates collected during the survey formed a subcluster with *M. eumusae* indicating that the isolates were more closely related to *M. eumusae.* All the three species along with the 50 isolates formed a monophyletic group indicated that the major

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leaf spot pathogens of banana might have been derived from a common ancestor (Devi and Thangavelu, 2014). Therefore the molecular characterization of the ITS region of the isolates revealed that the pathogen inciting Sigatoka leaf spot in Kerala was identified and confirmed as *Mycosphaerella eumusae* Crous & Mourichon (anamorph *Pseudocercospora eumusae* Crous & Mourichon).

The host plant resistance was evaluated by screening the banana accessions belonging to different genotypes such as AA, AAA, AB, AAB, ABB, AAAA and AAAB to assess their disease severity and were categorized into six groups based on their disease reaction. The results revealed that the banana accessions with genotype AAA were more susceptible to Sigatoka leaf spot disease while the varieties which were resistant or moderately resistant to Sigatoka leaf spot disease belonged to AB, AAB, ABB and AAAB group. The resistance of the cultivar with genotype AB, AAB, ABB and AAAB might be due to the presence of 'B' genome content, which contributed to high resistance of the varieties to Sigatoka leaf spot disease. These findings were in line with the reports of Meredith (1970).

The anatomical studies conducted in selected resistant (Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23) and susceptible (Nendran, Robusta, Moris, Grand Naine and Kadali) accessions of banana indicated that, the resistant accessions were characterized by higher cuticle and epidermal thickness both in the upper and the lower surface of the leaf. While the susceptible accessions exhibited lower cuticle and epidermal thickness on both surface. Therefore from the present investigations it was clear that increased epidermal cell layer thickness and cuticular thickness occur in resistant accessions might have acted as first line of defense to prevent invasion of pathogen. These observations were in line with the reports of Mayee and Apet (1995) and Sunkad and Kulkarni (2006) who reported that, genotypes of groundnut resistant to leaf rust had thicker epidermis cum cuticle. Thus, thicker epidermis cum cuticle acts as the physical barrier for the entry of the pathogen thereby imparting resistance to genotypes. It was also noticed during the study that, the infected leaves showed an increase in thickness of cuticle and the epidermis in resistant accessions while no variations in thickness of the cuticle and epidermis

were recorded in susceptible accessions on the upper surface. While on the lower surface of infected leaves, an increase in cuticle and epidermal thickness was noticed in resistant accessions but a decrease in thickness of epidermis and cuticle was observed in susceptible accessions. The increase in thickness of the cuticle and epidermis in resistant accessions might acts as a barrier to entry of the pathogen while decrease in thickness in susceptible accessions might be due to the destruction of the layers by the pathogen. Similar types of responses were recorded by Kim *et al.* (2007) in chilli pepper fruit when infected by *Colletotrichum gloeosporioides.*

The thickness of the mesophyll tissues *i.e.,* palisade and spongy tissues were found to be more in resistant accessions when compared with susceptible accessions both in the healthy as well as in infected leaves. In the present study it was also noticed that in infected leaves, the thickness of the mesophyll tissues increased in resistant accessions which might be due to the extensive deposition of callus like material around the mesophyll tissue. While the thickness of the mesophyll tissue decreased in susceptible varieties which might be due to the disruption of mesophyll tissues during the inavasion of the pathogen. Similar observations were reported by Lazorovits and Higins (1976) in tomato during the penetration of *Claosporium fulvum* and Silva *et al.* (2006) in coffee cultivars affected with leaf rust and coffee berry disease.

The analysis on number of stomata and stomatal pore width concluded that the resistant accessions had lesser number of stomata and less stomatal pore width when compared with the susceptible accessions. However, it was observed that the number of stomata was comparatively more on the abaxial surface than that of the adaxial surface in all the varieties, which acted as main avenue for the entry of the pathogen into leaf tissue. The observations on stomatal pore width revealed that the size of stomatal pore was less in resistant accessions when compared with susceptible accessions. It was also noted that, the highest stomatal pore width was recorded on the lower surface than on the upper surface of the leaves. Therefore the more number of stomata and highest stomatal pore width in susceptible varieties might have provided higher opportunity for penetration by pathogens and resulted

in high disease severity than resistant ones. These results are in agreement with the reports of different workers (Mayee and Apet, 1995, Benagi, 1995 and Sunkad and Kulkarni, 2006).

Since the entry of the pathogen inciting Sigatoka leaf spot disesase is mainly through the lower surface, the presence of thicker cuticle and epidermis, low stomatal density and small stomatal pore width on the lower surface in the resistant accessions might be responsible for the restricted entry of the pathogen in the resistant accessions. These results were in accordance with the reports of Banu (2001).

Biochemical basis of resistance in plants is considered to be an important aspect in host plant resistance as it prevented the invasion of pathogen into the plants. The biochemical basis of resistance is characterized by the presence or absence of a particular chemical substance or a group of substances in the host plants which affects the growth and multiplication of the pathogen inside the host. Through the production and reactions of the biochemical compounds the host inactivates the pathogen or its toxins or kills the pathogen before infection. Therefore it is very important to understand the role of biochemical compounds in host defense mechanism. In the present investigation, the role of phenols, reducing and non reducing sugars, the activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in host resistance were studied.

The role of phenols in imparting resistance in host plants against the invasion of the pathogen was reported by many scientists. Phenols act as an antifungal, antibacterial and antiviral compounds which inhibit mycelial growth (Tourneau, 1966) fungal spore germination and fungal enzymes (Vidhyasekaran, 1975) and production of toxins by pathogens (Tamari and Kaji, 1966). The first line of defense in plant against the pathogens was by the rapid synthesis of phenolics and their polymerization in the plant cell wall (Matern and Kneusel, 1988).

The results of the present study indicated that the presence of an increased level of phenolic content in the resistant accessions when compared with the susceptible accessions. Such increase in phenolic content were reported by Jayapal and Mahadevan (1968) in the resistant banana variety Kattuvazhi than that of the susceptible banana variety Monthan during the attack by *Cercospora musae.* The phenolic substances formed in the plants played an important role in plants by acting as protective compounds against the attack of fungi, bacteria and viruses (Howell *et al.,* 1976). Their study also revealed that the resistant varieties contained more phenolics than that of the susceptible reactions to defend itself against the pathogen. Similar observations were noted by Dagade (2003) and Singh *et al.* (2006) in resistant *Piper columbrinum* and *Brassica* sp.

The present study also revealed that the phenolic content of all selected accessions showed a reduction in the phenolic content when infected with the disease, which might be due to the reduction of the phenols into antimicrobial compounds. Higher reduction in phenolic content was noticed in resistant accession while a slight reduction in the phenolic content was observed in the susceptible accessions. These findings were in tune with the reports of Chattopadhyay and Bera (1980) and Takahama (2004), they noticed the conversion of phenols to quinones in resistant rice cultivar CH13 when infected by *Helminosporium oryzae* and in onions. Beckman (2000) observed that the cells storing phenols undergo decompartmentation causing the rapid oxidation of their phenolic content thereby ensuring lignification and suberization of cells and cell death which seal off infections or injuries at the site. Therefore in the present study it was concluded that the resistant variety showed an increase in levels of phenolic content than that of the susceptible variety while a general reduction in phenolic content were observed in the resistant and susceptible cultivars during infection (Fig 5.1). These findings were in line with the reports of Banu (2001).

In healthy leaves, the quantity of reducing sugars was observed to be more in resistant accessions when compared with the susceptible accessions (Fig 5.2). This might be because sugars present in the plants plays an important role in

resistance by enhancing oxidative burst at early stages of infection, increasing lignification of cell walls, stimulating the synthesis of flavonoids and by inducing certain PR proteins. Some sugars also act as priming agents inducing higher plant resistance to pathogens. Therefore the present study concluded that the presence of high levels of sugars in plants during plant fungal interaction enhances plant resistance against the pathogen. This result are in accordance with the reports of Jayapal and Mahadevan (1968) who reported the presence of higher levels of reducing sugars in the resistant banana leaves of Kattuvazhi than that of the susceptible cultivars against *C. musae*. The reports by Abraham (1986), Paul (1998), Mahajan (1999), Bera *et al.* (1999) and Morkunas and Ratajczak (2014) revealed the presence of higher levels of sugars in the resistant cultivars acted as the mechanism of host defense against the entry of the pathogen.

The infected leaves exhibited a reduction in reducing sugar content both in the resistant and in the susceptible accessions (Fig 5.2). This might be because in higher plants the pathogen uses the sugars present in plant for their own needs which in turn forced the plant to modify their sugar content into antimicrobial compounds there by offering resistance against the pathogen (Tauzin and Giardin, 2014). Similar observations were reported in banana leaves of resistant and susceptible cultivars by Jayapal and Mahadevan (1968) and Banu (2001) during the infection by *C. musae* and Veermohan *et al.,* (1994) in capsicum leaves infected with *Alternaria solani.*

Not much variations were observed in the amount of non reducing sugar content on healthy leaves between the resistant and susceptible accessions. During infection an increase in the amount of non reducing sugar content were noticed in the resistant accessions while a reduction in non reducing sugar content was in susceptible accessions except for Kadali and Robusta were a slight increase in non reducing sugar content were noticed (Fig 5.3). Jayapal and Mahadevan (1968) and Veermohan *et al.* (1994) also reported an increase in non reducing sugar content in resistant cultivars during the infection by *C. musae* and *A. solani* in banana and chilli leaves respectively.

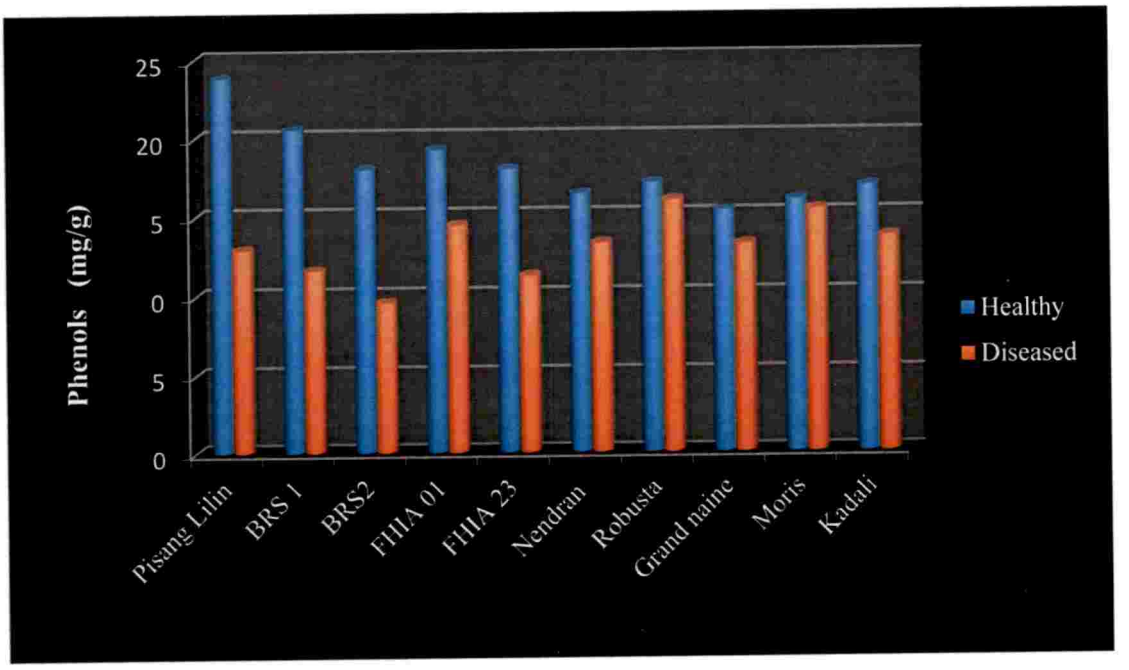

Fig 5.1: Phenolic content on resistant and susceptible accessions

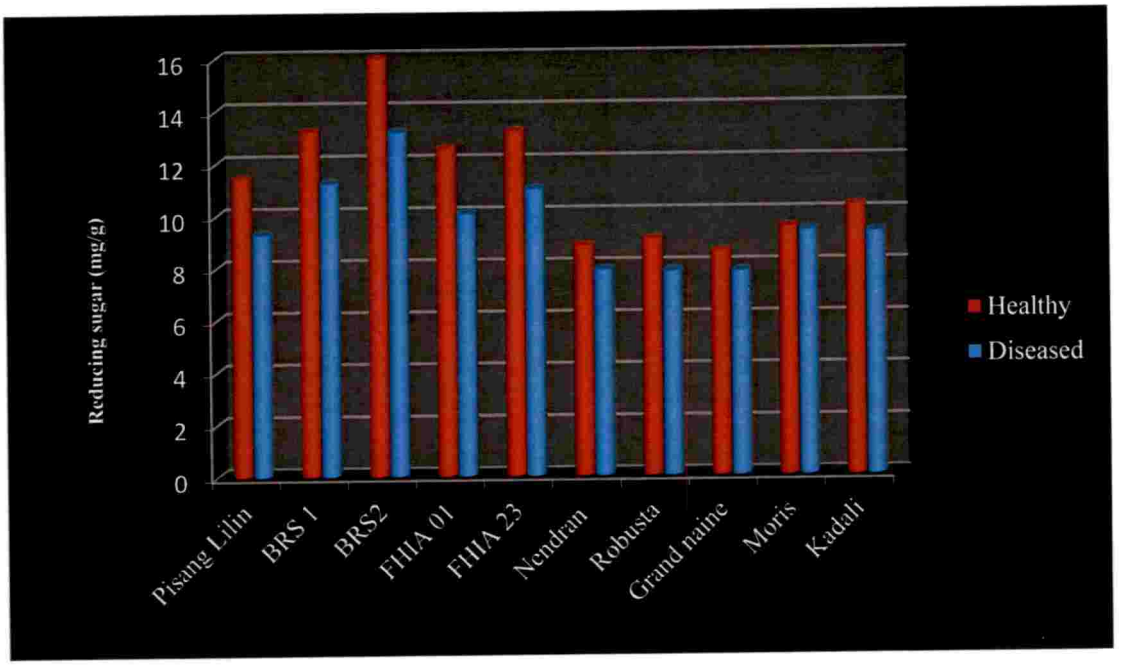

Fig 5.2 : Quantity of reducing sugar on resistant and susceptible accessions

Co- existence of both phenols and sugars resulted in glycolysation forming phenolic glycosides which were found to be more soluble in the cell sap thereby conferring disease resistance more efficiently (Walker, 1975).

The defense related enzymes such as peroxidase, polyphenol oxidase and phenylalanine lyase had a positive correlation with disease resistance in plants. These enzymes were involved in the oxidation of phenols into quinones which acts as a fungitoxic compound. Hence, the increased activity of the defense related enzymes might be responsible for disease resistance in plants.

Higher activity of peroxidase enzyme was noticed in the healthy leaves of resistant accessions while the activity was low in the susceptible accessions. In infected leaves, an increase in peroxidase activity was recorded on the resistant accessions while a decrease in enzyme activity was recorded in susceptible accessions (Fig 5.4). These observations were similar with the observations reported by Saharan and Kadian (1984) in cluster beans resistant against Alternaria blight and Kortekamp and Zyprian (2003) in resistant grapevine cultivar against downy mildew pathogen. This might be because peroxidases secreted by the plants during the attack of the pathogen have the ability to catalyze the generation of reactive oxygen species (ROS) coupled with oxidation of plant hormone indole-3-acetic acid (IAA) and defense-related compounds salicylic acid (SA), aromatic monoamines (AMAs) and chitooligosaccharides (COSs) which in turn stimulate the intracellular Ca^{2+} signaling and caused the polymerization of cell wall lignin thus help in plant defense response defense response (Kawano, 2003 and Fagerstedt *et al.*, 2010).

The activity of the polyphenol oxidase was observed to be highest in healthy leaves of the resistant accessions than that of the susceptible accessions. Trandafirescu *et al.* (1999) reported a positive correlation with the activity of polyphenol oxidase and resistant cultivars of *Stereum purpureum.* During infection of the leaves by pathogen, an increased level of polyphenol oxidase activity were observed in the resistant accessions as well as susceptible accessions. Though an

increase in activity was observed in both resistant and susceptible accessions, the activity of the enzyme was more in the resistant accessions when compared with susceptible ones (Fig 5.5). The results of the present investigations were in accordance with the results reported by Khirbat and Jalai (1999), Banu (2001), Li Li and Steffens (2002) and Raj *et al.,* 2006 they also noted an increased in levels polyphenol oxidase in the diseased leaves than in the healthy leaves.

The rapid stimulation of phenylalanine ammonia lyase (PAL) mRNA synthesis was observed as early defense response leading to accumulation of phenylpropanoid derived phytoalexin (Edwards *et al.,* 1985) and act as the precursors for the production of salicylic acid (Mani and Slusarenko, 1996) thereby inducing resistance against the disease in resistant cultivars. In the present study, it was observed that, comparatively higher levels of PAL activity were recorded in the resistant accessions than the susceptible cultivars (Fig 5.6). This observation were similar to the observations recorded by Chandra *et al.* (2007) they reported an increased levels of PAL activity in resistant cowpea cultivars when compared to the susceptible varieties. When the resistant and susceptible cultivars were infected by the pathogen a rapid increase in PAL were noted in resistant and susceptible cultivars, but an increased level of activity was noticed in the resistant cultivar compared to the susceptible ones. Banu (2001), Vanitha *et al.* (2009) and Ngadze *et al.* (2012) also reported similar responses in banana, tomato leaves and potato plants infected with *C. musae, Ralstonia solanacearum* and soft rot pathogen respectively.

The studies on molecular basis of disease resistance against Sigatoka leaf spot disease revealed that the amplification of DNA isolated from the selected resistant and susceptible accessions using the validated SCAR primers yielded an amplicon of size 644bp in resistant accessions and were absent in susceptible accessions. The sequences analysis of the amplicons in NCBI database using BLASTn analysis revealed 92% sequence homology to SCAR markers that were closely linked to Sigatoka leaf spot resistant gene of Kanthali clone1 (KJ801649.1). This result was similar to the results obtained by Das *et al.* (2016). In this study,

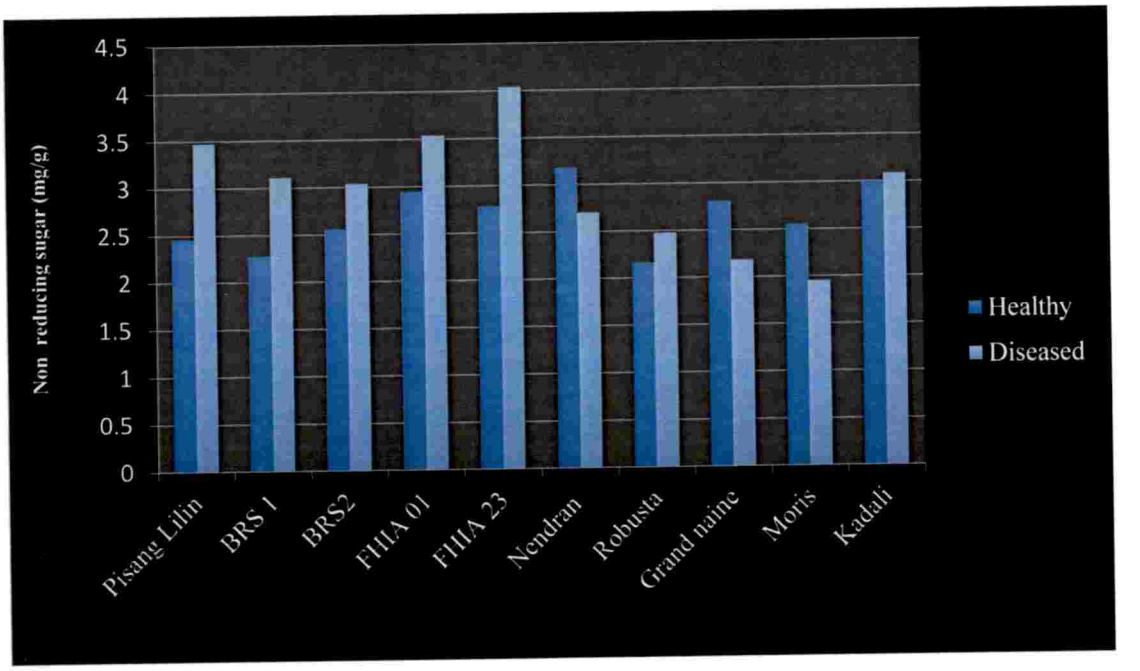

Fig 5.3: Quantity of non reducing sugar content on resistant and susceptible accessions

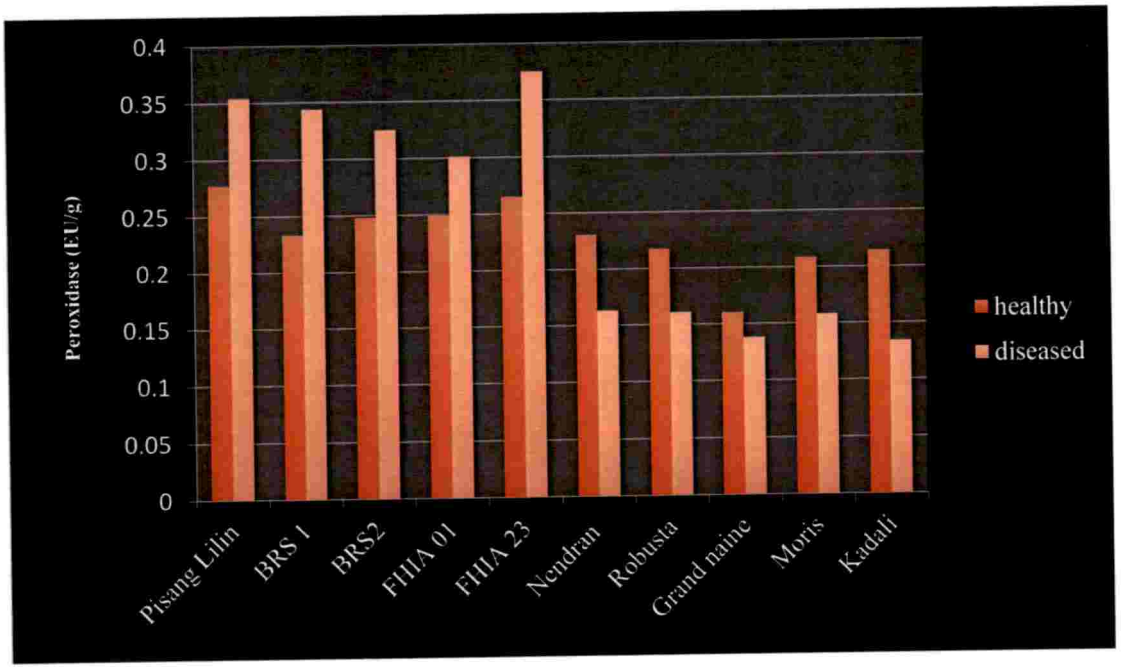

Fig 5.4: Activity of peroxidase enzyme on resistant and susceptible accessions

the RAPD-SCAR marker correctly amplified the desired fragment of 644bp in the selected five resistant accessions but were absent in the susceptible accessions. Therefore SCAR marker used in the study showed a strong association with Sigatoka leaf spot resistance and can be used effectively in marker assisted breeding programs.

Two separate field experiments were laid at Banana Research Station, Kannara with an objective to develop an effective management strategy for bringing down the severity of Sigatoka leaf spot disease by ecologically safe, environmentally viable and adoptable technology. Six selected chemical fungicides and eight bioagents / organic / inorganic preparations were given at the ten leaf spot stage of lowest leaf subsequently three sprays at fortnightly intervals. The results of the study revealed that all the treatments (six chemical fungicides and eight bioagents / organic / inorganic preparations) were significantly superior to the untreated controls.

The results on field evaluation of Sigatoka leaf spot disease with chemicals fungicides revealed that the plants sprayed with trifloxystrobin (25%) + tebuconazole (50%), 0.4g/l recorded the lowest mean per cent disease severity (PDS) of 15.43 % followed by T2 (copper hydroxide, 2g/l), T4 (hexaconazole + captan, 2g/l), T6 (Bordeaux mixture, 1%), T3 (propiconazole, 1ml/l), T1 pyraclostrobin, 1g/l), with a per cent disease severity of 16.65 %, 17.27%, 17.92%, 18.74% and 19.09% respectively. The PDS was noticed highest in unsprayed control (32.70 %). Hence, the maximum reduction in disease severity was recorded in plants sprayed with trifloxystrobin + tebuconazole, 0.4g/l. Pardeshi *et al.* (2016) reported that when the banana plants were sprayed with trifloxystrobin + tebuconazole, 0.7g/l recorded least disease severity of Sigatoka leaf spot disease (8.6%). Ruth and Nagalakshmi (2017) observed that banana plants sprayed with propiconazole (0.1%) and hexaconazole + captan (2g/l) were effective in management of Sigatoka leaf spot disease in banana. The time taken for development of disease and the youngest leaf spotted were highest in the plants sprayed with trifloxystrobin + tebuconazole.

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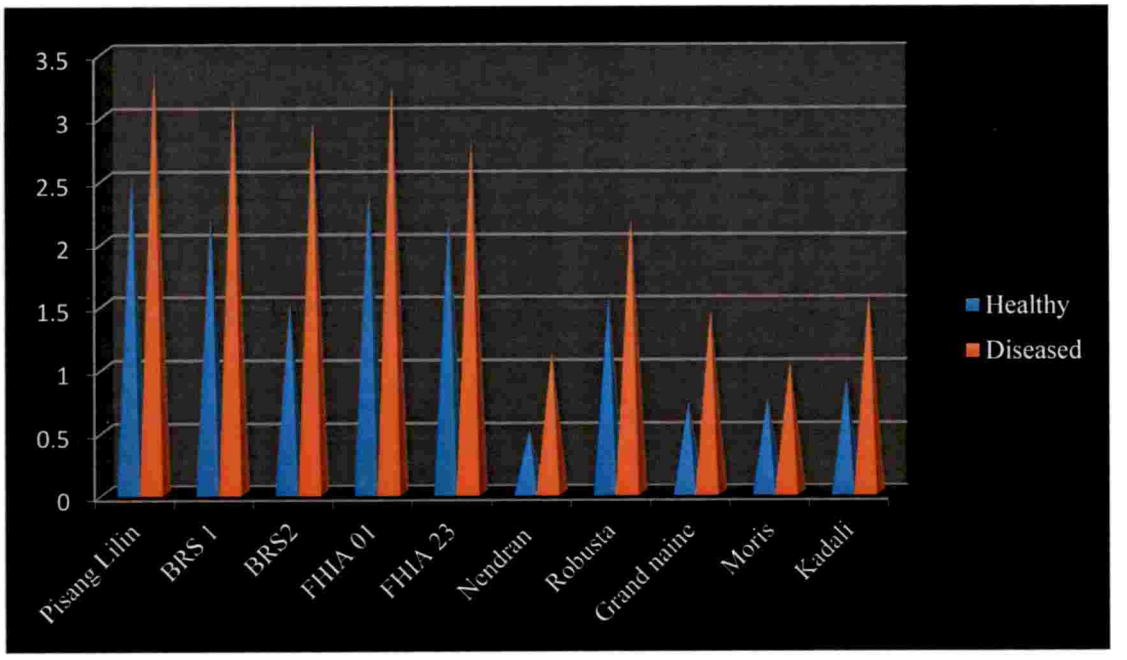

Fig 5.5: Activity of polyphenol oxidase on resistant and susceptible accessions

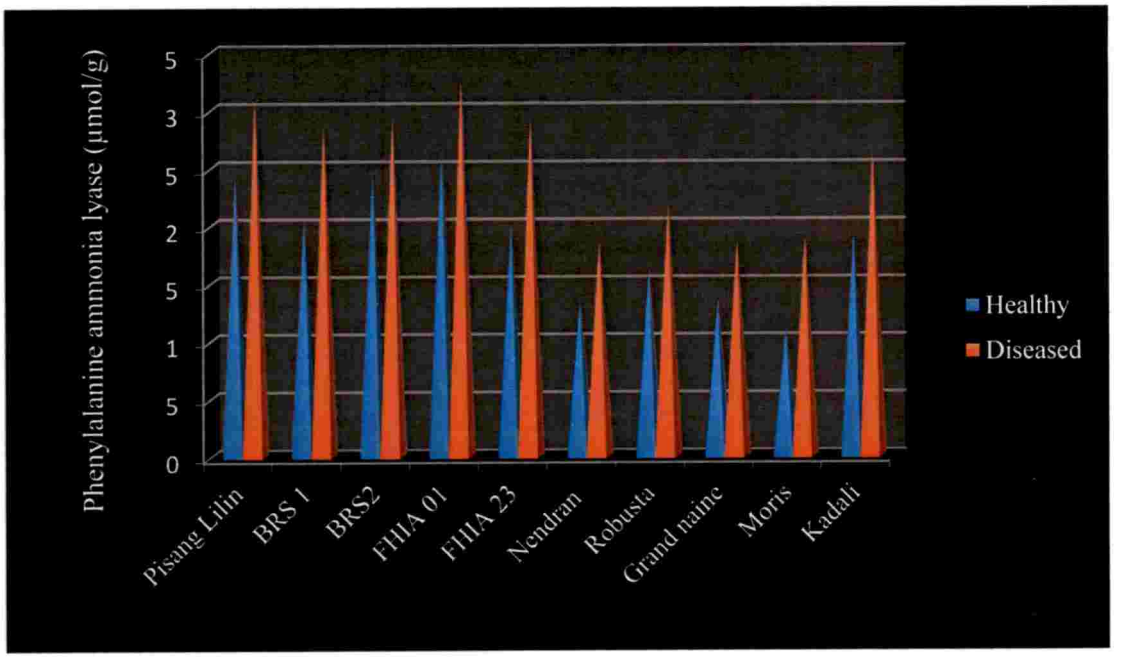

Fig 5.6: Activity of phenylalanine ammonia lyase on resistant and susceptible acessions

The use of bioagents /organic /inorganic preparations is an old age practices in India intended to provide cheap and effective options to the farmers in the country for effective management of diseases. The foliar application of selected bioagents/ organic/ inorganic preparations was effective in management of the Sigatoka leaf spot disease. Among the treatments, PDS of 20.84% was recorded in plants sprayed with Bordeaux mixture (1%) which serves as a chemical check. Similar recommendations were given by KAU (2011) in the package of practices, which recommended five to six sprayings with 1% Bordeaux mixture at fornightly interval soon after the appearance of the initial symptoms of Sigatoka leaf spot were effective in the management of the disease. This was followed by T2 (PGPR mix II, 2% , consortium of *P. fluorescens* + *B. subtilis*), T3 (*P. fluorescens,* 2%), T1 (cowdung extract + *P. fluorescens* (1%), T5 (salicylic acid, 25 ppm), T7 (petroleum based mineral oil, 0.1%), T4 (turmeric powder + baking soda mixture , 5:1), T6 (KAU micronutrient mix, 1%) with PDS of 20.93%, 21.72%, 22.74%, 22.76%, 23.44%, 23.79% and 24.53 % respectively. The highest PDS (32.70%) was recorded in plants without any treatment (T9). These observations were in agreement with the findings recorded by other scientists. The application of biocontrol agents such as *P. fluorescens* and *B. subtilis* were effective in reducing the disease pressure caused by *M. musicola* thereby increasing the yield of the plant (Hedge and Mesta, 2014). The assessment of inhibitory effect of salicylic acid on *M. fijiensis* revealed that the application of salicylic acid inhibited the mycelial growth and spore germination of the pathogen thereby effectively used in the management of the disease (Elisee *et al.,* 2014). Similarly Thammaiah and Shirol (2008) and Ruth and Nagalakshmi (2017) noted that petroleum based mineral oil was effective in the management of the disease by inhibiting the spore germination of the pathogen. In the present study, it was observed that the disease development time and youngest leaf spotted were highest in plants applied with PGPR mix II.

The studies on the residue analysis of the harvested fruits sprayed with different fungicides such as pyraclostrobin, trifloxystrobin + tebuconazole and hexaconazole + captan revealed that the fungicides left no residues on the banana

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fruits. Therefore the plants sprayed with the fungicides were efficient for the management of the disease as well as the fruits were safe to consume. Similar observations were reported by Mohapatra and Ajithakumar (2014) on banana fruits and soil sprayed with trifloxystobin + tebuconazole suggesting that this chemical can be used for the management of the disease considering the efficacy and safety of the fungicides.

Therefore the present investigation concluded that the pathogen associated with Sigatoka leaf spot disease of banana in Kerala is identified as *M. emusae* based on the symptomatology, cultural, morphological and molecular characterization of the pathogen. The screening of anatomical, biochemical and molecular basis of resistance of the host plant resistance revealed that the resistant accessions exhibited thicker cuticle, epidermis and mesophyll tissues on both the surface which acted as a first line of defense to the entry of the pathogen. The presence of less number of stomata and stomatal aperature on the lower surface of the leaves in resistant accessions restricted the entry of pathogen to the host tissue. The biochemical parameters such as phenols, reducing, non reducing sugars and the activity of defense related enzymes showed high values in resistant accessions when compared with the susceptible accessions which imparted resistance in resistant accessions. The presence of resistant gene in resistant accessions also contributed to the host plant resistance in the resistant cultivars. Therefore this gene could be used in marker assisted breeding programs in order to develop banana cultivar resistant to Sigatoka leaf spot disease. The studies on the management of the disease chemicals and organic/ inorganic treatments concluded that prophylactic spraying of chemical fungicides such as trifloxystrobin (25%) + tebuconazole (50%) (0.4g/l), copper hydroxide (2g/l), Bordeaux mixture (1%) and the bioagents such as PGPR mix II (20g/l) and *P. fluorescens* (20g/l) could bring down the disease severity to significantly to lower levels assuring effective management of the disease without leaving any residues on banana fruit and in the ecosystem.

The studies on the molecular basis of host pathogen interaction, population structure analysis of the pathogen, development of resistant varieties using the

identified resistant gene through marker assisted breeding and the popularization of disease management recommendations among the farmers can be included as the future line of work.

Summary

6. SUMMARY

Banana (*Musa* spp.) identified as a tropical treasure is grown extensively in the tropical as well as subtropical countries. Though the crop is grown under diverse agroclimatic conditions, its cultivation is often threatened by the incidence of an array of diseases. Among the various fungal diseases, Sigatoka leaf spot disease complex caused by *Mycosphaerella* spp. is a serious constraint to banana cultivation worldwide. In India, the Sigatoka disease is more prevalent in states of Kerala, Tamil Nadu, Karnataka, Maharastra, Gujarat, West Bengal and Tripura where the maximum disease severity of 20 - 40% was reported on commercially grown cultivars such as Nendran, Grand Naine and Njalipoovan *etc*. Though studies has been carried out, only little information is available on the molecular characterization of the pathogen associated with Sigatoka leaf spot disease complex, anatomical, biochemical and molecular basis of host plant resistance and the management of the disease using safer new molecule chemicals and bioagents/organic preparations. In this background, the present investigation "Characterization of *Mycosphaerella* spp. causing Sigatoka leaf spot disease complex of banana in Kerala and its management" was taken up to identify and characterize the pathogen associated with Sigatoka leaf spot disease in Kerala, to study the anatomical, biochemical and molecular basis of host plant resistance and to develop an effective integrated package using chemicals and organic/inorganic preparations to manage the disease in a sustainable manner.

Purposive sampling surveys at different agroclimatic zones of Kerala during two seasons *i.e.,* pre monsoon and post monsoon season revealed that the severity of the disease varied with the type of cultivars, the age of the plant, location and the season. The highest per cent disease severity (PDS) of 43.90% was recorded on nine months old banana var. Palayankodan at Pudukkad panchayath of Thrissur district during post monsoon survey and the lowest PDS of 3.33% was recorded during pre monsoon survey on three months old banana var. Njalipoovan at Thalikulam panchayath of Thrissur district.

The correlation analysis of per cent disease severity (PDS) with weather parameters such as rainfall, temperature and age of the plant revealed that PDS was positively correlated with rainfall and age of the plants but was negatively correlated with temperature. Therefore the incidence of the Sigatoka leaf spot disease was recorded to be more severe during rainy season indicated high rainfall, low temperature and high humidity favored the development of the disease.

The observations on variations in symptoms at different locations under field conditions revealed that there were six types of symptoms recorded on banana var. Nendran (AAB), two types each on Palayankodan (AAB) and Njalipoovan (AB), while only one type of symptom was recorded on Robusta (AAA) and Kadali (AA).

The stages of symptom expression on commercially grown cultivars *viz.*, Nendran, Grand Naine and Njalipoovan were categorized into six stages. The symptoms initially appeared as small light green to yellow dashes on the lower surface of the leaf which was visible only when the leaf was held against sunlight. This later changed into faint brown visible streaks. During the next stage, these streaks turned rusty brown on the adaxial surface of the leaves, which then developed into oval or elliptical brown spots with greyish centre surrounded by definite dark brown border with black pin head like fruiting bodies embedded in it. Upon heavy infection, the spots coalesced leading to complete necrosis of the leaves thereby destroying the functional green tissues of the leaves. Though the expression and stages of symptom development recorded during the two seasons *i.e.*, summer and rainy season were similar, the lesions were much darker in colour during the rainy season compared to the summer season giving a black appearance to the foliage and the spots were observed to get coalesced before maturation. The studies on the lesion size and the time taken to shift from stage 1 to stage 6 of the three cultivars during summer and rainy season revealed that the size of the lesions were smaller and the days required for symptom development was more during the summer season than in the rainy season.

The pathogen associated with Sigatoka leaf spot disease was isolated on

potato dextrose agar medium by leaf bit isolation technique. The fungal growth appeared 7- 10 days after isolation when incubated at 24°C. The colony was raised having irregular margins with greyish velvety appearance. No sporulation was observed in the culture. Among the different solid media such as potato dextrose agar (PDA), carrot agar (CA), V8-juice agar and banana leaf extract agar tested, the best media for the growth of the fungus was recorded as PDA while the least growth of the fungus was recorded in carrot agar.

The morphological studies revealed the presence of asexual structures and sexual fruiting bodies associated with different stages of lesions. The hyphae were brown coloured and sepatated which exhibited anastomosis or fusion of hyphae. The conidiophores were septate and emerged from conidiogenous cell. The conidia were septate, hyaline to olivaceous brown coloured without any scar and measured about 24.29 - 71.89 µm length x 0.91 - 2.40 µm width. The sexual fruiting body perithecia were flasked shaped, ostiolated and were dark brown in colour. The brusting of the mature perithecia released ascospores which were hyaline and 2- 3 septated. Based on the cultural and morphological characters of the pathogen was identified as *Mycosphaerella* spp. The molecular characterization of the pathogen by amplification of ITS- rDNA region using ITS 1 and ITS 4 primers yielded amplicons of 540 – 580 bp. *In silico* analysis of the nucleotide sequences of the amplicons at NCBI GeneBank using BLASTn analysis showed 99 - 100% sequence homology to *Mycosphaerella emusae* reported from CIRAD. Hence the pathogen inciting Sigatoka leaf spot disease in Kerala was identified and characterized as *Mycosphaerella emusae.*

Among the 100 accessions screened at the field genebank of Banana Research Station, Kannara, the most resistant accession was recorded as Calcutta 4 (AA) having PDS of 2.37% while the most susceptible was observed to be Grand Naine (AAA) (47.85%). The accessions belonging to AAA group were observed to be susceptible to Sigatoka leaf spot disease while the accessions belonging to AB, AAB, ABB and AAAB groups were observed to be resistant or moderately resistant to Sigatoka leaf spot disease. Five highly resistant accessions *viz.,* Pisang Lilin,

BRS 1, BRS 2, FHIA 01 and FHIA 23 and susceptible accessions *viz.,* Nendran, Grand Naine, Robusta, Moris and Kadali were selected for evaluation of anatomical, biochemical and molecular basis of host plant resistance.

The resistant accessions were characterized by thicker cuticle and epidermis on both lower and upper surface of the leaves, while the susceptible accessions had less cuticle and epidermal thickness on both the surface of the leaves. The thickness of the mesophyll tissues *viz.,* spongy and palisade tissue were found to be more in the resistant accessions than the susceptible accessions.

During infection by the pathogen, the infected leaves of resistant accessions recorded an increase in thickness of epidermis and the cuticle while no variations in thickness of the cuticle and epidermis were recorded on the upper surface but a decrease in cuticle and epidermal thickness was recorded on the lower surface of the susceptible accessions. The thickness of the mesophyll tissues increased in resistant accessions while a decrease in thickness was recorded in susceptible accessions.

The maximum number of the stomata and the stomatal pore width was recorded on susceptible accessions than that of the resistant ones. However, the number of stomata and the stomatal pore width was found to be more on the lower surface of both healthy and infected leaves of resistant and susceptible accessions than the upper surface. The stomatal pore width was observed to be minimum in the resistant accessions when compared with susceptible accessions where highest stomatal pore width was recorded.

The biochemical parameters such as phenolic content and the quantity of reducing and non reducing sugars were observed to be maximum in the healthy leaves of resistant accessions. While in infected leaves, a reduction in phenolic and reducing sugars was noticed in both resistant and susceptible accessions. In infected leaves, the amount of non reducing sugar increased in resistant accessions whereas a gradual reduction in non reducing sugar content was observed in susceptible accessions.

The studies on the activity of defense related enzymes revealed that the activity of the defense related enzymes such as peroxidase, polyphenol oxidase and phenylalanine ammonia lyase were highest in the resistant accessions than that of the susceptible accessions. During infection the activity of peroxidase enzyme increased in resistant accessions but the activity decreased in the susceptible accessions. The activity of polyphenol oxidase and phenylalanine lyase increased in both the resistant and susceptible accessions while the increase was more in the resistant accessions than that of the susceptible accessions.

The SCAR (Sequence characterized amplified regions) markers that were tightly linked to Sigatoka leaf spot disease resistance were used to identify the disease resistant gene associated with Sigatoka leaf spot disease in selected resistant and the susceptible accessions. The genomic DNA isolated from selected accessions were amplified using SCAR markers (SCAR F and SCAR R) which yielded an amplicon of size 644bp in resistant accessions *viz.,* Pisang Lilin, BRS1, BRS2, FHIA 01 and FHIA 23 while the amplicon was absent in the susceptible accessions such as Nendran, Grand Naine, Robusta, Moris and Kadali. Hence, the study revealed the presence of Sigatoka leaf spot disease resistant gene in resistant acessions while it was absent in susceptible accessions.

Two field experiments was conducted at Banana Research Station, Kannara to evaluate the efficiency of selected chemicals and bioagents /organic / inoragnic preparations for the management of Sigatoka leaf spot disease in banana. The results revealed that all the treatments were effective in the management of the disease. The plants sprayed with chemical fungicides showed less disease severity than that of bioagents /organic preparations. Among the fungicides used, the plants sprayed with trifloxystrobin (25%) + tebuconazole (50%) recorded the lowest per cent disease severity of 15.43 % followed by T2 (copper hydroxide), T4 (hexaconazole + captan, 2g/l), T6 (Bordeaux mixture,1%), T3 (propiconazole, 1ml/l), T1 (pyraclostrobin) with a per cent disease severity of 16. 65%, 17.27%, 17.92%, 18.74% and 19.09% respectively.

The studies on the effect of chemical fungicides on youngest leaf spotted (YLS) and disease development time (DDT) revealed that the highest YLS (8.00) was recorded in T5 (trifloxystrobin (25%) + tebuconazole (50%)) followed T3 (propiconazole), T2 (copper hydroxide) and T4 (hexaconazole (5%) + captan (70%)) with YLS of 7.54, 7.29 and 7.16 respectively. While the lowest YLS (5.18) was recorded in T7 (unsprayed control). In case of DDT the maximum DDT (50.66 days) was recorded in plants sprayed with T5 (trifloxystrobin (25%) + tebuconazole (50%), 0.4g/l) followed by T2 (copper hydroxide, 2g/l) and T4 (hexaconazole (5%) + captan (70%), 2g/l) having DDT of 49.33 days for both. The lowest DDT (32.66 days) was recorded in unsprayed control plants

The effect of chemical fungicides on vegetative and yield characters revealed that no significant differences were noticed in plant height and plant girth but the maximum number of functional green leaves were observed in plants sprayed with trifloxystrobin + tebuconazole and the lowest number of green leaves was noticed in plants without any treatment. The maximum bunch weight was recorded in plants treated with copper hydroxide and Bordeaux mixture having a bunch weight of 9.52 kg.

Among the bioagents/ organic/ inorganic preparations used, the PDS was lowest in plants sprayed with Bordeaux mixture (1%) which served as a chemical check. This was followed by T2 (PGPR mix II, 2%), T3 (*P. fluorescens,* 2%), T1 (cowdung extract + *P. fluorescens* (1%), T5 (salicylic acid, 25 ppm), T7 (petroleum based mineral oil, 0.1%), T4 (turmeric powder + baking soda mixture , 5:1), T6 (KAU micronutrient mix, 1%) with PDS of 20.93%, 21.72%, 22.74%, 22.76%, 23.44%, 23.79% and 24.53 % respectively. The highest PDS (32.70%) was recorded in plants without any treatment (T9).

The effect of bioagents/ organic / inorganic preparations on YLS and DDT revealed that the highest YLS (6.87) was recorded in T3 (*P. fluorescens,* 2%) as well as in T8 (Bordeaux mixture, chemical check). These treatments were followed by T3 (PGPR mix II, 2%), T7 (petroleum based mineral oil, 0.1%), T5

(salicylic acid, 25 ppm), T4 (turmeric powder + baking soda mixture, 5:1), T6 (KAU micronutrient mix, 1%) and T1 (cowdung extract + *P. fluorescens*, 1%) with YLS of 6.69, 6.67, 6.41, 6.37, 6.15 and 6.12 respectively. While the lowest YLS (5.16) was recorded in T9 (untreated control).

The highest DDT (44.00 days) was recorded in plants sprayed with Bordeaux mixture (chemical check). This was followed by T4 (turmeric powder + baking soda mixture), T2 (PGPR mix II), T6 (KAU micronutrient mix), T3 (*P. fluorescens*), T5 (salicylic acid), T1(cowdung extract + *P. fluorescens*) and T7 (petroleum based mineral oil) having DDT of 43, 40, 39.66, 38.66, 38, 37.33, 35.33 days. The lowest DDT (32.66 days) was noticed in plants without any treatment (T9)

The effect of organic/ inorganic preparations on vegetative and yield characters revealed that no significant differences were recorded in plant height but the maximum plant girth and number of functional leaves were noted in plants applied with PGPR mix II (2%) and salicylic acid (25 ppm). Also the yield was found to be maximum in plants sprayed with *P. fluorescens* (2%) having a bunch weight of 8.47 kg.

The studies on the economic analysis of various treatments revealed that at the market price of Rs 40/kg B: C ratio was highest in plants sprayed with Bordeaux mixture in both the experiments while the minimum B:C ratio was recorded in the untreated control .

The analysis of fungicide residue on fruits revealed that there was no residues of the chemicals left in fruit when the spraying was stopped at the shooting stage indicated that the chemicals were safe to use and could be included in the integrated management practices for the control of the disease.

Therefore the present study revealed that foliar application of systemic fungicide trifloxystrobin (25%) + tebuconazole (50%) (0.4g/l), protectant fungicide Bordeaux mixture (1%) and bioagents PGPR mix II (consortium of *P. fluorescens*

and *Bacillus subtilis*) (2%) starting when ten leaf spot stage was visible on the lowest leaves of majority of plants and subsequently three to four sprays at fortnightly intervals could effectively manage Sigatoka leaf spot disease without leaving any residues of the chemicals on the harvested fruits.

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Appendix

APPENDIX – 1

COMPOSITION OF THE MEDIA USED

1.Potato Dextrose Agar

Potato	- 200g
Agar- agar	- 20g
Dextrose	- 20g
Distilled water	- 1000 ml

2.Carrot Agar

Carrot	- 200g
Agar – agar	- 20g
Dextrose	- 20g
Distilled water	- 1000ml

3. Banana leaf extract agar

Banana leaf	- 200g
Agar –agar	- 20g
Distilled water	- 1000ml

4. V8- j ice agar

V8- juice	- 100ml
L- Asparagine	- 10g
Yeast extract	- 2g
Calcium carbonate	- 2g
Glucose	- 2g
Agar	- 20g
Distilled water	- 1000ml

APPENDIX – II

PREPARATION OF ELECTROPHORESIS BUFFER

TAE (Tris Acetate EDTA) BUFFER (10 X)

Tris buffer	- 24.2 g
Glacial acetic acid	- 5.71 ml
0.5M EDTA (pH 8.0)	- 100ml

TBE (Tris Borate EDTA) BUFFER (10X)

Tris base	- 108 g
Boric acid	- 55 g
20mM EDTA (pH 8.0)	- 100 ml

APPENDIX III

NUCLEOTIDE SEQUENCE OF THE ISOLATES COLLECTED DURING SURVEY

>S1
AGTTCCGTAGGGTGGACCTGCGGAGGGATCATTACTGAGTGAGGGCTCACGCCCGAC
CTCCAACCCTCTGTGAACCACACTTGTTGCTTCGGGGGCGACCCTGCCGGCGAACTCG
TCGCCGGGCGCCCCCGGAGGTCTTCTAAACACTGCATCTTTGCGTCGGAGTTCAAAAC
AAATCGAACAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC
AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGA
ACGCACATTGCGCCCTTTGGTATTCCGAAGGGCATGCCTGTTCGAGCGTCATTTCACC
ACTCAAGCCTGGCTTGGTATTGGGCGTCGCGGTGTTTCGCGCGCCTTAAAGTCTTCCG
GCTGAGCTGTCCGTCTCCTAGCGTTGTGGATTCTTCAATTCGCTTCGGAGTGCGGGCG
GCCGCGGCCGTTAAATCTTTATTGAAAGGTTGACCTCGGATCAGGTAGGGATACCCGC
TGAACTTAAGCATATCAATAAGCGGA

>S2
CTCCGTAGGGGTGACCTGCGGAGGGATCATTACTGAGTGAGGGCTCACGCCCGACCT
CCAACCCTCTGTGAACCACACTTGTTGCTTCGGGGGCGACCCTGCCGGCGAACTCGTC
GCCGGGCGCCCCCGGAGGTCTTCTAAACACTGCATCTTTGCGTCGGAGTTCAAAACAA
ATCGAACAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC
GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
ACATTGCGCCCTTTGGTATTCCGAAGGGCATGCCTGTTCGAGCGTCATTTCACCACTC
AAGCCTGGCTTGGTATTGGGCGTCGCGGTGTTTCGCGCGCCTTAAAGTCTTCCGGCTG
AGCTGTCCGTCTCCTAGCGTTGTGGATTCTTCAATTCGCTTCGGAGTGCGGGCGGCCG
CGGCCGTTAAATCTTTATTGAAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAA
CTTAAGCATATCAA

>S3
TTCCGTAGGGGTGACCTGCGGAGGGATCATTACTGAGTGAGGGCTCACGCCCGACCTC
CAACCCTCTGTGAACCACACTTGTTGCTTCGGGGGCGACCCTGCCGGCGAACTCGTCG
CCGGGCGCCCCCGGAGGTCTTCTAAACACTGCATCTTTGCGTCGGAGTTCAAAACAAA
TCGAACAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG
AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCA
CATTGCGCCCTTTGGTATTCCGAAGGGCATGCCTGTTCGAGCGTCATTTCACCACTCA
AGCCTGGCTTGGTATTGGGCGTCGCGGTGTTTCGCGCGCCTTAAAGTCTTCCGGCTGA
GCTGTCCGTCTCCTAGCGTTGTGGATTCTTCAATTCGCTTCGGAGTGCGGGCGGCCGC
GGCCGTTAAATCTTTATTGAAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAAC
TTAAGCATATCAA

>S4
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>S35
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>S36
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APPENDIX IV

NUCLEOTIDE SEQUENCE OF THE *Alternaria* sp. ISOLATES

>S1
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>S2
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CHARACTERIZATION OF *Mycosphaerella* spp. CAUSING SIGATOKA LEAF SPOT DISEASE COMPLEX OF BANANA IN KERALA AND ITS MANAGEMENT

by

Milsha George
(2015-21-009)

ABSTRACT OF THE THESIS

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DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA

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ABSTRACT

Sigatoka leaf spot disease complex caused by *Mycosphaerella* spp. is a serious constraint to banana cultivation in Kerala. The present study was undertaken to characterize the associated pathogen and to develop an integrated management package against this disease.

The project initiated with purposive sampling surveys conducted in various districts representing different agroclimatic zones of Kerala *viz.* Malappuram (Northern zone), Palakkad (Northern zone), Thrissur (Central zone), Ernakulam (Central zone), Wayanad (High range zone) and Trivandrum (Southern zone). The percent disease severity (PDS) ranged from 3.33 to 43.90%. The correlation analysis of PDS with weather parameters showed a positive correlation with rainfall however, it was found to be negatively correlated with temperature.

The study on symptomatology revealed that there were six types of symptoms noticed on banana var. Nendran (AAB), two types each on Palayankodan (AAB) and Njalipoovan (AB), while only one type of symptom was recorded on Robusta (AAA) and Kadali (AA).

Isolation of the pathogen could be achieved on potato dextrose agar medium after 10- 12 days of incubation at very specific conditions. The fungal colony was slightly raised with irregular margin and greyish velvety appearance. Studies on morphological structures revealed that the conidia were hyaline to olivaceous brown in colour which measured about 24.29 - 71.89 µm length x 0.91 - 2.40 µm width with 3-8 septations. The sexual structures were flask shaped perithecia containing long asci bearing eight ascospores. Based on cultural and morphological characters, the pathogen was identified as *Mycosphaerella eumusae*. The identity of the pathogen was further confirmed by PCR based molecular characterization. *In silico* analysis of the sequences of the isolates showed 99 -100% homology to *Mycosphaerella eumusae*. Hence, it is concluded that the pathogen associated with Sigatoka leaf spot disease of banana in Kerala is

identified as *Mycosphaerella eumusae* Crous & Mourichon (anamorph *Pseudocercospora eumusae* Crous & Mourichon).

The screening of accessions maintained in the Germplasm of Banana Research Station, Kannara was done to assess their disease reaction and were grouped into six categories. Five resistant varieties *viz.,* Pisang Lilin, BRS 1, BRS 2, FHIA 01 and FHIA 23 and susceptible varieties *viz.,* Nendran, Grand Naine, Robusta, Moris and Kadali were further selected to investigate the anatomical, biochemical and molecular basis of host plant resistance.

Anatomical studies revealed that the resistant varieties were characterized by thicker cuticle, epidermis and mesophyll tissues compared to the susceptible varieties. The number of stomata and the stomatal pore width were more in susceptible varieties compared to the resistant varieties.

The biochemical basis of resistance was assessed by quantifying phenols, reducing sugars, non reducing sugars and the activity of defense related enzymes *viz.,* peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in both resistant and susceptible varieties. The results revealed that the phenols, reducing sugars and the defense related enzymes were higher in resistant varieties compared to susceptible ones.

The amplification of genomic DNA of resistant and susceptible varieties using Sequence characterized amplified region (SCAR) markers yielded an amplicon of size 644bp in resistant cultivars while the bands were absent in susceptible cultivar. The BLASTn analysis of the sequence of the amplicons showed 90 per cent sequence homology to genomic sequences of Kanthali SCAR marker OPA1363 which is tightly linked to Sigatoka leaf spot disease resistance.

Field experiments were conducted to evaluate the efficacy of chemical fungicides and organic / inorganic preparations for disease management. Among the various chemical fungicides, foliar spraying with trifloxystrobin (25%) + tebuconazole (50%), 0.4g/l) was found to be the best followed by copper

hydroxide, hexaconazole (5%) + captan (70%), 2g/l) and Bordeaux mixture (1%), without leaving any toxic residues in the harvested fruits. The results of the experiment on disease management using organic / inorganic preparations revealed the effectiveness of PGPR mix II (consortium of *Pseudomonas fluorescens* and *Bacillus subtilis*), 2% followed by *Pseudomonas fluorescens* (2%) given as foliar spray. It is concluded that the present study has enlightened our knowledge on the etiology and management of Sigatoka leaf spot disease of banana cultivation in Kerala.